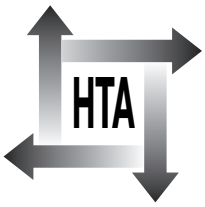
Health Technology Assessment 2007; Vol. 11: No. 36

A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food

I Abubakar, L Irvine, CF Aldus, GM Wyatt, R Fordham, S Schelenz, L Shepstone, A Howe, M Peck and PR Hunter

September 2007

Health Technology Assessment
NHS R&D HTA Programme
www.hta.ac.uk



How to obtain copies of this and other HTA Programme reports.
An electronic version of this publication, in Adobe Acrobat format, is available for downloading free of charge for personal use from the HTA website (http://www.hta.ac.uk). A fully searchable CD-ROM is also available (see below).

Printed copies of HTA monographs cost £20 each (post and packing free in the UK) to both public **and** private sector purchasers from our Despatch Agents.

Non-UK purchasers will have to pay a small fee for post and packing. For European countries the cost is £2 per monograph and for the rest of the world £3 per monograph.

You can order HTA monographs from our Despatch Agents:

– fax (with **credit card** or **official purchase order**)
– post (with **credit card** or **official purchase order** or **cheque**)
– phone during office hours (**credit card** only).

Additionally the HTA website allows you **either** to pay securely by credit card **or** to print out your order and then post or fax it.

Contact details are as follows:

HTA Despatch
c/o Direct Mail Works Ltd
4 Oakwood Business Centre
Downley, HAVANT PO9 2NP, UK

Email: orders@hta.ac.uk
Tel: 02392 492 000
Fax: 02392 478 555
Fax from outside the UK: +44 2392 478 555

NHS libraries can subscribe free of charge. Public libraries can subscribe at a very reduced cost of £100 for each volume (normally comprising 30–40 titles). The commercial subscription rate is £300 per volume. Please see our website for details. Subscriptions can only be purchased for the current or forthcoming volume.

Payment methods

Paying by cheque
If you pay by cheque, the cheque must be in **pounds sterling**, made payable to *Direct Mail Works Ltd* and drawn on a bank with a UK address.

Paying by credit card
The following cards are accepted by phone, fax, post or via the website ordering pages: Delta, Eurocard, Mastercard, Solo, Switch and Visa. We advise against sending credit card details in a plain email.

Paying by official purchase order
You can post or fax these, but they must be from public bodies (i.e. NHS or universities) within the UK. We cannot at present accept purchase orders from commercial companies or from outside the UK.

How do I get a copy of *HTA on CD*?

Please use the form on the HTA website (www.hta.ac.uk/htacd.htm). Or contact Direct Mail Works (see contact details above) by email, post, fax or phone. *HTA on CD* is currently free of charge worldwide.

The website also provides information about the HTA Programme and lists the membership of the various committees.

A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food

I Abubakar,[1*] L Irvine,[1] CF Aldus,[2] GM Wyatt,[2] R Fordham,[1] S Schelenz,[3] L Shepstone,[1] A Howe,[1] M Peck[2] and PR Hunter[1]

[1] School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK

[2] Institute of Food Research, Norwich Research Park, Norwich, UK

[3] Department of Microbiology, Norfolk and Norwich University Hospital, Norwich, UK

* Corresponding author

Declared competing interests of authors: none

Published September 2007

This report should be referenced as follows:

Abubakar I, Irvine L, Aldus CF, Wyatt GM, Fordham R, Schelenz S, *et al*. A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. *Health Technol Assess* 2007;**11**(36).

Health Technology Assessment is indexed and abstracted in *Index Medicus*/MEDLINE, *Excerpta Medica*/EMBASE and *Science Citation Index Expanded (SciSearch®)* and *Current Contents®*/Clinical Medicine.

NIHR Health Technology Assessment Programme

The Health Technology Assessment (HTA) programme, now part of the National Institute for Health Research (NIHR), was set up in 1993. It produces high-quality research information on the costs, effectiveness and broader impact of health technologies for those who use, manage and provide care in the NHS. 'Health technologies' are broadly defined to include all interventions used to promote health, prevent and treat disease, and improve rehabilitation and long-term care, rather than settings of care.

The research findings from the HTA Programme directly influence decision-making bodies such as the National Institute for Health and Clinical Excellence (NICE) and the National Screening Committee (NSC). HTA findings also help to improve the quality of clinical practice in the NHS indirectly in that they form a key component of the 'National Knowledge Service'.

The HTA Programme is needs-led in that it fills gaps in the evidence needed by the NHS. There are three routes to the start of projects.

First is the commissioned route. Suggestions for research are actively sought from people working in the NHS, the public and consumer groups and professional bodies such as royal colleges and NHS trusts. These suggestions are carefully prioritised by panels of independent experts (including NHS service users). The HTA Programme then commissions the research by competitive tender.

Secondly, the HTA Programme provides grants for clinical trials for researchers who identify research questions. These are assessed for importance to patients and the NHS, and scientific rigour.

Thirdly, through its Technology Assessment Report (TAR) call-off contract, the HTA Programme commissions bespoke reports, principally for NICE, but also for other policy-makers. TARs bring together evidence on the value of specific technologies.

Some HTA research projects, including TARs, may take only months, others need several years. They can cost from as little as £40,000 to over £1 million, and may involve synthesising existing evidence, undertaking a trial, or other research collecting new data to answer a research problem.

The final reports from HTA projects are peer-reviewed by a number of independent expert referees before publication in the widely read monograph series *Health Technology Assessment*.

Criteria for inclusion in the HTA monograph series

Reports are published in the HTA monograph series if (1) they have resulted from work for the HTA Programme, and (2) they are of a sufficiently high scientific quality as assessed by the referees and editors.

Reviews in *Health Technology Assessment* are termed 'systematic' when the account of the search, appraisal and synthesis methods (to minimise biases and random errors) would, in theory, permit the replication of the review by others.

The research reported in this monograph was commissioned by the HTA Programme as project number 03/40/03. The contractual start date was in April 2005. The draft report began editorial review in May 2006 and was accepted for publication in January 2007. As the funder, by devising a commissioning brief, the HTA Programme specified the research question and study design. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The HTA editors and publisher have tried to ensure the accuracy of the authors' report and would like to thank the referees for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this report.

The views expressed in this publication are those of the authors and not necessarily those of the HTA Programme or the Department of Health.

ISSN 1366-5278

Published by Gray Publishing, Tunbridge Wells, Kent, on behalf of NCCHTA.
Printed on acid-free paper in the UK by St Edmundsbury Press Ltd, Bury St Edmunds, Suffolk.

Abstract

A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food

I Abubakar,[1*] L Irvine,[1] CF Aldus,[2] GM Wyatt,[2] R Fordham,[1] S Schelenz,[3] L Shepstone,[1] A Howe,[1] M Peck[2] and PR Hunter[1]

[1] School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK

[2] Institute of Food Research, Norwich Research Park, Norwich, UK

[3] Department of Microbiology, Norfolk and Norwich University Hospital, Norwich, UK

* Corresponding author

Objectives: To determine the diagnostic accuracy of tests for the rapid diagnosis of bacterial food poisoning in clinical and public health practice and to estimate the cost-effectiveness of these assays in a hypothetical population in order to inform policy on the use of these tests.

Data sources: Studies evaluating diagnostic accuracy of rapid tests were retrieved using electronic databases and handsearching reference lists and key journals. Hospital laboratories and test manufacturers were contacted for cost data, and clinicians involved in the care of patients with food poisoning were invited to discuss the conclusions of this review using the nominal group technique.

Review methods: A systematic review of the current medical literature on assays used for the rapid diagnosis of bacterial food poisoning was carried out. Specific organisms under review were *Salmonella*, *Campylobacter*, *Escherichia coli* O157, *Staphylococcus aureus*, *Clostridium perfringens* and *Bacillus cereus*. Data extraction was undertaken using standardised data extraction forms. Where a sufficient number of studies evaluating comparable tests were identified, meta-analysis was performed. A decision analytic model was developed, using effectiveness data from the review and cost data from hospitals and manufacturers, which contributed to an assessment of the cost-effectiveness of rapid tests in a hypothetical UK population. Finally, diagnostic accuracy and cost-effectiveness results were presented to a focus group of GPs, microbiologists and consultants in communicable disease control, to assess professional opinion on the use of rapid tests in the diagnosis of food poisoning.

Results: Good test performance levels were observed with rapid test methods, especially for polymerase chain reaction (PCR) assays. The estimated levels of diagnostic accuracy using the area under the curve of the summary receiver operating characteristic curve was very high. Indeed, although traditional culture is the natural reference test to use for comparative statistical analysis, on many occasions the rapid test outperforms culture, detecting additional 'truly' positive cases of food-borne illness. The significance of these additional positives requires further investigation. Economic modelling suggests that adoption of rapid tests in combination with routine culture is unlikely to be cost-effective, however, as the cost of rapid technologies decreases; total replacement with rapid technologies may be feasible.

Conclusions: Despite the relatively poor quality of reporting of studies evaluating rapid detection methods, the reviewed evidence shows that PCR for *Campylobacter*, *Salmonella* and *E. coli* O157 is potentially very successful in identifying pathogens, possibly detecting more than the number currently reported using culture. Less is known about the benefits of testing for *B. cereus*, *C. perfringens* and *S. aureus*. Further investigation is needed on how clinical outcomes may be altered if test results are available more quickly and at a greater precision than in the current practice of bacterial culture.

Contents

List of abbreviations

ACMSF	Advisory Committee on the Microbiological Safety of Food
ANS	appropriate non-standard
AUC	area under the curve
CCDA	charcoal cefoperazone desoxycholate agar
CCDC	Consultant in Communicable Disease Control
CER	cost-effectiveness ratio
cfu	colony-forming units
CHO	chinese hamster ovary (assay)
CI	confidence interval
DOR	diagnostic odds ratio
EHEC	enterohaemorrhagic *Escherichia coli*
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FIA	fluorescence immunoassay
FN	false negative
FP	false positive
FPR	false positive rate
FSA	Food Standards Agency
FSML	Food Safety Microbiology Laboratory
GBS	Guillain–Barré syndrome
HGMF	hydrophobic grid membrane filters
HUS	haemolytic uraemic syndrome
ICER	incremental cost-effectiveness ratio
IID	infectious intestinal disease
IMS	immunomagnetic separation
MPN	most probable number
MRSA	methicillin-resistant *Staphylococcus aureus*
NAA	nucleic acid amplification
NGT	nominal group technique
NPV	negative predictive value
OR	odds ratio
PCR	polymerase chain reaction
POC	point of care
PPV	positive predictive value
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
QUORUM	Quality of Reporting of Meta-analyses
RPLA	reverse passive latex agglutination
rtPCR	real-time polymerase chain reaction
SD	standard deviation
SE	standard error
SMAC	sorbitol MacConkey agar
SROC	summary receiver operating characteristic
STARD	standards for reporting studies of diagnostic accuracy
STEC	shiga toxin-producing *Escherichia coli*
TN	true negative
TP	true positive
TPR	true positive rate
VCA	Vero cell toxicity assay
VTEC	verocytotoxin-producing *Escherichia coli*

All abbreviations that have been used in this report are listed here unless the abbreviation is well known (e.g. NHS), or it has been used only once, or it is a non-standard abbreviation used only in figures/tables/appendices in which case the abbreviation is defined in the figure legend or at the end of the table.

Executive summary

Background

Technological advances have increased the speed of diagnostic testing for many diseases. However, for bacterial food poisoning, stool culture, which can take up to 1 week, is still the only method routinely used for diagnosis in most UK microbiology laboratories.

The principle methodologies emerging for the rapid diagnosis of food poisoning are immunoassays, which detect antigens or antibodies from pathogens, and polymerase chain reaction (PCR), a commonly used technique to amplify and detect pathogenic DNA/RNA. Both techniques may significantly reduce the detection time for pathogens in faecal or food samples, compared with traditional culture methods.

This systematic review focused on the use of rapid tests for six bacterial food-borne pathogens: *Salmonella*, *Campylobacter*, *Escherichia coli* O157, *Clostridium perfringens*, *Staphylococcus aureus* and *Bacillus cereus*. Diagnostic accuracy was assessed, and an economic model was subsequently developed, assessing costs and cost-effectiveness of PCR and immunoassays, compared with culture.

Methods

Standard systematic review methods were applied. Literature was identified from electronic databases and further handsearching. Study findings were extracted using a predesigned and piloted tool in duplicate to avoid errors. The methodological quality of studies was assessed using a standard tool.

Data synthesis

Sensitivity, specificity and diagnostic odds ratios were presented in forest plots. Studies within clinically appropriate groups were subjected to meta-analysis. Evidence for heterogeneity was assessed using a χ^2 test and the I^2 statistic. Where correlation between sensitivity and specificity was evident (measured using Spearman's ρ), a summary receiver operating characteristic (SROC) curve was generated. Area under the curve (AUC) was the main measure of diagnostic accuracy. In the absence of correlation, pooled estimates of sensitivity and specificity were presented. Evidence of publication bias was examined using funnel plots of log odds ratios.

Results

The electronic search identified 1853 studies, 87 of which were included in this review. The quality of studies was variable for studies included in meta-analysis; however, in studies discussed narratively (principally for toxin-inducing pathogens), reporting was generally poor.

Clinical effectiveness

Campylobacter

SROC analysis was performed on six studies, evaluating PCR for the 16s rRNA gene. Combining 4495 samples, AUC was 0.987 [95% confidence interval (CI) 0.984 to 0.989]. Four studies (2078 samples) evaluated the ProSpecT immunoassay (Alexon-Trend), and reported an overall AUC of 0.862 (95% CI 0.568 to 1.000).

Salmonella

Identified test methods included PCR, Wellcolex Colour agar, MUCAP test, Wampole Bactigen and AutoMicroBic identification system. Combining 2134 samples (from seven studies), the AUC value for PCR was 0.995 (95% CI 0.985 to 1.000); however, publication bias was evident. Other tests exhibited very high diagnostic odds ratios (DORs), ranging from 264 (95% CI 116.9 to 597.6) (Wampole Bactigen) to 2951 (95% CI 710.9 to 12000) (Wellcolex Colour).

E. coli

SROC analysis for PCR assays showed very high diagnostic accuracy (AUC 0.996, 95% CI 0.990 to 1.000); however, publication bias was evident, compared with VTEC-Screen reverse passive latex agglutination (RPLA) results (AUC 0.994, 95% CI 0.982 to 1.000), which was not affected by publication bias. The Premier enterohaemorrhagic *Escherichia coli* (EHEC) immunoassay had high pooled sensitivity and specificity values (0.935 and 0.997, respectively), which were not correlated. Other entrohaemorrhagic *E. coli* tests evaluated included ProSpecT, Duopath Verotoxin, ImmunoCard Stat and RidaScreen Verotoxin.

A very limited number of studies evaluated rapid diagnostic methods against an appropriate reference standard for *C. perfringens*, *B. cereus* and staphylococcal food poisoning. Therefore, it was not possible to assess effectiveness using statistical methods.

Although traditional culture is the logical reference test to use, on many occasions the rapid test outperformed culture, detecting more positive cases of food-borne illness. Immunological and PCR tests may be useful for 'multiplexing', thereby providing simultaneous speciation or characterisation.

Cost-effectiveness

Cost estimates for each test method were derived from published sources, contact with manufacturers and discussion with laboratory staff. A decision analytic model was developed to assess their cost-effectiveness and the sensitivity of these results to changes in various parameters in the model was assessed.

Evidence about the relative costs of implementing rapid diagnostic methods in practice is sparse and highly uncertain. The isolation rate of the reviewed pathogens is low in laboratories. This implies that the provision of routine tests can be very expensive. At the baseline, testing one sample for *Campylobacter*, *Salmonella* and *E. coli* will cost £18.85 with PCR, £15.66 with immunoassays and £15.01 by culture methods. The most sensitive parameter in the decision analytic model is the isolation rate for each pathogen. Adoption of rapid tests in combination with routine culture is unlikely to be cost-effective; however, as the cost of rapid technologies decreases, total replacement with rapid technologies may be feasible. With multiplex PCR tests, if multiple pathogens could be simultaneously detected in the same reaction tube, molecular diagnosis may prove very cost-effective; however, there are insufficient published evaluations of these assays at present.

Conclusions

Evidence from this systematic review suggests that rapid diagnostic assays, especially PCR, for *Salmonella*, *Campylobacter* and *E. coli* O157 are highly accurate. Less is known about the benefits of testing for toxin-producing pathogens and the significance of additional positives detected by these assays. It is unclear whether the additional benefits derived from early diagnosis and more sensitive detection can justify the large set-up costs of rapid tests, particularly if they remain diagnostic adjuncts to culture. Any decisions regarding the use of these assays must consider the speed of diagnosis (including transportation and reporting delays), effect on clinical outcome and costs of implementation simultaneously.

Implications for research

The effectiveness and cost-effectiveness of emerging tests for more than one organism at a time, such as multiplex PCR and DNA microarrays technologies, require further investigation.

Substantial evidence suggests that rapid assays may be more sensitive than culture methods. Attempting to evaluate diagnostic tests in the absence of a true gold standard creates methodological challenges.

Implications for practice

The feasibility of conversion to rapid methods is dependent on localised considerations, including the community prevalence rates for specific pathogens, the skill base and subsequent training costs for laboratory staff and spare capacity available to ensure adequate laboratory space for new equipment. Although these tests show good promise for the future, further studies are needed to assess their immediate use in practice.

Chapter 1

Aim of the review

Technological advances in the past two decades have substantially increased the possibility of rapid diagnostic testing for many diseases. However, for bacterial pathogens which cause food-borne infections or food poisoning, traditional culture methods, which can take up to 1 week, are still the only method routinely used for diagnosis in many microbiology laboratories throughout the UK.

This systematic review of rapid tests for food-borne pathogens was commissioned by the NHS R&D Health Technology Assessment (HTA) programme, in order to assess the accuracy and cost-effectiveness of these emerging technologies. A principal aim of this review was to address important questions regarding the reliability, accuracy and cost-effectiveness of the tests from both clinical and public health viewpoints. The review also sought to address the feasibility of such tests being adopted more widely by microbiology laboratories throughout the UK.

Rapid diagnostic tests may be more expensive than traditional culture methods. However, this potential for an increase in laboratory costs needs to be balanced against a possible reduction in overall costs, such as those that may result from delays in identification of a pathogen with culture. Clinical staff such as microbiologists and GPs may also vary in their views of how much clinical impact a change in test usage might have, and changes of health service technologies will need to take into account not only clinical but also human and practical factors.

Key research aims

Key research aims were the following:

1. to identify studies on rapid diagnostic methods for food poisoning due to *Salmonella* spp., *Campylobacter*, *Escherichia coli* O157, *Clostridium perfringens*, *Staphylococcus aureus* and *Bacillus cereus* relevant to both the food chain and clinical samples
2. to assess and summarise the sensitivity and specificity of each diagnostic test for each organism compared to a gold standard
3. for tests designed and/or currently applied only to food samples, to assess usefulness for transfer to clinical testing
4. to assess the time for full laboratory analysis and reporting for each diagnostic test
5. to develop a decision analytic model to assess the cost and cost-effectiveness of each diagnostic test in a clinical setting and in the management of outbreaks
6. to make recommendations for future research based on this systematic review of evidence.

Chapter 2
Background

Description of the underlying health problem

Food poisoning or food-borne illness may occur after the consumption of food containing toxins or organisms that multiply to cause disease.[1] The Advisory Committee on the Microbiological Safety of Food (ACMSF) has defined food poisoning as "any disease of an infectious or toxic nature caused by or thought to be caused by the consumption of food or water".[2] The Food Standards Agency (FSA) has set a target to reduce cases of food poisoning by 20% between 2001 and 2006. The FSA chose to focus on microorganisms that cause the greatest number of cases of food poisoning, *Salmonella*, *Campylobacter* and *C. perfringens*, and those that cause severe disease: *Escherichia coli* O157 and *Listeria*.

The largest study of infectious intestinal disease (IID) carried out in the UK to date[3] estimated that 20% of the population of England suffer from food poisoning (approximately 10 million persons) each year. It was estimated that, at 1993–5 prices, IID in England costs some £750 million per year. About 20% of these results were directly due to the consumption of food. The contribution of individual pathogens as causative agents appears to have changed over the last decade, with developments in food technology and changes in both dietary habits and certain methods of food retailing combining to bring about a resurgence of some food-borne infections. The importance of food poisoning as a significant cause of morbidity remains high. *Table 1* includes the number of laboratory reports in 1995 and 2005.

Food-borne infection, for the majority of sufferers, does not require medical treatment. However, it is important not to underestimate its significance. A factor to consider when investigating food-borne pathogens is the potential for outbreaks of the disease to occur. A food-borne outbreak is defined as an occurrence of two or more cases of a similar illness resulting from the ingestion of a common food. In addition, the consequences of food-borne

TABLE 1 *Features of the six bacterial pathogens included in this review*

	Usual incubation period	Usual duration of symptoms	Common clinical features	Common mode of transmission	Laboratory reports in England and Wales	
					1995	2005
Bacterial intestinal infection						
Campylobacter spp.	2–5 days	2 days–1 week	D, P, Fe, B	F, W, An	43,876	44,342
Salmonella (non-typhoid)	12–72 hours	<3 weeks	V, D, Fe	F, X	29,314	6,639
E. coli O157[a]	1–6 days	4–6 days (not HUS)	D, B, HUS	F, X, W, An	792	946
Toxin-induced food poisoning						
Clostridium perfringens	12–18 hours	24 hours	D, P	F	342	41
Bacillus cereus – emetic	1–5 hours	24 hours	N, V, D, P	F	NA	NA
Bacillus cereus – diarrhoeal	8–16 hours	24 hours	D, V, N, P	F	87	NA
Staphylococcus aureus	2–4 hours	<12–48 hours	V, P, Fe	F	59	0

An, animal contact; B, blood in stool; D, diarrhoea; F, food; Fe, fever; HUS, haemolytic uraemic syndrome; N, nausea; P, abdominal pain; V, vomiting; W, water; X, person to person (faecal–oral).

[a] The nomenclature of enterohaemorrhagic *E. coli* is still confusing. The toxins are referred to as 'verocytotoxins' or 'shiga-toxins', and the toxin-producing strains verocytotoxin-producing *Escherichia coli* (VTEC) or shiga toxin-producing *Escherichia coli* (STEC). The term enterohaemorrhagic *E. coli* (EHEC) refers to a subset of VTEC strains belonging to serogroup O157 and to a few other serogroups that cause a clinical illness similar to that caused by *E. coli* O157.

Source: *Report of the Study of Infectious Intestinal Disease in England (2001)*. London: Food Standards Agency and Centre for Infections Statistics: 2001.

infection are often not confined to the primary patient alone, owing to the risk of person-to-person spread. In this sense, there is an increased urgency in identifying and confirming the cause of gastrointestinal illness. Delay in diagnosis may cause prolongation of the period during which a case remains infectious in the community. Most food-borne illnesses are self-limiting, particularly those caused by toxins formed in the food before consumption. However, a proportion of patients, especially those who are particularly vulnerable or infected with certain pathogens, may require a specific therapy and a delay in diagnosis may result in delayed treatment, with consequential increases in morbidity. Occasionally, food-borne infections may progress to more severe conditions such as septicaemia,[4] meningitis,[5] haemolytic uraemic syndrome,[6] reactive arthritis[7] and Guillain–Barré syndrome (GBS).[8] The appropriate identification of the aetiologic agent of infectious gastroenteritis is important, since there are major differences in the treatment required for the different agents.[9]

It is difficult to differentiate potential causes of enteric illness based on clinical features alone. Therefore, when a sample is microbiologically investigated, a collection of pathogens must be screened for.

Bacterial food-borne illnesses can be classed in two main groups: gastrointestinal infections, leading to proliferation of pathogenic microbes in the infected host (e.g. salmonellosis), and toxin-induced food poisoning, due to the presence of a preformed toxin resulting from bacterial growth (e.g. *B. cereus*) in the food. The mechanism of pathogenicity for some gastrointestinal infections also involves toxin production *in vivo* after consumption of the organism (e.g. *C. perfringens*).

There is a wide variety of food-borne pathogenic microorganisms and natural toxins. This systematic review will concentrate on six bacterial causes of food poisoning: *Salmonella* spp., *Campylobacter jejuni* and *E. coli* O157, which all cause gastrointestinal infection, *S. aureus* and *B. cereus*, which cause food-borne intoxications, and *C. perfringens*, which can act as an infection or an intoxication. These organisms were selected mainly because they are the commonest causes of bacterial food poisoning and outbreaks of food poisoning in the UK.[10] The inclusion of VTEC is necessary due to the severity of the disease it causes, and *S. aureus* and *B. cereus* because, like *C. perfringens*, they are diagnosed with toxin detection methods. Other organisms were not included individually either because they are uncommon, not relevant in community or caused primarily by food poisoning (*Clostridium difficile*), or predominantly transmitted through other routes (*Shigella* spp.). Viral food poisoning and food-borne disease relating to parasites are substantially different from bacterial causes, and are excluded from this review.

Shigella infection has decreased rapidly since the peak incidence period of 1950–69, when 20,000–40,000 cases per annum were reported in the UK. Most years since 1980 have seen less than 5000 reports. Humans are the only significant reservoir of infection, and food-borne outbreaks are relatively uncommon.[11]

Table 1 summarises the aetiological features of the six bacterial pathogens included in this review. Trends in microbiological identification are summarised in *Table 2* and reporting trends are illustrated in *Figure 1*.

As indicated, the estimated volume of food-borne illness each year far outweighs the number of laboratory reports recorded. It is impracticable and unnecessary to refer all patients with acute diarrhoea for microbiological investigation. The existence of a 'reporting pyramid' is notable with food-borne illnesses. It is estimated that for every 1000 cases of food-borne illness in the community, 160 present to their GP, 45 have a stool sent for routine microbiological examination and only 10 have positive results reported. Only a fraction of all food-borne illnesses are ever diagnosed and officially reported, or can be traced to a certain vehicle or a specific causative agent.[3]

Campylobacter jejuni

Bacteriological background

Campylobacters are Gram-negative bacteria which are important animal and human pathogens. Although there are 11 different species in total, this review focuses on *C. jejuni*, the cause of most reported *Campylobacter*-related human illnesses. Most laboratories routinely use selective media designed for *C. jejuni* alone, hence incidence of the less common species may be under-reported.[12]

Incidence

Campylobacter is the most commonly identified food-borne bacterial infection encountered in the world. In 2000, approximately 56,000 cases were formally recorded in UK laboratory reports, with as many as 400,000 expected to occur in total.[10] The number of laboratory reports has decreased

TABLE 2 *Trends in microbiological identification of* Campylobacter jejuni, Salmonella enteriditis *and* Escherichia coli *O157: 1995–2005*

Year	*C. jejuni*	*S. enteriditis*	*E. coli* O157
1995	43,449	15,691	792
1996	43,978	17,880	660
1997	51,360	22,254	1,087
1998	56,852	16,048	890
1999	56,254	10,454	1,084
2000	57,674	8,267	896
2001	54,917	10,491	768
2002	47,834	9,505	595
2003	46,178	9,767	675
2004	42,146	8,203	701
2005	44,342	6,639	946

Source: Health Protection Agency laboratory reports.

slightly in recent years, but a high degree of under-reporting is still expected. Although some outbreaks have been reported, most cases occur sporadically. It is most commonly transmitted by raw poultry, raw milk and water contaminated by animal faeces.

Symptoms

Campylobacteriosis is an acute bacterial enteric disease ranging from asymptomatic to severe, with diarrhoea, nausea, vomiting, fever and abdominal pain, with illness usually lasting 2–5 days. Although *Campylobacter* is in itself a relatively harmless pathogen, it can cause post-infectious complications which are potentially very serious. *Campylobacter* is believed to be a leading cause of GBS, an autoimmune reaction that causes paralysis and kills between 5 and 10% of its victims. Approximately 1 in 1000 cases of *C. jejuni* develops into GBS after 7–21 days of infection.[13] Reitter's syndrome, a form of reactive arthopathy, can also occur in up to 1% of *campylobacteriosis* patients.[9]

Treatment

In the general population, campylobacteriosis is a self-limiting disease, for which antimicrobial therapy is not required.[14] However, as with many intestinal infections, infants and immunocompromised individuals are at higher risk of developing more severe infection.

Current diagnosis

Identification methods for *Campylobacter* have traditionally involved the use of selective culture media which contain several antimicrobial agents to suppress the growth of other bacteria. This process is followed by biochemical tests such as nitrate/nitrite reduction, hippurate hydrolysis and nalidixic acid susceptibility. Although selective media are very useful for the initial isolation of *Campylobacter*, biochemical methods for identification are often tedious and may give ambiguous results. Isolation of the organism requires inoculation of faecal samples on a selective medium, followed by microaerobic incubation at 37 or 42°C for 48 hours. A further 24–48 hours are required for full phenotypic identification.[15]

Salmonella

Incidence

Salmonellae have been some of the most frequently reported aetiological agents in fresh produce-associated outbreaks of human infections in recent years, with over 15,000 laboratory diagnoses in 2000 arising from an estimated 41,616 cases.[10] The highest incidence rates occur in patients aged under 1 year and in individuals older than 70 years.

More prudent food hygiene regulations have seen the reported cases of *Salmonella enteritis* PT4 (long associated with egg consumption) decline considerably. However, other PT4 strains have been slowly and steadily increasing in incidence. In 2005 there were 1771 reported cases of *S. enteridis* PT4 and 4868 reported cases of *S. enteridis* (other PTs) in the UK.

Transmission

Most infections are acquired by eating contaminated poultry, eggs or dairy products. According to some estimates, almost three-quarters of all broiler chickens are contaminated

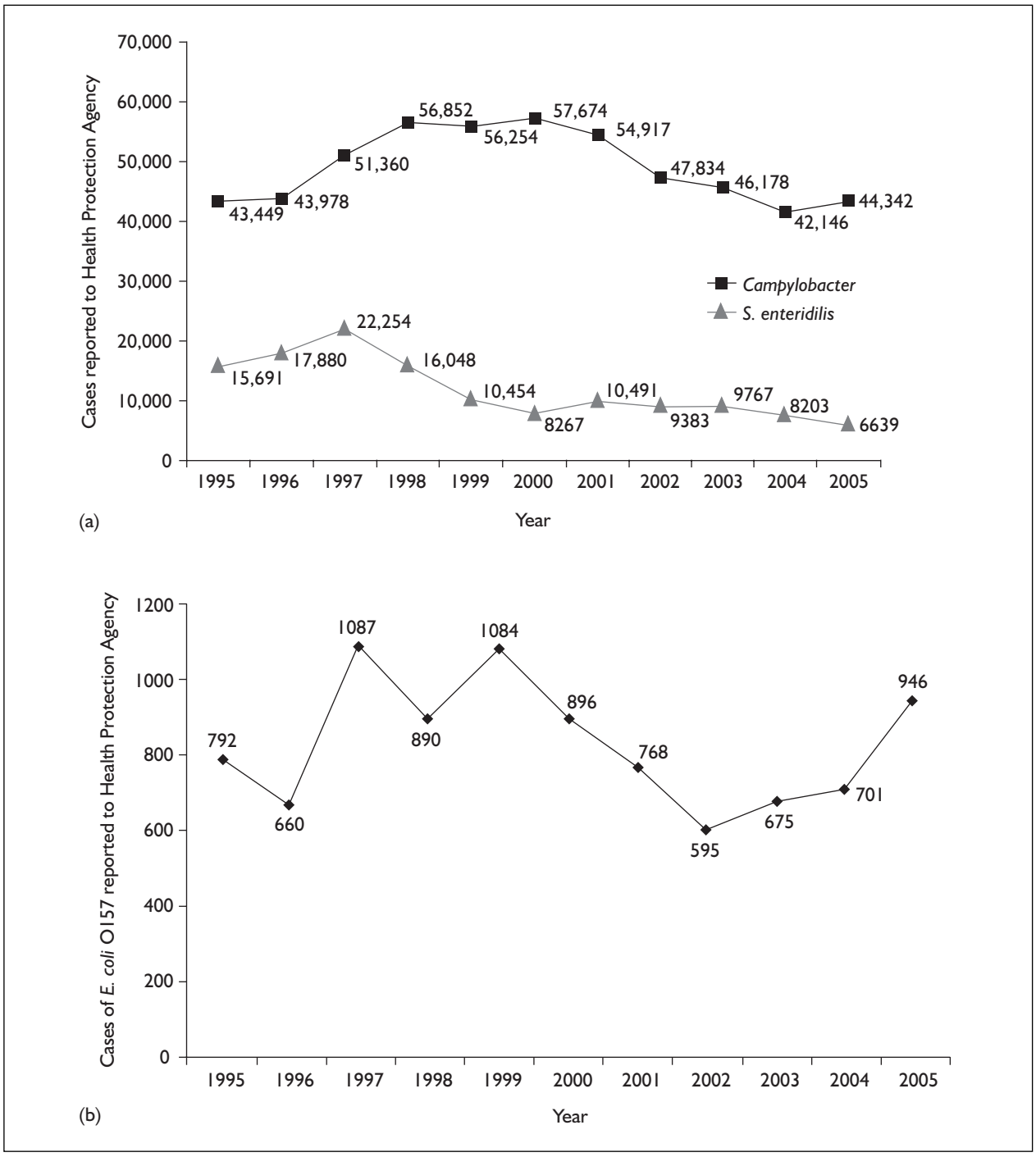


FIGURE 1 *Reporting trends in England and Wales for (a)* Campylobacter *and* Salmonella *and (b)* E. coli *O157, 1995–2005. Source: Health Protection Agency laboratory reports.*

with *Salmonella* during defeathering, slaughtering and evisceration, when faeces splatter the skin.[16] The *Salmonella* also spreads easily from raw or undercooked poultry to innocent vegetables, fruit or other foods via contaminated hands, knives, countertops or cutting boards. Due to the ability of *Salmonella* to multiply in a wide variety of foods, it is important to be able to isolate the organisms even when present in very small numbers in the faeces.

Symptoms

The symptoms of *Salmonella* infection are abdominal pain, diarrhoea, mild fever, chills, headache, nausea and vomiting, developing 12–72 hours (but occasionally as long as 7 days)

after infection. The discomfort generally lasts a few days. It can be dangerous for the elderly, infants and the immunocompromised, who may become extremely ill. *Salmonella* is also one of the leading predictors for reactive arthritis, a painful, chronic and potentially debilitating condition that causes joint inflammation.[17]

Treatment

Salmonella infection in older children and adults is usually a self-limiting disease (presenting as acute gastroenteritis), and therapy should mainly be directed at preventing dehydration. A recent Cochrane review[18] of antibiotic treatment for *Salmonella* gut infections suggested that they provided no clinical benefits to otherwise healthy children and adults with non-severe cases. Antibiotic administration may, in fact, prolong *Salmonella*.[19] However, it is justified to use antimicrobial therapy for infants under 3 months old with *Salmonella* gastroenteritis, and also in immunocompromised patients and patients with septicaemia. In these patients, antibiotic treatment will be most successful in the early stages of illness, and delaying treatment may result in septicaemia-related dehydration and renal failure.[9] Because the early stage is often clinically difficult to determine, these patients might benefit from rapid tests that can be done quickly in the place where the patient is receiving care.

Traditional methods for the isolation of *Salmonellase* use enrichment and selective media followed by serological and biochemical identification. These methods require a large amount of technical expertise and are labour intensive. Positive identification may be time consuming, with a positive result taking up to 3 days before confirmation. There are several selective plating media for the isolation of *Salmonellae* from human faeces and other specimens, but their sensitivity and specificity vary considerably. Due to the high rates of false-positive results, screening of stool samples for *Salmonella* becomes labour intensive, with additional costs for subsequent identification.

Escherichia coli O157

Background

The genus *Escherichia* consists of five species, of which *E. coli* is the most common and clinically most important. Since its recognition in 1982, *E. coli* O157:H7 has been noted as one of the most dangerous pathogens, as only very small numbers of the organism may be required to cause illness. For this reason, suspect colonies must be handled with care. All tests need to be carried out in a safety cabinet, usually in a biohazard room. It is the only pathogen in this discussion classed as 'category three' risk.

Incidence

The incidence of *E. coli* O157 tends to fluctuate, reflecting the outbreak-specific nature of disease. The highest levels (1087 cases) in the UK were recorded in 1997, largely associated with a highly publicised outbreak in Central Scotland.[20] On average there are approximately 600–800 confirmed cases each year, with 946 recorded in 2005, linked to a large outbreak in South Wales.

Transmission

Cattle are the principal reservoir of enterohaemorrhagic *E. coli* (EHEC) and the majority of large outbreaks have been food-borne.[21] A major source of *E. coli* O157 is ground beef; other sources include consumption of unpasteurised milk and juice, sprouts, lettuce and salami and contact with cattle. The organism is easily transmitted from person to person and has been difficult to control in schools and nurseries. Rapid detection of the causative pathogen is therefore an important contribution to the effective prevention of infection.[22]

Symptoms

E. coli O157 can cause acute bloody diarrhoea and abdominal cramps. Persons who only have diarrhoea usually recover completely, without antibiotics or other specific treatment, in 5–10 days. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics may precipitate kidney complications. Antidiarrhoeal agents, such as loperamide (Imodium®), should also be avoided.

Complications

Certain patients, particularly infants, the elderly and the immunocompromised, are at higher risk of developing secondary complications, which can substantially increase the potential risks of *E. coli* infection. EHEC has been strongly associated with haemorrhagic colitis and the more severe complications of haemolytic uraemic syndrome (HUS),[23] the major reason for an acute loss of kidney function in childhood. Blood transfusions and kidney dialysis are often required. Patients who develop HUS often require prolonged hospitalisation, dialysis and long-term follow-up. With intensive care, the death rate for HUS is 3–5%. Although reported cases of *E. coli* O157 are

less frequent than those of other enteric pathogens, such as *Campylobacter* and *Salmonella*, due to the morbidity and mortality associated with secondary complication, this pathogen is now considered a major public health problem of worldwide importance.

Diagnosis

Vero cell cytotoxicity assays are probably the most sensitive method of detecting shiga toxin *E. coli* strains such as O157:H7. However, most hospital laboratories would not routinely perform tissue culture work with Vero cell monolayers available on demand. Moreover, Vero cell assay results are generally not available for 48–72 hours. Instead, sorbitol MacConkey agar is currently used to detect *E. coli* O157 in UK laboratories. Isolation of *E. coli* O157:H7 from water and other environmental samples is laborious. Culture is problematic due to the large numbers of other flora that either overgrow or mimic the non-sorbitol-fermenting *E. coli* O157:H7.[24]

Non-O157 strains

Although several EHEC serotypes have been associated with human infection, recent well-publicised outbreaks of infection with *E. coli* O157:H7 have resulted in a focus on the development of methods for the identification of the specific EHEC serotype. The number of documented infections with *stec* other than O157:H7 is probably an underestimate, due to the use of serotype-specific methods.[25] This single serotype-directed effort is in part justifiable, in that the majority of HUS cases are caused by *E. coli* O157:H7. However, this may lead to a failure in the assessment of the prevalence of other EHEC isolates associated with human disease and also may leave health authorities unprepared for the emergence of new clones of these organisms.

Clostridium perfringens

C. perfringens has been shown to be a common, although often under-reported, cause of bacterial food poisoning, with its enterotoxin being responsible for the disease symptoms of diarrhoea and abdominal pain. Diagnosis and outbreak analysis of *C. perfringens* food poisoning can often be difficult, since this organism can exist as part of the normal gut flora in humans.

These pathogens are considered of economic importance, even in the cases of mild, self-limiting illnesses, due to the high sanitary costs and also their negative repercussions in the food processing industry. *C. perfringens* is one of the most easily preventable food-borne pathogens, as temperature abuse in prepared foods is the most common cause of infection. The refrigeration of food after preparation prevents the production of enterotoxin. Alternatively, reheating of the food can destroy the heat-labile enterotoxin. Good food handling practices will also reduce the risk of disease.

Diarrhoea due to *C. perfringens* may arise from consumption of contaminated food, be associated with antibiotic treatment or be the result of spread of the organism by person-to-person transmission among residents of institutions. Diarrhoea is caused by enterotoxin, which is released in the intestine during sporulation.

Incidence

Food-borne *C. perfringens* intoxication is a relatively common but underappreciated bacterial disease. The estimated total number of cases of *C. perfringens* was as high as 276,266 in 1992, although only 806 of these were confirmed by laboratory diagnosis. By 2000, the annual number of cases had fallen to 84,081, and the number of laboratory reports also decreased substantially to 245.[10] It is ubiquitous in the environment and frequently occurs in the intestinal tract; however, a large number of vegetative cells (more than 10^6) need to be ingested before infection occurs.

Foods commonly associated with *C. perfringens* food-borne disease are cooked meats or poultry held at improper temperatures. Infection is most likely to occur when large quantities of food are prepared several hours before serving, such as in institutions such as school cafeterias and nursing homes.

Symptoms

C. perfringens food poisoning may cause mild to acute gastroenteritis. Symptoms, including watery diarrhoea and intense abdominal cramps, are usually experienced within 8–22 hours after eating contaminated food. The illness is usually self-limiting, lasting less than 24 hours.

Individuals most at risk include pregnant women, newborn babies, the elderly and the immunocompromised.[26] Elderly people, in particular, may experience prolonged or severe symptoms. In the past 5 years, 58% of patients admitted to hospital with food-borne *C. perfringens* intoxication were aged 75 years and above, with duration of hospital stay as long as 80 days. On the whole, fatal cases of *C. perfringens* are very rare.

Current diagnosis

C. perfringens serotyping is available in only a few laboratories worldwide. This organism loses viability rapidly in foods that have been stored refrigerated, and faecal counts can still be high in elderly people in the absence of illness. Detection of enterotoxin may be the only test available when investigating an outbreak of food poisoning. Unfortunately, *C. perfringens* does not readily produce toxin *in vitro* and no single method has been found to induce toxin production in all strains. Because of this, testing isolates for toxin production is not performed routinely, indicated by the very low proportion of suspected cases which are formally identified. If specifically requested by the sending clinician, serological assays are used for detecting enterotoxin in the faeces of patients. Bacteriological confirmation can also be possible by finding extremely large numbers of the causative bacteria in implicated foods or in the faeces of patients.

Bacillus cereus

Bacterial background

B. cereus is a Gram-negative, spore-forming, motile, aerobic rod that also grows well anaerobically. It has been recognised as an opportunistic pathogen of increasing importance. Two types of illness have been attributed to the consumption of food contaminated with *B. cereus*: emetic and diarrhoeal food poisoning syndromes, each formed from separate toxins (see *Table 3*). For both types of food poisoning, the food involved has usually been heat treated, and surviving spores germinate to produce somatic cells and toxins. Some food types that are preferentially contaminated with *B. cereus* are crude cereals, starchy food, dairy products, meat, dehydrated foods and spices. *B. cereus* grows well after cooking and cooling (<48°C).

Symptoms

B. cereus food poisoning is under-reported as both types of illness are relatively mild and usually last for less than 24 hours. However, occasional reports have described fatal incidents associated with emetic toxins.[27]

Emetic strains

The emetic syndrome is caused by toxin formed in food. The number of organisms needed for illness is thought to be in the region of $\geqslant 10^5$ colony-forming units (cfu) g^{-1} food. Emetic activity is extremely stable, being unaffected by heating or by extremes of pH.

Diarrhoeal strains

Diarrhoeal *B. cereus* syndrome is caused by enterotoxin which is released in the intestine and may also be preformed in foods. The number of organisms needed to cause illness is thought to be at least 10^5 cfu g^{-1} food. Laboratory confirmation of *B. cereus* diarrhoeal food poisoning requires demonstration of $\geqslant$105 cfu g^{-1} of food or faeces, or the detection of enterotoxin in food or faeces. *B. cereus* occurs widely in pasteurised dairy products and a significant proportion of these isolates is capable of psychotrophic growth and enterotoxin production,[28] which has prompted an interest in the examination of strains for the ability to produce toxin.

Conventional procedures for the detection of *B. cereus* involve the plate count method and the most probable number (MPN) method. *B. cereus* does not ferment mannitol, and most strains

TABLE 3 *Characteristics of the two types of disease caused by* Bacillus cereus

	Diarrhoeal syndrome	Emetic syndrome
Infective dose (cells g^{-1})	10^5–10^7 (total)	10^5–10^8
Toxin produced	In small intestine of host	Preformed in cells
Type of toxin	Protein	Cyclic peptide
Incubation period (hours)	8–16 (occasionally >24)	0.5–5
Duration of illness (hours)	12–24 (occasionally several days)	6–24
Symptoms	Abdominal pain, watery diarrhoea and occasionally nausea	Nausea, vomiting and malaise (sometimes followed by diarrhoea, due to additional enterotoxin diarrhoea)
Foods most frequently implicated	Meat products, soups, vegetables, puddings/sauces and milk/milk products	Fried and cooked rice, pasta, pastry and noodles

develop a positive egg yolk reaction. Detection of low numbers of *B. cereus* is especially difficult if the foods are heavily contaminated with other microorganisms.

Staphylococcus aureus

Incidence

Staphylococcal food poisoning is caused by ingestion of enterotoxins that are produced in foods by some strains of S. aureus. The most common toxins implicated in staphylococcal food poisoning are *sea* to *sej*, which cause 95% of all outbreaks.[29] Since most people recover within 1–2 days, many do not seek medical advice, and the incidence of disease is thought to be under-reported. In 1995 there were an estimated 13,989 cases of infectious intestinal illness caused by *S. aureus* in England and Wales, yet only 59 laboratory reports were recorded.[10]

Symptoms

The most common symptoms experienced with staphylococcal food poisoning are nausea, vomiting, diarrhoea and abdominal cramping, and illness is commonly confused with that of emetic *B. cereus*. These symptoms usually appear within 1–6 hours of consuming infected food; however, the onset and severity of illness are usually dependent on the amount of contaminated food eaten, the amount of toxin ingested and the individual's susceptibility to it. Growth of enterotoxigenic strains to $\geqslant 10^5$ cfu g^{-1} food is generally considered necessary to produce enough to induce illness. Detection of staphylococcal enterotoxin in a suspect food is very strong evidence of involvement of that food in an outbreak.

Illness usually lasts 1–2 days, including abrupt, sometimes violent, onset with nausea, cramps, vomiting, diarrhoea, hypotension and prostration.[11]

Transmission

A wide variety of foods have been associated with staphylococcal food poisoning, including meat, eggs, bakery and dairy products. *S. aureus* usually contaminate the food during the handling stage after cooking.

Current diagnosis

Due to the self-limiting nature of the illness, diagnosis of staphylococcal food poisoning is usually determined by clinical presentation. Faecal testing for *S. aureus* is performed after specific requests from the sending clinician. In these cases, a commercially available reverse passive latex agglutination assay (SET-RPLA) is the most commonly used assay for *S. aureus* detection. The production of a sufficient amount of toxin is essential for successful detection. Subsequently, the propensity for this test to produce false-negative results is elevated.

Current service provision

Current diagnosis of infectious intestinal disease in the UK, and most other countries, still relies on traditional culture methods. Although considerable research has been carried out to develop new detection techniques, little advance has been made to implement them on a routine basis. The Health Protection Agency Laboratory of Enteric Pathogens (LEP) is a reference laboratory service which provides further phenotyping and epidemiological referencing for culture-positive samples from UK and abroad. Although the methods used have the potential to provide rapid results, this service is not primarily used as a way to achieve faster diagnosis, but rather as a confirmatory reference service using the most sensitive typing techniques currently available.

In-surgery laboratory services are usually limited to dipstick urine testing, pregnancy tests and finger-stick blood glucose determinations. Point of care testing for food poisoning is unavailable in the UK. Testing on faecal samples does not routinely occur outside of the microbiology laboratory, nor is it likely to in the future. Instead, the local hospital or regional Health Protection Agency laboratory provides this service.

Chapter 3

Diagnostic test background

Conventional culture methods

For over a century, the detection of enteric pathogens has relied on culture techniques to isolate bacteria. A variety of selective, non-selective and differential media, and also enrichment broths, are traditionally used by clinical microbiology laboratories for the screening of stool cultures. Suspect colonies, screened using various media, are selected for further confirmatory biochemical tests. When a pathogen is detected, serological typing and more detailed biochemical testing are performed, and data from these tests facilitate epidemiological analyses. Although these conventional methods are very valuable, they are both time and material consuming and may be unsuitable in outbreak situations. One of the key criticisms of conventional methods is that results are available relatively late in the clinical illness, limiting the overall value of the test. Treatment decisions are usually based on clinical severity of illness prior to receipt of culture confirmation of microbial cause. This lengthy time taken to diagnose food-borne illness by culture methods may have an impact on the clinical route for each patient.

Pre-enrichment and enrichment of samples

Organisms are traditionally cultured from stool by inoculating the specimen on to a combination of enteric selective and differential media. One of the reasons why culture methods are time consuming is the pre-enrichment and enrichment process required, which is usually performed overnight. Pre-enrichment involves the use of a non-selective medium which allows the recovery of stressed cells in food or faeces. This is followed by an enrichment step in a second broth, which is usually selective due to inclusion of antimicrobials against non-target organisms in their formulation. Pre-enrichment ensures that stressed cells are competent to grow in the relatively toxic conditions of the selective enrichment. Without the recovery (pre-enrichment) stage, growth can be inhibited in the selective broths. A comparative study of five plating media for *Salmonella* spp. suggests that the sensitivity of assays will be substantially increased if the sample is pre-enriched, with sensitivity ranging from 0.365 to 0.784 for direct plating and from 0.909 to 0.932 after enrichment in selenite broth.[30] This suggests that the increase in accuracy gained from pre-enriching samples would justify the increased time taken to report back results.

Culture is by no means 'perfect'. For instance, Hektoen Enteric Agar, although showing high sensitivity in the detection of *Salmonella*, is not very specific,[31] and a high level of false-positive results require time-consuming complementary testing to identify or, in most cases, to exclude the presence of *Salmonella* colonies. Problems with using culture are experienced with other enteric pathogens also, such as non-O157 strains of EHEC.[32,33] These EHEC ferment sorbitol and therefore, on diagnostic agars, are visually different from *E. coli* O157 strains, and therefore may not be identified, even though they have the potential to cause the same disease.

Standard culture tests usually require 3–7 days to issue final results because bacterial identification requires further biochemical confirmatory tests.

Rapid technologies

'Rapid diagnosis' is an umbrella term describing a wide range of novel testing procedures which can significantly reduce the reporting time compared with that of conventional bacterial culture. With more rapid laboratory diagnosis, the clinician could be informed of the microbial cause of food poisoning prior to making treatment decisions, in addition serving as a trigger for a suspected food-borne outbreak if further cases are present. This could be exceptionally important in, for example, the case of EHEC infection where the use of antibiotics is contraindicated and rapid treatment is essential to prevent kidney damage in infected individuals.

From a public health perspective, faster detection times are essential to prevent the spread of infectious diseases or the identification of a continuing source of infection. Extensive research has been carried out to develop rapid tests for food-borne pathogens and/or bacterial toxins, but it remains to be seen whether they can make a

significant diagnostic impact by being implemented in routine practice.

Rapid methods developed include a variety of assays such as highly specific nucleic acid-based methods, antibody-based tests, simple miniaturised biochemical assays and physicochemical tests that measure bacterial metabolites such as bioluminescence and fluorescence.

Antigen detection

An antibody is a large protein produced in an animal in response to an invasion by a 'foreign' molecule, i.e. one not recognised as 'self'. The antibody binds to the foreign structure, often with a very high affinity, as part of the general immune response, which leads to neutralisation of the 'invader'. Although binding of an antibody to the analyte (e.g. bacterial cell, toxin) is the key event in the technique, recognition that this event has taken place is needed to discover if the analyte is present. This is done by labelling (or 'tagging') the antibody with another molecule which can produce a measurable signal. The signal generated by the tag can be a colour change, production of light or fluorescence, an electrical or optical output or by simple visual recognition. In many cases the signal is quantifiable. The test formats include:

- **Enzyme-linked immunosorbent assay (ELISA)**: ELISA is the most common format, in which the tag is an enzyme, usually one which catalyses the formation of a coloured product from a colourless substrate to give the assay endpoint. ELISAs can take several forms, the most widespread of which is based on a 96-well polystyrene microplate. Within this assay format there are several types. In one, for example, antibodies are immobilised on the surface of the well and samples are added; the target analyte in the sample is bound by the antibody. Unbound material is washed away and a second, enzyme-labelled, antibody is then added. After a further washing stage, substrate is added and the colour change recorded, usually with a dedicated spectrophotometer. Tags other than enzymes are used in some assays, and such tags can offer the possibility of multiplexing the assay, for example, in fluorescence immunoassays (FIAs).
- **Lateral flow**: These assays are housed in a completely self-contained cassette device. They require less technical knowledge to interpret, and could be used 'in the field' or, potentially, for 'bedside' testing, and are formulated as a one-off, disposable, single-sample test.
- **Latex agglutination**: The third common format relies on the ability of antibodies to form cross-linked complexes with the target cells or toxins in a process known as agglutination. In some forms of agglutination tests, the antibody is in solution; in others, it is coated on the surface of latex particles. The sample is mixed with the antibody or coated particles on a solid surface, such as a tray or microscope slide, and observed for the formation of visible agglutinated clumps in the liquid, usually within a few minutes. Multiplex detection of different cells or toxins in the same sample by formation of separate coloured complexes is possible. Due to the high volume of background flora in faecal samples, without pre-enrichment all detection methods face inhibitors which drastically reduce the sensitivity and specificity of each test.
- **Immunomagnetic separation (IMS)**: This technique uses binding properties of antibodies to separate and/or concentrate bacterial cells from a dilute or complex sample. Antibodies are immobilised on magnetic microparticles and these are added to the sample. After a suitable incubation period in which cells bind to the antibodies, the particles are removed from the sample with a powerful magnet and washed to remove unbound cells and matrix material (e.g. food). They can then be transferred to an analytical system or culture medium for further analysis or manipulation.

Although ELISA tests can increase the speed of detection, it is a very labour-intensive technique. As laboratory budgets are tightened and diagnostic capabilities advance, laboratory managers will aim for the minimum technician time spent while retaining high sensitivity and specificity. Immunoassay techniques, which rely on repetitive washing cycles, may become redundant as automated, large-scale tests become available.

Nucleic acid-based detection methods

The genetic material of each living organism – plant or animal, bacterium or virus – possesses sequences of its nucleotide building blocks (usually DNA, sometimes RNA) that are uniquely and specifically present only in its own species. Provided that at least partial sequences of the DNA target are known *a priori*, these sequences can be used to design synthetic oligonucleotide primers that hybridise specifically to target sequences. Various nucleic acid-based techniques have been developed, including direct DNA probes, polymerase chain reaction (PCR), amplification of the hybridising

probes (e.g. ligase chain reaction and Q-beta replicase amplification), amplification of the signals generated from hybridising probes (e.g. branched DNA and hybrid capture) and transcription-based amplification (e.g. nucleic acid sequence-based amplification and transcription-mediated amplification). PCR is the most versatile and widely used amplification technique.

There are three basic steps in PCR:

1. The target genetic material must be denatured – unwinding the strands of its double-helix. This is accomplished by heating to 90–96°C.
2. Hybridisation or annealing: primers bind to their complementary bases on the now single-stranded DNA.
3. DNA synthesis by a polymerase: starting from the primer, the polymerase can read a template strand and match it with complementary nucleotides very quickly. The result is two helixes in place of the first.

These steps are repeated, usually for 25–30 cycles. The creation of new copies of the original DNA strand is exponential, so that within a short period (usually 1 hour) there is enough of the target DNA to detect. The DNA product of amplification is of a fixed length (i.e. covering the distance between the two primers), and detection of this defined product indicates a positive PCR result.

PCR tests are considered especially attractive due to their relative ease of use, low cost and potential application in large-scale screening programmes by means of automated technologies. As nucleic acid-based methods are exquisitely sensitive, the use of an imperfect gold standard is likely to undermine their true diagnostic accuracy. Although there is always a risk that dead/non-viable cells are detected in PCR assays, nucleic acid-based methods are generally more sensitive than culture, and so pathogens detected by PCR but not culture (defined here as 'false positives') may be 'true' positives (*Figure 2*).

- **Choosing suitable targets**: The most important aspect of PCR is the analyst's choice of genetic target to be amplified. As sequencing of genomes of infectious pathogens becomes more widespread, researchers can begin to understand which primer sets would provide the most clinically useful diagnostic test. Frequently, there are minor sequence differences between strains of a pathogen, even within a species, so careful study of all available sequences of the target organism is necessary when designing primer sets to ensure that all the desired coverage is achieved.
- **Commercial and 'in-house' PCR test**: With the increasing number of genomes of infectious pathogens being sequenced, there is opportunity for any research scientist to attempt to design a diagnostic test based on any segment of a genetic sequence. Over the past decade, a vast number of laboratories worldwide have developed their own 'in-house' PCR assays for food-borne pathogens. As a result, the test methods and primers used are heterogeneous, and validation and optimisation of in-house assays need further study. On the other hand, there are a very limited number of commercial tests available for the detection of enteric pathogens.
- **Conventional and quantitative real-time PCR (rtPCR)**: Most conventional first-generation PCR assays have cumbersome procedures for detecting amplification products; after initial DNA purification, there is a 2-hour PCR stage, followed by gel electrophoresis and ethidium bromide staining. In addition, many lack sequence-specific identification of the gene being amplified. In comparison, quantitative real-time PCR involves the amplification and detection of amplified products coupled in a single reaction vessel, greatly increasing the speed of detection. This allows for the direct detection of the PCR product during the exponential phase of the amplification reaction, combining amplification and detection in one step. Unlike conventional PCR methods, rtPCR does not require post-PCR sample handling, preventing carry-over contamination and resulting in much faster and higher throughput assays. Measurement of the rate of increase in amplification product in real time also offers the possibility of extrapolating back to the starting DNA concentration, thus offering the possibility of quantification.
- **Multiplex PCR**: A disadvantage of nucleic acid diagnostic kits and many user-developed tests is that they are narrow in scope. Current organism-specific PCR methods assume that the microbiologist knows which pathogen is causing the disease – an assumption which, if true, makes the test useful only as an expensive confirmation for the clinical diagnosis. Significant interest therefore rests on **multiplex** PCR, which uses numerous primers within a single reaction tube in order to amplify nucleic acid fragments from different targets. Specific nucleic acid amplification (NAA) should occur if the appropriate target DNA is present in the sample tested. It has been argued that the costs

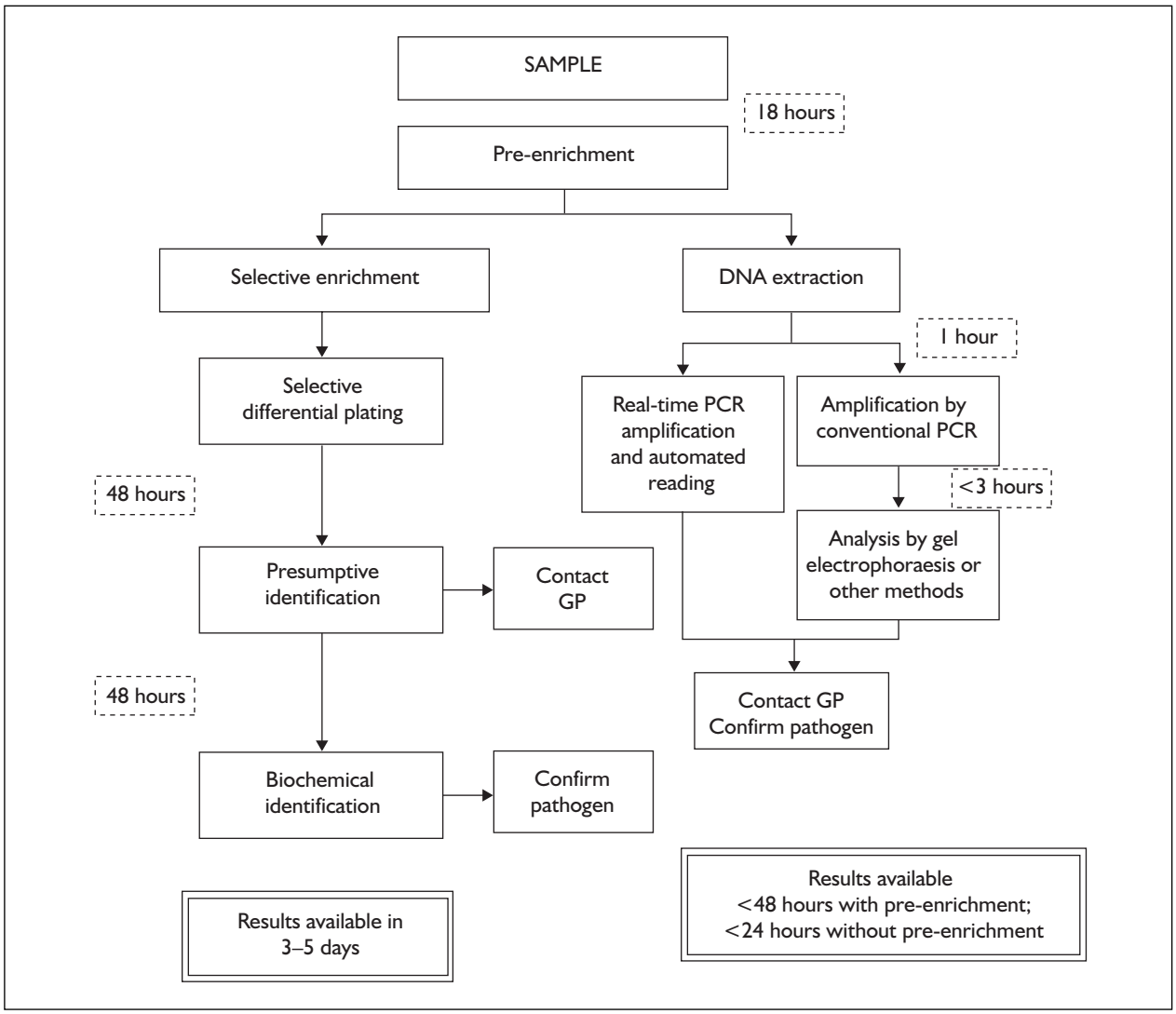


FIGURE 2 *Simplified flowchart of culture methods versus PCR detection (all times approximated; several other algorithms possible)*

of reagents and the preparation time are less in multiplex PCR than in systems where several tubes of uniplex PCR are used. However, the primers used in multiplex reactions must be designed carefully to have similar annealing temperatures and to lack complementarity. Extensive empirical testing is often needed and, as yet, multiplex PCR tests for enteric pathogens are not commercially available.

Limitations

PCR methods (particularly rtPCR) potentially offer faster detection time and increased accuracy compared with traditional culture. PCR tests are attractive due to their ease of use, relatively low cost in terms of laboratory manpower, rapid turnaround time and potential to be fully automated. However, some problems still exist with nucleic acid diagnosis.

- **Costs**: A high level of investment is required for PCR testing, in terms of both initial technician training and high capital costs, particularly for real-time equipment.
- **Inhibitors in sample**: NAA for faecal specimens can be particularly difficult due to the high level of background flora present that PCR may fail to detect in samples where the presence of unusually high concentrations of inhibitory compounds that were not sufficiently reduced by the level of dilution used.
- **DNA extraction**: Although extraction of DNA can eliminate inhibitory substances in faeces, these procedures are also labour intensive and expensive. Because of the heterogeneous nature of faecal specimens, it is very difficult to develop a DNA extraction method that will successfully remove inhibitors that may be present in various amounts in different samples

to ensure that DNA of comparable quality is extracted from every sample.

- **Laboratory set-up**: False-positive results may be caused by contamination, and very strict guidelines must be adhered to, including the separation of pre- and post-amplification samples within the laboratory suite.
- **Lack of an isolate**: A further limitation is that a cultured specimen may still be required for identification at the species level and for epidemiological typing. Enrichment culture of the faecal specimen would provide a simple specimen preparation and a cultured isolate available for subsequent confirmation.

Chapter 4

Systematic review methods

This section summarises the methods used for the systematic review and meta-analyses. There were five stages of article appraisal:

1. search of databases and handsearching
2. abstract appraisal
3. full article appraisal of relevant evaluative papers
4. data extraction and quality assessment
5. meta-analysis for homogeneous rapid test methods and narrative synthesis of heterogenous studies.

Search strategy

A search strategy was developed to find studies that covered the main subject areas addressed in this systematic review: infectious intestinal disease, rapid diagnostic test methods and diagnostic accuracy information. Literature was identified from several sources, including electronic databases and other sources. The following databases were searched:

- MEDLINE (1966 to April 2005)
- EMBASE (1980 to April 2005)
- BIOSIS (1969 to April 2005)
- Web of Science (Science Citation Index) (1945 to April 2005)
- CINAHL (Cumulative Index to Nursing and Allied Health Literature) (1982 to April 2005)
- AOAC Method Validation Programme (1992 to April 2005).

The search strategy was developed initially for MEDLINE. The MEDLINE search strategy was subsequently modified for use in EMBASE, CINAHL and BIOSIS databases. Details of algorithms used are described in Appendix 1.

A review of reference lists from all included articles was undertaken. Attempts to identify unpublished literature included contacting manufacturers and searching the National Research Register. Abstracts from conference proceedings were included only if additional information was available from the authors or from other publications from that group. International and national experts working the fields relevant to each organism were contacted to check the completeness of the searches conducted.

The following journals were also handsearched from January 2000 to June 2005 to validate electronic searching:

- *Journal of Clinical Microbiology*
- *European Journal of Clinical Microbiology and Infectious Disease*
- *Applied and Environmental Microbiology*
- *Journal of Medical Microbiology*.

Updates to electronic databases were checked throughout the data extraction period to ensure inclusion of studies up to September 2005.

A database of relevant articles was constructed using Reference Manager 11.

Selection of papers: inclusion/exclusion criteria

Abstracts of all papers were appraised independently by four members of the review team (LI/IA for studies on rapid diagnostic methods conducted on clinical samples and CFA/GMW for food-based assays).

The criteria for study inclusion in the systematic review were as follows:

- **Outcome measures**: The main focus of this review was to assess test accuracy. As a minimum, included studies had to report summary accuracy statistics (sensitivity and specificity), or present sufficient raw data to allow these statistics to be calculated when compared with a suitable gold standard. Some articles were identified that did not necessarily meet the appraisal criteria, but contained detailed descriptions of novel diagnostic techniques and their technical efficiency, or referred to economic costs of such methods. These were obtained at the abstract appraisal stage for potential use as background information.
- **Tests performed on either human faecal samples or food**. A systematic review of clinical diagnostic studies was performed at the

University of East Anglia, with a supporting review of rapid test technologies for food samples undertaken at the Institute of Food Research, Norwich. Tests evaluated on veterinary samples were excluded from this review.

- **Related to food poisoning**: As some of the organisms included in this review can cause other clinical manifestations and syndromes outside the intestinal tract, a significant number of studies mixed the pool of samples so that faecal specimens were only a fraction of those tested. This was particularly evident with *S. aureus* studies, where most studies evaluated nasal or wound specimens. Despite an attempt to eliminate studies focused on methicillin-resistant strains in our search strategy, a large number of staphylococcal diagnostic studies identified were not relevant to our review. All studies in which food-borne illness was not the primary concern were excluded.
- **Setting**: Studies conducted in microbiology laboratories were included in the review.
- **Study design**: All studies that compared a new test or strategy with an established reference test in patients suspected of having the target disorder were included. This could take the form of inter-laboratory collaborative trials or evaluation in a single laboratory. Both retrospective and prospective study designs were included, with their appropriateness considered during quality assessment.
- **Eligibility assessment**: Full copies of articles were obtained for papers meeting all three criteria. When it was unclear whether one or more of these criteria were satisfied, full articles were also obtained to ensure search completeness. When single articles evaluated more than one test method, or evaluated them within more than one study population, the component evaluations were processed individually.

All clinical studies were assessed for inclusion by two reviewers (LI and IA), and disagreements were resolved by consensus. Following abstract appraisal, it became apparent that very old studies were generally of poor quality or technically irrelevant. The decision was then made to include only assays evaluated within the past 20 years (that is, to exclude all studies pre-1985).

Appendix 5 details all papers which were excluded after initial review, with justifications.

Quality assessment criteria

Quality issues in diagnostic test studies are considerably different from those in effectiveness studies.[34] Certain types of study designs are likely to produce results that are more favourable to new technologies than they should be. Non-randomised, non-blinded trials commonly overestimate the diagnostic accuracy of index tests,[35] and may lead to incorrect inferences. The preferred study design for assessing test accuracy is one which prospectively recruits all eligible participants, uses a reference test to confirm or refute the presence of disease, determines the accuracy with which the index test identifies disease and reports all results explicitly to allow computation of summary statistics (recently, guidelines for diagnostic evaluations have been produced by the TDR Diagnostic Evaluation Expert Panel[36,37]). Quality assessment must take account of these, and other factors, when measuring the validity and accountability of results from a wide range of evaluation studies. The Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool was used to assess the quality of diagnostic accuracy studies:[236]

	QUADAS tool	Yes	No	Unclear
1.	Was the spectrum of patients representative of the patients who will receive the test in practice?	( )	( )	( )
2.	Were selection criteria clearly described?	( )	( )	( )
3.	Is the reference standard likely to correctly classify the target condition?	( )	( )	( )
4.	Is the period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	( )	( )	( )
5.	Did the whole sample, or a random selection of the sample, receive verification using a reference standard of diagnosis?	( )	( )	( )
6.	Did patients receive the same reference standard regardless of the index test result?	( )	( )	( )
7.	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	( )	( )	( )
8.	Was the execution of the index test described in sufficient detail to permit replication of the test?	( )	( )	( )
9.	Was the execution of the reference standard described in sufficient detail to permit its replication?	( )	( )	( )
10.	Were the index test results interpreted without knowledge of the results of the reference standard?	( )	( )	( )

11. Were the reference standard results interpreted without knowledge of the results of the index test?	( )	( )	( )
12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	( )	( )	( )
13. Were uninterpretable/ intermediate test results reported?	( )	( )	( )
14. Were withdrawals from the study explained?	( )	( )	( )

Data extraction strategy

Extraction of study findings was conducted in duplicate (by IA and LI) using a predesigned and piloted data extraction form. Any disagreements between reviewers were resolved by consensus. The detailed forms used for each step are included in Appendix 3. The form was used to collect information on study design, methods, participants, testing procedures and accuracy details.

Extracted data from selected studies were entered into a separate Excel spreadsheet independently by two reviewers. Reviewers were not blinded to the names of study authors, institutions or publications. Where raw outcome data could not be extracted from a paper, the authors were contacted, with varying degrees of success.

Basic statistical analysis

Diagnostic performance indices (sensitivity, specificity, predictive values) were extracted from each study. The number of false positives, true positives, false negatives and true negatives were used to recalculate measures of accuracy for each test compared with an appropriate reference test:

		Reference test +	Reference test –	
Index test	+	a = TP	b = FP	$A + b$
	–	c = FN	d = TN	$C + d$
		A + c	$B + d$	$A + b + c + d$
		TPR = $a/(a + c)$	1– FPR = $d/(b + d)$	

a = TP = the number of true positives, defined as the number of individuals for whom the test is positive and who actually have the disease

b = FP = the number of false positives, defined as the number of individuals for whom the test is positive but who do not have the disease

c = FN = the number of false negatives, defined as the number of individuals for whom the test is negative but who actually have the disease

d = TN = the number of true negatives, defined as the number of individuals for whom the test is negative but who do not have the disease.

TPR = sensitivity, defined as $a/(a + c)$

FPR = specificity, defined as $d/(b + d)$

PPV = positive predictive value, defined as $a/(a + b)$

NPV = negative predictive value, defined as $d/(c + d)$

Diagnostic odds ratio (DOR) = ad/bc

Studies reporting test accuracy were grouped according to the index test evaluated, and the sensitivity, specificity, DOR were calculated for each evaluation. Due to the high sensitivity of most NAA tests, a large proportion of cells in the two-by-two table had zero entries. As is accepted practice, 0.5 was added to each cell for studies with either 100% sensitivity or specificity to conduct meta-analyses.

With very high diagnostic accuracy estimates for many rapid tests, generation of confidence intervals (CIs) by conventional methods was not appropriate. In this instance, the Wilson score method was applied, to provide more precise estimates of two-sided CIs for proportions.[38,39]

All data were presented in forest plots, with horizontal lines representing 95% CIs for estimates and size of points reflecting total sample size. Statistical pooling was used where appropriate (see below). Where there was evidence of significant clinical heterogeneity, narrative synthesis was used and a meta-analysis was not conducted.

Statistical heterogeneity and use of random/fixed-effect models

Correlation of sensitivity and specificity

With diagnostic accuracy studies, the decision to pool sensitivity and specificity results is dependent on whether they are correlated. Spearman's rank correlation test can be applied to assess this. For highly correlated data (estimated as Spearman's ϵ of $|0.5|$ or above), sensitivity and specificity estimates were combined using summary receiving

operating characteristic (SROC) curve analysis. Where they were not correlated (i.e. Spearman's ϵ |0.5| or less), separate pooled measures of sensitivity and specificity were presented in forest plots.

Random or fixed-effect model

Where it was reasonable to assume that the underlying diagnostic accuracy was the same in all studies, and that the observed variation in sensitivity or specificity is due entirely to sampling variation, a fixed-effect model was applied. Where heterogeneity existed, statistical analysis involved a random-effects model.

If differences in the results cannot be attributed to known sources of variation, pooling of the results should not be attempted because it will not be possible to interpret the summary estimate.[40] A χ^2 test (or Fisher's exact test for small studies) was used to test the hypothesis that there is no statistically significant evidence of heterogeneity in the DOR. This statistical test for differences in proportions provides a conservative test of the null hypothesis that the study results are homogeneous. In addition, the I^2 statistic was used to assess heterogeneity.[41]

Summary receiver operating characteristic curve and area under the curve analysis

Within clinically appropriate groups, meta-analytic methods were used to combine diagnostic studies [all meta-analyses were carried out using SAS (Version 9) and STATA (Version 9.0)]. There is no universally accepted measure of test accuracy in meta-analyses of screening and diagnostic data. An SROC curve was generated for each comparison and the area under the curve (AUC) was used as the main measure of overall diagnostic accuracy. The greater the AUC value, the higher is the estimate of test accuracy. Summary estimates of AUC were produced with 95% CIs.

Paired sensitivity and 1 – specificity results for each study were plotted in the ROC plane to display the trade-off between sensitivity and specificity, detect heterogeneity, and identify outliers.

Assuming the logarithm of the DOR = D:

$$D = \ln(\text{DOR}) = \ln[\text{TPR}/(1 - \text{TPR})] \times [(1 - \text{FPR})/\text{FPR}]$$

and logarithm of S, a measure of test threshold:

$$S = \ln[\text{TPR}/(1 - \text{TPR})] \times [\text{FPR}/(1 - \text{FPR})]$$

D was plotted against S for each study and a line ($D = a + bS$) was fitted to the data. Weighted least-squares was used to fit this regression line. Although extensions to this model exist (incorporating other factors to explain heterogeneity in the DOR) and more complex models have been suggested utilising Bayesian methods, this model seems appropriate, avoiding unnecessary complexity particularly with relatively small sample sizes.

The regression model used to fit the SROC curve was used to test for potential threshold effects. The logarithmically transformed DORs (lnDOR) will be symmetrical around the line sensitivity = specificity where studies are homogeneous, so any variation in these end-points between studies can be attributed to a threshold effect.

Where there were three or fewer studies for a particular test, SROC analysis was not presented graphically; however, AUC values are still reported in the text if this mode of analysis is most appropriate (i.e. where sensitivity and specificity are correlated).

Publication bias

Meta-analytic systematic reviews must assess the magnitude of publication bias. There is considerable emphasis on 'significant' ($p < 0.05$) in academic journals, so studies which lack the statistical power to detect a clinically important effect may remain unpublished. Additionally, trials of lower quality may yield exaggerated estimates of diagnostic accuracy. The existence of publication bias was examined graphically by the use of funnel plots of log odds ratio (OR). These were only presented for meta-analyses with four or more studies, as visualisation of asymmetry is difficult with a small number of studies. The Begg and Mazumdar adjusted rank correlation test for publication bias was used to assess statistical evidence of publication bias; this estimate was recorded for all meta-analyses.[42] p-Value estimates from the more powerful Egger's test[43] were also presented.

Rapidity

There are two important aspects when evaluating the rapidity of new diagnostic tests. Primarily, they must detect a pathogen significantly faster than the standard method. In addition, the hands-on time requirement for technicians working with the

assay should ideally be shorter than current practice. The purpose of calculating 'hands-on' time was to allow separate calculation of technician activity required and therefore the accurate estimation of labour costs.

Where quantitative time estimates were reported, these were extracted and used to derive summary data, based on median values for time to detect and hands-on time for each test method. For all studies, qualitative assessments of detection time and technician time requirements were provided. Although many different testing algorithms could be assessed, this research is based on a representative assessment of time taken to identify each pathogen.

Chapter 5

Evidence of clinical effectiveness

Quantity of research available

The titles and abstracts of 1853 papers were screened for eligible studies, the full text of 153 papers were retrieved for more detailed evaluation and 87 studies of test accuracy were ultimately included. *Figure 3* shows a flowchart of the study selection process. The reasons for exclusion of studies are provided in Appendix 5.

Almost all relevant studies evaluated tests for *E. coli*, *Campylobacter* or *Salmonella*. Very few studies evaluating clinical diagnostic tests for intestinal *C. perfringens*, *B. cereus* and *S. aureus* were identified.

Non-English language papers

The standardised search strategy identified 211 foreign language papers. A more stringent inclusion criterion was applied to non-English language papers. In an effort to review comparable test methods, only papers which evaluated assays and provided sufficiently comparable data such as evaluation of commercial tests were reviewed. Full translation was not carried out on tests which were developed in-house or are no longer commercially available. In total, six non-English language articles were included in the review.

Main test methods studied

Twenty-eight of the accepted studies concentrated on antibody-based serological methods. Thirty-two evaluated nucleic acid-based method and five evaluated improvements to the traditional culture technique. (This review may not be a reliable indication of the volume of nucleic acid-based tests being developed. Several studies were identified in the search describing a new nucleic acid-based test method; however, they were not evaluated against any reference standard. These studies make up a large proportion of excluded studies, detailed in Appendix 5, and it is highly likely that many more nucleic acid-based tests have been developed without an assessment of diagnostic effectiveness, which therefore would have been missed by the search strategy.) Less frequent test methods included oligonucleotide assay, DNA hybridisation and hydrophobic grid membrane filters (HGMFs), results of which have been assessed in a narrative evaluation.

Setting and population

The study setting was not always reported, but where it was, studies were conducted in either hospital laboratories or publicly funded research institutions. Five studies were produced from collaborations between a number of laboratory centres, the results of which may be more valid than single-location evaluations, as reproducibility and repeatability of test methods can be measured.

A limited number of clinical studies were found from developing countries, most of which did not meet the criteria of comparison against a gold standard. Many of these assays were technologically out of date. The review group decided that it was inappropriate to use these for the UK setting.

Study design

Given the low isolation rates for the organisms studied, designing prospective evaluations of novel rapid methods may prove prohibitively expensive for some laboratories. The majority of studies identified have been evaluated retrospectively, using banked reference strains which have been inoculated into healthy stools. Nucleic acid-based detection methods in particular use this study design. Seventeen prospective study designs were identified – these involved large-scale, relatively long-term evaluations, usually being sponsored or partly funded by the manufacturer of the test product involved.

The review of studies on clinical isolates was restricted to the detection of enteric pathogens in faecal samples, but there has been some research carried out on the detection of antibodies from saliva.[44]

Quality of included studies

The information gained in quality assessment is important in determining the strength of inferences. Quality differences may explain heterogeneity in study results. The majority of studies were retrospective. Problems with the quality of data from diagnostic test papers are compounded by how poorly they are reported.

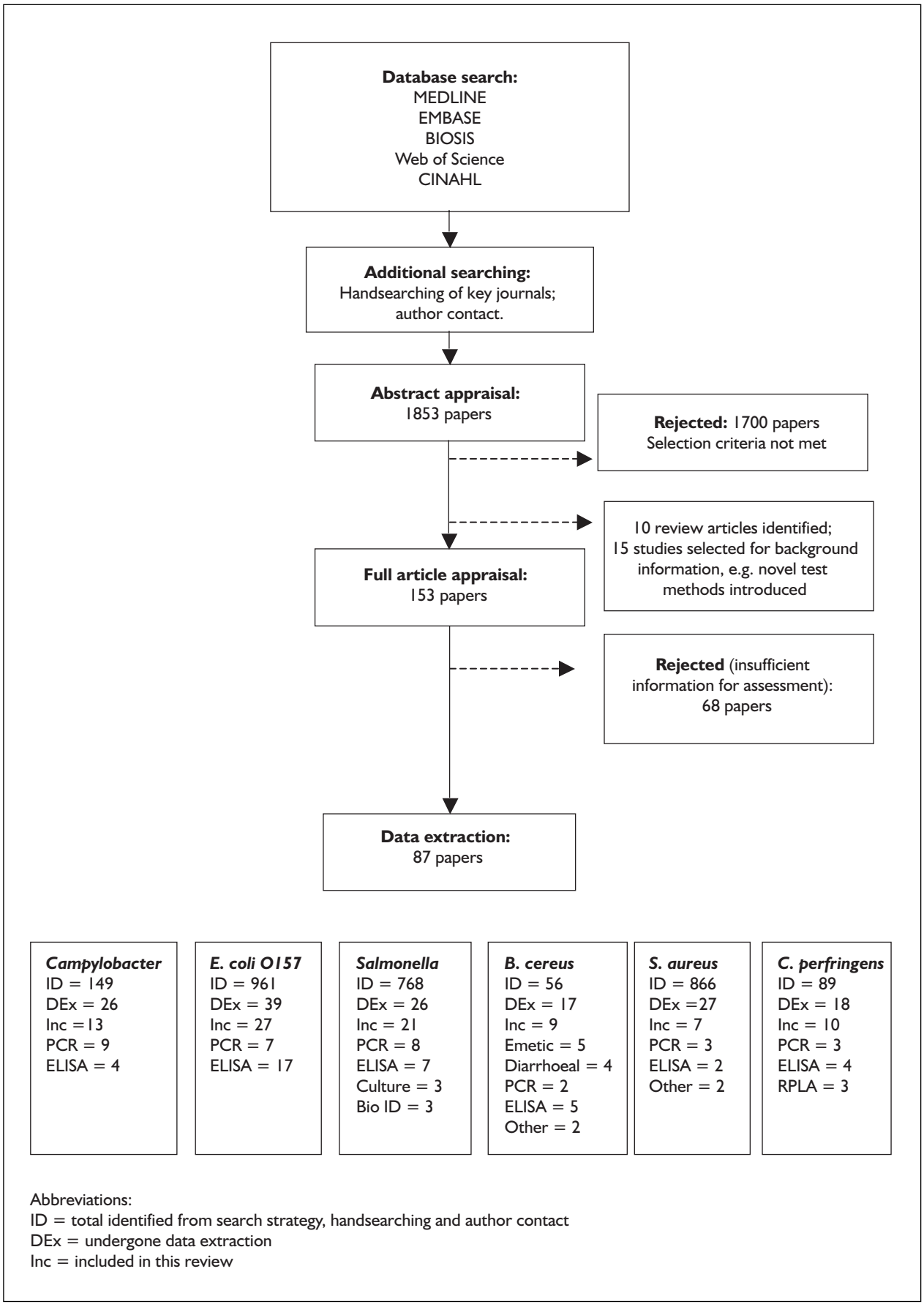


FIGURE 3 *Flow diagram of the study selection process*

TABLE 4 *QUADAS scores for included studies*

	All *Campylobacter* papers (*n* = 13)	All *E. coli* papers (*n* = 24)	All *Salmonella* papers (*n* = 20)	All *B. cereus* papers (*n* = 10)	All *S. aureus* papers (*n* = 6)	All *C. perfringens* papers (*n* = 15)
Was the spectrum of patients representative of the patients who will receive the test in practice?	8	18	12	1	0	6
Were selection criteria clearly described?	9	11	12	0	0	5
Is the reference standard likely to correctly classify the target condition?	11	24	19	1	3	6
Is the period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	4	18	14	1	3	0
Did the whole sample, or a random selection of the sample, receive verification using a reference standard of diagnosis?	13	21	20	1	4	6
Did patients receive the same reference standard regardless of the index test result?	13	24	20	2	2	3
Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	13	21	17	3	3	6
Was the execution of the index test described in sufficient detail to permit replication of the test?	11	19	11	9	5	8
Was the execution of the reference standard described in sufficient detail to permit its replication?	8	18	10	3	4	2
Were the index test results interpreted without knowledge of the results of the reference standard?	12	18	17	10	1	7
Were the reference standard results interpreted without knowledge of the results of the index test?	13	18	10	10	0	3
Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	10	18	7	0	0	5
Were uninterruptible/intermediate test results reported?	8	17	7	5	5	1
Were withdrawals from the study explained?	2	8	11	0	0	1

TABLE 5 *Average hands-on and total time*

	Mean (standard deviation) (hours)
Total time rapid methods	38.70 (20.90)
Hands-on time rapid	1.16 (0.50)
Total time traditional	72.00 (22.80)
Hands-on time traditional	0.88 (0.23)

Few studies report any detailed information about the patients being tested, or how reliable and reproducible the test is among different populations.

An analysis of papers using the QUADAS tool is presented in *Table 4*.

Analysis of hands-on and total time

As stated earlier, the specific methods used in various assays differed significantly. *Table 5* reports pooled estimates of the average hands-on time and total time for rapid and traditional culture methods from included studies. The actual time taken to perform each individual assay differs considerably. Therefore, these data are presented to illustrate the potentially shorter time required for rapid assays in general rather than to give an accurate comparison of the methods.

Campylobacter

Number of studies

A total of 149 studies relating to rapid diagnostic tests for *Campylobacter* food poisoning were identified from the initial search strategy. Following abstract appraisal, relevant data were extracted from 26 studies, and 13 of these were included in this review. Basic information for all included studies is provided in *Table 6*.

Methodological quality of studies

Quality of reporting was high (QUADAS >11) for six studies and medium (QUADAS 6–10) for the remaining seven studies included in this review. However, data was extracted from several studies (n = 12) which did not report full diagnostic accuracy information and were of poor methodological quality. These papers were excluded from analysis (see Appendix 5).

Correlation between sensitivity and specificity, and tests for heterogeneity

Table 6 shows a very high correlation between sensitivity and specificity for all rapid assays evaluated for the diagnosis of campylobacterosis. In view of this, summary estimates of sensitivity and specificity were not generated using meta-analysis. Where appropriate, pooled DORs and the AUC of an SROC curve are shown.

There were three sets of studies with sufficient data to allow meta-analysis. These evaluated the antibody-based ProSpecT *Campylobacter* microplate assay, PCR detection using 16s rRNA primer and PCR detection using hippuricase gene (*HipO*). For each of these three groups of studies, a single, large study accounted for more than half of the combined sample. As a result, pooled accuracy measures link very closely with results from the largest included study.

Meta-analysis

Nucleic acid-based test methods

The majority of studies identified evaluated PCR assays for *Campylobacter* food poisoning. Ten studies reviewed NAA tests compared with a suitable reference test and provided enough diagnostic accuracy information to permit a meta-analysis of results. PCR tests for *Campylobacter* were comprised mainly (9/10 studies) of in-house-designed assays.

Of evaluated PCR assays, the housekeeping 16s rRNA gene was the predominant target for *Campylobacter* species, with *HipO* gene regularly used for the more specific detection of *C. jejuni*. A summary of diagnostic accuracy values for 16s rRNA *Campylobacter* PCR is shown in *Table 7*, *Figure 4* (forest plot of sensitivity and specificity) and *Figure 5* (forest plot of DORs). A fixed-effect model was used to pool the DORs from each group of PCR assay studies.

SROC analysis

Figure 6 shows a symmetrical SROC curve ($|b| < 0.001$, $p = 0.999$) for PCR assays using the 16s rRNA primer to detect *Campylobacter*. A total of 4495 samples were tested. The homogeneous AUC from the SROC curve was 0.987 (95% CI 0.984 to 0.989).

HipO* PCR for *C. jejuni

Nucleic acid-based detection specifically for *C. jejuni* targeted the hippuricase (*HipO*) genes. A summary of diagnostic accuracy from individual studies of *HipO Campylobacter* PCR is shown in

TABLE 6 *Characteristics of studies evaluating rapid assays for the diagnosis of* Campylobacter *food poisoning*

Study	Basic test details					Diagnostic accuracy		*Quality:* QUADAS score	Time issues	
	Test name	Test type	Direct or from isolate	Reference test	Sample size	Sensitivity (95% CI)	Specificity (95% CI)		Result within 24 hours	Reduced hands-on time
Endtz, 2000[164]	ProSpecT *Campylobacter*	EIA	1 hour incubation	Modified CCDA	78	0.800 (0.627 to 0.905)	1.000 (0.926 to 1)	12	Yes	Unclear
Tolcin, 2000[181]	ProSpecT *Campylobacter*	EIA	1 hour incubation	Modified CCDA	164	0.960 (0.865 to 0.989)	0.991 (0.952 to 0.998)	9	Yes	Unclear
Dediste, 2003[162]	ProSpecT *Campylobacter*	EIA	1 hour incubation	Modified CCDA	1205	0.891 (0.815 to 0.938)	0.977 (0.967 to 0.985)	13	Yes	Unclear
Hindiyeh, 2000[168]	ProSpecT *Campylobacter*	EIA	1 hour incubation	Modified CCDA	631	0.889 (0.672 to 0.969)	0.993 (0.983 to 0.998)	10	Yes	Unclear
Iijima, 2004[117]	Real time Multiplex PCR	*yphC* and *gyrA*	Direct	Strains only	161	0.842 (0.624 to 0.945)	0.951 (0.902 to 0.976)	9	Yes	Yes
Amar, 2004[45]	Real time PCR	Commercial	Isolate	Modified CCDA	91	0.833 (0.437 to 0.970)	0.988 (0.937 to 0.998)	12	Yes	Unclear
Collins, 2001[160]	PCR	16s rRNA	Direct	Modified CCDA	42	0.900 (0.744 to 0.965)	1.000 (0.758 to 1.000)	9	Yes	Unclear
La Gier, 2004[170]	Real time PCR	*HipO*	Direct	Strains only	65	1.000 (0.912 to 1.000)	1.000 (0.867 to 1.000)	9	Yes	Yes
Lawson, 1998[47]	PCR	16s rRNA	Isolate	Modified CCDA	200	0.938 (0.717 to 0.989)	0.978 (0.945 to 0.992)	8	Yes	No
Lawson, 1999[48]	PCR	16s rRNA	Direct	Modified CCDA	3738	0.890 (0.858 to 0.915)	0.976 (0.970 to 0.981)	12	Unclear	Probably not
Linton, 1997[15]	PCR	16s rRNA and *HipO*	Isolate	Modified CCDA	43	1.000 (0.824 to 1.000)	1.000 (0.867 to 1.000)	8	Unclear	Unclear
Maher, 2003[172]	PCR	16s rRNA	Isolate	Modified CCDA	119	0.944 (0.742 to 0.990)	0.594 (0.497 to 0.685)	11	Unclear	Unclear
Kulkarni, 2002[12]	PCR	16s rRNA	Direct	Modified CCDA	343	0.882 (0.657 to 0.967)	0.985 (0.965 to 0.993)	13	Yes	Probably not

CCDA, charcoal cefoperazone desoxycholate agar; EIA, enzyme immunoassay; PCR, polymerase chain reaction.

TABLE 7 *Correlation between sensitivity and specificity, and tests for heterogeneity for studies evaluating rapid assays for the diagnosis of* Campylobacter *food poisoning*

	Correlation			Heterogeneity		
Test	**No. of studies**	**Spearman's ϵ**	***p*** **(ϵ = 0)**	**OR Pearson χ^2**	***p***	**I^2 statistic (%)**
16s rRNA PCR	6	0.714	0.111	1.05	0.958	0
HipO PCR	3	−1.000	–	2.41	0.300	17
ProSpecT EIA	4	0.738	0.262	3.93	0.270	23.6

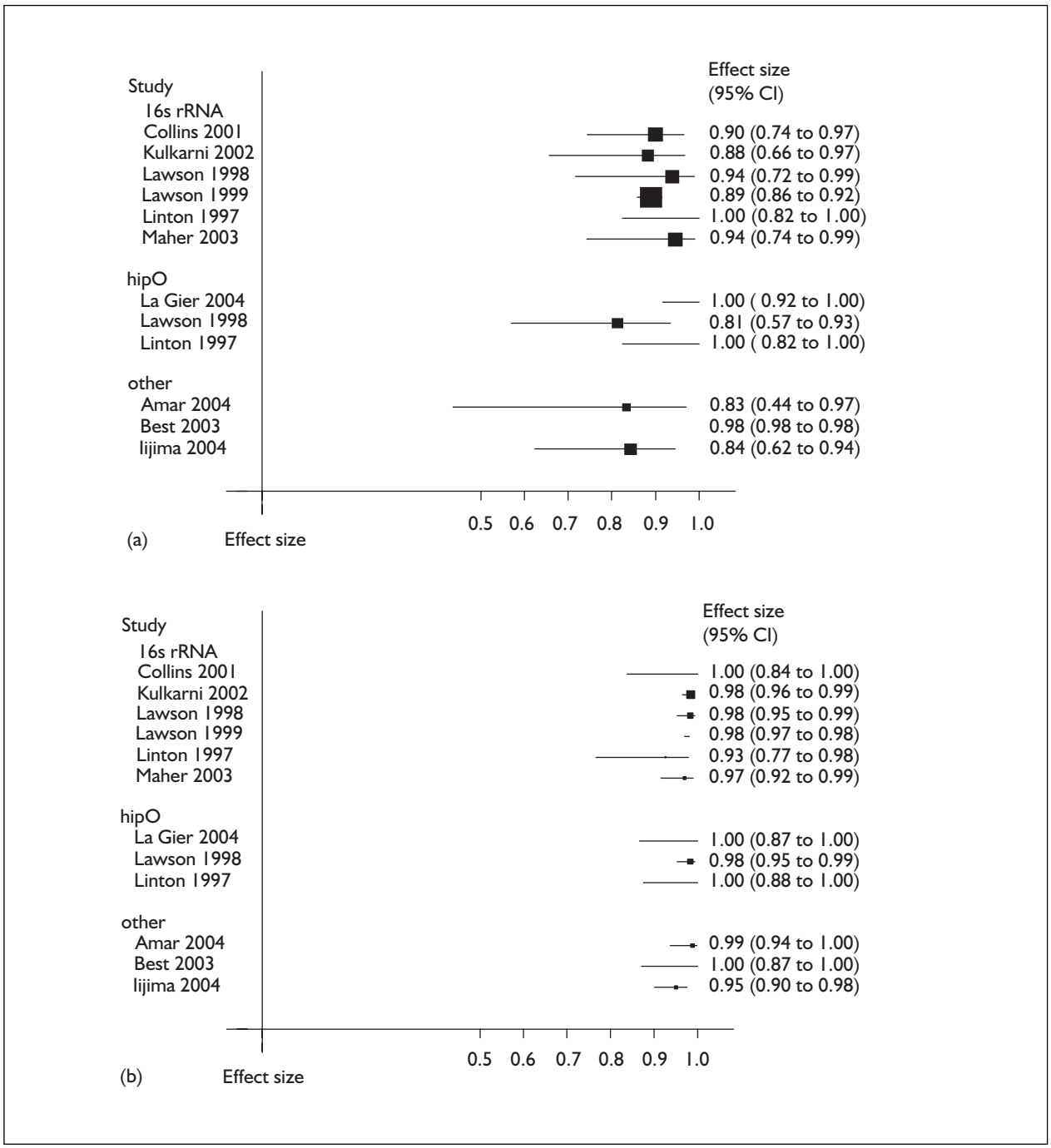


FIGURE 4 *Forest plots of studies showing (a) the sensitivity and (b) the specificity of PCR assays for* Campylobacter *detection*

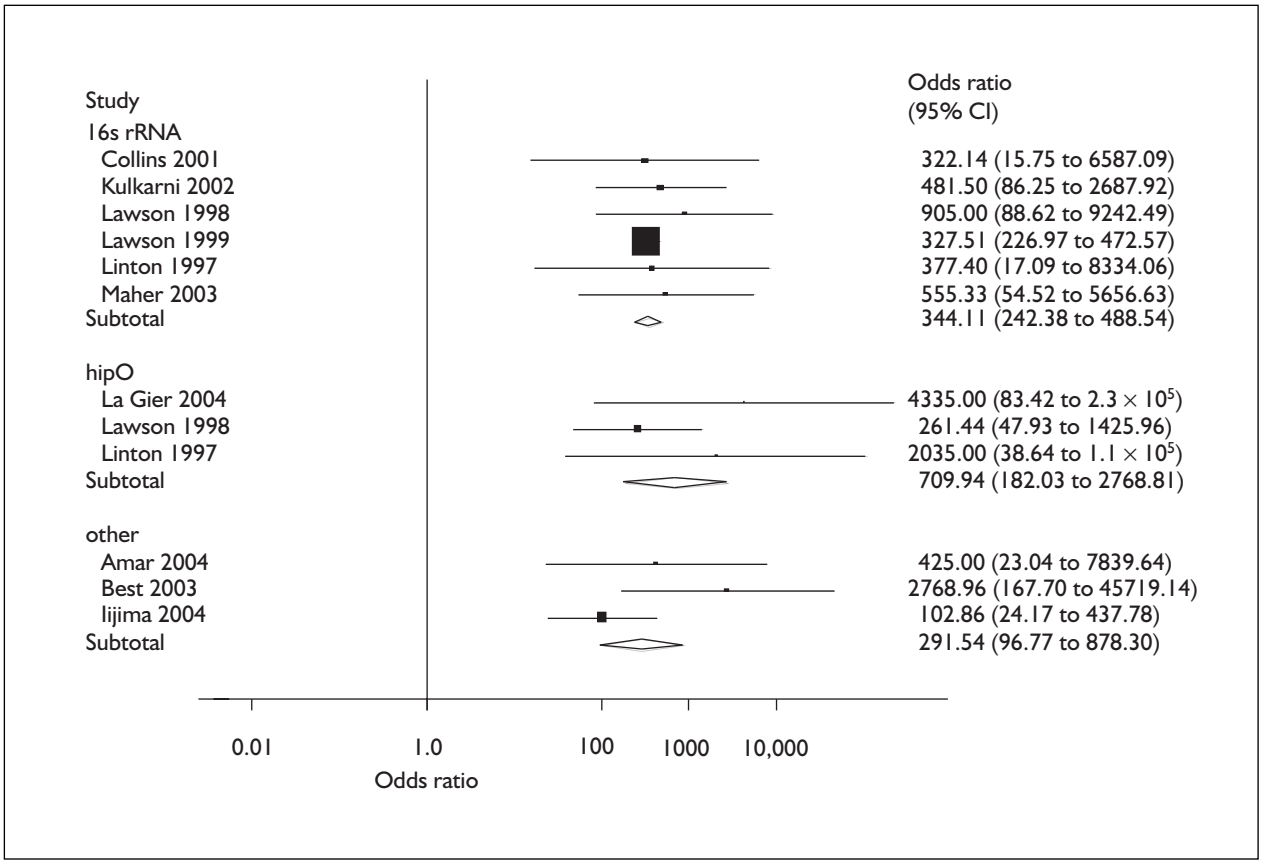


FIGURE 5 *Forest plot of DORs for PCR assays for* Campylobacter *food poisoning*

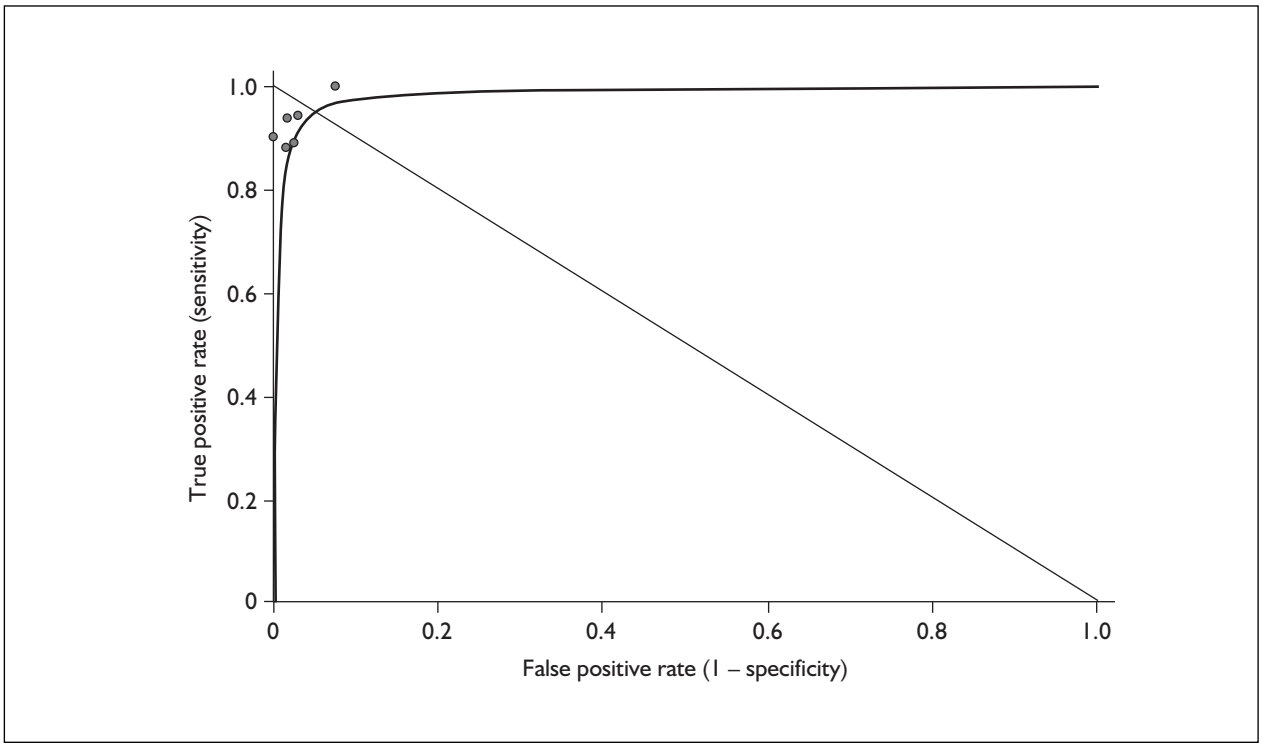


FIGURE 6 *SROC curve for PCR assays using the 16s rRNA primer*

Table 7. A heterogeneous AUC ($b = 1.001$, $p = 0.032$) for the SROC curve for PCR targeting the *HipO* gene was 0.997 (95% CI 0.997 to 0.998).

Other PCR assays

Two other studies evaluated PCR assays for *Campylobacter*. A commercially available PCR test kit, RealArt *Campylobacter* PCR kit, produced by Artus[45] was found to detect *Campylobacter* in five out of six samples positive by culture and in one additional sample. It was negative in all 86 other samples. The test performed well with the small number of samples on which it was tested; however, the kit is produced for research use only, and is not available for routine diagnostic use. In another study,[46] 11 previously developed PCR assays for *C. jejuni*, *C. coli* and *C. lari* were reviewed. Although accuracy values were not explicitly recorded, the specificity of assays varied from 84 to 100% and the sensitivity ranged from 88 to 100%. No assay was 100% accurate; tests that yielded amplicons from all *C. jejuni* strains proved to be the least specific.

Publication bias

Figure 7 shows the funnel plot for PCR studies with 16s rRNA primers. The adjusted Kendall's score obtained from Begg's test for publication bias was 1.0 ($p = 0.851$) and 1.0 ($p = 0.602$) and the Eggers test results were 202.17 ($p = 0.139$) and 2533.76 ($p = 0.276$) for 16s rRNA and *HipO* PCR studies, respectively, indicating no evidence of significant publication bias.

Antibody-based test methods

Only one commercial antibody-based test for *Campylobacter* was identified in clinical studies. Four studies evaluating the ProSpecT *Campylobacter* microplate assay (Remel) provided enough diagnostic accuracy information to be included in meta-analysis.

Figures 8 and *9* show the results of studies evaluating this assay. The estimates of sensitivity were high and the minimum specificity from the studies was 0.98. The pooled DOR was 462 (95% CI 228.4 to 934.9) from a fixed-effect model (*Figure 9*).

SROC analysis

A symmetrical SROC curve ($|b| < 0.001$, $p = 0.188$) for the ProSpecT *Campylobacter* microplate assay is shown in *Figure 10*. The AUC of the SROC curve was 0.862 (95% CI 0.568 to 1.000). There did not appear to be uncertainty over false positive results for these antibody-based

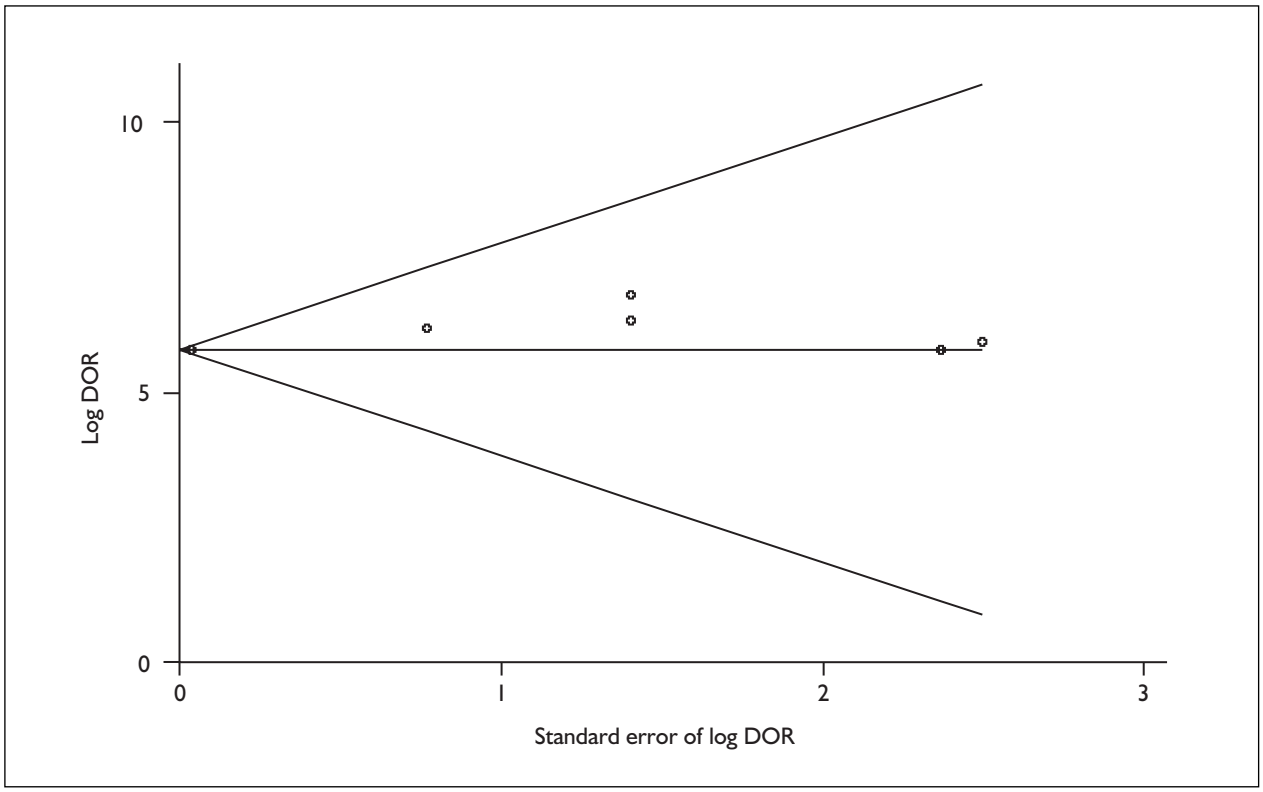


FIGURE 7 *Begg's funnel plot of log DOR of PCR assay studies with 16s rRNA primer, with pseudo 95% CIs*

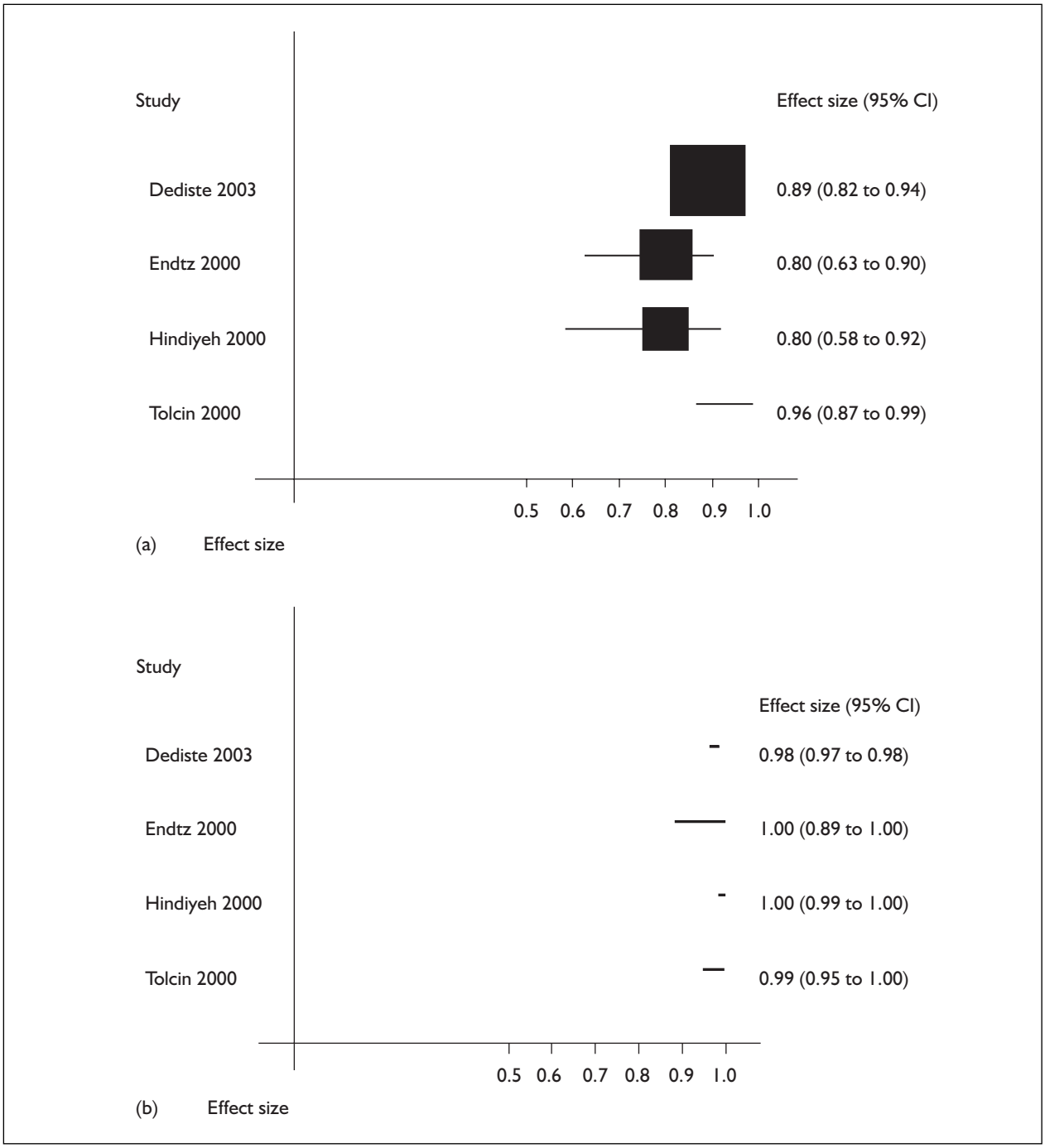


FIGURE 8 *Forest plots of studies showing (a) the sensitivity and (b) the specificity of ProSpecT assay for* Campylobacter *detection*

tests, such as those which may hinder understanding of nucleic acid-based test effectiveness. Culture methods, in this instance, appear to be a suitable gold standard.

Publication bias

There was no statistical evidence of publication bias (Begg's test, Kendall's score = –2, $p = 0.624$) (*Figure 11*) and Eggers test results (slope 1270, $p = 0.333$).

Discussion

The majority of tests for *Campylobacter* focus on the detection of the two most common species, *C. jejuni* and *C. coli*. However, various evaluation studies suggest that that the prominence of *C. jejuni* and *C. coli* over other species may be exaggerated. A major drawback of culture methods for *Campylobacter*, on epidemiological grounds, is that the pre-enrichments necessary to detect *C. jejuni* and *C. coli* eliminate the other

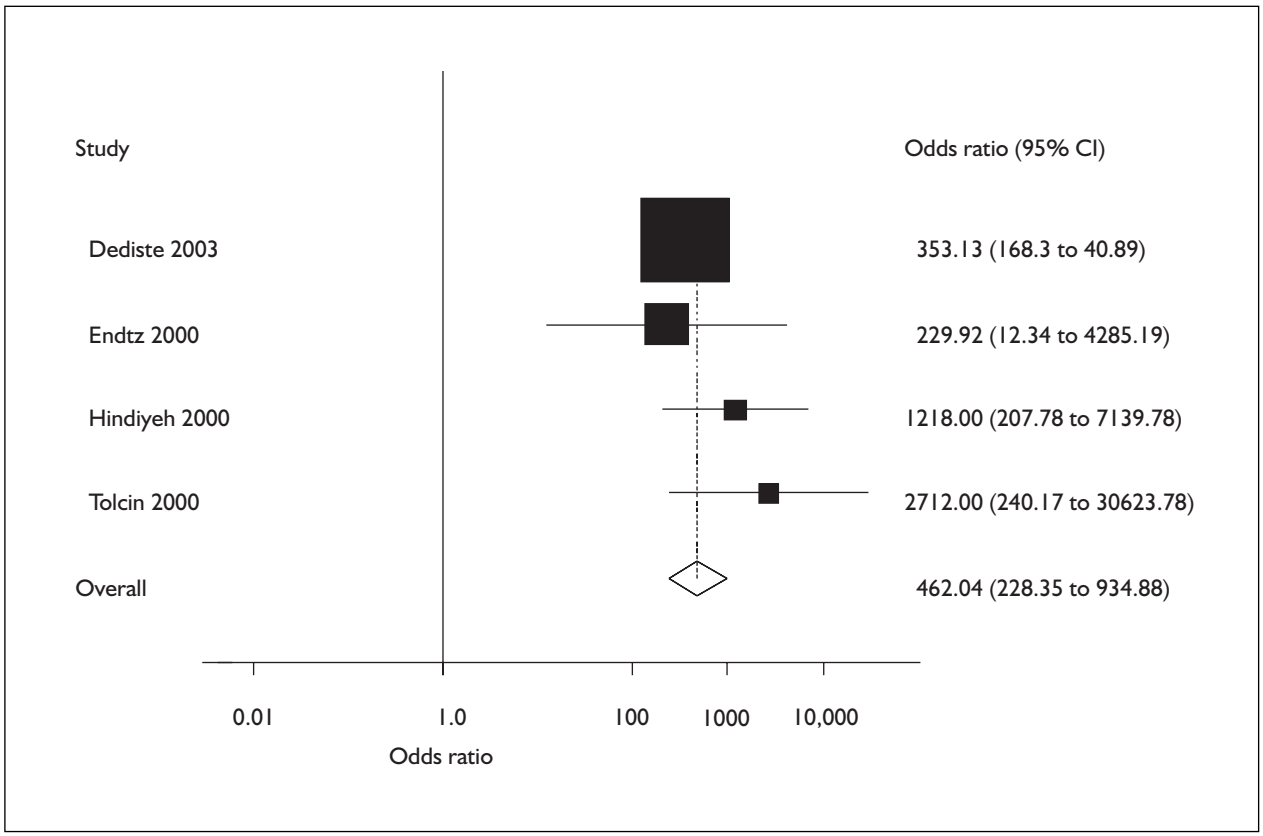


FIGURE 9 *Forest plot of DORs for PCR assays for* Campylobacter *food poisoning*

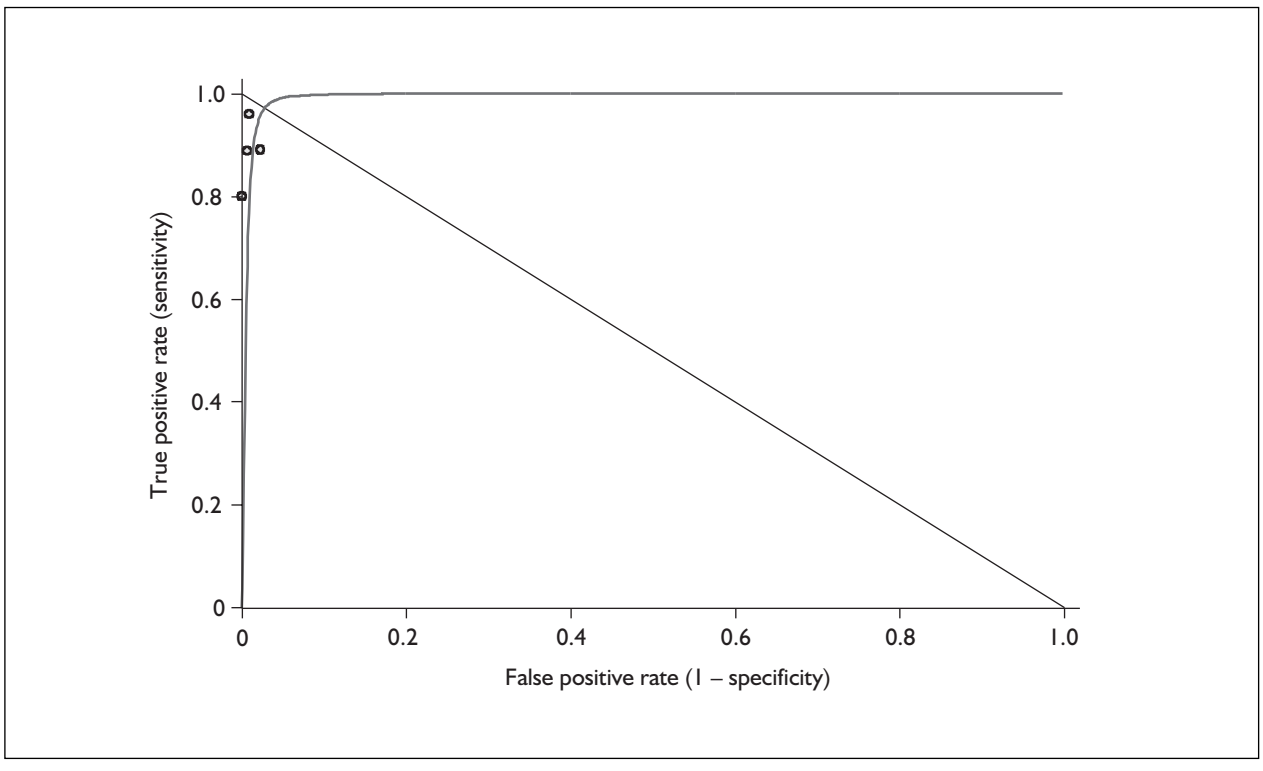


FIGURE 10 *SROC curve for the ProSpecT* Campylobacter *microplate enzyme immunoassay*

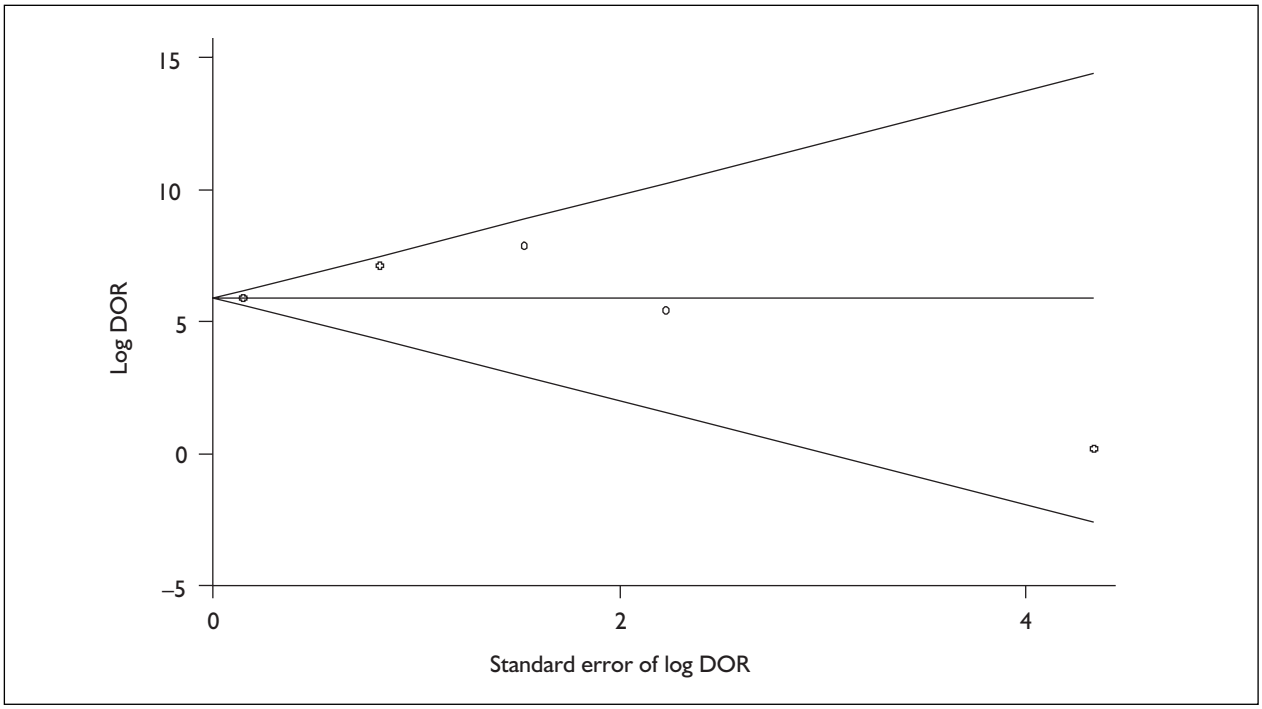


FIGURE 11 *Begg's funnel plot of log DOR for studies evaluating the ProSpecT enzyme immunoassay for* Campylobacter, *with* pseudo *95% CIs*

species. A number of studies using PCR detection methods[12,47,48] predict that *C. upsaliensis* and *C. hypointestinalis* are being missed in routine culture.

The overall sensitivity for 16s rRNA PCR was lowered considerably by one study,[48] a large-scale collaborative trial of UK laboratories which constituted the largest NAA-based evaluation study performed on *Campylobacter* to date. The authors reported that the time lag of 10 days between culture and PCR detection may have artificially reduced the sensitivity of nucleic acid-based methods. It is therefore likely that in a routine investigative circumstance, when samples would not sit for so long before testing, the sensitivity of the test would be higher. This would increase the summary estimate of 16s rRNA test sensitivity.

It is important to note that although the numbers of studies providing full evaluation with a reference test are limited, a large number of studies describe novel assays in development which have not yet been validated by comparing diagnostic accuracy with another test method. These studies have been excluded from the final review (Appendix 5). One excluded study[49] developed a rapid duplex rtPCR assay for speciation of *C. jejuni* and *C. coli* directly from culture plates. This was a very large study (involving over 6000 samples), but evaluation against culture was not performed, and negative samples were not included.

Salmonella

Number of studies

The original search strategy identified 768 studies relating to rapid diagnostic tests for *Salmonella*. The majority of these were eliminated by reviewing titles and abstracts. Most studies identified on methods for *Salmonella* detection are focused on food or veterinary samples. Following appraisal of abstracts, 26 studies were subjected to full text review and data extraction. Twelve clinical studies, primarily describing PCR assays for *Salmonella* detection, were excluded (Appendix 5). Twenty-two studies were ultimately included in this review. Basic information for all included studies is provided in *Table 8*.

Methodological quality of studies

The methodological quality of studies evaluating *Salmonella* detection methods was high. Nine studies scored QUADAS >10 (out of 14) and the remaining 11 scored QUADAS 6–10. None of the studies evaluating tests of *Salmonella* scored QUADAS of ⩽5.

TABLE 8 *Characteristics of studies evaluating rapid assays for the diagnosis of* Salmonella

Study	Basic test details				Diagnostic accuracy			Time issues	
	Test type/ target gene	Direct or from isolate	Reference test	Sample size	Sensitivity (95% CI)	Specificity (95% CI)	*Quality:* QUADAS score	Result within 24 hours	Reduced hands-on time
PCR assays									
Davis, 2003[161]	PCR (BAX system)	Isolates	*Salmonella* culture	78	1.000 (0.566 to 1.000)	0.986 (0.926 to 0.998)	8	Yes	Yes
Chiu, 1996[50]	PCR (*spvC* and *invA*)	12–18 hours incubation	XLD and SS agar	57	0.909 (0.722 to 0.975)	0.486 (0.330 to 0.644)	11	No	Yes
Alvarez, 2004[157]	PCR (DT104)	4 hours incubation	XLD, Hektoen, TSI agar	120	0.933 (0.787 to 0.982)	1.000 (0.959 to 1.000)	13	Yes	Yes
Luk, 1997[51]	PCR (*rfbS*)	Isolates	*Salmonella* culture	203	0.566 (0.484 to 0.643)	1.000 (0.938 to 1.000)	8	Yes	No
Iijima, 2004[117]	PCR (*yphC* and *gyrA*)	Direct	Strains only	161	0.818 (0.523 to 0.949)	0.960 (0.915 to 0.982)	9	Yes	Yes
Farrell, 2003[166]	PCR (*prgK* gene)	Isolate	Lysine iron agar and TSI agar	298	1.000 (0.910 to 1.000)	0.996 (0.978 to 0.999)	11	Yes	Yes
Amar, 2004[45]	PCR commercial	Isolate	Modified CCDA	91	0.750 (0.301 to 0.954)	1.000 (0.958 to 1.000)	12	Yes	Unclear
Malorney, 2003[173]	PCR (*invA* gene)	DNA sample	Strains only	1204	0.959 (0.939 to 0.973)	0.990 (0.979 to 0.995)	8	Unclear	Unclear
Wellcolex									
Rohner, 1992[179]	LAT	Overnight incubation	MacConkey and Hektoen agar	1010	0.872 (0.733 to 0.944)	0.998 (0.993 to 0.999)	7	Unclear	Yes
Bouvet, 1992[159]	LAT	Overnight incubation	MacConkey and Hektoen agar	193	0.984 (0.953 to 0.994)	1.000 (0.741 to 1.000)	8	Unclear	Yes
Hansen, 1993[167]	LAT	Overnight incubation	XLD and MacConkey agar	702	1.000 (0.910 to 1.000)	0.983 (0.971 to 0.991)	13	Unclear	Yes
MUCAP test									
Manafi, 1992	Improved culture	Isolates	Pure strains only	96	0.767(0.591 to 0.882)	1.000 (0.945 to 1.000)	6	Unclear	Unclear
Abdalla, 1994[55]	Improved culture	Isolated colonies	Rambach agar	50	1.000 (0.862 to 1.000)	0.577 (0.389 to 0.745)	8	Unclear	Unclear
Aguirre, 1990[30]	Improved culture	Isolated colonies	MacConkey or SS agar	432	0.952 (0.883 to 0.981)	0.900 (0.864 to 0.927)	7	Unclear	Unclear

continued

TABLE 8 *Characteristics of studies evaluating rapid assays for the diagnosis of* Salmonella *(cont'd)*

Study	Basic test details				Diagnostic accuracy		*Quality:* QUADAS score	Time issues	
	Test type/ target gene	Direct or from isolate	Reference test	Sample size	Sensitivity (95% CI)	Specificity (95% CI)		Result within 24 hours	Reduced hands-on time
Munoz, 1993[174]	Improved culture	Isolates	MacConkey, BG, or SS agar	976	1.000 (0.979 to 1.000)	0.9189 (0.898 to 0.936)	11	Unclear	Unclear
AutoMicrobic EPS									
Geers, 1988[52]	Biochemical ID	Colonies only	Biochemical test	484	0.870 (0.679 to 0.955)	0.855 (0.820 to 0.884)	7	Unclear	Yes
Villasante, 1987[54]	Biochemical ID	Colonies only	Biochemical test	800	0.997 (0.988 to 0.999)	0.877 (0.826 to 0.915)	8	Unclear	Yes
Imperatrice, 1993[53]	Biochemical ID	Colonies only	Biochemical test	265	0.995 (0.970 to 0.999)	0.901 (0.817 to 0.949)	8	Unclear	Yes
Wampole Bactigen									
Metzler, 1998[175]	LAT	Incubated overnight	Standard culture	1128	0.862 (0.694 to 0.945)	0.965 (0.953 to 0.975)	11	Unclear	Unclear
McGowan, 1989[142]	LAT	Incubated overnight	Standard culture	2382	0.859 (0.777 to 0.914)	0.973 (0.965 to 0.979)	12	Yes	Yes
Fedorka, 1989[166]	LAT	Incubated overnight	Standard culture	822	1.000 (0.816 to 1.000)	0.935 (0.916 to 0.950)	11	Yes	Yes
Geers, 1988[52]	LAT	Colonies only	Biochemical test	481	1.000 (0.839 to 1.000)	0.991 (0.978 to 0.997)	7	Yes	Unclear

BG, Brilliant Green; LAT, latex agglutination test; SS, *Salmonella–Shigella*; XLD, xylose lysine desoxycholate.

Correlation between sensitivity and specificity, and tests for heterogeneity

Where there is evidence of correlation (Spearman's $\epsilon > 0.5$), sensitivity and specificity estimates from each study were presented but not pooled. *Table 9* shows Spearman's ϵ and Pearson's χ^2 test results for rapid diagnostic assays for *Salmonella* infection. Correlation was evident in PCR and Wampole Bactigen results, therefore meta-analyses were carried out for these assays, including AUC of a SROC curve analysis. Due to the presence of significant heterogeneity (*Table 9*) for all assays based on the χ^2 test (and the exact test where appropriate), random-effect models were used.

Meta-analysis

Nucleic acid-based test methods

In total, there were seven in-house-designed PCR assays for use on clinical samples with sufficient data for meta-analysis. The characteristics of studies evaluating these assays are summarised in *Table 8*. Four were reported to perform worse than culture, one was equivalent and in two studies authors concluded that PCR assays performed significantly better than the traditional culture method. More research is required to determine whether the additional cases detected by PCR are indeed true isolates.

Figure 12 shows forest plots of sensitivity and specificity of *Salmonella* studies with a random-effect model. The pooled estimate of sensitivity was 0.85 (95% CI 0.75 to 0.97) and the specificity was 0.98 (95% CI 0.96 to 1.00). One study[50] had very low specificity, and the PCR assay by Luk and colleagues[51] had the lowest sensitivity. Both studies were good-quality studies based on the QUADAS score (*Table 8*). The Luk assay was developed to detect Serogroup D *Salmonella* only, so although a high proportion of false negatives were reported, the assay succeeded in its more specific objective. Such a narrow focus is not appropriate in the routine diagnostic laboratory. Although the quality of the Chiu study was high (QUADAS = 11), it had a notably small sample size ($n = 57$).

Figure 13 shows the DORs from these studies. The DOR values for PCR were the most varied, reflecting the greatest variation in sample sizes for these studies (range 57–1204). All the studies had very high DORs.

Publication bias

There was no statistical evidence of publication bias (*Figure 14*, Begg's test, Kendall's score = 7, $p = 0.293$; Egger's test bias = 1063, $p = 0.714$).

Non-molecular-based methods

The characteristics of the studies evaluating non-molecular-based tests are summarised in *Table 8*.

Wampole Bactigen

The Wampole Bactigen latex agglutination test was evaluated in four studies. All these studies were carried out in the late 1980s in the USA, and the current UK availability of this kit remains unclear. The kit is used as an early screen for *Salmonella* and *Shigella*, with negative samples eliminated within 24 hours, and a further 24 hours needed to identify positive samples biochemically. All studies concluded that the *Salmonella* test may be useful as an enrichment broth screening test to detect *Salmonella* spp.; however, given the low positive predictive value of the kit, parallel primary plating of samples is used. The sensitivity and specificity estimates are shown in a forest plot (in *Figure 15*). The sensitivities and specificities of the assay from each study were very high. The summary estimate of sensitivity was 0.932 (95% CI 0.850 to 1.015) and that for specificity was 0.967 (95% CI 0.950 to 0.985) using a random-effects model. A pooled estimate of the DOR (264.3, 95% CI 116.9 to 597.6) was determined (*Figure 16*).

Publication bias

Begg's test for Wampole Bactigen assays produced a Kendall's score of 6 ($p = 0.089$), and Eggers test produced bias 1653 ($p = 0.303$), hence no publication bias was evident.

Wellcolex Colour *Salmonella* test

The Wellcolex Colour *Salmonella* test is a rapid latex agglutination test designed to detect *Salmonella* antigens in stool enrichment broth or from culture plates. Latex particles are colour coded with antibodies specific for *Salmonella* serogroups A–E and G, and Vi antigen, which makes it more specific than other commercial latex test systems. This review found that the above test was most commonly assessed following enrichment in selenite F broth, and compared with traditional culture methods. Measures of diagnostic accuracy from each of the three evaluative studies are shown in forest plots (*Figures 15* and *16*). The summary DOR was 2951 (95% CI 710.9 to 12,000) using a fixed-effect model (no evidence of heterogeneity, $I^2 = 0\%$). The Wellcolex Colour *Salmonella* test produced the highest pooled DOR. SROC analysis was not carried out because there are only three eligible studies.

AutoMicrobic Enteric Pathogen Screen cards

Three studies evaluated the AutoMicrobic EPS system against a reference standard of

TABLE 9 *Correlation between sensitivity and specificity, and tests for heterogeneity for studies evaluating rapid assays for the diagnosis of* Salmonella *infection*

	Correlation			Heterogeneity		
Test	**No. of studies**	**Spearman's ϵ**	***p*** **(ϵ = 0)**	**OR Pearson χ^2**	***p***	**I^2 statistic (%)**
PCR	7	0.296	0.518	45.66	0.001	86.9
MUCAP	4	0.889	0.111	4.38	0.223	31.5
Wellcolex	3	0.500	0.667	0.45	0.797	0
AutoMicroBic	3	–0.500	0.667	15.4	0.001	87.0
Wampole Bactigen	4	0.105	0.895	4.4	0.221	31.5

FPR, false positive rate; TPR, true positive rate.

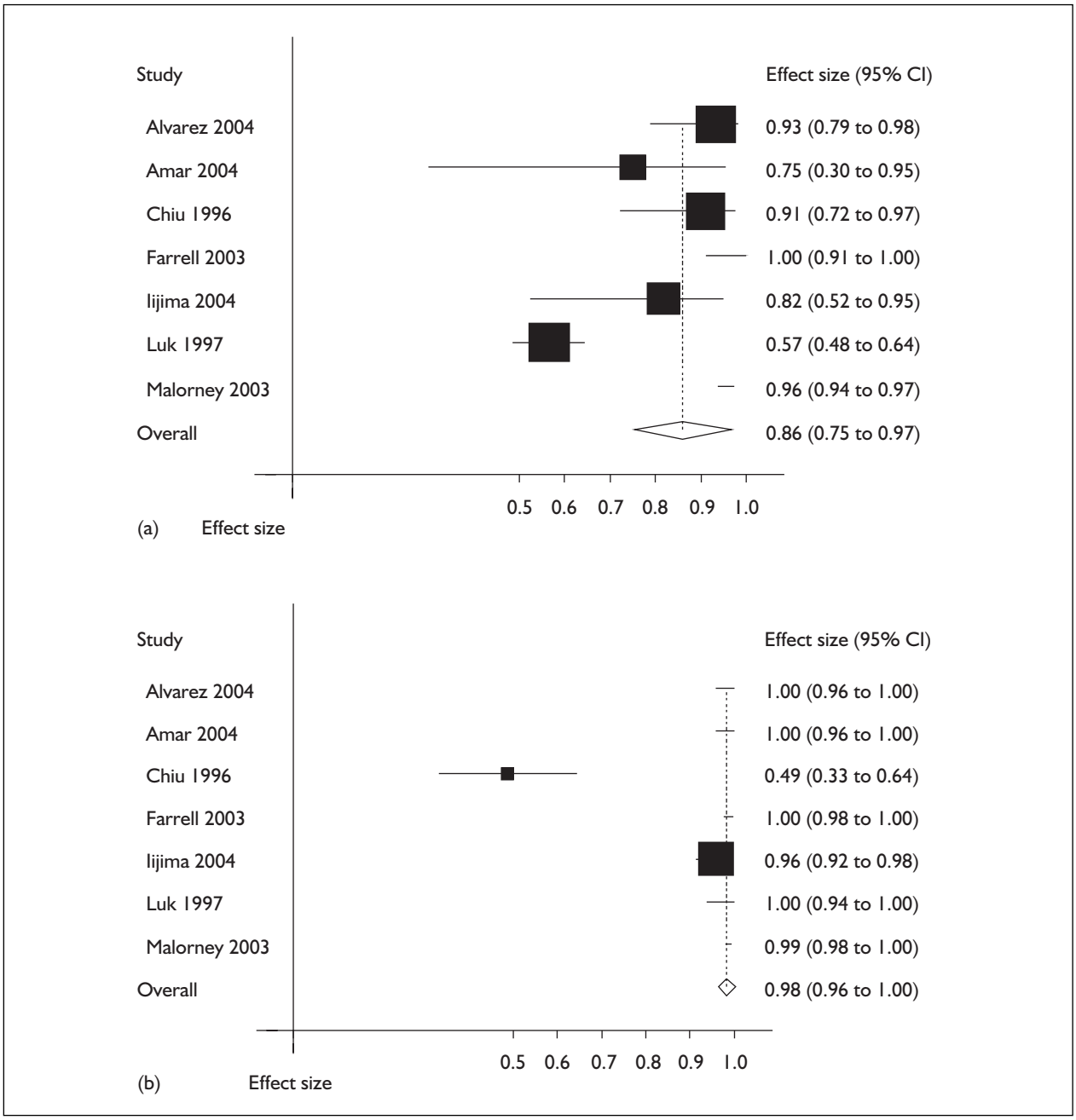


FIGURE 12 *Forest plots of studies showing (a) the sensitivity and (b) the specificity of evaluating PCR assays for* Salmonella *detection*

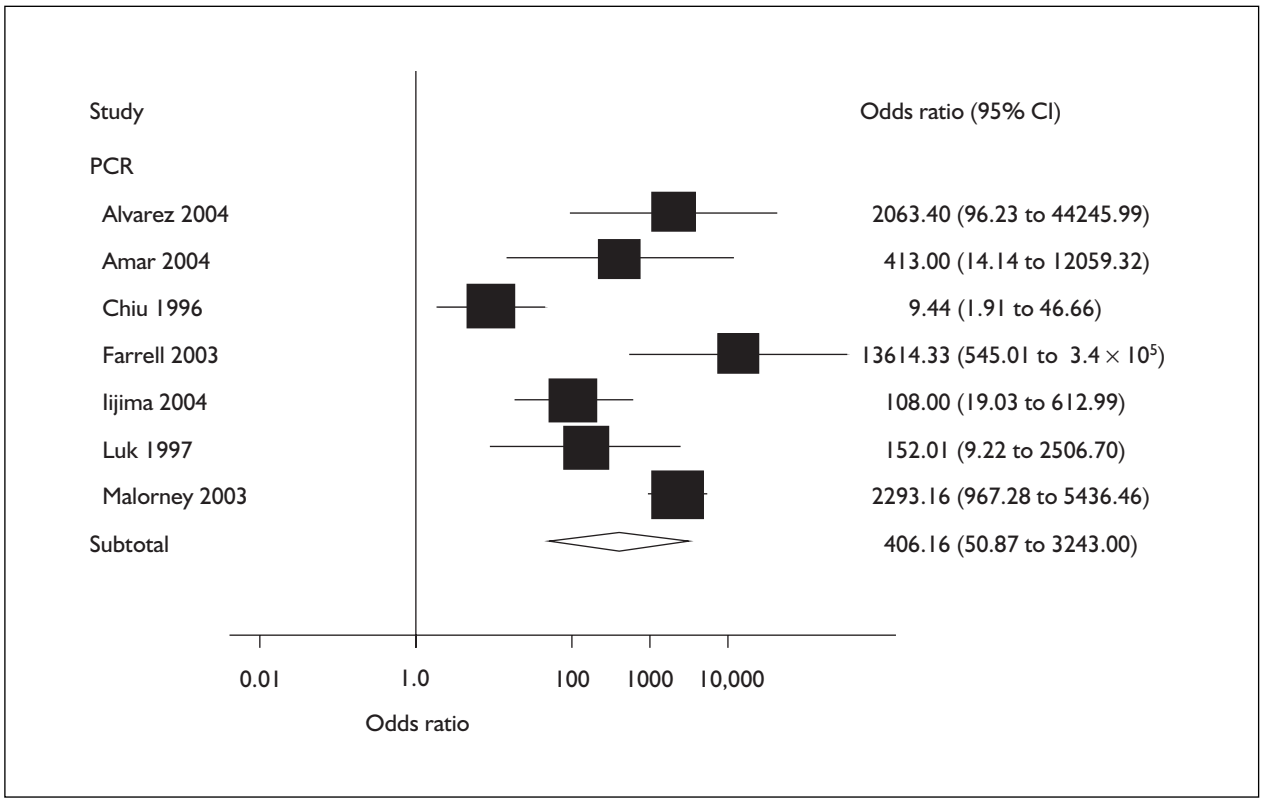


FIGURE 13 *Forest plot of DORs for PCR assays for* Salmonella

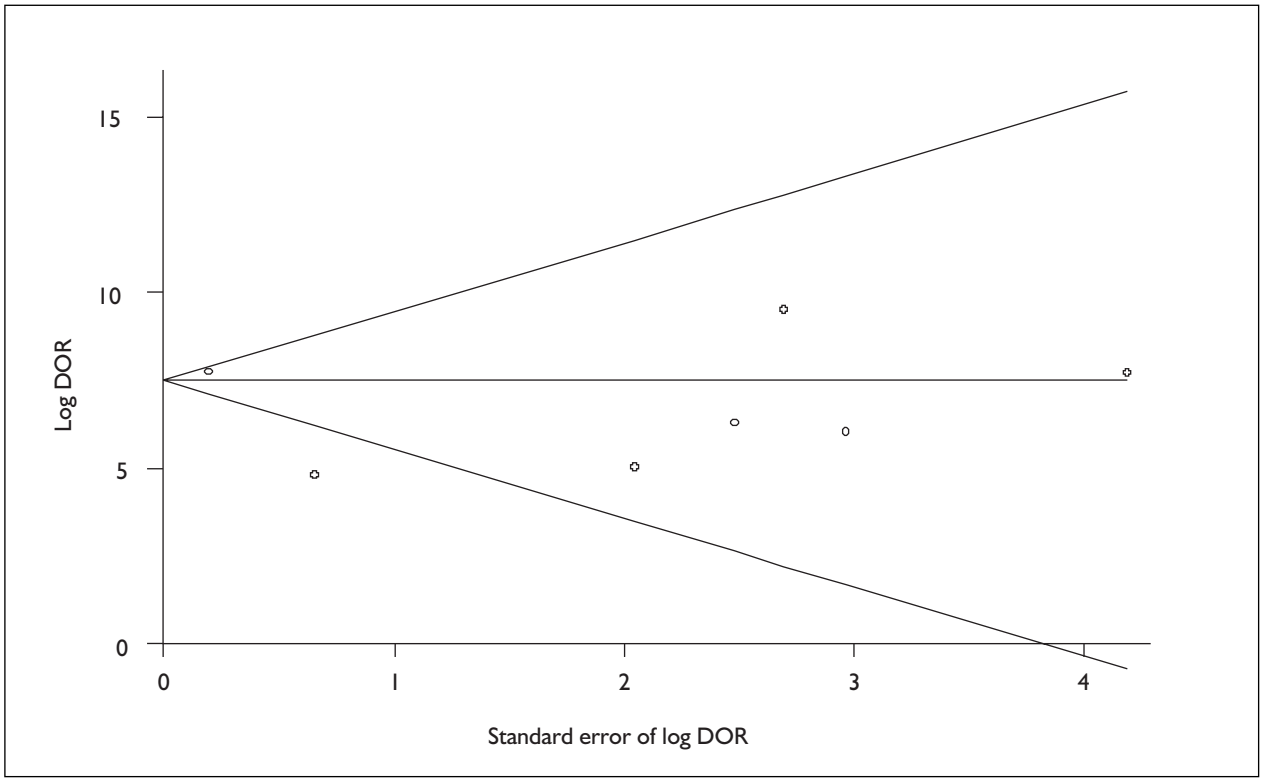


FIGURE 14 *Begg's funnel plot of log DOR for studies evaluating the PCR detection of* Salmonella*, with 95% CIs*

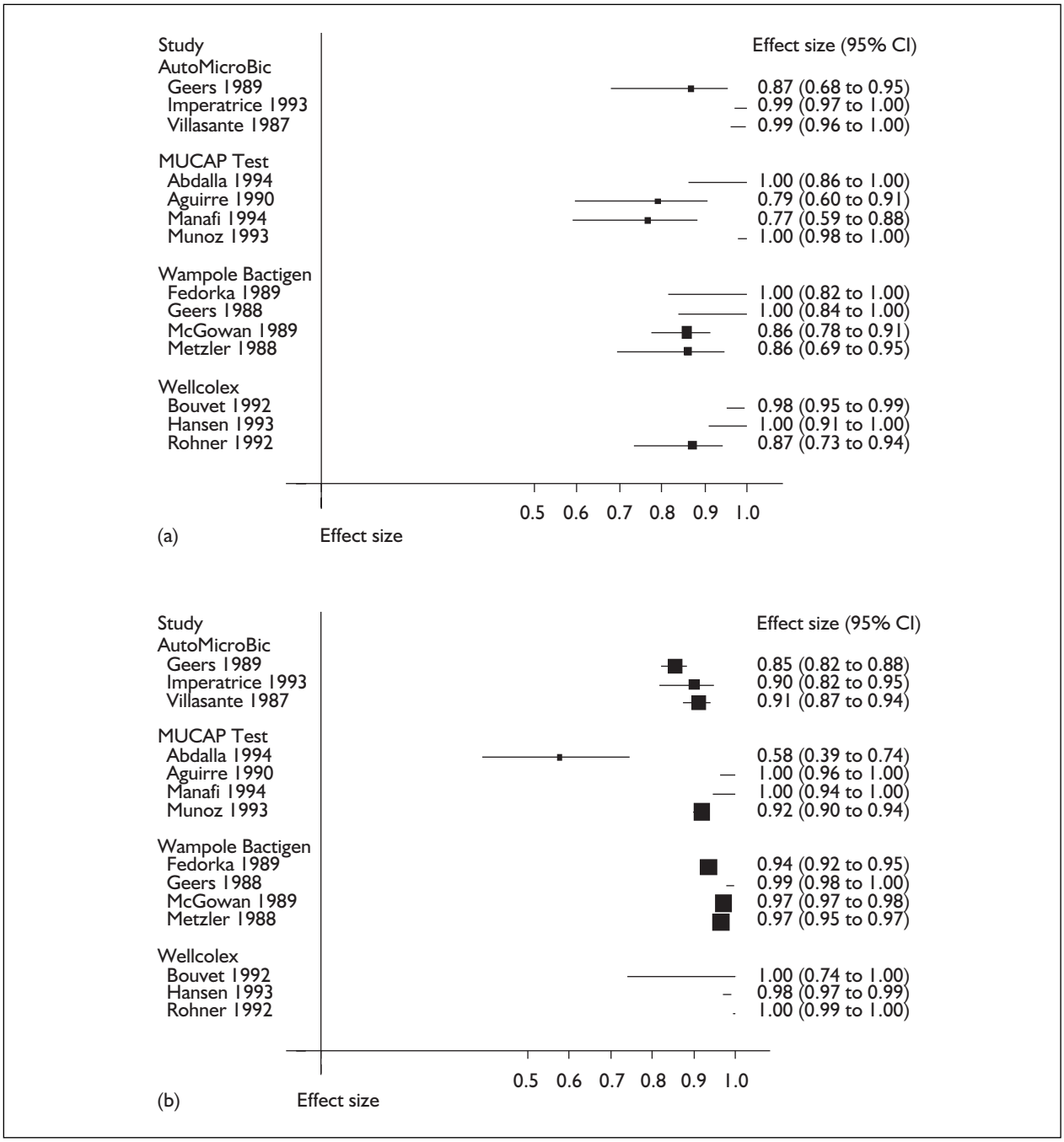


FIGURE 15 *Forest plots of studies showing (a) the sensitivity and (b) the specificity of the Wellcolex Colour, MUCAP, Wampole Bactigen and AntiMicrobic EPS assays for* Salmonella

conventional biochemical test media.[52–54] (Notably, all studies retrieved for EPS were carried out in the late 1980s. The system is still commercially available in the UK from BioMerieux-Vitek, but several modifications may have taken place since then. Unfortunately, evaluations of current systems used on clinical samples could not be found. It would be useful to know if the level of false positive results has improved since these studies took place.) This system is able to detect *Salmonella*, and also the less common pathogens *Shigella*, *Yersinia enterocolitica* and *Edwardsiella* spp. A preliminary diagnosis can be achieved within 6 hours of incubation, allowing correct clinical decisions to be made the next day from receiving samples (24 hours earlier than conventional methods). The sensitivity and specificity estimates from these studies are summarised in *Figure 15*. *Figure 16* shows the meta analysis of DORs (365.5, 95% CI 30.2 to 4421.1) from the three included studies. Due to the limited number of studies, an SROC analysis was not carried out.

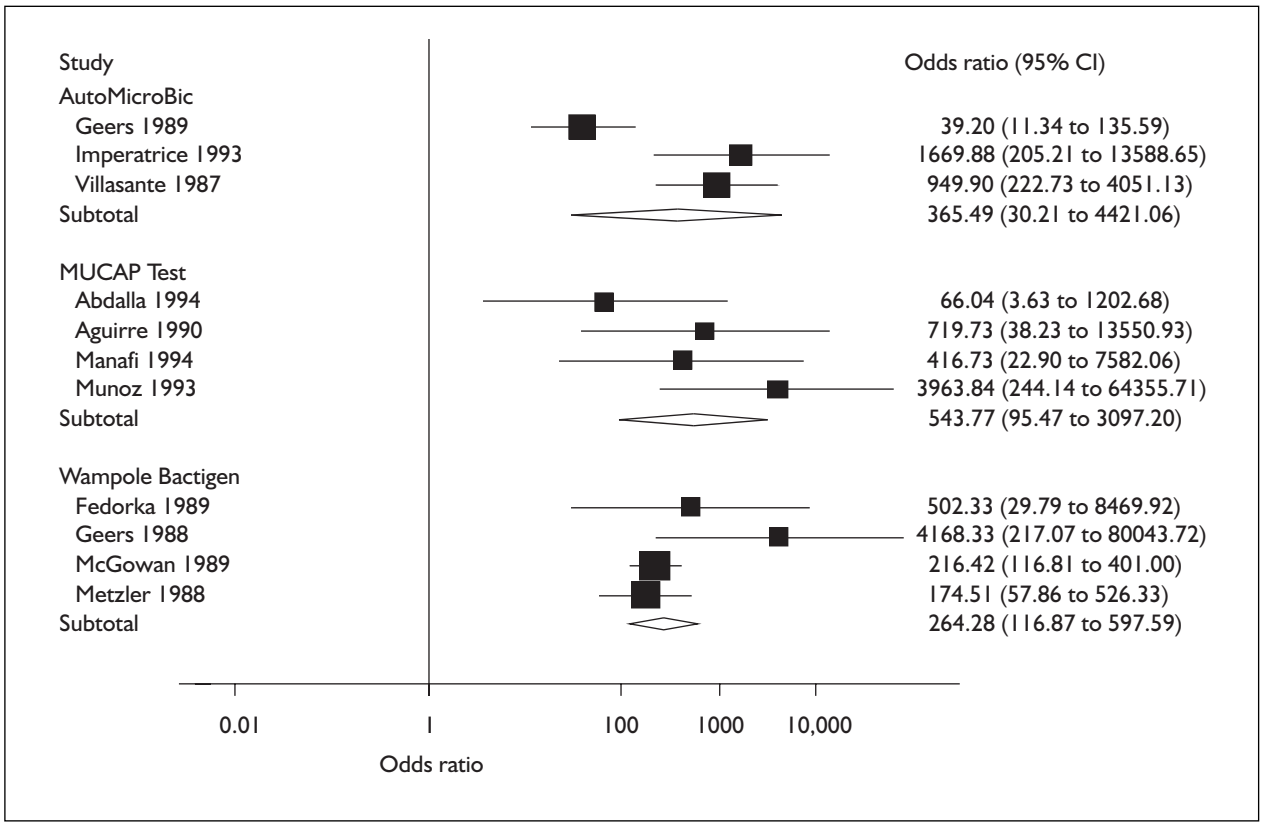


FIGURE 16 *Forest plot of DORs for non-molecular based assays for* Salmonella*. Studies evaluating the Wellcolex assay are not presented in this figure because they were pooled using a fixed-effect model.*

MUCAP test

The MUCAP test is a rapid method for the presumptive detection of *Salmonella* spp. which can potentially reduce the work and material involved in testing by selecting samples requiring further assessment. A total of eight comparisons were carried out on the MUCAP test. *Figure 15* shows estimates of sensitivity and specificity from each study, all of which are high except the study by Abdalla and colleagues,[55] where the specificity was 0.58 (95% CI 0.39 to 0.74). A high pooled DOR of 543.8 (95% CI 95.5 to 3097.2) was obtained (*Figure 16*).

Publication bias

There was no statistical evidence of publication bias using Begg's test (Wellcolex test Kendall's score = –1, $p = 0.602$, MUCAP test Kendall's score = 2, $p = 0.805$, and AutoMicrobic EPS Kendall's score = 1, $p = 0.602$,). Egger's test was similarly not significant for Wellcolex test (–857.5, $p = 0.835$), MUCAP test (0.0051, $p = 0.067$) and AutoMicrobic EPS (3704.1, $p = 0.257$).

Culture-based methods

A large proportion of studies focusing on *Salmonella* detection compared different culture-based methods, as opposed to evaluating 'rapid' methods such as PCR or immunoassays. This review identified at least 10 studies evaluating commercially available chromogenic agar plates (see Appendix 5). These assays use a combination of chromogenic substrates and conventional biochemical tests, and are more specific than traditional culture methods. Chromogenic agar plates can reduce the workload with regard to unnecessary examination of suspect colonies, saving time, supplies and money; however, they do not reduce the time needed to detect isolates by more than 1 day, and therefore cannot be considered 'rapid'.

Discussion

In view of the high prevalence and strong media attention given to *Salmonella* infections, it is surprising that evaluative studies for rapid diagnostic test methods are relatively scarce. This review identified three key latex agglutination kits, evaluations of which had all been carried out in the late 1980s. Studies evaluating nucleic acid detection methods have been published more frequently in recent years, but the number of developmental, non-evaluative studies far outweighs those in which the PCR assay has been evaluated against a suitable reference test. The most well developed and methodologically sound

research on *Salmonella* detection relates to improvements in culture methods, which, although useful to a laboratory user, do not significantly improve the speed of microbiological detection.

Escherichia coli O157 and other shiga toxin-producing *E. coli*

Number of studies

A total of 916 studies relating to rapid diagnostic tests for *E. coli* were identified from the initial search strategy. Of these, 123 related specifically to the *E. coli* O157 strain and 153 focused on all shiga toxin-producing *E. coli* (STEC) strains. Following appraisal of abstracts, 40 published articles were reviewed as background information only, relevant data were extracted from 39 studies and 27 of these were included in this review. Basic information for all included studies is provided in *Table 10*.

Of the 27 studies included in this review, 10 used PCR methods and 12 used antibody-based techniques. PCR and antibody-based tests were compared in five studies. Fourteen studies were suitable for meta-analysis.

Methodological quality of studies

The methodological quality of studies evaluating *E. coli* rapid assays was relatively high compared with that for other pathogens. Seventeen of the 26 studies were of 'high' quality (QUADAS >11) quality, seven were of medium quality (QUADAS = 6–10), and only two were of poor quality (QUADAS <5).

Correlation between sensitivity and specificity, and tests for heterogeneity

Significant correlation between sensitivity and specificity was observed except for Premier EHEC (*Table 11*). The sensitivity and specificity of studies evaluating Premier EHEC assay were therefore pooled. Random effects models were used for the meta-analysis where appropriate because the studies all showed significant evidence of heterogeneity.

Meta-analysis

Nucleic acid amplification tests

PCR diagnosis of *E. coli* was predominantly centred on detection of shiga toxin genes, often referred to as *stx1* and *stx2* present in STEC. The review identified 12 studies in which novel assays were developed around *stx1* and *stx2*: eight of these contained sufficient comparative statistical data to enable meta-analysis. PCR for the detection of the *eae* gene was additionally evaluated in four studies.

Figure 17 shows forest plots of sensitivity and specificity of *E. coli* O157 studies with random-effect model.

A total of 4746 samples were tested with stx PCR assays. Further details are provided in *Table 10*. Sensitivity estimates for EHEC studies were consistently high (lowest 0.82); however, they had wide 95% CIs. Of the three test methods evaluated, PCR assays provided the highest sensitivity value and narrowest 95% CI.

Figure 18 shows DORs from these studies. There was some variation in DOR values for PCR assays for EHEC. The lowest DOR value was observed in the study by Paton and colleagues,[56] with a sample size of 183, whereas the very large study by Welinder-Olsson and colleagues[57] had a DOR of 290,000. This illustrates the problem of using DOR as a summary estimate when specificity is very high and sample sizes vary widely.

The SROC curve (*Figure 19*) is symmetrical ($|b| < 0.001$, $p = 0.141$). Therefore, a homogeneous estimate of the AUC was calculated. The AUC of the SROC curve was 0.996 (95% CI 0.990 to 1.000).

Publication bias

Meta-analysis of studies evaluating the PCR detection of stx genes in *E. coli* shows evidence of asymmetry (*Figure 20*). Begg's test found statistically significant evidence of publication bias (Kendall's score = 15, $p = 0.024$). In contrast, the result of Egger's test was not significant (87,824, $p = 0.771$).

Antibody-based tests

This review identified 17 shiga-like toxin ELISAs that are commercially available in kit form. Of these, five were evaluated against a suitable reference test.

Tests of pure isolates show that the specificities of the various *stx* ELISAs are in close agreement with the results of Vero cell cytotoxicity assays (VCAs), the most appropriate reference test for the detection of STEC. On clinical samples, ELISAs were generally less sensitive than VCAs. Broadly comparing all immunoassay tests included in the meta-analysis, testing almost 4000 clinical samples, individual sensitivities ranged from 0.824 to 1.000 and specificities ranged from 0.667 to 1.000.

TABLE 10 *Characteristics of studies evaluating rapid assays for the diagnosis of* E. coli *food poisoning*

Study	Basic test details					Diagnostic accuracy		*Quality*: QUADAS score	Time issues	
	Test name	Test type	Direct or from isolate	Reference test	Sample size	Sensitivity (95% CI)	Specificity (95% CI)		Result within 24 hours	Reduced hands-on time
Welinder-Olsson, 2000[57]	Conventional PCR	*stx1*, *stx2* and eaeA	Isolate	SMAC culture	3948	1.000 (0.935 to 1.000)	0.999 (0.999 to 1.000)	13	Yes	Unclear
Welinder-Olsson, 2004[21]	Conventional PCR	*stx1* and *stx2*	Isolate	SMAC culture	59	1.000 (0.701 to 1000)	0.960 (0.865 to 0.989)	13	Yes	Unclear
Belanger, 2002[158]	Real-time PCR	*stx1* and *stx2*	Isolate	SMAC culture	43	0.917 (0.871 to 1.000)	1.000 (0.646 to 0.985)	7	Yes	No
Ramotar, 1995[178]	Conventional PCR	*stx1* and *stx2*	Direct	SMAC culture	100	0.950 (0.764 to 0.991)	0.970 (0.916 to 0.989)	8	Unclear	Unclear
Beutin, 2002[63]	Conventional PCR	*stx1* and *stx2*	Direct	SMAC culture	234	0.932 (0.838 to 0.973)	0.954 (0.912 to 0.976)	11	Yes	Yes
Paton, 1998[177]	Conventional PCR	*stx1*, *sx2*, eaeA and hlyA	Isolate	Banked strains	92	1.000 (0.939 to 1.000)	1.000 (0.893 to 1.000)	5	Yes	Unclear
Paton, 2005[56]	Conventional PCR	*stx1*, *stx2* and subA	Isolate	Banked strains	183	1.000 (0.978 to 1.000)	1.000 (0.956 to 1.000)	5	Yes	Unclear
Davis, 2003[161]	BAX System	Commercial PCR	Isolates	SMAC culture	18	1.000 (0.675 to 1.000)	1.000 (0.824 to 1.000)	8	Yes	Yes
Pulz, 2003[22]	Real-time PCR	*stx1*, *stx2* and *stx2e*	Overnight incubation	Conventional PCR		1.000 (0.923 to 1.000)	0.980 (0.954 to 0.991)	12	Yes	Yes
Reischl, 2002[71]	Real-time PCR	*stx1* and *stx2*	Isolates	Conventional PCR	622	0.964 (0.935 to 0.980)	1.000 (0.989 to 1.000)	9	Yes	Yes
Beutin, 2002[63]	VTEC Screen	RPLA	Overnight incubation	SMAC culture	234	0.898 (0.795 to 0.953)	0.994 (0.968 to 0.999)	11	Yes	Yes
Chart, 2001[64]	VTEC Screen	RPLA	Overnight incubation	Vero cell	15	0.889 (0.565 to 0.980)	0.667 (0.300 to 0.903)	9	Yes	Yes
Bettelheim, 2001[62]	VTEC Screen	RPLA	Direct	Culture	239	1.000 (0.935 to 1.000)	0.982 (0.906 to 0.982)	9	Yes	Yes
Kai, 1997[65]	VTEC Screen	RPLA	Direct	Vero cell	178	1.000 (0.975 to 1.000)	1.000 (0.889 to 1.000)	7	No	Yes
Carroll, 2003[58]	VTEC Screen	RPLA	Overnight incubation	SMAC culture	554	1.000 (0.510 to 1.000)	0.978 (0.962 to 0.987)	13	Yes	Yes
Klein, 2002[59]	Premier EHEC	EIA	Direct	SMAC culture	1851	0.893 (0.728 to 0.963)	1.000 (0.998 to 1)	13	Yes	Yes

continued

TABLE 10 *Characteristics of studies evaluating rapid assays for the diagnosis of* E. coli *food poisoning (cont'd)*

Study	Basic test details					Diagnostic accuracy		*Quality:* QUADAS score	Time issues	
	Test name	Test type	Direct or from isolate	Reference test	Sample size	Sensitivity (95% CI)	Specificity (95% CI)		Result within 24 hours	Reduced hands-on time
Novicki, 2000[60]	Premier EHEC	EIA	Overnight incubation	SMAC culture	488	0.824 (0.589 to 0.938)	1.000 (0.992 to 1.000)	12	Yes	Yes
Carroll, 2003[58]	Premier EHEC	EIA	Overnight incubation	SMAC culture	554	1.000 (0.510 to 1.000)	0.984 (0.969 to 0.991)	13	Yes	Yes
Kehl, 1997[32]	Premier EHEC	EIA	Overnight incubation	SMAC and cytotoxicity assay	972	1.000 (0.772 to 1.000)	0.997 (0.991 to 0.998)	12	Yes	Yes
Mackenzie, 1998[33]	Premier EHEC	EIA	Broth	SMAC culture	876	0.893 (0.785 to 0.950)	0.923 (0.903 to 0.939)	13	Yes	Yes
Vidal, 2002[61]	Premier EHEC	EIA	Broth	PCR	78	0.833 (0.437 to 0.970)	0.819 (0.715 to 0.891)	10	Yes	Yes
Mackenzie, 1998[33]	Premier *E. coli* O157	EIA	Isolate	SMAC culture	876	0.864 (0.761 to 0.926)	0.984 (0.973 to 0.991)	12	Yes	Yes
Park, 1998[176]	LMD Laboratories	EIA	Direct	SMAC culture	605	0.912 (0.770 to 0.969)	0.995 (0.985 to 0.998)	12	Yes	Yes
Dylla, 2003[163]	LMD Laboratories	EIA	Direct	SMAC culture	185	1.000 (0.701 to 1.000)	0.989 (0.959 - 0.996)	12	Yes	Yes
Law, 1994[171]	In house	EIA	Isolate	SMAC culture	475	1.000 (0.916 to 1.000)	0.972 (0.952 to 0.984)	11	Unclear	No
Karmali, 1999[169]	Verotox-F assay	EIA	Isolate	Vero cell phenotyping assay	165	1.000 (0.944 to 1.000)	1.000 (0.963 to 1.000)	12	Unclear	Yes
Gavin, 2004[66]	ProSpecT assay	EIA	Isolate	SMAC culture	2603	1.000 (0.898 to 1.000)	0.999 (0.997 to 0.999)	12	Yes	Yes
Park, 2003[70]	Duopath Verotoxin	Immuno-chromato-graphic test	Isolate	Premier EHEC (EIA)	291	1.000 (0.918 to 1.000)	1.000 (0.971 to 0.997)	12	Yes	Yes
Mackenzie, 2000[67]	ImmunoCard STAT *E. coli* O157	EIA	isolate	SMAC culture	277	0.929 (0.685 to 0.987)	1.000 (0.986 to 1.000)	11	Yes	Yes
Pulz, 2003[22]	RidaScreen Verotoxin	EIA	Isolate	Conventional PCR	295	0.674 (0.529 to 0.791)	0.968 (0.937 to 0.983)	12	Yes	Yes

SMAC, sorbitol MacConkey agar.

TABLE 11 *Correlation between sensitivity and specificity, and tests for heterogeneity for studies evaluating rapid assays for the diagnosis of* E. coli *food poisoning*

	Correlation			Heterogeneity		
Test	**No. of studies**	**Spearman's ϵ**	***p*** **(ϵ = 0)**	**OR Pearson χ^2**	***p***	**I^2 statistic (%)**
stx PCR	7	0.647	0.116	15.2	0.019	60.5
Premier EHEC	5	0.177	0.776	7.28	0.122	45.1
VTEC Screen	5	–0.671	0.215	14.7	0.005	72.8

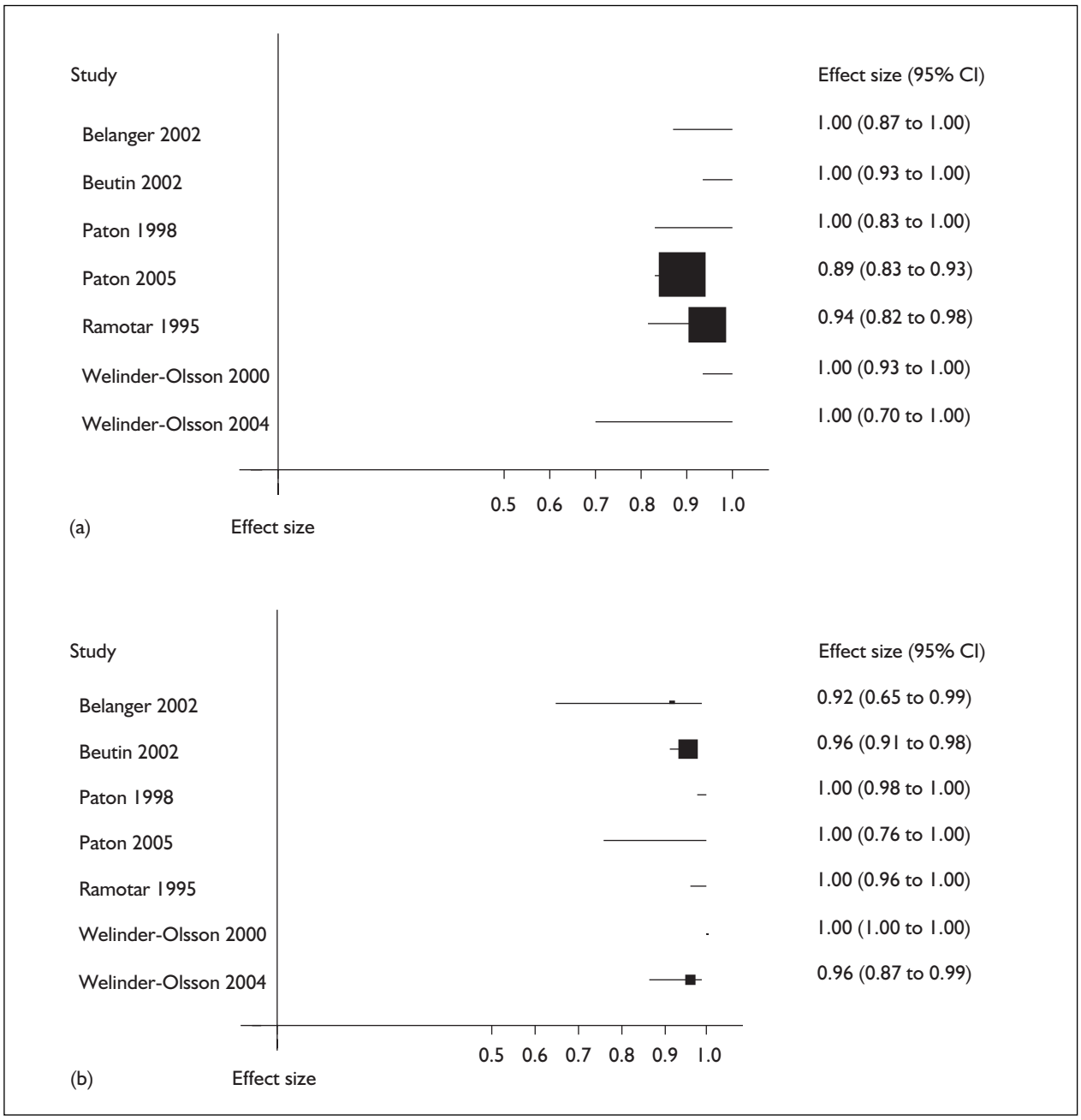


FIGURE 17 *Forest plots of studies showing (a) the sensitivity and (b) the specificity of* stx *PCR for* E. coli *detection*

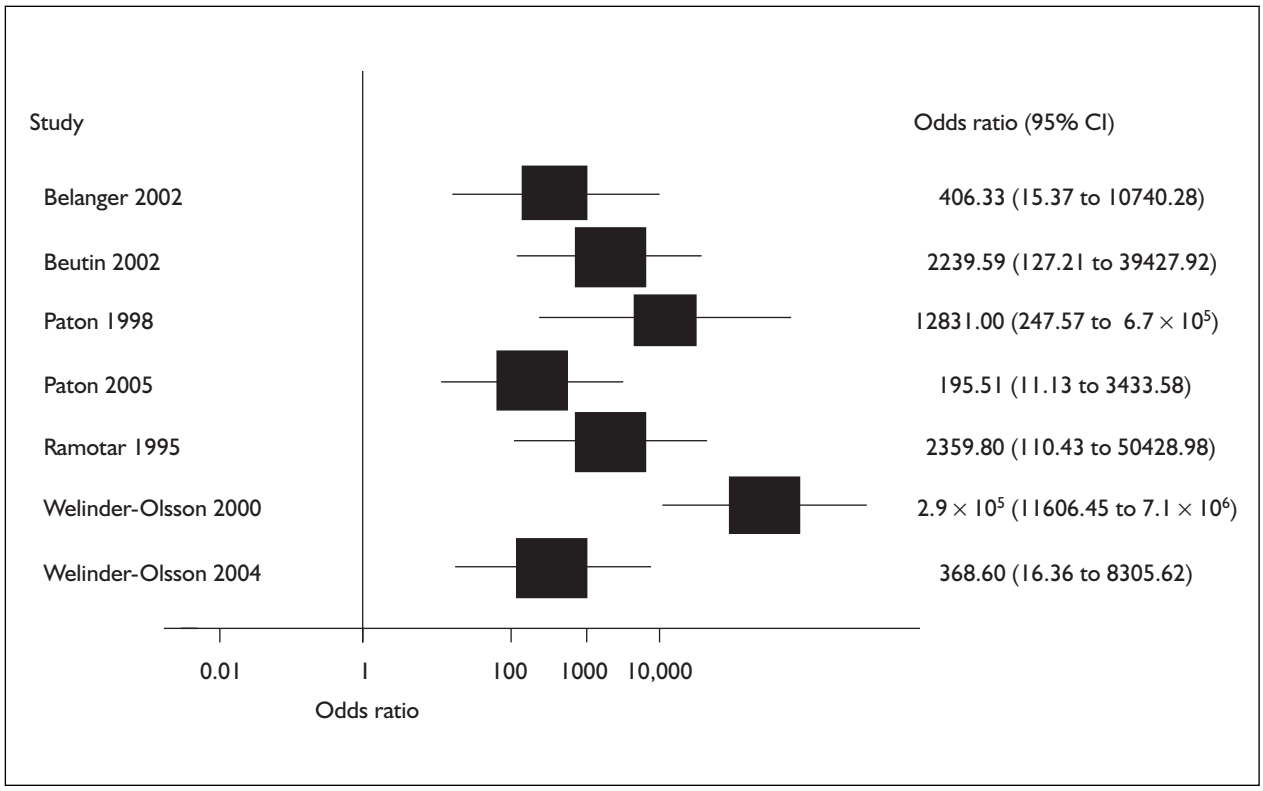


FIGURE 18 *Forest plot of DORs for* stx *PCR assays for* E. coli *food poisoning*

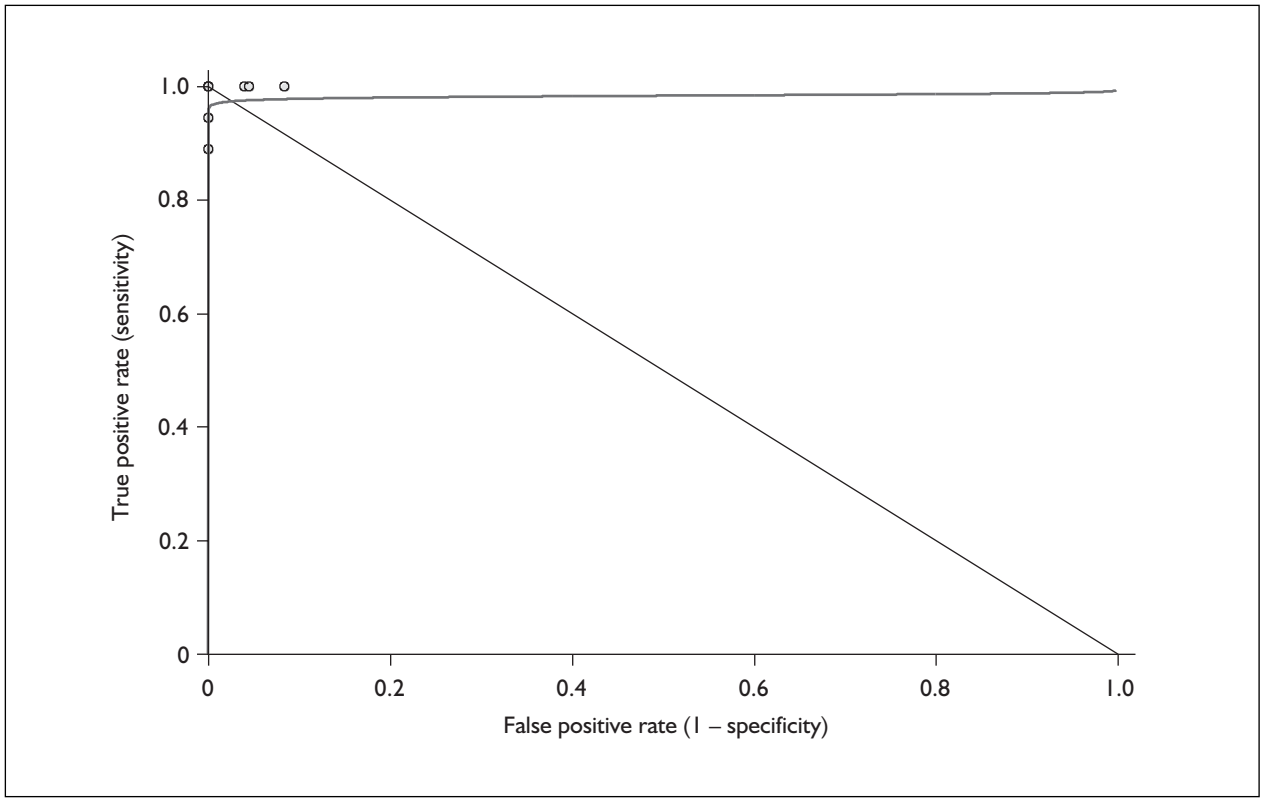


FIGURE 19 *SROC curve for* stx *PCR tests for* E. coli

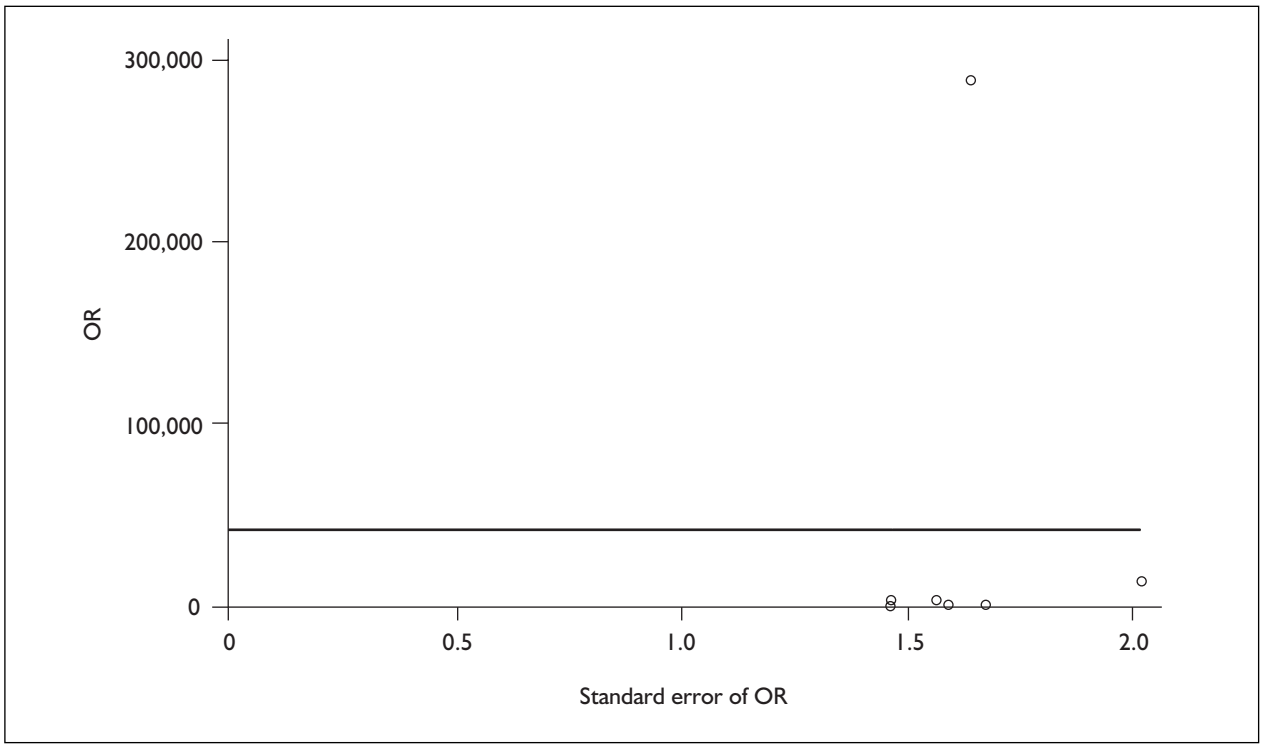


FIGURE 20 *Begg's funnel plot of DORs for* stx *PCR assays, with 95% CIs*

Forest plots for sensitivity, specificity and DORs for antigen-based tests for EHEC are provided in *Figures 21* and *22*). Sensitivity estimates for Premier EHEC and VTEC Screen assays were generally high (lowest estimate 0.82). There was one notable outlier in specificity for EHEC non-molecular assays. Chart and colleagues,[64] evaluating VTEC Screen assay, had a specificity estimate of 0.67, which is 0.25 lower than the second lowest. The quality of this study was medium (QUADAS = 9); however, it had a very small sample size (n = 15).

Premier EHEC (Meridian Diagnostics)
Meridian Diagnostic's Premier EHEC test kit was evaluated against SMAC culture and a Vero cell cytotoxicity assay in six studies.[32,33,58–61] Based on evidence of its diagnostic performance, the Premier EHEC kit provides an alternative test method when cytotoxin assay or PCR is neither feasible nor accessible. A total of 4741 samples were tested in the four studies. Pooled estimates of sensitivity and specificity were calculated using random-effect models. The pooled estimate of sensitivity and specificity for this assay were 0.926 (95% CI 0.867 to 0.985) and 0.998 (95% CI 0.996 to 1.001), respectively. The pooled DOR was similarly high (3755.0, 95% CI 795.5 to 17726.2).

VTEC Screen (Denka Seiken)
Five studies evaluating Denka Seiken's VTEC Screen were identified.[58,62–65] This commercial test kit uses reversed passive latex agglutination to detect shiga-like toxins. The reference test used was verocell cytoxicity, and in some cases additional *stx* gene-specific PCR. Promising in terms of ease of use and performance, the VTEC Screen test kit has considerable potential to improve STEC detection and isolation rates in routine clinical diagnostic laboratories, especially for non-O157 STEC and sorbitol-fermenting *E. coli* O157. The VTEC Screen assay was evaluated in five studies (*Table 10*). The pooled DOR was high [1224.1 (95% CI 117.5 to 13,000)].

A symmetrical SROC curve (b = 0.172, p = 0.906) was plotted (*Figure 23*) and a homogeneous AUC of the SROC curve was calculated [AUC = 0.994 (95% CI 0.982 to 1.000). There was no statistical evidence of publication bias (Begg's test Kendall's score = 2, p = 0.624).

Assays ineligible for meta-analysis
***ProSpecT Shiga Toxin* E. coli**
The ProSpecT Shiga Toxin *E. coli* microplate (Oxoid) was evaluated in one study. Gavin and colleagues[66] conducted a large-scale prospective evaluation of this commercial ELISA kit over two consecutive summers. They concluded that the kit

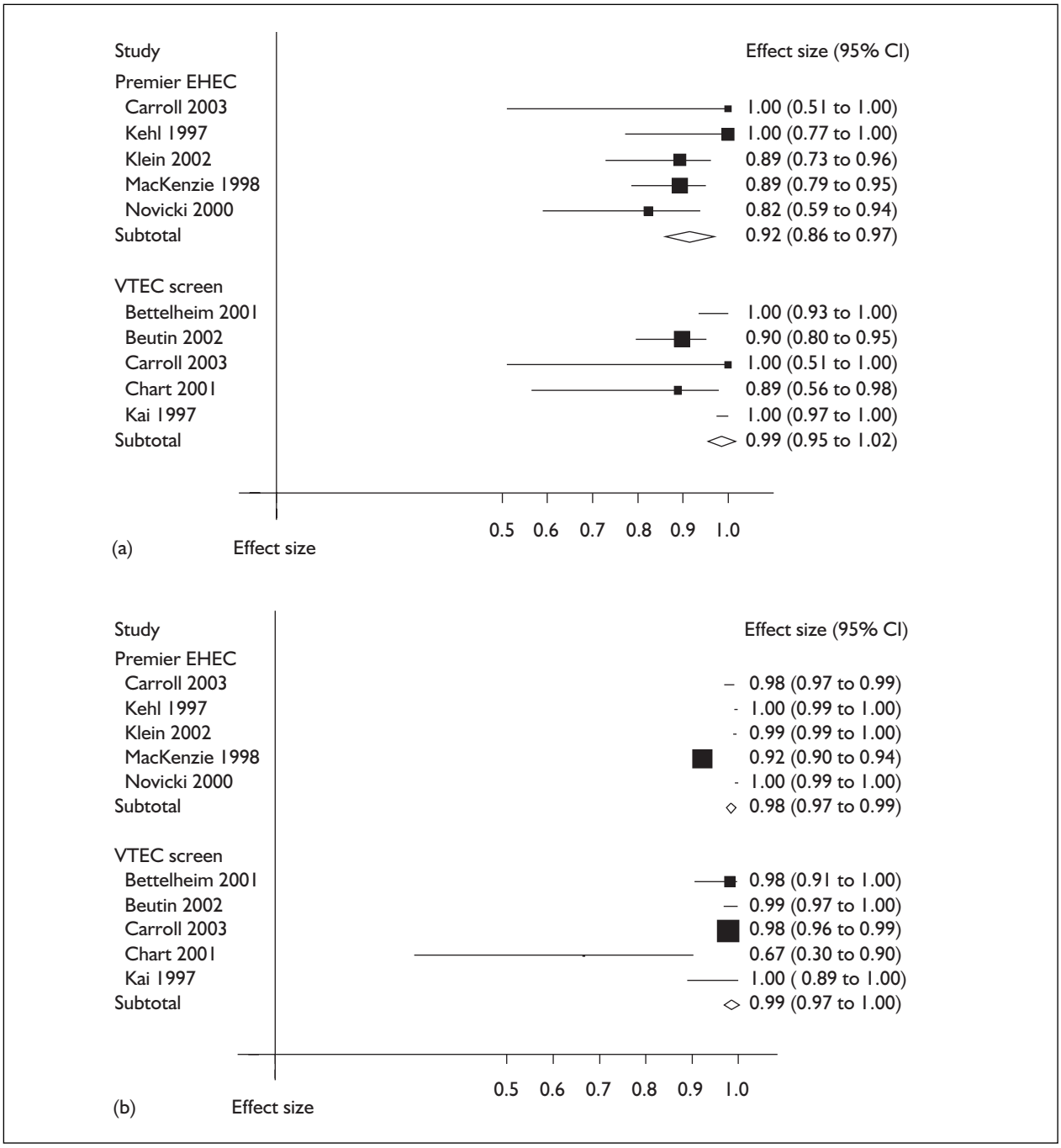


FIGURE 21 *Forest plots of studies showing (a) the sensitivity and (b) the specificity of non-molecular assays for* E. coli *detection*

could detect equal numbers of *E. coli* O157 as SMAC culture, with the potential to detect twice as many non-O157 STEC. This study recommends adoption of virulence factor-based tests such as the ProSpecT kit for routine diagnostic use.

ImmunoCard STAT! O157:H7

The ImmunoCard STAT! (ICS) test (Meridian Diagnostics) attempts to detect *E. coli* by targeting O157:H7 strains only, which are most commonly associated with severe secondary illnesses such as HUS. In this sense, the most appropriate reference test to use is culture with SMAC, as this method also attempts to isolate the O157 strain to the exclusion of others. Authors have evaluated the ICS test accordingly. The test kits performed equally well as culture;[67,68] however, in a larger retrospective component, the sensitivity of the test was relatively low (81%) compared with other methods.

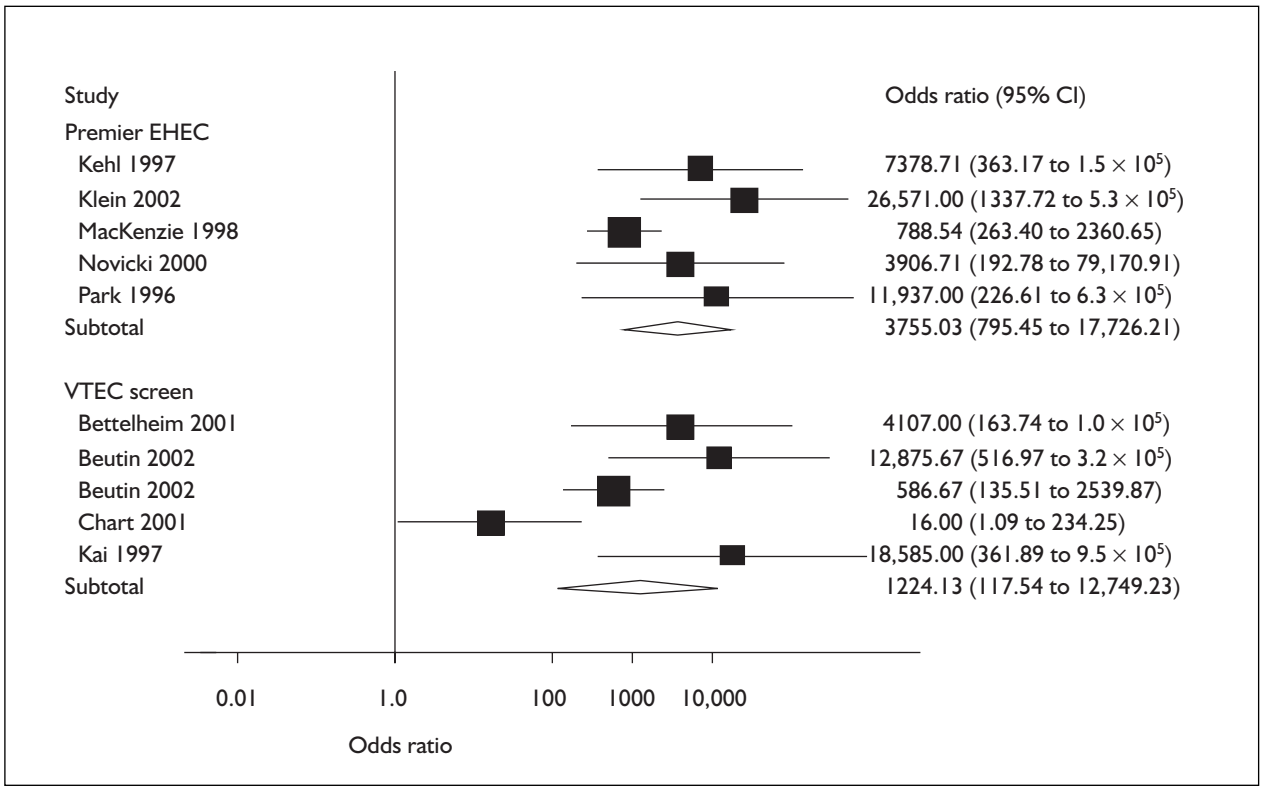


FIGURE 22 *Forest plot of DORs for antibody tests for* E. coli *food poisoning*

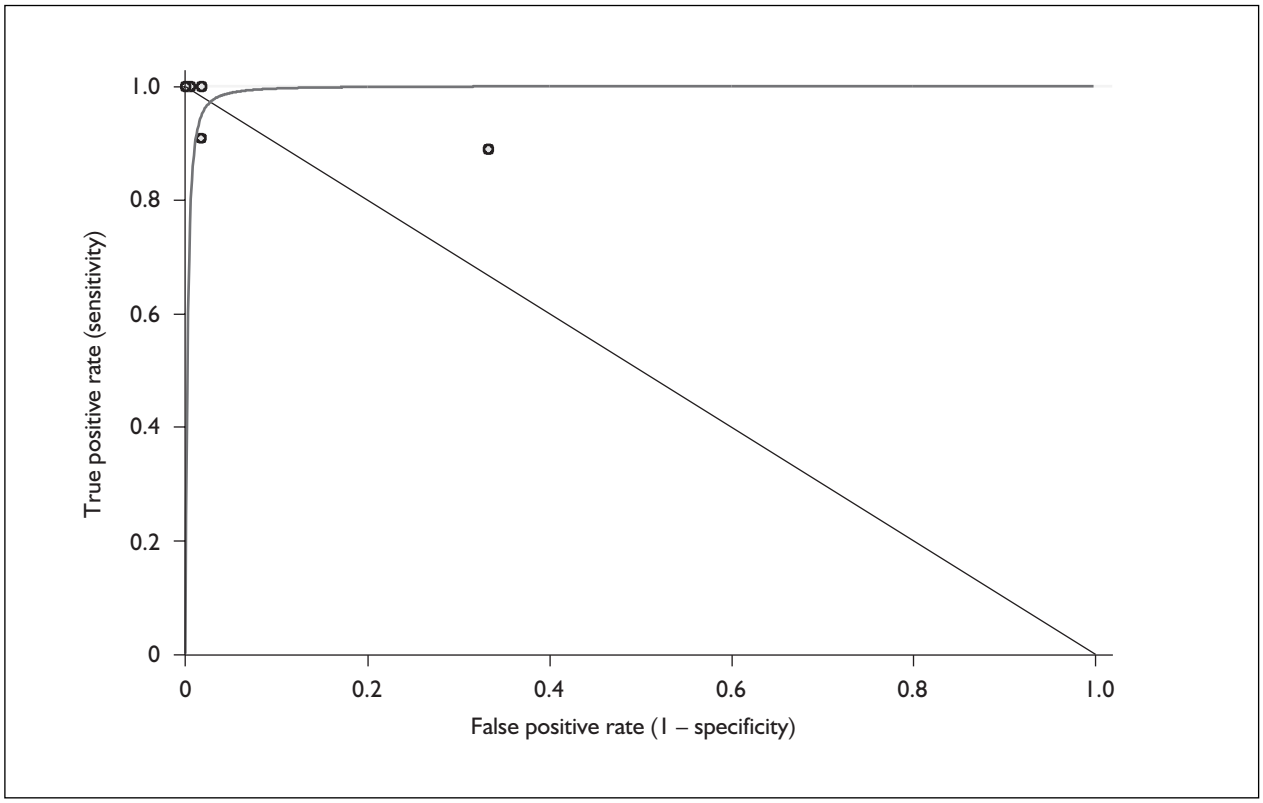


FIGURE 23 *SROC curve for studies evaluating the VTEC Screen (Denka Seiken) for the detection of* E. coli

Immunochromogenic assays

The most comprehensive investigation of the efficacy of the IMS method for the isolation of *E. coli* O157 is that of Karch and colleagues.[69] This method involves a selective enrichment step using O157-specific antibodies attached to paramagnetic particles to enhance detection. Thirty children with HUS were examined. EHEC O157 strains were isolated from stool samples of 18 patients and non-O157 EHEC strains were isolated from five patients. The IMS method detected EHEC O157 in all 18 samples compared with seven using sorbitol SMAC and cefixime–tellurite SMAC. However, this method did not detect non-O157 *E. coli* strains.

Duopath Verotoxin

Illustrating the potential benefits of applying methods from the food industry to clinical practice, Park and colleagues[70] evaluated the clinical use of Duopath Verotoxin, an immunochromographic test developed for confirmation of STEC strains from food products. Clinical stool samples were retrospectively and prospectively tested by the Premier EHEC assay as the reference test for determination of the performance of the Duopath Verotoxin test. Concordance between the two methods was very high.

Real-time versus conventional PCR: concordance

To test the comparability of results between rtPCR and conventional PCR assays, Reischl and colleagues[71] developed two duplex assays (*stx*1 and *stx*2; *eae* and *E-hly*) for use with the LightCycler instrument. From 622 reference strains, concordance with conventional equipment was observed in all but 10 specimens, which were not of human origin. By automating the methods, time requirements were reduced by an estimated 4–4.5 hours.

ELISA versus conventional PCR versus rtPCR

Pulz and colleagues[22] compared the diagnostic accuracy of a novel PCR assay using LightCycler instrumentation to that of both conventional PCR and immunoassay in a prospective evaluation of 295 faecal samples. rtPCR performed slightly better than the conventional methods. Comparing both PCR methods with the immunoassay (RIDASCREEN Verotoxin ELISA, R-Biopharm), significantly more STEC-positive stool specimens were identified by the nucleic acid-based techniques.

Discussion

Considerable controversy exists as to whether SMAC culture methods are appropriate for the detection of *E. coli*.[72] The selective enrichment involved with SMAC is designed predominantly for the O157 strain, and may have an inhibitory effect on other (non-O157) strains producing shiga-like toxins. In choosing a rapid test to detect *E. coli*, it is important for policy makers to ascertain the significance of these non-O157 strains.[73] For example, Karch and colleagues[69] found that sole reliance on SMAC agar would underdetect 28% of STEC, and sole reliance on enzyme immunoassay (EIA) would underdetect 11% of *E. coli* O157:H7 organisms. The choice of a rapid test for *E. coli* depends on the significance and prevalence of non-O157 strains. Within the UK, serious complications, and subsequent media coverage, have largely been associated with *E. coli* O157 strains. There is, however, evidence from some other European countries that non-O157 strains may be equally harmful. In Germany, Gunzer and colleagues[74] recovered 44 isolates of shiga-like-toxin (SLT) producing non-O157 *E. coli* strains and only 18 isolates belonging to serogroup O157 from 668 diarrhoeal stool specimens. Given the low sensitivity associated with SMAC for non-O157 strains, it is likely that several of these may have been missed in routine investigation, leaving the true prevalence hard to estimate. New test methods with improved detection of non-O157 strains may have the potential to bridge this gap in knowledge, delivering not only faster diagnosis, but increasing the likelihood of pathogen detection in routine investigation.

Some of the studies included have a number of limitations. For instance, Kehl and colleagues sought toxins on frozen stools.[32] Due to freezing and thawing potentially liberating toxin, and the lack of use of freezing and thawing in the protocols of currently used tests, this study was excluded in a sensitivity analysis but the overall results remain stable. Some studies, such as that of Park and colleagues,[70] did not perform a rapid test on stool. Stool samples were inoculated into broth, and the broth was subsequently tested for the presence of shiga toxin. These assays may not be truly 'rapid' because standard culture could also be positive that day, and a rapid antigen test on a suspect sorbitol non-fermenting colony (identifying *E. coli* O157:H7) could obtain the same information as the toxin assay.

The incidence of *E. coli*, and hence the isolation rate in routine culture, are considerably lower than for other food-borne pathogens, such as *Campylobacter*, *Salmonella* and *Yersinia*. Occasionally, research has prompted the use of testing strategies when stool samples are submitted for culture, such as only testing for *E. coli* in infant samples or if

blood is detected in the stool. Although this may appear attractive in terms of laboratory resource use and labour requirements, it is unclear if this would prove cost-effective in the long run. If more expensive rapid test methods are adopted, the use of triage may be advisable.

Clostridium perfringens

Illness due to *C. perfringens* results from ingestion of large numbers of vegetative cells, which subsequently produce an enterotoxin (*cpe*) after sporulating in the gut. Spore count should be interpreted with caution and the presence of enterotoxin may be more informative.[75]
C. perfringens enterotoxin is not usually produced in foods; therefore, assays for *cpe* in foods are not very meaningful.

Number of studies

The initial search strategy identified 89 studies evaluating rapid detection tests for *C. perfringens* in clinical specimens. Following abstract appraisal, 19 were subjected to full critical appraisal and data extraction. Ten of these have been included in this review. Basic information for all included studies is provided in *Table 12*.

Contemporary research on *C. perfringens* detection has focused on environmental and veterinary testing.[76,77] There has been scarce published research on clostridial food poisoning in clinical laboratories in the last decade. Of the 10 studies included in this review, three developed PCR assays and seven investigated serological methods. Four studies evaluated the rapid tests with a reference standard and six could measure diagnostic accuracy through the use of known reference strains.

Methodological quality of studies

Studies evaluating rapid detection of *C. perfringens* enterotoxin were of varying quality. Only four studies were identified in the review in which a rapid assay was evaluated against a suitable reference standard using a reasonably large sample size (>100). A number of studies which did not include a full diagnostic comparison are included in this review, to allow a fuller understanding of research in the area.

Antibody-based tests

Commercial kits identified to test for *C. perfringens* enterotoxin include PET-RPLA (Oxoid), which uses RPLA, and the TechLab *C. perfringens* test, based on an EIA technique.

TechLab immunoassay was evaluated, using an in-house ELISA test previously designed by the Food Safety Microbiology Laboratory (FSML) as a reference.[78] The authors found the commercial kit to be significantly less sensitive than the in-house method (33.9% compared with 100%). No other evaluations of this product were identified, and its performance characteristics have not been established.

Oxoid's PET-RPLA was compared with an in-house designed ELISA in one study,[79] testing 131 faecal specimens from food poisoning outbreaks. A high (94%) concordance rate was reported between the two methods, with discrepancies ascribed to either a low toxin concentration or non-specific interference in the PET-RPLA. The same authors later tested 392 faecal specimens from food poisoning outbreaks by Vero cell assay, PET-RPLA and in-house ELISA methods.[79] The Vero cell assay was the least sensitive and reproducible method, detecting only 30.00% of all enterotoxins. ELISA and PET-RPLA sensitivity rates were only slightly better, detecting 40% and 42% of all enterotoxins, respectively. In a subsequent study,[80] PET-RPLA was compared with a Vero cell assay in a case–control study of a *C. perfringens* food poisoning outbreak in Italy. The Vero cell assay detected only 8/15 (53.30%) of PCR-confirmed cases, whereas the PET-RPLA could detect 13/15 (86.70%).

C. perfringens enterotoxin is only produced in sporation, therefore it may be difficult to attain in usual culture media. This has led to some uncertainty regarding the accuracy of the SET-RPLA. Mpamugo and colleagues[81] used the Oxoid RPLA kit to establish the incidence of cases of sporadic diarrhoea associated with *C. perfringens*, using a previously designed ELISA test[82] for confirmation over a 2-year period. The authors concluded that an evaluation of diagnostic accuracy for PET-RPLA is needed, although no further studies have been identified which do this. The lack of any well-defined reference test makes it hard to evaluate the PET-RPLA fully, but a number of studies suggest that it may fail to detect a considerable proportion (21.00–45.00%) of enterotoxigenic cells. The preferred method currently used at FSML is the in-house-designed ELISA test reviewed here.[82]

Four in-house-designed immunoassay tests were identified testing for *C. perfringens* type A enterotoxin.[82–85] Results from all studies could be read within 24 hours of sample delivery. The largest investigation[82] tested 515 faecal and 21

TABLE 12 *Characteristics of studies evaluating rapid assays for the diagnosis of* C. perfringens *food poisoning*

Study	Basic test details					Diagnostic accuracy		*Quality:* QUADAS score	Time issues	
	Test name	Test type	Direct or from isolate	Reference test	Sample size	Sensitivity (95% CI)	Specificity (95% CI)		Result within 24 hours	Reduced hands-on time
Augustynowicz, 2002[90]	In-house	PCR	Direct	Oxoid PET-RPLA	30	1.000 (0.8064 to 1.000)	NA	8	Yes	Yes
Fach, 1993[165]	In-house	PCR	Direct	NA	Unclear	NA	NA	2	Yes	Yes
Tansuphasiri, 2002[91]	In-house	PCR	Direct	Oxoid PET-RPLA	477	NA	NA	4	Yes	Yes
Bartholomew, 1985[82]	In-house	ELISA	Direct	Double gel infusion	44	1.000 (0.8865 to 1.000)	0.357 (0.163 to 0.612)	9	Yes	Unclear
Cudjoe, 1991[83]	In-house	IMS-ELISA		NA	25	NA	NA	2	Yes	Unclear
Germani, 1990[84]	In-house	ERIA	Direct	ELISA	100	1.000 (0.439 to 1.000)	0.938 (0.872 to 0.971)	9	Yes	Yes
Forward, 2003[78]	In-house	ELISA	Isolate	TechLab EIA	843	1.000 (0.8454 to 1.000)	0.950 (0.933 to 0.963)	10	Yes	Unclear
Berry, 1988[79]	In-house (Bartholomew)	ELISA	ELISA	Oxoid RPLA and Vero cell assay	392	40 (157 to 392)	NA	7	No	Yes
Brett, 1992[75]	Oxoid	RPLA	Direct	In-house ELISA	818	NA	NA	9	No	Unclear
Mpamugo, 1995[81]	Oxoid	RPLA	Direct	ELISA	370	NA	NA	7	Unclear	Yes

ERIA, erythroimmunoassay; NA, not applicable.

food samples in a multi-centred investigation throughout the UK. The authors highlighted the importance of fast delivery to the laboratory to ensure that enterotoxins were still viable in the specimen, as the ELISA assay detected 228/294 (78%) of specimens tested within the first 2 days of *C. perfringens* outbreaks. However, for samples collected later than this, the test detection rate dropped to 74/233 (32%). When results from the ELISA assay were compared with double gel diffusion and counter-immunoelectrophoresis, the ELISA was recorded as detecting significantly more than its comparators (ELISA sensitivity 89% compared with 68% and 66% of 44 positive enterotoxin samples tested).

PCR assays

Only three of the six nucleic acid-based detection studies identified compared the assay with a suitable reference test. Most PCR detection targets either the entertoxin gene (*cpe*) or phospholipase C (*plc*) genes.

An early PCR-based assay was developed by Saito and colleagues;[86] however, as their methods required 48 hours of pre-enrichment of each sample, this was not considered a rapid test. Kato and Kato[87] evaluated their in-house PCR assay with a combination of banked reference strains and clinical isolates, reporting one false positive and no missed strains from a total of 107 isolates. Fach and Popoff [88] evaluated an in-house-designed duplex PCR against an in-house-designed slide latex agglutination assay. Testing 23 human faecal samples, they concluded the latter assay produced one false negative and one false positive compared with PCR. The PCR, targeting the *plc* and *cpe* genes, was presented as a very useful test for both faecal and food samples. The method was repeated by Augustynowicz and colleagues,[89,90] who reported that at least one of the two PCR targets (*plc* and *cpe*) were detected in all 30 stool samples, whereas the PET-RPLA method detected only 16 *C. perfringens*-positive stool samples. A similar duplex PCR has since been developed in Japan by Tansuphasiri and colleagues,[91] who found that all reference strains with previously known enterotoxigenicity produced the expected results. The same group has recently reduced the pre-enrichment time for this assay to 4 hours, which increases its appeal in the investigation of clostridial food poisoning.[92]

Discussion

The volume of research attributed to diagnostic test methods for *C. perfringens* is substantially less than that for *Campylobacter*, *Salmonella* and *E. coli* assays. The reason for this may include the natural history of the disease, limited duration of ill health and low complication rate of disease due to *C. perfringens*.

Problems associated with artificially growing *C. perfringens* enterotoxin on plating media have resulted in ambiguous results for many serological assays. The use of PCR may alleviate this issue, but due to a lack of comparison of the two methods this has not been established. More comparative research is required against a well-established reference standard.

Screening for enterotoxigenic *C. perfringens* isolated from primary faecal spore isolation cultures may increase the knowledge base for this pathogen, and lead to improved patient outcomes, particularly in elderly patients with food-borne diarrhoea.

Bacillus cereus

Number of studies

The standardised search strategy identified 56 clinical studies for *B. cereus*. After initial abstract appraisal, nine studies reporting detection methods for either emetic or diarrhoeal forms of *B. cereus* were included in this review. Basic information for all included studies is provided in *Table 13*.

Methodological quality of studies

Studies evaluating *B. cereus* detection methods on prospectively collected human stool samples could not be found. All tests were used on banked strains of the pathogen, and the sample size for each study did not exceed 14. Due to the small number of studies which evaluated rapid detection methods against a suitable reference test, the reviewers were unable to assess the effectiveness of the majority of assays described. The results of the remaining seven studies without a comparator are also reported. Similarly, rigorous quality assessment could not be applied due to the limited information available.

Diarrhoeal strains

Rapid detection methods for diarrhoeal strains of *B. cereus* were the focus of five identified studies (*Table 13*). Two commercially available serological kits were identified, the BCET-RPLA (*B. cereus* enterotoxin-reverse passive latex agglutination, Oxoid) and VIA (Tecra). Both were developed to test food samples, but have since been applied in clinical practice. These kits do not detect the same

TABLE 13 *Characteristics of studies evaluating rapid assays for the diagnosis of* B. cereus *food poisoning*

Study	Basic test details					Diagnostic accuracy		*Quality:* QUADAS score	Time issues	
	Test name	Test type	Direct or from isolate	Reference test	Sample size	Sensitivity (95% CI)	Specificity (95% CI)		Result within 24 hours	Reduced hands-on time
Beecher, 1994[93]	Oxoid BCET	RPLA	Strains only	Tecra	2	NA	NA	3	Unclear	Unclear
Buchanan, 1994[28]	Oxoid BCET	RPLA		Strains only	12	NA	NA	3	Unclear	Unclear
Buchanan, 1994[28]	Tecra VIA	ELISA		Strains only	12	NA	NA	3	Unclear	Unclear
Day, 1994[94]	Oxoid BCET	RPLA		Strains	14	43.75	NA	7	Unclear	Unclear
Day, 1994[94]	Tecra	ELISA		Strains	14	87.5	NA	7	Unclear	Unclear
Tan, 1987[96]	Tecra BCET	RPLA	Isolate	Isolates	28			10	Unclear	Unclear
Haggblom, 2002[99]	Liquid chromatography		Isolate	Boar spermatozoan motility assay	5	NA	NA	4	No	Unclear
Finlay, 1999[100]	Metabolic staining assay		Strains	Hep-2 cell assay	13	NA	NA	6	Unclear	Yes
Andersson, 1998[102]	In-house	Bioassay	Toxin	NA	5 strains	2/2	3/3	4	Unclear	Unclear
Ehling-Schulz, 2004[98]	In-house	PCR	Isolate	NA	178	NA	NA	3	No	Unclear
Scraft, 1995[180]	In-house	PCR	Isolate	NA	56	NA	NA	3	Unclear	Unclear

NA, not applicable.

antigen, and comparisons between them have led to mixed conclusions. The two were given parallel evaluations in four studies.[28,93–95] BCET-RPLA (Oxoid) is the most commonly cited test method for diarrhoeal *B. cereus*, identified in 12 evaluative and non-evaluative studies in the wider search. Fletcher and Logan[95] compared the BCET-RPLA (Oxoid) and BDE-VIA (*Bacillus* diarrhoeal enterotoxin, Tecra) with an improved McCoy cell culture cytotoxicity assay. They found the cytotoxicity assay was more sensitive than the Oxoid kit and, unlike the Tecra kit, did not give false positive results. Beecher and Wong [93] were similarly unclear as to the usefulness of the two commercial kits, although Day and colleagues[94] suggested that the Tecra kit is more reliable, having detected 87.5% (14/16) strains, compared with the Oxoid kit, which detected only 43.75% (7/16). Another study which examined foods and faeces using Tecra EIA detected the enterotoxin in seven out of 34 foods and 10 out of 15 faecal specimens from outbreaks of food poisoning. One normal faecal specimen also gave a positive result.[96] The Chinese hamster ovary (CHO) cytotoxicity assay[97] is a common detection technique for diarrhoeal *B. cereus* strains; however, as results become available only after 3 days, this is not considered a 'rapid' method.

Emetic strains

Of the toxins produced by *B. cereus*, the emetic toxin is likely the most serious but, due to lack of a suitable assay, the least well known.[28] In this review, no commercial kits for the detection of emetic toxin were identified. This review identified six novel assays developed for emetic (cereulide-producing) strains; however, none of these were compared with a suitable reference test. One study[98] assessed a novel PCR assay to detect emetic strains of *B. cereus*. Using banked bacterial isolate, the assay was found to be accurate with 30 cereulide-producing (i.e. emetic) strains correctly identified, whereas all 148 non-cereulide producing strains gave no PCR signal. This appears to be a high-performance diagnostic tool for emetic strains of *B. cereus*; however, a larger, prospectively designed study is required. Other detection methods which were developed, but not fully evaluated, include liquid chromatography[99] and MMT assay.[100] In addition, tests developed to test food samples include PCR[101] and bioassay.[102]

Discussion

There is currently insufficient evidence to assess the diagnostic accuracy of rapid methods in the detection of *B. cereus*. The main diagnostic process with *B. cereus* in practice relies on clinical history. As the duration of illness for both emetic and diarrhoeal strains of *B. cereus* does not usually exceed 24 hours, it is reasonable to suggest that the urgency for rapid tests of *B. cereus* is somewhat diminished, in comparison with potentially more serious infections such as with *E. coli* or *Campylobacter*. Although comparisons with reference tests were not performed, results from the studies suggest that to measure the food poisoning risk of *B. cereus*, the toxin content must be measured. From the evidence identified in this systematic review, the diagnostic accuracy of test methods has not been fully assessed. Current provision of tests for *B. cereus* may underestimate the role of emetic strains, due to the lack of a suitable assay. In an epidemiological profiling investigation to determine the aetiology of food-borne outbreaks in which no pathogen was isolated by routine laboratory testing, Hall and colleagues[103] used pathogenic-specific profiles and estimated that as many as 18.8% of all reported food-borne outbreaks in the USA between 1982 and 1989 were caused by either *B. cereus* or *S. aureus* vomiting toxin outbreaks. From a research perspective, there may be additional value in exploring novel assay techniques for emetic strains further, to assess the level of under-reporting of these pathogens.

Staphylococcus aureus

Number of studies

The initial standardised search strategy identified 2593 studies relating to detection of *S. aureus*. When an attempt was made to limit methicillin-resistant strains [focusing on methicillin-resistant *S. aureus* (MRSA) and hospital-acquired infection], 727 were eliminated. Several MRSA-related papers were subsequently eliminated in the abstract appraisal process, suggesting that the electronic search lacked specificity. Twenty-seven studies were considered potentially eligible and data was extracted using the standard proforma. Of these, only four provided summary statistics of diagnostic accuracy when testing on faecal samples. Basic information for all included studies is provided in *Table 14*.

Methodological quality of studies

The methodological quality of studies evaluating diagnostic tests for *S. aureus* was considerably low.

Most *S. aureus* studies were not excluded on grounds of insufficient quality or data, but rather that they were not directly linked to staphylococcal food poisoning. The review identified several

studies developing novel detection techniques for blood cultures, including DNA hybridisation[104] and fibre-optic-based biosensors.[105] Application to faecal samples (i.e. pertaining directly to intestinal toxins) used agglutination, immunoassay (three studies) or PCR (six studies) techniques.

Very few of the studies assessed were ultimately included in this review. As discussed in an earlier section of this report, *S. aureus* is ubiquitous in the environment, and enterotoxigenic forms of the organism account for only a small fraction of its species. As such, most of the current research on its detection does not concentrate on *staphylococcal* enterotoxins. Very few studies evaluated the rapid method against a suitable reference test. However, some non-evaluative studies have been included in this review to identify potentially relevant assays to assess the availability of rapid assays to improve the identification of *S. aureus*. Some of the molecular assays included utilise isolates grown from non-faecal samples. In addition, none of the studies was carried out directly on faecal samples.

PCR assays

The characteristics and results of studies evaluating PCR assays for the detection of *S. aureus* are summarised in *Table 15*. Two multiplex PCR assays were developed and evaluated against the current standard phenotypic test, SET-RPLA (Oxoid). Klotz and colleagues[106] recorded their in-house-designed PCR as being more sensitive than SET-RPLA, with the reference test detecting only 75.9% of toxins detected by PCR. Sharma and colleagues[107] found similarly favourable results for nucleic acid-based detection of all staphylococcal enterotoxins in the same multiplex assay, unusually without the need for bacterial pre-enrichment of samples, with results available within 3–4 hours. Using a mixture of environmental and clinical strains, they reported that the SET-RPLA produced two false negative results (sensitivity 98.7%) whereas PCR detected all toxin types. This PCR multiplex assay was subsequently adapted by Letertre and colleagues,[29] to include *seg* to *sej*. They found that compared with both rtPCR and conventional PCR, SET-RPLA missed 13 out of 68 enterotoxigenic strains of *S. aureus*, with sensitivity at 80.8%, whereas PCR techniques were found to be fully reliable. In previous non-evaluative studies, Tsen and colleagues[108] and Monday and Bohach[109] developed individual PCR assays for each of the staphylococcal enterotoxin groups A–E. Given the expanding number of enterotoxin groups being discovered, the development of multiplex assays is a considerable progression.

Antibody-based tests

This review identified three studies in which EIAs were used to detect *S. aureus* enterotoxins, only one of which was evaluated against a reference test. Guardati and colleagues[110] developed a novel ELISA assay for the identification of *S. aureus* strains of human and animal origin, and found that although it missed no positive strains, the commercial kits against which it was compared rated poorly, detecting between 90.2% (Bactident Staph) and 96% (Bacto Staph Latex) of isolates. The ELISA also produced 100% specificity, compared with a range of 90.8% (Staphyslide Test and Sero-StTAT Staph) to 93.7% (Pastorex Staph). Fukuda and colleagues[111] developed a bioluminescent EIA in which detection of protein A-bearing *S. aureus* is possible within 7 hours, including culture. Kijek and colleagues[112] developed a rapid IMS–electrochemiluminescence assay to detect staphyloccocal enterotoxin B, using the ORIGEN Immunoassay System (IMS) (Igen, Rockville, MD, USA). Both studies concluded that the methods were successful in detecting the specific toxins of interest (*sea* and *seb*). However, as staphylococcal food poisoning may be due to a wide range of toxins, it is unlikely that these assays would be applied in routine practice.

Four studies[113–116] compared commercial agglutination tests designed for the identification of *S. aureus*; however, the strain collections for these evaluations rarely came from faecal samples.

Discussion

Studies evaluating tests for the identification of *S. aureus* are limited and of poor quality. It is not possible to make any evidence-based recommendations for practice based on the limited nature of the available data. Further studies to develop rapid assays for *S. aureus* and subsequent evaluation in trials are recommended.

Assays detecting multiple organisms

One of the limitations of the many test formats is that for the majority of assays, users must test for each bacterial species separately. The development of nucleic acid-based methods in which probes for multiple organisms may be used in the same reaction tube signals significant advances in detection technology. Testing for a wide range of pathogens in a single reaction is likely to result in long-term cost saving, in terms of technician time and reagent use, compared with plating for multiple organisms on individual selective agars.

TABLE 14 *Characteristics of studies evaluating rapid assays for the diagnosis of* S. aureus *food poisoning*

Study	Basic test details					Diagnostic accuracy			Time issues	
	Test name	Test type	Direct or from isolate	Reference test	Sample size	Sensitivity	Specificity	*Quality:* QUADAS score	Result within 24 hours	Reduced hands-on time
Fukuda, 2002	Bioluminescent enzyme immunoassay (BLEIA)	EIA	Isolate	Previously isolated strains	24 + 58 + 38	100	–	3	Yes	Marginally
Guardati, 1993	Immunoenzymatic assay based on monoclonal antibody MAb C1–10/11	Immunoassay	Isolate	Previously isolated strains	655	100	100	2	Yes	Marginally
Klotz, 2003	Real-time fluorescence PCR assay	PCR	Isolate	SET-RPLA	93	100	92.45	8	Yes	Yes
Letertre, 2003	rtPCR assay	PCR	Isolate	Conventional PCR	100				Yes	Yes
Sharma, 2000	Multiplex PCR	PCR	Isolate	SET-RPLA	157	100	98.7	6	Yes	Yes
Araj, 1997	4 commercial kits: Slidex Staph-Kit; Avistaph; Staphylase; Pastorex Staph-Plus	LAT	Isolate	Previously isolated strains	367	96.6 96.6 96.6 96.6	91.0 67.0 94.0 94.0	4	Yes	Yes
Kijek, 2000	ORIGEN immunoassay system	IMS–electro-chemiluminescence detection	Direct	Spiked samples	111	98	100	2	Yes	Yes

Research is broadly centred on two techniques: multiplex PCR and microarray technology. A limited number of studies were identified using these techniques for the detection of food-borne pathogens. These are outlined below.

Multiplex PCR

Iijima and colleagues[117] reported a multiplex assay demonstrated using 161 clinical stool samples, with the ability to detect *S. enterica*, *Vibrio parahaemolyticus*, *C. jejuni* and STEC infections in a single reaction. Unusually, this rtPCR assay did not require sample pre-enrichment, so results were available within 3 hours from receipt of sample. Comparing PCR detection with conventional culture results, the authors found the PCR to be more sensitive than its reference standard for *S. enterica* and *V. parahaemolyticus*, but slightly less sensitive for *C. jejuni*. No STEC isolates were found.

Fukushima and colleagues[118] developed a 20-primer rtPCR assay to detect 17 species of food- and water-borne pathogens in faecal samples. They reported that eight species could be detected without pre-enrichment, making results available within 2 hours or less; however, for pathogens with an infective dose of 10^4 cfu g^{-1}, an overnight enrichment step is still required.

In the food industry, multiplex testing has been developed more widely. Gilbert and colleagues[119,120] developed a multiplex PCR assay for the simultaneous detection of *C. jejuni*, *Salmonella* and *E. coli* O157 in raw and ready-to-eat foods, by successfully combining the methods from separately published PCR assays for each of the organisms. Similar multiplex assays have also been developed for *Salmonella* and STEC[121] and for *Salmonella*, *Listeria monocytogenes*, and *S. aureus*.[122] Hence the simplex assays discussed in this review may potentially be combined to allow the cost-effective detection of all three bacterial pathogens in one reaction tube.

Microarray technology

Microarray technology is a powerful tool that can be used for the simultaneous detection of thousands of genes of target DNA on a glass slide. Importantly, selecting groups of oligonucleotide probes for each microarray can allow the detection of several bacterial species without significantly increasing the complexity or cost, so a wide range of pathogens may be tested for using a single microarray slide. As this is still very much at the development stage, studies tend to have very small sample sizes, with specimens from only two or three patients (often examined in triplicate to ensure validity). As yet, no large-scale evaluation of the methods has been reported for the detection of food-borne pathogens, but exploratory research in the area suggests that this technology shows very promising accuracy. In two of the larger studies relating to intestinal pathogens, Hong and colleagues[123] described the rapid detection of 14 common pathogenic bacteria in food-borne infections using oligonucleotide array technology, and Chiang and colleagues[124] reported a detection rate of more than 98% when 182 randomly selected strains of *Bacillus* spp., *E. coli*, *Salmonella* spp., *Staphylococcus* spp. and *Vibrio* spp. were assayed.

Chapter 6

Systematic review of studies testing food samples

Introduction

This chapter reviews tests that are designed and/or currently applied only to food samples and assesses the potential usefulness for transfer to clinical testing. It comprises summary descriptions of methods used for food studies and results by organism. The analyses reported aim to identify tests with potential for further study in the clinical microbiology setting. This chapter is therefore limited to a simple assessment of the technical feasibility of the assays and comparison of their sensitivity with a reference standard where available. More detailed data are appended.

Methods

A search strategy was constructed for MEDLINE, EMBASE, Web of Science and Science Citation Index Expanded (SCI-EXPANDED). The search strategy was designed to identify studies describing rapid methods of detection for the six organisms relevant to this study and having comparison of the rapid method(s) with a reference standard. The key groups of search terms and sequence of filters used to obtain studies for further consideration are given in Appendix 1. Searches were not restricted by language and the search was last updated at the end of September 2005.

Selection of papers

All study titles were reviewed by CA to assess the likely relevance of the study to the review and then further exclusion was based on abstract appraisal and publication date if studies:

- were pre-1995 (except *B. cereus* and *C. perfringens*, for which there were few studies)
- were not food-related
- were method reviews
- had no comparison of method performance with an appropriate reference standard
- could not be considered more rapid than traditional methods.

Full texts of selected studies were evaluated and further inclusion and exclusion criteria were applied. Further studies were excluded if:

- Food matrices were not relevant to this study.
- Data did not fit extraction criteria.
- The study was of poor quality. (Systematic reviews of food pathogen detection studies have only recently emerged, and there is a lack of any validated quality assessment instruments in the food industry. A subjective decision was taken between CA and GW as to the quality of study methods, based on several years of experience in the field of microbial detection of food-borne pathogens. Notably, all included studies appear in peer-reviewed journals.)

In addition, for studies which were described in more than one paper, e.g. development and validation, only the most relevant publication was included. References were managed and stored using the Reference Manager program (version 11). An overview of the search results is provided in *Table 15*. Data were extracted, stored and managed using an Excel spreadsheet proforma (see Appendix 2).

Quality assurance for search strategy

The AOAC database of Performance Tested Methods (Microbiological test section) was searched to determine that all available relevant publications had been retrieved by the previous search strategy. This source also indicates that a test kit's performance has been reviewed by AOAC

TABLE 15 *Search results for diagnostic studies of food-based assays*

Organism	*Campylobacter*	*Salmonella*	*C. perfringens*	*B. cereus*	*S. aureus*	VTEC	Total
	7,073	24,256	2,422	28,358	29,347	5,887	97,343
Filter 1	851	2,834	273	1,885	2,351	842	9,036
Filter 2	140	512	45	75	372	123	1,267
Final	8	22	1	18	3	18	70

Research Institute and found to perform to the manufacturer's specifications.

Reference methods named or indicated by authors of the literature under examination were checked against formal standards obtained from standards agencies [e.g. International Standards Organization (ISO), Bacteriological Analytical Manual (BAM)] to check stated methodology or to determine unstated methodology.

Reviewers CA and GMW were authors of one of the papers included in the review.[125] CA entered data from this publication according to the prescribed procedure.

Summary of analysis for all food-based studies

For the food-based studies, the following provides descriptive analyses of the data and data sources accessed, evidence of the efficacy of tests described and evidence for the rapidity with which tests can be conducted.

In this report, analysis of food-based studies was carried out separately from clinical studies due mainly to the difference in priorities of the two areas. Priorities for food-based studies are, largely, to determine the absence of a single organism in an aliquot of a food (which is likely to be either negative for the target organism or contaminated at very low concentration), or to identify or enumerate putative pathogens. Additionally, food microbiologists may be interested in a particular phenotypic characteristic, e.g. psychrotolerant bacillus species[126] in, for example, ready meals. Priorities for clinical studies are to determine the cause of disease (presence of the likely causative agent) from matrices positive for a disease agent. They may also prioritise identifying pathogenic species by, e.g., nucleic acid analyses of pathogenic determinants. As a consequence of this, data extraction (and therefore results) for the food-based studies analysed in this report is treated in a slightly different format to that for clinical data.

It is important to note that data suitable for statistical analyses for *C. perfringens* were extremely limited and therefore are presented as narrative. One reason for this may be the prioritisation of strategy and/or funds (and therefore research) towards a particular pathogen or group of pathogens. This is well illustrated in the food arena, where in the relatively recent past there has been great effort directed towards developing methods for *E. coli* O157 (in relative terms, an emerging pathogen) but minimal effort in developing methods for detecting *C. perfringens* (a relatively well-established pathogen in terms of methodology). Instead, resources have been directed towards control of *C. perfringens* with the development of predictive models (e.g. the Perfringens Predictor predictive microbiology programme) and public information awareness campaigns.

The language of all selected studies was English.

Study types

The major study types selected for analysis in this report were those in which one or more methods were compared (often simultaneously) against a panel of previously known or unknown sample types. Methods compared were usually a novel method (the test method) against a method considered to be a standard method (the reference method). Where a number of methods considered rapid were tested against a pre-evaluated panel of test materials or bacterial strains without simultaneous testing using a standard method, the pre-evaluated panel were assumed 'true' or 'standard-based' results and the rapid methods were compared against these. Where a number of rapid tests were evaluated within a single study, results were statistically evaluated against each other.

Presentation of results

Methods for each of the six organisms under evaluation were considered separately. Full tabulation of results includes identification of the study by author and year, general method format, e.g. PCR, ELISA, the target analyte, kit type where possible, reference method used, study size and sample type and analyses. For each of the studies tabulated there is additional information regarding the test, supplier's details where applicable and other relevant comments.

Tests were carried out on a number of different foods spiked at different concentrations, each of which is a single 'record' (row) in the data extraction form. Tabulated method details are given in Appendix 3.

Campylobacter species food studies

One study described an antigen detection method and nine studies described one or more nucleic acid-based methods. No other method types were evaluated. Studies described an antigen detection method in ELISA format and nucleic acid-based methods in PCR, multiplex PCR (mPCR), rtPCR and PCR hybridisation (also sometimes called PCR–ELISA) format. Analytes for detection included cell surface antigens, a number of

TABLE 16 Campylobacter *method performance summary for food studies*

	Significantly different with fewer presumptive positives[a]	Not significantly different	Significantly different with more presumptive positives[b]	Total
Antigen detection	0	1	0	1
Nucleic acid-based	3	6	6	15
Traditional	0	0	0	0
All	3	7	6	16

[a] Inferior: significantly different by χ^2 and a lower proportion of presumptive positives, $p = 0.05$.
[b] Superior: significantly different by χ^2 and a higher proportion of presumptive positives, $p = 0.05$.

different genes, conserved DNA and 16S rRNA. Test materials were mainly poultry-based but also comprised a wide range of relevant foods, including red meats, vegetables and dairy products. Two proprietary methods were evaluated (EiaFoss ELISA, BAX PCR) and, although proprietary component(s) were used in several of the methods, all other methods were considered non-proprietary at the point of publication of the study.

Historically, at least in the UK, there has been little agreement on the way forward for a standard methodology for the detection or enumeration of *Campylobacter* species and this may be reflected in the fact that only one study utilises the International Standard method (ISO12072:1995) for the detection of thermotolerant *Campylobacter* species from food and animal feeding stuffs.

Traditional methods are lengthy and complex, often comprising a non-selective or semi-selective pre-enrichment stage followed by supplementation with further selective agents and two phases of incubation (at different temperatures) for the initial 48 hours and then plating to two highly selective agars or one selective and one non-selective agar (made physically selective via a 45-µm pore size filter, through which *Campylobacter* spp. can selectively migrate) prior to incubation for up to 3 days under appropriate microaerophilic conditions.

Performance

In summary, seven evaluations identified test methods as equivalent to standard reference methods using χ^2 and nine as significantly different to reference methods (*Table 16*).

Rapidity

A number of authors described methods as rapid. *Campylobacter* detection is a long process by traditional methods (4–5 days) and a number of *Campylobacter* study authors claim to be able to reduce this time by between 1 and 3 days. Rapid methods could be performed in 3.9–49.85 hours (total time to presumptive positive result). Traditional methods also require substantial hands-on time with addition of selective supplements to basal media and in some cases during incubation.

Salmonella species food studies

Twelve studies described antigen detection methods, 13 described nucleic acid-based methods and three described traditional methods. Two studies described methods dependent on organism motility[127,128] and no studies described bioassays. Studies described antigen detection methods in different formats [$n = 20$, RPLA, ELISA, IMS, automated IMS (AIMS), PCR-ELISA, FIA and lateral flow immunoassay (LFIA)] and nucleic acid-based methods ($n = 20$, PCR and rtPCR with or without BAX, VIDAS and Taqman systems) and the remainder described rapid or standard traditional-style methods ($n = 5$). Analytes for detection included *Salmonella* cells, cell surface antigens, a number of genes and ribosomal RNA. Analyses were performed in a wide range of relevant foods including cake mix, cheese, coconut, egg, seafood (fish and shrimps) meat (beef, pork, poultry and frankfurter), milk, chocolate, peanut butter, flour and yeast. Assay outcomes were generally lines on gel or on the basal membrane of LFIA devices, colour development, fluorescence intensity, analysis of melting point versus temperature (BAX software), zone of turbidity [on modified semisolid Rappaport-Vassiliadis (MSRV) agar] and colonies on plates.

Performance

The relative performances of assays for the detection of *Salmonella* in food are shown in *Table 17*.

TABLE 17 Salmonella *method performance summary for food studies*

	Significantly different with fewer presumptive positives[a]	Not significantly different	Significantly different with more presumptive positives[b]	Total
Antigen detection	12	3	4	19
Nucleic acid-based	1	8	12	21
Traditional	3	0	0	3
All	16	11	16	43

[a] Significantly different by χ^2 and a lower proportion of presumptive positives, $p = 0.05$.
[b] Significantly different by χ^2 and a higher proportion of presumptive positives, $p = 0.05$.

TABLE 18 E. coli *method performance summary for food studies*

	Significantly different with fewer presumptive positives[a]	Not significantly different	Significantly different with more presumptive positives[b]	Total
Antigen detection	10	13	4	29
Nucleic acid-based	1	11	4	16
Traditional	1	6	1	7

[a] Significantly different by χ^2 and a lower proportion of presumptive positives, $p = 0.05$.
[b] Significantly different by χ^2 and a higher proportion of presumptive positives, $p = 0.05$.

Rapidity

For rapid methods, pre-enrichment was performed for all studies (100%) and for reference methods described pre-enrichment was performed for all but one (95%, quantitative plating) method. Standard methods take between 36.45 and 97 hours to presumptive positive detection (0.45–1.0 hours for hands-on) and rapid methods described take between 7.55 and 73 hours to presumptive positive (0.65–2.55 hours for hands-on).

Verocytotoxin-producing *E. coli* food studies

Fourteen studies described antigen detection methods ($n = 28$), 13 described nucleic acid-based methods [$n = 20$; one of which was a combined nucleic acid–antibody based (NA–AB) assay] and four described traditional (TRAD) or enhanced traditional (eTRAD) methods. One study described a bioassay (the Vero cell assay). Studies described antigen detection methods in four different formats (ELISA, IMS, LFIA and IMS–plate) and nucleic acid-based methods in two basic formats, PCR and rtPCR. Analytes for detection included *E. coli* cells, cell surface antigens [mainly O157 lipopolysaccharide (LPS)], a number of virulence genes, *eae*, *stx1*, *stx2*, and verotoxins VT1, VT2 and VT2c. Analyses were performed in a wide range of relevant foods.

The performance of the methods compared with a reference method is summarised in *Table 18*. It should be noted that much of the work for these studies was carried out prior to the publication and wide dissemination of the validated BS EN ISO 16654 (2001) method for *E. coli* O157 and therefore many of the comparative methods are listed as ANS (appropriate non-standard; Appendix 3).

Rapidity summary

For rapid methods, pre-enrichment was performed for all studies (100%) and for reference methods described pre-enrichment was performed for all but one (95%, quantitative plating) method. Standard methods took between 36.45 and 97 hours to presumptive positive detection (0.45–1.0 hours for hands-on) and rapid methods described took between 7.55 and 73 hours to presumptive positive (0.65–2.55 hours for hands-on).

Clostridium perfringens food studies

Like *B. cereus*, *C. perfringens* grows readily (1 day to presumptive positive; ISO/DIS 7937:2002) in traditional culture from naturally contaminated

samples, provided that it is handled in an anaerobic manner. Maximum growth rate in ideal conditions can be higher than 1.0 log increase per hour (doubling every 0.3 hours) in meat medium at 45°C at pH 8.0 (www.combase.cc) (using the ComBase Predictor Program).

For *C. perfringens*, it is also important to consider the route of food-borne infection; it is usually associated with disease after consumption of cooked meats. It may be present, usually in low concentration, in raw meats. A recent survey indicated that 1.4% of retail raw meats in the USA were *cpe* gene, *C. perfringens* positive. *C. perfringens* may be present as vegetative cells or as spores. Spores can survive thermal treatment given to some products and may subsequently germinate and outgrow very rapidly during the cooling process, giving rise to high numbers of cells and therefore toxin. For this reason, in recent years, a large proportion of the UK and US research budget in this area has been targeted toward establishing the growth kinetics of *C. perfringens* during cooling, and a number of mathematical models and predictive modelling tools have been developed (see www.combase.cc).

A number of studies have been carried out on the detection or enumeration of *C. perfringens* and its associated toxins in foods. Results retrieved indicated that standard methods were developed and evaluated during the period 1974–85 with early molecular research in areas such as identification of genes of virulence factors reported from the late 1980s (e.g. by Titball and colleagues[237]).

Some early studies evaluated methods for toxin detection. As an example, Harmon and Kautter[238] described a 15-laboratory collaborative study to estimate *C. perfringens* population using an indirect semi-quantitative method for quantifying the concentration of alpha toxin in cooked beef. This method was proposed as "adequate for estimating population levels of *C. perfringens* in foods associated with food-borne disease outbreaks" and was proposed as an improvement to the AOAC Official First Action Method 41.019–41.023, which reportedly underestimated numbers from foods that had been frozen or refrigerated.

Gene sequences for the four major toxins of *C. perfringens* (alpha, beta, gamma and epsilon) have been published (1989–93) and since then several PCR methods have been developed. It is important to note that only the alpha toxin is implicated in human food-borne illness or gas gangrene. *C. perfringens* can grow exceedingly quickly,[239] which may mean that minimal time is required for pre-enrichment. Where *C. perfringens* is implicated in food poisoning, it is usually present in high numbers in food or faecal samples. This means that short incubation or even no pre-enrichment is required.

C. perfringens method performance summary

Combined results for six studies describe tryptose sulfite cycloserine (TSC) as superior to lactose sulphite medium (LS), sulphite cycloserine (SC), Shahidi-Ferguson perfringens (agar) (SFP) and a PCR method, equivalent to DNA hybridisation and iron milk medium (IMM) MPN and inferior to rapid perfringens medium (RPM). In one study, RPM is described as superior to perfringens enrichment medium (PEM) and TSC and in another as equivalent to LS. LS is suggested as a growth medium for its confirmatory qualities but is found to be poor as a confirmatory agent when compared with other confirmatory methods.

Quantification of cells by toxin presence is unlikely to be useful, as different strains are now known to produce different concentrations of toxin in response to different stimuli.

C. perfringens can be presumptively detected or enumerated with good accuracy using traditional methods within a 1-day time frame. Evidence evaluated suggests that it may be feasible to provide PCR or ELISA determinations, with a degree of characterisation, using a 4-hour enrichment period, within one working day. This may be beneficial in quality assurance and positive-release scenarios.

Bacillus cereus food studies

Methods for *B. cereus*; *B. cereus* enterotoxin or species of the *B. cereus* group

Four studies described antigen detection methods and two studies described nucleic acid-based methods. One study evaluated an enhanced traditional method and one evaluated a novel bioassay. Studies described antigen detection methods in ELISA and RPLA format. Nucleic acid-based methods were both in PCR format. Analytes for detection included cell surface antigens, emetic and diarrhoeal toxins, genes and 16s rRNA. Relevant test materials were mainly dry products, dairy products, vegetables and spices.

TABLE 19 B. cereus *method performance summary for food studies*

	Significantly different with fewer presumptive positives[a]	Not significantly different	Significantly different with more presumptive positives[b]	Total
Antigen detection	1	3	7	11
Nucleic acid-based	0	1	2	3
Bioassay	0	1		1
Traditional	0	1	0	1
All	1	6	9	16

[a] Significantly different by χ^2 and a lower proportion of presumptive positives, $p = 0.05$.
[b] Significantly different by χ^2 and a higher proportion of presumptive positives, $p = 0.05$.

TABLE 20 S. aureus *method performance summary for food studies*

	Significantly different with fewer presumptive positives[a]	Not significantly different	Significantly different with more presumptive positives[b]	Total
Antigen detection	10	0	0	10
Nucleic acid-based	0	0	2	2
Bioassay	0	0	0	0
Biochemical gallery	1	0	1	2
Traditional	1	6	1	8
All	12	6	4	22

[a] Significantly different by χ^2 and a lower proportion of presumptive positives, $p = 0.05$.
[b] Significantly different by χ^2 and a higher proportion of presumptive positives, $p = 0.05$.

Three proprietary methods were evaluated [BCET-RPLA (*Bacillus cereu*s enterotoxin–reverse passive latex agglutination), Oxoid; BDE-ELISA (*Bacillus* diarrhoeal enterotoxin, or VIA), Tecra; and *Bacillus* chromogenic medium (BCM® Biosynth], and although proprietary component(s) were used in several of the methods, all other methods were considered non-proprietary at the point of publication of the study. Only one study utilises the International Standard method (ISO 7932:2004) as a reference standard.

Performance

In summary, six evaluations identified test methods as equivalent to standard reference methods, 10 as significantly different to the reference method, one with fewer presumptive positives than the reference method and nine with more (*Table 19*).

Rapidity

B. cereus detection is a relatively quick process by traditional methods and presumptive positive detection/enumeration may be made within 24 hours. A further enrichment of positive isolates for 18 to 24 hours can result in detection of toxin using, for example, the RPLA method. There is no requirement for substantial hands-on time for detection of this organism or its toxins. However, some selective supplementation of the media is required. Simultaneous detection and characterisation such as those which could be obtained by PCR may be beneficial.

Staphylococcus aureus food studies

Staphylococcal food poisoning is an intoxication caused by ingestion of toxins produced in foods by *S. aureus* cells. It may be appropriate, therefore, to detect either cells or toxin for this organism.

Broad conclusions are that petrifilm methodology is equivalent to Baird Parker agar (BPA), as evidenced in three studies, and RPFA is equivalent to BPA, as evidenced in two studies. API STAPH is superior to ID32 STAPH (one study only). The VIDAS SET2 method is superior to VIDAS SET and may be useful for a wide range of dairy products. *Table 20* summarises method performance for all studies considered statistically.

TABLE 21 *Method performance summary for all methods and all organisms, identifying number (percentage) of test methods having lower, equal or higher presumptive positive results compared with reference methods*

	Lower	Equal	Higher	Total
Antigen detection	43[a] (61.4)	7 (10)	20 (28.6)	70
Nucleic acid-based	14 (23)	19 (31.1)	28 (45.9)	61
Traditional	7 (35)	10 (50)	3 (15)	20
Other	3 (75)	1 (25)	0 (0)	4
All	67 (43.2)	37 (23.9)	51 (32.9)	155

[a] Two records are for mixed antibody-nucleic acid tests.

Rapidity

RPLA is considered more rapid than BPA. Petrifilms are considered more rapid than either of the traditional methods. Toxin analysis by ELISA is more rapid than cell detection. Extraction procedures and the confirmation of colonies should be taken into consideration when estimating timeliness of a method, especially as some methods are not highly specific and therefore require relatively more confirmation.

Assessment of food-based methods

Summary statistics for all methods

Method evaluations (n = 155) described in 67 articles were statistically evaluated and a further 39 studies were used for non-statistical evaluation of methods. Summary results for all statistically evaluated methods are given in *Table 21*.

The sensitivity and specificity for all methods are most easily visualised by grouping test methods according to whether lower or higher presumptive positive results compared with the reference method results are obtained. A 'lower' result indicates a <100/100 sensitivity/specificity scenario, an 'equal' result indicates an equivalent (e.g. 100/100) scenario and a higher result indicates a 100/<100 scenario.

Results indicate that there were a relatively high proportion of antigen detection methods in the 'lower' category (61.4% compared with 43.2% for all methods). There appeared to be a higher proportion of nucleic acid-based tests in the 'higher' category (45.9% compared with 32.9%). Traditional methods were most abundant in the 'equal' category. This result is unsurprising as most of the traditional or enhanced traditional methods were very similar to the reference method in format.

Despite the relatively high false positive rate (the 'higher' classification), nucleic acid-based methods display fewer significant differences from standard methods with respect to disparate results as detailed by summary statistics (*Table 21*) for significant differences identified between test and reference methods. Antibody-based methods exhibit a significant difference at $p = 0.05$ or lower in 49.9% of tests and nucleic acid-based methods exhibit difference in 42.6% of tests. Antigen detection and nucleic acid-based tests showed no significant difference (34.3 and 45.9%, respectively; $p = 0.05$) or 50 and 62.3%, respectively, at $p = 0.01$).

Conclusions for the food-based method review

- Nucleic acid-based methods may be more valuable for the detection of organisms that are relatively difficult to culture (e.g. *Campylobacter* as opposed to *B. cereus*).
- Immunological and nucleic acid-based tests may be better for 'multiplexing' thereby providing simultaneous speciation or characterisation (e.g. Hsu and colleagues[129]).
- Traditional methods will allow the examination of the live target organism. However, there is some evidence that prolonged pre-enrichment may be counterproductive for some organisms, for example missed detection of *E. coli* O157 due to being outcompeted by other autochthonous flora.[130]
- Nucleic acid-based and immunological methods are, in general, more rapid to presumptive positive. Some authors indicate that substantial savings in time to results can be achieved.[129]
- Rapid characterisation may be of particular benefit in the medical arena for rapid identification of EHEC. This would allow informed, rapid and appropriate treatment, thereby reducing the chance of serious kidney damage or death.
- For *Campylobacter* detection, rapid methods would be of immense benefit, as this organism is very difficult to culture and sub-type.

Chapter 7

Economic evaluation

With over 800,000 stool tests for gastroenteritis ordered each year in England, progressive introduction of new diagnostic technologies could lead to substantial increases in costs, especially if new rapid tests were to be used as an 'add-on' to existing culture methods. Furthermore, widespread implementation of these rapid tests could have substantial implications for the organisation of treatment services and training of laboratory staff. Any introduction of new diagnostic technologies should include careful consideration of the costs and benefits of different testing strategies before their implementation.[131] Economic evaluation is crucial to determining circumstances under which new tests should be performed, and what level of capital investment is justified.

It is increasingly recognised that clinical decisions must take into account the economic costs of a given intervention. Cost containment must be balanced with public health objectives, and attempts to evaluate diagnostic test methods economically must address multifaceted issues:

- specifying the relevant diagnostic alternatives
- measuring diagnostic accuracy
- measuring diagnostic costs
- specifying the measured outcomes of the diagnostic process.

If a case is to be made for the cost-effectiveness of new diagnostic tests, much more work is required to identify and quantify the potential intermediate effects and to predict the likely impact on health outcomes. However, the impact on health outcomes is extremely difficult to quantify.[132] The measurement of such outcomes would require large-scale trials, which are unlikely to be feasible, so a modelling approach is needed to extrapolate from intermediate outcomes and incorporate variations in epidemiological, costing and effectiveness data. A decision analytic model was constructed to assess the financial costs and potential clinical benefits gained from implementing various rapid testing strategies in a hypothetical NHS microbiology department. Data on isolation rates for each pathogen and costs of microbiological examination were based on published studies, laboratory user information, manufacturers' pricing quotations and expert opinion, where possible. It must be noted that a high degree of uncertainty in both data input and model structure is inevitable when assessing new technologies, so where possible sensitivity analysis has been applied to account for any problems encountered in parameter estimations.

Plan of investigation

For the purpose of this report, the following assumptions were made:

- Testing period was set at 1 year.
- Analysis is based on the NHS viewpoint, involving the cost of microbiological investigation only. As it is unclear how treatment costs (e.g. antibiotic therapy or hospital bed days) may change as a result of rapid diagnosis, there are insufficient data to model further the impact new testing strategies may have on these costs and subsequent health outcomes.
- Testing was carried out Monday–Friday, 9 am–6 pm, for all methods.
- Setting was a hypothetical UK microbiology laboratory testing 10,000 faecal samples for enteric pathogens per year.
- For the purposes of the model, testing in the laboratories during the stated time period will require a steady flow of samples to be constant. To account for outbreak scenarios, the isolation rate can be artificially increased, as would occur as more of the specimens being sent in would produce positive results.
- No inconclusive results were recorded, and no tests were repeated on the same sample.
- The costs of diagnostic tests used within the economic model are average costs and include elements of capital and overheads of providing these services.
- The effect of cases averted due to the prevention of person to person transmission was considered minimal for food-borne pathogens.

Cost-effectiveness analysis of competing test strategies was twofold. Initially, the majority of analyses worked on the assumption that the

sensitivity and specificity for the reference test, bacterial culture, equalled one (i.e. perfect accuracy). In the second part of decision modelling, this assumption was relaxed, to determine the cost per additional case detected associated with each strategy if culture methods were sub-optimal.

Test strategies

It is unlikely that bacterial culture methods will soon be replaced as the routine diagnostic procedure for suspected food-borne illness. Growth of culture has enjoyed a 100-year history in microbiology laboratories, with reasonable diagnostic accuracy at a fairly low cost. The introduction of rapid diagnostic technology would probably be additive, rather than a substitute for culture methods, in the first instance. However, decision analysis can play an important role in predicting the outcomes associated with numerous strategies, irrespective of the likelihood that they will be adopted. We therefore address the option that bacterial culture will be fully replaced by rapid methods, although we acknowledge that this is an unlikely scenario for the immediate future.

As discussed earlier in this report, it is impractical to assume that every incident of food-borne illness will undergo microbiological investigation. Under-reporting by affected individuals is the primary reason for this, but also GPs may put in place a system of triage whereby mild cases in otherwise healthy adults do not need laboratory analysis. Likewise, it is highly probable that any decision to order an additional test to enable faster diagnosis will involve prioritising those cases for which rapid detection will bring the greatest benefit. Food-borne illness is an important cause of cross-infection among certain patient groups, including neonates and the elderly. For *E. coli* in particular, serious infection leading to hospitalisation is most pertinent with younger populations.[133] There is some empirical evidence to suggest that children are more susceptible than adults to O157 strains. In an economic review of the 1994 outbreak of *E. coli* O157 in Midlothian, Scotland, GP and hospital surveys confirmed that 49.3% of all cases (35/71) were aged under 5 years, accounting for 50% (12/24) of hospitalisations.[134] The need for more prompt diagnosis and patient management for children highlights the potential benefits of rapid testing for this patient group. Consequentially, we have constructed various testing strategies in which the detection of microorganisms in high-risk patients is prioritised.

Four broad testing strategies were examined (*Table 22*), as outlined below.

Status quo – routine culture

Costs and cost-effectiveness of the current testing strategy were assessed, namely where all samples are cultured for *Campylobacter*, *Salmonella* and *E. coli* O157. Enrichment broths and selective agars used for each organism are in line with those from UK National Standard Operating Procedures.

Routine culture combined with additional rapid tests for high-risk patients

'High-risk' patients include pregnant women, aged under 5 or over 65 years, immunocompromised or anyone presenting with severe symptoms, such as bloody diarrhoea. Although there is no clear evidence that the pathogen isolation rate is higher for these patients, the consequences of prolonged undiagnosed food poisoning may be more serious, such as an increased risk of renal failure. For modelling purposes, it is assumed 25% of patients are 'high risk'. Sensitivity analysis was carried out to assess the additional cost and cost-effectiveness outcomes if up to 50% of all samples were deemed 'high risk', such as may be the case if a suspected food-borne outbreak were to occur in a nursing home or primary school.

Routine culture combined with additional rapid tests for enterohaemorrhagic E. coli

Given the importance that health professionals place on *E. coli* O157, irrespective of its low prevalence compared with some other enteric pathogens, various testing strategies were developed in which only rapid tests for *E. coli* were carried out. It is assumed that the rapid test kits used for *E. coli* could detect all enterohaemorrhagic strains, thus potentially reporting more true cases of infection than SMAC culture methods, which may miss sorbitol-fermenting non-O157 strains.[25] In this instance, additional cases detected can be used as a suitable outcome measure.

Complete replacement of routine culture with rapid tests for all samples

The most radical strategies modelled involve the total replacement of bacterial culture methods with rapid testing. Although this is unlikely to be adopted in the very near future, there is evidence to suggest that new technologies, particularly involving molecular detection methods, may evolve and replace existing procedures in the next few decades. This strategy is a forward-looking assessment of how microbiological investigation of food-borne illness may look in the future.

TABLE 22 *Summary of testing strategies*

Strategy	Test	Organisms	Patient group	Total samples	Total rapid tests	Description of strategy
Status quo						
Routine culture only, no rapid testing	Culture	All		10,000	30,000	Standard culture in local laboratory
High-risk patients, rapid						
Culture and rtPCR for high-risk groups only	rtPCR	All	High-risk	2,500	7,500	Test high-risk samples for all three organisms by rtPCR
Culture and ELISA for high-risk groups only	ELISA	All	High-risk	2,500	7,500	Test high-risk samples for all three organisms by ELISA
E. coli *rapid texts*						
Culture and rtPCR testing for *E. coli* only	rtPCR	*E. coli*	All	10,000	10,000	Test all samples for *E. coli* by rtPCR
Culture and ELISA testing for *E. coli* only	ELISA	*E. coli*	All	10,000	10,000	Test all samples for *E. coli* by ELISA
Complete replacement of culture methods						
Complete replacement of culture with rtPCR	rtPCR	All	All	10,000	30,000	Test all samples for all three organisms by rtPCR
Complete replacement of culture with ELISA	ELISA	All	All	10,000	30,000	Test all samples for all three organisms by ELISA

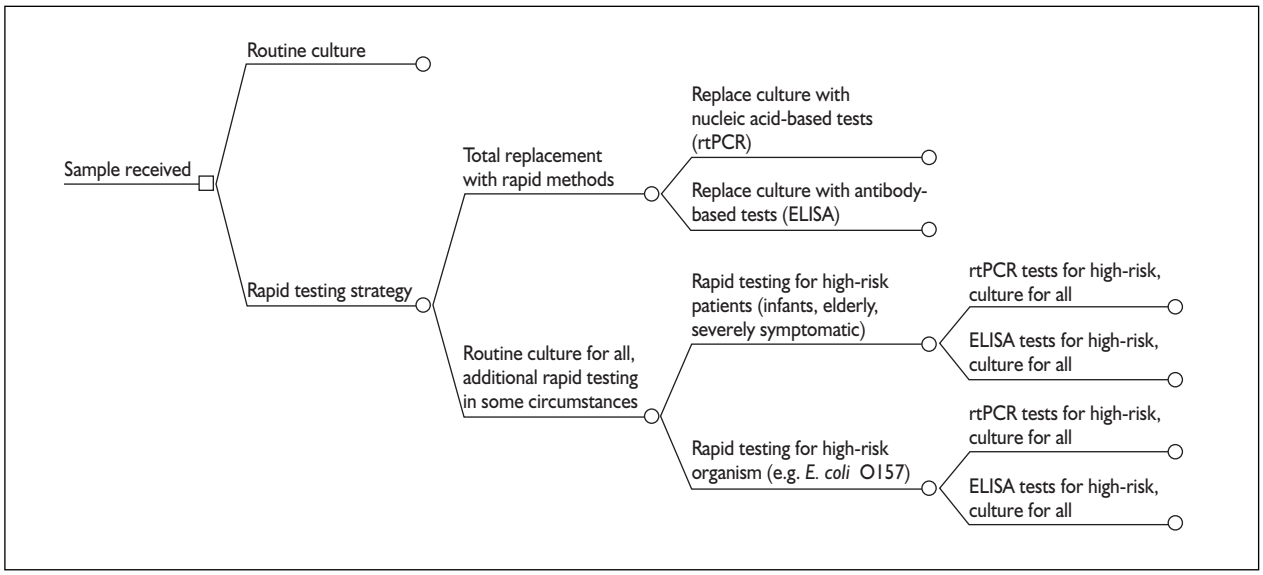


FIGURE 24 *Decision tree outline of potential strategies*

This decision model (*Figure 24*) concentrates on the three testing procedures for which a sufficient level of data is available to establish diagnostic accuracy and cost estimates. These are bacterial culture (status quo), antibody-based assays and PCR. It is assumed that commercial testing kits will be used in applying rapid methods.

Outcome measures

For the purpose of this report, the cost-effectiveness of diagnostic tests for food-borne illness is expressed as **cost per case detected**. The cost per case finding is a concept borrowed from the economic evaluation of screening procedures. Where clinical evidence can show that the rapid method is more sensitive than traditional culture methods, incremental cases detected and cost per additional case detected have also been calculated.

Quality of life considerations have not been incorporated into this analysis because the quality of life literature with regard to food-borne illness has not yet been investigated, and is beyond the scope of this review. As the course of gastrointestinal illness usually lasts no longer than 1 week, and patients are usually treated symptomatically, it is unlikely that the quality of life impact would be significant for the majority of patients. However, for the small minority of patients who go on to develop secondary complications such as HUS or GBS, quality of life estimates are likely to be significant.[134] There is not enough literature currently available to deduce the impact that rapid diagnosis would have on the course of illness in these cases, and therefore it is unclear what impact quality of life considerations may have on cost-effectiveness estimates for the rapid diagnosis of food-borne illnesses. More research is needed in this area, but the difficulties in developing such evaluative studies of diagnostic procedures have been noted.[132,135]

Review of economic literature and construction of the model

An attempt was made to collect previously published cost and cost-effectiveness evidence. Although considerable investigation has been carried out on the costs of food-borne disease,[136–139] at the time of writing, no economic studies were identified on the cost-effectiveness of test methods for the rapid diagnosis of food poisoning. Where economic considerations were mentioned in the included articles, this information is recorded in the study characteristics in Appendix 3. In should be noted, however, that these studies focused only on cost analysis, and as such, information regarding relative efficiency could not be gained. Interpretation of these studies was further complicated as none were conducted within the UK and baseline years for cost estimation varied widely.

Decision analytic models are formulated using three key fields of information:

- effectiveness data
- epidemiological data
- cost data.

TABLE 23 *Baseline values and sensitivity range for diagnostic accuracy of nucleic acid-based methods (PCR tests)*

Organism	Baseline values		Upper limit (+10%)		Lower limit (–10%)	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Campylobacter	0.90	0.97	0.99	1.00	0.81	0.87
Salmonella	0.88	0.98	0.97	1.00	0.79	0.88
E. coli O157	0.99	1.00	1.00	1.00	0.89	0.90

TABLE 24 *Baseline values and sensitivity range for diagnostic accuracy of antibody-based methods (ELISA tests)*

Organism	Baseline values		Upper limit (+10%)		Lower limit (–10%)	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Campylobacter	0.89	0.98	0.98	1.00	0.81	0.89
Salmonella	0.96	0.96	1.00	1.00	0.86	0.86
E. coli O157	0.95	0.98	1.00	1.00	0.86	0.88

Effectiveness data

No diagnostic test method is 'perfect'. The economic cost of diagnostic errors must be incorporated into any decision model on clinical effectiveness. With food-borne illness, the cost of incorrect positive diagnosis, in particular, may be extensive when public health measures to prevent further spread of infection are considered.

Effectiveness data were collected through a systematic review of evidence evaluating rapid test methods for *Campylobacter*, *Salmonella*, and *E. coli* O157. Due to the lack of substantiating evidence of rapid technologies being applied to toxin-inducing food-borne pathogens, tests for *C. perfringens*, *S. aureus* and *B. cereus* were excluded from the decision analytic model. This is in keeping with current laboratory practice, as toxin-inducing food-borne pathogens are usually only tested for after a specific request from the clinician.

Rapid diagnosis

An overall summary of clinical effectiveness for nucleic acid-based (PCR) (as previously described, there are a number of inherent problems in using this summary figure; the majority of studies assessing nucleic acid-based methods were carried out using in-house-designed PCR, as opposed to commercially available kits, and, as such, questions of reproducibility and heterogeneity must be addressed) and immunological diagnosis was calculated from the body of evidence reviewed. Data were derived from pooled sensitivity and specificity estimates, as reported in forest plots (Chapter 5). The baseline values for diagnostic accuracy of PCR and immunoassays for each pathogen are given in *Tables 23* and *24*. Due to the intrinsic uncertainty surrounding these values, a 10% upper/lower confidence range has been applied for sensitivity analysis.

Traditional culture

Attempts to quantify the diagnostic accuracy of rapid test methods are confounded by the lack of a robust reference test. In most cases, traditional culture methods are assumed to be 'correct'; however, a growing body of evidence comparing culture with new rapid techniques (such as nucleic acid-based detection) suggests that sensitivity values are significantly lower than 100%. In particular, evidence suggests that sensitivity of culture for *Salmonella* and EHEC may vary in the range from 30–80% compared with PCR, ELISA or full biochemical identification.[25] The culture method is more robust for *Campylobacter* detection, with the sensitivity range varying between 83 and 100%. It is probable that these samples include a mix of under-reporting by culture and misinterpretation by rapid methods (e.g. due to PCR contamination); however the impact of under-reporting may be assessed through sensitivity analysis. As it is the reference standard used for the majority of rapid tests, the baseline value for culture methods was set at 100% sensitivity and specificity. However, a lower limit of 80% sensitivity was modelled.

Epidemiological data

Annualised data detailing the number of positive laboratory results for enteric pathogens are widely

available from the Health Protection Agency. However, without knowing the volume of stool tests carried out each year, it is impossible to estimate the isolation rate for each pathogen. It is notoriously difficult to estimate this value.

Published evidence

The most comprehensive research carried out on food-borne pathogens in the UK to date was commissioned by the FSA in the late 1990s. The Report of the Study of Infectious Intestinal Disease in England[3] preceded its full investigation with an enumeration study in 1994, which estimated the incidence of IID presenting to GPs and the proportion of samples routinely sent for microbiological investigation. Data relating to the volume of tests performed are summarised in *Table 25*.

A GP case–control component was also included in the Study of Infectious Intestinal Disease in England,[3] to assess the incidence of IID presenting to GPs. The number and proportion of pathogens isolated from a total of 2893 stool samples tested are given in *Table 26*.

Using the information provided by the above study[3] and annual reports from the Health Protection Agency, the percentage of positive samples for *Campylobacter*, *Salmonella* and *E. coli* O157 can be extrapolated to provide the estimates of the proportion of positive samples given in *Table 27*.

Isolation rates for *Campylobacter* and *Salmonella* appear to have decreased dramatically since the IID study results were recorded. Given the importance of accuracy in epidemiological data for decision analysis, attempts were made to obtain a clearer picture of the volume of laboratory tests today, by conducting a small validation survey.

Validation survey

In order to test the validity of the IID data in current practice, information was obtained on faecal workload and positive isolates by sending questionnaires to randomly selected microbiology laboratories in England and Wales. There was a reply from 7/20 (35%) of laboratories. Data were pooled to increase the statistical power of isolation

TABLE 25 *Total number of samples and pathogen detection rate in the Study of Infectious Intestinal Disease in England*[3]

	Total stools examined in 1994	Total stools positive for any target isolate	Proportion positive (%)
All laboratories in England (n = 178)	878,247	108,180	12.3

TABLE 26 *Pathogen detection in stool samples from patients presenting to GPs in the Study of Infectious Intestinal Disease in England*[3]

Organism	Cases of IID presenting to GP		
	No. identified	No. tested	Proportion identified (%)
Campylobacter	354	2893	12.2
Salmonella	146	2893	5.0
E. coli O157	3	2893	0.1
Other organisms	1085	2893	37.5
No organism identified	1305	2893	45.1

TABLE 27 *Estimates of the proportion of positive samples in 2005*

Organism	Estimated stools examined[a]	Total laboratory reports recorded in 2005	Proportion positive (%)
Campylobacter	878,247	44,342	5.05
Salmonella	878,247	6,639	0.76
E. coli O157	878,247	946	0.11

[a] Volume of tests based on value derived in the IID enumeration study.[3]

TABLE 28 *Results of a survey of seven hospitals in England and Wales to estimate current workload and isolation rates*

Organism	Total stools examined in 2004	Total stools positive for isolate	Proportion positive (%)	Upper value–lower value
Campylobacter	58,964	2562	4.35	16.94–2.98
Salmonella	58,964	584	0.99	7.91–0.65
E. coli O157	58,432	37	0.06	0.51–0.01

Source: Survey of faecal workload and positive isolates in randomly selected laboratories in England and Wales.

rate estimates, which are given in *Table 28* along with 95% CIs, highest estimates and lowest estimates.

Statistical significance of summary estimates

There does not appear to be a statistically significant variation between the estimated isolation rates derived from published 2005 data and actual rate of positive test results recorded between 2004 and 2005 in replies from laboratories.

Summary of epidemiological data

Table 29 details baseline values (generated from published evidence), along with upper and lower values for sensitivity analysis (as reported in the validation survey). Upper and lower values were used instead of CIs of isolation rates to investigate the effect of a large outbreak on laboratory services. The much higher upper value for *E. coli* O157 was derived using empirical evidence from the South Wales *E. coli* O157 outbreak in autumn 2005. In a subsequent review commissioned by the Chief Medical Officer for Wales,[140] it was reported that 2100 samples were tested during the outbreak, with 158 persons (7.52%) positively identified as infected.

Costing details

This economic evaluation was undertaken from the perspective of the NHS. Therefore, only direct medical costs paid for by the NHS bodies and funding laboratory tests are included. It is unclear how indirect costs such as time costs incurred by patients or their over-the-counter medical treatment costs would change if rapid tests were implemented, and as such these values are excluded from analysis.

Initial costs of implementing programme

A decision model was developed to convey how rapid test methods could be adopted in a medium-sized NHS laboratory. Initial one-off costs in starting up the programme include financing additional training in nucleic acid-based methods.[141] A hypothetical training scenario was developed, with the estimated cost of technician training estimated at £6600, based on hourly wage rates for laboratory staff (2005 prices). Results are shown in Appendix 6, *Table 49*.

Finding spare capacity within the laboratory building to accommodate new equipment may be extremely difficult. For strategies in which tests are provided in addition to culture, this is likely to have a significant impact on the overall costs of developing a rapid diagnostic service. PCR suites, in particular, may demand a large area of laboratory space to ensure that contamination will not lead to false positive reporting. However, these costs are likely to vary widely between laboratories, depending on the size and capacity levels in each centre. To minimise the subjectivity involved in measuring these start-up costs, these were excluded from this analysis. Each laboratory

TABLE 29 *Summary of epidemiological data*

Organism	Isolation rate (% positive samples)	Upper value	Lower value
Campylobacter	5.05	16.94[a]	2.98
Salmonella	0.76	7.91[a]	0.65
E. coli O157	0.11	7.52[b]	0.01

[a] Derived from outlier values reported in the laboratory survey.
[b] Estimated isolation rate experienced during South Wales *E. coli* O157 outbreak in 2005.

manager should assess their unique laboratory requirements carefully before deciding if rapid testing could be implemented.

Labour costs

Staff costs contribute significantly to the total cost of running a diagnostic laboratory. Traditional culture methods, with their time-consuming and labour-intensive procedures, have relatively high staff costs. Labour costs include not only the time taken to perform the test, but also the time for preparation of the extract, which may include a lengthy extraction or concentration step.

Under the assumptions of the decision analytic model, all tests are carried out by a medical laboratory assistant. Results are read and interpreted by a biomedical scientist. Salary costs have been taken from pay scales in effect for laboratory personnel in 2005, with the time for analysis, administration and reporting calculated (*Table 30*). All calculations are based on a working week of 37.5 hours, with 17% on-costs included to estimates.

The hands-on time for each test was calculated from the commencement of handling the specimens and controls in the laboratory to the final absorbance results. Biochemical tests are required for all culture-positive samples, and staff time for this was calculated according to the proportion of culture-positive samples received. Full staff cost analysis is reported in Appendix 6, *Table 48*.

Nucleic acid-based diagnosis

Attempts to attach a singular value to the cost of nucleic acid-based diagnosis are confounded by three major issues:

- Conventional PCR tests have a significantly different cost structure to automated rtPCR tests.
- A breakdown of costs for in-house-designed PCR tests is difficult to administer. Once the high initial costs of developing the assay have been accrued, the marginal costs of routinely using an in-house-designed PCR test may be significantly lower than continuing to purchase commercial kits. There is insufficient evidence in the published literature to validate the true costs of molecular assay development.
- The total cost per case is dependent on the throughput per 'run' of PCR equipment.

Application of commercial real-time PCR test kits versus in-house-designed methods

rtPCR tests can either be developed by the testing laboratory themselves ('in-house') or purchased from a commercial supplier. Good in-house assays include appropriate controls in order to monitor their reproducibility and effectiveness, with the results of validation studies of in-house assays being submitted for publication in peer-reviewed journals.

In the absence of reliable information on the costs of in-house-designed PCR assays, the unit cost listed for commercially available kits was used. Although the list price was set at £5.25, substantial economies of scale are usually available, and following consultation with the supplier, a price of £3.75 was agreed.

Problems remain with the use of commercial tests, including variability between batches; however, for economic modelling purposes, it is assumed a commercial rtPCR test kit will be used to detect pathogens by nucleic acid-based methods. Justifications for this assumption include:

- The methodologies and primer selection of in-house-designed PCR tests are very heterogeneous, and most of the in-house tests have not undergone sufficient validation which

TABLE 30 *Estimates of staff cost using 2005 NHS pay scales*

Test method	Staff grade[a]	Time taken	Staff cost per test (£)
Campylobacter test culture	MLA/MLSO to report	8 minutes test plus 10 minutes for positives	1.40
Salmonella test culture	MLA/MLSO to report	8 minutes test plus 10 minutes for positives	1.31
E. coli O157 test culture	MLA/MLSO to report	10 minutes test plus 10 minutes for positives	1.59
PCR – any organism	MLA/MLSO to report	6 minutes test	0.94
ELISA – any organism	MLSO	7 minutes test	1.40

MLA, Medical Laboratory Assistant; MLSO, Medical Laboratory Scientific Officer.
[a] Based on 2005 NHS pay scales.

commercial test kits must pass to ensure reproducibility and efficacy.
- Staff training is considerably reduced when using commercial test kits compared with in-house designs, as they are easy to use. If implementing nucleic acid-based methods in regional laboratories, the additional training costs to use the commercial kits would be much less than training a laboratory scientist how to replicate an in-house-designed assay.
- There is reduced risk of background DNA contamination with quantitative rtPCR methods, as the process is fully automated.
- Quantitative rtPCR technology is expanding at such a rate that some authors predict that conventional PCR methods will soon become obsolete.

Reagent costs per test

Unit costs of commercially available kits vary widely. Volume buying can reduce the cost by up to 40% and 'bundling' of different kits from the same manufacturer could result in substantial discounts. The unit cost of Applied Biosystems rtPCR kits was valued at £5.25. A discounted price of £3.75 per test was available to moderate- and high-volume laboratories (information gained through direct contact with manufacturer). This includes sample reaction mixes, positive and negative control reaction mixes and reagents used for quality control. A 25% sensitivity range was placed on these to account for other manufacturers' prices (from the evidence available at the time of analysis, the Applied Biosystems kits were the lowest cost commercial rtPCR assays on the market; however, as the technique becomes more popular, it is likely that costs may decrease).

Impact of PCR capital equipment throughput on price per test

Traditional culture and antibody-based testing can be performed in a laboratory of any size, irrespective of the throughput of faecal samples that it may have. Due to the significant start-up costs of purchasing the necessary equipment, PCR tests are likely to require development in a larger laboratory setting.

Nucleic acid-based test methods, particularly rtPCR, are associated with very high capital costs. As previously described, thermal cycler machines are used to amplify DNA for nucleic acid-based detection. Most have a maximum capacity of 96 wells, which can hold up to 92 samples (excluding positive and negative controls). The most up-to-date technology can perform DNA amplification and interpret results within 40 minutes. It is estimated that to make optimal use of this expensive equipment, each PCR run must have a high throughput of tests administered.

The decision to implement nucleic acid-based tests must take account of the volume of samples being sent to the laboratory each day. For example, in many smaller laboratories, it may not be possible to achieve adequate economies of scale to justify the large sunk cost of PCR capital equipment.

Using the testing strategies outlined, the throughput of tests per day for nucleic acid-based detection is estimated in *Table 31*.

Despite the large volume of tests carried out each year, there may still be sub-optimal usage of rtPCR equipment. Ideally, given that each thermal cycler can test 92 samples simultaneously, and three or four cycles can be run per day, the volume of testing would be higher. However, the cost of commercially available reagents would limit usage.

rtPCR needs a large capital outlay, with fully automated thermal cyclers costing within the range £20,000–30,000. NHS procurement of expensive medical equipment usually permits substantial reductions in the unit price. Through discussion with manufacturers, we have assumed the baseline cost to NHS laboratories to be £20,000. The cost range for sensitivity analysis (*Table 32*) is set at £16,500–23,500 (based on estimates quoted by Applied Biosystems). The

TABLE 31 *Estimated throughput of tests per day for nucleic acid-based assays*

Organisms	Patient groups	PCR capacity	Annual samples	Throughput (annum)	Throughput (day)	Runs/day
All	All	96 (92)	10,000	30,000	115	1/2
All	High-risk (25%)	96 (92)	2,500	7,500	29	0/1
All	High-risk (50%)	96 (92)	5,000	15,000	58	0/1
E. coli	All	96 (92)	10,000	10,000	38	0/1

TABLE 32 *Capital cost (£) of nucleic acid-based detection methods based on different testing strategies*

	£20,000 over 3 years	£16,500 over 5 years	£23,500 over 3 years
Annualised capital costs	6,666.67	3,300.00	7,833.33
Capital cost per test			
rtPCR replacement strategy	0.22	0.11	0.26
25% high-risk patients, all organisms	0.89	0.37	1.74
50% high-risk patients, all organisms	0.44	0.22	0.52
All samples, *E. coli* only	0.67	0.33	0.78
ELISA test	0.05	0.03	0.10
Culture	0.02	0.02	0.05

TABLE 33 *Cost estimates (£) for antibody-based tests*

	Baseline[a]	Min. unit cost (–10%)	Max. unit cost (+20%)
Campylobacter ProSpecT kit	3.64	3.28	4.37
Salmonella Wellcolex kit	1.41	1.27	1.69
E. coli Premier EHEC kit	3.65	3.28	4.38

[a] Cost estimates obtained through direct contact with manufacturers.

equipment is assumed to have a useful lifespan of either 3 years (minimum value and baseline) or 5 years (maximum value) years (there is no consensus on the lifespan of medical equipment – 10 years is commonly used, but for rapidly evolving technologies this can be much shorter; a 3–5-year lifespan was chosen after discussion with laboratory users regarding the 'useful lifespan' of equipment in their workplaces).

For the six rtPCR testing strategies applied to this decision analysis, baseline capital costs associated with each strategy range from £0.22 per test for full rtPCR replacement, compared with £0.89, if only samples from high-risk patients are included. When sensitivity analysis is included, this range widens from £0.11 to £1.74. This considerable variation suggests that the throughput of tests is an important factor when implementing rtPCR tests for enteric pathogens. Full details of variation in rtPCR capital costs are given in Appendix 6, *Table 51*.

Antibody-based tests: immunoassays

The cost of a test includes the direct costs of reagents, equipment and labour. The shelf-life of each test and volume of throughput may affect the cost per test. Kits with a shorter shelf-life (e.g. 6 months) may not be used before the expiry date if there is insufficient throughput of specimens. Similarly, a microtitre format of a batch of eight or 96 will be expensive for testing on a single specimen. Although it is difficult to quantify how strongly these may impact the cost per test for antibody-based tests, as a precautionary measure a wide upper sensitivity range of 20% is applied to all costs, while the lower value remains at 10% (*Table 33*).

Culture-based methods

As most UK laboratories now use prepoured plates to culture food-borne pathogens, costs for culture are based on this method.

In most hospital laboratories, the use of an enrichment broth increases the overall cost and turnaround time of the stool culture. Unfortunately, conventional approaches are time consuming and costly. Eliminating culture broths may be one strategy for reducing these costs; however, it is likely that the sensitivity of these tests would be sacrificed as a result.[142] Costs used in this economic model were based on current laboratory costs computed at a moderately large hospital laboratory, and through contact with Oxoid, the leading supplier of bacterial culture reagents in UK.

Breakdown of unit costs for each testing strategy

A summary of unit costs of tests is given in *Table 34*.

A detailed summary of how these costs were built up is given in Appendix 6, *Tables 50–52*. In

TABLE 34 *Summary of unit costs of tests (£)*

	Baseline unit costs	Min. value	Max. value
Campylobacter culture	5.21	4.17	6.25
Campylobacter ELISA	7.54	6.03	9.05
Campylobacter PCR	7.83	6.81	10.13
Salmonella culture	4.81	3.84	5.77
Salmonella ELISA	2.67	2.14	3.20
Salmonella PCR	5.35	4.82	7.15
E. coli O157 culture	4.99	4.00	5.99
E. coli O157 ELISA	5.45	4.36	6.54
E. coli rtPCR	5.67	5.08	7.53

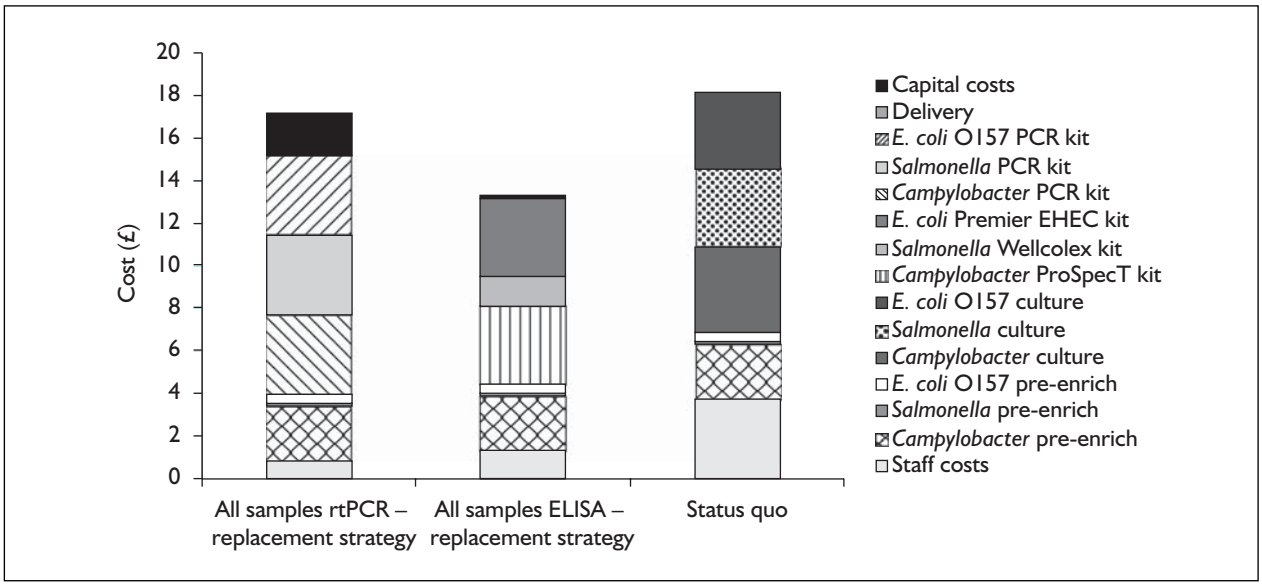


FIGURE 25 *Breakdown of unit costs per sample for each testing strategy: replacement strategies versus status quo*

summary, the composite values for each testing strategy are shown in *Figures 25* and *26*.

Notably, the unit costs of bacterial culture are slightly higher than that of full-scale rtPCR detection or similarly replacement with ELISA methods. rtPCR assays demand a large capital outlay, but these costs may be offset by a reduction in technical staff costs associated with labour-intensive bacteriological culture methods. It is worth noting that due to the ability of commercial rtPCR test kits to automate DNA extraction and analysis of results, the majority (78%) of staff costs with PCR occur due to the need for initial pre-enrichment. Should direct sampling become available in the future, this would have a dramatic impact not only on the timeliness of PCR, but also on labour costs incurred.

Additive costs incurred when testing for all three pathogens are higher than for those strategies which test for only *E. coli* strains. It is important to assess whether these lower costs relate to more efficient outcomes. This can be achieved through cost-effectiveness analysis.

Cost-effectiveness results

Base-case results

The base-case test parameter values represent the best estimate of sensitivity and specificity for each type of test. Because the study designs used to obtain measured values of sensitivity and specificity vary widely, and the degree of available evidence varies across the different types of test under consideration, reasonable upper and lower

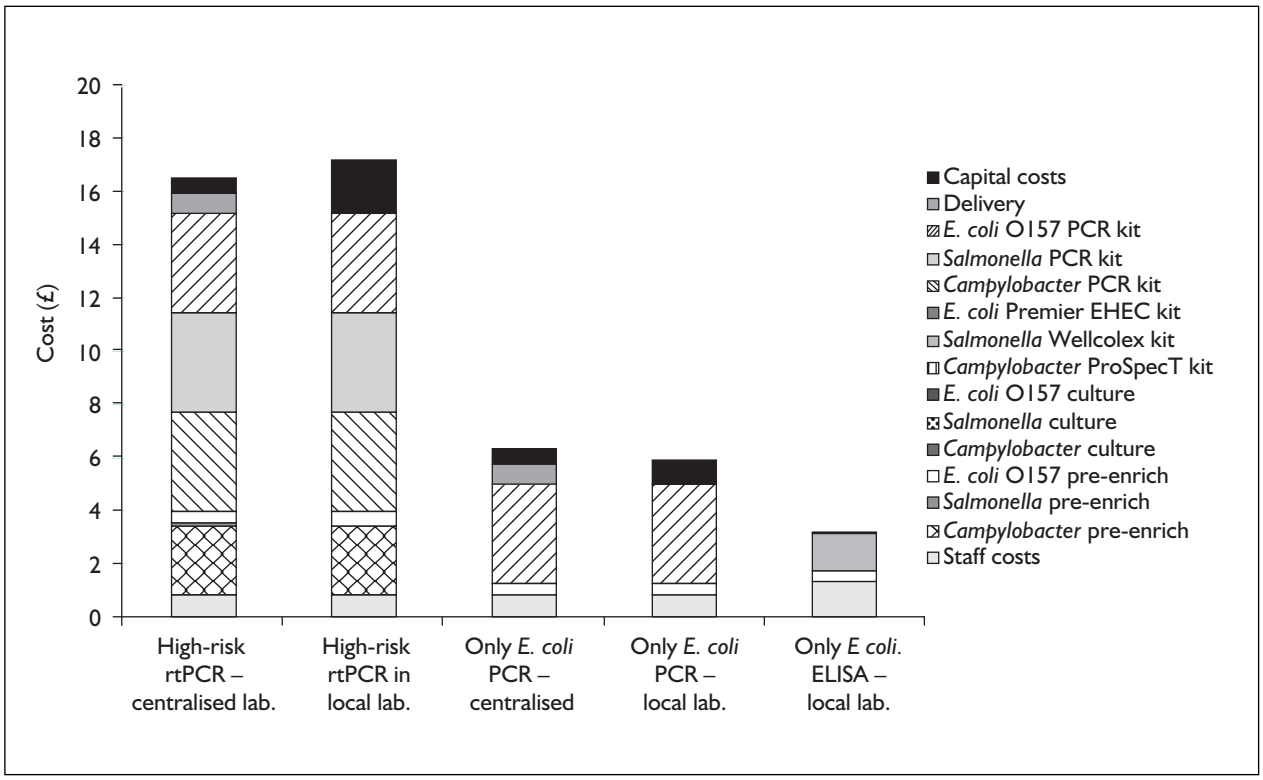


FIGURE 26 *Breakdown of additional costs per sample for diagnostic adjunct strategies*

uncertainty boundaries are specified for the diagnostic accuracy of each test. The results show a series of simulations to assess the cost-effectiveness of various testing strategies (*Table 35*).

Baseline results from this decision model suggest that if rapid test strategies were to replace fully bacterial culture as the standard test, long-run cost savings may potentially be enjoyed. However, the implementation of rapid test methods as a diagnostic adjunct would require significantly more funding. The use of ELISA test kits is a marginally less expensive strategy than rtPCR testing; however, it is likely that rtPCR tests are available sooner than antibody-based tests. When diagnostic accuracy estimates are varied in the model, it is suggested that the greatest uncertainty surrounds the sensitivity of culture methods. At the baseline 100% sensitivity, culture may detect 591 cases of food-borne infection in every 10,000 samples (using baseline epidemiological data, based on 2005 reported cases of *Salmonella*, *Campylobacter* and *E. coli* O157), but when the lowest estimate is applied (see *Table 35*), potentially 118 cases may be missed. Speed of diagnosis, rates of diagnostic error and costs of implementation should be considered simultaneously when deciding which strategy is optimal.

Sensitivity analysis 1: variation in costs of implementation

To assess the robustness of optimal testing strategies to potential changes in prices, a 25% sensitivity range was placed on unit costs for each strategy. *Table 36* shows the unit price of each method.

Sensitivity analysis 2: variation in isolation rates

Cost-effectiveness results for individual organisms according to minimum and maximum recorded isolation rates from laboratory survey are presented in *Tables 37–39*.

Sensitivity analysis 3: outbreak of food-borne illness

In September 2005, a large outbreak of *E. coli* O157 was reported in South Wales, with the original source implicated identified as a local school meat supplier. A large-scale investigation was mounted, with an increased volume of tests carried out. A report from this investigation stated that 2100 stool samples were taken, with 158 cases positively identified. In accordance with this empirical evidence, an isolation rate of 7.52% was applied to the economic model to account for a hypothetical outbreak of *E. coli* O157, and the cost-effectiveness of each strategy in this setting (*Table 40*).

TABLE 35 *Baseline cost of implementation with time taken to conduct the test*

Strategy	Baseline cases detected per 10,000 samples	Minimum TP cases detected (assume culture misses 20% diagnoses)	Maximum TP cases detected (assume culture 100%)	Cost of implementation (£)	Time taken to diagnose (days)	% correct diagnoses [(TP + TN)/N]
Culture (status quo)	591	473	591	188,100.00	3–4	1
Replace culture by PCR	534	478	584	178,966.67	1	0.98
Replace culture by ELISA	534	483	581	162,100.00	1–2	0.98
Culture all and PCR for high-risk patients	591	473	591	236,175.00	1 for high-risk, 3–4 for all other patients	0.98
Culture all and ELISA for high-risk patients	591	473	591	228,625.00	1–2 days for high-risk, 3–4 for all other patients	0.98
Culture all, PCR *E. coli* only	591	473	591	248,288.89	1 for *E. coli* results, 3–4 for all *Campylobacter* and *Salmonella*	0.98
Culture all, ELISA *E. coli* only	591	473	591	243,500.00	1–2 for *E. coli* results, 3–4 for all *Campylobacter* and *Salmonella*	0.99

TN, true negative; TP, true positive.

TABLE 36 *Summary unit costs for each test method, by organism (costs per sample)*

Strategy	Baseline unit costs (£)	Min. value (£)	Max. value (£)
Campylobacter culture	5.21	4.17	6.25
Campylobacter ELISA	7.54	6.03	9.05
Campylobacter PCR	7.83	6.81	10.13
Salmonella culture	4.81	3.84	5.77
Salmonella ELISA	2.67	2.14	3.20
Salmonella PCR	5.35	4.82	7.15
E. coli O157 culture	4.99	4.00	5.99
E. coli O157 ELISA	5.45	4.36	6.54
E. coli rtPCR	5.67	5.08	7.53

TABLE 37 *Cost-effectiveness of different strategies for detecting* Campylobacter

Strategy	Cost per sample (£)	Baseline isolation – 5.05%	Lowest isolation – 2.98%	Highest isolation – 16.94%
Status quo – cost of culture	5.21	158.65	268.79	47.28
rtPCR replacement – all samples	7.38	164.50	278.70	49.03
ELISA replacement of culture	7.59	170.27	288.49	50.75
rtPCR for high-risk patients	7.82	174.23	288.49	51.93

TABLE 38 *Cost-effectiveness of different strategies for detecting* Salmonella

Strategy	Cost per sample (£)	Baseline isolation – 0.76%	Lowest isolation – 0.65%	Highest isolation – 7.91%
Status quo – cost of culture	4.81	677.30	787.69	64.73
rtPCR replacement of culture	4.90	755.27	878.36	72.18
ELISA replacement of culture	2.88	411.07	478.06	39.28
rtPCR for high-risk patients	5.34	821.97	955.94	78.55

TABLE 39 *Cost-effectiveness of different strategies for detecting* E. coli

Strategy	Cost per sample (£)	Baseline isolation – 0.11%	Lowest isolation – 0.01%	Highest isolation – 0.51%
Status quo – cost of culture	4.99	5,273.20	56,800.00	1,113.73
rtPCR replacement of culture	5.22	5,025.18	54,128.46	1,061.34
ELISA replacement of culture	5.44	5,405.38	58,223.86	1,141.64
rtPCR for high-risk patients	5.66	5,306.20	58,623.25	1,149.48
ELISA for high-risk patients	5.44	5,343.75	58,223.86	1,141.64
rtPCR for all patients, *E. coli* only	5.89	5,514.38	60,870.64	1,193.54

Owing to the similarity in diagnostic accuracy values and unit costs, the cost per case detected was remarkably similar for all strategies. However, as the 'additive' strategies convey only the **incremental** costs of choosing rapid test methods, and not the full costs of these **in addition to** routine culture costs, these options are considerably more expensive.

Sensitivity analysis 4: suboptimal detection with bacterial culture

Implications of routine use of culture

An interesting finding from this review concerns not just the diagnostic error experienced with new rapid technologies, but also that of routine culture. As bacterial culture is the current testing procedure nationwide, it is likely that the

TABLE 40 *Cost per case detected in outbreak scenario (*E. coli *isolation rate 7.52%)*

Strategy	Cost/sample (£)	Cost/case detected (£)	Implementation (£)
Status quo – cost of culture	4.99	68.48	158,100
rtPCR replacement of culture	5.67	71.13	178,966
ELISA replacement of culture	5.45	73.62	162,100
'Add-on' rapid strategies			
rtPCR for high-risk patients	5.66 add-on	85.23	80,166 add-on
ELISA for high-risk patients	5.45 add-on	75.62	54,365 add-on
rtPCR for all patients, *E. coli* only	5.89 add-on	79.99	60,188 add-on

published epidemiological data underestimates the identification of pathogens in submitted samples. The public health implications of these findings may be considerable, and further research in this area is warranted.

Implications of under-reporting by culture on costs per case detected

The most serious implications of diagnostic errors in culture methods are likely to be experienced with *E. coli* infections. Evidence from clinical effectiveness studies suggests that sensitivity of SMAC in detecting all EHEC (i.e. including non-O157 strains) may be as low as 32%,[25] when compared with a 'gold standard' of SMAC culture **and** cytotoxicity assays. Instead of concentrating on the biochemical characteristics of the *E. coli* O157 strain, rapid detection technologies target all shiga toxins, and as such can encompass O157 and non-O157 strains equally. It is therefore possible to estimate the level of under-reporting for **all** EHEC associated with the use of SMAC culture. Keeping rapid test sensitivity values as estimated from meta-analysis of studies, and changing the culture sensitivity for EHEC to 80%, there are considerably more cases detected by rapid techniques. The cost per additional case detected, further to what would be found in culture, is shown in *Table 41*.

Replacing culture

It is not assumed at present that rapid test methods will replace bacteriological culture, but rather that they should be a diagnostic adjunct to improve patient outcomes when speed of response is crucial in preventing further outbreak or worsening of patient conditions. However, with much research being invested in the development of rapid methods, these tests may well be viable as replacements to traditional culture in the future. This hypothetical analysis illustrates that if microbiology laboratories were to switch from bacterial culture to molecular methods, the increase in costs per sample for rapid methods compared with culture is not significant.

Summary of cost-effectiveness results

In terms of economic efficiency, strategies to implement rapid detection methods as a diagnostic adjunct to culture were strictly dominated by strategies which fully replace culture by either serological or nucleic acid-based tests. However, in the light of the apprehension shared among many microbiologists over abandoning bacterial culture, 'double testing' strategies remained in analysis. For 'adjunct' strategies, the implicit cost-effectiveness of carrying out the additional rapid tests would in most cases be low. For 'replacement' strategies, the cost per case detected varied widely with sensitivity analysis; however, the overall costs for each strategy were relatively similar. Results were most sensitive to changes in isolation rate. Incremental changes in cost estimates made a moderate impact on overall costs. Naturally, implementing rapid detection for all organisms would cost significantly more than if performing rapid detection for EHEC only. However, using cost per case detected as a suitable

TABLE 41 *Cost per additional case of EHEC detected by rapid methods compared with bacterial culture*

Strategy	Cost per sample (£)	EHEC cases detected per 10,000 tests	Cost per EHEC case detected (£)
rtPCR	5.22	18	2933.91
ELISA	5.44	17	3206.25
Culture	4.99	11	4536.20

outcome measure, testing for all three organisms is associated with much more attractive cost-effectiveness outcomes.

One of the initial assumptions of the model was that the sensitivity and specificity of bacterial culture equalled unity. Relaxing this assumption would be expected to lead to improvements in the cost-effectiveness of rapid detection methods relative to standard testing. With a culture underperformance modelled at 80% sensitivity, both nucleic acid-based and serological assays could detect an additional seven cases of EHEC infections per year. Given the severity of illness, and the speed at which an *E. coli* outbreak can spread, switching to an alternative testing strategy for this pathogen may have substantial benefits.

A subgroup of high-risk patients (i.e. those with severe symptoms or the very old and young) were assumed to account for 20% of all samples submitted. Two-way sensitivity analysis was performed to assess the incremental cost-effectiveness of testing this patient group with rapid detection methods, assuming that the isolation rate will now be higher (given the presence of severe symptoms, e.g. bloody diarrhoea). It is worth noting, however, that triage may have a cost to the laboratory. Several alternative testing algorithms will have to be in place and any lack of information accompanying the sample will have to be sought by telephone before testing strategies can be decided.

Discussion

This modelling exercise relies heavily on knowing precise data estimates for three key parameters: isolation rates for each pathogen, costs of implementation and diagnostic accuracy for each test. Sensitivity analysis was performed on each of these parameters to assess the impact that changes in these estimates may have on outcomes for each. The model predicts that whereas small changes in diagnostic accuracy and cost estimates do not vary the overall cost per case detected for each strategy, a marginal change in isolation rates results in relatively large changes in the outcomes. Outcomes are highly sensitive to the overall prevalence of each organism, which may explain why the cost per case detected for *Campylobacter*, the most commonly reported food-borne illness, is substantially lower than that for *E. coli*, which occurs rarely, but is given high priority due to the serious medical complications that may ensue. The sensitivity of isolation rates will have widespread implications for these results with respect to geographical variations in community prevalence. By using minimum and maximum isolation rates recorded from a prospective nationwide laboratory survey, authors have attempted to account for this regional variability. However, policy makers should take account of their localised community prevalence when assessing the need for rapid test methods.

Whereas sensitivity analysis to overall diagnostic accuracy values did not significantly alter outcomes for each testing strategy, the implications of diagnostic errors with bacterial culture had a greater impact. Under-reporting by culture methods may suggest that the number of positive cases recorded annually may indeed be lower than the 'true' volume of pathogenic samples submitted, given the current reliance on culture methods throughout the UK. Much more research is required in this area.

Availability of data

Although stringent attempts were made to secure the most accurate data for this economic evaluation, there are potential shortcomings with several of the input values for the model. By using the most recently published annual figures for disease incidence, we are confident that the volume of positive cases is relevant; however, the only figure we could identify regarding the total volume of tests carried out per year (used to measure the isolation rate) was last recorded in 1994. A small-scale laboratory survey was carried out in an attempt to validate this figure, which somewhat agreed with the published value; however, uncertainty still remains around the actual volume of tests carried out nationally in current practice.

Applicability to smaller laboratories

One criticism of this hypothetical model may relate to the moderately large volume of tests performed annually (10,000 samples). Many hospital laboratories carry out much fewer tests for food-borne pathogens each year. However, due to the substantial equipment costs associated with nucleic acid-based tests, and the need for a high throughput of samples to lower the average cost per test, this level of investment would be infeasible. One option worth exploration could be the introduction of a centralised PCR testing centre in each region, where samples could be sent for rapid diagnosis. However, it is worth noting that the cost savings enjoyed from economies of scale are forgone when additional expenses are considered, such as delivery charges to the centre.

It is also noted that such a system would require meticulous organisation and planning. These are illustrated in Appendix 6, with a breakdown of costs in a hypothetical centralised laboratory testing 50,000 samples annually (*Table 54*).

Exclusion of latex agglutination tests from analysis

A notable omission from this cost-effectiveness model is the role of antibody-based screening tests. Without adequate diagnostic accuracy data, these tests could not be included in decision analysis; however, their merits have been noted.[142] The diagnostic accuracy of these tests is fair, with alternative detection methods performing better, but at a higher cost. The main cost savings arise from the potential to eliminate further workup of pathogens. These assays are faster, cheap, easy to perform, widely available and can cut down on staff time. Because 85–99% of the total number of specimens tested have negative results for specific organisms, proponents argue that the use of cheap, fast kits could decrease the expense and overall turnaround time for most stool cultures performed. The cost savings include the cost of subculture plates, biochemical tests used to screen colonies and technologist time to perform the assays. Although they are not routinely used in UK laboratories, other countries have adopted them for routine testing (for example, the Wellcolex Colour *Salmonella* test has been adopted widely in Germany as a screening device). To appreciate fully the potential cost savings, it is necessary to look at the volume of subculture plates that receive further workup. Usually a high proportion (50–70%) of biochemical screens are performed on cultures that eventually have negative results. McGowan and Rubenstein[142] estimated that in their institution, eliminating the workup of colonies from Gram negative subculture plates would result in a media saving of US$124.00 per 100 stool specimens cultured and a time saving of approximately 20 hours per 100 stool specimens cultured.

Cost per case detected as an outcome measure

Cost per case detected is usually consigned to evaluations of screening programmes, defined as when tests are performed on people at risk of developing a certain disease, even if they have no symptoms. Screening tests can predict the likelihood of someone having or developing a particular disease, and hence the added value of 'detecting' one person at risk is an economic result in itself. However, as previously discussed, using this cost-effectiveness measure fails to link diagnostic performance to long-term health outcomes. There is research potential to investigate the 'end-point' impact of these tests, possibly through a prospectively designed randomised controlled trial.

Although the impact of timeliness is central to the implementation of new test methods, without available data on patient outcomes it is unclear how speed of detection may impact the overall effectiveness, and subsequent cost-effectiveness, of each strategy. An assessment of clinicians' willingness to pay for faster diagnosis may also contribute to understanding.

Chapter 8

Nominal group analysis with clinicians

To develop a valid diagnostic model based on the reviewed literature and assessment of cost-effectiveness, it is important to seek a clinical evaluation of the impact of different testing options. The qualitative arm of this research involved exploring disagreement and concordance of ideas between health professionals involved in the diagnostic process for food-borne illness. Following ethical approval, a focus group with three GPs, four microbiologists and one Consultant in Communicable Disease Control (CCDC) was organised, applying the nominal group technique (NGT). This approach was first described in the 1960s as a procedure to facilitate effective group decision-making in social psychological research,[143] and has been used in many settings to gain a ranking of expert views on important impacts of different strategic interventions.[144,145] Its three most typical applications have been problem identification, development of solutions and establishing priorities all of which have relevance to our study aims.

Nominal group process

The NGT consists of two rounds in which panellists rate, discuss and then re-rate a series of items or questions. The meeting was structured as follows:

- Reviews of the relevant literature are provided to participants before the meeting.
- Participants spend several minutes writing down their views about each topic in question.
- Each participant, in turn, contributes one idea to the facilitator, who records it on a flip chart.
- Similar suggestions are grouped together, where appropriate. There is a group discussion to clarify and evaluate each idea.
- Each participant privately ranks each idea (round 1).
- The ranking is tabulated and presented.
- The overall ranking is discussed and re-ranked (round 2).
- The final rankings are tabulated and the results fed back to the participants.

Alongside the consensus process, we will have a non-participant observer (research associate) collecting qualitative data on the nominal group. This is similar to a focus group. However, the NGT focuses on a single goal.

Following an overview of research findings from the study investigator (IA), the NGT facilitator (AH) addressed participants with three key research questions:

1. Based on clinical and cost-effectiveness data provided, would you choose rapid tests for:
   - an individual case of food-borne illness?
   - a suspected outbreak of food-borne illness?
2. For which organism, if any, should rapid testing be prioritised?
3. What, if any, testing strategy should be implemented when ordering or using rapid tests for food-borne illness?

Clinical aspects

For individual cases of food-borne illness, participants from all professional groups agreed that the decision to order rapid testing must depend on clinical criteria: these criteria were severity of symptoms and belonging to a 'high-risk' patient group. All participants suggested that if rapid tests were to be adopted, priority would be given to test children aged under 5 years. Elderly, pregnant or immunocompromised patients were also judged as in greater need of rapid tests than the wider community. Additionally, one GP suggested that they would only consider rapid testing in patients who were clinically very unwell or with serious illnesses which put them at risk of sepsis, e.g. diabetes or other chronic disease. Other priorities were to make diagnostic distinctions of food-borne illness from other sources of acute abdominal pain, e.g. appendicitis.

There was a general consensus among the group that not all patients would warrant selection for rapid testing, i.e. that triage of patients would be advisable. One GP noted that in reality the decision to order microbiological examinations, whether rapid or routine, is usually empirically based. All GPs argued that in terms of patient outcomes, correct patient management is more valuable than microbiological identification *per se*.

It was noted that the decision to order laboratory tests may rest on funding issues, particularly if both standard culture and rapid tests are available, but with a large cost variation, as this would act as a financial disincentive in spite of apparent clinical priorities.

Some disagreement was expressed among the microbiologists regarding the need for rapid tests in individual case settings, for example between the benefits of quicker results in terms of excluding a significant problem, while another microbiologist was not convinced of the need for rapid tests except in the context of a specific outbreak.

Organisms under investigation

Perhaps the most interesting findings from the NGT session centred around the participants' perception of which organism held greatest importance. Having been presented with UK isolation rates for each organism, costs per individual test and, crucially, cost per case detected, most participants still felt that *E. coli* was the organism for which rapid testing could bring about most positive change. Irrespective of the high cost of detection and low prevalence of the disease, most participants believed that *E. coli* testing must be prioritised due to its severe secondary complications.

Only one participant (a GP) argued that tests should be prioritised based on prevalence, that is, that tests for *Campylobacter*, *Salmonella* and then *E. coli* should be introduced. The same GP argued that nearly all GP cases of food poisoning that were confirmed microbiologically were reported as *Campylobacter*; and *Salmonella*, which has a longer duration of illness, was also an important pathogen. Contrastingly, a CCDC did not think that rapid tests for *Campylobacter* should take precedence, due to its self-limiting nature and the low risk of outbreak. Given the mixed opinions expressed within this small group of health professionals, more research is needed to uncover which pathogens should be prioritised.

Public health aspects

For suspected outbreaks of food poisoning, microbiologists would be much more inclined to use rapid tests than for individual cases. Notably, however, there was some variation between professions in what is deemed an 'outbreak'. GPs argue that the outbreak setting is important – for example, outbreaks in old people's homes are much more likely to cause serious illness than in secondary schools, where one can assume subjects are stronger. If there is a clear causal path of the outbreak, such as several infected people in the same restaurant, or perhaps in a non-food-borne source such as children visiting a petting farm, rapid tests may also be applied to reveal the scale of outbreak. For restaurant outbreaks, the CCDC present believed rapid detection methods may be useful in citing the source of infection – when waiting for culture results, the food source is most likely to be thrown out/finished before confirmation of infection can be given.

Interestingly, rapid detection as a tool to curbing secondary infection was not an overly important issue to any clinicians. The CCDC suggested that rapid tests may be useful for contacts with high risk of passing on infection, such as food handlers, children and people with learning difficulties, but for the general population participants did not express the opinion that rapid tests could limit secondary infection.

Timeliness issues

A key area of concern for many participants was organisational factors surrounding implementation of rapid tests. Participants suggested that rapidity could be reduced by variations at patient and service level; for example, patient non-compliance in returning a specimen to their surgery, or the time delay between the sample arriving in local hospital and larger laboratories receiving the sample. All participants agreed that very careful planning is required if such a testing strategy was to be developed, to ensure that any significant benefits were maintained in 'real-life' settings. Service implementation needs to ensure that the 'actual' time from when a patient seeks medical attention to when results are reported back is significantly faster – GPs were dubious that this would always happen unless a very well-organised delivery/reporting system was developed. There was agreement among all professional groups involved that food-borne outbreak control lacks definitive standards on the duration between confirmation of laboratory results and reporting to a clinician.

Choice of rapid tests

Antibody-based tests did not receive as much interest from participants as molecular methods.

This outcome was expected, as economic modelling showed that antibody test kits have almost equivalent costs to rtPCR kits, but with longer detection times. The CCDC suggested that he may choose the 'cheapest test' first and, once this organism is eliminated, would go on to use the more expensive methods. This view was not shared among other group members.

All microbiologists observed that molecular diagnosis is coming to the fore in routine pathological examination. One suggested that future developments may include a 'molecular suite' in which any pathogen, irrespective of taxonomy, may be detected within the same quantitative rtPCR laboratory, catering for all infectious diseases, such as sexually transmitted diseases, meningitis or respiratory infections. The potential benefits of multiplex testing for food-borne pathogens were also highlighted by another microbiologist, who suggested that if it were possible to test for multiple enteric pathogens in the one run, rtPCR tests would certainly be useful, but until these methods are validated, it remains most crucial to exclude *E. coli* O157.

With the suggestion that full-scale molecular detection is foreseeable within the next few decades, one microbiologist argued that PCR as a diagnostic adjunct is a very expensive strategy. If molecular diagnosis were to **fully replace** culture, certain measures could still be put in place to keep a record of epidemiological information or data on antibiotic resistance. Suggestions included using rtPCR for all samples, but additionally culturing the *n*th stool for reference purposes, or storing half of a stool sample for 24 hours (until PCR results are known), then culturing every positively identified specimen. It is important to note, however, that this opinion was not shared by all microbiologists. More research is required to assess professional opinion on the future directions of molecular diagnostics.

Ease of use issues

The NGT session highlighted problems around the technical skills required for microbiological investigations. One microbiologist discussed how, in recent years, it has become increasingly difficult to employ laboratory technicians with adequate skills to carry out tests. More technically demanding tests increase the likelihood of human error, such as missed diagnosis or incorrect reporting of a disease. Culture is relatively technically demanding. Antibody-based methods will rely on culture to a certain extent. In this microbiologist's opinion, quantitative rtPCR shows the greatest potential to be carried out by less skilled technical staff. The use of commercial kits may further cut down training required. If the pattern of skill shortages continues in the future, healthcare providers may find that simple-to-use, easy-to-interpret methods such as rtPCR are necessary to ensure diagnostic accuracy. The appropriate level of skill in using tests can only be achieved in two respects: through adequate training and also through adequate practice. In this respect, the larger the laboratory, that is, the higher the throughput, the more likely it will be that human error will be reduced.

Key recommendations from nominal group session

- More research is required to define clinical guidelines for which rapid tests should be advocated.
- Any strategy to introduce rapid tests would require meticulous organisation, particularly if a centralised nucleic acid-based testing laboratory was created.
- Ease of use is a very important consideration with regard to the current shortage of appropriately skilled laboratory staff. In this respect, commercially available rtPCR kits, which eliminate the need for technically demanding culture, may prove the most suitable.
- Most clinicians believe that excluding the most dangerous pathogens is the greatest concern, as opposed to those (such as *Campylobacter*) with the greatest prevalence.
- The most benefit could be derived from a single test which could be used to detect all the possible pathogens simultaneously – that is, multiplex PCR.

Chapter 9
Discussion

This research was conducted in an attempt to synthesise a mixed and heterogeneous body of evidence regarding the diagnostic accuracy and cost-effectiveness of rapid diagnostic tests for bacterial food poisoning. Through a systematic review of evidence, using meta-analytic techniques where appropriate, this report is the first comprehensive evaluation of study results to date produced in the UK.

Summary of diagnostic accuracy evidence

Food-borne infections

In general, very good test performance levels of sensitivity and specificity were observed with rapid test methods. From meta-analysis of the published evidence, nucleic acid-based tests appear to perform marginally better than the ProSpecT EIA kit for detection of *Campylobacter*. There are no statistically significant differences between rapid diagnostic accuracy values for any of the rapid test methods for *Salmonella* food poisoning, although adaptions to traditional methods, namely the use of chromogenic medium and automated minaturised bacterial identification systems, performed less well. From the available evidence on the diagnostic accuracy of commercial serological testing kits for *E. coli*, the VTEC Screen assay appears to be more sensitive than the Premier EHEC kit, with comparable high specificities. Nucleic acid-based tests outperformed serological kits for all EHEC strains.

Toxin-induced food poisoning

For toxin-induced food poisoning, this review could not identify a sufficient number of comparable studies to warrant meta-analysis. From a narrative review of methods identified, there is mixed evidence regarding the potential benefits of rapid tests. The reference tests in diagnostic comparisons for *C. perfringens*, *B. cereus* and *S. aureus* are most commonly the commercially available RPLA kits marketed by Oxoid. Several authors note that these may not be optimal methods. The limited evidence available suggests that nucleic acid-based detection methods perform well for *C. perfringens*, *Staphylococcal* enterotoxins and emetic strains of *B. cereus*, in most cases detecting more cases of illness than the current reference methods.

Methodological aspects of review

Search strategy

The pre-piloted search strategy identified a sizeable number of papers claiming to evaluate rapid tests; however, despite a variety of commands to locate evaluative studies, most studies were eliminated due to a lack of comparison with a reference test. Lack of specificity in electronic searches has been problematic in other reviews of this nature, and researchers have cited insufficient MESH indexing as a common problem with evaluations of diagnostic technology.[146] In addition, a small proportion of relevant studies subsequently found through handsearching reference lists and key journals were not identified in the standardised search, indicting sub-optimal sensitivity. These factors made searching for evaluations difficult and could mean that some papers are still to be discovered. It is also likely that some evaluations were presented as conference posters but the data from these are less easy to trace.

Inclusion criteria

A stringent study inclusion criteria was developed using findings from the initial piloted search strategy. This included the prerequisite that all included studies must compare the rapid test with a suitable reference standard. Subsequently, when it became clear that very few evaluations of rapid toxin detection methods have been published, this criteria was relaxed to allow for a more narrative discussion of developments in rapid diagnosis of *B. cereus*, *S. aureus* and *C. perfringens*. A systematic appraisal of the diagnostic accuracy of detection methods for these enterotoxin-producing pathogens was not possible due to a lack of evaluative studies on many occasions. There is a larger evidence base for food-borne bacterial infections, namely *Campylobacter*, *Salmonella* and *E. coli* O157.

Statistical issues

Availability of data for statistical analysis

Planned statistical analysis included attempts to model the variability due to other sources, such as meta-regression of patient characteristics, study quality and characteristics such as inclusion criteria and measurement of outcomes in the regression model. Due to the small number of studies in each category, this was not possible with the data currently available.

Lack of true gold standard

Although traditional culture is the natural reference test to use for comparative statistical analysis, on many occasions the rapid test may outperform culture. A major problem in most studies concerned the reporting of diagnostic accuracy in the absence of a true gold standard. The most accurate verification of disease status in a faecal specimen uses biochemical identification, an expensive and labour-intensive method which is usually only applied to those samples in which food-borne pathogens are presumptively detected by culture. Sensitive phenotypic reference tests were used by some authors, and in many cases these were collinear with novel rapid techniques to a greater extent than conventional culture. In other cases, variation in the choice of bacterial agar plating may have an impact on results recorded with culture. The application of different gold standard methods makes it difficult to assess whether evaluation results for different tests are truly comparable. Standard methods and a high-quality evaluation design need to be applied so that accurate comparisons can be made.

To complicate further the issue of 'true' identification of pathogenic isolates, it is possible that a significant proportion of microbes detected by nucleic acid-based methods may indeed be false positives.[50] In view of this possibility, stringent measures are needed when performing the PCR assay to avoid false positive reactions. Much improvement of methodology is needed, as through the use of an imperfect 'gold' standard,[147] studies in this area may be inherently statistically flawed. In reality, most diagnostic tests are evaluated using architecture subject to immense bias.

Assessment of heterogeneity

Limitations of the meta-analysis in this review relate to the lack of appropriate techniques for pooling the results of diagnostic accuracy studies. This review utilised the simplest techniques available to facilitate access to data. A systematic approach to the choice of meta-analysis method was taken based on the assessment of correlation between sensitivity and specificity, evaluation of heterogeneity with a χ^2 test and the I^2 statistic, graphical display of results and, where appropriate, SROC analysis. The SROC analysis technique used in this review includes the lack of an average estimate of sensitivity/specificity and the use of a regression model with the explanatory variable estimated with error. This makes the standard error estimated less suitable for assessing sources of heterogeneity. However, formal assessment of sources of heterogeneity was either done through subgroup analyses or not undertaken due to the limited number of studies in each subgroup. Alternative models were fitted in the statistical software SAS and the results were consistent with the reported results. There is no consensus as to the standard statistical approach to use in pooling diagnostic accuracy studies and investigating heterogeneity. The methods used in this report were based on the limitations of available data and the particular clinical question that the report addressed.

Quality of included studies

The methodological quality of studies included in this review varied considerably. The QUADAS tool,[236] was used to assess the quality of diagnostic accuracy studies included in this systematic review. Limitations of studies related to poor reporting of test results and the blinding of their interpretation. Although most of the selected studies provided enough data to estimate sensitivity, specificity and predictive values, subgroup analysis was not attempted by any authors, and reproducibility of the test was rarely formally assessed. For several studies, particularly those evaluating nucleic acid-based assays, the length of time between reference test and index test was extensive (up to 2 weeks in some instances), and analysts could not be reasonably sure that the pathogenic state of each specimen did not change between the two tests.

Variability of the results from one study to another demonstrates the necessity to standardise the different methodologies in the future. Several evaluations could benefit from design improvements. The applicability of methodological guidance such as the STARD (standards for reporting studies of diagnostic accuracy) initiative should be highlighted for future research.

Prospective versus retrospective studies

As food-borne pathogens are generally associated with very low (<5%) isolation rates, prospectively designed studies must include a large sample size to permit the identification of a sufficient number of true positive results. This may be prohibitively expensive for several test centres. Although the use of a retrospective ('banked samples') study design is not usually encouraged when evaluating new technologies, in this instance, the resources needed to conduct sufficiently large prospective studies are sizeable and may not be justifiable in view of opportunity costs. The studies reviewed here were mainly of a retrospective design, with the notable exception of *E. coli* diagnostic evaluations, which may reflect the additional weight given due to the severity of the illness that it causes. Only one prospective study was identified for toxin-inducing food-borne illness.[78] The use of banked reference samples may lead to the test performance appearing better than may have been experienced using fresh patient samples.

Laboratory proficiency

Laboratory proficiency may have a significant impact on the diagnostic error rate experienced with relatively complex microbiological testing methods. It is likely that research teams who have embarked on developing rapid detection methods would have adequate experience in the techniques to minimise any human error. Therefore, the sensitivity and specificity values reported in these studies may be higher than what could be experienced in practice in a smaller, less experienced laboratory setting. This factor further highlights the need for easy-to-use, highly reproducible tests. This requirement could most likely be met through the use of commercial kits, as opposed to in-house-designed assays.

Cost and cost-effectiveness

Costing issues

No previous economic evaluations of rapid diagnostic testing for food poisoning could be identified from the literature search. Only six of the clinical studies included in the review contained any information on comparative costs of implementing rapid tests, and owing to the wide variation in country and year of measurement for these cost data, reliability as an estimate of current UK costs was limited.

Economic analysis was further hindered by difficulties in valuing the costs involved in in-house-designed tests. Although the large proportion of included studies were novel assays which were as yet not commercially available, cost estimates for these methods are very difficult to quantify, and are likely to vary widely. Decision analysis was based on the assumption that commercially available test kits were used, as unit costs could be applied to these with greater confidence. Discrepancy may exist between the diagnostic accuracy reported with commercial kits and those from in-house-designed assays. In particular, for published literature on nucleic acid-based studies, in-house-designed methods were more widely reported than evaluations of commercial kits, and the accuracy of kits may vary from that reported and used in economic analysis. However, as PCR reported consistently high sensitivity and specificity values irrespective of assay format, and sensitivity analysis around diagnostic accuracy did not alter the findings to a large degree, it is unlikely that this will cause a serious miscalculation of correct diagnoses or alter subsequent cost per case detected values.

Cost-effectiveness issues

Robustness of cost-effectiveness results

The economic model was most sensitive to changes in isolation rates and less sensitive to small changes in costs or diagnostic accuracy. As community prevalence is likely to vary in different regions, policy makers must carefully assess the potential benefits gained from implementing rapid diagnostic tests in each setting.

Effectiveness data

Effectiveness data for the diagnostic tests came from the systematic review and meta-analysis. The results show that the cost per case detected is robust to changes in diagnostic accuracy. This is not surprising, considering the very high sensitivity and specificity values that the review of published studies identified. More revealing was the potential impact that suboptimal culture methods may result in. As the standard detection method for almost a century, it may have been taken for granted that bacterial culture produced 'perfect' accuracy values. However, with the advent of completely new detection techniques, this hypothesis is starting to be challenged. Additional cases detected of EHEC, *Salmonella* and *Campylobacter* could be substantial. This is an under-researched area, which could benefit greatly from a larger prospectively designed study to validate the emerging theory. However, culture is useful for other reasons, such as phage typing and sequencing of genes to identify related clusters and for antibiotic resistance testing.

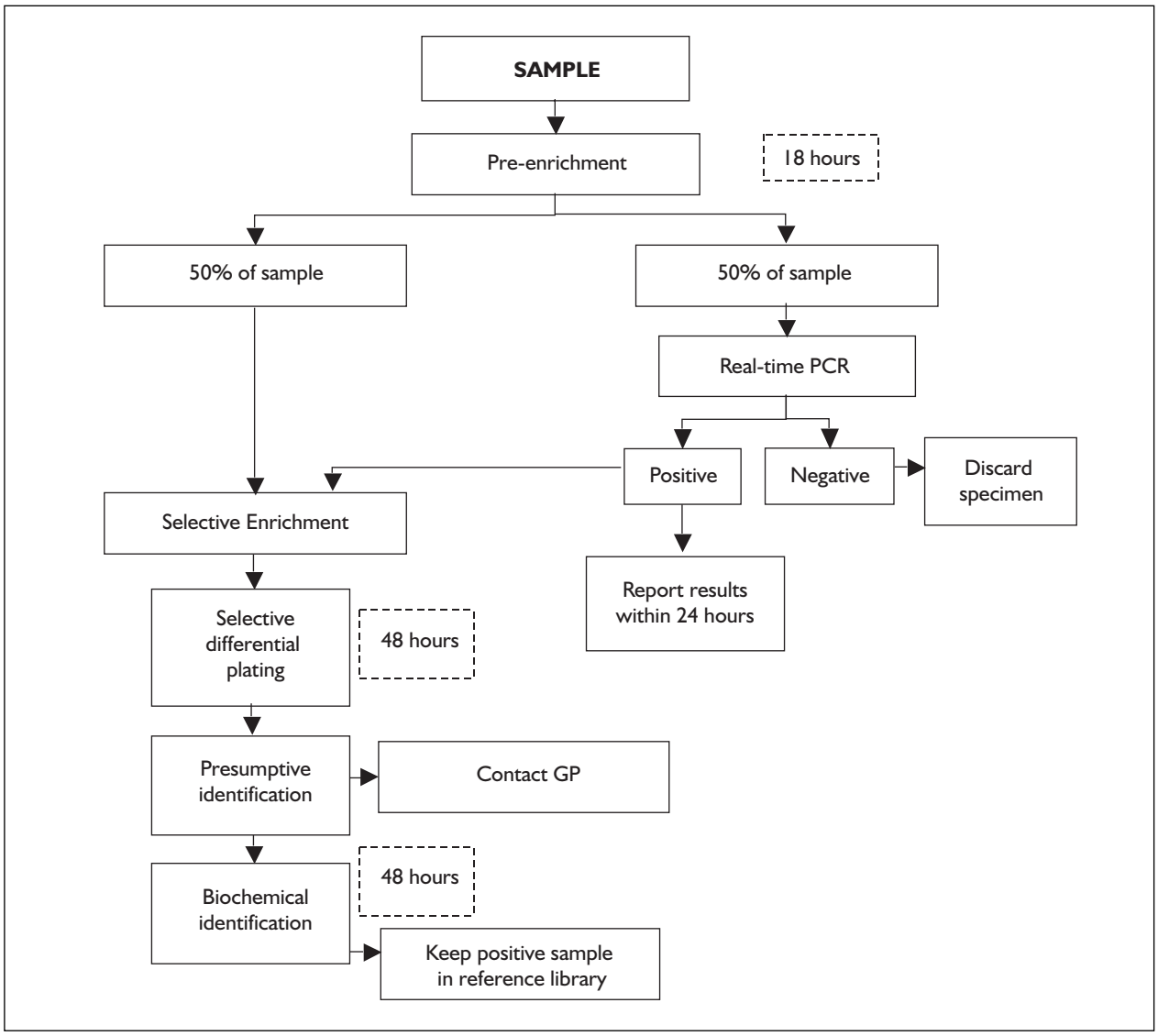


FIGURE 27 *Alternative methods for the rapid diagnosis of food poisoning*

Cost data

The availability of data to estimate confidently the cost-effectiveness of each method was limited. As tests become more widespread, unit prices are likely to decrease for all methods apart from culture, where they have been stable for decades. This analysis is likely to underestimate potential cost-effectiveness of rapid alternatives as a result.

Diagnostic adjunct versus future PCR replacement of culture

When modelling cost-effectiveness, costs to test all with PCR compared with testing all by traditional culture are equivalent, and when the diagnostic accuracy of culture methods is measured to be less than optimal, nucleic acid-based methods may actually be more cost-effective, despite the high capital costs involved. Given that PCR results are available quicker than for culture, and are equivalent in price, this suggests that PCR could be a viable replacement to culture in the future. One method of achieving this would be to keep the stool sample in storage media until the results of PCR detection are known, at which point positive samples can be cultured using standard methods and the isolate can be retained for reference purposes. An outline of this is shown in *Figure 27*.

Outbreak setting

There is insufficient evidence available to assess the impact of rapid diagnostic testing in an outbreak situation. The clinical significance of additional positive results or faster diagnosis remains unclear. The data presented in this report

do not conclusively suggest that faster diagnosis will lead to a better clinical outcome for the patient. Ideally, in the absence of primary studies, these potential effects could be assessed using a mathematical transmission model. However, in the absence of data to parameterise such a model, it was not possible to model mathematically how public health measures may change as a result of rapid diagnosis. The impact of more prompt interventions within outbreaks, brought forward through rapid testing, needs to be examined in future studies.

Issues for implementation

Acceptance and application of rapid methods in the analysis of bacterial food-borne pathogens have been slow. There are a number of factors to be considered when determining which testing system is most effective. These factors include the initial investment that is required, operating costs, technician time, range of organisms under investigation and the ability of the test to identify correctly food pathogens of interest.

Other testing, such as for viruses and parasites, may be performed on samples collected from cases or outbreaks of gastroenteritis. Viral gastroenteritis is mainly diagnosed using molecular methods and as such PCR equipment is already available, expertise in place and training already performed. Common procedures such as nucleic acid extraction will reduce the costs of tests for individual targets.

Improvements to speed apart from rapid laboratory technologies

To ensure faster reporting of results, it may be worthwhile investigating the means of collecting, storing and transporting specimens collected in the primary sector. UK laboratories currently lack a means of providing primary care providers with real-time electronic access to laboratory test results. Prompt delivery of results relies on compliance from patients, efficient administration and well-organised general practice. Electronic patient records and connecting for health may improve the timeliness of results in the future.

Seven days per week laboratory service

Another consideration regarding time management of laboratories involves the assumption that tests are carried out from Monday to Friday only. This in itself will leave a significant proportion (around 20%) of samples left over the weekend period, and therefore not treated 'rapidly'. Since 15–30% of results are delayed by 24–48 hours due to reduced laboratory staffing at weekends, the turnaround time could be improved if laboratories would offer rapid test services 7 days per week. However, a 7-day working week may impact the costing model in terms of overtime charges and additional recruitment needed to cope with longer opening hours. Further potential problems may occur if relevant core staff and medical specialists are not available at all times during laboratory working hours.

Funding issues

The initial financial investment required for introducing rapid methods may be a significant drawback, particularly for smaller laboratories. rtPCR requires a quantitative thermal cycler, costing in the region of £16,000–24,000. In addition, the cost of educating and training personnel to familiarise them with new technologies and procedures may be substantial. Whereas laboratories with a high volume of throughput may recover set-up costs quickly through the use of automated test methods, smaller laboratories may struggle to fund initial training and capital costs with their limited budgets.

Further microbiological issues

Drug sensitivity testing in culture versus rapid methods

Culture methods may hold an advantage over rapid tests when deducing the best possible antibiotic course to provide to the patient. With culture methods, once the organism has been identified, the growth can be further tested to determine its sensitivity to the antibiotic treatments that the clinician may want to administer. If a pathogen is susceptible to a particular antibiotic, then it may be used for therapy. Most standard medical textbooks[148] do not recommend the use of antibiotics for *Salmonella* and *Campylobacter* and there is evidence that the use of antibiotics in patients infected with *E. coli* increases the risk of HUS.[149] Restricting their use contributes to the control of antibiotic resistance. In the rapid assays evaluated in this review, sensitivity testing for different antibiotic treatment options was not integrated. However, as antibiotic resistance and toxin production are a consequence of gene expression in bacteria, specific PCR probes for resistance genes may be developed. Currently, if rapid assays are used, culture will still be needed for phage typing and gene sequencing. This will increase costs in terms of equipment and labour intensity.

Multiple infections and asymptomatic infections

The identification of multiple pathogens is a frequent occurrence in population-based studies of gastrointestinal illness.[150,151] As more sensitive assays are developed, it may become increasingly difficult to ascertain the true causes of disease. For example, the presence of PCR evidence of an organism does not always mean that organism is causing disease. The importance of additional positives identified by molecular methods requires further investigation to ascertain that they are true positives (using Amplicon sequencing RNA-based PCR). In addition, the clinical significance of the detection of multiple pathogens from a patient requires further investigation.

Conclusions and implications for healthcare and research

Emerging test methods

Multiplex PCR detection transferable to clinical samples should be considered, because the costs of reagents and the preparation time are less than in systems where several tubes of uniplex PCR are used. Faced with a sample which, for example, has come from a patient with non-organism-specific gastroenteric symptoms, it seems likely that there could be large savings in both costs (economic benefit) and time (clinical benefit) if a single test could determine which pathogen is causing the problem, rather than running a number of tests consecutively to eliminate causes one by one. One multiple pathogen rtPCR test method was evaluated in this review.[117]

This systematic review identified PCR as the key methodology in nucleic acid-based detection. However, developments in other molecular techniques are expected. The microarray method allows the simultaneous analysis of multiple genetic characteristics of target organisms in one experiment.[152,153] Unlike PCR methods, the glass microarray chips analyse several genes, and several sequences for each gene, simultaneously. Thus, identification is made on the basis of multiple genetic characteristics, which limits the probability of both false positive and false negative results. A Danish research team headed by Keramas[154,155] has developed a DNA microarray to detect *C. jejuni* and *C. coli* in environmental samples. Their results thus far indicate very high diagnostic accuracy, which is likely to be transferable to clinical samples. Further research in the area of DNA microarrays for *Campylobacter* is recommended. Many similar pieces of work are under way but no formal trials or assessments have yet been done, so the currently available data cannot inform the recommendations of this report. However, it does seem that considerable progress is being made and this type of approach should certainly be assessed again for its usefulness in the near future.

Rapid diagnostics symbolise a hugely expanding industry, with potentially faster and more accurate techniques continually being developed. This review identified evaluative studies up to September 2005; however, in subsequent months several novel assays were described, yet no independent evaluation was available during the period of this review. It is likely that systematic reviews such as this will need to be updated on a regular basis, to take account of the most up-to-date techniques and their accuracy compared with a suitable reference test.

Point of care testing

As yet, rapid diagnostic tests for enteric pathogens have not been miniaturised to allow point of care (POC) or mobile testing. However, POC diagnostics are advancing at a very fast rate, notably spurred on by the contemporary threat of biological warfare. This is likely to be a very significant advance in the future.[9,26,156] As POC tests evolve, their role in the diagnosis and management of patients with food-borne illness and their impact on clinical outcomes will become better defined. Even if POC tests for enteric pathogens do become available, health and safety concerns over handling and disposing of potentially infected stool samples may limit their uptake.

Clinical outcomes

Research on the improvement of patient outcomes from using rapid tests is notably lacking. As most evaluations have been laboratory based, the follow-up of patients (as opposed to just their specimens) is limited. If health authorities were to consider seriously implementing the rapid methods in practice, it would be very useful to ascertain the impact of these tests on clinical outcome.

An important finding from the NGT session was that GPs would only request a rapid test if there is clinical evidence to suggest that they improve patient outcomes. Although we may hypothesise that faster confirmation would lead to a greater knowledge base from which to treat patients and inform outbreak management, there is no specific evidence, from measuring patient outcomes, that this is the case.

There is some evidence from the USA that even slightly adapting testing strategies for enteric pathogens may lead to improved clinical outcomes and greater cost-effectiveness of microbiological investigation.[9,26,156] Positive results from the USA are contrasted to those from recent randomised controlled trials carried out in Europe. In The Netherlands, Bruins and colleagues[132] found no beneficial financial or clinical impact of shortened microbiological procedures for either hospitalised patients overall or for patients with bacteraemia. Although this is not directly linked with food poisoning, it does highlight the need for full investigation of outcomes prior to the adoption of new technology.

Ultimately, the decisions about the cost-effectiveness of strategies involving rapid diagnostic tests rely on information not only about diagnostic performance but also on subsequent costs and effects on treatment. Relatively robust data can be obtained on, for example, the incremental cost per accurate diagnosis. Such data are of limited value as a basis for decisions about allocative efficiency. This review has been unable to identify any data which consider long-term costs and consequences of faster diagnosis of food-borne illness. Without this information, it is unlikely that hospital providers can justify the additional costs incurred with using both culture and rapid detection techniques. The diagnostic accuracy and cost-effectiveness of rapid methods may warrant their implementation as full replacements to routine culture; however, it is doubtful that the technology to support such a policy change will be available in the near future.

Chapter 10
Conclusions

What the studies tell us

- Although several rapid tests have been developed to detect food pathogens in clinical samples, relatively few compare their results with an appropriate reference test.
- Significant research has been directed in the clinical and food industries to develop new rapid diagnostic tests for *Campylobacter*, *Salmonella* and *E. coli* O157. For tests which have been evaluated against an appropriate reference standard (usually culture), diagnostic accuracy is generally very high.
- There has been little research to date on detection methods for *Clostridium perfringens*, *staphylococcal* food poisoning or *Bacillus cereus*.

What the studies cannot tell us

- Inconclusive evidence exists regarding whether disparent results are caused by the lack of sensitivity of bacterial culture or lack of specificity of rapid test.
- There is inconclusive evidence on the implications of these rapid assays for clinical practice, as it is not possible to ascertain the significance of additional positive samples identified.

What we can infer from modelling

- There is sufficient epidemiological data to measure the cost and cost-effectiveness of rapid diagnostic testing compared with current methods using annualised estimates of laboratory activity.
- Although cost-effectiveness results appear robust to changes in assay costs or diagnostic accuracy, changes in the isolation rates for each pathogen will significantly alter the estimated cost per case detected.
- It is likely that the high capital costs of implementing NAA testing currently outweigh the potential benefits of molecular epidemiology and faster diagnosis. However, as rtPCR techniques continue to develop and costs fall, NAA testing may provide a suitable alternative to bacterial culture.

What we cannot infer from modelling

- No literature on the effectiveness of rapid diagnostic tests on the management of outbreaks was available to allow the assessment of cost-effectiveness in an outbreak setting.
- It is unclear from the reviewed studies or other published literature if rapid diagnosis of food poisoning would prevent deaths in a clinical setting. It is therefore impossible to estimate the impact that rapid tests may have on mortality rates. Any discussion on this aspect would have to rely on mathematical/transmission models of outbreaks and be purely hypothetical because of limited data. The mortality rate for food poisoning is very low already, and it was not possible to incorporate mortality factors into the economic model.

Implications for health policy makers

- Costly and time-consuming diagnostic tests are becoming increasingly difficult to justify, and not all stool specimens can or should be processed to screen for all potential pathogens. *Salmonella*, *Campylobacter* and *E. coli* O157 continue to be pathogens that most clinical laboratories routinely test for in all stool and faecal specimens. Less is known about the benefits of testing for *B. cereus*, *C. perfringens* and *S. aureus*.
- Evidence from studies shows that PCR is potentially very successful in identifying pathogens, possibly more than the number currently detected through culture. Therefore, the proportion of infectious intestinal disease due to food poisoning with **no** organism detected is likely to decrease from the current 40%. A limitation of PCR assays is that they do not detect toxin formation but rather toxin genes. Only antigen detection methods can detect the actual toxin protein.
- Cost-effectiveness modelling shows that all methods of microbiological detection will be expensive. However, using cost per case detected as an intermediate outcome, the costs of total replacement of bacterial culture with

rapid tests are not excessive when compared with current practice, as culture methods also have a high cost.
- The feasibility of conversion to rapid methods is dependent on localised considerations, including the community prevalence rates for specific pathogens, the skill base and subsequent training costs for laboratory staff, and spare capacity available to ensure adequate laboratory space for new equipment.
- Insistence on retaining bacterial culture methods will mean that costs for rapid methods will be artificially raised. It is unlikely that the implementation of rapid test methods, in addition to bacterial culture, will be efficient on a cost per case detected basis; however, more research is needed to assess the impact of timeliness of results on patient outcomes.
- Evidence of speed of diagnosis, rates of diagnostic error and costs of implementation should be considered simultaneously when deciding which strategy is optimal.

Implications for research

In order of priority these are as follows:

- Further research is needed on the effectiveness and cost-effectiveness of emerging rapid diagnostic procedures in food-borne infection, particularly those being developed to test for more than one organism at a time, such as multiplex PCR and DNA microarray technologies.
- The hypothesis that nucleic acid and serological assays can detect more true positive cases of food-borne infection than traditional culture methods should be validated through further research. Specifically, the under-reporting of non-O157 EHEC by SMAC should be addressed prospectively.
- Special attention should be paid to new methods for the detection of *Campylobacter* (currently the major cause of food-borne disease in the UK) because of the technical difficulties with cultural methods for this organism, and the high likelihood that newly emerging potentially pathogenic species such as *C. upsaliensis* and *C. concisus* are missed by current techniques.
- The role of rapid detection methods for toxin-induced food-borne illnesses, namely *C. perfringens*, *S. aureus* and *B. cereus*, remains unclear.
- Multiple pathogens will be detected in a significant number of samples if more sensitive methods are employed. Therefore, tests to determine the agent responsible for the symptoms will need to be developed.
- Due to the large volume of research currently emerging in the field of rapid diagnostics, systematic reviews such as this should be regularly updated to account for potentially faster, more accurate technologies being developed.
- Methodological and statistical issues are profoundly difficult to address when attempting to evaluate diagnostic tests in the absence of a true gold standard.
- The implications of rapid techniques, if more sensitive than current methods, will impact significantly on burden of disease studies.

Implications for practice

- Based on the evidence summarised, the reviewers do not feel it is appropriate to draw a firm conclusion either recommending the use of these assays or excluding their use in all cases. Further studies are needed.
- Economic feasibility of conversion to rapid methods is dependent on localised considerations, including the community prevalence rates for specific pathogens, the skill base and subsequent training costs for laboratory staff and spare capacity available to ensure adequate laboratory space for new equipment.
- Adoption of rapid tests in combination with routine culture is unlikely to be cost-effective; however, as the costs of rapid technologies decrease, total replacement with rapid technologies may be feasible.

Acknowledgements

We would like to thank all participants at the NGT analysis session, various manufacturers for access to cost data (including Oxoid, Applied Biosystems, Meridien and Remel) and microbiologists who responded to the survey of isolation rates. We are grateful to Ed Wilson and Richard Smith for advice on the economic analyses and Lee Hooper and Fujian Song for advice on systematic review methodology. We also wish to thank various national and international experts and authors who contributed by either checking our search strategy and search results or by providing additional data from their studies.

Contribution of authors

All authors saw and approved the manuscript. Lisa Irvine (Research Associate) and Ibrahim Abubakar (Senior Clinical Lecturer) conducted the analysis and wrote the systematic review of clinical papers. Clare Aldus (Research Scientist) and Gary Wyatt (Senior Research Scientist) conducted the analysis and wrote the systematic review of diagnostic developments in the food industry. The initial research proposal was suggested by Ibrahim Abubakar, Paul Hunter (Professor of Health Protection), and Michael Peck (Professor of Food Safety Microbiology). Paul Hunter contributed to the design, conduct and supervision of the writing of the report. Mike Peck contributed to the study design and supervised the synthesis of the results of food-based assays. Clare Aldus, Gary Wyatt and Silke Schelenz (Clinical Microbiologist) contributed to interpretation of microbiological data, and Lee Shepstone (Reader in Medical Statistics) provided support for statistical analysis. Amanda Howe (Professor of Primary Care) performed the nominal group analysis among health professionals, and Richard Fordham (Senior Lecturer: Health Economics) provided support for economic analysis. All authors contributed to the development of the study design and writing the report.

References

1. Mims CA. *Medical microbiology*. 3rd ed. Edinburgh: Elsevier Science; 2004.
2. Chief Medical Officer. *Definition of food poisoning*. London: Department of Health; 1992.
3. Food Standards Agency. *A report of the study of infectious intestinal disease in England*. London: Stationery Office; 2000.
4. Humphreys H, Keane CT, Hone R, Pomeroy H, Russell RJ, Arbuthnott JP, *et al*. Enterotoxin production by *Staphylococcus aureus* isolates from cases of septicaemia and from healthy carriers. *J Med Microbiol* 1989;**28**:163–72.
5. Hardy C, Bansal A, Lowes JA, George CF. *Salmonella* meningitis following treatment of enteritis with neomycin. *Postgrad Med J* 1984;**60**:284–6.
6. Bolton FJ, Aird H. Verocytotoxin-producing *Escherichia coli* O157: public health and microbiological significance. *Br J Biomed Sci* 1998; **55**:127–35.
7. Hannu T, Mattila L, Rautelin H, Pelkonen P, Lahdenne P, Siitonen A, *et al*. *Campylobacter*-triggered reactive arthritis: a population-based study. *Rheumatology (Oxford)* 2002;**41**:312–18.
8. Tam CC, Rodrigues LC, O'Brien SJ. Guillain–Barré syndrome associated with *Campylobacter jejuni* infection in England, 2000–2001. *Clin Infect Dis* 2003;**37**:307–10.
9. Murray PR, Baron EJ, American Society for Microbiology. *Manual of clinical microbiology*. 8th ed. Washington, DC: ASM Press; 2003.
10. Adak GK, Long SM, O'Brien SJ. Trends in indigenous foodborne disease and deaths, England and Wales: 1992 to 2000. *Gut* 2002;**51**:832–41.
11. Hawker J. *Communicable disease control handbook*. 2nd ed. Oxford: Blackwell Science; 2005.
12. Kulkarni SP, Lever S, Logan JM, Lawson AJ, Stanley J, Shafi MS. Detection of campylobacter species: a comparison of culture and polymerase chain reaction based methods. *J Clin Pathol* 2002;**55**:749–53.
13. Allos BM. Association between *Campylobacter* infection and Guillain–Barré syndrome. *J Infect Dis* 1997;**176** Suppl 2:S125–8.
14. Mandal BK, Ellis ME, Dunbar EM, Whale K. Double-blind placebo-controlled trial of erythromycin in the treatment of clinical *Campylobacter* infection. *J Antimicrob Chemother* 1984;**13**:619–23.
15. Linton D, Lawson AJ, Owen RJ, Stanley J. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. *J Clin Microbiol* 1997;**35**:2568–72.
16. World Health Organization, Food and Agriculture Organization of the United Nations. *Risk assessments of Salmonella in eggs and broiler chickens*. Geneva: World Health Organization; 2002.
17. Hill Gaston JS, Lillicrap MS. Arthritis associated with enteric infection. *Best Pract Res Clin Rheumatol* 2003;**17**:219–39.
18. Sirinavin S, Garner P. Antibiotics for treating *Salmonella* gut infections. *Cochrane Database Syst Rev* 2000;(2):CD001167.
19. Wistrom J, Norrby SR. Fluoroquinolones and bacterial enteritis, when and for whom? *J Antimicrob Chemother* 1995;**36**:23–39.
20. Cowden JM, Ahmed S, Donaghy M, Riley A. Epidemiological investigation of the central Scotland outbreak of *Escherichia coli* O157 infection, November to December 1996. *Epidemiol Infect* 2001;**126**:335–41.
21. Welinder-Olsson C, Stenqvist K, Badenfors M, Brandberg A, Floren K, Holm M, *et al*. EHEC outbreak among staff at a children's hospital – use of PCR for verocytotoxin detection and PFGE for epidemiological investigation. *Epidemiol Infect* 2004;**132**:43–9.
22. Pulz M, Matussek A, Monazahian M, Tittel A, Nikolic E, Hartmann M, *et al*. Comparison of a shiga toxin enzyme-linked immunosorbent assay and two types of PCR for detection of shiga toxin-producing *Escherichia coli* in human stool specimens. *J Clin Microbiol* 2003;**41**:4671–5.
23. Boyce TG, Swerdlow DL, Griffin PM. *Escherichia coli* O157:H7 and the hemolytic–uremic syndrome. *N Engl J Med* 1995;**333**:364–8.
24. Bopp DJ, Sauders BD, Waring AL, Ackelsberg J, Dumas N, Braun-Howland E, *et al*. Detection, isolation, and molecular subtyping of *Escherichia coli* O157:H7 and *Campylobacter jejuni* associated with a large waterborne outbreak. *J Clin Microbiol* 2003;**41**:174–80.
25. Kehl SC. Role of the laboratory in the diagnosis of enterohemorrhagic *Escherichia coli* infections. *J Clin Microbiol* 2002;**40**:2711–15.
26. Meer RR, Songer JG, Park DL. Human disease associated with *Clostridium perfringens* enterotoxin. *Rev Environ Contam Toxicol* 1997;**150**:75–94.

27. Dierick K, Van CE, Swiecicka I, Meyfroidt G, Devlieger H, Meulemans A, *et al*. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. *J Clin Microbiol* 2005;**43**:4277–9.

28. Buchanan RL, Schultz FJ. Comparison of the Tecra VIA kit, Oxoid BCET-RPLA kit and CHO cell culture assay for the detection of *Bacillus cereus* diarrhoeal enterotoxin. *Lett Appl Microbiol* 1994; **19**:353–6.

29. Letertre C, Perelle S, Dilasser F, Fach P. Detection and genotyping by real-time PCR of the staphylococcal enterotoxin genes sea to sej. *Mol Cell Probes* 2003;**17**:139–47.

30. Aguirre PM, Cacho JB, Folgueira L, Lopez M, Garcia J, Velasco AC. Rapid fluorescence method for screening *Salmonella* spp. from enteric differential agars. *J Clin Microbiol* 1990;**28**:148–9.

31. Dusch H, Altwegg M. Comparison of Rambach agar, SM-ID medium, and Hektoen Enteric agar for primary isolation of non-typhi *Salmonellae* from stool samples. *J Clin Microbiol* 1993;**31**:410–12.

32. Kehl KS, Havens P, Behnke CE, Acheson DW. Evaluation of the premier EHEC assay for detection of shiga toxin-producing *Escherichia coli*. *J Clin Microbiol* 1997;**35**:2051–4.

33. Mackenzie AM, Lebel P, Orrbine E, Rowe PC, Hyde L, Chan F, *et al*. Sensitivities and specificities of premier *E. coli* O157 and premier EHEC enzyme immunoassays for diagnosis of infection with verotoxin (shiga-like toxin)-producing *Escherichia coli*. The SYNSORB Pk Study investigators. *J Clin Microbiol* 1998;**36**:1608–11.

34. Begg CB. Biases in the assessment of diagnostic tests. *Stat Med* 1987;**6**:411–23.

35. Song F, Eastwood AJ, Gilbody S, Duley L, Sutton AJ. Publication and related biases. *Health Technol Assess* 2000;**4**(10).

36. Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, Poole F, *et al*. Evaluation of diagnostic tests for infectious diseases: general principles. *Nat Rev Microbiol* 2006;**4**(9 Suppl):S21–31.

37. Peeling RW, Smith PG, Bossuyt PM. A guide for diagnostic evaluations. *Nat Rev Microbiol* 2006;**4**(9 Suppl):S2–6.

38. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med* 1998;**17**:857–72.

39. Zhou XH, Qin G. Improved confidence intervals for the sensitivity at a fixed level of specificity of a continuous-scale diagnostic test. *Stat Med* 2005;**24**:465–77.

40. Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. *Stat Med* 2002; **21**:1525–37.

41. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327**:557–60.

42. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;**50**:1088–101.

43. Hayashino Y, Noguchi Y, Fukui T. Systematic evaluation and comparison of statistical tests for publication bias. *J Epidemiol* 2005;**15**:235–43.

44. Chart H, Perry NT, Willshaw GA, Cheasty T. Analysis of saliva for antibodies to the LPS of *Escherichia coli* O157 in patients with serum antibodies to *E. coli* O157 LPS. *J Med Microbiol* 2003;**52**(Pt 7):569–72.

45. Amar CF, East C, Maclure E, McLauchlin J, Jenkins C, Duncanson P, *et al*. Blinded application of microscopy, bacteriological culture, immunoassays and PCR to detect gastrointestinal pathogens from faecal samples of patients with community-acquired diarrhoea. *Eur J Clin Microbiol Infect Dis* 2004;**23**:529–34.

46. On SL. Identification methods for campylobacters, helicobacters, and related organisms. *Clin Microbiol Rev* 1996;**9**:405–22.

47. Lawson AJ, Shafi MS, Pathak K, Stanley J. Detection of campylobacter in gastroenteritis: comparison of direct PCR assay of faecal samples with selective culture. *Epidemiol Infect* 1998;**121**:547–53.

48. Lawson AJ, Logan JM, O'neill GL, Desai M, Stanley J. Large-scale survey of *Campylobacter* species in human gastroenteritis by PCR and PCR–enzyme-linked immunosorbent assay. *J Clin Microbiol* 1999;**37**:3860–4.

49. Best EL, Powell EJ, Swift C, Grant KA, Frost JA. Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. *FEMS Microbiol Lett* 2003;**229**:237–41.

50. Chiu CH, Ou JT. Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture-multiplex PCR combination assay. *J Clin Microbiol* 1996;**34**:2619–22.

51. Luk JM, Kongmuang U, Tsang RS, Lindberg AA. An enzyme-linked immunosorbent assay to detect PCR products of the rfbS gene from serogroup D salmonellae: a rapid screening prototype. *J Clin Microbiol* 1997;**35**:714–18.

52. Geers TA, Backes BA. Evaluation of two rapid methods to screen pathogens from stool specimens. *Am J Clin Pathol* 1989;**91**:327–30.

53. Imperatrice CA, Nachamkin I. Evaluation of the Vitek EPS enteric pathogen screen card for detecting *Salmonella*, *Shigella*, and *Yersinia* spp. *J Clin Microbiol* 1993;**31**:433–5.

54. Villasante PA, Agulla A, Merino FJ, Perez T, Ladron de GC, Velasco AC. Rapid automated

method for screening of enteric pathogens from stool specimens. *J Clin Microbiol* 1987;**25**:584–5.

55. Abdalla S, Vila J, Jimenez de Anta MT. Identification of *Salmonella* spp. with Rambach agar in conjunction with the 4-methylumbelliferyl caprylate (MUCAP) fluorescence test. *Br J Biomed Sci* 1994;**51**:5–8.

56. Paton AW, Paton JC. Multiplex PCR for direct detection of shiga toxigenic *Escherichia coli* strains producing the novel subtilase cytotoxin. *J Clin Microbiol* 2005;**43**:2944–7.

57. Welinder-Olsson C, Kjellin E, Badenfors M, Kaijser B. Improved microbiological techniques using the polymerase chain reaction and pulsed-field gel electrophoresis for diagnosis and follow-up of enterohaemorrhagic *Escherichia coli* infection. *Eur J Clin Microbiol Infect Dis* 2000;**19**:843–51.

58. Carroll KC, Adamson K, Korgenski K, Croft A, Hankemeier R, Daly J, *et al*. Comparison of a commercial reversed passive latex agglutination assay to an enzyme immunoassay for the detection of shiga toxin-producing *Escherichia coli*. *Eur J Clin Microbiol Infect Dis* 2003;**22**:689–92.

59. Klein EJ, Stapp JR, Clausen CR, Boster DR, Wells JG, Qin X, *et al*. Shiga toxin-producing *Escherichia coli* in children with diarrhea: a prospective point-of-care study. *J Pediatr* 2002;**141**:172–7.

60. Novicki TJ, Daly JA, Mottice SL, Carroll KC. Comparison of sorbitol MacConkey agar and a two-step method which utilizes enzyme-linked immunosorbent assay toxin testing and a chromogenic agar to detect and isolate enterohemorrhagic *Escherichia coli*. *J Clin Microbiol* 2000;**38**:547–51.

61. Vidal M, Carreno M, Vidal R, Arellano C, Solari V, Prado V. [Evaluation of molecular and immunoenzymatic assays for detecting enterohemorrhagic *E. coli* in food-borne outbreaks]. *Rev Med Chil* 2002;**130**:603–9.

62. Bettelheim KA. Development of a rapid method for the detection of verocytotoxin-producing *Escherichia coli* (VTEC). *Lett Appl Microbiol* 2001;**33**:31–5.

63. Beutin L, Zimmermann S, Gleier K. Evaluation of the VTEC-Screen "Seiken" test for detection of different types of shiga toxin (verotoxin)-producing *Escherichia coli* (STEC) in human stool samples. *Diagn Microbiol Infect Dis* 2002;**42**:1–8.

64. Chart H, Willshaw GA, Cheasty T. Evaluation of a reversed passive latex agglutination test for the detection of verocytotoxin (VT) expressed by strains of VT-producing *Escherichia coli*. *Lett Appl Microbiol* 2001;**32**:370–4.

65. Kai A, Obata H, Hatakeyama K, Igarashi H, Itoh T, Kudoh Y. [Evaluation of a latex agglutination method for detecting and characterizing verotoxin (VT) produced by *Escherichia coli*]. *Kansenshogaku Zasshi* 1997;**71**:248–54.

66. Gavin PJ, Peterson LR, Pasquariello AC, Blackburn J, Hamming MG, Kuo KJ, *et al*. Evaluation of performance and potential clinical impact of ProSpecT shiga toxin *Escherichia coli* microplate assay for detection of shiga toxin-producing *E. coli* in stool samples. *J Clin Microbiol* 2004;**42**:1652–6.

67. Mackenzie A, Orrbine E, Hyde L, Benoit M, Chan F, Park C, *et al*. Performance of the ImmunoCard STAT! *E. coli* O157:H7 test for detection of *Escherichia coli* O157:H7 in stools. *J Clin Microbiol* 2000;**38**:1866–8.

68. Stapp JR, Jelacic S, Yea YL, Klein EJ, Fischer M, Clausen CR, *et al*. Comparison of *Escherichia coli* O157:H7 antigen detection in stool and broth cultures to that in sorbitol–MacConkey agar stool cultures. *J Clin Microbiol* 2000;**38**:3404–6.

69. Karch H, Janetzki-Mittmann C, Aleksic S, Datz M. Isolation of enterohemorrhagic *Escherichia coli* O157 strains from patients with hemolytic–uremic syndrome by using immunomagnetic separation, DNA-based methods, and direct culture. *J Clin Microbiol* 1996;**34**:516–9.

70. Park CH, Kim HJ, Hixon DL, Bubert A. Evaluation of the Duopath verotoxin test for detection of shiga toxins in cultures of human stools. *J Clin Microbiol* 2003;**41**:2650–3.

71. Reischl U, Youssef MT, Kilwinski J, Lehn N, Zhang WL, Karch H, *et al*. Real-time fluorescence PCR assays for detection and characterization of shiga toxin, intimin, and enterohemolysin genes from shiga toxin-producing *Escherichia coli*. *J Clin Microbiol* 2002;**40**:2555–65.

72. Klein EJ, Stapp JR, Neill MA, Besser JM, Osterholm MT, Tarr PI. Shiga toxin antigen detection should not replace sorbitol MacConkey agar screening of stool specimens. *J Clin Microbiol* 2004;**42**:4416–17.

73. Park CH, Gates KM, Vandel NM, Hixon DL. Isolation of shiga-like toxin producing *Escherichia coli* (O157 and non-O157) in a community hospital. *Diagn Microbiol Infect Dis* 1996;**26**:69–72.

74. Gunzer F, Bohm H, Russmann H, Bitzan M, Aleksic S, Karch H. Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. *J Clin Microbiol* 1992;**30**:1807–10.

75. Brett MM, Rodhouse JC, Donovan TJ, Tebbutt GM, Hutchinson DN. Detection of *Clostridium perfringens* and its enterotoxin in cases of sporadic diarrhoea. *J Clin Pathol* 1992;**45**:609–11.

76. McCourt MT, Finlay DA, Laird C, Smyth JA, Bell C, Ball HJ. Sandwich ELISA detection of *Clostridium perfringens* cells and alpha-toxin from field cases of necrotic enteritis of poultry. *Vet Microbiol* 2005;**106**:259–64.

77. Wise MG, Siragusa GR. Quantitative detection of *Clostridium perfringens* in the broiler fowl

gastrointestinal tract by real-time PCR. *Appl Environ Microbiol* 2005;**71**:3911–16.

78. Forward LJ, Tompkins DS, Brett MM. Detection of *Clostridium difficile* cytotoxin and *Clostridium perfringens* enterotoxin in cases of diarrhoea in the community. *J Med Microbiol* 2003;**52**(Pt 9):753–7.
79. Berry PR, Rodhouse JC, Hughes S, Bartholomew BA, Gilbert RJ. Evaluation of ELISA, RPLA, and Vero cell assays for detecting *Clostridium perfringens* enterotoxin in faecal specimens. *J Clin Pathol* 1988;**41**:458–61.
80. Fontana C, Jezzi T, Testore GP, Dainelli B. Differentiation of *Clostridium difficile*, *Clostridium bifermentans*, *Clostridium sordellii*, and *Clostridium perfringens* from diarrheal stool by API ZYM and API LRA oxidase test. *Microbiol Immunol* 1995; **39**:231–5.
81. Mpamugo O, Donovan T, Brett MM. Enterotoxigenic *Clostridium perfringens* as a cause of sporadic cases of diarrhoea. *J Med Microbiol* 1995;**43**:442–5.
82. Bartholomew BA, Stringer MF, Watson GN, Gilbert RJ. Development and application of an enzyme linked immunosorbent assay for *Clostridium perfringens* type A enterotoxin. *J Clin Pathol* 1985;**38**:222–8.
83. Cudjoe KS, Thorsen LI, Sorensen T, Reseland J, Olsvik O, Granum PE. Detection of *Clostridium perfringens* type A enterotoxin in faecal and food samples using immunomagnetic separation (IMS)–ELISA. *Int J Food Microbiol* 1991;**12**:313–21.
84. Germani Y, Popoff MR, Begaud E, Guesdon JL. Competitive erythroimmunoassay for detecting *Clostridium perfringens* type A enterotoxin in stool specimens. *Res Microbiol* 1990;**141**:563–71.
85. McClane BA, Strouse RJ. Rapid detection of *Clostridium perfringens* type A enterotoxin by enzyme-linked immunosorbent assay. *J Clin Microbiol* 1984;**19**:112–15.
86. Saito M, Matsumoto M, Funabashi M. Detection of *Clostridium perfringens* enterotoxin gene by the polymerase chain reaction amplification procedure. *Int J Food Microbiol* 1992;**17**:47–55.
87. Kato N, Kato H. Human diseases caused by exotoxins produced by anaerobes and their rapid detection. *Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi* 1998;**9**:97–104.
88. Fach P, Popoff MR. Detection of enterotoxigenic *Clostridium perfringens* in food and fecal samples with a duplex PCR and the slide latex agglutination test. *Appl Environ Microbiol* 1997;**63**:4232–6.
89. Augustynowicz E, Gzyl A, Slusarczyk J. Molecular epidemiology survey of toxinogenic *Clostridium perfringens* strain types by multiplex PCR. *Scand J Infect Dis* 2000;**32**:637–41.
90. Augustynowicz E, Gzyl A, Slusarczyk J. Detection of enterotoxigenic *Clostridium perfringens* with a duplex PCR. *J Med Microbiol* 2002;**51**:169–72.
91. Tansuphasiri U, Wongsuvan G, Eampokalap B. PCR detection and prevalence of enterotoxin (cpe) gene in *Clostridium perfringens* isolated from diarrhea patients. *J Med Assoc Thai* 2002;**85**:624–33.
92. Tansuphasiri U, Chanyasanha C, Cheaochantanakij N. An enrichment broth culture–duplex PCR combination assay for the rapid detection of enterotoxigenic *Clostridium perfringens* in fecal specimens. *Southeast Asian J Trop Med Public Health* 2005;**36**:1229–38.
93. Beecher DJ, Wong AC. Identification and analysis of the antigens detected by two commercial *Bacillus cereus* diarrheal enterotoxin immunoassay kits. *Appl Environ Microbiol* 1994;**60**:4614–16.
94. Day TL, Tatani SR, Notermans S, Bennett RW. A comparison of ELISA and RPLA for detection of *Bacillus cereus* diarrheal enterotoxin. *J Appl Bacteriol* 1994;**77**:9–13.
95. Fletcher P, Logan NA. Improved cytotoxicity assay for *Bacillus cereus* diarrhoeal enterotoxin. *Lett Appl Microbiol* 1999;**28**:394–400.
96. Tan A, Heaton S, Farr L, Bates J. The use of *Bacillus* diarrhoeal enterotoxin (BDE) detection using an ELISA technique in the confirmation of the aetiology of *Bacillus*-mediated diarrhoea. *J Appl Microbiol* 1997;**82**:677–82.
97. Buchanan RL, Schultz FJ. Comparison of the Tecra VIA kit, Oxoid BCET-RPLA kit and CHO cell culture assay for the detection of *Bacillus cereus* diarrhoeal enterotoxin. *Lett Appl Microbiol* 1994;**19**;353–6.
98. Ehling-Schulz M, Fricker M, Scherer S. Identification of emetic toxin producing *Bacillus cereus* strains by a novel molecular assay. *FEMS Microbiol Lett* 2004;**232**:189–95.
99. Haggblom MM, Apetroaie C, Andersson MA, Salkinoja-Salonen MS. Quantitative analysis of cereulide, the emetic toxin of *Bacillus cereus*, produced under various conditions. *Appl Environ Microbiol* 2002;**68**:2479–83.
100. Finlay WJ, Logan NA, Sutherland AD. Semiautomated metabolic staining assay for Bacillus cereus emetic toxin. *Appl Environ Microbiol* 1999;**65**:1811–12.
101. Nakano S, Maeshima H, Matsumura A, Ohno K, Ueda S, Kuwabara Y, *et al*. A PCR assay based on a sequence-characterized amplified region marker for detection of emetic *Bacillus cereus*. *J Food Protect* 2004;**67**:1694–701.
102. Andersson MA, Mikkola R, Helin J, Andersson MC, Salkinoja-Salonen M. A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin and related depsipeptide ionophores. *Appl Environ Microbiol* 1998;**64**:1338–43.
103. Hall JA, Goulding JS, Bean NH, Tauxe RV, Hedberg CW. Epidemiologic profiling: evaluating foodborne outbreaks for which no pathogen was

isolated by routine laboratory testing: United States, 1982–9. *Epidemiol Infect* 2001;**127**:381–7.

104. Gonzalez V, Padilla E, Gimenez M, Vilaplana C, Perez A, Fernandez G, *et al*. Rapid diagnosis of *Staphylococcus aureus* bacteremia using *S. aureus* PNA FISH. *Eur J Clin Microbiol Infect Dis* 2004;**23**:396–8.
105. Chang YH, Chang TC, Kao EF, Chou C. Detection of protein A produced by *Staphylococcus aureus* with a fiber-optic-based biosensor. *Biosci Biotechnol Biochem* 1996;**60**:1571–4.
106. Klotz M, Opper S, Heeg K, Zimmermann S. Detection of *Staphylococcus aureus* enterotoxins A to D by real-time fluorescence PCR assay. *J Clin Microbiol* 2003;**41**:4683–7.
107. Sharma NK, Rees CE, Dodd CE. Development of a single-reaction multiplex PCR toxin typing assay for *Staphylococcus aureus* strains. *Appl Environ Microbiol* 2000;**66**:1347–53.
108. Tsen HY, Chen ML, Hsieh YM, Sheu SJ, Chen YL. *Bacillus cereus* group strains, their hemolysin BL activity, and their detection in foods using a 16S RNA and hemolysin BL gene-targeted multiplex polymerase chain reaction system. *J Food Protect* 2000;**63**:1496–502.
109. Monday SR, Bohach GA. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. *J Clin Microbiol* 1999;**37**:3411–14.
110. Guardati MC, Guzman CA, Piatti G, Pruzzo C. Rapid methods for identification of *Staphylococcus aureus* when both human and animal staphylo cocci are tested: comparison with a new immunoenzymatic assay. *J Clin Microbiol* 1993; **31**:1606–8.
111. Fukuda S, Tatsumi H, Igarashi H, Igimi S. Rapid detection of *Staphylococcus aureus* using bioluminescent enzyme immunoassay. *Lett Appl Microbiol* 2000;**31**:134–8.
112. Kijek TM, Rossi CA, Moss D, Parker RW, Henchal EA. Rapid and sensitive immunomagnetic–electrochemiluminescent detection of staphyloccocal enterotoxin B. *J Immunol Methods* 2000;**236**:9–17.
113. Araj GF, Atamian RB. Reliability of rapid kits for Staphylococcus aureus identification. *Lab Med* 1997;**28**:126–30.
114. Holliday MG, Ford M, Perry JD, Gould FK. Rapid identification of *Staphylococcus aureus* by using fluorescent staphylocoagulase assays. *J Clin Microbiol* 1999;**37**:1190–2.
115. Pennell DR, Rott-Petri JA, Kurzynski TA. Evaluation of three commercial agglutination tests for the identification of *Staphylococcus aureus*. *J Clin Microbiol* 1984;**20**:614–17.
116. van Griethuysen A, Buiting A, Goessens W, van Keulen P, Wintermans R, Kluytmans J. Multicenter evaluation of a modified protocol for the RAPIDEC staph system for direct identification of *Staphylococcus aureus* in blood cultures. *J Clin Microbiol* 1998;**36**:3707–9.
117. Iijima Y, Asako NT, Aihara M, Hayashi K. Improvement in the detection rate of diarrhoeagenic bacteria in human stool specimens by a rapid real-time PCR assay. *J Med Microbiol* 2004;**53**(Pt 7):617–22.
118. Fukushima H, Tsunomori Y, Seki R. Duplex real-time SYBR green PCR assays for detection of 17 species of food- or waterborne pathogens in stools. *J Clin Microbiol* 2003;**41**:5134–46.
119. Gilbert C, Winters D, O'Leary A, Slavik M. Development of a triplex PCR assay for the specific detection of *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli* O157:H7. *Mol Cell Probes* 2003;**17**:135–8.
120. Gilbert C, O'Leary A, Winters D, Slavik M. Development of a multiplex PCR assay for the specific detection of *Salmonella*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. *J Rapid Methods Autom Microbiol* 2003;**11**:61–74.
121. Chen J, Griffiths MW. Detection of *Salmonella* and simultaneous detection of *Salmonella* and shiga-like toxin-producing *Escherichia coli* using the magnetic capture hybridization polymerase chain reaction. *Lett Appl Microbiol* 2001;**32**:7–11.
122. Alarcon B, Garcia-Canas V, Cifuentes A, Gonzalez R, Aznar R. Simultaneous and sensitive detection of three foodborne pathogens by multiplex PCR, capillary gel electrophoresis, and laser-induced fluorescence. *J Agric Food Chem* 2004;**52**:7180–6.
123. Hong Y, Berrang ME, Liu T, Hofacre CL, Sanchez S, Wang L, *et al*. Rapid detection of *Campylobacter coli*, *C. jejuni* and *Salmonella enterica* on poultry carcasses by using PCR–enzyme-linked immunosorbent assay. *Appl Environ Microbiol* 2003;**69**:3492–9.
124. Chiang YC, Yang CY, Li C, Ho YC, Lin CK, Tsen HY. Identification of *Bacillus* spp., *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp. and *Vibrio* spp. with 16S ribosomal DNA-based oligonucleotide array hybridization. *Int J Food Microbiol* 2006;**107**:131–7.
125. Capps KL, McLaughlin EM, Murray AW, Aldus CF, Wyatt GM, Peck MW, *et al*. Validation of three rapid screening methods for detection of verotoxin-producing *Escherichia coli* in foods: interlaboratory study. *J AOAC Int* 2004;**87**:68–77.
126. Francois P, Bento M, Vaudaux P, Schrenzel J. Comparison of fluorescence and resonance light scattering for highly sensitive microarray detection of bacterial pathogens. *J Microbiol Methods* 2003;**55**:755–62.

127. Afflu L, Gyles CL. A comparison of procedures involving single step Salmonella, 1–2 test, and modified semisolid Rappaport–Vassiliadis medium for detection of *Salmonella* in ground beef. *Int J Food Microbiol* 1997;**37**:241–4.

128. Hanai K, Satake M, Nakanishi H, Venkateswaran K. Comparison of commercially available kits with standard methods for detection of *Salmonella* strains in foods. *Appl Environ Microbiol* 1997; **63**:775–8.

129. Hsu CF, Tsai TY, Pan TM. Use of the duplex TaqMan PCR system for detection of shiga-like toxin-producing *Escherichia coli* O157. *J Clin Microbiol* 2005;**43**:2668–73.

130. Chapman PA, Ellin M, Ashton R. Evaluation of a novel enrichment procedure for the isolation of *Escherichia coli* O157 from naturally contaminated raw beef, lamb and mixed meat products. *Food Microbiol* 2001;**18**:471–8.

131. Wagner JL. The feasibility of economic evaluation of diagnostic procedures. *Soc Sci Med* 1983;**17**:861–9.

132. Bruins M, Oord H, Bloembergen P, Wolfhagen M, Casparie A, Degener J, *et al*. Lack of effect of shorter turnaround time of microbiological procedures on clinical outcomes: a randomised controlled trial among hospitalised patients in The Netherlands. *Eur J Clin Microbiol Infect Dis* 2005;**24**:305–13.

133. de Wit MAS, Koopmans MPG, Kortbeek LM, Wannet WJB, Vinje J, van Leusden F, *et al*. Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. *Am J Epidemiol* 2001;**154**:666–74.

134. Roberts JA, Upton PA, Azene G. *Escherichia coli* O157:H7; an economic assessment of an outbreak. *J Public Health Med* 2000;**22**:99–107.

135. Fryback DG, Thornbury JR. The efficacy of diagnostic imaging. *Med Decis Making* 1991;**11**:88–94.

136. Roberts JA, Cumberland P, Sockett PN, Wheeler J, Rodrigues LC, Sethi D, *et al*. The study of infectious intestinal disease in England: socio-economic impact. *Epidemiol Infect* 2003;**130**:1–11.

137. Roberts CL, Mshar PA, Cartter ML, Hadler JL, Sosin DM, Hayes PS, *et al*. The role of heightened surveillance in an outbreak of *Escherichia coli* O157.H7. *Epidemiol Infect* 1995;**115**:447–54.

138. Buzby JC, Allos BM, Roberts T. The economic burden of *Campylobacter*-associated Guillain–Barré syndrome. *J Infect Dis* 1997;**176** Suppl 2:S192–7.

139. Buzby JC, Roberts T. Economic costs and trade impacts of microbial foodborne illness. *World Health Stat Q* 1997;**50**:57–66.

140. Office of the Chief Medical Officer, Welsh Assembly Government. *South Wales E. coli 0157 Outbreak – September 2005*. Cardiff: Welsh Assembly Government; 2006.

141. Page B, Petrich A, Stevens D, Luinstra K, Callery S, Gafni A, *et al*. Training costs and investment payback of implementing molecular diagnostics for identification of vancomycin resistant enterococci in a clinical microbiology laboratory. *Diagn Microbiol Infect Dis* 2002;**42**:91–7.

142. McGowan KL, Rubenstein MT. Use of a rapid latex agglutination test to detect *Salmonella* and *Shigella* antigens from Gram-negative enrichment broth. *Am J Clin Pathol* 1989;**92**:679–82.

143. Delbecq AL, Vandeven AH. Group process model for problem identification and program planning. *J Appl Behavl Sci* 1971;**7**:466–92.

144. Howe A. Patient-centred medicine through student-centred teaching: a student perspective on the key impacts of community-based learning in undergraduate medical education. *Med Educ* 2001;**35**:666–72.

145. Lloyd-Jones G, Fowell S, Bligh JG. The use of the nominal group technique as an evaluative tool in medical undergraduate education. *Med Educ* 1999;**33**:8–13.

146. Deville WL, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, *et al*. Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002;**2**:9.

147. Valenstein PN. Evaluating diagnostic tests with imperfect standards. *Am J Clin Pathol* 1990; **93**:252–8.

148. Clark ML, Kumar PJ. *Kumar & Clark clinical medicine*. 6th ed. Edinburgh: Elsevier Saunders; 2005.

149. Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI. The risk of the hemolytic–uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* 2000;**342**:1930–6.

150. Albert S, Weber B, Schafer V, Rosenthal P, Simonsohn M, Doerr HW. Six enteropathogens isolated from a case of acute gastroenteritis. *Infection* 1990;**18**:381–2.

151. Roman E, Wilhelmi I, Colomina J, Villar J, Cilleruelo ML, Nebreda V, *et al*. Acute viral gastroenteritis: proportion and clinical relevance of multiple infections in Spanish children. *J Med Microbiol* 2003;**52**(Pt 5):435–40.

152. Wilson WJ, Strout CL, DeSantis TZ, Stilwell JL, Carrano AV, Andersen GL. Sequence-specific identification of 18 pathogenic microorganisms using microarray technology. *Mol Cell Probes* 2002;**16**:119–27.

153. Warsen AE, Krug MJ, LaFrentz S, Stanek DR, Loge FJ, Call DR. Simultaneous discrimination between 15 fish pathogens by using 16S ribosomal DNA PCR and DNA microarrays. *Appl Environ Microbiol* 2004;**70**:4216–21.

154. Keramas G, Perozziello G, Geschke O, Christensen CB. Development of a multiplex microarray microsystem. *Lab Chip* 2004;**4**:152–8.

155. Keramas G, Bang DD, Lund M, Madsen M, Rasmussen SE, Bunkenborg H, *et al*. Development

of a sensitive DNA microarray suitable for rapid detection of *Campylobacter* spp. *Mol Cell Probes* 2003;**17**:187–96.

156. Barenfanger J, Drake C, Kacich G. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *J Clin Microbiol* 1999;**37**:1415–18.

157. Alvarez J, Sota M, Vivanco AB, Perales I, Cisterna R, Rementeria A, *et al*. Development of a multiplex PCR technique for detection and epidemiological typing of *Salmonella* in human clinical samples. *J Clin Microbiol* 2004 2004;**42**:1734–8.

158. Belanger SD, Boissinot M, Menard C, Picard FJ, Bergeron MG. Rapid detection of shiga toxin-producing bacteria in feces by multiplex PCR with molecular beacons on the smart cycler. *J Clin Microbiol* 2002;**40**:1436–40.

159. Bouvet PJ, Jeanjean S. Evaluation of two colored latex kits, the Wellcolex Colour Salmonella Test and the Wellcolex Colour Shigella Test, for serological grouping of *Salmonella* and *Shigella* species. *J Clin Microbiol* 1992;**30**:2184–6.

160. Collins E, Glennon M, Hanley S, Murray AM, Cormican M, Smith T, *et al*. Evaluation of a PCR/DNA probe colorimetric membrane assay for identification of *Campylobacter* spp. in human stool specimens. *J Clin Microbiol* 2001;**39**:4163–5.

161. Davis J, Cichon M, George H, Fontana J. An evaluation of the BAX(R) system for rapid screening of stool specimens for *Salmonella* species and *E. coli* O157:H7. *Abstracts of the General Meeting of the American Society for Microbiology* 2003;**103**:C-208.

162. Dediste A, Vandenberg O, Vlaes L, Ebraert A, Douat N, Bahwere P, *et al*. Evaluation of the ProSpecT microplate assay for detection of *Campylobacter*: a routine laboratory perspective. *Clin Microbiol Infect* 2003;**9**:1085–90.

163. Dylla BL, Vetter EA, Hughes JG, Cockerill FR, III. Evaluation of an immunoassay for direct detection of *Escherichia coli* O157 in stool specimens. *J Clin Microbiol* 1995;**33**:222–4.

164. Endtz HP, Ang CW, van den Braak N, Luijendijk A, Jacobs BC, de Man P, *et al*. Evaluation of a new commercial immunoassay for rapid detection of *Campylobacter jejuni* in stool samples. *Eur J Clin Microbiol Infect Dis* 2000;**19**:794–7.

165. Fach P, Guillou JP. Detection by *in vitro* amplification of the alpha-toxin (phospholipase C) gene from *Clostridium perfringens*. *J Appl Bacteriol* 1993;**74**:61–6.

166. Fedorko DP, Lehman SM, Yu PK, Germer JJ, Anhalt JP. Increased efficiency of stool culture for the detection of *Salmonella* and *Shigella*. *Diagn Microbiol Infect Dis* 1989;**12**:463–6.

167. Hansen W, Freney J. Comparative evaluation of a latex agglutination test for the detection and presumptive serogroup identification of *Salmonella* spp. *J Microbiol Methods* 1993;**17**:227–32.

168. Hindiyeh M, Jense S, Hohmann S, Benett H, Edwards C, Aldeen W, *et al*. Rapid detection of *Campylobacter jejuni* in stool specimens by an enzyme immunoassay and surveillance for *Campylobacter upsaliensis* in the greater Salt Lake City area. *J Clin Microbiol* 2000;**38**:3076–9.

169. Karmali MA, Petric M, Bielaszewska M. Evaluation of a microplate latex agglutination method (Verotox-F assay) for detecting and characterizing verotoxins (shiga toxins) in *Escherichia coli*. *J Clin Microbiol* 1999;**37**:396–9.

170. LaGier MJ, Joseph LA, Passaretti TV, Musser KA, Cirino NM. A real-time multiplexed PCR assay for rapid detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli*. *Mol Cell Probes* 2004;**18**:275–82.

171. Law D, Hamour AA, Acheson DW, Panigrahi H, Ganguli LA, Denning DW. Diagnosis of infections with shiga-like toxin-producing *Escherichia coli* by use of enzyme-linked immunosorbent assays for shiga-like toxins on cultured stool samples. *J Med Microbiol* 1994;**40**:241–5.

172. Maher M, Finnegan C, Collins E, Ward B, Carroll C, Cormican M. Evaluation of culture methods and a DNA probe-based PCR assay for detection of *Campylobacter* species in clinical specimens of feces. *J Clin Microbiol* 2003;**41**:2980–6.

173. Malorny B, Hoorfar J, Bunge C, Helmuth R. Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. *Appl Environ Microbiol* 2003;**69**:290–6.

174. Munoz P, Diaz MD, Cercenado E, Rodriguez-Creixems M, Berenguer J, Bouza E. Rapid screening of Salmonella species from stool cultures. *Am J Clin Pathol* 1993;**100**:404–6.

175. Metzler J, Nachamkin I. Evaluation of a latex agglutination test for the detection of *Salmonella* and *shigella* spp. by using broth enrichment. *J Clin Microbiol* 1988;**26**:2501–4.

176. Park CH, Vandel NM, Hixon DL. Rapid Immunoassay for detection of *Escherichia coli* O157 directly from stool specimens. *J Clin Microbiol* 1996;**34**:988–90.

177. Paton AW, Paton JC. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. *J Clin Microbiol* 1998;**36**:598–602.

178. Ramotar K, Waldhart B, Church D, Szumski R, Louie TJ. Direct detection of verotoxin-producing *Escherichia coli* in stool samples by PCR. *J Clin Microbiol* 1995;**33**:519–24.

179. Rohner P, Dharan S, Auckenthaler R. Evaluation of the Wellcolex Colour Salmonella Test for detection of *Salmonella* spp. in enrichment broths. *J Clin Microbiol* 1992;**30**:3274–6.

180. Schraft H, Griffiths MW. Specific oligonucleotide primers for detection of lecithinase-positive *Bacillus* spp. by PCR. *Appl Environ Microbiol* 1995; **61**:98–102.

181. Tolcin R, LaSalvia MM, Kirkley BA, Vetter EA, Cockerill FR III, Procop GW. Evaluation of the Alexon-trend ProSpecT *Campylobacter* microplate assay. *J Clin Microbiol* 2000;**38**:3853–5.

182. Bennett AR, MacPhee S, Betts RP. The isolation and detection of *Escherichia coli* O157 by use of immunomagnetic separation and immunoassay procedures. *Lett Appl Microbiol* 1996;**22**:237–43.

183. Chapman PA, Siddons CA. Evaluation of a commercial enzyme immunoassay (EHEC-Tek) for detecting *Escherichia coli* O157 in beef and beef products. *Food Microbiol* 1996;**13**:175–82.

184. Johnson JL, Brooke CL, Fritschel SJ. Comparison of the BAX for screening *E. coli* O157:H7 method with conventional methods for detection of extremely low levels of *Escherichia coli* O157:H7 in ground beef. *Appl Environ Microbiol* 1998;**64**:4390–5.

185. Sharma VK, an-Nystrom EA, Casey TA. Semi-automated fluorogenic PCR assays (TaqMan) for rapid detection of *Escherichia coli* O157:H7 and other shiga toxigenic *E. coli*. *Mol Cell Probes* 1999;**13**:291–302.

186. Sharma VK, Carlson SA. Simultaneous detection of *Salmonella* strains and *Escherichia coli* O157:H7 with fluorogenic PCR and single-enrichment-broth culture. *Appl Environ Microbiol* 2000;**66**:5472–6.

187. Chapman PA, Ellin M, Ashton R. A comparison of immunomagnetic separation and culture, Reveal(TM) and VIPTM for the detection of *E. coli* O157 in enrichment cultures of naturally-contaminated raw beef, lamb and mixed meat products. *Lett Appl Microbiol* 2001;**32**:171–5.

188. Chapman PA, Ellin M, Ashton R, Shafique W. Comparison of culture, PCR and immunoassays for detecting *Escherichia coli* O157 following enrichment culture and immunomagnetic separation performed on naturally contaminated raw meat products. *Int J Food Microbiol* 2001;**68**:11–20.

189. Chapman PA, Cudjoe KS. Evaluation of Beadretriever (TM), an automated system for concentration of *Escherichia coli* O157 from enrichment cultures by immunomagnetic separation. *J Rapid Methods Autom Microbiol* 2001;**9**:203–14.

190. Henry YM, Natrajan N, Lauer WF. Deter for detection of *Escherichia coli* O157 in raw ground beef and raw ground poultry. *J AOAC Int* 2001;**84**:752–60.

191. Kerr P, Finlay D, Thomson-Carter F, Ball HJ. A comparison of a monoclonal antibody-based sandwich ELISA and immunomagnetic bead selective enrichment for the detection of *Escherichia coli* O157 from bovine faeces. *J Appl Microbiol* 2001;**91**:933–6.

192. Manafi M, Kremsmaier B. Comparative evaluation of different chromogenic/fluorogenic media for detecting *Escherichia coli* O157:H7 in food. *Int J Food Microbiol* 2001;**71**:257–62.

193. Feldsine PT, Kerr DE, Leung SC, Lienau AH, Moser RF, Mui LA. Visual immunoprecipitate assay eight hour method for detection of enterohemorrhagic *Escherichia coli* O157:H7 in raw and cooked beef (modification of AOAC official method 996.09): collaborative study. *J AOAC Int* 2002;**85**:1029–36.

194. Sharma VK. Detection and quantitation of enterohemorrhagic *Escherichia coli* O157, O111, and O26 in beef and bovine feces by real-time polymerase chain reaction. *J Food Protect* 2002; **65**:1371–80.

195. Chapman PA, Ashton R. An evaluation of rapid methods for detecting *Escherichia coli* O157 on beef carcasses. *Int J Food Microbiol* 2003;**87**:279–85.

196. Abdulmawjood A, Bulte M, Roth S, Schonenbrucher H, Cook N, Heuvelink AE, *et al*. Development, validation, and standardization of polymerase chain reaction-based detection of *E. coli* O157. *J AOAC Int* 2004;**87**:596–603.

197. Bono JL, Keen JE, Miller LC, Fox JM, Chitko-McKown CG, Heaton MP, *et al*. Evaluation of a real-time PCR kit for detecting *Escherichia coli* O157 in bovine fecal samples. *Appl Environ Microbiol* 2004;**70**:1855–7.

198. Capps KL, McLaughlin EM, Murray AWA, Aldus CF, Wyatt GM, Peck MW, *et al*. Validation of three rapid screening methods for detection of verotoxin-producing *Escherichia coli* in foods: interlaboratory study. *J AOAC Int* 2004;**87**:68–77.

199. Arthur TM, Bosilevac JM, Nou XW, Koohmaraie M. Evaluation of culture- and PCR-based detection methods for *Escherichia coli* O157:H7 in inoculated ground beef. *J Food Protect* 2005;**68**:1566–74.

200. Bettelheim KA. Reliability of O157:H7 ID agar (O157 H7 ID-F) for the detection and isolation of verocytotoxigenic strains of *Escherichia coli* belonging to serogroup O157. *J Appl Microbiol* 2005;**99**:408–10.

201. Ellingson JLE, Koziczkowski JJ, Anderson JL, Carlson SA, Sharma VK. Rapid PCR detection of enterohemorrhagic *Escherichia coli* (EHEC) in bovine food products and feces. *Mol Cell Probes* 2005;**19**:213–17.

202. Winters DK, Slavik MF. Evaluation of a PCR based assay for specific detection of *campylobacter jejuni* in chicken washes. *Mol Cell Probes* 1995;**9**:307–10.

203. Lilja L, Hanninen ML. Evaluation of a commercial automated ELISA and PCR-method for rapid detection and identification of *Campylobacter jejuni* and *C. coli* in poultry products. *Food Microbiol* 2001;**18**:205–9.

204. Bang DD, Wedderkopp A, Pedersen K, Madsen M. Rapid PCR using nested primers of the 16S rRNA and the hippuricase (hipO) genes to detect *Campylobacter jejuni* and *Campylobacter coli* in

environmental samples. *Mol Cell Probes* 2002;**16**:359–69.

205. Sails AD, Bolton FJ, Fox AJ, Wareing DRA, Greenway DLA. Detection of *Campylobacter jejuni* and *Campylobacter coli* in environmental waters by PCR enzyme-linked immunosorbent assay. *Appl Environ Microbiol* 2002;**68**:1319–24.
206. Manfreda G, De Cesare A, Bondioli V, Franchini A. Comparison of the BAX (R) System with a multiplex PCR method for simultaneous detection and identification of *Campylobacter jejuni* and *Campylobacter coli* in environmental samples. *Int J Food Microbiol* 2003;**87**:271–8.
207. Sails AD, Fox AJ, Bolton FJ, Wareing DR, Greenway DL. A real-time PCR assay for the detection of *Campylobacter jejuni* in foods after enrichment culture. *Appl Environ Microbiol* 2003;**69**:1383–90.
208. Mateo E, Carcamo J, Urquijo M, Perales I, Fernandez-Astorga A. Evaluation of a PCR assay for the detection and identification of *Campylobacter jejuni* and *Campylobacter coli* in retail poultry products. *Res Microbiol* 2005;**156**:568–74.
209. Ng SP, Tsui CO, Roberts D, Chau PY, Ng MH. Detection and serogroup differentiation of *Salmonella* spp. in food within 30 hours by enrichment-immunoassay with a T6 monoclonal antibody capture enzyme-linked immunosorbent assay. *Appl Environ Microbiol* 1996;**62**:2294–302.
210. Kimura B, Kawasaki S, Fujii T, Kusunoki J, Itoh T, Flood SJ. Evaluation of TaqMan PCR assay for detecting *Salmonella* in raw meat and shrimp. *J Food Protect* 1999;**62**:329–35.
211. Richter J, Becker H, Martlbauer E. Improvement in *Salmonella* detection in milk and dairy products: comparison between the ISO method and the Oxoid SPRINT *Salmonella* test. *Lett Appl Microbiol* 2000;**31**:443–8.
212. Walker RL, Kinde H, Anderson RJ, Brown AE. Comparison of VIDAS enzyme-linked fluorescent immunoassay using Moore swab sampling and conventional culture method for *Salmonella* detection in bulk tank milk and in-line milk filters in California dairies. *Int J Food Microbiol* 2001; **67**:123–9.
213. Fang Q, Brockmann S, Botzenhart K, Wiedenmann A. Improved detection of *Salmonella* spp. in foods by fluorescent *in situ* hybridization with 23S rRNA probes: a comparison with conventional culture methods. *J Food Protect* 2003;**66**:723–31.
214. Fratamico PM. Comparison of culture, polymerase chain reaction (PCR), TaqMan *Salmonella*, and Transia Card *Salmonella* assays for detection of *Salmonella* spp. in naturally-contaminated ground chicken, ground turkey, and ground beef. *Mol Cell Probes* 2003;**17**:215–21.
215. Silbernagel KM, Jechorek RP, Carver CN, Horter BL, Lindberg KG. 3M (TM) Petrifilm (TM) Staph Express Count plate method for the enumeration of *Staphylococcus aureus* in selected types of processed and prepared foods: collaborative study. *J AOAC Int* 2003;**86**:954–62.
216. Van Kessel JS, Karns JS, Perdue ML. Using a portable real-time PCR assay to detect *Salmonella* in raw milk. *J Food Protect* 2003;**66**:1762–7.
217. Briggs J, Dailianis A, Hughes D, Garthwaite I. Validation study to demonstrate the equivalence of a minor modification (TECRA (R) ULTIMA (TM) Protocol) to AOAC method 998.09 (TECRA (R) *Salmonella* Visual Immunoassay) with the cultural reference method. *J AOAC Int* 2004;**87**:374–9.
218. Croci L, Delibato E, Volpe G, De Medici D, Palleschi G. Comparison of PCR, electrochemical enzyme-linked immunosorbent assays, and the standard culture method for detecting *Salmonella* in meat products. *Appl Environ Microbiol* 2004; **70**:1393–6.
219. Ellingson JLE, Anderson JL, Carlson SA, Sharma VK. Twelve hour real-time PCR technique for the sensitive and specific detection of *Salmonella* in raw and ready-to-eat meat products. *Mol Cell Probes* 2004;**18**:51–7.
220. Lynch MJ, Leon-Velarde CG, McEwen S, Odumeru JA. Evaluation of an automated immunomagnetic separation method for the rapid detection of *Salmonella* species in poultry environmental samples. *J Microbiol Methods* 2004;**58**:285–8.
221. Malorny B, Cook N, D'Agostino M, De Medici D, Croci L, Abdulmawjood, A *et al*. Multicenter validation of PCR-based method for detection of *Salmonella* in chicken and pig samples. *J AOAC Int* 2004;**87**:861–6.
222. McMahon WA, Schultz AM, Johnson RL. Evaluation of VIDAS (R) immuno-concentration *Salmonella* (ICS) plus selective plate method (Hektoen Enteric, bismuth sulfite, *Salmonella* identification) for detection of *Salmonella* in selected foods (method modification 2001.07): collaborative study. *J AOAC Int* 2004;**87**:380–4.
223. McMahon WA, Schultz AM, Johnson RL. Evaluation of VIDAS *Salmonella* (SLM) immunoassay method with Rappaport–Vassiliadis (RV) medium for detection of *Salmonella* in foods: collaborative study. *J AOAC Int* 2004;**87**:175A.
224. Perelle S, Dilasser F, Malorny B, Grout J, Hoorfar J, Fach P. Comparison of PCR–ELISA and LightCycler real-time PCR assays for detecting *Salmonella* spp. in milk and meat samples. *Mol Cell Probes* 2004;**18**:409–20.
225. Touron A, Berthe T, Pawlak B, Petit F. Detection of *Salmonella* in environmental water and sediment by a nested-multiplex polymerase chain reaction assay. *Res Microbiol* 2005;**156**:541–53.
226. Jackson SG. Rapid screening-test for enterotoxin-producing *Bacillus cereus*. *J Clin Microbiol* 1993;**31**:972–4.

227. Mantynen V, Lindstrom K. A rapid PCR-based DNA test for enterotoxic *Bacillus cereus*. *Appl Environ Microbiol* 1998;**64**:1634–9.

228. Chen CH, Ding HC, Chang TC. Rapid identification of *Bacillus cereus* based on the detection of a 28.5-kilodalton cell surface antigen. *J Food Protect* 2001;**64**:348–54.

229. Peng H, Ford V, Frampton EW, Restaino L, Shelef LA, Spitz H. Isolation and enumeration of *Bacillus cereus* from foods on a novel chromogenic plating medium. *Food Microbiol* 2001;**18**:231–8.

230. Chen CH, Ding HC. A colony blot immunoassay for the rapid identification of *Bacillus cereus*. *J Food Protect* 2004;**67**:387–90.

231. Mach PA, Lindberg KG, Lund ME. Evaluation of a dry, rehydratable film method for rapid enumeration of *Staphylococcus aureus*. *J AOAC Int* 2000;**83**:1096–107.

232. Atanassova V, Meindl A, Ring C. Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw pork and uncooked smoked ham – a comparison of classical culturing detection and RFLP–PCR. *Int J Food Microbiol* 2001;**68**:105–13.

233. Silbernagel KM, Lindberg KG. Petrifilm(TM) rapid *S. aureus* count plate method for rapid enumeration of *Staphylococcus aureus* in selected foods: collaborative study. *J AOAC Int* 2001;**84**:1431–43.

234. Ingham SC, Becker KL, Fanslau MA. Comparison of the Baird–Parker agar and 3M Petrifilm Staph Express count plate methods for enumeration of *Staphylococcus aureus* in naturally and artificially contaminated foods. *J Food Protect* 2003;**66**:2151–5.

235. Zschöck M, Nesseler A, Sudarwanto I. Evaluation of six commercial identification kits for the identification of *Staphylococcus aureus* isolated from bovine mastitis. *J Appl Microbiol* 2005;**98**:450–5.

236. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003;**3**:25.

237. Titball RW, Hunter SE, Martin KL, Morris BC, Shuttleworth AD, Rubidge T, *et al*. Molecular cloning and nucleotide sequence of the alpha-toxin (phospholipase C) of *Clostridium perfringens*. *Infect Immun* 1989;**57**:367–76.

238. Harmon SM, Kautter DA. Collaborative study of alpha-toxin method for estimating population levels of *Clostridium perfringens* in food. *Journal of the Association of Official Analytical Chemists* 1974;**57**:91–4.

239. Brynestad S, Granum PE. *Clostridium perfringens* and foodborne infections. *Int J Food Microbiol* 2002;**74**:195–202.

240. Lin Z, Kurazono H, Yamasaki S, Takeda Y. Detection of various variant verotoxin genes in *Escherichia coli* by polymerase chain reaction. *Microbiol Immunol* 1993;**37**:543–8.

241. Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA. Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. *Nature* 1986;**324**(6093):163–6.

242. Farrell. Prospective validation of real time PCR detection of Salmonella in culture from clinical stool specimens. *Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy* 2003.

243. Yamasaki S, Lin Z, Shirai H, Terai A, Oku Y, Ito H, *et al*. Typing of verotoxins by DNA colony hybridization with poly- and oligonucleotide probes, a bead-enzyme-linked immunosorbent assay, and polymerase chain reaction. *Microbiol Immunol* 1996;**40**:345–52.

244. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. *Infect Immun* 1977;**8**:775–9.

245. Karmali MA. Infection by verocytotoxin-producing *Escherichia coli*. *Clin Microbiol Rev* 1989;**2**:15–38.

246. Law D, Ganguli LA, Donohue-Rolfe A, Acheson DW. Detection by ELISA of low numbers of Shiga-like toxin-producing *Escherichia coli* in mixed cultures after growth in the presence of mitomycin C. *J Med Microbiol* 1992;**36**:198–202.

247. Rahn K, De Grandis SA, Clarke RC, McEwen SA, Galan JE, Ginocchio C *et al*. Amplification of an invA gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of Salmonella. *Mol Cell Probes* 1992;**6**:271–9.

248. Soumet C, Ermel G, Salvat G, Colin P. Detection of Salmonella spp. in food products by polymerase chain reaction and hybridization assay in microplate format. *Lett Appl Microbiol* 1997;**24**:113–16.

249. Park CE, Akhtar M, Rayman MK. Evaluation of a commercial enzyme-immunoassay kit (Ridascreen) for detection of staphylococcal enterotoxins a, b, c, d, and e in foods. *Appl Environ Microbiol* 1994;**60**:677–81.

250. Burriel AR. A comparison of the micromethods API Staph and ID32 STAPH in the identification of coagulase-negative staphylococci isolated from sheep. *J Microbiol Methods* 1997;**28**:109–12.

251. de Buyser ML, Audinet N, Delbart MO, Maire M, Francoise F. Comparison of selective culture media to enumerate coagulase-positive staphylococci in cheeses made from raw milk. *Food Microbiol* 1998;**15**:339–46.

252. de Buyser ML, Lombard B, Schulten SM, In't Veld PH, Scotter SL, Rollier P *et al*. Validation of EN ISO standard methods 6888 Part 1 and Part 2: 1999–enumeration of coagulase-positive staphylococci in foods. *Int J Food Microbiol* 2003;**83**:185–94.

Appendix 1

Search strategy

The following sources were searched for studies relating to diagnostic accuracy for each of the organisms being evaluated. A range of free text and subject headings was used, as appropriate. Details of the search strategies are given below.

Initially the search strategy was not limited by date. However, at an early stage it became evident that developments in rapid testing for food poisoning only really came to the fore since the early 1990s. It was therefore felt that a date restriction could be applied relatively arbitrarily at 1985, as diagnostic test methods are rapidly evolving and it was thought that papers before this date would not be relevant to current practices in microbiology. No language restrictions were applied to the searches.

MEDLINE (1985 to April week 4 2005)

1. exp "sensitivity and specificity" or "evaluation studies".sh or ("false positive" or "false negative" or "predictive value").ti,ab,sh.
2. exp diagnosis/ or (detect$ or diagnos$).ti,ab,sh.
3. 1 or 2
4. time factors.sh. or (rapid$ or fast$ or quick$).ti,ab,sh.
5. 3 and 4
6. *Salmonella*.mp
7. *Campylobacter jejuni*.mp
8. *Escherichia coli* O157.mp or enterohaemorrhagic.ti,ab,sh
9. C*lostridium perfringens*.mp
10. *Staphylococcus aureus*.mp
11. (resistan$ or Methicillan or Nosocomial).ti,ab,sh
12. 10 not 11
13. *Bacillus cereus*.mp
14. 6 or 7 or 8 or 9 or 12 or 13
15. limit 14 to humans
16. 4 and 15

EMBASE (1985 to 2005 week 17) and CINAHL (1985 to April week 4 2005)

1. exp "sensitivity and specificity" or "evaluation studies".sh or ("false positive" or "false negative" or "predictive value").ti,ab,sh
2. exp diagnosis/ or (detect$ or diagnos$).ti,ab,sh.
3. 1 or 2
4. (rapid$ or fast$ or quick$).ti,ab,sh
5. 3 and 4
6. *Salmonella/*
7. *Campylobacter jejuni/*
8. *Escherichia coli/*
9. *Clostridium perfringens/*
10. *Staphylococcus aureus/*
11. (resistan$ or Nosocomial).ti,ab,sh
12. 10 not 11
13. *Bacillus cereus/*
14. 5 or 6 or 7 or 8 or 11 or 12
15. limit 14 to humans,
16. 4 and 15

Web of Science (1945 to 2005)

1. TS=sensitivity
2. TS=compare
3. TS=rapid
4. TS=detect
5. (#1 or #2) and #3 and #4
6. TS=Food Poisoning
7. TS=*Salmonella*
8. TS=*Campylobacter jejuni*
9. TS=*Escherichia coli*
10. TS=*E. coli*
11. TS=*Clostridium perfringens*
12. TS=*Bacillus cereus*
13. TS=*Staphylococcus aureus*
14. TS=MRSA
15. TS=Methicillin-Resistant *Staphylococcus aureus*
16. (#6 or #7 or #8 or #9 or #10 or #11 or #12 or #13) not (#14 or #15)
17. 5 and 16

Abstracts selected by search strategy

- MEDLINE – 1279
- EMBASE – 793
- BIOSIS – 17
- Web of Science – 32
- CINAHL – 25

Appendix 2

Data extraction table

General Information				
ID for reviewer			Source (Journal, Conference)	
Author				
Article Title				
Year of Publication			Year of Study	
Country of Study			Language	
Source of Funding			Corresponding Author	
Patient Characteristics			**Exposure: Organism(s)**	
Age			**Gastrointestinal infection**	
Co-morbidity			*Salmonella*	O
Patient Selection			*Campylobacter jejuni*	O
Test Setting			*Escherichia coli* O157	O
			Toxin Induced Food Poisoning	
Study Design			*Staphylococcus aureus*	O
			Bacillus cereus	O
Blinded			*Clostridium perfringens*	O
Study Design			**Other (Specify)**	
Feasible in Local Lab?			**Analyte/Primer (if applic)**	
			Amplification size (PCR)	
Detection: Description of Test Method				
Type of Test	Nucleic Acid	O		
	Immunoassay	O		
	Agglutination	O		
Pre-enrichment required?	Yes/no			
Name			Manufacturer	
Qualitative/Quantitative			Outcome Measure	
Threshold Sensitivity				
records indeterminate results			Positive/Negative control provided	
Comparison: Description of Reference Method				
Specific Test Details				
Name			Manufacturer	
Outcome Measure			Full or partial validation?	
Measured Independently?	Yes/no		Appropriate comparator?	
Interpreted Blindly?	Yes/no			
Accuracy				
Calculated:			**Recorded:**	
True Positives			Reproducibility	
True Negatives			Repeatability	
False Positives			Sensitivity	
False Negatives			Specificity	
			Positive Predictive Value	
Number of Test Materials			Negative Predictive Value	
Additional notes on concordance				
Statistical tests used				
Detection Times				
Rapid Test			**Reference Case**	
Pre-enrichment: time and reagents			Hands-on Time	
Incubation time			Total Time to Results	
Amplification time (if PCR)				
Hands-on time				
Total time to results			Difference in reporting times	
Additional Information				
Notes on costs or cost-effectiveness				
Authors' conclusions				
Original Search Strategy	Yes/no		Likely to include?	

Appendix 3

Study characteristics for clinical studies

Authors (year):	Abdalla *et al*. (1994)[55]
Journal:	*British Journal of Biomedical Science*
Title:	Identification of *Salmonella* spp. with Rambach agar in conjunction with the 4-methylumbelliferyl caprylate (MUCAP) fluorescence test
Notes:	
Setting:	Hospital laboratory, Spain **Design:** Prospective
Sample size:	50 propylene glycol and β-galactosidase-negative strains
Target organism:	*Salmonella* sp.
Rapid test	**Methods**
MUCAP	Based on a rapid detection of C8 esterase enzyme by using a fluorogenic 4-methylumbelliferone-conjugated substrate. Test performed with Rambach cultured strains as described by manufacturer
Reference test	**Methods**
Standard culture	Cultured on Rambach agar using standard methods

Reported accuracy data:

Sensitivity	100	**TP**	24
Specificity	57.69	**FP**	11
PPV	68.57	**FN**	0
NPV	100	**TN**	15

Agreement between methods:	MUCAP test able to detect *Salmonellae* strains missed by Rambach agar, including *S. typi* and *S. paratypi A*. No positive reaction occurred with other members of the family Enterbacteriaceae
Economic evaluation:	None provided
What the authors conclude:	MUCAP test found to be a useful and fast adjunct to Rambach agar in the identification of all strains of *Salmonellae*
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Aguirre *et al*. (1990)[30]
Journal:	*Journal of Clinical Microbiology*
Title:	Rapid fluorescence method for screening *Salmonella* spp. from enteric differential agars
Notes:	Results obtained on different selective media pooled for meta-analysis
Setting:	Hospital laboratory, Spain **Design:**
Sample characteristics:	
Sample size:	125 and 307
Target organism:	*Salmonella* spp.
Rapid test MUCAP	**Methods** Based on a rapid detection of C8 esterase enzyme by using a fluorogenic 4-methylumbelliferone-conjugated substrate.
Reference test Standard culture	**Methods** 125 samples cultured on MacConkey agar and 307 cultured on SS agar using standard methods

Reported accuracy data: Mcconkey agar				**Reported accuracy data: SS agar**			
Sensitivity	79.17	**TP**	19	**Sensitivity**	66.66	**TP**	60
Specificity	100	**FP**	0	**Specificity**	98.16	**FP**	4
PPV	100	**FN**	5	**PPV**	93.75	**FN**	30
NPV	95.28	**TN**	101	**NPV**	87.65	**TN**	213

Economic evaluation:	None provided
What the authors conclude:	The MUCAP test is an easy, rapid, and sensitive method for the screening of colonies suspected of being *Salmonella* spp, reducing the number of biochemical tests needed
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Alvarez *et al*. (2004)[157]
Journal:	*Journal of Clinical Microbiology*
Title:	Development of a Multiplex PCR Technique for Detection and Epidemiological Typing of *Salmonella* in Human Clinical Samples
Notes:	
Setting:	University laboratory, Spain **Design:** Prospective
Sample characteristics:	Consecutive human stool samples obtained from Spanish hospital
Sample size:	120
Target organism:	*Salmonella*
Rapid test PCR	**Methods** Selenite–cystine broth incubated for 4 hours at 37°C. DNA extracted
Reference test Culture and AB 120E	**Methods** XLD, MacConkey and Hektoen agars inoculated in selenite–cystine broth. *Salmonella* colonies confirmed with triple sugar iron agar, API 20E strips and serotyped (Kauffma–White scheme)

Reported accuracy data:			
Sensitivity	98.93	**TP**	28
Specificity	95.76	**FP**	9
PPV	87.68	**FN**	2
NPV	99.66	**TN**	81

Economic evaluation:	None provided
What the authors conclude:	Simple, inexpensive, and sensitive and enables the quick and precise detection of most prevalent serotypes of *Salmonella* in human clinical samples
Assessment of authors' conclusions:	Appropriate conclusions. High-quality paper; larger scale investigation may be beneficial

Authors (year):	Amar *et al*. (2004)[45]
Journal:	*European Journal of Clinical Microbiology and Infectious Diseases*
Title:	Blinded application of microscopy
Notes:	
Setting:	PHLS Laboratory, UK **Design:** Blinded, multicentre trial
Sample characteristics:	Unselected faecal samples (age range 0.8–81 years), submitted between 24 July 2002 and 25 October 2002
Sample size:	92
Target organism:	Wide range, including *Campylobacter*, *Salmonella* and *Clostridium perfringens*
Rapid test PCR	**Method** *Salmonella* detected by rtPCR using ABI PRISM Sequence detector 7700 and RealArt *Salmonella* TM PCR Kit (Artus). *Campylobacter* detected using ABI PRISM Sequence detector 7700 and RealArt *Campylobacter* TM PCR Kit (Artus). A gel-based duplex PCR performed for detection of *C. perfringens* phospholipase C and enterotoxin genes
Reference test Culture	**Methods** Standard operating procedures of PHLS were followed

Reported accuracy data: *Salmonella*				**Reported accuracy data: *C. jejuni***			
Sensitivity	75.00	**TP**	3	**Sensitivity**	83.33	**TP**	5
Specificity	100.00	**FP**	0	**Specificity**	98.84	**FP**	1
PPV	100.00	**FN**	1	**PPV**	83.33	**FN**	1
NPV	98.88	**TN**	88	**NPV**	98.84	**TN**	85

Clostridium perfringens	3 positive by both methods – equivalence
Major confounders or bias:	Low-sensitivity results for *Salmonella* and *Campylobacter* may be attributable to low numbers of positive isolates. For rarer pathogens, including *Cryptosporidium* and non-EHEC forms of *E. coli*, PCR was considerably more sensitive
Economic evaluation:	None provided
What the authors conclude:	The results of the present study demonstrate the potential for PCR to improve the detection of enteric pathogens in the faecal sample from patients with diarrhoea
Assessment of authors' conclusions:	Well-executed small study. One of the few studies reviewed to discuss multiple pathogens and asymptomatic infection

Authors (year):	Andersson *et al*. (1998)[102]
Journal:	*Applied and Environmental Microbiology*
Title:	A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin and related depsipeptide ionophores
Notes:	
Setting:	National Reference Laboratory **Design:** Assay development
Sample characteristics:	Reference *B. cereus* strains obtained from various laboratories
Sample size:	2 emetic strains, 3 non-emetic strains and other bacteria
Target organism:	*B. cereus* emetic strain
Rapid test	**Methods** Bioassay for detection of the emetic mitochondrial toxin, by all criteria identical with cereulide based on the loss of motility of boar spermatozoa
Reference test	**Methods** No comparison with another assay
Economic evaluation:	No economic data presented
What the authors conclude:	The test is a sensitive, inexpensive and rapid bioassay for detection of the emetic toxin of *B. cereus*
Assessment of authors' conclusions:	No comparison group, therefore diagnostic accuracy for this assay is not available.

Authors (year): Araj and Atamian (1997)[113]
Journal: *Laboratory Medicine*
Title: Reliability of rapid kits for *Staphylococcus aureus* identification
Notes:
Setting: University hospital laboratory, Lebanon
Design: Diagnostic comparison study
Sample characteristics: Various clinical specimens – not all faecal
Sample size: 240 *S. aureus* and 127 coagulase-negative stapylococci (CNS) clinical isolates

Target organism: *Staphylococcus aureus*

Rapid test
Comparison of various commercial kits, using banked samples.
No reference test provided

Methods
Slidex Staph-Kit (BioMerieux, France); Avistaph (Omega Diagnostics, UK); Staphylase (Oxoid, UK); Pastorex Staph-Plus (Sanofi Diagnostics Pasteur, France) all used according to manufacturers' instructions. Agglutination scored as positive (+), equivecol (±)

Reported accuracy data:

Slidex		Avistaph		Staphylase		Staph-Plus	
Sensitivity	96.60	**Sensitivity**	96.60	**Sensitivity**	96.60	**Sensitivity**	96.60
Specificity	91.00	**Specificity**	67.00	**Specificity**	94.00	**Specificity**	94.00
PPV	96.00	**PPV**	86.00	**PPV**	97.00	**PPV**	97.00
NPV	99.00	**NPV**	99.00	**NPV**	99.00	**NPV**	99.00
Uninterpretable	1.60	**Uninterpretable**	2.70	**Uninterpretable**	3.00	**Uninterpretable**	0.30

Economic evaluation: Not provided

Authors (year): Augustynowicz *et al*. (2002)[90]
Journal: *Journal of Medical Microbiology*
Title: Detection of enterotoxigenic *Clostridium perfringens* with a duplex PCR
Notes:
Setting: Reference laboratory, Poland **Design:** Assay development
Sample characteristics: 64 isolates, 30 of which were stool samples

Target organism: *Clostridium perfringens*

Rapid test
PCR

Methods
Two sets of primers designed to detect *Clostridium perfringens* phospholipase C (*plc*) and enterotoxin (*cpe*) genes in a single PCR reaction

Reference test
RPLA

Methods
Oxoid PET-RPLA used according to manufacturer's instructions

Reported accuracy data:

Sensitivity	100.00	**TP**	16
Specificity	NA	**FP**	14
PPV	100.00	**FN**	0
NPV	NA	**TN**	0

Economic evaluation: None provided

What the authors conclude: Duplex PCR for diagnosis of enterotoxigenic *C. perfringens* from vegetative cultures can be a useful tool as fresh isolates often sporulate poorly or not all, giving rise to the possibility of false negative results by serological analysis

Assessment of authors' conclusions: Appropriate conclusions

Authors (year):	Bartholomew *et al*. (1985)[82]
Journal:	*Journal of Clinical Pathology*
Title:	Development and application of an enzyme linked immunosorbent assay for *Clostridium perfringens* type A enterotoxin
Notes:	
Setting:	National reference laboratory, UK
Design:	Interlaboratory collaborative trial
Sample size:	515 faecal samples, 12 food samples from outbreaks of *C. perfringens* food poisoning. 44 known positive samples In order to compare ELISA technique with other standard immunological tests a group of 44 specimens were assayed for toxin by ELISA, double gel diffusion and counterimmunoelectrophoresis
Target organism:	*Clostridium perfringens*
Rapid test ELISA	**Methods** Double antibody ELISA. In-house design using rabbit antienterotoxin IgG
Reference test Double gel diffusion and counterimmuno-electrophoresis	**Methods** Citation to earlier description of methods. 79 faecal specimens from control groups tested. Blinded

Reported accuracy data: sandwich ELISA vs double gel infusion:

Sensitivity	100.00	**TP**	30
Specificity	35.71	**FP**	9
PPV	76.92	**FN**	0
NPV	100.00	**TN**	5

Sensitivity vs reference method:	Assay confirmed 47 of 50 (94%) of outbreaks compared with 32 of 48 (67%) confirmed by serotyping
Turnaround time vs reference method	Performed within 24 hours
Economic evaluation:	None provided
What the authors conclude:	The ELISA is recommended as a valuable tool in the investigation of *C. perfringens* food-borne illness
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Beecher and Wong (1994)[93]
Journal:	*Applied and Environmental Microbiology*
Title:	Identification and analysis of antigens detected by two commercial *Bacillus cereus* diarrhoeal enterotoxin immunoassay kits
Notes:	
Setting:	University laboratory **Design:** Evaluation of two commercial assays
Sample characteristics:	*B. cereus* strains obtained from various laboratories
Sample size:	2 *B. cereus* strains
Target organism:	*B. cereus*
Rapid test	**Methods** Commercial immunoassays (*Bacillus cereus* Enterotoxin-Reverse Passive Latex Agglutination kit from Oxoid and *Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay from Tecra)
Reference test	**Methods** Comparison with each other
Reported accuracy data:	
Sensitivity	**TP**
Specificity	**FP**
PPV	**FN**
NPV	**TN**
Agreement between methods:	The study aimed to determine the antigen detected by each assay and determine if they are part of a diarrhoea-causing enterotoxin
Economic evaluation:	No economic data presented
What the authors conclude:	The Oxoid BCET RPLA kit detects the L2 component of haemolysin BL enterotoxin while the Tecra assay detects two other proteins that are not part of the enterotoxin
Assessment of authors' conclusions:	The results of this paper highlight the need to validate commercial assays before they are adopted for widespread use. It is unclear whether the proteins detected by the Tecra assay have a role in diarrhoea

Authors (year):	Belanger *et al*. (2002)[158]
Journal:	*Journal of Clinical Microbiology*
Title:	Rapid detection of shiga toxin-producing bacteria in faeces by multiplex PCR with molecular beacons on the Smart Cycler
Notes:	
Setting:	Hospital laboratory, Canada **Design:** Diagnostic comparison
Sample characteristics:	In patients
Sample size:	38 faecal samples from 27 patients
Target organism:	EHEC
Rapid test rtPCR	**Methods** rtPCR for the detection of *stx1* and *stx2*. Prior to clinical evaluation, the assay was tested on 23 STEC strains and 20 non-STEC to ensure adequate sensitivity and specificity
Reference test SMAC culture	**Methods** Culture on SMAC agar. Sorbitol-negative colonies were then tested for the O157 antigen by VTEC-RPLA (Denka Seiken)
Reported accuracy data:	
Sensitivity	100 **TP** 26
Specificity	92 **FP** 1
PPV	96 **FN** 0
NPV	100 **TN** 11
Economic evaluation:	Not carried out
What the authors conclude:	This is a simple, rapid, sensitive and specific method and allows detection of all shiga toxin-producing bacteria directly from faecal samples, irrespective of their serotypes
Assessment of authors' conclusions:	Assay requires validation with a larger pool of clinical samples; details of sources of bias not given

Authors (year):	Berry *et al*. (1988)[79]
Journal:	*Journal of Clinical Pathology*
Title:	Evaluation of ELISA, RPLA and Vero cell assays for detecting *Clostridium perfringens* enterotoxin in faecal specimens
Notes:	
Setting:	PHLS Laboratory, UK **Design:**
Sample characteristics:	
Sample size:	392 faecal specimens
Target organism:	*Clostridium difficile* and *Clostridium perfringens*
Rapid test TechLab EIA	**Methods** Performed according to manufacturer's instructions. Optical density read at 450 and 620 nm. All specimens that gave equivocal or positive readings were repeated
Rapid test Vero cell assay	**Methods** 96-well microtitre tray seeded with suspension of Vero cells in Eagle's minimal essential medium and incubated overnight at 37°C. Faecal extracts prepared and added. Plate covered and incubated overnight at 37°C, then cell cultures examined for cytotoxicity
Reference test In-house ELISA	**Methods** PHLS FSML in-house EIA performed as prescribed by Bartholomew *et al*.[82]

Reported accuracy data: TechLab EIA			**Reported accuracy data: Vero cell**		
Sensitivity	**TP**	21	**Sensitivity**	**TP**	11
Specificity	**FP**	0	**Specificity**	**FP**	
PPV	**FN**	41	**PPV**	**FN**	
NPV	**TN**	781	**NPV**	**TN**	

Major confounders or bias:	RPLA = good sensitivity, problems with specificity
Economic evaluation:	Approximate cost (UK£ 1988)
What the authors conclude:	In-house FSML EIA and RPLA tests are both more sensitive than Vero cell assays for detection of *C. perfringens*
Assessment of authors' conclusions:	ELISA is a much more sensitive technique than Vero cell assay

Authors (year):	Bettelheim (2001)[62]
Journal:	*Letters in Applied Microbiology*
Title:	Development of a rapid method for the detection of verocytotoxin-producing *Escherichia coli* (VTEC)
Notes:	
Setting:	University research laboratory **Design:** Retrospective evaluation
Sample characteristics:	Reference strains only
Sample size:	239 isolates: results reported here correspond to all 111 from human sources
Target organism:	EHEC
Rapid test VTEC Screen (Denka Seiken)	**Methods** Only parts of the VTEC screen were used, including polymyxin B solution, dilutent, sensitised latex, control latex and positive control. Same-day results (2–4 h) reported with >98% accuracy
Reference test Standard culture	**Methods** Strains previously isolated by culture

Reported accuracy data (results from human sources only)			
Sensitivity	100	**TP**	55
Specificity	98.21	**FP**	1
PPV	98.21	**FN**	0
NPV	100	**TN**	55

Major confounders or bias:	Use of reference strains, instead of fresh clinical samples, may not accurately measure how the test works in routine practice
Economic evaluation:	None provided
What the authors conclude:	Result available on same working day
Assessment of authors' conclusions:	Appropriate conclusions – reducing time for incubation before performing RPLA test should be investigated further

Authors (year):	Beutin *et al*. (2002)[63]
Journal:	*Diagnostic Microbiology and Infectious Disease*
Title:	Evaluation of the VTEC-Screen 'Seiken' test for detection of different types of shiga toxin (verotoxin)-producing *Escherichia coli* (STEC) in human stool samples
Notes:	
Setting:	National reference laboratory, Germany **Design:** Diagnostic comparison
Sample characteristics:	Stool samples analysed at national centre from collaborating laboratories over 1 year from patients with gastrointestinal disease, including four samples from HUS patients
Sample size:	234 samples
Target organism:	EHEC
Rapid test 1 VTEC Screen (Denka Seiken)	**Methods** VTEC Screen (Denka-Seiken) performed according to manufacturer's instructions. Positive and negative controls provided.
Rapid test 2 PCR for *stx*1 and *stx*2 genes	**Methods** Following previously published methods developed by Lin *et al*.[240]
Reference test SMAC culture	**Methods** 1, culture on SMAC media; 2, VCA assay

Reported accuracy data: VTEC Screen				**Reported accuracy data: PCR**			
Sensitivity	89.83	**TP**	53	**Sensitivity**	93.2203	**TP**	55
Specificity	99.43	**FP**	1	**Specificity**	95.43	**FP**	8
PPV	98.15	**FN**	6	**PPV**	87.3	**FN**	4
NPV	96.66	**TN**	174	**NPV**	97.66	**TN**	167

Economic evaluation:	Not carried out
What the authors conclude:	The test was found to be accurate, rapid and easy to perform, thus being suitable for the routine screening of clinical stool specimens for STEC
Assessment of authors' conclusions:	Large study. Characteristics of study population not given. Assessment of study quality hampered by lack of details

Authors (year):	Bouvet and Jeanjean, (1992)[159]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of two coloured latex kits, the Wellcolex Colour *Salmonella* Test and the Wellcolex Colour Shigella Test, for serological grouping of *Salmonella* and *Shigella* species
Notes:	Reporting only *Salmonella* results
Setting:	National reference laboratory, France **Design:** Retrospective diagnostic comparison study
Sample characteristics:	Mixed samples/strains
Sample size:	193 randomly chosen pure cultures received at a reference laboratory – human, veterinary, and miscellaneous sources
Target organism:	*Salmonella*
Rapid test Wellcolex Colour *Salmonella*	**Methods** Coloured latex kit performed on non-lactose-fermenting colonies growing in primary culture on selective media. Based on agglutination of antibody-coated coloured latex particles in the presence of homologous antigens. Performed to manufacturer's instructions. Positive controls supplied with kit
Reference test Standard culture	**Methods** Details not provided

Reported accuracy data:			
Sensitivity	98.35	**TP**	179
Specificity	100	**FP**	0
PPV	100	**FN**	3
NPV	78.57	**TN**	11

Economic evaluation:	Not provided
What the authors conclude:	Easy to perform, accurate and easy to interpret when pure cultures were tested
Assessment of authors' conclusions:	Suitable conclusions

Authors (year):	Brett *et al*. (1992)[75]
Journal:	*Journal of Clinical Pathology*
Title:	Detection of *Clostridium perfringens* and its enterotoxin in cases of sporadic diarrhoea
Notes:	
Setting:	Hospital laboratories, UK **Design:** Intercollaborative trial
Sample characteristics:	All sporadic cases of diarrhoea submitted between September and December 1988. Age range 3 months to 89 years (most patients over 60 years)
Sample size:	818 samples
Target organism:	*Clostridium perfringens*
Rapid test Oxoid RPLA kit with ELISA confirmation	**Methods** Methods used similar to those by Berry *et al*.[79] Positive extracts referred to national reference laboratory for confirmation by ELISA
Reference test Confirmation of positive samples with ELISA	**Methods** ELISA performed in reference laboratory using standard methods. Pure cultures of *C. perfringens* isolated from enterotoxin-positive faeces serologically typed using set of 143 antisera
Reported accuracy data:	
Sensitivity	unclear **TP** 56
Specificity	unclear **FP** 8
PPV	0.875 **FN** unclear
NPV	unclear **TN** unclear
Economic evaluation:	None provided
What the authors conclude:	Diagnostic laboratories should perhaps consider screening for *C. perfringens* enterotoxin in cases of sporadic diarrhoea, particularly in elderly patients
Assessment of authors' conclusions:	High-quality epidemiological investigation study. Focus on non-food-related diarrhoea; however, test could successfully be applied with suspected food-borne infection also

Authors (year):	Buchanan and Schultz (1994)[28]
Journal:	*Letters in Applied Microbiology*
Title:	Comparison of the Tecra VIA kit, Oxoid BCET-RPLA kit and CHO cell culture assay for the detection of *Bacillus cereus* diarrhoeal enterotoxin
Notes:	
Setting:	Reference laboratory **Design:** Comparison of three assays
Sample characteristics:	*B. cereus* strains obtained from various laboratories
Sample size:	11 *B.cereus* strains and 1 *B. thuringiensis*
Target organism:	*B. cereus*
Rapid test	**Methods** Commercial immunoassays (*Bacillus cereus* Enterotoxin-Reverse Passive Latex Agglutination kit from Oxoid and Bacillus Diarrhoeal Enterotoxin Visual Immunoassay from Tecra) and the Chinese Hamster Ovary Cell Cytotoxicity assay
Reference test	**Methods** Comparison with each other
Reported accuracy data:	
Sensitivity	**TP**
Specificity	**FP**
PPV	**FN**
NPV	**TN**
Agreement between methods:	The study aimed to evaluate the efficacy of the Tecra assay and compare it with the Oxoid kit and CHO cell assay. Tecra assay correlated better with the CHO assay
Economic evaluation:	No economic data presented
What the authors conclude:	The CHO assay provided more accurate results for assaying cultures for *B. cereus* enterotoxin, both in sensitivity and its direct relationship with biological activity
Assessment of authors' conclusions:	Further limitations of the two commercial assays were identified by this study

Authors (year):	Carroll *et al*. (2003)[58]
Journal:	*European Journal of Clinical Microbiology and Infectious Disease*
Title:	Comparison of a commercial reversed passive latex agglutination assay to an enzyme immunoassay for the detection of shiga toxin-producing *Escherichia coli*
Notes:	
Setting:	Paediatric hospitals, USA **Design:** Prospective
Sample size:	554 diarrhoeal stool samples
Target organism:	EHEC
Rapid test 1 VTEC Screen (Denka Seiken)	**Methods** Reagents added according to manufacturer's instructions. Incubation for at least 16 hours. Agglutination patterns read manually
Rapid test 2 Premier EHEC (Meridian Diagnostics)	**Methods** Performed according to manufacturer's instructions using the positive and negative controls supplied in kit
Reference Test SMAC culture	**Methods** Inoculated to sorbitol MacConkey agar plates according to standard microbiology procedures. Incubated at 35°C and read at 24 and 48 hours for presence of sorbitol non-fermenters

Reported accuracy data: VTEC Screen				**Reported accuracy data: Premier EHEC**			
Sensitivity	100.00	**TP**	4	**Sensitivity**	100.00	**TP**	4
Specificity	97.82	**FP**	12	**Specificity**	98.36	**FP**	9
PPV	75.00	**FN**	0	**PPV**	69.23	**FN**	0
NPV	100.00	**TN**	538	**NPV**	100.00	**TN**	541

Additional statistical analysis:	99% agreement between VTEC Screen and Premier EHEC ($\kappa = 0.823$)
Major confounders or bias:	VTEC screen results delayed by 1 day compared with Meridian EHEC assay
Economic evaluation:	VTEC screen costs less than current toxin kits marketed in USA, hence some laboratories may implement routine shiga toxin screening in the future
What the authors conclude:	VTEC screen test is easy to perform and does not require special equipment. Results were comparable with those of Premier EHEC, but there were more unconfirmed positives
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Chiu and Ou (1996)[50]
Journal:	*Journal of Clinical Microbiology*
Title:	Rapid identification of *Salmonella* serovars in faeces by specific detection of virulence genes, *invA* and *spvC*, by an enrichment broth culture–multiplex PCR combination assay
Notes:	
Setting:	Paediatric hospital, Taiwan **Design:** Prospective
Sample characteristics:	Faecal specimen from 57 children with mucoid and/or bloody diarrhoea were admitted to a Taiwanese hospital
Sample size:	57 samples
Target organism:	*Salmonella*
Rapid test Multiplex-PCR	**Methods** 6 hours pre-enrichment, multiplex PCR targeting *spvC* and *invA* genes. Amplified DNA fragments in gel were visualised and photographed under UV illumination
Reference test Culture	**Methods** Growth on xylose–lysine–sodium deoxycholate agar (Difco), *Salmonella–Shigella* agar following enrichment in Gram-negative broth
Reported accuracy data:	
Sensitivity	90.91 **TP** 20
Specificity	48.57 **FP** 18
PPV	52.63 **FN** 2
NPV	89.47 **TN** 17
Agreement between methods:	PCR more sensitive than culture. From 40 banked positive samples, PCR detected 38 whereas culture methods reported only 24
Economic evaluation:	Not carried out
What the authors conclude:	A sensitive, rapid and efficient test that will cause only an incremental increase in the cost of stool processing
Assessment of authors' conclusions:	Clearly highlights the potential increased sensitivity when using PCR rather than culture; however, no discussion is provided on the potential for PCR to produce false positives

Authors (year):	Collins *et al*. (2001)[160]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of a PCR/DNA probe colorimetric membrane assay for identification of *Campylobacter* spp. in human stool specimens
Notes:	
Setting:	University microbiology laboratory, Ireland
Design:	
Sample characteristics:	Specifically selected stool specimens from community and inpatient cases of acute diarrhoeal disease
Sample size:	42–30 culture positive stool specimens, 12 culture-negative
Target organism:	*Campylobacter jejuni* and *Campylobacter coli*
Rapid test PCR, post-PCR hybridisation and colorimetric membrane assay	**Methods** DNA extraction using QIAamp DNA stool minikit, followed by post-PCR hybridisation to DNA probes specific for *Campylobacter* genus, *C. jejuni* and *C. coli* in a colorimetric membrane assay. Can be achieved in same working day
Reference test Standard culture	**Methods** Cultured on modified CCDA. 48 hours to identify to genus level, which must be followed by additional biochemical tests to identify species

Reported accuracy data:

Sensitivity	90.00	**TP**	27
Specificity	100.00	**FP**	0
PPV	100.00	**FN**	3
NPV	80.00	**TN**	12

Major confounders or bias:	Selection of samples for PCR detection was not random. However, as this was a small preliminary study, this may be justified
Economic evaluation:	None provided
What the authors conclude:	Focus of paper not with 'rapid' tests but with sensitivity of PCR after stool has been sitting around for a long time. Authors appear to suggest that culture is 'gold' standard for immediate testing, but if test is carried out 2 days later, PCR method is likely to 'rescue' additional positive results that culture will miss
Assessment of authors' conclusions:	1. Although this technique provided good sensitivity and specificity when compared with traditional culture methods, the labour intensity of the method would limit this to research use only, and routine laboratory use is unlikely. 2. Results recorded in data set relate to Set A (culture positive, tested immediately) and Set C (originally culture negative) as this would best represent practice in a non-research environment

Authors (year):	Cudjoe *et al*. (1991)[83]
Journal:	*International Journal of Food Microbiology*
Title:	Detection of *Clostridium perfringens* type A enterotoxin in faecal and food samples using immunomagnetic separation (IMS)–ELISA
Notes:	
Setting:	Research laboratory, Norway **Design:** Assay development
Sample characteristics:	12 spiked faecal samples, 12 healthy faecal samples, one faecal sample from suspected case of *C. perfringens*
Sample size:	25 faecal samples
Target organism:	*Clostridium perfringens*
Rapid test IMS-ELISA	**Methods** IMS–ELISA developed for detection and quantitation of *C. perfringens* type A enterotoxin from faecal and food extracts. Minimal 3 hours coating of immunomagnetic particles (Dynabeads M-280). Specificity was confirmed by both crossed immunoelectrophoresis and Western immunoblotting techniques
Reported accuracy data:	Not reported
Economic evaluation:	Not provided
What the authors conclude:	A simple, rapid and sensitive immunoassay
Assessment of authors' conclusions:	Full evaluation needed with appropriate comparator

Authors (year):	Davis *et al*. (2003)[161]
Journal:	*Abstracts from the General Meeting of American Society of Microbiologists*
Title:	An evaluation of the BAX system for rapid screening of stool specimens for *Salmonella* species and *E. coli* O157:H7
Notes:	Data derived from published abstract and contact with corresponding author (J Fontana)
Setting:	Public health laboratory, USA
Design:	Prospective case–control
Sample characteristics:	Fresh human stool samples
Sample size:	78 samples tested for *Salmonella* – 18 of these also tested for *E. coli*
Target organism:	*Salmonella* species and *E. coli* O157
Rapid test BAX system (Dupont Qualicon)	**Methods** Overnight enrichment in tethrathionate broth for *Salmonella* and MacConkey broth for *E. coli* O157 samples
Reference test Culture	**Methods** Conventional culture methods used

Reported accuracy data: *Salmonella* results				**Reported accuracy data: *E. coli* O157 results**			
Sensitivity	100	**TP**	5	**Sensitivity**	100	**TP**	4
Specificity	98.6	**FP**	1	**Specificity**	100	**FP**	0
PPV	83.3	**FN**	0	**PPV**	100	**FN**	0
NPV	100	**TN**	72	**NPV**	100	**TN**	14

Economic evaluation:	None performed
What the authors conclude:	If the BAX system is validated for screening of *Salmonella* spp. and *E. coli* O157:H7 from stool samples, it will reduce the time to implement outbreak control measures by at least 2 days
Assessment of authors' conclusions:	This is one of the few studies in which a well-recognised commercial test developed for the food industry has been applied for testing on clinical samples. Results from this small study suggest the BAX system could successfully be used on stool specimens

Authors (year):	Day *et al*. (1994)[94]
Journal:	*Journal of Applied Bacteriology*
Title:	A comparison of ELISA and RPLA for detection of *Bacillus cereus* diarrhoeal enterotoxin
Notes:	
Setting:	Reference laboratory, USA **Design:** Comparison of two commercial assays
Sample characteristics:	*B. cereus* strains obtained from various laboratories
Sample size:	14 *B.cereus* strains
Target organism:	*B. cereus*
Rapid test 1 Oxoid BCET-RPLA	**Methods** Commercial immunoassays (*Bacillus cereus* Enterotoxin-Reverse Passive Latex Agglutination kit from Oxoid and *Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay from Tecra)
Rapid test 2 Tecra ELISA	**Methods** Comparison with each other

Reported accuracy data: Oxoid RPLA				**Reported accuracy data: Tecra ELISA**			
Sensitivity	43.75	**TP**	6	**Sensitivity**	87.50	**TP**	13
Specificity	NA	**FP**	NA	**Specificity**	NA	**FP**	NA
PPV	NA	**FN**	8	**PPV**	NA	**FN**	1
NPV	NA	**TN**	NA	**NPV**	NA	**TN**	NA

Agreement between methods:	The study aimed to evaluate both assays for diarrhoeal toxin in cultures of *B. cereus* isolated from food-borne outbreaks. Of 14 samples, 12 cultures were positive by the Tecra ELISA and six by RPLA. One was negative to both
Economic evaluation:	No economic data presented
What the authors conclude:	Tecra ELISA assay is a more reliable indicator of diarrhoeaogenic enterotoxin of *B. cereus* than Oxoid BCET-RPLA
Assessment of authors' conclusions:	Limitations of the two commercial assays were discussed. Properly designed diagnostic evaluation studies are required

Authors (year):	Dediste *et al*. (2003)[162]
Journal:	*Clinical Microbiology and Infection*
Title:	Evaluation of the ProSpecT Microplate assay for the detection of *Campylobacter*: a routine laboratory perspective
Notes:	
Setting:	Hospital laboratory, Belgium **Design:** Consecutive
Sample characteristics:	All samples over a 4-month period from non-hospitalised and HIV-positive adult and paediatric patients
Sample size:	1205
Target organism:	*Campylobacter*
Rapid test ProSpecT Microplate ELISA assay	**Methods** Commercial enzyme immunoassay (Alexon-Trend, USA), performed according to instructions of the manufacturer. Plates read both visually and spectrophotometrically. Positive and negative controls provided
Reference test Culture	**Methods** Culture on Mueller Hinton agar supplemented with cefoperazone, rifampicin and amphotericin B. Plates incubated for 3 days at 25°C in a micro-aerobic atmosphere and examined daily.

Reported accuracy data:			
Sensitivity	89.10	**TP**	90
Specificity	97.70	**FP**	25
PPV	78.26	**FN**	11
NPV	98.99	**TN**	1079

Additional statistical analysis:	Standard errors calculated for accuracy data. The χ^2 test was used to compare performance of EIA between stools preserved in Cary–Blair transport medium and fresh stool specimens
Economic evaluation:	Not carried out
What the authors conclude:	A rapid 2-hour assay. Best used for patients requiring antibiotic therapy, cases of prolonged illness, in pregnancy and for immunocompromised patients
Assessment of authors' conclusions:	Very clear presentation of diagnostic accuracy statistics. Appropriate conclusions

Authors (year):	Dylla *et al*. (1995)[163]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of an immunoassay for direct detection of *Escherichia coli* O157 in stool specimens
Notes:	
Setting:	Hospital microbiology laboratory **Design:** Diagnostic comparison
Sample characteristics:	Stool samples from inpatients and outpatients seen at Mayo Clinic
Sample size:	185 samples from 161 patients
Target organism:	*E. coli* O157
Rapid test	**Methods** ELISA (LMD laboratories)
Reference test	**Methods** Culture on SMAC agar and 5% sheep blood agar
Reported accuracy data:	
Sensitivity	98.86 **TP** 9
Specificity	100 **FP** 0
PPV	100 **FN** 2
NPV	81.82 **TN** 174
Economic evaluation:	Not carried out
What the authors conclude:	An accurate, easy-to-read screening method for the detection of E. coli O157 in faecal specimens
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Ehling-Schulz *et al.* (2004)[98]
Journal:	*FEMS Microbiology Letters*
Title:	Identification of emetic toxin producing *Bacillus cereus* strains by a novel molecular assay
Notes:	
Setting:	Reference laboratory **Design:** Development of a new assay
Sample characteristics:	Reference *B. cereus* strains obtained from various laboratories
Sample size:	178 bacterial isolates include 100 *B. cereus* isolates from various sources
Target organism:	*B. cereus* emetic strain
Rapid test PCR	**Methods** PCR assay for emetic strain of *B. cereus*. Not truly rapid, requires 24 hours after isolation
Reference test	**Methods** No comparison with another assay
Reported accuracy data:	
Sensitivity	30/30 (100%) **TP** NA
Specificity	NA **FP** NA
PPV	NA **FN** NA
NPV	NA **TN** NA
Economic evaluation:	No economic data presented
What the authors conclude:	This simple and rapid PCR assay represents an attractive alternative for the detection of the emetic toxin via cytotoxicity assay, HPLC–MS and using a sperm-based bioassay
Assessment of authors' conclusions:	This appears to be a sensitive and test strain specific molecular assay for the detection of emetic strain of *B. cereus*, but there was no comparison test in the study.

Authors (year):	Endtz *et al*. (2000)[164]
Journal:	*European Journal of Clinical Microbiology and Infectious Diseases*
Title:	Evaluation of a new commercial immunoassay for rapid detection of *campylobacter jejuni* in stool samples
Notes:	
Setting:	Hospital laboratory, The Netherlands **Design:** Diagnostic comparison
Sample characteristics:	30 consecutive culture-positive for *Campylobacter* stool specimens, submitted between January and April 1999. 30 *Campylobacter* culture-negative specimens and 18 *Salmonella* culture-positive samples were used to test specificity
Sample size:	78
Target organism:	*C. jejuni* and *C. coli*
Rapid test ProSpecT *Campylobacter* microplate assay	**Methods** Commercial enzyme immunoassay (Alexon-Trend, USA), performed according to instructions of the manufacturer
Reference test Culture on modified CCDA	**Methods** Not reported
Reported accuracy data:	
Sensitivity	80.00 **TP** 24
Specificity	100.00 **FP** 0
PPV	100.00 **FN** 6
NPV	88.89 **TN** 48
Major confounders or bias:	Significant time delay between culture test and EIA may have affected sample. Blinding not possible
Economic evaluation:	Not carried out
What the authors conclude:	A rapid, easy-to-perform test to detect *C. jejuni* in stool samples
Assessment of authors' conclusions:	Data suggest that the test's sensitivity and specificity are promising, but this needs to be confirmed in a larger, prospective study

Authors (year):	Fach and Guillou (1993)[165]
Journal:	*Journal of Applied Bacteriology*
Title:	Detection by *in vitro* amplification of the alpha-toxin (phospholipase C) gene from *Clostridium perfringens*
Setting:	Research laboratory, France **Design:** Assay development
Sample characteristics:	Strains only
Sample size:	Unclear
Target organism:	*Clostridium perfringens*
Rapid test	A gel-based duplex PCR performed for detection of *C. perfringens* phospholipase C and enterotoxin genes. Method first used by Saiki and colleagues[241]
Reference test	Assay development only: No comparator
Reported accuracy data:	Not provided
Economic evaluation:	Not provided
What the authors conclude:	This PCR satisfies the criteria of specificity, sensitivity and rapidity required for a useful tool in epidemiology and for the diagnosis of *C. perfringens* as it may be used directly on stool samples
Assessment of authors' conclusions:	Methods clearly reported; however, full evaluation with suitable comparator necessary

Authors (year):	Farrell (2003)[242]
Journal:	*Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy*
Title:	Prospective validation of real time PCR detection of *Salmonella* in culture from clinical stool specimens
Notes:	Poster presentation only
Setting:	Hospital laboratory, USA **Design:** Prospective
Sample characteristics:	All bacterial colonies from stool samples screened for *Salmonella* between 6 January 2003 and 6 May 2003
Sample size:	298 colonies (from 170 patients)
Target organism:	*Salmonella enterica*
Rapid test rtPCR for the *prgK* gene	**Methods** LightCycler platform used. Study states real-time PCR performed in 3–4 hours
Reference test Culture	**Methods** Cultured on lysine iron agar and triple sugar iron agar. Study states conventional screening and identification protocols require 2–3 days

Reported accuracy data:			
Sensitivity	99.62	**TP**	38
Specificity	100	**FP**	1
PPV	97.44	**FN**	0
NPV	100	**TN**	259

Economic evaluation:	None reported
What the authors conclude:	PCR equivalent to culture-based methods of detecting and identifying *Salmonella*; however, real-time PCR identification can be performed in 3–4 hours, as opposed to conventional screening and identification protocols, which require 2–3 days
Assessment of authors' conclusions:	Methods appear correct

Authors (year):	Fedorka *et al*. (1989)[166]
Journal:	*Diagnostic Microbiology and Infectious Disease*
Title:	Increased efficiency of stool culture for the detection of *Salmonella* and *Shigella*
Notes:	
Setting:	Research laboratory, USA **Design:** Diagnostic comparison
Sample size:	822 consecutive stool samples
Target organism:	*Salmonella* and *Shigella*
Rapid test Wampole Bactigen	**Methods** Wampole Bactigen *Salmonella–Shigella* latex agglutination test. Three reagents used: (1) coated with goat antibodies to more than 80 common *Salmonella* serogroups; (2) layered with rabbit antibodies to *Shigella* group B and D; (3) bears rabbit antibodies to *Shigella* groups A and C.
Reference test Standard culture	**Methods** SS culture with subculture of selenite broths

Reported accuracy data:			
Sensitivity	100	**TP**	17
Specificity	93.54	**FP**	52
PPV	24.64	**FN**	0
NPV	100	**TN**	753

Economic evaluation:	None provided
What the authors conclude:	The (Wampole) SSLA test is a useful screening test for *Salmonella*. By eliminating unnecessary subcultures of selenite broth, it reduces turnaround by 24 hours for negative stool cultures
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Finlay *et al*. (1999)[100]
Journal:	*Applied and Environmental Microbiology*
Title:	Semiautomated metabolic staining assay for *Bacillus cereus* emetic toxin
Notes:	
Setting:	University laboratory **Design:** Development of a new assay
Sample characteristics:	Reference *B. cereus* strains obtained from various laboratories
Sample size:	13 *B.cereus* strains
Target organism:	*B. cereus* emetic strain
Rapid test	**Methods** Metabolic staining assay based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) conversion
Reference test	**Methods** Comparison with Hep-2 cell assay
Reported accuracy data:	Not given
Major confounders or bias:	None examined
Economic evaluation:	No economic data presented, but authors state it is cheaper than the reference standard
What the authors conclude:	A significant improvement on current methods of emetic toxin assay as it is cheaper and considerably less labour intensive than animal challenge assays, requires no specialised laboratory apparatus, eliminates personal visual assessment and appears to be specific for the *B. cereus* emetic toxin
Assessment of authors' conclusions:	The lack of comparison data limits the assessment of this assay

Authors (year):	Fletcher and Logan (1999)[95]
Journal:	*Letters in Applied Microbiology*
Title:	Improved cytotoxicity assay for *Bacillus* diarrhoeal enterotoxin
Notes:	
Setting:	University laboratory **Design:** Evaluation of a new assay
Sample characteristics:	Reference *B. cereus* strains obtained from various laboratories
Sample size:	71 *B.cereus* strains
Target organism:	*B. cereus* diarrheoal strains
Rapid test	**Methods** McCoy cell-based assay
Reported accuracy data:	Not given
Agreement between methods:	O14 food poisoning samples, 10 were positive on ICA, 13 on Tecra and 7 on Oxoid
Economic evaluation:	No economic data presented
What the authors conclude:	The cytotoxicity assay is more sensitive than the Oxoid kit and unlike the Tecra kit did not give false positive results with supernatant samples heat treated to destroy the toxin
Assessment of authors' conclusions:	This assays appears to be as good as the commercial kits available

Authors (year):	Forward *et al*. (2003)[78]
Journal:	*Journal of Medical Microbiology*
Title:	Detection of *Clostridium difficile* cytotoxin and *Clostridium perfringens* enterotoxin in cases of diarrhoea in the community
Notes:	
Setting:	PHLS Laboratory, UK **Design:** Diagnostic comparison
Sample size:	843 cases of diarrhoea in the community between November 1999 and April 2000
Target organism:	*C. difficile* and *C. perfringens*
Rapid test TechLab EIA	**Methods** Performed according to manufacturer's instructions. Optical density read at 450 and 620 nm. All specimens that gave equivocal or positive readings were repeated
Rapid test Vero cell assay	**Methods** 96-well microtitre tray seeded with suspension of Vero cells in Eagle's minimal essential medium and incubated overnight at 37°C. Faecal extracts prepared and added. Plate covered and incubated overnight at 37°C, then cell cultures examined for cytotoxicity
Reference test In-house ELISA	**Methods** PHLS FSML in-house EIA performed as prescribed by Bartholomew *et al*.[82]

Reported accuracy data: TechLab EIA				**Reported accuracy data: Vero cell**		
Sensitivity	100.00	**TP**	21	**Sensitivity**	**TP**	11
Specificity	33.87	**FP**	41	**Specificity**	**FP**	
PPV	95.01	**FN**	0	**PPV**	**FN**	
NPV	100.00	**TN**	781	**NPV**	**TN**	

Economic evaluation:	Not provided
What the authors conclude:	TechLab EIA was less sensitive than FSML EIA. A Vero cell assay for CPE is too insensitive for routine use
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Fukuda *et al*. (2000)[111]
Journal:	*Letters in Applied Microbiology*
Title:	Rapid detection of *Staphylococcus aureus* using bioluminescent enzyme immunoassay
Notes:	
Setting:	Commercial research laboratory (Kikkoman Corp, Japan)
Target organism:	*S. aureus*
Rapid test Checklite BH *Staphylococcus aureus* screening test (Kikkoman)	**Methods** Bioluminescent enzyme immunoassay (BLEIA) for detecting protein A-bearing *S. aureus* using biotylinated firefly luciferase. Stored strains in broth were subjected to the assay. The test was not carried out directly on stool samples

Reference test	**Positive/negative controls**	**Blinding**
Test performed on known *S. aureus*-positive samples	NA	No

Reported accuracy data:			
Sensitivity	100	**TP**	24
Specificity	86	**FP**	50
PPV	–	**FN**	0
NPV	100	**TN**	–

Turnaround time vs reference method:	Detection of protein-A bearing *S. aureus* is possible within 7 hours including culture
Economic evaluation:	Not provided
What the authors conclude:	This system could be adapted to detect other food-borne pathogens or toxins using appropriate immunological reagents
Assessment of authors' conclusions:	Given the increased speed of detection associated with the BLEIA, further research regarding its use with other food-borne pathogens may be justified

Authors (year):	Gavin *et al*. (2004)[66]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of performance and potential clinical impact of ProSpecT shiga toxin *Escherichia coli* microplate assay for detection of shiga toxin-producing *E. coli* in stool samples
Notes:	Included due to availability of economic analysis
Setting:	Reference laboratory, USA **Design:** Partial prospective diagnostic accuracy study
Sample characteristics:	Fresh human stool samples collected over two consecutive summers
Sample size:	2060 samples tested using ProSpecT kit. 543 of these also routinely tested using SMAC culture, remainder only colonised if ProSpecT kit produced positive result
Target organism:	Shiga toxin *E. coli*
Rapid test	**Methods**
ProSpecT Shiga toxin *E. coli* (Alexon Trend)	Microplate immunoassay using rabbit polyclonal anti-Shiga toxin 1 and 2 capture antibodies and a horseradish peroxidase-labelled monoclonal mouse anti-Shiga toxin 1 and 2 conjugate. Assay performed according to manufacturer's instructions

Reference test	**Methods**	**Positive/negative controls**	**Blinding**
	SMAC	Both	Yes

Reported accuracy data: full validation (pilot sample)				**Reported accuracy data: partial validation (larger sample)**			
Sensitivity	100	**TP**	7	**Sensitivity**	100	**TP**	27
Specificity	100	**FP**	0	**Specificity**	100	**FP**	2
PPV	100	**FN**	0	**PPV**	93.1	**FN**	0
NPV	100	**TN**	536	**NPV**	100	**TN**	2031

Economic evaluation:	Materials and labour cost of ProSpecT assay calculated at $16 per test, based on list price for the assay and average labour cost of $20/hour. Over the two summers, screening 2060 stool specimens for the presence of Shiga toxins by ProSpecT assay cost $32,960
What the authors conclude:	ProSpecT assay demonstrated 100% sensitivity and specificity for detection of O157 serotype STEC. Lack of true gold standard for non-O157 serotypes prohibits evaluation of ProSpecT for all other STEC
Assessment of authors' conclusions:	Study highlights under-reporting of non-O157 strains resulting from reliance on SMAC culture

Authors (year):	Geers and Backes (1989)[52]
Journal:	*American Journal of Clinical Pathology*
Title:	Evaluation of two rapid methods to screen pathogens from stool specimens
Notes:	
Setting:	Hospital laboratory, USA **Design:** Retrospective
Sample size:	125 *Salmonella*, *Shigella* or *Yersinia* isolates and 81 non-enteric pathogens tested with EPS card; 300 stool cultures with suspicious colonies tested with TSI–urea screens
Target organism:	*Salmonella*, *Shigella* or *Yersinia*
Rapid test AutoMicroBic EPS	**Methods** Enteric Pathogen Screen cards of the AutoMicroBic system (Vitek Systems)
Rapid test Bactigen *Salmonella-Shigella* latex agglutination	**Methods: Bactigen *Salmonella–Shigella* latex agglutination** Three reagents used: (1) coated with goat antibodies to more than 80 common *Salmonella* serogroups; (2) layered with rabbit antibodies to *Shigella* group B and D; (3) bears rabbit antibodies to *Shigella* groups A and C
Reference test Biochemical test media	**Methods** Triple sugar iron and urea agar. Indole tests performed on organisms displaying characteristic reactions on these media. Definitive identification achieved with AMS Gram-negative identification cards and confirmed by sera testing

Reported accuracy data: EPS				**Reported accuracy data: Bactigen SS latex agglutination**			
Sensitivity	86.96	**TP**	20	**Sensitivity**	100	**TP**	20
Specificity	85.47	**FP**	67	**Specificity**	98.25	**FP**	4
PPV	22.99	**FN**	3	**PPV**	83.33	**FN**	0
NPV	99.24	**TN**	394	**NPV**	100	**TN**	457

Economic evaluation:	Not provided
What the authors conclude:	Because the (Wampole) LPA method is faster, eliminates more clinically irrelevant organisms from further testing and does not require the use of an expensive identification system, the authors believe that it is better suited for direct screening for *Salmonella* and *Shigella* for most clinical laboratories
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Germani *et al*. (1990)[84]
Journal:	*Research in Microbiology*
Title:	Competitive erythroimmunoassay for detecting *Clostridium perfringens* type a enterotoxin in stool specimens
Notes:	
Setting:	Research laboratory, France **Design:** Prospective
Sample characteristics:	Retrieved from children's hospital where patients suffering from diarrhoea. Causes were food-borne intoxication (5 cases) or antibiotic-associated colitis or unknown (95 cases)
Sample size:	100 samples
Target organism:	*C. perfringens*
Rapid test Competitive erythroimmunoassay (ERIA)	**Methods** Immunoassay technique which assesses erythrocyte attachment to wells as assessed by eye. Methods described previously
Reference test ELISA	**Methods** Sheep anti-CPE Ig in a 1:400 dilution in carbonate buffer, incubated overnight at room temperature. Washing process takes place over 5.5 hours

Reported accuracy data:

Sensitivity	100	**TP**	3
Specificity	93.8	**FP**	6
PPV	33.3	**FN**	0
NPV	100	**TN**	91

Sensitivity vs reference method:	ELISA reported two false negatives – therefore cannot be assumed as best reference. ERIA as sensitive as other serological assays and more sensitive than the ELISA
Turnaround time vs reference method:	Authors claim ERIA is quicker than ELISA
Economic evaluation:	Authors claim ERIA is cheaper than ELISA, but no evaluation provided
What the authors conclude:	ERIA recommended in diagnosis for poorly equipped laboratories
Assessment of authors' conclusions:	Quality of methods sounds; however, evaluation may be dated

Authors (year):	Guardati *et al*. (1993)[110]
Journal:	*Journal of Clinical Microbiology*
Title:	Rapid methods for identification of *Staphylococcus aureus* when both human and animal staphylococci are tested: comparison with a new immunoenzymatic assay
Notes:	
Setting:	University laboratories, Switzerland and Italy
Sample characteristics:	Of human and animal origin
Sample size:	275 *S. aureus* and 380 non-*S. aureus* reference strains
Target organism:	*S. aureus*
Rapid test	**Methods**
Immunoassay	Based on monoclonal antibody Mab C1-10/11, prepared against SaG, an enzyme produced by all isolates of this species
Reference test:	
Six commercially available:	Staphaurex (Wellcome Diagnostics), Pastorex Staph (Diagnostic Pasteur), Staphyslide test (bio Merieux), Sero-STAT (Scott Laboratories), Bacto Staph Latex (Difco) and Bactident (Merck)

Reported accuracy data: Immunoassay				**Reported accuracy data: Staphaureux (one of 6 commercial kits evaluated)**			
Sensitivity	100	**TP**	275	**Sensitivity**	92	**TP**	253
Specificity	100	**FP**	0	**Specificity**	91.1	**FP**	34
PPV	100	**FN**	0	**PPV**	88.2	**FN**	22
NPV	100	**TN**	380	**NPV**	94	**TN**	346

Sensitivity vs reference method:	EIA more sensitive and specific than all commercial kits
Turnaround time vs reference method	Unclear
Economic evaluation:	None provided
What the authors conclude:	The EIA is a useful test for isolates where identification is doubtful and for epidemiological studies
Assessment of authors' conclusions:	Conclusion is appropriately cautious

Authors (year):	Haggblom *et al*. (2002)[99]
Journal:	*Applied and Environmental Microbiology*
Title:	Quantitative analysis of cereulide, the emetic toxin of *Bacillus cereus* produced under various conditions
Notes:	
Setting:	University laboratory **Design:** Development of a new assay
Sample characteristics:	Reference *B. cereus* strains obtained from various laboratories
Sample size:	5 *B. cereus* strains
Target organism:	*B. cereus* emetic strain
Rapid test	**Methods**
	A quantitative and sensitive chemical assay for cereulide based on liquid chromatography connected to ion trap mass spectrometry. Requires 24 hours after isolation
Reference test	**Methods**
	Comparison with boar spermatozoan motility assay
Reported accuracy data:	Not presented
Major confounders or bias:	None examined
Economic evaluation:	No economic data presented
What the authors conclude:	A sensitive and rapid chemical assay for cereulide was developed which correlated well with the sperm motility assay
Assessment of authors' conclusions:	Diagnostic accuracy data not presented. We are therefore unable to assess the effectiveness of this assay

Authors (year):	Hansen and Freney (1993)[167]
Journal:	*Journal of Microbiological Methods*
Title:	Comparative evaluation of a latex agglutination test for the detection and presumptive serogroup identification of *Salmonella* spp.
Notes:	
Setting:	Hospital laboratory, Belgium **Design:** Diagnostic comparison
Sample size:	All 702 diarrhoeal stool samples, selected from 4953 collected at microbiology laboratory between 1 January 1990 and 31 August 1990
Target organism:	*Salmonella* species
Rapid test Wellcolex Colour *Salmonella*	**Methods** RPLA test designed to detect *Salmonella* antigens, using overnight selenite broth cultures. Test performed as recommended by manufacturer
Reference test Standard culture	**Methods** Stool samples inoculated on to XLD agar, MacConkey agar heart infusion agar, and selenite enrichment broth. Subcultures of selenite broth made after 24 hours at 36°C on to XLD and MacConkey agar plates; these media also incubated overnight at 36°C
Reported accuracy data:	
Sensitivity	100 **TP** 39
Specificity	98.34 **FP** 11
PPV	78 **FN** 0
NPV	100 **TN** 652
Economic evaluation:	None provided
What the authors conclude:	Wellcolex Colour test provides a simple and rapid procedure for detection and presumptive serogroup identification of *Salmonella* spp. in enrichment broths
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Hindiyeh *et al*. (2000)[168]
Journal:	*Journal of Clinical Microbiology*
Title:	Rapid detection of *Campylobacter jejuni* in stool specimens by an enzyme immunoassay and surveillance for *Campylobacter upsaliensis* in the Greater Salt Lake City area
Notes:	
Setting:	Hospital laboratory with samples from four centres
Design:	Diagnostic comparison
Sample characteristics:	Patients with suspected bacterial diarrhoea having liquid or non-formed stools were included
Sample size:	631 stool samples
Target organism:	*C. jejuni*
Rapid test ProSpecT Microplate assay	**Methods** Commercial EIA (Alexon-Trend, USA), performed according to instructions of the manufacturer. Incubated at room temperature for 1 hour, wells washed, enzyme conjugate added, samples incubated for further 30 minutes.Wells washed and incubated with colour substrate at room temperature for 10 minutes. Results read spectrophotometrically at 450 nm
Reference test Culture	**Methods** Culture on a Campy-CVA plate (Remel, Lenexa, KS, USA) and blood-free *Campylobacter* agar with cefoperazone (20 μg ml^{-1}), amphotericin B (10 μg ml^{-1}) and teicoplanin (4 μg ml^{-1})

Reported accuracy data:			
Sensitivity	88.89	**TP**	16
Specificity	99.67	**FP**	4
PPV	80.00	**FN**	2
NPV	99.35	**TN**	609

Agreement between methods:	
Additional statistical analysis:	Observed difference between results of EIA compared with results from culture media were not statistically significant (by McNemar's test, $p > 0.25$)
Economic evaluation:	Cost-effectiveness of this assay requires evaluation since the direct cost of the EIA is $8 more than that of culture
What the authors conclude:	Assay is less sensitive than culture, has high specificity and results are available within 24 hours
Assessment of authors' conclusions:	Appropriate conclusion

Authors (year):	Iijima *et al*. (2004)[117]
Journal:	*Journal of Medical Microbiology*
Title:	Improvement in the detection rate of diarrhoeagenic bacteria in human stool specimens by a rapid real-time PCR assay
Notes:	
Setting:	Hospital laboratory, Japan **Design:** Prospective, consecutive
Sample characteristics:	Stool specimens from 16 healthy people, 40 people linked to food poisoning incidents and 105 outpatients receiving treatment for diarrhoea and/or abdominal pain
Sample size:	161 stool specimens in total
Target organism:	*Salmonella enterica*, *Vibrio parahaemolyticus*, *Campylobacter jejuni* and shiga toxin-producing *Escherichia coli*
Rapid test Multiplex rtPCR detection	**Methods** Based on *invA* gene for *S. enterica*, *yphC* and *gyrA* genes for *C. jejuni*, and *stx*1, *stx*2 and *eae* genes for STEC. Diagnosis, including DNA extraction and rtPCR, within 3 hours. PCR cycle performed within 70 minutes
Reference test Standard culture	**Methods** Standard methods used, referencing *Manual for Clinical Microbiology*, 7th ed.

Reported accuracy data: *S. enterica*				**Reported accuracy data: *C. jejuni***			
Sensitivity	81.82	**TP**	9	**Sensitivity**	84.21	**TP**	16
Specificity	96.00	**FP**	6	**Specificity**	95.07	**FP**	7
PPV	60.00	**FN**	2	**PPV**	69.56	**FN**	3
NPV	98.63	**TN**	144	**NPV**	97.83	**TN**	135

Additional analysis:	Retest of PCR-positive, culture-negative by the PCR method produced identical results
Economic evaluation:	None given
What the authors conclude:	This PCR-based method contributes to improved rapid diagnosis of enteric bacterial infections, while yielding higher detection rates of causative agents
Assessment of authors' conclusions:	Very promising assay which could allow multiplex reactions, for the simultaneous detection of multiple pathogens

Authors (year):	Imperatrice and Nachamkin (1993)[53]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of the Vitek EPS Enteric Pathogen Screen card for detecting *Salmonella*, *Shigella* and *Yersinia* spp.
Notes:	
Setting:	Hospital laboratory, USA **Design:** Retrospective
Sample size:	125 *Salmonella*, *Shigella* or *Yersinia* isolates and 81 non-enteric pathogens tested with EPS card; 300 stool cultures with suspicious colonies tested with TSI–urea screens
Target organism:	*Salmonella*, *Shigella* or *Yersinia*
Rapid test AutoMicroBic EPS	**Methods** Enteric Pathogen Screen cards of the AutoMicroBic system (Vitek Systems)
Reference test Biochemical test media	**Methods** Triple sugar iron and urea agar

Reported accuracy data: EPS				**Reported accuracy data: TSI–urea screen**			
Sensitivity	99.46	**TP**	183	**Sensitivity**	Unclear	**TP**	Unclear
Specificity	90.12	**FP**	8	**Specificity**	32.24	**FP**	166
PPV	95.81	**FN**	1	**PPV**	Unclear	**FN**	Unclear
NPV	98.65	**TN**	73	**NPV**	Unclear	**TN**	79

Major confounders or bias:	Uses updated versions of Vitek software analysis program to Geers and Backes,[52] so results may not be directly comparable
Economic evaluation:	In an analysis of the cost of 100 cultures with a positive screen, the EPS card cost $288 versus $432 for the TSI–urea test and subsequent biochemical identification in our laboratory (US$ 1992)
What the authors conclude:	The Vitek EPS Screen card was both sensitive in detecting the spectrum of organisms isolated in the patient population and much more specific than our conventional TSI–urea screen
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Kai *et al*. (1997)[65]
Journal:	*Kansenshogaku Zasshi (Journal of the Japanese Association for Infectious Diseases)*
Title:	Evaluation of a latex agglutination method for detecting and characterising verotoxin (VT) produced by *Escherichia coli*
Notes:	Japanese translation
Setting:	**Design:**
Sample characteristics:	
Sample size:	178 human strains – 147 VTEC, 31 controls
Target organism:	*E. coli* (EHEC and VTEC)
Rapid test 1 PCR	**Methods** Cultured in trypticase soy broth at 37°C for 18–24 hours. PCR detection of *stx*1 and *stx*2 genes following methods first described by Yamasaki *et al*.[243]
Rapid test 2 Latex agglutination and ELISA	**Methods** Followed instructions published in Denka Seiken kit product insert. Uses monoclonal antibody and polyclonal antibody of vt1 and vt2 toxins
Reference test Vero cell assay	**Methods** Cultured in trypticase soy broth at 37°C for 18–24 hours. VT producability investigated following methods first described by Konowalchuk *et al*.[244]

Reported accuracy data: PCR (human samples)				**Reported accuracy data: Latex agglutination (human samples)**			
Sensitivity	100.00	**TP**	147	**Sensitivity**	100.00	**TP**	147
Specificity	100.00	**FP**	0	**Specificity**	100.00	**FP**	0
PPV	100.00	**FN**	0	**PPV**	100.00	**FN**	0
NPV	100.00	**TN**	31	**NPV**	100.00	**TN**	31

Agreement between methods:	100% concordance for all tests on humans; however, lower concordance (97.5%) experienced when 158 animal strains were tested using similar methods
Economic evaluation:	None given
What the authors conclude:	Sensitivity, specificity and concordance rate were all 100% for Vero cell culture, PCR method and VTEC Screen test
Assessment of authors' conclusions:	Authors' conclusions correct

Authors (year):	Karmali *et al*. (1999)[169]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of a microplate latex agglutination method (Verotox-F assay) for detecting and characterising verotoxins (shiga toxins) in *Escherichia coli*
Notes:	
Setting:	Pediatric hospital, Canada, and microbiology department, Czech Republic
Design:	
Sample size:	165 banked reference strains – 68 VT positive (65 human isolates) and 104 VT negative (100 human isolates)
Target organism:	Verotoxin (shiga toxin) *E. coli*

Rapid test	**Methods**
Verotox-F assay	RPLA performed according to manufacturer's instructions. Examined for latex agglutination after 20–24 hours

Reference test	**Methods**	**Positive/negative controls**	**Blinding**
Vero cell assay included in kit	"Performed as described previously" (Karmali)[245]	Positive and negative controls	

Reported accuracy data:			
Sensitivity	100	**TP**	65
Specificity	100	**FP**	0
PPV	100	**FN**	0
NPV	100	**TN**	100

Sensitivity vs reference method:	Equivalent
Turnaround time vs reference method:	Results in 1 day compared with at least 3 days' incubation for Vero cell assay
Economic evaluation:	None provided
What the authors conclude:	Rapid, reliable and easy to perform
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Kehl *et al*. (1997)[32]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of the Premier EHEC assay for detection of shiga toxin-producing *Escherichia coli*
Notes:	Manufacturer funded, plus Government grant
Setting:	Paediatric hospital laboratory, USA
Design:	Prospective evaluation study
Sample characteristics:	Consecutive samples from children's hospitals (inpatients and outpatients)
Sample size:	974 with Premier EHEC and culture
Target organism:	*E. coli*
Rapid test Premier EHEC (Meridian Diagnostics)	**Methods** Performed according to manufacturer's recommendations. Tested on overnight growth from MacConkey's broth. Spectrophotometric reading set at 450 nm, optical density >0.150
Reference test SMAC culture, cytotoxicity tests, and immunoblot assay	**Methods** All samples cultured using standard method, incubated for 18–24 hours. Samples positive by any test (n = 16) confirmed by cytoxicity testing. Positive and negative controls provided

Reported accuracy data:

Sensitivity	100	**TP**	13
Specificity	99.69	**FP**	3
PPV	81.25	**FN**	0
NPV	100	**TN**	956

Agreement between methods:	Premier EHEC assay detected 40% more STEC than conventional SMAC culture. STEC other than O157 accounted for 20% of STEC disease in children
Economic evaluation:	The cost of the assay may limit its widespread use. Good criteria would allow for the cost-effective utilisation of this assay to be determined
What the authors conclude:	Premier EHEC assay is a sensitive and specific method for the detection of all STEC isolates. Routine use would improve the detection of *E. coli* O157:H7 and allow for determination of the true incidence of STEC other than O157:H7
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Klein *et al*. (2002)[59]
Journal:	*Journal of Pediatrics*
Title:	Shiga toxin-producing *Escherichia coli* in children with diarrhoea: a prospective point-of-care study
Notes:	
Setting:	Paediatric hospital laboratory, USA
Design:	Prospective evaluation study
Sample characteristics:	Consecutive samples from children's hospitals (inpatients and outpatients)
Sample size:	1851 with Premier EHEC and culture
Target organism:	*E. coli*
Rapid test Premier EHEC (Meridian Diagnostics)	**Methods** Performed according to manufacturer's recommendations. Tested on overnight growth from MacConkey's broth. Spectrophotometric reading set at 450 nm, optical density >0.150
Reference test SMAC culture, cytotoxicity tests and immunoblot assay	**Methods** All samples cultured using standard method, incubated for 18–24 hours. Samples positive by any test (n = 16) confirmed by cytoxicity testing. Positive and negative controls provided

Reported accuracy data:

Sensitivity	89.29	**TP**	25
Specificity	100.00	**FP**	0
PPV	100.00	**FN**	3
NPV	99.84	**TN**	1823

Agreement between methods:

Economic evaluation:	The cost of the assay may limit its widespread use. Good criteria would allow for the cost-effective utilisation of this assay to be determined
What the authors conclude:	Premier EHEC assay is a sensitive and specific method for the detection of all STEC isolates. Routine use would improve the detection of *E. coli* O157:H7 and allow for determination of the true incidence of STEC other than O157:H7
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Klotz *et al*. (2003)[106]
Journal:	*Journal of Clinical Microbiology*
Title:	Detection of *Staphylococcus aureus* enterotoxins A to D by real-time fluorescence PCR assay
Notes:	
Setting:	University research laboratory, Germany
Design:	Diagnostic comparison
Sample characteristics/size:	93 banked reference strains
Target organism:	*S. aureus* enterotoxins A to D

Rapid test	**Methods**
Real-time fluorescence PCR	Detection of *S. aureus* enterotoxins A to D by real-time fluorescence PCR assay. *mecA* gene encoding methicillin resistance and the *femB* gene (a specific genomic marker for *S. aureus*) were also used

Reference test	**Methods**	**Positive/negative controls**	**Blinding**
SET-RPLA (Oxoid)	Commercially available reverse-passive latex agglutination kit used according to manufacturer's instructions	Positive controls used	Unclear

Reported accuracy data:			
Sensitivity	100	**TP**	40
Specificity	92.45	**FP**	4
PPV	90.91	**FN**	0
NPV	100	**TN**	49

Sensitivity vs reference method:	More sensitive than SET-RPLA
Turnaround time vs reference method:	rtPCR results available within 6 hours after isolation of *S. aureus*, compared with 2.5 days for SET-RPLA
Economic evaluation:	Costs per strain are about $12 for SET-RPLA and about $8 for rtPCR
What the authors conclude:	Faster, less expensive and less labour intensive than reference standard
Assessment of authors' conclusions:	Appropriate conclusions. This promising assay requires further assessment with a larger sample study

Authors (year):	Kulkarni *et al*. (2002)[12]
Journal:	*Journal of Clinical Pathology*
Title:	Detection of *Campylobacter* species: a comparison of culture and polymerase chain reaction based methods
Notes:	Shares part-authorship with Lawson *et al*.[48]
Setting:	Public Health Laboratory, UK
Design:	Prospective diagnostic accuracy study
Sample characteristics:	All stool samples received in laboratory over a 10-week period from August to October 1998. Culture and DNA extraction performed on all samples within 24 hours of receipt by laboratory
Sample size:	343 stool samples
Target organism:	*Campylobacter* species
Rapid test PCR screen and PCR–ELISA identification based on 16s rRNA gene	**Methods** PCR screening performed on all samples using RoboCycler thermocycler. Samples positive for *C. jejuni–C. coli* undergo further PCR–ELISA to identify genetic composition – specifically hip gene of *C. jejuni* and *asp* gene of *C. coli*. Initial screening of all samples using PCR technique can be performed in approximately 90 minutes, following DNA extraction. Gel electrophoresis performed to analyse sample, taking additional 30 minutes. Further workup on positive samples to confirm speciation by PCR–ELISA would increase time to results; however, this is not recorded. All PCR results available on the same day the assays were performed
Reference test Standard culture	**Methods** Selective culture performed with CCDA plates. Plates incubated microanaerobically for 2 days
Reported accuracy data:	
Sensitivity	88.24% **TP** 15
Specificity	98.47% **FP** 5
PPV	75.00% **FN** 2
NPV	99.38% **TN** 321
Additional statistical analysis:	McNemar's test used to compare alternative detection methods. No statistical differences between PCR and culture in detection of *C. jejuni–C. coli*
Major confounders or bias:	Unclear if blinding was used. By setting policy that DNA extraction must take place within 24 hours of sample arriving in the laboratory, the potential for cell degradation is reduced.
Economic evaluation:	Hypothesises "using PCR in the enteric laboratory for the detection of campylobacters is labour intensive and not cost-effective"; "advantages do not outweigh the expense". No formal economic evaluation is carried out
What the authors conclude:	The optimal method for the detection of *Campylobacters* spp. from stool samples in the diagnostic laboratory remains selective culture
Assessment of authors' conclusions:	One of the few studies to provide an economic critique on test options. PCR methods used in this study, including screening by PCR and further PCR–ELISA for identification, are somewhat more labour intensive than most current PCR techniques

Authors (year):	LaGier *et al*. (2004)[170]
Journal:	*Molecular and Cellular Probes*
Title:	A real-time multiplexed PCR assay for rapid detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli*
Notes:	
Setting:	Research laboratory, USA **Design:** Retrospective
Sample characteristics:	Strains from bacteria isolate archive, originally isolated from primary patient specimens
Sample size:	67 strains – 25 known negative, 12 known *C. coli*, 30 known *C. jejuni*
Target organism:	*C. jejuni* and *C. coli*
Rapid test Real-time multiplex PCR detecting hipO for *C. jejuni* and glyA for *C. coli*	**Methods** In-house multiplex *C. jejuni–C. coli* species-specific primer and TaqMan probe set designed with Primer Express software. Qiagen DNA stool Mini Kit used for DNA extraction. Roche LightCycler–FastStart DNA Master Hybridization Probe Kit used for rtPCR assay, which followed format from Bopp *et al*.[24] Turnaround time for PCR assay, including DNA extractions, stated as 3.5–4.0 hours
Reference test Standard culture	**Methods** Strains previously confirmed. Time taken for gold standard culture methods stated as 2–5 days
Reported accuracy data:	
Sensitivity	100.00 **TP** 40
Specificity	100.00 **FP** 0
PPV	100.00 **FN** 0
NPV	100.00 **TN** 25
Major confounders or bias:	Prohibited by sole use of reference strains – more applicable to routine diagnostic use if assay could be tested prospectively
Economic evaluation:	Increased productivity highlighted as decreased turnaround time will free up laboratory personnel working hours for other tasks
What the authors conclude:	This assay is the first successful attempt to identify and differentiate *C. jejuni* and *C. coli* directly from clinical isolates in a single reaction
Assessment of authors' conclusions:	Paper very strong on analytical methodology, but to assess diagnostic accuracy more fully a larger sample size, tested prospectively, would be required

Authors (year):	Law *et al*. (1994)[171]
Journal:	*Journal of Medical Microbiology*
Title:	Diagnosis of infections with shiga-like toxin-producing *Escherichia coli* by use of enzyme-linked immunosorbent assays for shiga-like toxins on cultured stool samples
Notes:	
Setting:	Hospital Laboratory, UK **Design:**
Sample characteristics:	Unselected cases, although they tend to have more severe illness
Sample size:	475 stool specimens from 457 patients were examined
Target organism:	STEC
Rapid test	**Methods**
ELISA	In-house-designed ELISA, as described in Law[246]
Reference test	**Methods**
SMAC culture	Cultured on SMAC, plus slide agglutination performed with *E. coli* O157 antiserum
Reported accuracy data:	

Sensitivity	100	**TP**	42
Specificity	97.2	**FP**	12
PPV	77.8	**FN**	0
NPV	100	**TN**	421

Sensitivity vs reference method:	Detects more than culture
Turnaround time vs reference method:	Faster
Economic evaluation:	None provided
What the authors conclude:	Rapid and sensitive technique, especially where low numbers of organisms are present in faeces or for non-O157 serotypes

Authors (year):	Lawson *et al*. (1998)[47]
Journal:	*Epidemiology and Infection*
Title:	Detection of *Campylobacter* in gastroenteritis: comparison of direct PCR assay of faecal samples with selective culture
Notes:	
Setting:	PHLS Laboratory and national reference laboratory, UK
Design:	Blinded evaluation
Sample characteristics:	Unselected consecutive faecal samples
Sample size:	200
Target organism:	*Campylobacter species*
Rapid test	**Methods**
Conventional PCR	
Rapid test	**Methods**
Reference test	**Methods**
Culture	Cultured on modified CCDA agar
Reported accuracy data:	

Sensitivity	93.75	**TP**	15	PCR positive	19
Specificity	97.83	**FP**	4	Culture positive	16
PPV	78.95	**FN**	1		
NPV	99.44	**TN**	180		

Economic evaluation:	"As presently configured, PCR is relatively labour intensive, and costly compared to culture, and thus is as yet unlikely to provide an alternative to culture diagnosis for *C. jejeuni* and *C. coli*"
What the authors conclude:	16 samples (8%) of 200 unselected faecal samples sent for testing at a clinical laboratory proved to be culture positive for *Campylobacter* whereas 19 (9.5%) were positive by PCR
Assessment of authors' conclusions:	Study highlights benefits of PCR when studying rarer forms of *Campylobacter*

Authors (year):	Lawson *et al*. (1999)[48]
Journal:	*Journal of Clinical Microbiology*
Title:	Large-scale survey of *Campylobacter* species in human gastroenteritis by PCR and PCR–enzyme-linked immunosorbent assay
Notes:	Based on methods first described by Linton *et al*.[15] (1997) and Lawson *et al*.[47]
Setting:	Central Public Health Laboratory, UK
Design:	Blinded prospective diagnostic accuracy study
Sample characteristics:	Fresh stool samples submitted from outpatients, GPs or environmental health officer within seven PHLS laboratories over a 2-year period
Sample size:	3738 faecal samples
Target organism:	*Campylobacter* species, including *C. jejuni*, *C. coli*, *C. upsaliensis*, *C. hypointestinalis* and *C. lari*
Rapid test PCR and PCR-ELISA detecting the 16S rDNA gene	**Methods** PCR screening performed on all samples using RoboCycler thermocycler. Samples positive for *C. jejuni–C. coli* undergo further PCR–ELISA to identify genetic composition – specifically *hip* gene of *C. jejuni* and *asp* gene of *C. coli*. Initial screening of all samples using PCR technique can be performed in approximately 90 minutes, following DNA extraction. Further workup on positive samples to confirm speciation by PCR–ELISA would increase time to results; however, this is not recorded
Reference test Standard culture	**Methods** Five laboratories cultured on *Campylobacter* Blood Free Selective Agar Base and CCDA supplement, one cultured with cefoperazone–amphotericin B–teicoplanin supplement and one used a cefoperazone and amphotericin B supplement. All plates were incubated for 48 hours under microaerobic conditions. Isolates were identified to genus level by morphology and Gram staining; the time taken for this is not recorded

Reported accuracy data:

Sensitivity	89.01	**TP**	413
Specificity	97.59	**FP**	79
PPV	83.94	**FN**	51
NPV	98.43	**TN**	3195

Agreement between methods:	0.78
Additional statistical analysis:	McNemar's test used to compare alternative detection methods. No statistical differences between PCR and culture in detection of *C. jejuni–C. coli*
Major confounders or bias:	Time between culture and receipt of faecal sample for DNA extraction was up to 10 days. Culture-positive only samples may have been PCR negative due to degradation of *Campylobacter* cells in this period
Economic evaluation:	None
What the authors conclude:	1. Key area of concern for authors in the under-reporting of some *Campylobacter* species due to culture methods designed to detect only *C. jejuni* or *C. coli*. In particular, PCR detection of previously unreported *C. upsaliensis* is highlighted. 2. There are more positive samples by PCR–ELISA than by culture alone
Assessment of authors' conclusions:	1. Focuses on epidemiological context, allowing full identification to a species level. 2. PCR assays designed for groups of enteropathogenic species rather than individual species-specific PCRs show advancement within the field and may lead to development of full multiplex design

Authors (year):	Letertre *et al*. (2003)[29]
Journal:	*Molecular and Cellular Probes*
Title:	Detection and genotyping by real-time PCR of the staphylococcal enterotoxin genes *sea* to *sej*
Notes:	
Setting:	Research laboratory, France **Design:**
Sample size:	Collection of 100 isolations – 83 *S. aureus* and 17 other bacteria
Target organism:	*S. aureus sea* to *sej*
Rapid test	**Methods**
rtPCR	In-house-designed rtPCR detecting *sea* to *sej* genes performed using SYBR Green in LightCycler system

Reference test	**Methods**	**Positive/negative controls**	**Blinding**
Conventional block cycler PCR and RPLA test	PCR using Staur4 and Staur6 primers to target sequence of 23S rRNA. RPLA test by Oxoid	All banked strains – 17 non-*S. aureus* and 83 known *S. aureus*	Unclear

Reported accuracy data:		
Sensitivity	**TP**	57
Specificity	**FP**	11
PPV	**FN**	0
NPV	**TN**	32

Sensitivity vs reference method:	Full correlation between conventional and rtPCR
Turnaround time vs reference method	Yields data within 1 hour versus 5 hours with conventional block cycler PCR and gel electrophoresis
Economic evaluation:	None given
What the authors conclude:	A very quick, reliable and specific alternative to conventional block cycler PCRs to identify the enterotoxin profile of toxigenic *S. aureus*
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Linton *et al*. (1997)[15]
Journal:	*Journal of Clinical Microbiology*
Title:	PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrhoeic samples
Notes:	
Setting:	PHLS Laboratory, UK **Design:** Retrospective
Sample characteristics:	DNA extracted from 18 culture-positive diarrhoeic stools, 5 culture-negative diarrhoeic stools, 20 culture-negative healthy stools
Sample size:	43 faecal samples
Target organism:	*C. jejuni* (16s rRNA, hip) and *C. coli* (16S rRNA, asp)
Rapid test PCR	**Methods** 16s rRNA PCR annealing temperature 58°C, generating an amplicon of 853 bp. *HipO* PCR annealing temperature 66°C, generating an amplicon of 735 bp
Reference test Culture	**Methods** Culture on modified CCDA, incubated microaerobically at 37°C for 48 hours. Identified biochemically by Gram stain, oxidase and catalase activities, hippurate hydrolysis, indoxyl acetate hydrolysis, hydrogen sulfide production from triple sugar iron agar and susceptibility to nalidixic acid and cephalothin

Reported accuracy: 16s rRNA gene				**Reported accuracy: *hipO* gene**			
Sensitivity	100.00	**TP**	18	**Sensitivity**	100.00	**TP**	18
Specificity	100.00	**FP**	0	**Specificity**	100.00	**FP**	0
PPV	100.00	**FN**	0	**PPV**	100.00	**FN**	0
NPV	100.00	**TN**	25	**NPV**	100.00	**TN**	25

Major confounders or bias:	Study not blinded as culture results already known
Economic evaluation:	None provided
What the authors conclude:	This study assessed the ability of PCR to detect *Campylobacter* in stools that were already culture positive. Coidentification of *C. hypointestinalis* in one *C. jejuni* sample where this was missed by culture indicates PCR may be more sensitive for all *Campylobacter* strains
Assessment of authors' conclusions:	Small ($n = 45$) study. Focus of paper on epidemiological study of *Campylobacter* spp., including differentiation of *coli*/*jejuni*/less common species

Authors (year):	Luk *et al*. (1997)[51]
Journal:	*Journal of Clinical Microbiology*
Title:	An enzyme-linked immunosorbent assay to detect PCR products of the rfbS gene from serogroup D *Salmonellae*: a rapid screening prototype
Notes:	
Setting:	Hospital laboratory, Sweden **Design:** Prospective
Sample size:	203 consecutive stool samples from patients with diarrhoea
Target organism:	*Salmonella* serotype D
Rapid test DIG-ELISA following a PCR	**Methods** Samples inoculated into enrichment Rappaport broth overnight at 37°C. DNA extracted, followed by PCR and DIG–ELISA
Reference test *Salmonella* culture	**Methods** Standard procedures as described by Murray *et al*.[9]

Reported accuracy data:			
Sensitivity	56.55	**TP**	82
Specificity	100	**FP**	0
PPV	100	**FN**	63
NPV	52.07	**TN**	58

Time allocation:	Assay takes approximately 6 hours (PCR 4 hours; ELISA 2 hours) along with brief enrichment cultivation of the samples (from 4 to 16 hours)
Economic evaluation:	None provided
What the authors conclude	A fast, accurate, semiquantitative means of detecting infectious agents such as *Salmonellae*, and future robotic automation is possible
Assessment of authors' conclusions:	Appropriate conclusions. Assay only designed to detect serotype D so low sensitivity for species overall. Diagnostic value limited to serotype D only

Authors (year):	Mackenzie *et al*. (1998)[33]
Journal:	*Journal of Clinical Microbiology*
Title:	Sensitivities and specificities of premier *E. coli* O157 and premier EHEC enzyme immunoassays for diagnosis of infection with verotoxin (shiga-like toxin)-producing *Escherichia coli*. The SYNSORB Pk Study investigators
Notes:	2 × 2 data derived from 'field' cases (i.e. non-resolved) only, to mirror diagnostic practice
Setting:	Paediatric emergency setting, Canada
Design:	Prospective evaluation
Sample characteristics:	876 children aged between 1 month and 8 years presenting with acute diarrhoea to emergency centres in Canada between 1 June 1996 and 31 October 1996
Sample size:	877 samples
Target organism:	*E. coli* O157 and all enterohaemorrhagic strains of *E. coli*
Rapid test Premier *E. coli* O157 and Premier EHEC	**Methods** Premier *E. coli* O157 evaluated against SMAC culture. Premier EHEC evaluated against initial Premier *E. coli* O157 results to assess prevalence of non-O157 strains. Results read using spectrophotometer at 0.3 U at 450 nm. Both assays performed according to manufacturer's instructions (Meridian Diagnostics)
Reference test SMAC culture	**Methods** Stools cultured for *E.coli* O157 and for other bacterial stool pathogens, each centre using its own routine procedure. All sites used SMAC

Reported accuracy data: *E. coli* O157				**Reported accuracy data: EHEC**			
Sensitivity	86.36	**TP**	57	**Sensitivity**	89	**TP**	50
Specificity	98.4	**FP**	13	**Specificity**	92.32	**FP**	63
PPV	81.43	**FN**	9	**PPV**	44.25	**FN**	6
NPV	98.88	**TN**	798	**NPV**	99.21	**TN**	757

Agreement between methods:	Discordant results tested in independent laboratory using cytoxicity and non-O157 verotoxin-producing *E. coli* culture
Economic evaluation:	"At this time, rapid immunological tests are the most economical way" to address problem that SMAC culture can only confidently identify O157:H7 strains.
What the authors conclude:	The availability of a simple test for the presence of verotoxin will determine the true prevalence of non-O157 VTEC and of toxin production by organisms other than *E. coli*
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Mackenzie *et al*. (2000)[67]
Journal:	*Journal of Clinical Microbiology*
Title:	Performance of the ImmunoCard STAT! *E. coli* O157:H7 test for detection of *Escherichia coli* O157:H7 in stools
Notes:	2 × 2 data derived from prospective evaluation only, to mirror diagnostic practice
Setting:	Multi-site evaluation, Canada
Design:	Prospective and retrospective
Sample characteristics:	277 samples in prospective study; 522 stored stool specimens in retrospective study
Sample size:	277 samples prospective; 522 retrospective
Target organism:	*E. coli* O157 strain only
Rapid test ImmunoCard STAT! EIA	**Methods** ImmunoCard STAT! O157:H7 test evaluated against SMAC culture. Assay performed according to manufacturers' instructions (ICS, Meridian Diagnostics)
Reference test SMAC culture	**Methods** Stools cultured for E.coli O157 on SMAC and sorbitol-negative colonies were identified as O157 by latex agglutination

Reported accuracy data: Prospective				**Reported accuracy data: Retrospective**			
Sensitivity	92.86	**TP**	13	**Sensitivity**	81.29	**TP**	339
Specificity	100	**FP**	0	**Specificity**	95.15	**FP**	5
PPV	100	**FN**	1	**PPV**	98.55	**FN**	78
NPV	99.62	**TN**	263	**NPV**	55.68	**TN**	98

Economic evaluation:	None provided
What the authors conclude:	The ImmunoCard STAT! O157:H7 test has a high sensitivity and specificity. It is simple to perform, the direct test gives a result within 10 minutes and the test will be of particular value in areas where *E. coli* O157:H7 is the predominant VTEC serotype
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Maher *et al*. (2003)[172]
Journal:	*Journal of Medical Microbiology*
Title:	Evaluation of culture methods and a DNA probe-based PCR assay for detection of *Campylobacter* species in clinical specimens of faeces
Notes:	
Setting:	Research laboratory, Ireland
Sample characteristics:	Faecal samples submitted from hospital and community
Sample size:	320 stool samples
Target organism:	*Campylobacter* species
Rapid test PCR/DNA probe membrane-based colorimetric assay, using 16S/23S DNA	**Methods** DNA extracted with QIAamp DNA stool Minikit
Reference test Standard culture	**Methods** Direct inoculation of faeces on to modified CCDA, comprising of blood-free selective agar with *Campylobacter*-selective supplement and *Campylobacter* growth supplement. Enrichment in modified Preston broth for 48 hours at 37°C prior to plating
Reported accuracy data:	
Sensitivity	94.44 **TP** 17
Specificity	59.41 **FP** 41
PPV	29.31 **FN** 1
NPV	98.36 **TN** 60
Additional statistical analysis:	This study also provided comparison of direct CCDA culture and culture with pre-enrichment, using a sample of 320 faecal specimens
Major confounders or bias:	From the subset of 127 samples used to evaluate PCR methods against culture, it was not possible to extract DNA from eight samples, one of which was culture positive
Economic evaluation:	None provided
What the authors conclude:	Molecular diagnosis useful for more obscure *Campylobacter* species
Assessment of authors' conclusions:	This assay detects *Campylobacter* species in 41 (38%) of culture-negative specimens. A second 16s PCR/DNA probe assay confirmed that 35 of these were true positives. This proves that 35 out of the total 320 (11%), of samples would remain undiagnosed using conventional culture methods

Authors (year):	Malorny *et al*. (2003)[173]
Journal:	*Applied and Environmental Microbiology*
Title:	Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard
Notes:	
Setting:	International collaborative study **Design:** Validation study
Sample size:	12 *Salmonella* and 16 non-*Salmonella* coded (blind) DNA samples sent to 16 international laboratories
Target organism:	*Salmonella* species
Rapid test PCR targeting *invA* gene	**Methods** Validation study based on PCR assay with primer set 139–141, designed by Rahn *et al*.[247]
Reference test DNA from known strains	**Methods** Strains grown aerobically without shaking at 37°C for 16 hours in Luria–Bertani medium. DNA extraction by conventional methods
Reported accuracy data:	
Sensitivity	95.93 **TP** 495
Specificity	98.98 **FP** 7
PPV	98.61 **FN** 21
NPV	97.01 **TN** 681
Economic evaluation:	None provided
What the authors conclude:	The collaborative study showed a high sensitivity and reproducibility of the PCR assay among the 16 international laboratories when identical batches of reagents were used
Assessment of authors' conclusions:	Appropriate conclusions; however, validation with fresh clinical samples is advised

Authors (year):	Mpamugo *et al*. (1995)[81]
Journal:	*Journal of Medical Microbiology*
Title:	Enterotoxigenic *Clostridium perfringens* as a cause of sporadic cases of diarrhoea
Notes:	
Setting:	Reference laboratory, UK **Design:** Prospective
Sample characteristics:	Consecutive stool samples collected in 2-month period
Sample size:	370 specimens
Target organism:	*C. perfringens*
Rapid test	**Methods** Oxoid RPLA performed according to manufacturer's instructions
Reference test	**Methods** Confirmation of positive cases with ELISA
Reported accuracy data:	
Sensitivity	Unclear **TP** 65 positive cases
Specificity	Unclear **FP** Unclear
PPV	Unclear **FN** Unclear
NPV	Unclear **TN** 305 negative cases
Economic evaluation:	None provided
What the authors conclude:	Diagnosis should be confirmed by the detection of enterotoxin, but further work is still required to assess whether an acceptable accuracy is obtained with RPLA kit or whether ELISA is needed in all cases
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	McGowan and Rubenstein (1989)[142]
Journal:	*American Journal of Clinical Pathology*
Title:	Use of a rapid latex agglutination test to detect *Salmonella* and *Shigella* antigens from Gram-negative enrichment
Notes:	
Setting:	Children's hospital, USA **Design:** Consecutive, prospective
Sample characteristics:	Children recruited from December to June 1988
Sample size:	2481 rectal or faecal swabs
Target organism:	*Salmonella* and *Shigella*
Rapid test Wampole Bactigen	**Methods** After primary culture and incubation (max. 18 hours) in Gram-negative broth, Wampole Bactigen *Salmonella–Shigella* latex agglutination test used. Three reagents used: (1) coated with goat antibodies to more than 80 common *Salmonella* serogroups; (2) layered with rabbit antibodies to *Shigella* group B and D; (3) bears rabbit antibodies to *Shigella* groups A and C
Reference test Standard culture	**Methods** Samples inoculated on a primary media culture and then placed in a Gram-negative broth. After incubation, sample subcultured to XLD and MacConkey agars, which were aerobically incubated at 35°C and examined every 24 hours for a total of 48 hours
Reported accuracy data:	
Sensitivity	85.86 **TP** 85
Specificity	97.27 **FP** 65
PPV	56.67 **FN** 14
NPV	99.39 **TN** 2317
Economic evaluation:	"In our institution, eliminating the workup of colonies from GNB subculture plates would result in a media saving of $124 per 100 stool specimens cultured and a time saving of approximately 20 hours per 100 stool specimens cultured" (US$ 1998)
What the authors conclude:	Tests such as the Bactigen latex test can decrease the overall turnaround time for a specimen, particularly those with negative results
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Metzler and Nachamkin (1988)[175]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of a latex agglutination test for the detection of *Salmonella* and *Shigella* spp. by using broth enrichment
Notes:	
Setting:	University laboratory, USA **Design:** Consecutive, prospective
Sample size:	1128 stool samples
Target organism:	*Salmonella* and *Shigella*
Rapid test Bactigen *Salmonella–Shigella* latex agglutination	**Methods** Three reagents used: (1) coated with goat antibodies to more than 80 common *Salmonella* serogroups; (2) layered with rabbit antibodies to *Shigella* group B and D; (3) bears rabbit antibodies to *Shigella* groups A and C.
Reference test Standard culture	**Methods** Samples inoculated on a primary media culture and then placed in a Gram-negative broth
Reported accuracy data:	
Sensitivity	86.21 **TP** 25
Specificity	96.54 **FP** 38
PPV	39.68 **FN** 4
NPV	99.62 **TN** 1061
Economic evaluation:	None provided
What the authors conclude:	*Salmonella* test may be useful as an enrichment broth screening test
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Munoz (1993)[174]
Journal:	*Clinical Microbiology and Infectious Diseases*
Title:	Rapid screening of *Salmonella* species from stool cultures
Notes:	Results obtained on different selective media pooled for meta-analysis
Setting:	Hospital laboratory, Spain **Design:** Prospective, consecutive
Sample characteristics:	All clinical stool specimens received into laboratory in 3-month period
Sample size:	976 suspected colonies from 555 clinical samples and 480 patients
Target organism:	*Salmonella* spp.
Rapid test	**Methods**
MUCAP (Biolife, Italy)	Based on a rapid detection of C8 esterase enzyme by using a fluorogenic 4-methylumbelliferone-conjugated substrate
Reference test	**Methods**
Standard culture	MacConkey Agar, *Salmonella–Shigella* agar, and Brilliant Green agar, inoculated overnight. All suspect colonies overwent Triple Sugar Iron agar test and subculture
Reported accuracy data:	
Sensitivity	100 **TP** 176
Specificity	91.87 **FP** 65
PPV	73.03 **FN** 0
NPV	100 **TN** 735
Economic evaluation:	"In Spain, the estimated cost is $1.00 per day (US$ 1993). Laboratories with a lower incidence of *Salmonella* should determine whether it is worthwhile to incorporate the test in routine procedures or reserve it for special circumstances"
What the authors conclude:	MUCAP can enhance the rate of recognition of *Salmonella* colonies in the presence of mixed-lactose-fermenting bacteria on the agar surface. It also facilitates the detection of rare colonies with uncommon biochemical characteristics
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Novicki *et al*. (2000)[60]
Journal:	*Journal of Clinical Microbiology*
Title:	Comparison of sorbitol MacConkey agar and a two-step method which utilises enzyme-linked immunosorbent assay toxin testing and a chromogenic agar to detect and isolate enterohemorrhagic *Escherichia coli*
Notes:	Shares authorship with Carroll[58]
Setting:	University research laboratory **Design:** Prospective evaluation
Sample size:	488 stool samples from adults and children
Target organism:	EHEC
Rapid test	**Methods**
Premier EHEC ELISA	Two-step method utilising chromogenic selective-differential medium (Rainbow Agar O157) for the isolation of *E. coli* together with Premier EHEC ELISA to detect *stx1* and *stx2*.
Reference test	**Methods**
SMAC culture	Using accepted biochemical and phenotypic tests. Discrepancy resolution by PCR method in independent laboratory
Reported accuracy data:	
Sensitivity	82.4 **TP** 14
Specificity	100 **FP** 0
PPV	100 **FN** 3
NPV	99.37 **TN** 471
Sensitivity vs reference method:	Compared with a Vero cell assay, SMAC had sensitivities of 23.5% for the identification of EHEC serotypes and 50.0% for the identification of O157:H7 alone. The two-step method had sensitivities of 76.5% and 100%, respectively. The Premier EHEC alone had a sensitivity of 82.4% in the detection of *stx1* and *stx2*.
Economic evaluation:	Comparative costs of materials provided
What the authors conclude:	ELISA–Rainbow Agar method proved superior to SMAC in isolating both O157:H7 and other EHEC serotypes
Assessment of authors' conclusions:	Well-structured paper

Authors (year):	Park *et al*. (1996)[176]
Journal:	*Journal of Clinical Microbiology*
Title:	Rapid immunoassay for detection of *Escherichia coli* O157 directly from stool specimens
Notes:	
Setting:	Hospital laboratory, USA **Design:** Prospective comparison
Sample characteristics:	601 stool samples collected over 4-year period in hospital laboratory, plus four known positive specimens
Sample size:	605
Target organism:	*E. coli* O157
Rapid test *E. coli* O157 antigen detection test	**Methods** Kit developed by LMD Laboratories (NB no longer commercially available)
Reference test SMAC culture	**Methods** Standard SMAC plate – if organism not recovered in primary culture SMAC plate swept tube of MacConkey broth used. Incubated at 35°C for 18–24 hours
Reported accuracy data:	
Sensitivity	91.1765 **TP** 31
Specificity	99.4746 **FP** 3
PPV	91.1765 **FN** 3
NPV	99.4746 **TN** 568
Agreement between methods:	Immunofluorescence stain used to arbitrate differences between SMAC culture and ELISA results
Economic evaluation:	"The cost of materials is higher for screening stool samples by ELISA than it is when conventional culture methods are used. The average cost per ELISA is approximately $2 to $3; however, its use saves the additional costs of technologist time associated with follow-up testing of sorbitol-negative non-*E. coli* colonies from SMAC. The cost benefit in using ELISA comes from the rapid turnaround time it provides"
What the authors conclude:	The assay is accurate and rapid (<1 hour) for the detection of serotype O157 only
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Park *et al*. (2003)[70]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of the Duopath Verotoxin test for detection of shiga toxins in cultures of human stools
Notes:	
Setting:	Hospital laboratory, USA **Design:** Retrospective and prospective evaluation
Sample characteristics:	41 frozen stool samples known to contain STEC isolates (O157:H7 and non-O157:H7
Target organism:	Shiga toxin-producing *E. coli*
Rapid test	**Methods**
Duopath Verotoxin (Merck)	Immunochromogenic test designed to detect *stx* in food samples – applied to clinical specimens for the first time. Samples previously isolated using SMAC (18–24 hours)

Reference test	**Methods**	**Positive/negative controls**	**Blinding**
Premier EHEC assay (Meridian Bioscience)	Suspended in MacConkey broth and incubated for 18–24 hours. Procedure then performed according to instructions of manufacturer	41 known positive samples included	Yes

Reported accuracy data:			
Sensitivity	100	**TP**	43
Specificity	100	**FP**	0
PPV	100	**FN**	0
NPV	100	**TN**	248

Sensitivity vs reference method:	Equivalence
Turnaround time vs reference method	Provides a turnaround time of 24 hours
Economic evaluation:	None provided
What the authors conclude:	Simple to perform and easy to interpret. Potential for clinical application
Assessment of authors' conclusions:	Suitable conclusions

Authors (year):	Paton and Paton (1998)[177]
Journal:	*Journal of Clinical Microbiology*
Title:	Detection and characterisation of shiga toxigenic *Escherichia coli* using multiplex PCR assays for *stx1*, *stx2*, *eaeA*, enterohemorrhagic *E. coli hlyA*, *rfb*O111, and *rfb*157
Setting:	Hospital laboratory **Design:** Diagnostic comparison
Sample characteristics:	Banked isolates from 28 human, 7 animal and 17 food sources; 8 culture-positive clinical STEC cases and 32 STEC culture-negative clinical samples
Sample size:	92 (52 strains and 40 fresh samples)
Target organism:	EHEC
Rapid test	**Methods**
Multiplex PCR	Detects *stx1*, *stx2*, *eaeA* and *hlyA* genes. Results read with electrophoresis
Reference test	**Methods**
	Traditional culture

Reported accuracy data:			
Sensitivity	100	**TP**	60
Specificity	100	**FP**	0
PPV	100	**FN**	32
NPV	100	**TN**	0

Major confounders or bias:	Use of banked reference strains may not demonstrate real-life diagnostic utility of this assay
Economic evaluation:	Not carried out
What the authors conclude:	The assay can be used for determining the toxin genotype of STEC isolates, and also for direct detection of toxin genes in primary faecal culture extracts
Assessment of authors' conclusions:	Appropriate conclusion. Further prospective evaluation required

Authors (year):	Paton and Paton (2005)[56]
Journal:	*Journal of Clinical Microbiology*
Title:	Multiplex PCR for direct detection of shiga toxigenic *Escherichia coli* strains producing the novel subtilase cytotoxin
Notes:	
Setting:	University laboratory, Australia **Design:**
Sample characteristics:	171 primary faecal cultures from patients with diarrhoea, plus 12 healthy controls
Sample size:	183 samples
Target organism:	STEC
Rapid test PCR	**Methods** Detection of toxin A subunit gene *subA*, *stx1* and *stx2*, with amplification products of 556, 180, and 255 bp, respectively. Assay first used with 44 STEC strains to test efficacy, followed by analysis of primary faecal culture extracts
Reference test Standard culture	**Methods** Methods not provided
Reported accuracy data:	
Sensitivity	100.00 **TP** 171
Specificity	100.00 **FP** 0
PPV	100.00 **FN** 0
NPV	100.00 **TN** 83
Major confounders or bias:	Time delay of up to 4 years between primary culture and PCR detection. Samples stored at –15°C, but unclear how this affects pathogenicity
Economic evaluation:	None provided
What the authors conclude:	Main focus of paper examines the prevalence of potent AB5 cytotoxin (SubAB) in STEC strains. The combination of *stx1*, *stx2* and subAB target primers in this assay can successfully identify shiga toxins in all 171 faecal cultures, and does not produce any false positives among 12 negative controls
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Pulz *et al*. (2003)[22]
Journal:	*Journal of Clinical Microbiology*
Title:	Comparison of a shiga toxin enzyme-linked immunosorbent assay and two types of PCR for detection of shiga toxin-producing *Escherichia coli* in human stool specimens
Sample size:	295 stool specimens
Target organism:	Shiga toxin-producing *E. coli*
Rapid test 1 rtPCR	**Methods** Detection of *stx* genes with LightCycler instrument performed with a single capillary tube using melting point analysis
Rapid test 2 RIDASCREEN Verotoxin ELISA (BioPharm)	**Methods** Detects presence of *stx1* and *stx2*. Absorbances measured at 450 nm using a spectrophotometer. Positive and negative controls run with each test

Reference test	**Methods**	**Positive/negative controls**	**Blinding**
Conventional PCR	Block cycler PCR followed by gel electrophoresis	STEC strain EDL933 used as positive control	

Reported accuracy data: ELISA vs Conventional PCR				**Reported accuracy data: rtPCR vs Conventional PCR**			
Sensitivity	67.40	**TP**	31	**Sensitivity**	100.00	**TP**	46
Specificity	96.80	**FP**	8	**Specificity**	80.00	**FP**	5
PPV	79.50	**FN**	15	**PPV**	90.10	**FN**	0
NPV	94.10	**TN**	241	**NPV**	100.00	**TN**	244

Sensitivity vs reference method:	rtPCR better, immunoassay worse
Turnaround time vs reference method	Faster
Economic evaluation:	None provided
What the authors conclude:	The detection of STEC by molecular methods is significantly more effective than detection by a licensed commercially available immunoassay
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Ramotar *et al*. (1995)[178]
Journal:	*Journal of Clinical Microbiology*
Title:	Direct detection of verotoxin-producing *Escherichia coli* in stool samples by PCR
Notes:	
Setting:	Hospital laboratory, Canada **Design:** Diagnostic comparison
Sample characteristics:	Patients presenting at the outpatient clinics of the Calgary General Hospital (July to September 1990, April to September 1993), the Alberta Children's Hospital (May to August 1992), and the Calgary Medical Laboratories
Sample size:	121 faecal samples
Target organism:	**EHEC**
Rapid test PCR for *stx1* and *stx2* genes	**Methods** Processed in Perkin-Elmer Cetus thermal cycler. Positive and negative controls provided. Stool samples showing positive results by PCR were retested on two further occasions several days and 4–8 weeks later to examine the reproducibility of PCR testing
Reference test SMAC culture	**Methods** Culture on STAC or by colony blots with gene probes. 2 × 2 data below correspond to evaluation against SMAC culture only
Reported accuracy data:	
Sensitivity	95.00 **TP** 19
Specificity	97.03 **FP** 3
PPV	86.36 **FN** 1
NPV	98.99 **TN** 98
Economic evaluation:	Not carried out
What the authors conclude:	PCR of DNA extracted directly from stool samples provides a rapid method for the detection of stool samples containing verotoxin-producing *E. coli* compared with colony blot testing
Assessment of authors' conclusions:	Appropriate conclusions. Well-designed study

Authors (year):	Reischl *et al*. (2002)[71]
Journal:	*Journal of Clinical Microbiology*
Title:	Real-time fluorescence PCR assays for detection and characterisation of shiga toxin, intimin, and enterohemolysin genes from shiga toxin-producing *Escherichia coli*
Notes:	
Setting:	Research laboratory, Germany **Design:**
Sample characteristics:	Banked reference strains
Sample size:	504
Target organism:	Shiga toxin-producing *E. coli*
Rapid test	**Methods**
rtPCR	Duplex real-time fluorescence PCR Assay detecting *stx1* and *stx2* and intimin (*eae*) and enterohemolysin (*E-hly*) genes. rtPCR performed on LightCycler machine

Reference test	**Methods**	**Posistive/negative controls**	**Blinding**
Conventional PCR		Both	Unclear

Reported accuracy data:			
Sensitivity	100	**TP**	272
Specificity	100	**FP**	100
PPV	100	**FN**	100
NPV	100	**TN**	350

Sensitivity vs reference method:	Equivalent
Turnaround time vs reference method:	Faster
Economic evaluation:	None performed
What the authors conclude:	Although they are currently more expensive to perform than block cycler PCR assays, the speed, greater information and reliability of the results make the LC-PCR assays attractive alternatives to conventional block cycler PCR assays for the detection and characterisation of STEC
Assessment of authors' conclusions:	Useful study to show equivalence of conventional and quantitative PCR techniques

Authors (year):	Rohner *et al*. (1992)[179]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of the Wellcolex Colour *Salmonella* test for detection of *Salmonella* spp. in enrichment broths
Notes:	
Setting:	Hospital laboratory, Belgium **Design:** Diagnostic comparison
Sample size:	1010 routine stool samples from patients with diarrhoeal illness.
Target organism:	*Salmonella* species
Rapid test	**Methods**
Wellcolex Colour *Salmonella*	Latex agglutination method with Selenite F Broth. 18–24 hours incubation necessary. Test performed as recommended by manufacturer. Two latex reagents and three positive controls included in kit.
Reference test	**Methods**
Standard culture	Stool samples inoculated on to MacConkey agar, Hektoen enteric agar and 10 ml of Gram-negative broth. Incubated for 18–24 hours at 35°C

Reported accuracy data:			
Sensitivity	87.18	**TP**	34
Specificity	99.79	**FP**	2
PPV	94.44	**FN**	5
NPV	99.49	**TN**	969

Economic evaluation:	None provided
What the authors conclude:	Simple to use, and positive results are easily interpretable. Allows the early diagnosis of salmonellosis
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Schraft and Griffiths (1995)[180]
Journal:	*Applied and Environmental Microbiology*
Title:	Specific oligonucleotide primers for detection of lecithinase-positive *Bacillus* spp. by PCR
Notes:	
Setting:	University laboratory **Design:** Evaluation of a new assay
Sample characteristics:	*B. cereus* strains obtained from various laboratories
Sample size:	56 *B. cereus* strains, of which 35 were food poisoning outbreak isolates
Target organism:	*B. cereus*
Rapid test	**Methods** PCR assay and PCR–hybridisation assay. Requires isolation followed by 24 hours
Reference test	**Methods** Comparison with previous isolation
Reported accuracy data:	Not given
Economic evaluation:	No economic data presented
What the authors conclude:	The assay has high specificity based on three oligonucleotides for isolates of the *B. cereus* group. The detection limit for *B. cereus* in artificially contaminated milk was 1 cfu ml^{-1} of milk using a combined PCR–hybridisation assay
Assessment of authors' conclusions:	Test requires evaluation of diagnostic accuracy

Authors (year):	Sharma *et al*. (2000)[107]		
Journal:	*Applied and Environmental Microbiology*		
Title:	Development of a single-reaction multiplex PCR toxin typing assay for *Staphylococcus aureus* strains		
Notes:			
Setting:	University research laboratory, UK		
Design:	Diagnostic accuracy comparison		
Sample size:	257 reference strains of *S. aureus* from various environmental sources, including 39 human strains		
Target organism:	*S. aureus* strains		
Rapid test Multiplex PCR	**Methods** Multiplex PCR for staphylococcal enterotoxins A to E. Results within 3–4 hours		
Reference test SET-RPLA (Oxoid)	**Methods** Used according to manufacturer's instructions		
Reported accuracy data:			
Sensitivity	100	**TP**	155
Specificity	98.73	**FP**	2
PPV	100	**FN**	0
NPV	100	**TN**	0
Economic evaluation:	None provided		
What the authors conclude:	Recommend use as a screening test for presence of enterotoxin genes		

Authors (year):	Tan *et al*. (1997)[96]
Journal:	*Journal of Applied Microbiology*
Title:	The use of *Bacillus* diarrhoeal enterotoxin detection using an ELISA technique in the confirmation of the aetiology of *Bacillus* mediated diarrhoea
Notes:	
Setting:	Public Health Laboratories **Design:** Evaluation of a commercial assay
Sample characteristics:	Reference *B. cereus* strains obtained from various laboratories
Sample size:	28 faecal samples, 34 food samples and 41 isolates
Target organism:	*B. cereus* diarrhoeal strain
Rapid test	**Methods** Tecra Bacillus Diarrhoea Enterotoxin Visual Immunoassay ELISA kit
Reference test	**Methods** Comparison with isolation of *B. cereus* for food samples and with food poisoning history for faecal samples
Reported accuracy data:	

	Faecal	Food	
Sensitivity	(10/15)	(7/9)	**TP**
Specificity	(1/13)	(0/8)	**FP**
PPV			**FN**
NPV			**TN**

Economic evaluation:	No economic data presented
What the authors conclude:	The study shows the usefulness of the Tecra Bacillus ELISA test for the diagnosis of diarrhoeal *B. cereus* food poisoning
Assessment of authors' conclusions:	This study demonstrates the usefulness of ELISA as an added test for supporting the diagnosis of *B. cereus* food poisoning

Authors (year):	Tansuphasiri *et al*. (2002)[91]
Journal:	*Journal of the Medical Association of Thailand*
Title:	PCR detection and prevalence of enterotoxin (cpe) gene in *Clostridium perfringens* isolated from diarrhoea patients
Notes:	
Setting:	Reference laboratory, Thailand **Design:** Assay development
Sample characteristics:	Consecutive 477 faecal colonies from 233 patients
Target organism:	*C. perfringens*
Rapid test PCR	**Methods** Duplex PCR using two sets of primers which amplify in the same reaction two different gene fragments: the phospholipase C (*plc*, alpha-toxin) and the enterotoxin (*cpe*) genes in *C. perfringens*
Reference test Culture	**Methods** Primary cultures of TSC–egg yolk agar and additional testing with Oxoid RPLA kit
Reported accuracy data:	Not provided
Economic evaluation:	"PCR assay is faster, less expensive and more suitable for large-scale use in epidemiological studies than conventional procedures"
What the authors conclude:	Recommend this assay to screen for enterotoxigenic *C. perfringens* isolates from primary faecal spore isolation cultures, particularly in elderly patients with food-borne diarrhoea and non-food related diarrhoea
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Tolcin *et al*. (2000)[181]
Journal:	*Journal of Clinical Microbiology*
Title:	Evaluation of the Alexon-Trend ProSpecT *Campylobacter* microplate assay
Notes:	
Setting:	Hospital laboratory with samples from three institutions
Design:	Diagnostic comparison
Sample characteristics:	Clinical stool samples
Sample size:	164 stool samples from 164 individuals
Target organism:	*Campylobacter*
Rapid test ProSpecT Microplate assay	**Methods** Commercial enzyme immunoassay (Alexon-Trend, USA), performed according to instructions of the manufacturer. Reactions read visually and spectrophotometrically in a single-wavelength spectrophotometer at 450 nm
Reference test Culture	**Methods** Not reported (referenced *Manual of Clinical Microbiology*, 7th ed.)
Reported accuracy data:	
Sensitivity	96.00 **TP** 48
Specificity	99.12 **FP** 1
PPV	97.96 **FN** 2
NPV	98.26 **TN** 113
Additional statistical analysis:	"Excellent" interobserver agreement in both the visual and spectrophotometric test interpretations
Major confounders or bias:	Blinding not possible as culture results known previously
Economic evaluation:	Not carried out
What the authors conclude:	The assay demonstrated 96% sensitivity and 99% specificity and is an acceptable alternative method of *Campylobacter* detection
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Villasante *et al*. (1987)[54]
Journal:	*Journal of Clinical Microbiology*
Title:	Rapid automated method for screening of enteric pathogens from stool specimens
Notes:	
Setting:	Hospital laboratory, Spain **Design:** Diagnostic comparison
Sample size:	800 colonies on stool differential agar media with characteristic biochemical activity of *Salmonella*, *Shigella* or *Yersinia*
Target organism:	*Salmonella*, *Shigella* or *Yersinia*
Rapid test AutoMicroBic EPS	**Methods** Entero Pathogen Screen cards of the AutoMicrobic system (Vitek Systems)
Reference test Biochemical test media	**Methods** Triple sugar iron agar, urea agar, and phenylalanine agar
Reported accuracy data:	
Sensitivity	98.93 **TP** 185
Specificity	95.76 **FP** 26
PPV	87.68 **FN** 2
NPV	99.66 **TN** 587
Economic evaluation:	None provided
What the authors conclude:	A fast, easy and sensitive method for screening for *Salmonella*, *Shigella* or *Yersinia* species
Assessment of authors' conclusions:	Appropriate conclusions

Authors (year):	Welinder-Olsson *et al*. (2004)[21]
Journal:	*Epidemiology and Infection*
Title:	EHEC outbreak among staff at a children's hospital – use of PCR for verocytotoxin detection and PFGE for epidemiological investigation
Notes:	
Setting:	Community **Design:** Case report
Sample characteristics:	Nursing staff at a children's hospital with approximately 1600 employees
Sample size:	58
Target organism:	*E. coli*
Rapid test	**Methods** PCR
Reference test	**Methods** SMAC agar for culture
Reported accuracy data:	
Sensitivity	100 **TP** 9
Specificity	96 **FP** 2
PPV	81.81 **FN** 0
NPV	100 **TN** 48
Economic evaluation:	Not carried out
What the authors conclude:	Propose routinely performed screening for EHEC using PCR for patients suffering from diarrhoea
Assessment of authors' conclusions:	Well executed evaluation of EHEC outbreak. Appropriate conclusions

Authors (year):	Welinder-Olsson *et al*. (2000)[57]						
Journal:	*European Journal of Clinical Microbiology and Infectious Diseases*						
Title:	Improved microbiological techniques using the polymerase chain reaction and pulse-field gel electrophoresis for diagnosis and follow-up of enterohaemorrhagic *Escherichia coli* Infection						
Notes:							
Setting:	Hospital laboratory, Sweden **Design:** Large-scale prospective						
Sample characteristics:	Samples collected during 1997–8. All samples from under 15-year-olds with diarrhoea and older patients with a reported diagnosis of bloody diarrhoea were sent to university hospital for PCR detection						
Sample size:	3948 fresh clinical samples						
Target organism:	EHEC						
Rapid test PCR detection of EHEC using primers for *stx1*, *stx2* and *eae* genes	**Methods** Suitable reference strains used for positive and negative controls. Gel electrophoresis run time of 22 hours. Amplified in Perkin-Elmer DNA thermal cycler 9600, showing the expected fragments of 130, 298, and 376 bp						
Reference test Culture, toxin test, verocytotoxin test, exposure or clinical symptoms	**Methods** All samples cultured on SMAC using standard methods. Partial verification also carried out on PCR-positive culture-negative samples, namely 15 samples undergo additional verocytotoxin test and four undergo additional direct toxin test						
Reported accuracy data:	**All patients**			**EHEC-positive patients**			
Sensitivity	100	**TP**	55	**Sensitivity**	70	**TP**	55
Specificity	99.97	**FP**	1	**Specificity**	100	**FP**	24
PPV	98	**FN**	0	**PPV**	100	**FN**	0
NPV	100	**TN**	3893	**NPV**	61	**TN**	37
Economic evaluation:	None provided						
What the authors conclude:	PCR is more sensitive than culture for detecting EHEC in the gut						
Assessment of authors' conclusions:	1. This study highlights attempts to use 'true' gold standard – by including toxin and verocytotoxin tests to reference standard, a more accurate view of disease status is recorded. 2. Well-executed study, including full patient characteristics and clear outline of methods used						

Appendix 4

Study characteristics for food studies

TABLE 42 *Performance of VTEC methods on food samples*

Study	Method format	Target analyte	Kit type	Reference method	Sample size and test material type	Sensitivity/specificity	Proportion of presumptive positives (relative to standard)
Bennett, 1996[182]	ELISA	O157 cells	TECRA	ANS	30 (spiked minced beef)	100/83	Higher
	ELISA	O157 cells	EHEC-Tek	ANS	30 (spiked minced beef)	87/100	Equivalent
	IMS–plate	O157 cells	Dynabeads	ANS	30 (spiked minced beef)	100/100	Equivalent
	IMS–plate	O157 cells	Dynabeads	ANS	30 (spiked minced beef)	100/67	Higher
Chapman, 1996[183]	ELISA	O157 cells	EHEC-Tek	ANS	108 (spiked beefburgers)	100/97.5	Higher
Johnson, 1998[184]	PCR	O157 cells	BAX	ANS	200 (spiked ground beef)	96.5/100	Higher
	LFIA	O157 cells	VIA	ANS	200 (spiked ground beef)	71.5/100	Higher
	TRAD	O157 cells	NP	ANS	200 (spiked ground beef)	39/100	Lower
	TRAD	O157 cells	NP	ANS	200 (spiked ground beef)	28/100	Lower
Sharma, 1999[185]	RT–mPCR	*eaeA*, *stx1*, *stx2*	NP	ANS	47 (pure culture)	100/92	Higher
	RT PCR	*eaeA*	NP	ANS	47 (pure culture)	76/90	Lower
	RT PCR	*Stx1*	NP	ANS	47 (pure culture)	100/100	Equal
	RT PCR	*Stx* (except *stx1*)	NP	ANS	47 (pure culture)	100/100	Equal
Sharma, 2000[186]	RT PCR	eae gene	NP	ANS	66 (pure culture)	100/95.8	Higher
Chapman, 2001a[130]	LFIA	O157 cells (24 h)	REVEAL	ANS	100 (naturally contaminated meat)	77/100	Lower
Chapman, 2001a[130]	LFIA	O157 cells (24 h)	VIP	ANS	100 (naturally contaminated meat)	34.2/100	Inferior
Chapman, 2001b[187]	LFIA	O157 cells (8 h)	REVEAL	ANS	40 (naturally contaminated meat)	100/100	Equal
Chapman, 2001b[187]	LFIA	O157 cells (8 h)	VIP	ANS	40 (naturally contaminated meat)	19.2/100	Lower
Chapman, 2001c[188]	PCR	O157 cells	NP	ANS	120 (naturally contaminated meat)	100/78	Higher
Chapman, 2001c[188]	LFIA	O157 cells	BioSign	ANS	120 (naturally contaminated meat)	100/98	Higher
Chapman, 2001c[188]	LFIA	O157 cells	Path-Stik	ANS	120 (naturally contaminated meat)	97/100	Lower
Chapman, 2001d[188]	eTRAD	O157 cells	SPRINT	ANS	120 (naturally contaminated meat)	100/65	Higher
Henry, 2001[190]	ELISA	O157 LPS	Detex	USDA/FSIS	264 (spiked meat)	98/100	Lower

continued

TABLE 42 *Performance of VTEC methods on food samples (cont'd)*

Study	Method format	Target analyte	Kit type	Reference method	Sample size and test material type	Sensitivity/specificity	Proportion of presumptive positives (relative to standard)
Kerr, 2001[191]	ELISA	O157 cells	NP	ANS	354 (bovine faeces)	100/97.3	Higher
Manafi, 2001[192]	LFIA	O157 cells	GLISA	ANS	46 (pure culture)	97/91.6	Lower
	TRAD	O157 cells	SMAC	ANS	46 (pure culture)	94.1/91.6	Equal
	TRAD	O157 cells	Fluorocult HC	ANS	46 (pure culture)	94.1/100	Equal
	TRAD	O157 cells	Rainbow	ANS	46 (pure culture)	91.1/91.6	Equal
	TRAD	O157 cells	BCM	ANS	46 (pure culture)	97.0/91.6	Equal
Feldsine, 2002[193]	LFIA	O157 cells	VIP	USDA/FSIS	396 (spiked foods)	100/84	Higher
	ELISA	O157 cells	Assurance	USDA/FSIS	396 (spiked foods)	100/81.9	Higher
Sharma, 2002[194]	RT–mPCR	*stx1*, *stx2*	NP	ANS	67 (pure culture)	95.4/100	Lower
	RT–mPCR	*eae* (O26, O111, O157)	NP	ANS	67 (pure culture)	88/100	Lower
Chapman, 2003[195]	LFIA	O157 cells	REVEAL	ANS	96 (spiked meats)	77.6/100	Lower
	LFIA	O157 cells	VIP	ANS	96 (spiked meats)	36/100	Lower
	LFIA	O157 cells	STAT	ANS	96 (spiked meats)	12.9/100	Lower
	LFIA	O157 cells	REVEAL	ANS	96 (spiked meats)	92.7/100	Lower
	LFIA	O157 cells	REVEAL	ANS	96 (spiked meats)	95.5/100	Lower
Gilbert, 2003[119,120]	mPCR	O157 gene	NP	ANS	45 (spiked foods)	100/100	Equal
Abdulmawjood, 2004[196]	PCR	O157 rfbE gene	NP	ANS	155 (pure cultures)	100/100	Equal
Bono, 2004[197]	RT PCR	O157:gene	RAPID	ANS	75 (bovine faecal)	100/32.6	Higher
	RT PCR	O157:gene	RAPID	ANS	75 (bovine faecal)	100/37	Higher
Capps, 2004[198]	LFIA	O157 cells	NP	ISO	1635 (spiked foods)	93.5/92.3	Lower
	LFIA	Toxin	NP	ISO/VCA	1659 (spiked foods)	55.4/94.1	Lower
	ELISA	Toxin	NP	ISO/VCA	1168 (spiked foods)	88.9/98.4	Lower
	PCR	*stx1*, *stx2* genes	NP	ISO/VCA	1010 (spiked foods)	72.1/98.9	Lower
	VCA	Toxin	NP	ISO	390 (spiked foods)	89.3/100	Lower
Arthur, 2005[199]	IMS–PCR	O157:H7 gene	Assurance GDS	US MRU	136 (spiked beef)	53/100	Lower
	IMS–PCR	O157:H7 gene	Assurance GDS	US MRU	136 (spiked beef)	73/100	Lower
	RT PCR	O157:H7 gene	BAX	US MRU	134 (spiked beef)	66/100	Lower
	RT PCR	*eae* gene	Light cycler	US MRU	126 (spiked beef)	77/100	Lower
	RT PCR	*eae* gene	Light cycler	US MRU	57 (spiked beef)	98/100	Lower
	AB–plate	O157 gene	Pathatrix	US MRU	57 (spiked beef)	98/100	Lower
Bettelheim, 2005[200]	eTRAD	O157 cells	O157:H7 ID	ANS	347 (pure cultures)	93.7/100	Lower

continued

TABLE 42 *Performance of VTEC methods on food samples (cont'd)*

Study	Method format	Target analyte	Kit type	Reference method	Sample size and test material type	Sensitivity/specificity	Proportion of presumptive positives (relative to standard)
Ellingson, 2005[201]	RT PCR	*eae* gene	NP	ANS	50 (spiked meat or faeces)	100/100	Equal
	RT PCR	*eae* gene	NP	ANS	33 (pure culture)	80/100	Lower
Hsu, 2005[129]	RT–mPCR	O157 gene	TaqMan	ANS	217 (pure cultures)	100/100	Equal
	RT–mPCR	*stx2* gene	TaqMan	ANS	217 (pure cultures)	100/100	Equal

AB, antibody; eTRAD, enhanced traditional method (very similar to traditional method); ANS, appropriate non-standard method (comprises acceptable broth type followed by plating to acceptable agar type with or without IMS); GLISA, gold-linked immunosorbent assay; ISO, International Standards Organization Standard Method; LFIA, lateral flow immunoassay; mPCR, multiplex PCR; NP, non-proprietary; RT, real-time; TRAD, traditional; USDA/FSIS, United States Department of Agriculture/Food Safety Inspection Service Standard Method; US MRU, United States Meat Research Unit (a standard method for a department within the Meat and Animal Research Centre). Equal = methods not statistically significantly different ($p = 0.05$). Inferior = methods are significantly different ($p = 0.05$) and judged as inferior if fewer presumptive positives were identified, compared with reference method. Superior = methods are significantly different ($p = 0.05$) and judged as superior if more presumptive positives were identified, compared with reference method.

TABLE 43 *Performance of* Campylobacter sp. *detection methods in food studies*

Study	Method format	Target analyte	Kit type	Reference method	Sample size and test material type	Sensitivity/specificity	Proportion of presumptive positives (relative to standard)
Winters, 1995[202]	PCR	DNA	NP	ANS	60 (poultry)	85/100	Lower
Lilja, 2001[203]	ELISA	Thermotolerant *Campylobacter* sp.	EiaFoss	ANS	60 (poultry)	100/100	Higher
	PCR	Thermotolerant. *Campylobacter* sp.	NP	ANS	60 (poultry)	100/100	Equal
Bang, 2002[204]	mPCR	16S rRNA	NP	NCFA	269 (poultry environmental)	100/71.1	>>higher
	mPCR	HipO	NP	NCFA	269 (poultry environmental)	100/70.3	>>higher
Sails, 2002[205]	PCR–hybrid[a]	Hippuricase gene	NP	ANS	69 (environmental water)	100/00	Equal
Gilbert, 2003[119,120]	PCR	Conserved genomic DNA	NP	ANS	45 (meat, vegetables and dairy foods)	100/100	Equal
Hong, 2003[123]	PCR–hybrid	*ceuE*	NP	ANS	32 (poultry/poultry environmental)	–/–	Higher
Manfreda, 2003[206]	mPCR	Genes	BAX	ANS	56 (poultry environmental)	75/100	Lower
	mPCR	Genes	BAX	ANS	56 (poultry environmental)	95.8/100	Lower
	mPCR	Genes	NP	ANS	56 (poultry environmental)	25/100	Lower
	mPCR	Genes	NP	ANS	56 (poultry environmental)	83/100	Lower
Sails, 2003[207]	RT PCR	ORF-C	NP	ANS	97 (seafood, meat, dairy)	100/85	Higher
Mateo, 2005[208]	PCR	16S rRNA	NP	ISO	73 (poultry)	100/73.9	Higher
	PCR[b]	16S rRNA	NP	ISO	68 (poultry)	100/77.7	Higher

ANS, appropriate non-standard method (comprises acceptable broth type followed by plating to acceptable agar type); ISO, International Standards Organization Standard method; mPCR, multiplex; NP, non-proprietary PCR; NCFA, Nordic Committee on Food Analysis; NP, non-proprietary; –, not stated; RT, real-time.
[a] Also called PCR-ELISA.
[b] Direct PCR compared against direct plating method.

TABLE 44 *Performance of* Salmonella *sp. detection methods in food samples*

Study	Method format	Target analyte	Kit type	Reference method	Sample size and test material type	Sensitivity/specificity	Proportion of presumptive positives (relative to standard)
Ng *et al.*, 1996[209]	ELISA	LPS	NP	ISO	219 (pure culture)	99/100	Lower
Soumet *et al.*, 1997[248]	PCR–hybrid	Gene	NP	ANS	207	93.2/100	Lower
Hanai *et al.*, 1997[128]	eTRAD	Cells	OSRT	USFDA	60 (a mixture of pure culture, spiked or naturally contaminated foods)	66/100	Lower
	LFIA	Cells	Suncoli	USFDA	60 (a mixture of pure culture, spiked or naturally contaminated foods)	87.2/100	Lower
	ELISA	Cells	Unique	USFDA	60 (a mixture of pure culture, spiked or naturally contaminated foods)	87.2/100	Lower
	ELISA	Cells	Locate	USFDA	60 (a mixture of pure culture, spiked or naturally contaminated foods)	100/0	Lower
	Immunocapture	Cells	1–2 test	USFDA	60 (a mixture of pure culture, spiked or naturally contaminated foods)	78.7/100	Lower
	IMS–plate	Cells	Dynabeads	USFDA	60 (a mixture of pure culture, spiked or naturally contaminated foods)	97.9/100	Lower
	TRAD (JP std)	cells	NP	USFDA	60 (a mixture of pure culture, spiked or naturally contaminated foods)	82.9/100	Lower
Afflu and Gyles, 1997[127]	LFIA	Cells	SSS	ANS (MSRV)	45	100/–	Equal
	Immunocapture	Motile cells		ANS (MSRV)	45	46.6/–	Lower
	Immunocapture	Motile cells		ANS (MSRV)	45	80/–	Lower
Kimura *et al.*, 1999[210]	PCR[a]	Gene	TaqMan	ANS	100 (naturally contaminated meat)	100/95.6	Higher
	PCR[b]	Gene	TaqMan	ANS	100 (naturally contaminated meat)	100/95.6	Higher
Richter *et al.*, 2000[211]	eTRAD	Cells	SPRINT	ISO	348 (spiked dairy)	89/100	Inferior
Walker *et al.*, 2001[212]	FIA	Cells	VIDAS	BAM	300 (natural dairy)	100/96.9	Higher
Walker *et al.*, 2001[212]	FIA[c]	Cells	VIDAS	BAM	200 (natural dairy)	100/93.6	Higher
Fang *et al.*, 2003[213]	FISH[d]	Gene	NP	ISO	225	100/88.2	Higher
	FISH	Gene	NP	ISO	225	100/86.7	Higher
	FISH	Gene	NP	ISO	225	100/97.4	Higher

continued

TABLE 44 *Performance of* Salmonella *sp. detection methods in food samples (cont'd)*

Study	Method format	Target analyte	Kit type	Reference method	Sample size and test material type	Sensitivity/specificity	Proportion of presumptive positives (relative to standard)
Fratamico, 2003[214]	PCR (*invA*)	*invA* gene	NP	ISO	488 (naturally contaminated meat)	100/92.5	Superior
	PCR	Gene	TaqMan	ISO	488 (naturally contaminated meat)	100/87.5	Superior
	LFIA	Cells	Transia card	ISO	488 (naturally contaminated meat)	100/85.3	Superior
Silbernagel *et al.*, 2003[215*]	PCR	Gene	BAX	AOAC/BAM	1188	100/94.1	Higher
Gilbert *et al.*, 2003[119,120]	mPCR[e]		NP	ANS	45 (meat, vegetable, dairy)	100/100	Equal
	PCR[f]		NP	ANS	45 (meat, vegetable, dairy)	100/100	Equal
Van Kessel *et al.*, 2003[216]	PCR	Gene	RAPID	ANS	200 (naturally contaminated milk)	100/82.9	Higher
Briggs *et al.*, 2004[217]	ELISA		Tecra-VIA	AOAC/BAM	150 spiked/unspiked foods	99.1/100	Lower
Croci *et al.*, 2004[218]	FIA	Cells	NP	ISO	30 (naturally contaminated meat)	100/100	Equal
	PCR	Gene	NP	ISO	30 (naturally contaminated meat)	100/100	Equal
Ellingson *et al.*, 2004[219]	RT PCR		Light cycler	VIP then AOAC/BAM	26 9 (pure culture)	100/100	Equal
Lynch *et al.*, 2004[220]	IMS		AIMS	HC	250 (naturally contaminated meat)	100/92.8	Higher
Malorny *et al.*, 2004[221]	PCR	Gene	NP	ISO	165 (spiked/unspiked chicken)	100/91.7	Higher
Malorny *et al.*, 2004,[221]	PCR	Gene	NP	ISO	165 (spiked/unspiked pig swab)	100/91.7	Higher
McMahon *et al.*, 2004a[222]	IMS–plate		VIDAS	AOAC/FDA BAM	540 (spiked/unspiked foods)	96/100	Lower
McMahon *et al.*, 2004a[222]	IMS–plate		VIDAS	AOAC/FDA BAM	540 (spiked/unspiked foods)	96/100	Lower
McMahon *et al.*, 2004b[223]	IC–ELISA		VIDAS	AOAC/FDA BAM	540 (spiked/unspiked foods)	98.6/100	Lower
Perelle *et al.*, 2004[224**]	PCR–hybrid	*invA gene*	NP	ISO	92 (naturally contaminated meat and milk)	100/100	Equal
	RTPCR	*invA gene*	Light cycler	ISO	92 (naturally contaminated meat and milk)	100/100	Equal

continued

TABLE 44 *Performance of* Salmonella *sp. detection methods in food samples (cont'd)*

Study	Method format	Target analyte	Kit type	Reference method	Sample size and test material type	Sensitivity/specificity	Proportion of presumptive positives (relative to standard)
Hong *et al.*, 2003[123]	mPCR–direct	*invA gene*		ANS	120	87/100	Equal
	mPCR–enriched[k]	*invA* gene		ANS	60	100/90	Equal
Touron *et al.*, 2005[225]	nmPCR	*fliC* gene++	NP	ANS	132 (naturally contaminated water and sediment)	100/80.9	Higher

ANS, appropriate non-standard method; AOAC/FDA, BAM, Association of Analytical Communities/Food and Drug Administration, Bacteriological Analytical Manual; eTRAD, enhanced traditional; FIA, fluorescent immunoassay; HC, Health Canada Standard method; IC, immunocapture; ISO, International Standards Organization; LFIA, lateral flow immunoassay; mPCR, multiplex PCR; MSRV, modified semi-solid Rappaport-Vassiliadis medium; nmPCR, nested multiplex PCR; NP, non-proprietary; USFDA, United States Food and Drug Administration.

* For cheese samples, the BAX method appeared superior to the reference method.

** IAC included and some inhibition reported

[a] Chelex extraction.

[b] Enviroamp extraction (PE Applied Biosystems).

[c] Slight changes to the manufacturer's protocol.

[d] Method using individual *Salmonella* primers.

[e] Method using combined *Salmonella*, *E. coli* and *Campylobacter* primers.

[f] Method using *Salmonella* primers only.

TABLE 45 *Performance of* B. cereus *methods for food studies*

Study	Method format	Target analyte	Kit type	Reference method	Sample size and test material type	Sensitivity/specificity	Proportion of presumptive positives (relative to standard)
Jackson, 1993[226]	BIO	Enterotoxin	NP	FIA[5]	59 (culture s/nate)	100/100	Equal
Buchanan, 1994[28]	RPLA	Enterotoxin	BCET-RPLA	BIO[6]	12 (f/s culture s/nate)	83/–	Lower
	ELISA	Enterotoxin	BDE-ELISA	BIO[6]	12 (f/s culture s/nate)	100/–	Equal
	RPLA	Enterotoxin	BCET-RPLA	BIO[6]	12 (f/s boiled culture s/nate)	100/50	Higher
	ELISA	Enterotoxin	BDE-ELISA	BIO[6]	12 (f/s boiled culture s/nate)	33/83	Lower
Day, 1994[94]	ELISA	Diarrhoeal toxin	BDE-ELISA	BCET-RPLA	14 (centrifuged s/nate)	100/12.5	Higher
Mantynen, 1998[227]	PCR	*HblA* gene	NP	BCET-RPLA	80 (culture s/nate)	100/94.1	Higher
	ELISA	Diarrhoeal toxin	BDE-ELISA	BCET-RPLA	80 (culture s/nate)	58.6/100	Lower
Tsen, 2000[108]	PCR	16S rRNA	NP	BIO	122 (culture s/nate)	100/89	Higher
	PCR	Haemolysin BL	NP	BCET-RPLA	122 (culture s/nate)	100/100	Equal
Chen, 2001[228]	ELISA	28.5 kDa antigen	NP	ANS	165 (pure bc/non-bc)	100/88.2	Higher
	ELISA	28.5 kDa antigen	NP	ANS	165 (pure bcg/non-bcg)	100/99.1	Higher
	ELISA	20 kDa antigen	NP	ANS	165 (pure bc/non-bc)	100/87.4	Higher
	ELISA	20 kDa antigen	NP	ANS	165 (pure bcg/non-bcg)	100/98.2	Higher
	ELISA	28.5 kDa antigen	NP	ANS	15 (relevant unspiked foods)	–/–	Higher
Peng, 2001[229]	TRAD	Cells/phospholipase C	BCM	ISO	51 (relevant unspiked foods)	100/96	Higher
Chen, 2004[230]	Colony blot	28.5 kDa antigen[3]	NP	ANS	100 (pure cultures)	98.4/98.4	Lower

bc, *Bacillus cereus*; bcg, *Bacillus cereus*-group; BIO, biological assay (McCoy cell monolayer); FIA, fluorescent immunoassay; f/s, filter sterile; ISO, International Standards Organization; non-bc, non-*Bacillus cereus*; NP, non-proprietary; s/n, supernate; TRAD, traditional.

TABLE 46 *Performance of* S. aureus *detection methods in food studies*

Study	Method format	Target analyte	Kit type	Reference method	Sample size and test material type	Sensitivity/specificity	Proportion of presumptive positives (relative to standard)
Park, 1994[249]	ELISA	Toxins a–e	Ridascreen-SET	ANS	36 (spiked foods)	93.3/100	Lower
Burriel, 1997[250]	Biochem	Various	Staph-ID32	ANS	86 (pure culture)	66/–	NA
	Biochem	Various	API-Staph	ANS	86 (pure culture)	62.5/–	NA
De Buyser, 1998[251]	TRAD	Cells	RPFA (Oxoid)	ANS	57 (raw milk cheese)	–	Equal
	TRAD	Cells	RPFA (Biokar)	ANS	57 (raw milk cheese)	–	Equal
Mach, 2000[231]	eTRAD	Cells	Petrifilm RSA	ANS (API)	216 (pure culture)	87.6/97.5	Lower
	TRAD	Cells	NP	ANS (API)	216 (pure culture)	97.9/95	Equal
	eTRAD	Cells	Petrifilm RSA	ANS	216 (pure culture)	100/89.8	Higher
Atanassova, 2001[232]	PCR	Cells	NP	ANS + RPLA	135 (pork during curing)	100/66	Higher
	PCR	Toxin	NP	ANS + RPLA	135 (pork during curing)	100/82.4	Higher
Silbernagel, 2001[233]	eTRAD	Cells	Petrifilm RSA	AOAC	936 (spiked foods)	–	Equal
De Buyser, 2003[252]	TRAD	Cells	NP (RPFA)	ISO	576 (spiked foods)	–	Equal[a]
Ingham, 2003[234]	eTRAD	Cells/toxin	Petrifilm SE	ISO	>120 (spiked/unspiked foods)	–	Equal[a]
Vernozy 2004	ELISA	Toxins	VIDAS SET	NA	143 (toxin-spiked foods)	92.3/–	Lower
	ELISA	Toxins	VIDAS SET2	NA	143 (toxin-spiked foods)	100/–	Lower
	ELISA	Toxins	TRANSIA	NA	143 (toxin-spiked foods)	91.6/–	Lower
Zschöck, 2005[235]	RPLA	Cells	Masta-Staph	ANS + PFGE	141 (pure culture)	86.7/90.1	Lower
	RPLA	Cells	Staphylase test	ANS + PFGE	141 (pure culture)	78.4/85.1	Lower
	RPLA	Cells	Staphtect-plus	ANS + PFGE	141 (pure culture)	81.1/86.5	Lower
	RPLA	Cells	Staphyloslide latex	ANS + PFGE	141 (pure culture)	77.8/84.4	Lower
	RPLA	Cells	Slidex Staph Plus	ANS + PFGE	141 (pure culture)	77.8/84.4	Lower
	RPLA	Cells	Dry Spot Staphytect plus	ANS + PFGE	141 (pure culture)	75.6/83.0	Lower

ANS, appropriate non-standard method [comprises plating to Baird-Parker agar (possibly followed by another method) or standard biochemical confirmation techniques or using the API biochemical gallery]; AOAC, Association of Analytical Communities; Biochem, indicates that a biochemical gallery was tested; eTRAD, enhanced traditional [in each case this was a thin agar layer method (Petrifilm)]; – or NA, statistic not applicable; ISO, International Standards Organization; PFGE, pulsed field gel electrophoresis; TRAD, traditional.

[a] Characteristics for the test were statistically equivalent as described by the authors.

Appendix 5

Excluded studies

Organism	Test	Study	Not food poisoning	Mixture faecal/food/env samples	Strains only	Banked/spiked samples only	Technical – no clinical information	No comparator/ref standard	Inadequate data for 2 × 2 tables	Did not use tests of interest	Non-rapid
E. coli	Multi-PCR	Pan, 2002	–	–	+		+	+	+	–	–
E. coli	MLVA	Noller, 2003	–	–	+		–	–	+	+	–
E. coli	Multi–PCR	Gannon, 1997	–	+	–		+	+	+	–	–
E. coli	Multi–PCR	Fratmanico, 1995	–	–	+		+	+	+	–	–
E. coli	Multi–PCR	Cebula, 1995	–	–	+		–	+	–	–	–
E. coli	PCR	Abdulmawjood, 2003	–	+	+		+	+	+	–	–
E. coli	Complex typing	Milch, 1997	–	+	+	+	+	+	+	–	–
E. coli	Unclear	Gunzberg, 1993	–	–	+	?	+	+	+	+	–
E. coli	CoA	Ram, 1995	–	–	?	–	+	+	+	–	–
E. coli	CoA	Ram, 1993	–	–	–	–	+	–	–	–	–
E. coli	PCR	Okamoto, 1999	?	–	–	–	?	–	+	+	–
E. coli	PCR	Li, 2004	–	+	–	–	+	+	+	+	–
E. coli	PCR	Dutta, 2001	–	–	–	–	–	–	–	+	–
E. coli	PCR	Thomas, 1994	–	–	–	–	–	+	+	–	–
E. coli and *Salmonella*	PCR	Naravaneni, 2005	–	+	+	+	+	+	+	–	–
S. aureus	Chrom	Carricajo, 2001	+	+	–		–	–	–	–	–
S. aureus	PCR-RFLP	Marcos, 1999	–	+	+		+	+	+	–	–
S. aureus	PCR	Stuhlmeier, 2003	+	+	+		+	+	+	–	–
S. aureus	Chrom	Samra, 2004	+	+	–		–	+	+	–	–
S. aureus	Chrom	Gaillot, 2000	–	–	+	+	+	+	+	–	–
Salmonella	Plating media	Ruiz, 1996	–	–	–		–	–	–	+	–
Salmonella	Stool-processing methods	Kongmuang, 1994	–	–	–		–	–	+	+	-
Salmonella	Media plating	Dusch, 1993	–	–	–		–	–	–	+	–
Salmonella	Biochemical	Ryck, 1994	–	+	+		+	+	+	–	–
Salmonella	CoA	Sanborn, 1980	–	–	–		–	–	–	–	–
Salmonella	Media plating	Manafi, 1994	–	–	+		–	–	–	?	–
Salmonella	LAT	Benge, 1989	–	–	–		–	–	–	?	–
Salmonella	ELISA, CoA, and more	Rahman, 1991	–	–	+	+	–	+	+	?	–
Salmonella	PCR	Widjojoatmodjo, 1992	–	–	–	+	+	+	+	–	–
Salmonella	EIA	Luk, 1991	–	+	–	+	+	+	+	–	–
Salmonella	PCR	Pathmanathan, 2003	–	?	+	+	+	+	+	–	–
Salmonella	ELISA	Pelton, 1994	+	+	–	–	–	–	–	–	–
Salmonella	ELISA	Quang, 1997	+	+	–	–	–	–	–	–	–
Salmonella, *E. coli*, *S. aureus* and others	Dot immunogold	Dykman, 2000	–	–	–	–	?	–	?	–	–
Salmonella	Selective enrichment methods	Spanova, 2001	–	+	–	–	–	–	+	–	–

continued

Organism	Test	Study	Not food poisoning	Mixture faecal/food/env samples	Strains only	Banked/spiked samples only	Technical – no clinical information	No comparator/ref standard	Inadequate data for 2 × 2 tables	Did not use tests of interest	Non-rapid
Salmonella/typhoid	Serodiagnosis	Olsen, 2004	+	+	–	–	–	–	–	–	–
Campylobacter	PCR–ELISA	Metherell, 1999	–	–	–	+	–	+	+	–	–
Campylobacter	PCR–ELISA	Sails, 2001	–	–	+	+	+	+	+	–	–
Campylobacter	Duplex PCR	Misawa, 2002	–	–	–	+	+	+	+	–	–
Campylobacter	DIG-ELISA	Gunnarsson, 1998	–	–	–	–	+	+	+	–	–
Campylobacter	PCR	Stonnet, 1995	–	–	–	–	–	–	+	–	–
Campylobacter	Chrom	Bar, 1987	–	–	+	+	+	–	+	+	–
Campylobacter	CoA	Chattopadhyay, 2002	+	–	–	–	–	–	–	–	–
Campylobacter	PCR	Mahendru, 1997	–	–	–	–	+	+	+	+	–
Campylobacter	PCR	Englen, 2003	–	–	–	–	+	+	+	–	–
Campylobacter	PCR	Persson, 2005	–	–	+	+	+	+	+	–	–
Campylobacter	ELISA	Strid, 2001	–	–	–	–	+	+	+	–	–
Campylobacter	PCR	Best, 2003	–	–	+	+	–	–	+	–	–
C. perfringens	Culture/DIG	Giugliano, 1983	–	+	+	+	–	–	+	+	–
C. perfringens	DNA probe	Vela, 1999	–	–	–	–	+	+	+	–	–
C. perfringens	PCR	Yoo, 1997	+	+	–	–	+	+	+	–	–
C. perfringens	PCR	Saito, 1992	–	–	–	–	–	–	–	–	+

Appendix 6

Costing data

Summary of testing strategies

TABLE 47 *Testing strategies for a moderately sized (10,000 tests/year) NHS laboratory*

Strategy	Test	Organisms	Patient group	Total samples	Total tests	Description
PCR 1	rtPCR	All	All	10,000	30,000	rtPCR test all samples for all three organisms
PCR 2	rtPCR	All	High-risk	2,500	7,500	Test high-risk samples for all three organisms
PCR 3	rtPCR	E. coli	All	10,000	10,000	Test all samples for *E. coli* only
ELISA 1	ELISA	All	All	10,000	30,000	ELISA test all samples for all three organisms
ELISA 2	ELISA	Local	High-risk	2,500	7,500	ELISA test all samples for *E. coli* only
Culture	Culture	Local	All	10,000	30,000	Standard culture

TABLE 48 *Detailed breakdown of staff costs*

	Medical laboratory assistant (MLA) (£)[a]						
Staff costs plus on-costs 17%	**Min.**	**Max.**	**Biomedical scientist (£)[b]**	**Hands-on time taken (minutes)**	**Isolation rates[c]**	**Total staff cost, 10,000 tests (£)[d]**	**Average staff costs per test (£)[e]**
Salary (including 17% indirect/on-costs)	13,898.43	17,244.63	26,123.76				
Hourly rate	7.13	8.84	13.40				
Per minute	0.12	0.15	0.22	(Hourly rate/60)			
Campylobacter							
Sample preparation culture (all)	0.59	0.74		5		7,369.50	
Campylobacter test culture	0.36	0.44		3		4,421.70	
Campylobacter workup positives		2.23		10	5.04892132	1,127.32	
				Per positive sample			
Reporting			0.11	0.5		1,116.40	
						14,034.92	**1.40**
Salmonella							
Sample preparation culture (all)	0.59	0.74		5		7,369.50	
Salmonella test culture	0.36	0.44		3		4,421.70	
Salmonella workup positives			2.23	10	0.75593768	168.79	
				Per positive sample			
Reporting			0.11	0.5		1,116.40	
						13,076.39	**1.31**

continued

TABLE 48 *Detailed breakdown of staff costs*

Staff costs plus on-costs 17%	Medical laboratory assistant (MLA) (£)[a] Min.	Max.	Biomedical scientist (£)[b]	Hands-on time taken (minutes)	Isolation rates[c]	Total staff cost, 10,000 tests (£)[d]	Average staff costs per test (£)[e]
***E. coli* O157**							
Sample preparation culture (all)	0.59	0.74		5		7,369.50	
E. coli O157 test culture	0.59	0.74		5		7,369.50	
E. coli O157 workup positives	0.12	0.15	2.23	1 + 10 Per positive sample	0.10771457	25.64	
Reporting			0.11	0.5		1,116.40	
						15,881.04	**1.59**
PCR test							
Sample pre-enrichment	0.59	0.74		5		7,369.50	
Sample loading PCR (60 minutes for 92 samples)	0.08	0.10		0.65[f]		961.24	
Reading and reporting		0.11		0.5		1,116.40	
						9,447.14	**0.94**
ELISA test							
Sample pre-enrichment	0.59	0.74		5		7,369.50	
ELISA (load, wash, add antibody, wash, add reagent, wash, read) per run			13.40	60			
ELISA per sample			0.5	0.67		6,666.67	
						14,036.17	**1.40**

[a] Basic salary MLA grade, NHS Careers accessed November 2005.
[b] Basic salary Biomedical Scientist grade, NHS Careers accessed November 2005.
[c] Based on HPA data for 2005 isolation rates.
[d] Based on hypothetical, moderately sized laboratory testing 10,000 samples per year.
[e] Average staff costs per test = [MLA cost per minute × (time taken to prepare sample + time taken to plate on culture dish] + [BioMedical Scientist cost per minute × (Time taken to work-up positives/isolation rate/100)].
[f] 60 minutes/92 samples = 0.65 minutes per sample.

Potential start-up costs to consider

TABLE 49 *Potential PCR training costs when implemented in routine practice: assume 1 biomedical scientist trains 9 laboratory assistants (3 groups of 3) for 30 hours each*

Grade	Hourly rate (£)[a]	Training time (hours)	Total cost (£)
Biomedical scientist	13.40	90	1,205.71
Laboratory assistant	8.84	30	2,387.72
		Training equipment/supplies	3,000.00
			6,593.43

[a] Basic salary MLA and biomedical scientist grades, NHS Careers, accessed November 2005.

Per sample costs

TABLE 50 *Breakdown of costs of culture for hypothetical laboratory testing of 10,000 stool samples per year*

Cost	Breakdown	Baseline (£)	Max. value (£)	Min. value (£)
Staff costs	*Campylobacter* culture	1.22	0.98	1.46
	Salmonella culture	1.14	0.91	1.37
	E. coli O157 culture	1.36	1.09	1.63
Materials	*Campylobacter* culture	3.99	3.19	4.79
	Salmonella culture	3.67	2.93	4.40
	E. coli O157 culture	3.63	2.91	4.36
Capital costs	Culture capital costs	0.02	0.02	0.05
Total cost of *Campylobacter* culture per sample		5.21	4.17	6.25
Total cost of *Salmonella* culture test per sample		4.81	3.84	5.77
Total cost of *E. coli* O157 culture test per sample		4.99	4.00	5.99
Total cost per sample of routine culture tests		15.01	12.01	18.01
Campylobacter culture on 10,000 samples		52,119.86	41,695.89	62,543.83
Salmonella culture test on 10,000 samples		48,051.09	38,440.87	57,661.31
E. coli O157 culture test on 10,000 samples		49,941.10	39,952.88	59,929.31
Total cost of routine culture for 10,000 samples		150,112.05	120,089.64	180,134.46

TABLE 51 *Breakdown of costs of PCR for hypothetical laboratory testing of 10,000 stool samples per year*

Cost	Breakdown	Baseline (£)	Max. value (£)	Min. value (£)
Staff costs	PCR test, staff costs	0.81	0.65	0.97
Pre-enrichment	Campylobacter pre-enrichment	2.60	2.08	3.12
	Salmonella pre-enrichment	0.12	0.10	0.14
	E. coli O157 pre-enrichment	0.44	0.35	0.53
Materials	*Campylobacter* PCR kit	3.75	3.75	5.25
	Salmonella PCR kit	3.75	3.75	5.25
	E. coli O157 PCR kit	3.75	3.75	5.25
Capital costs	rtPCR thermal cycler	20,000 over 3 years	16,500 over 5 years	23,500 over 3 years
	Annualised capital costs	6,666.67	3,300.00	7,833.33
	All samples, 3 organisms	0.22	0.11	0.26
	25% high-risk patients	0.89	0.37	1.74
	50% high-risk patients	0.44	0.22	0.52
	All samples, *E. coli* only	0.67	0.33	0.78
Total PCR costs per sample received	All samples, 3 organisms	17.51	16.05	23.24
	All samples, *E. coli* only	5.67	5.08	7.53
	Triage patients, all organisms	19.51	16.82	27.68
Total PCR costs implementation	All samples, 3 organisms	175,066.67	160,520.00	232,413.33
	All samples, *E. coli* only	56,666.67	50,800.00	75,333.33
	Triage patients, all organisms	146,300.00	151,398.00	124,561.00
Total cost per sample of implementing PCR tests		17.51	16.05	23.24
Total replacement with PCR for 10,000 samples		175,066.67	160,520.00	232,413.33

TABLE 52 *Breakdown of costs of ELISA for hypothetical laboratory testing of 10,000 stool samples per year*

Cost	Breakdown	Baseline (£)	Max. value (£)	Min. value (£)
Staff costs	ELISA test, staff costs	1.30	1.04	1.56
Pre-enrichment	*Campylobacter* pre-enrichment	2.60	2.08	3.12
	Salmonella pre-enrichment	0.12	0.10	0.14
	E. coli O157 pre-enrichment	0.44	0.35	0.53
Materials	*Campylobacter* ProSpecT kit	3.64	3.64	4.37
	Salmonella Wellcolex kit	1.41	1.41	1.66
	E. coli Premier EHEC kit	3.65	3.65	4.30
Capital costs	ELISA test, capital costs	0.05	0.03	0.10
Total cost of *Campylobacter* ELISA test per sample		7.54	6.03	9.05
Total cost of *Salmonella* ELISA test per sample		2.67	2.14	3.20
Total cost of EHEC ELISA test per sample		5.45	4.36	6.54
Total cost per sample of implementing ELISA tests		15.66	12.53	18.79
Campylobacter ELISA test on 10,000 samples		75,400.00		
Salmonella ELISA test on 10,000 samples		26,700.00		
EHEC ELISA test on 10,000 samples		54,489.36		
Total replacement with ELISA for 10,000 samples		156,589.36	125,271.49	187,907.23

Options to centralise PCR testing

TABLE 53 *Testing strategies for a large centralised laboratory testing 50,000 samples through rtPCR each year*

Strategy	Organisms	Patient group	Total samples	Total tests	Strategy description
PCR 1	All	All	50,000	150,000	Send all samples to large laboratory: test for all three organisms
PCR 2	All	High-risk	12,500	375,000	Send high-risk samples to large laboratory: test for all three organisms
PCR 3	*E. coli*	High-risk	12,500	12,500	Send high-risk samples to large laboratory: test for *E. coli* only

TABLE 54 *Breakdown of costs for a centralised PCR testing unit catering for 50,000 stool samples per year*

	Cost		**Baseline (£)**	**Max. value (£)**	**Min. value (£)**
Staff	PCR test	Staff costs	0.81	0.65	0.97
	Campylobacter pre-enrichment	*Campylobacter* pre-enrichment	2.60	2.08	3.12
Pre-enrichment	*Salmonella* pre-enrichment	*Salmonella* pre-enrichment	0.12	0.10	0.14
	E. coli O157 pre-enrichment	*E. coli* O157 pre-enrichment	0.44	0.35	0.53
	PCR test	*Campylobacter* PCR kit	3.75		
PCR kit	PCR test	*Salmonella* PCR kit	3.75		
	PCR test	*E. coli* O157 PCR kit	3.75	3.75	5.25
Courier	Delivery costs	Delivery	0.75	0.5	1
	All samples, 3 organisms		150,000		
	Samples sent		500,00		
	Triage of patients – 25%		125,00	15,000	7,500
	Capital cost		20,000 over 3 years	16,500 over 5 years	23,500 over 3 years
	Annualised capital costs		6,666.67	3,300.00	7,833.33
Capital costs	All samples, 3 organisms	Capital costs	0.09	0.04	0.10
	Triage patients, all organisms	Capital costs	0.18	0.07	0.35
	Triage patients, *E. coli* only	Capital costs	0.53	0.22	1.04
	All samples, 3 organisms		17.86	16.35	23.77
	Triage patients, all organisms		18.12	16.44	24.50
Total PCR costs	Triage patients, *E. coli* only		6.28	5.47	8.79
	All samples, 3 organisms		892,833.33	817700.00	1,384,011.11
Total PCR costs implementation	Triage patients, all organisms		226,541.67	246,630.00	183,768.33
	Triage patients, *E. coli* only		78,541.67	82,050.00	65,958.33

Appendix 7

Original protocol

This is the original protocol that was submitted on 1 February 2005.

Summary

This review will answer key questions on rapid diagnostic tests for food poisoning in line with HTA objectives.

1. Does it work? For whom? How does it compare with alternatives?

We will conduct a systematic review of the literature to determine the diagnostic accuracy of tests for rapid diagnosis of food poisoning in clinical and public health practice.

Search strategy

A systematic search of MEDLINE, EMBASE, BIOSIS and other databases will be used to assemble published literature. Other studies will be identified from handsearching key journals, screening reference lists of retrieved articles and contacting clinical experts and test manufacturers.

Review strategy

Two reviewers will screen studies for relevance independently. Study inclusion, data extraction and quality assessment will be carried out by two reviewers. Studies will be quality assessed using the quality assessment tool developed for this project based on standard methods. Meta-analysis will be used to produce summary receiver operating curves but if we detect significant heterogeneity a narrative synthesis will be presented.

2. At what cost?

Economic evaluations will be reviewed and a decision model will be developed to estimate the cost-effectiveness of alternative tests. The usefulness of the results in practice will be explored using nominal group analysis of GPs' and CCDCs' opinion.

1.0 Background

1.1 Definition

Food poisoning occurs after the consumption of food containing toxins or organisms that multiply to cause disease.[1] The Advisory Committee on the Microbiological Safety of Food (ACMSF) defined food poisoning as "any disease of an infectious or toxic nature caused by or thought to be caused by the consumption of food or water". This definition was circulated by the Chief Medical Officer in 1992.[2] The terms 'food-borne infections' and 'food-borne intoxications' are widely used to highlight the different pathological mechanisms of the two principal groups of food poisoning bacteria.

1.2 Incidence

The commonest causes of bacterial food poisoning outbreaks include *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* O157, *Clostridium perfringens*, *Bacillus cereus*, *Shigella* spp. and *Staphylococcus aureus*.[3] A total of 26,528 cases of food poisoning were notified to the Health Protection Agency and laboratory reports of isolation of 14,844 cases of *Salmonella*, 55,887 cases of *Campylobacter*, 896 cases of *E. coli* 0157 and 1983 cases of *Norovirus* in England and Wales in 2000.

Organisms

There is a wide variety of food-borne pathogenic microorganisms and natural toxins.[4] The majority of cases of food poisoning and outbreaks of food poisoning are caused by a limited number of these organisms. A list of organisms causing food associated disease is summarised below.

Pathogenic bacteria causing food poisoning include *Salmonella* spp. and *Campylobacter jejuni, Escherichia coli* O157, *Clostridium perfringens*, *Bacillus cereus*, *Shigella* spp., *Staphylococcus aureus*, *Clostridium botulinum*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Listeria monocytogenes*, *Vibrio cholera*e O1, *Vibrio cholerae* non-O1, *Vibrio vulnificus*, *Aeromonas hydrophila* and other spp., *Plesiomonas shigelloides*, *Miscellaneous enterics*, *Streptococcus* and *Escherichia coli* – enterotoxigenic, enteropathogenic and enteroinvasive.

Parasitic protozoa and worms causing food-associated disease include: *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Anisakis* spp. The common viral causes of food associated disease include: *Rotavirus*, *Norovirus*, and hepatitis A virus. Several

natural toxins cause food-associated disease. However, they are uncommon and are usually investigated at the National Reference Laboratory. The causes include ciguatera poisoning, shellfish toxins, scombroid poisoning, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohaemagglutinin and crayanotoxin.

The systematic review will concentrate on six bacterial causes of food poisoning – *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* O157, *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus*. These organisms were selected mainly because they are the commonest causes of food poisoning and outbreaks of food poisoning in the UK. We included *E. coli* 0157 because of the severity of the disease it causes and *S. aureus* and *B. cereus* because they are diagnosed with toxin detection methods. Other organisms were not included individually either because they are uncommon, not relevant in community setting (*C. difficile*), predominantly transmitted through other routes (*Norovirus*, *Shigella* spp.), or self-limiting of illness (*Norovirus*). However a broader search covering all other organisms will be conducted. A separate analysis will be conducted for each diagnostic method/organism if literature on a particularly efficient and effective method is identified.

The diagnostic tests will be those that can be used in clinical laboratories. The majority of rapid methods have mainly been tried on food and animal samples.[4] We will review the diagnostic methods applied in both human and food settings and relate these to the industrial production/commercial settings in which food is prepared, served and consumed.

2.0 How the project has changed since the outline proposal was submitted

In view of the comments from the reviewers we have made a number of changes to the protocol. We have clarified how we are going to deal with poor quality studies, heterogeneity and included improvements in the search strategy. We have replaced the planned GP survey with a GP and Consultants in Communicable Disease Control nominal group analysis, a qualitative technique that will enable us to obtain useful information at a cheaper rate. A Senior Lecturer in statistics has joined the review group as a co-applicant.

3.0 Planned investigation

3.1 Research objectives

1. To identify studies on rapid diagnostic methods for food poisoning due to *Salmonella* spp., *Campylobacter*, *Escherichia coli* O157, *Clostridium perfringens*, *Staphylococcus aureus* and *Bacillus cereus* relevant to both the food chain and clinical samples.
2. To identify studies on rapid diagnostic methods for all other causes of food poisoning, with relevance as above.
3. To assess and summarise the sensitivity, specificity and positive predictive values of each diagnostic test for each organism compared to a gold standard.
4. For tests designed and/or currently applied only to food samples, to assess usefulness for transfer to clinical testing.
5. To assess the time for full laboratory analysis and reporting for each diagnostic test.
6. To assess based on identified studies the cost and cost-effectiveness of each diagnostic test in a clinical setting and in the management of outbreaks.
7. To use a model to assess the impact of each diagnostic test on the clinical care of individual patients and on public health.

3.2 Existing research

Various methods have been tried for the rapid diagnosis of organisms commonly causing food poisoning. However, no systematic review of the evidence of which tests are effective in practice was identified. Several diagnostic studies were identified using a sensitive search strategy. A total of 750–1000 articles of varying quality were found. Based on initial review of abstracts, about one-third of these articles will be directly relevant and require further assessment and possible inclusion in a review.

Rapid diagnostic methods for *Salmonella* spp. include automated rapid enzyme immunoassay,[5] polymerase chain reaction (PCR),[6–9] random amplified polymorphic DNA (RAPD)[10] and latex particle agglutination.[11] Methods for the rapid diagnosis of *Campylobacter jejuni* include PCR,[12,13] DNA hybridisation, enzyme immunoassays (EIAs)[14] and hydrophobic grid membrane filters (HGMFs).[15] *E. coli* 0157 can be diagnosed rapidly using latex agglutination[16] and PCR.[17] Recently, rapid antibody-based dipstick detection methods for O157 and other verotoxigenic serotypes have been described by one of the co-applicants.[18,19] Rapid methods for the diagnosis of *Clostridium perfringens* include PCR.[20] Rapid methods for the

diagnosis of *Staphylococcus aureus* include real-time fluorescence PCR assay,[21,22] detection of enterotoxins,[23] ELISA[24] and latex agglutination tests.[25] The methods for the rapid diagnosis of *Bacillus cereus* include PCR identification of emetic toxin,[26] HEp-2 cell assay[27] and reversed passive latex agglutination and ELISA.[28]

3.3 Research methods

This review will include a broad and comprehensive search for and a critical assessment of studies on the rapid diagnosis of food poisoning. The criteria for inclusion and exclusion of studies in the review are:

- Organisms and diagnostic investigations: we will review rapid diagnostic tests for common organisms and for less common causes of food poisoning, concentrating on comparison of tests between individuals.
- Types of studies: all types of diagnostic studies that compare a rapid test with a gold standard.
- Types of participants: patients with suspected food poisoning at home or in primary care with local laboratory diagnosis.
- Types of outcome measures: 1. diagnostic accuracy – sensitivity, specificity and diagnostic odds ratios; 2. impact on management of patients and prevention of wider outbreaks; 3. costs and cost-effectiveness.

3.3.1 Search strategy

We intend to combine a standardised search strategy which creates a large set of high-validity articles on diagnosis with the MESH term(s) for each organism.

A. Standardised search strategy (1. exp "sensitivity and specificity"/, 2. exp diagnostic errors, 3. (sensitivity or false positive or false negative or predict$ or observer variation).ti,ab,sh., 4. 1 or 2 or 3, 5. (blind$ or mask$ or compar$).ti,ab,sh., 6. 4 and 5

B. Organisms and MESH terms search. Two categories will be searched:

1. Common pathogenic bacteria – *Salmonella* spp. (MESH) or *Salmonella* food poisoning (MESH), *Campylobacter jejuni* (MESH), *Escherichia coli* O157 (MESH), *Clostridium perfringens*, *Staphylococcus aureus* (MESH), *Bacillus cereus* (MESH).
2. All organisms using the MESH term "Food Poisoning", including all subheadings.

The standardised search "AND" the organism MESH term(s) search will be combined.

We will apply the above strategy for each pathogen using OVID interface to search the following databases: (1) MEDLINE (1966 to date), (2) EMBASE (1974 to date) and (3) BIOSIS (1969 to date). Other databases will be searched including those that involve validation of the new tests against standard methods in collaborative trials, such as those in the AOAC Method Validation Program. We will also search CINAHL (Cumulative Index to Nursing and Allied Health Literature), NHS EED (NHS Economic Evaluation Database), Web of Knowledge, Dissertation Abstracts Online database, Database of Abstracts and Reviews and Conference Proceedings. Grey literature: diagnostic equipment manufacturers and individuals working in fields relevant to each organism will be contacted to identify grey literature. International and national experts on food poisoning will be contacted to check the completeness of any search conducted. Authors of published articles will be contacted to enquire about unpublished studies they may be aware of. We will ask for any additional unpublished, ongoing and planned studies from referees. The reference list of published articles including previous reviews will also be checked and authors contacted if unpublished papers are identified. Other databases that index grey literature such as SIGLE (System for Information on Grey Literature) and British National Bibliography for Report Literature will be searched.

We will include articles in all languages and studies carried out in humans or animals.

3.3.2 Review strategy

a. Study eligibility and application of inclusion and exclusion criteria

The titles and abstracts of papers identified will be screened by two independent reviewers. All articles that are considered to potentially meet the eligibility criteria outlined above by any of the reviewers will be selected. The assessment of study eligibility of this initial selection will not be blinded to publication details such as journal or author names.

b. Data extraction

Two reviewers will independently use standard forms to extract data from all identified papers. Key data items will include patient characteristics, organism(s), test used, characteristics of the tests (for instance PCR – type of probe, quantitative or qualitative), location, outcome measures and the source of funding. Other characteristics to be recorded include study quality, publication details, time for analysis, sensitivity, specificity and positive predictive values of each rapid diagnostic test compared with a gold standard (laboratory culture

and or electron microscopy for each organism). The underlying numbers used in calculating these measures of diagnostic accuracy will also be recorded.

c. Assessment of methodological quality
Two reviewers will independently assess the quality of identified studies by recording the characteristics of included studies on a standard form. A third reviewer will settle disagreements. The methodological quality of studies will be assessed using the criteria suggested by the Cochrane Methods Group on Screening and Diagnostic Tests.[29] Quality assessment criteria will not be over-utilised as suggested in the Cochrane Handbook as these studies are observational. We will deal with variation in methodological quality by exploring the effect of major sources of bias or variability in study quality and examine its effect on the summary receiver operating characteristic curve (see below). If analysis of the studies with a particular bias (for instance – independence of observations) does not produce a difference from that obtained with high-quality studies then they will be included to increase precision of summary estimates. However, if they differ we will restrict the analysis to studies of high quality directly applicable to diagnosis of food poisoning.

d. Statistical analysis – effectiveness
If meta-analysis is not found to be appropriate due to clinical heterogeneity we will limit the analysis to a qualitative narrative synthesis of the diagnostic research available.

Statistical analysis will follow that suggested by Lijmer.[30] Accuracy is usually presented in individual studies in terms of sensitivity and specificity, i.e. dichotomous data rather than differences in distributions. Standard meta-analytic techniques, that is, a simple pooled estimated of sensitivity and another of specificity, will be inappropriate as these two statistics are likely to be correlated. Therefore, we will summarise accuracy across studies using a Summary Receiver Operating Characteristic (SROC) curve. This will be accomplished through a meta-analytic regression model used to explain variability in study diagnostic odds ratios (DOR).[31] In particular, variability across studies due to the use of different thresholds to define positivity can be assessed and modelled using this approach. Variability due to other sources, e.g. patient characteristics (age), study quality and characteristics such as inclusion criteria and measurement of outcomes, can also be explicitly modelled. The modelled DORs can be transformed back into paired sensitivities and specificities. In the unlikely case of sensitivity and specificity appearing independent (as judged by Spearman's rank correlation, for example) then standard meta-analytic techniques will be applied. Heterogeneity can be assessed using the I^2 statistic.[32] We will conduct the analysis for each organism and diagnostic test.

Publication bias will be assessed using funnel plots of DORs. Galbraith plots will be used to identify outlying studies.

e. Costs and cost-effectiveness
The economic analysis will consist of reviewing published studies considered adequate to address and/or containing issues of cost and cost-effectiveness. These aspects will be abstracted along with the other variables outlined above by at least another Health Economic reviewer. The scope of the economic appraisal will be from a personal, social and health sector perspective, i.e. the 'societal' perspective. These costs will include (1) *inter alia* private purchases of OTC medication; lost family production (e.g. *carer time*); (2) work days lost; lost revenue from closures, shut-downs, sales, bad publicity, etc.; (3) testing, community infection control measures, clean-up work, monitoring, etc.; (4) related inpatient and outpatient episodes, GP visits and laboratory tests (including capital equipment, reagents, containers, administration and other consumables).

Cost information
Cost information will be obtained from a variety of sources. In the first instance the availability of cost data will be assessed from the appropriate publications identified by the systematic review. Some variability may be expected to occur in individual studies because (a) baseline years may differ, (b) the basis for costing is different, (c) costs and prices are reported interchangeably and (d) patterns of healthcare delivery may be the underlying cause of cost variation, not the procedure itself. An average estimate will be used where there is compatibility between the estimates. Also, relevant extreme values will be used in the model where appropriate for purposes of sensitivity analysis.

It is very likely that other sources of cost data will be required. The costs of the tests themselves (new and existing) can be obtained from manufacturers' specifications and relevant marketing information. However, these costs will be assessed for their underlying assumptions. In practice the total NHS

cost (staff time, equipment, transport, consumables, etc.) will need to be derived not only from discussions with suppliers but also with laboratory managers and user groups. Our team has experience in costing laboratory procedures, e.g. LBC systems for cervical screening and NAATs to detect *Chlamydia trachomatis*.

There will also be 'flow on' costs when treatment is enacted from diagnostic results. In the first instance these fall on healthcare providers (GPs, hospitals, etc.). These direct healthcare costs can be obtained from standard reference cost manuals such as the *NHS Reference Cost manual*[33] and other published sources, such as the PSSRU's *Unit Costs of Health and Social Care*.[34] We use these costs regularly in our health economic work and, where applicable, these will be included in the model. Where relevant, costs falling on commercial organisations from food poisoning alerts such as a disruption of production or service will be estimated from case reports and other literature. However, modelling events such as these may be better handled outside of the main model and estimates of their incidence and cost developed separately.

Costs falling on individuals will include the actual out-of-pocket personal care costs from contracting food poisoning, e.g. purchase of over-the-counter drugs; loss of wages; travel to a chemist/GP. We shall assume for the purposes of this model that time taken for personal care and lost leisure time has only a 'frictional' value (i.e. there is a very low value in the alternative use of time). The average number of lost days' productivity will be obtained from relevant Health and Safety statistics and an average cost per day used. In this way the 'indirect' or productivity costs can be estimated.

f. Impact on management of patients and outbreaks

This study will be unique in extrapolating the results of the review of effectiveness and cost-effectiveness to a hypothetical population the size of an average PCT. The most comprehensive of the results from the literature review will be used to assess the cost-effectiveness of selected tests. The cost-effectiveness end-point will be cost per case detected. The element of timeliness of testing will captured in this measure by defining cost per case detected within a pre-determined time-frame that is (a) faster than normal tests, (b) technically feasible and (c) and/or acceptable to decision-makers. A full sensitivity analysis of key variables considered to influence the cost-effectiveness will be undertaken.

g. Economic modelling

The model will be based on standard decision analysis (DA) theory. In the absence of a randomised controlled trial a DA approach is a useful means of determining the likely cost-effectiveness of alternative treatments/diagnostic pathways. It uses a decision tree approach that allows the synthesis of existing clinical evidence combined with other data sources (e.g. cost). For example, a model could compare the existing test with a new test taking into account the probability of a higher detection and treatment rate and lower re-test rate, etc. The relevant costs (tests, follow-up procedures, etc.) and expected outcomes (e.g. case detected) can be attached to these branches of the model. It is important to set up the model in a way that reflects current practice.

Unlike deterministic models (if X then Y), uncertainty is included in the DA approach (if X then pY) to reflect actual practice and variability in the outcomes and other factors (e.g. costs). Probability of Y is handled by including in the model a distribution of the likelihood of Y occurring. There may be several stages of the model at which uncertainty occurs. There may also be stages and feed-back loops in the model where elapsed time is represented when stages of treatment or diagnosis are repeated (referred to as a Markov transition probabilities). Model can be run using a simulation package to generate results from large numbers of hypothetical patients with the prescribed variance in inputs and outcomes. Once built, the model is capable of being interrogated in different ways (e.g. by changing the probabilities and the costs, etc.) and hence producing a sensitivity analysis of estimates within which the true cost-effectiveness is expected to be found.

h. Nominal group analysis of GPs and Consultants in Communicable Disease Control

The rationale for exploring GP opinion is that, to develop a valid model based on the literature, a clinical evaluation of the impact of different testing options will be important. For example, what difference will a test result that is available one or two days earlier than the current convention make in terms of patient contacts or prescriptions? Which would GPs and CCDCs consider a priority?

We now propose to test GP and CCDC opinion on aspects of the model using a modified focus group approach which will be more efficient than a survey, and will allow ranking of different factors for consideration in the modelling. This is the

'nominal group technique',[35] where relevant factors can be identified and validated by the group, and they then prioritise those most likely to be important to clinical and practical outcomes, from which likely behavioural modifications can be identified and costed. We shall run two groups of ten attendees, one for GPs and one for CCDCs.

3.4 Expected output

To produce a comprehensive report of the findings with recommendations to the NHS HTA including evidence of impact of rapid tests in clinical care and outbreak management of food poisoning, cost-effectiveness and feasibility of using rapid diagnostic methods in food poisoning. We will also identify the need for further research and the best way to answer questions arising from the review using primary research.

4.0 Ethical arrangements

The nominal group analysis to seek the opinion of healthcare professionals will require ethical approval. Approval will be sought from the Norwich Local Research Ethics Committee.

5.0 Timetable

The proposed start date of the study is 1 February 2005. We estimate that a 12-month period will be required to conduct the review and produce a report.

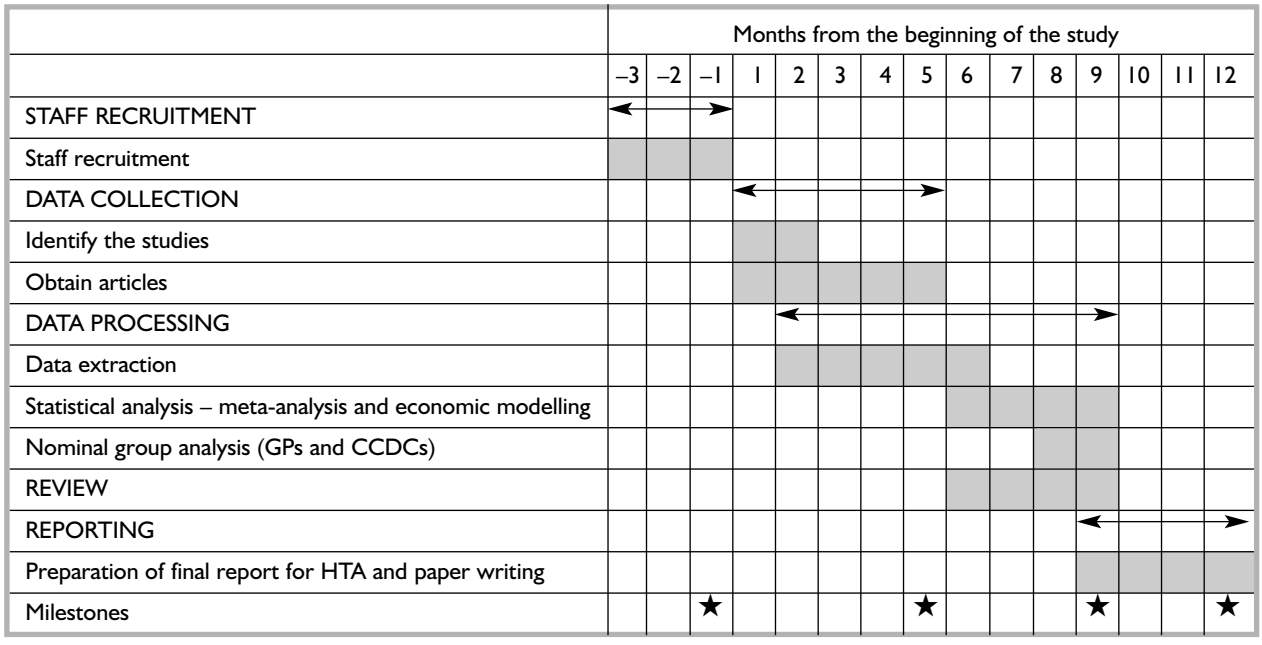
6.0 Expertise

Paul Hunter, Professor of Health Protection and Consultant Medical Microbiologist, will contribute a wide range of expertise including methodological issues around the design of the systematic review, knowledge and experience of using diagnostic microbiological methods in food poisoning, epidemiology and statistics. He chairs the HPA Advisory Committee on Water and has been involved in the investigation of many food and waterborne outbreaks.

Ibrahim Abubakar is a Clinical Lecturer in Health Protection. His main areas of expertise include communicable disease epidemiology, review methodology and coordination of the project. Ibrahim is leading a Cochrane Review on the prevention and treatment of cryptosporidiosis.

Ric Fordham is a Senior Lecturer in Health Economics and Director of the NHS Health Economics Support Programme. His expertise includes economic evaluation and modelling and he has an interest in the economic implications of technology adoption in medicine. Ric is currently involved in another HTA commissioned review on the treatment of warts.

Professor Michael Peck, Head of the Food Safety Microbiology and Computational Microbiology Group of the Institute of Food Research, Norwich, will contribute his expertise on rapid diagnostic tests. Amanda Howe, Professor of Primary Care,



will contribute to the nominal group analysis and knowledge of the application of diagnostic methods for food poisoning in primary care. Silke Schelenz is a Consultant Medical Microbiologist and her areas of expertise include the diagnosis of intestinal pathogens causing food poisoning in a clinical microbiology laboratory.

Lee Shepstone is a Senior Lecturer in Medical Statistics and will provide statistical expertise to the review.

7.0 Consumer involvement

The nominal group analysis of GPs and CCDCs will ensure that two key groups of potential users of the results of the diagnostic tests are involved in the assessment of the impact of rapid diagnostic testing.

8.0 Justification for support required

We propose that the project will require a study coordinator (research associate) based at the Medical School, University of East Anglia. The research associate will be a health economist responsible for modelling the data and will be directly responsible for daily supervision of the project. We will require the time and resources of an additional part-time research associate from the Institute of Food Research in Norwich to contribute to searching, collating and synthesising findings relevant to diagnostic methods in food, water, animals and the environment.

Reference list

1. Mims CA. *Medical microbiology*. 2nd ed. London: Mosby; 1998.
2. Chief Medical Officer. *Definition of food poisoning*. PL/CMO(92)14. London: Department of Health; 1992.
3. Marshall B, Wareing DR, Durband CA, Wright PA. Evolution of a laboratory based system for investigating outbreaks of infectious intestinal disease. *Commun Dis Public Health* 2000;**3**2:111–14.
4. FDA. *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook: Bad Bug Book*. Washington, DC: Food and Drug Administration, Centers for Disease Control and Prevention, USDA Food Safety Inspection Service and National Institutes of Health; 2003.
5. Yeh KS, Tsai CE, Chen SP, Liao CW. Comparison between VIDAS automatic enzyme-linked fluorescent immunoassay and culture method for *Salmonella* recovery from pork carcass sponge samples. *J Food Prot* 2002;**65**:1656–9.
6. Oliveira SD, Rodenbusch CR, Ce MC, Rocha SL, Canal CW. Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. *Lett Appl Microbiol* 2003;**36**:217–21.
7. Whyte P, McGill K, Collins JD, Gormley E. The prevalence and PCR detection of Salmonella contamination in raw poultry. *Vet Microbiol* 2002;**89**:53–60.
8. Chen S, Yee A, Griffiths M, Wu KY, Wang CN, Rahn K, *et al*. A rapid, sensitive and automated method for detection of *Salmonella* species in foods using AG-9600 AmpliSensor Analyzer. *J Appl Microbiol* 1997;**83**:314–21.
9. Chen S, Yee A, Griffiths M, Larkin C, Yamashiro CT, Behari R, *et al*. The evaluation of a fluorogenic polymerase chain reaction assay for the detection of *Salmonella* species in food commodities. *Int J Food Microbiol* 1997;**35**:239–50.
10. Dieckmann H, Dreesman J, Dieckmann H, Malorny B, Schroeter A, Pulz M. [Investigation of foodborne outbreak due to Salmonella infantis using epidemiological and microbiological methods]. *Gesundheitswesen* 1999;**61**:241–7.
11. Ruiz M, Rodriguez JC, Sirvent E, Escribano I, Cebrian L, Royo G. Usefulness of different techniques in the study of the epidemiology of salmonellosis. *APMIS* 2003;**111**:848–56.
12. Englen MD, Ladely SR, Fedorka-Cray PJ. Isolation of *Campylobacter* and identification by PCR. *Methods Mol Biol* 2003;**216**:109–21.
13. Englen MD, Fedorka-Cray PJ. Evaluation of a commercial diagnostic PCR for the identification of *Campylobacter jejuni* and *Campylobacter coli*. *Lett Appl Microbiol* 2002;**35**:353–6.
14. Wang H. Rapid methods for detection and enumeration of *Campylobacter* spp. in foods. *J AOAC Int* 2002;**85**:996–9.
15. Wang H, Boyle E, Farber J. Rapid and specific enzyme immunoassay on hydrophobic grid membrane filter for detection and enumeration of thermophilic *Campylobacter* spp. from milk and chicken rinses. *J Food Prot* 2000;**63**:489–94.
16. Komatsu M, Aihara M, Nagasaka Y, Nakajima H, Iwasaki M, Takahashi M, *et al*. [Rapid detection of *Escherichia coli* 0157:H7 in feces by latex agglutination and immunochromatographic assay]. *Kansenshogaku Zasshi* 1997;**71**:1124–30.
17. Wu CF, Valdes JJ, Bentley WE, Sekowski JW. DNA microarray for discrimination between pathogenic 0157:H7 EDL933 and non-pathogenic *Escherichia coli* strains. *Biosens Bioelectron* 2003;**19**:1–8.

18. Aldus CF, Van Amerongen A, Ariens RM, Peck MW, Wichers JH, Wyatt GM. Principles of some novel rapid dipstick methods for detection and characterization of verotoxigenic *Escherichia coli*. *J Appl Microbiol* 2003;**95**:380–9.

19. Capps KL, Murray AWA, Aldus CF, Wyatt GM, Peck MW, van Amerongen A, *et al*. Validation of three rapid screening methods for the detection of verotoxin-producing Escherichia coli in foods: an Interlaboratory Study. *J AOAC Int* 2004; **87**:68–77.

20. Song Y, Liu C, Molitoris D, Tomzynski TJ, Teague MM, Read E, *et al*. Use of 16S–23S rRNA spacer-region (SR)-PCR for identification of intestinal clostridia. *Syst Appl Microbiol* 2002;**25**: 528–35.

21. Stuhlmeier R, Stuhlmeier KM. Fast, simultaneous, and sensitive detection of staphylococci. *J Clin Pathol* 2003;**56**:782–5.

22. Klotz M, Opper S, Heeg K, Zimmermann S. Detection of *Staphylococcus aureus* enterotoxins A to D by real-time fluorescence PCR assay. *J Clin Microbiol* 2003;**41**:4683–7.

23. Lapeyre C, de Solan MN, Drouet X. Immunoenzymatic detection of staphylococcal enterotoxins: international interlaboratory study. *J AOAC Int* 1996;**79**:1095–101.

24. Shinagawa K, Watanabe K, Matsusaka N, Konuma H, Sugii S. Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in incriminated foods and clinical specimens from outbreaks of food poisoning. *Nippon Juigaku Zasshi* 1990;**52**:847–50.

25. van Griethuysen A, Bes M, Etienne J, Zbinden R, Kluytmans J. International multicenter evaluation of latex agglutination tests for identification of *Staphylococcus aureus*. *J Clin Microbiol* 2001;**39**:86–9.

26. Ehling-Schulz M, Fricker M, Scherer S. Identification of emetic toxin producing *Bacillus cereus* strains by a novel molecular assay. *FEMS Microbiol Lett* 2004;**232**:189–95.

27. Mikami T, Horikawa T, Murakami T, Matsumoto T, Yamakawa A, Murayama S, *et al*. An improved method for detecting cytostatic toxin (emetic toxin) of *Bacillus cereus* and its application to food samples. *FEMS Microbiol Lett* 1994;**119**:53–7.

28. Shinagawa K. Analytical methods for *Bacillus cereus* and other *Bacillus* species. *Int J Food Microbiol* 1990;**10**:125–41.

29. Cochrane Collaboration. *Cochrane Methods Group on Systematic Review of Screening and Diagnostic Tests: recommended methods*. URL: http://www.cochrane.org/docs/sadt.htm. Accessed 6 June 1996.

30. Lijmer J. *Evaluation of diagnostic test from accuracy to outcome*. Enschede: Print Partners; 2001.

31. Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. *Stat Med* 2002;**21**: 1525–37.

32. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327**:557–60.

33. Department of Health. URL: http://www.dh.gov.uk/PublicationsAndStatistics/Publications/PublicationsPolicyAndGuidance/PublicationsPolicyAndGuidanceArticle/fs/en?CONTENT_ID=4070195&chk=UzhHA3. Accessed 2004.

34. Netten A, Curtis L. *Unit costs of health and social care*. URL: http://www.pssru.ac.uk/uc/uc2003.htm. Accessed 2003.

35. Lloyd Jones G, Fowell S, Bligh J. The use of the nominal group technique as an evaluative tool in medical education. *Med Educ* 1999;**33**:8–13.

Annex: Data for a review of diagnostic test accuracy

1. CRITERIA FOR STUDY VALIDITY	
Was the test compared with a valid reference standard?	Studies categorised by reference standard used
Were the test and reference standard measured independently?	In both directions or one
Was the choice of patients who were assessed by the reference standard independent of the test's results? (Avoidance of verification bias)	
Was the test measured independently of all other clinical information?	
Was the reference standard measured before any interventions were started with knowledge of test results (Avoidance of treatment paradox)	
2. ADDITIONAL VALIDITY CRITERIA FOR STUDIES COMPARING TESTS	
Validity of design? Categories in order of decreasing validity are: All tests done independently on each person Different tests on randomly allocated individuals Test all but not independent assess Different test, not random selection of individuals	
3. CRITERIA RELEVANT TO THE APPLICABILITY OF THE RESULTS	
3.1. The clinical problem	
Spectrum of disease and non-disease	Inclusion and exclusion criteria
Duration of illness before testing.	
Previous tests/referral filter, i.e. to what clinical (including previous test) information is the test being evaluated	
Co-morbid conditions	
Demographic information: such as age	
3.2. The test	
Categories for how the test was done	e.g. types of PCR methods
State the explicit threshold used	
% excluded because test was infeasible or result indeterminate	
Test reproducibility	
4. INDIRECT MEASURES OF QUALITY AND APPLICABILITY	
Year of study (or publication)	
Disease prevalence	
Sample size	
Prospective or retrospective study design	

Health Technology Assessment reports published to date

Volume 1, 1997

No. 1
Home parenteral nutrition: a systematic review.
By Richards DM, Deeks JJ, Sheldon TA, Shaffer JL.

No. 2
Diagnosis, management and screening of early localised prostate cancer.
A review by Selley S, Donovan J, Faulkner A, Coast J, Gillatt D.

No. 3
The diagnosis, management, treatment and costs of prostate cancer in England and Wales.
A review by Chamberlain J, Melia J, Moss S, Brown J.

No. 4
Screening for fragile X syndrome.
A review by Murray J, Cuckle H, Taylor G, Hewison J.

No. 5
A review of near patient testing in primary care.
By Hobbs FDR, Delaney BC, Fitzmaurice DA, Wilson S, Hyde CJ, Thorpe GH, *et al*.

No. 6
Systematic review of outpatient services for chronic pain control.
By McQuay HJ, Moore RA, Eccleston C, Morley S, de C Williams AC.

No. 7
Neonatal screening for inborn errors of metabolism: cost, yield and outcome.
A review by Pollitt RJ, Green A, McCabe CJ, Booth A, Cooper NJ, Leonard JV, *et al*.

No. 8
Preschool vision screening.
A review by Snowdon SK, Stewart-Brown SL.

No. 9
Implications of socio-cultural contexts for the ethics of clinical trials.
A review by Ashcroft RE, Chadwick DW, Clark SRL, Edwards RHT, Frith L, Hutton JL.

No. 10
A critical review of the role of neonatal hearing screening in the detection of congenital hearing impairment.
By Davis A, Bamford J, Wilson I, Ramkalawan T, Forshaw M, Wright S.

No. 11
Newborn screening for inborn errors of metabolism: a systematic review.
By Seymour CA, Thomason MJ, Chalmers RA, Addison GM, Bain MD, Cockburn F, *et al*.

No. 12
Routine preoperative testing: a systematic review of the evidence.
By Munro J, Booth A, Nicholl J.

No. 13
Systematic review of the effectiveness of laxatives in the elderly.
By Petticrew M, Watt I, Sheldon T.

No. 14
When and how to assess fast-changing technologies: a comparative study of medical applications of four generic technologies.
A review by Mowatt G, Bower DJ, Brebner JA, Cairns JA, Grant AM, McKee L.

Volume 2, 1998

No. 1
Antenatal screening for Down's syndrome.
A review by Wald NJ, Kennard A, Hackshaw A, McGuire A.

No. 2
Screening for ovarian cancer: a systematic review.
By Bell R, Petticrew M, Luengo S, Sheldon TA.

No. 3
Consensus development methods, and their use in clinical guideline development.
A review by Murphy MK, Black NA, Lamping DL, McKee CM, Sanderson CFB, Askham J, *et al*.

No. 4
A cost–utility analysis of interferon beta for multiple sclerosis.
By Parkin D, McNamee P, Jacoby A, Miller P, Thomas S, Bates D.

No. 5
Effectiveness and efficiency of methods of dialysis therapy for end-stage renal disease: systematic reviews.
By MacLeod A, Grant A, Donaldson C, Khan I, Campbell M, Daly C, *et al*.

No. 6
Effectiveness of hip prostheses in primary total hip replacement: a critical review of evidence and an economic model.
By Faulkner A, Kennedy LG, Baxter K, Donovan J, Wilkinson M, Bevan G.

No. 7
Antimicrobial prophylaxis in colorectal surgery: a systematic review of randomised controlled trials.
By Song F, Glenny AM.

No. 8
Bone marrow and peripheral blood stem cell transplantation for malignancy.
A review by Johnson PWM, Simnett SJ, Sweetenham JW, Morgan GJ, Stewart LA.

No. 9
Screening for speech and language delay: a systematic review of the literature.
By Law J, Boyle J, Harris F, Harkness A, Nye C.

No. 10
Resource allocation for chronic stable angina: a systematic review of effectiveness, costs and cost-effectiveness of alternative interventions.
By Sculpher MJ, Petticrew M, Kelland JL, Elliott RA, Holdright DR, Buxton MJ.

No. 11
Detection, adherence and control of hypertension for the prevention of stroke: a systematic review.
By Ebrahim S.

No. 12
Postoperative analgesia and vomiting, with special reference to day-case surgery: a systematic review.
By McQuay HJ, Moore RA.

No. 13
Choosing between randomised and nonrandomised studies: a systematic review.
By Britton A, McKee M, Black N, McPherson K, Sanderson C, Bain C.

No. 14
Evaluating patient-based outcome measures for use in clinical trials.
A review by Fitzpatrick R, Davey C, Buxton MJ, Jones DR.

No. 15
Ethical issues in the design and conduct of randomised controlled trials.
A review by Edwards SJL, Lilford RJ, Braunholtz DA, Jackson JC, Hewison J, Thornton J.

No. 16
Qualitative research methods in health technology assessment: a review of the literature.
By Murphy E, Dingwall R, Greatbatch D, Parker S, Watson P.

No. 17
The costs and benefits of paramedic skills in pre-hospital trauma care.
By Nicholl J, Hughes S, Dixon S, Turner J, Yates D.

No. 18
Systematic review of endoscopic ultrasound in gastro-oesophageal cancer.
By Harris KM, Kelly S, Berry E, Hutton J, Roderick P, Cullingworth J, *et al*.

No. 19
Systematic reviews of trials and other studies.
By Sutton AJ, Abrams KR, Jones DR, Sheldon TA, Song F.

No. 20
Primary total hip replacement surgery: a systematic review of outcomes and modelling of cost-effectiveness associated with different prostheses.
A review by Fitzpatrick R, Shortall E, Sculpher M, Murray D, Morris R, Lodge M, *et al*.

Volume 3, 1999

No. 1
Informed decision making: an annotated bibliography and systematic review.
By Bekker H, Thornton JG, Airey CM, Connelly JB, Hewison J, Robinson MB, *et al*.

No. 2
Handling uncertainty when performing economic evaluation of healthcare interventions.
A review by Briggs AH, Gray AM.

No. 3
The role of expectancies in the placebo effect and their use in the delivery of health care: a systematic review.
By Crow R, Gage H, Hampson S, Hart J, Kimber A, Thomas H.

No. 4
A randomised controlled trial of different approaches to universal antenatal HIV testing: uptake and acceptability. Annex: Antenatal HIV testing – assessment of a routine voluntary approach.
By Simpson WM, Johnstone FD, Boyd FM, Goldberg DJ, Hart GJ, Gormley SM, *et al*.

No. 5
Methods for evaluating area-wide and organisation-based interventions in health and health care: a systematic review.
By Ukoumunne OC, Gulliford MC, Chinn S, Sterne JAC, Burney PGJ.

No. 6
Assessing the costs of healthcare technologies in clinical trials.
A review by Johnston K, Buxton MJ, Jones DR, Fitzpatrick R.

No. 7
Cooperatives and their primary care emergency centres: organisation and impact.
By Hallam L, Henthorne K.

No. 8
Screening for cystic fibrosis.
A review by Murray J, Cuckle H, Taylor G, Littlewood J, Hewison J.

No. 9
A review of the use of health status measures in economic evaluation.
By Brazier J, Deverill M, Green C, Harper R, Booth A.

No. 10
Methods for the analysis of quality-of-life and survival data in health technology assessment.
A review by Billingham LJ, Abrams KR, Jones DR.

No. 11
Antenatal and neonatal haemoglobinopathy screening in the UK: review and economic analysis.
By Zeuner D, Ades AE, Karnon J, Brown J, Dezateux C, Anionwu EN.

No. 12
Assessing the quality of reports of randomised trials: implications for the conduct of meta-analyses.
A review by Moher D, Cook DJ, Jadad AR, Tugwell P, Moher M, Jones A, *et al*.

No. 13
'Early warning systems' for identifying new healthcare technologies.
By Robert G, Stevens A, Gabbay J.

No. 14
A systematic review of the role of human papillomavirus testing within a cervical screening programme.
By Cuzick J, Sasieni P, Davies P, Adams J, Normand C, Frater A, *et al*.

No. 15
Near patient testing in diabetes clinics: appraising the costs and outcomes.
By Grieve R, Beech R, Vincent J, Mazurkiewicz J.

No. 16
Positron emission tomography: establishing priorities for health technology assessment.
A review by Robert G, Milne R.

No. 17 (Pt 1)
The debridement of chronic wounds: a systematic review.
By Bradley M, Cullum N, Sheldon T.

No. 17 (Pt 2)
Systematic reviews of wound care management: (2) Dressings and topical agents used in the healing of chronic wounds.
By Bradley M, Cullum N, Nelson EA, Petticrew M, Sheldon T, Torgerson D.

No. 18
A systematic literature review of spiral and electron beam computed tomography: with particular reference to clinical applications in hepatic lesions, pulmonary embolus and coronary artery disease.
By Berry E, Kelly S, Hutton J, Harris KM, Roderick P, Boyce JC, *et al*.

No. 19
What role for statins? A review and economic model.
By Ebrahim S, Davey Smith G, McCabe C, Payne N, Pickin M, Sheldon TA, *et al*.

No. 20
Factors that limit the quality, number and progress of randomised controlled trials.
A review by Prescott RJ, Counsell CE, Gillespie WJ, Grant AM, Russell IT, Kiauka S, *et al*.

No. 21
Antimicrobial prophylaxis in total hip replacement: a systematic review.
By Glenny AM, Song F.

No. 22
Health promoting schools and health promotion in schools: two systematic reviews.
By Lister-Sharp D, Chapman S, Stewart-Brown S, Sowden A.

No. 23
Economic evaluation of a primary care-based education programme for patients with osteoarthritis of the knee.
A review by Lord J, Victor C, Littlejohns P, Ross FM, Axford JS.

Volume 4, 2000

No. 1
The estimation of marginal time preference in a UK-wide sample (TEMPUS) project.
A review by Cairns JA, van der Pol MM.

No. 2
Geriatric rehabilitation following fractures in older people: a systematic review.
By Cameron I, Crotty M, Currie C, Finnegan T, Gillespie L, Gillespie W, *et al*.

No. 3
Screening for sickle cell disease and thalassaemia: a systematic review with supplementary research.
By Davies SC, Cronin E, Gill M, Greengross P, Hickman M, Normand C.

No. 4
Community provision of hearing aids and related audiology services.
A review by Reeves DJ, Alborz A, Hickson FS, Bamford JM.

No. 5
False-negative results in screening programmes: systematic review of impact and implications.
By Petticrew MP, Sowden AJ, Lister-Sharp D, Wright K.

No. 6
Costs and benefits of community postnatal support workers: a randomised controlled trial.
By Morrell CJ, Spiby H, Stewart P, Walters S, Morgan A.

No. 7
Implantable contraceptives (subdermal implants and hormonally impregnated intrauterine systems) versus other forms of reversible contraceptives: two systematic reviews to assess relative effectiveness, acceptability, tolerability and cost-effectiveness.
By French RS, Cowan FM, Mansour DJA, Morris S, Procter T, Hughes D, *et al*.

No. 8
An introduction to statistical methods for health technology assessment.
A review by White SJ, Ashby D, Brown PJ.

No. 9
Disease-modifying drugs for multiple sclerosis: a rapid and systematic review.
By Clegg A, Bryant J, Milne R.

No. 10
Publication and related biases.
A review by Song F, Eastwood AJ, Gilbody S, Duley L, Sutton AJ.

No. 11
Cost and outcome implications of the organisation of vascular services.
By Michaels J, Brazier J, Palfreyman S, Shackley P, Slack R.

No. 12
Monitoring blood glucose control in diabetes mellitus: a systematic review.
By Coster S, Gulliford MC, Seed PT, Powrie JK, Swaminathan R.

No. 13
The effectiveness of domiciliary health visiting: a systematic review of international studies and a selective review of the British literature.
By Elkan R, Kendrick D, Hewitt M, Robinson JJA, Tolley K, Blair M, *et al*.

No. 14
The determinants of screening uptake and interventions for increasing uptake: a systematic review.
By Jepson R, Clegg A, Forbes C, Lewis R, Sowden A, Kleijnen J.

No. 15
The effectiveness and cost-effectiveness of prophylactic removal of wisdom teeth.
A rapid review by Song F, O'Meara S, Wilson P, Golder S, Kleijnen J.

No. 16
Ultrasound screening in pregnancy: a systematic review of the clinical effectiveness, cost-effectiveness and women's views.
By Bricker L, Garcia J, Henderson J, Mugford M, Neilson J, Roberts T, *et al*.

No. 17
A rapid and systematic review of the effectiveness and cost-effectiveness of the taxanes used in the treatment of advanced breast and ovarian cancer.
By Lister-Sharp D, McDonagh MS, Khan KS, Kleijnen J.

No. 18
Liquid-based cytology in cervical screening: a rapid and systematic review.
By Payne N, Chilcott J, McGoogan E.

No. 19
Randomised controlled trial of non-directive counselling, cognitive–behaviour therapy and usual general practitioner care in the management of depression as well as mixed anxiety and depression in primary care.
By King M, Sibbald B, Ward E, Bower P, Lloyd M, Gabbay M, *et al*.

No. 20
Routine referral for radiography of patients presenting with low back pain: is patients' outcome influenced by GPs' referral for plain radiography?
By Kerry S, Hilton S, Patel S, Dundas D, Rink E, Lord J.

No. 21
Systematic reviews of wound care management: (3) antimicrobial agents for chronic wounds; (4) diabetic foot ulceration.
By O'Meara S, Cullum N, Majid M, Sheldon T.

No. 22
Using routine data to complement and enhance the results of randomised controlled trials.
By Lewsey JD, Leyland AH, Murray GD, Boddy FA.

No. 23
Coronary artery stents in the treatment of ischaemic heart disease: a rapid and systematic review.
By Meads C, Cummins C, Jolly K, Stevens A, Burls A, Hyde C.

No. 24
Outcome measures for adult critical care: a systematic review.
By Hayes JA, Black NA, Jenkinson C, Young JD, Rowan KM, Daly K, *et al*.

No. 25
A systematic review to evaluate the effectiveness of interventions to promote the initiation of breastfeeding.
By Fairbank L, O'Meara S, Renfrew MJ, Woolridge M, Sowden AJ, Lister-Sharp D.

No. 26
Implantable cardioverter defibrillators: arrhythmias. A rapid and systematic review.
By Parkes J, Bryant J, Milne R.

No. 27
Treatments for fatigue in multiple sclerosis: a rapid and systematic review.
By Brañas P, Jordan R, Fry-Smith A, Burls A, Hyde C.

No. 28
Early asthma prophylaxis, natural history, skeletal development and economy (EASE): a pilot randomised controlled trial.
By Baxter-Jones ADG, Helms PJ, Russell G, Grant A, Ross S, Cairns JA, *et al*.

No. 29
Screening for hypercholesterolaemia versus case finding for familial hypercholesterolaemia: a systematic review and cost-effectiveness analysis.
By Marks D, Wonderling D, Thorogood M, Lambert H, Humphries SE, Neil HAW.

No. 30
A rapid and systematic review of the clinical effectiveness and cost-effectiveness of glycoprotein IIb/IIIa antagonists in the medical management of unstable angina.
By McDonagh MS, Bachmann LM, Golder S, Kleijnen J, ter Riet G.

No. 31
A randomised controlled trial of prehospital intravenous fluid replacement therapy in serious trauma.
By Turner J, Nicholl J, Webber L, Cox H, Dixon S, Yates D.

No. 32
Intrathecal pumps for giving opioids in chronic pain: a systematic review.
By Williams JE, Louw G, Towlerton G.

No. 33
Combination therapy (interferon alfa and ribavirin) in the treatment of chronic hepatitis C: a rapid and systematic review.
By Shepherd J, Waugh N, Hewitson P.

No. 34
A systematic review of comparisons of effect sizes derived from randomised and non-randomised studies.
By MacLehose RR, Reeves BC, Harvey IM, Sheldon TA, Russell IT, Black AMS.

No. 35
Intravascular ultrasound-guided interventions in coronary artery disease: a systematic literature review, with decision-analytic modelling, of outcomes and cost-effectiveness.
By Berry E, Kelly S, Hutton J, Lindsay HSJ, Blaxill JM, Evans JA, *et al*.

No. 36
A randomised controlled trial to evaluate the effectiveness and cost-effectiveness of counselling patients with chronic depression.
By Simpson S, Corney R, Fitzgerald P, Beecham J.

No. 37
Systematic review of treatments for atopic eczema.
By Hoare C, Li Wan Po A, Williams H.

No. 38
Bayesian methods in health technology assessment: a review.
By Spiegelhalter DJ, Myles JP, Jones DR, Abrams KR.

No. 39
The management of dyspepsia: a systematic review.
By Delaney B, Moayyedi P, Deeks J, Innes M, Soo S, Barton P, *et al*.

No. 40
A systematic review of treatments for severe psoriasis.
By Griffiths CEM, Clark CM, Chalmers RJG, Li Wan Po A, Williams HC.

Volume 5, 2001

No. 1
Clinical and cost-effectiveness of donepezil, rivastigmine and galantamine for Alzheimer's disease: a rapid and systematic review.
By Clegg A, Bryant J, Nicholson T, McIntyre L, De Broe S, Gerard K, *et al*.

No. 2
The clinical effectiveness and cost-effectiveness of riluzole for motor neurone disease: a rapid and systematic review.
By Stewart A, Sandercock J, Bryan S, Hyde C, Barton PM, Fry-Smith A, *et al*.

No. 3
Equity and the economic evaluation of healthcare.
By Sassi F, Archard L, Le Grand J.

No. 4
Quality-of-life measures in chronic diseases of childhood.
By Eiser C, Morse R.

No. 5
Eliciting public preferences for healthcare: a systematic review of techniques.
By Ryan M, Scott DA, Reeves C, Bate A, van Teijlingen ER, Russell EM, *et al*.

No. 6
General health status measures for people with cognitive impairment: learning disability and acquired brain injury.
By Riemsma RP, Forbes CA, Glanville JM, Eastwood AJ, Kleijnen J.

No. 7
An assessment of screening strategies for fragile X syndrome in the UK.
By Pembrey ME, Barnicoat AJ, Carmichael B, Bobrow M, Turner G.

No. 8
Issues in methodological research: perspectives from researchers and commissioners.
By Lilford RJ, Richardson A, Stevens A, Fitzpatrick R, Edwards S, Rock F, *et al*.

No. 9
Systematic reviews of wound care management: (5) beds; (6) compression; (7) laser therapy, therapeutic ultrasound, electrotherapy and electromagnetic therapy.
By Cullum N, Nelson EA, Flemming K, Sheldon T.

No. 10
Effects of educational and psychosocial interventions for adolescents with diabetes mellitus: a systematic review.
By Hampson SE, Skinner TC, Hart J, Storey L, Gage H, Foxcroft D, *et al*.

No. 11
Effectiveness of autologous chondrocyte transplantation for hyaline cartilage defects in knees: a rapid and systematic review.
By Jobanputra P, Parry D, Fry-Smith A, Burls A.

No. 12
Statistical assessment of the learning curves of health technologies.
By Ramsay CR, Grant AM, Wallace SA, Garthwaite PH, Monk AF, Russell IT.

No. 13
The effectiveness and cost-effectiveness of temozolomide for the treatment of recurrent malignant glioma: a rapid and systematic review.
By Dinnes J, Cave C, Huang S, Major K, Milne R.

No. 14
A rapid and systematic review of the clinical effectiveness and cost-effectiveness of debriding agents in treating surgical wounds healing by secondary intention.
By Lewis R, Whiting P, ter Riet G, O'Meara S, Glanville J.

No. 15
Home treatment for mental health problems: a systematic review.
By Burns T, Knapp M, Catty J, Healey A, Henderson J, Watt H, *et al*.

No. 16
How to develop cost-conscious guidelines.
By Eccles M, Mason J.

No. 17
The role of specialist nurses in multiple sclerosis: a rapid and systematic review.
By De Broe S, Christopher F, Waugh N.

No. 18
A rapid and systematic review of the clinical effectiveness and cost-effectiveness of orlistat in the management of obesity.
By O'Meara S, Riemsma R, Shirran L, Mather L, ter Riet G.

No. 19
The clinical effectiveness and cost-effectiveness of pioglitazone for type 2 diabetes mellitus: a rapid and systematic review.
By Chilcott J, Wight J, Lloyd Jones M, Tappenden P.

No. 20
Extended scope of nursing practice: a multicentre randomised controlled trial of appropriately trained nurses and preregistration house officers in pre-operative assessment in elective general surgery.
By Kinley H, Czoski-Murray C, George S, McCabe C, Primrose J, Reilly C, *et al*.

No. 21
Systematic reviews of the effectiveness of day care for people with severe mental disorders: (1) Acute day hospital versus admission; (2) Vocational rehabilitation; (3) Day hospital versus outpatient care.
By Marshall M, Crowther R, Almaraz-Serrano A, Creed F, Sledge W, Kluiter H, *et al*.

No. 22
The measurement and monitoring of surgical adverse events.
By Bruce J, Russell EM, Mollison J, Krukowski ZH.

No. 23
Action research: a systematic review and guidance for assessment.
By Waterman H, Tillen D, Dickson R, de Koning K.

No. 24
A rapid and systematic review of the clinical effectiveness and cost-effectiveness of gemcitabine for the treatment of pancreatic cancer.
By Ward S, Morris E, Bansback N, Calvert N, Crellin A, Forman D, *et al*.

No. 25
A rapid and systematic review of the evidence for the clinical effectiveness and cost-effectiveness of irinotecan, oxaliplatin and raltitrexed for the treatment of advanced colorectal cancer.
By Lloyd Jones M, Hummel S, Bansback N, Orr B, Seymour M.

No. 26
Comparison of the effectiveness of inhaler devices in asthma and chronic obstructive airways disease: a systematic review of the literature.
By Brocklebank D, Ram F, Wright J, Barry P, Cates C, Davies L, *et al*.

No. 27
The cost-effectiveness of magnetic resonance imaging for investigation of the knee joint.
By Bryan S, Weatherburn G, Bungay H, Hatrick C, Salas C, Parry D, *et al*.

No. 28
A rapid and systematic review of the clinical effectiveness and cost-effectiveness of topotecan for ovarian cancer.
By Forbes C, Shirran L, Bagnall A-M, Duffy S, ter Riet G.

No. 29
Superseded by a report published in a later volume.

No. 30
The role of radiography in primary care patients with low back pain of at least 6 weeks duration: a randomised (unblinded) controlled trial.
By Kendrick D, Fielding K, Bentley E, Miller P, Kerslake R, Pringle M.

No. 31
Design and use of questionnaires: a review of best practice applicable to surveys of health service staff and patients.
By McColl E, Jacoby A, Thomas L, Soutter J, Bamford C, Steen N, *et al*.

No. 32
A rapid and systematic review of the clinical effectiveness and cost-effectiveness of paclitaxel, docetaxel, gemcitabine and vinorelbine in non-small-cell lung cancer.
By Clegg A, Scott DA, Sidhu M, Hewitson P, Waugh N.

No. 33
Subgroup analyses in randomised controlled trials: quantifying the risks of false-positives and false-negatives.
By Brookes ST, Whitley E, Peters TJ, Mulheran PA, Egger M, Davey Smith G.

No. 34
Depot antipsychotic medication in the treatment of patients with schizophrenia: (1) Meta-review; (2) Patient and nurse attitudes.
By David AS, Adams C.

No. 35
A systematic review of controlled trials of the effectiveness and cost-effectiveness of brief psychological treatments for depression.
By Churchill R, Hunot V, Corney R, Knapp M, McGuire H, Tylee A, *et al*.

No. 36
Cost analysis of child health surveillance.
By Sanderson D, Wright D, Acton C, Duree D.

Volume 6, 2002

No. 1
A study of the methods used to select review criteria for clinical audit.
By Hearnshaw H, Harker R, Cheater F, Baker R, Grimshaw G.

No. 2
Fludarabine as second-line therapy for B cell chronic lymphocytic leukaemia: a technology assessment.
By Hyde C, Wake B, Bryan S, Barton P, Fry-Smith A, Davenport C, *et al*.

No. 3
Rituximab as third-line treatment for refractory or recurrent Stage III or IV follicular non-Hodgkin's lymphoma: a systematic review and economic evaluation.
By Wake B, Hyde C, Bryan S, Barton P, Song F, Fry-Smith A, *et al*.

No. 4
A systematic review of discharge arrangements for older people.
By Parker SG, Peet SM, McPherson A, Cannaby AM, Baker R, Wilson A, *et al*.

No. 5
The clinical effectiveness and cost-effectiveness of inhaler devices used in the routine management of chronic asthma in older children: a systematic review and economic evaluation.
By Peters J, Stevenson M, Beverley C, Lim J, Smith S.

No. 6
The clinical effectiveness and cost-effectiveness of sibutramine in the management of obesity: a technology assessment.
By O'Meara S, Riemsma R, Shirran L, Mather L, ter Riet G.

No. 7
The cost-effectiveness of magnetic resonance angiography for carotid artery stenosis and peripheral vascular disease: a systematic review.
By Berry E, Kelly S, Westwood ME, Davies LM, Gough MJ, Bamford JM, *et al*.

No. 8
Promoting physical activity in South Asian Muslim women through 'exercise on prescription'.
By Carroll B, Ali N, Azam N.

No. 9
Zanamivir for the treatment of influenza in adults: a systematic review and economic evaluation.
By Burls A, Clark W, Stewart T, Preston C, Bryan S, Jefferson T, *et al*.

No. 10
A review of the natural history and epidemiology of multiple sclerosis: implications for resource allocation and health economic models.
By Richards RG, Sampson FC, Beard SM, Tappenden P.

No. 11
Screening for gestational diabetes: a systematic review and economic evaluation.
By Scott DA, Loveman E, McIntyre L, Waugh N.

No. 12
The clinical effectiveness and cost-effectiveness of surgery for people with morbid obesity: a systematic review and economic evaluation.
By Clegg AJ, Colquitt J, Sidhu MK, Royle P, Loveman E, Walker A.

No. 13
The clinical effectiveness of trastuzumab for breast cancer: a systematic review.
By Lewis R, Bagnall A-M, Forbes C, Shirran E, Duffy S, Kleijnen J, *et al*.

No. 14
The clinical effectiveness and cost-effectiveness of vinorelbine for breast cancer: a systematic review and economic evaluation.
By Lewis R, Bagnall A-M, King S, Woolacott N, Forbes C, Shirran L, *et al*.

No. 15
A systematic review of the effectiveness and cost-effectiveness of metal-on-metal hip resurfacing arthroplasty for treatment of hip disease.
By Vale L, Wyness L, McCormack K, McKenzie L, Brazzelli M, Stearns SC.

No. 16
The clinical effectiveness and cost-effectiveness of bupropion and nicotine replacement therapy for smoking cessation: a systematic review and economic evaluation.
By Woolacott NF, Jones L, Forbes CA, Mather LC, Sowden AJ, Song FJ, *et al*.

No. 17
A systematic review of effectiveness and economic evaluation of new drug treatments for juvenile idiopathic arthritis: etanercept.
By Cummins C, Connock M, Fry-Smith A, Burls A.

No. 18
Clinical effectiveness and cost-effectiveness of growth hormone in children: a systematic review and economic evaluation.
By Bryant J, Cave C, Mihaylova B, Chase D, McIntyre L, Gerard K, *et al*.

No. 19
Clinical effectiveness and cost-effectiveness of growth hormone in adults in relation to impact on quality of life: a systematic review and economic evaluation.
By Bryant J, Loveman E, Chase D, Mihaylova B, Cave C, Gerard K, *et al*.

No. 20
Clinical medication review by a pharmacist of patients on repeat prescriptions in general practice: a randomised controlled trial.
By Zermansky AG, Petty DR, Raynor DK, Lowe CJ, Freementle N, Vail A.

No. 21
The effectiveness of infliximab and etanercept for the treatment of rheumatoid arthritis: a systematic review and economic evaluation.
By Jobanputra P, Barton P, Bryan S, Burls A.

No. 22
A systematic review and economic evaluation of computerised cognitive behaviour therapy for depression and anxiety.
By Kaltenthaler E, Shackley P, Stevens K, Beverley C, Parry G, Chilcott J.

No. 23
A systematic review and economic evaluation of pegylated liposomal doxorubicin hydrochloride for ovarian cancer.
By Forbes C, Wilby J, Richardson G, Sculpher M, Mather L, Reimsma R.

No. 24
A systematic review of the effectiveness of interventions based on a stages-of-change approach to promote individual behaviour change.
By Riemsma RP, Pattenden J, Bridle C, Sowden AJ, Mather L, Watt IS, *et al*.

No. 25
A systematic review update of the clinical effectiveness and cost-effectiveness of glycoprotein IIb/IIIa antagonists.
By Robinson M, Ginnelly L, Sculpher M, Jones L, Riemsma R, Palmer S, *et al*.

No. 26
A systematic review of the effectiveness, cost-effectiveness and barriers to implementation of thrombolytic and neuroprotective therapy for acute ischaemic stroke in the NHS.
By Sandercock P, Berge E, Dennis M, Forbes J, Hand P, Kwan J, *et al*.

No. 27
A randomised controlled crossover trial of nurse practitioner versus doctor-led outpatient care in a bronchiectasis clinic.
By Caine N, Sharples LD, Hollingworth W, French J, Keogan M, Exley A, *et al*.

No. 28
Clinical effectiveness and cost – consequences of selective serotonin reuptake inhibitors in the treatment of sex offenders.
By Adi Y, Ashcroft D, Browne K, Beech A, Fry-Smith A, Hyde C.

No. 29
Treatment of established osteoporosis: a systematic review and cost–utility analysis.
By Kanis JA, Brazier JE, Stevenson M, Calvert NW, Lloyd Jones M.

No. 30
Which anaesthetic agents are cost-effective in day surgery? Literature review, national survey of practice and randomised controlled trial.
By Elliott RA Payne K, Moore JK, Davies LM, Harper NJN, St Leger AS, *et al*.

No. 31
Screening for hepatitis C among injecting drug users and in genitourinary medicine clinics: systematic reviews of effectiveness, modelling study and national survey of current practice.
By Stein K, Dalziel K, Walker A, McIntyre L, Jenkins B, Horne J, *et al*.

No. 32
The measurement of satisfaction with healthcare: implications for practice from a systematic review of the literature.
By Crow R, Gage H, Hampson S, Hart J, Kimber A, Storey L, *et al*.

No. 33
The effectiveness and cost-effectiveness of imatinib in chronic myeloid leukaemia: a systematic review.
By Garside R, Round A, Dalziel K, Stein K, Royle R.

No. 34
A comparative study of hypertonic saline, daily and alternate-day rhDNase in children with cystic fibrosis.
By Suri R, Wallis C, Bush A, Thompson S, Normand C, Flather M, *et al*.

No. 35
A systematic review of the costs and effectiveness of different models of paediatric home care.
By Parker G, Bhakta P, Lovett CA, Paisley S, Olsen R, Turner D, *et al*.

Volume 7, 2003

No. 1
How important are comprehensive literature searches and the assessment of trial quality in systematic reviews? Empirical study.
By Egger M, Jüni P, Bartlett C, Holenstein F, Sterne J.

No. 2
Systematic review of the effectiveness and cost-effectiveness, and economic evaluation, of home versus hospital or satellite unit haemodialysis for people with end-stage renal failure.
By Mowatt G, Vale L, Perez J, Wyness L, Fraser C, MacLeod A, *et al*.

No. 3
Systematic review and economic evaluation of the effectiveness of infliximab for the treatment of Crohn's disease.
By Clark W, Raftery J, Barton P, Song F, Fry-Smith A, Burls A.

No. 4
A review of the clinical effectiveness and cost-effectiveness of routine anti-D prophylaxis for pregnant women who are rhesus negative.
By Chilcott J, Lloyd Jones M, Wight J, Forman K, Wray J, Beverley C, *et al*.

No. 5
Systematic review and evaluation of the use of tumour markers in paediatric oncology: Ewing's sarcoma and neuroblastoma.
By Riley RD, Burchill SA, Abrams KR, Heney D, Lambert PC, Jones DR, *et al*.

No. 6
The cost-effectiveness of screening for *Helicobacter pylori* to reduce mortality and morbidity from gastric cancer and peptic ulcer disease: a discrete-event simulation model.
By Roderick P, Davies R, Raftery J, Crabbe D, Pearce R, Bhandari P, *et al*.

No. 7
The clinical effectiveness and cost-effectiveness of routine dental checks: a systematic review and economic evaluation.
By Davenport C, Elley K, Salas C, Taylor-Weetman CL, Fry-Smith A, Bryan S, *et al*.

No. 8
A multicentre randomised controlled trial assessing the costs and benefits of using structured information and analysis of women's preferences in the management of menorrhagia.
By Kennedy ADM, Sculpher MJ, Coulter A, Dwyer N, Rees M, Horsley S, *et al*.

No. 9
Clinical effectiveness and cost–utility of photodynamic therapy for wet age-related macular degeneration: a systematic review and economic evaluation.
By Meads C, Salas C, Roberts T, Moore D, Fry-Smith A, Hyde C.

No. 10
Evaluation of molecular tests for prenatal diagnosis of chromosome abnormalities.
By Grimshaw GM, Szczepura A, Hultén M, MacDonald F, Nevin NC, Sutton F, *et al*.

No. 11
First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS).
By Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM.

No. 12
The effectiveness and cost-effectiveness of ultrasound locating devices for central venous access: a systematic review and economic evaluation.
By Calvert N, Hind D, McWilliams RG, Thomas SM, Beverley C, Davidson A.

No. 13
A systematic review of atypical antipsychotics in schizophrenia.
By Bagnall A-M, Jones L, Lewis R, Ginnelly L, Glanville J, Torgerson D, *et al*.

No. 14
Prostate Testing for Cancer and Treatment (ProtecT) feasibility study.
By Donovan J, Hamdy F, Neal D, Peters T, Oliver S, Brindle L, *et al*.

No. 15
Early thrombolysis for the treatment of acute myocardial infarction: a systematic review and economic evaluation.
By Boland A, Dundar Y, Bagust A, Haycox A, Hill R, Mujica Mota R, *et al*.

No. 16
Screening for fragile X syndrome: a literature review and modelling.
By Song FJ, Barton P, Sleightholme V, Yao GL, Fry-Smith A.

No. 17
Systematic review of endoscopic sinus surgery for nasal polyps.
By Dalziel K, Stein K, Round A, Garside R, Royle P.

No. 18
Towards efficient guidelines: how to monitor guideline use in primary care.
By Hutchinson A, McIntosh A, Cox S, Gilbert C.

No. 19
Effectiveness and cost-effectiveness of acute hospital-based spinal cord injuries services: systematic review.
By Bagnall A-M, Jones L, Richardson G, Duffy S, Riemsma R.

No. 20
Prioritisation of health technology assessment. The PATHS model: methods and case studies.
By Townsend J, Buxton M, Harper G.

No. 21
Systematic review of the clinical effectiveness and cost-effectiveness of tension-free vaginal tape for treatment of urinary stress incontinence.
By Cody J, Wyness L, Wallace S, Glazener C, Kilonzo M, Stearns S, *et al*.

No. 22
The clinical and cost-effectiveness of patient education models for diabetes: a systematic review and economic evaluation.
By Loveman E, Cave C, Green C, Royle P, Dunn N, Waugh N.

No. 23
The role of modelling in prioritising and planning clinical trials.
By Chilcott J, Brennan A, Booth A, Karnon J, Tappenden P.

No. 24
Cost–benefit evaluation of routine influenza immunisation in people 65–74 years of age.
By Allsup S, Gosney M, Haycox A, Regan M.

No. 25
The clinical and cost-effectiveness of pulsatile machine perfusion versus cold storage of kidneys for transplantation retrieved from heart-beating and non-heart-beating donors.
By Wight J, Chilcott J, Holmes M, Brewer N.

No. 26
Can randomised trials rely on existing electronic data? A feasibility study to explore the value of routine data in health technology assessment.
By Williams JG, Cheung WY, Cohen DR, Hutchings HA, Longo MF, Russell IT.

No. 27
Evaluating non-randomised intervention studies.
By Deeks JJ, Dinnes J, D'Amico R, Sowden AJ, Sakarovitch C, Song F, *et al*.

No. 28
A randomised controlled trial to assess the impact of a package comprising a patient-orientated, evidence-based self-help guidebook and patient-centred consultations on disease management and satisfaction in inflammatory bowel disease.
By Kennedy A, Nelson E, Reeves D, Richardson G, Roberts C, Robinson A, *et al*.

No. 29
The effectiveness of diagnostic tests for the assessment of shoulder pain due to soft tissue disorders: a systematic review.
By Dinnes J, Loveman E, McIntyre L, Waugh N.

No. 30
The value of digital imaging in diabetic retinopathy.
By Sharp PF, Olson J, Strachan F, Hipwell J, Ludbrook A, O'Donnell M, *et al*.

No. 31
Lowering blood pressure to prevent myocardial infarction and stroke: a new preventive strategy.
By Law M, Wald N, Morris J.

No. 32
Clinical and cost-effectiveness of capecitabine and tegafur with uracil for the treatment of metastatic colorectal cancer: systematic review and economic evaluation.
By Ward S, Kaltenthaler E, Cowan J, Brewer N.

No. 33
Clinical and cost-effectiveness of new and emerging technologies for early localised prostate cancer: a systematic review.
By Hummel S, Paisley S, Morgan A, Currie E, Brewer N.

No. 34
Literature searching for clinical and cost-effectiveness studies used in health technology assessment reports carried out for the National Institute for Clinical Excellence appraisal system.
By Royle P, Waugh N.

No. 35
Systematic review and economic decision modelling for the prevention and treatment of influenza A and B.
By Turner D, Wailoo A, Nicholson K, Cooper N, Sutton A, Abrams K.

No. 36
A randomised controlled trial to evaluate the clinical and cost-effectiveness of Hickman line insertions in adult cancer patients by nurses.
By Boland A, Haycox A, Bagust A, Fitzsimmons L.

No. 37
Redesigning postnatal care: a randomised controlled trial of protocol-based midwifery-led care focused on individual women's physical and psychological health needs.
By MacArthur C, Winter HR, Bick DE, Lilford RJ, Lancashire RJ, Knowles H, *et al*.

No. 38
Estimating implied rates of discount in healthcare decision-making.
By West RR, McNabb R, Thompson AGH, Sheldon TA, Grimley Evans J.

No. 39
Systematic review of isolation policies in the hospital management of methicillin-resistant *Staphylococcus aureus*: a review of the literature with epidemiological and economic modelling.
By Cooper BS, Stone SP, Kibbler CC, Cookson BD, Roberts JA, Medley GF, *et al*.

No. 40
Treatments for spasticity and pain in multiple sclerosis: a systematic review.
By Beard S, Hunn A, Wight J.

No. 41
The inclusion of reports of randomised trials published in languages other than English in systematic reviews.
By Moher D, Pham B, Lawson ML, Klassen TP.

No. 42
The impact of screening on future health-promoting behaviours and health beliefs: a systematic review.
By Bankhead CR, Brett J, Bukach C, Webster P, Stewart-Brown S, Munafo M, *et al*.

Volume 8, 2004

No. 1
What is the best imaging strategy for acute stroke?
By Wardlaw JM, Keir SL, Seymour J, Lewis S, Sandercock PAG, Dennis MS, *et al*.

No. 2
Systematic review and modelling of the investigation of acute and chronic chest pain presenting in primary care.
By Mant J, McManus RJ, Oakes RAL, Delaney BC, Barton PM, Deeks JJ, *et al*.

No. 3
The effectiveness and cost-effectiveness of microwave and thermal balloon endometrial ablation for heavy menstrual bleeding: a systematic review and economic modelling.
By Garside R, Stein K, Wyatt K, Round A, Price A.

No. 4
A systematic review of the role of bisphosphonates in metastatic disease.
By Ross JR, Saunders Y, Edmonds PM, Patel S, Wonderling D, Normand C, *et al*.

No. 5
Systematic review of the clinical effectiveness and cost-effectiveness of capecitabine (Xeloda®) for locally advanced and/or metastatic breast cancer.
By Jones L, Hawkins N, Westwood M, Wright K, Richardson G, Riemsma R.

No. 6
Effectiveness and efficiency of guideline dissemination and implementation strategies.
By Grimshaw JM, Thomas RE, MacLennan G, Fraser C, Ramsay CR, Vale L, *et al*.

No. 7
Clinical effectiveness and costs of the Sugarbaker procedure for the treatment of pseudomyxoma peritonei.
By Bryant J, Clegg AJ, Sidhu MK, Brodin H, Royle P, Davidson P.

No. 8
Psychological treatment for insomnia in the regulation of long-term hypnotic drug use.
By Morgan K, Dixon S, Mathers N, Thompson J, Tomeny M.

No. 9
Improving the evaluation of therapeutic interventions in multiple sclerosis: development of a patient-based measure of outcome.
By Hobart JC, Riazi A, Lamping DL, Fitzpatrick R, Thompson AJ.

No. 10
A systematic review and economic evaluation of magnetic resonance cholangiopancreatography compared with diagnostic endoscopic retrograde cholangiopancreatography.
By Kaltenthaler E, Bravo Vergel Y, Chilcott J, Thomas S, Blakeborough T, Walters SJ, *et al*.

No. 11
The use of modelling to evaluate new drugs for patients with a chronic condition: the case of antibodies against tumour necrosis factor in rheumatoid arthritis.
By Barton P, Jobanputra P, Wilson J, Bryan S, Burls A.

No. 12
Clinical effectiveness and cost-effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry: a systematic review.
By Pandor A, Eastham J, Beverley C, Chilcott J, Paisley S.

No. 13
Clinical effectiveness and cost-effectiveness of pioglitazone and rosiglitazone in the treatment of type 2 diabetes: a systematic review and economic evaluation.
By Czoski-Murray C, Warren E, Chilcott J, Beverley C, Psyllaki MA, Cowan J.

No. 14
Routine examination of the newborn: the EMREN study. Evaluation of an extension of the midwife role including a randomised controlled trial of appropriately trained midwives and paediatric senior house officers.
By Townsend J, Wolke D, Hayes J, Davé S, Rogers C, Bloomfield L, *et al*.

No. 15
Involving consumers in research and development agenda setting for the NHS: developing an evidence-based approach.
By Oliver S, Clarke-Jones L, Rees R, Milne R, Buchanan P, Gabbay J, *et al*.

No. 16
A multi-centre randomised controlled trial of minimally invasive direct coronary bypass grafting versus percutaneous transluminal coronary angioplasty with stenting for proximal stenosis of the left anterior descending coronary artery.
By Reeves BC, Angelini GD, Bryan AJ, Taylor FC, Cripps T, Spyt TJ, *et al*.

No. 17
Does early magnetic resonance imaging influence management or improve outcome in patients referred to secondary care with low back pain? A pragmatic randomised controlled trial.
By Gilbert FJ, Grant AM, Gillan MGC, Vale L, Scott NW, Campbell MK, *et al*.

No. 18
The clinical and cost-effectiveness of anakinra for the treatment of rheumatoid arthritis in adults: a systematic review and economic analysis.
By Clark W, Jobanputra P, Barton P, Burls A.

No. 19
A rapid and systematic review and economic evaluation of the clinical and cost-effectiveness of newer drugs for treatment of mania associated with bipolar affective disorder.
By Bridle C, Palmer S, Bagnall A-M, Darba J, Duffy S, Sculpher M, *et al*.

No. 20
Liquid-based cytology in cervical screening: an updated rapid and systematic review and economic analysis.
By Karnon J, Peters J, Platt J, Chilcott J, McGoogan E, Brewer N.

No. 21
Systematic review of the long-term effects and economic consequences of treatments for obesity and implications for health improvement.
By Avenell A, Broom J, Brown TJ, Poobalan A, Aucott L, Stearns SC, *et al*.

No. 22
Autoantibody testing in children with newly diagnosed type 1 diabetes mellitus.
By Dretzke J, Cummins C, Sandercock J, Fry-Smith A, Barrett T, Burls A.

No. 23
Clinical effectiveness and cost-effectiveness of prehospital intravenous fluids in trauma patients.
By Dretzke J, Sandercock J, Bayliss S, Burls A.

No. 24
Newer hypnotic drugs for the short-term management of insomnia: a systematic review and economic evaluation.
By Dündar Y, Boland A, Strobl J, Dodd S, Haycox A, Bagust A, *et al*.

No. 25
Development and validation of methods for assessing the quality of diagnostic accuracy studies.
By Whiting P, Rutjes AWS, Dinnes J, Reitsma JB, Bossuyt PMM, Kleijnen J.

No. 26
EVALUATE hysterectomy trial: a multicentre randomised trial comparing abdominal, vaginal and laparoscopic methods of hysterectomy.
By Garry R, Fountain J, Brown J, Manca A, Mason S, Sculpher M, *et al*.

No. 27
Methods for expected value of information analysis in complex health economic models: developments on the health economics of interferon-β and glatiramer acetate for multiple sclerosis.
By Tappenden P, Chilcott JB, Eggington S, Oakley J, McCabe C.

No. 28
Effectiveness and cost-effectiveness of imatinib for first-line treatment of chronic myeloid leukaemia in chronic phase: a systematic review and economic analysis.
By Dalziel K, Round A, Stein K, Garside R, Price A.

No. 29
VenUS I: a randomised controlled trial of two types of bandage for treating venous leg ulcers.
By Iglesias C, Nelson EA, Cullum NA, Torgerson DJ on behalf of the VenUS Team.

No. 30
Systematic review of the effectiveness and cost-effectiveness, and economic evaluation, of myocardial perfusion scintigraphy for the diagnosis and management of angina and myocardial infarction.
By Mowatt G, Vale L, Brazzelli M, Hernandez R, Murray A, Scott N, *et al*.

No. 31
A pilot study on the use of decision theory and value of information analysis as part of the NHS Health Technology Assessment programme.
By Claxton K, Ginnelly L, Sculpher M, Philips Z, Palmer S.

No. 32
The Social Support and Family Health Study: a randomised controlled trial and economic evaluation of two alternative forms of postnatal support for mothers living in disadvantaged inner-city areas.
By Wiggins M, Oakley A, Roberts I, Turner H, Rajan L, Austerberry H, *et al*.

No. 33
Psychosocial aspects of genetic screening of pregnant women and newborns: a systematic review.
By Green JM, Hewison J, Bekker HL, Bryant, Cuckle HS.

No. 34
Evaluation of abnormal uterine bleeding: comparison of three outpatient procedures within cohorts defined by age and menopausal status.
By Critchley HOD, Warner P, Lee AJ, Brechin S, Guise J, Graham B.

No. 35
Coronary artery stents: a rapid systematic review and economic evaluation.
By Hill R, Bagust A, Bakhai A, Dickson R, Dündar Y, Haycox A, *et al*.

No. 36
Review of guidelines for good practice in decision-analytic modelling in health technology assessment.
By Philips Z, Ginnelly L, Sculpher M, Claxton K, Golder S, Riemsma R, *et al*.

No. 37
Rituximab (MabThera®) for aggressive non-Hodgkin's lymphoma: systematic review and economic evaluation.
By Knight C, Hind D, Brewer N, Abbott V.

No. 38
Clinical effectiveness and cost-effectiveness of clopidogrel and modified-release dipyridamole in the secondary prevention of occlusive vascular events: a systematic review and economic evaluation.
By Jones L, Griffin S, Palmer S, Main C, Orton V, Sculpher M, *et al.*

No. 39
Pegylated interferon α-2a and -2b in combination with ribavirin in the treatment of chronic hepatitis C: a systematic review and economic evaluation.
By Shepherd J, Brodin H, Cave C, Waugh N, Price A, Gabbay J.

No. 40
Clopidogrel used in combination with aspirin compared with aspirin alone in the treatment of non-ST-segment-elevation acute coronary syndromes: a systematic review and economic evaluation.
By Main C, Palmer S, Griffin S, Jones L, Orton V, Sculpher M, *et al.*

No. 41
Provision, uptake and cost of cardiac rehabilitation programmes: improving services to under-represented groups.
By Beswick AD, Rees K, Griebsch I, Taylor FC, Burke M, West RR, *et al.*

No. 42
Involving South Asian patients in clinical trials.
By Hussain-Gambles M, Leese B, Atkin K, Brown J, Mason S, Tovey P.

No. 43
Clinical and cost-effectiveness of continuous subcutaneous insulin infusion for diabetes.
By Colquitt JL, Green C, Sidhu MK, Hartwell D, Waugh N.

No. 44
Identification and assessment of ongoing trials in health technology assessment reviews.
By Song FJ, Fry-Smith A, Davenport C, Bayliss S, Adi Y, Wilson JS, *et al*.

No. 45
Systematic review and economic evaluation of a long-acting insulin analogue, insulin glargine
By Warren E, Weatherley-Jones E, Chilcott J, Beverley C.

No. 46
Supplementation of a home-based exercise programme with a class-based programme for people with osteoarthritis of the knees: a randomised controlled trial and health economic analysis.
By McCarthy CJ, Mills PM, Pullen R, Richardson G, Hawkins N, Roberts CR, *et al*.

No. 47
Clinical and cost-effectiveness of once-daily versus more frequent use of same potency topical corticosteroids for atopic eczema: a systematic review and economic evaluation.
By Green C, Colquitt JL, Kirby J, Davidson P, Payne E.

No. 48
Acupuncture of chronic headache disorders in primary care: randomised controlled trial and economic analysis.
By Vickers AJ, Rees RW, Zollman CE, McCarney R, Smith CM, Ellis N, *et al*.

No. 49
Generalisability in economic evaluation studies in healthcare: a review and case studies.
By Sculpher MJ, Pang FS, Manca A, Drummond MF, Golder S, Urdahl H, *et al*.

No. 50
Virtual outreach: a randomised controlled trial and economic evaluation of joint teleconferenced medical consultations.
By Wallace P, Barber J, Clayton W, Currell R, Fleming K, Garner P, *et al*.

Volume 9, 2005

No. 1
Randomised controlled multiple treatment comparison to provide a cost-effectiveness rationale for the selection of antimicrobial therapy in acne.
By Ozolins M, Eady EA, Avery A, Cunliffe WJ, O'Neill C, Simpson NB, *et al*.

No. 2
Do the findings of case series studies vary significantly according to methodological characteristics?
By Dalziel K, Round A, Stein K, Garside R, Castelnuovo E, Payne L.

No. 3
Improving the referral process for familial breast cancer genetic counselling: findings of three randomised controlled trials of two interventions.
By Wilson BJ, Torrance N, Mollison J, Wordsworth S, Gray JR, Haites NE, *et al*.

No. 4
Randomised evaluation of alternative electrosurgical modalities to treat bladder outflow obstruction in men with benign prostatic hyperplasia.
By Fowler C, McAllister W, Plail R, Karim O, Yang Q.

No. 5
A pragmatic randomised controlled trial of the cost-effectiveness of palliative therapies for patients with inoperable oesophageal cancer.
By Shenfine J, McNamee P, Steen N, Bond J, Griffin SM.

No. 6
Impact of computer-aided detection prompts on the sensitivity and specificity of screening mammography.
By Taylor P, Champness J, Given-Wilson R, Johnston K, Potts H.

No. 7
Issues in data monitoring and interim analysis of trials.
By Grant AM, Altman DG, Babiker AB, Campbell MK, Clemens FJ, Darbyshire JH, *et al*.

No. 8
Lay public's understanding of equipoise and randomisation in randomised controlled trials.
By Robinson EJ, Kerr CEP, Stevens AJ, Lilford RJ, Braunholtz DA, Edwards SJ, *et al*.

No. 9
Clinical and cost-effectiveness of electroconvulsive therapy for depressive illness, schizophrenia, catatonia and mania: systematic reviews and economic modelling studies.
By Greenhalgh J, Knight C, Hind D, Beverley C, Walters S.

No. 10
Measurement of health-related quality of life for people with dementia: development of a new instrument (DEMQOL) and an evaluation of current methodology.
By Smith SC, Lamping DL, Banerjee S, Harwood R, Foley B, Smith P, *et al*.

No. 11
Clinical effectiveness and cost-effectiveness of drotrecogin alfa (activated) (Xigris®) for the treatment of severe sepsis in adults: a systematic review and economic evaluation.
By Green C, Dinnes J, Takeda A, Shepherd J, Hartwell D, Cave C, *et al*.

No. 12
A methodological review of how heterogeneity has been examined in systematic reviews of diagnostic test accuracy.
By Dinnes J, Deeks J, Kirby J, Roderick P.

No. 13
Cervical screening programmes: can automation help? Evidence from systematic reviews, an economic analysis and a simulation modelling exercise applied to the UK.
By Willis BH, Barton P, Pearmain P, Bryan S, Hyde C.

No. 14
Laparoscopic surgery for inguinal hernia repair: systematic review of effectiveness and economic evaluation.
By McCormack K, Wake B, Perez J, Fraser C, Cook J, McIntosh E, *et al*.

No. 15
Clinical effectiveness, tolerability and cost-effectiveness of newer drugs for epilepsy in adults: a systematic review and economic evaluation.
By Wilby J, Kainth A, Hawkins N, Epstein D, McIntosh H, McDaid C, *et al*.

No. 16
A randomised controlled trial to compare the cost-effectiveness of tricyclic antidepressants, selective serotonin reuptake inhibitors and lofepramine.
By Peveler R, Kendrick T, Buxton M, Longworth L, Baldwin D, Moore M, *et al*.

No. 17
Clinical effectiveness and cost-effectiveness of immediate angioplasty for acute myocardial infarction: systematic review and economic evaluation.
By Hartwell D, Colquitt J, Loveman E, Clegg AJ, Brodin H, Waugh N, *et al*.

No. 18
A randomised controlled comparison of alternative strategies in stroke care.
By Kalra L, Evans A, Perez I, Knapp M, Swift C, Donaldson N.

No. 19
The investigation and analysis of critical incidents and adverse events in healthcare.
By Woloshynowych M, Rogers S, Taylor-Adams S, Vincent C.

No. 20
Potential use of routine databases in health technology assessment.
By Raftery J, Roderick P, Stevens A.

No. 21
Clinical and cost-effectiveness of newer immunosuppressive regimens in renal transplantation: a systematic review and modelling study.
By Woodroffe R, Yao GL, Meads C, Bayliss S, Ready A, Raftery J, *et al*.

No. 22
A systematic review and economic evaluation of alendronate, etidronate, risedronate, raloxifene and teriparatide for the prevention and treatment of postmenopausal osteoporosis.
By Stevenson M, Lloyd Jones M, De Nigris E, Brewer N, Davis S, Oakley J.

No. 23
A systematic review to examine the impact of psycho-educational interventions on health outcomes and costs in adults and children with difficult asthma.
By Smith JR, Mugford M, Holland R, Candy B, Noble MJ, Harrison BDW, *et al*.

No. 24
An evaluation of the costs, effectiveness and quality of renal replacement therapy provision in renal satellite units in England and Wales.
By Roderick P, Nicholson T, Armitage A, Mehta R, Mullee M, Gerard K, *et al*.

No. 25
Imatinib for the treatment of patients with unresectable and/or metastatic gastrointestinal stromal tumours: systematic review and economic evaluation.
By Wilson J, Connock M, Song F, Yao G, Fry-Smith A, Raftery J, *et al*.

No. 26
Indirect comparisons of competing interventions.
By Glenny AM, Altman DG, Song F, Sakarovitch C, Deeks JJ, D'Amico R, *et al*.

No. 27
Cost-effectiveness of alternative strategies for the initial medical management of non-ST elevation acute coronary syndrome: systematic review and decision-analytical modelling.
By Robinson M, Palmer S, Sculpher M, Philips Z, Ginnelly L, Bowens A, *et al*.

No. 28
Outcomes of electrically stimulated gracilis neosphincter surgery.
By Tillin T, Chambers M, Feldman R.

No. 29
The effectiveness and cost-effectiveness of pimecrolimus and tacrolimus for atopic eczema: a systematic review and economic evaluation.
By Garside R, Stein K, Castelnuovo E, Pitt M, Ashcroft D, Dimmock P, *et al*.

No. 30
Systematic review on urine albumin testing for early detection of diabetic complications.
By Newman DJ, Mattock MB, Dawnay ABS, Kerry S, McGuire A, Yaqoob M, *et al*.

No. 31
Randomised controlled trial of the cost-effectiveness of water-based therapy for lower limb osteoarthritis.
By Cochrane T, Davey RC, Matthes Edwards SM.

No. 32
Longer term clinical and economic benefits of offering acupuncture care to patients with chronic low back pain.
By Thomas KJ, MacPherson H, Ratcliffe J, Thorpe L, Brazier J, Campbell M, *et al*.

No. 33
Cost-effectiveness and safety of epidural steroids in the management of sciatica.
By Price C, Arden N, Coglan L, Rogers P.

No. 34
The British Rheumatoid Outcome Study Group (BROSG) randomised controlled trial to compare the effectiveness and cost-effectiveness of aggressive versus symptomatic therapy in established rheumatoid arthritis.
By Symmons D, Tricker K, Roberts C, Davies L, Dawes P, Scott DL.

No. 35
Conceptual framework and systematic review of the effects of participants' and professionals' preferences in randomised controlled trials.
By King M, Nazareth I, Lampe F, Bower P, Chandler M, Morou M, *et al*.

No. 36
The clinical and cost-effectiveness of implantable cardioverter defibrillators: a systematic review.
By Bryant J, Brodin H, Loveman E, Payne E, Clegg A.

No. 37
A trial of problem-solving by community mental health nurses for anxiety, depression and life difficulties among general practice patients. The CPN-GP study.
By Kendrick T, Simons L, Mynors-Wallis L, Gray A, Lathlean J, Pickering R, *et al*.

No. 38
The causes and effects of socio-demographic exclusions from clinical trials.
By Bartlett C, Doyal L, Ebrahim S, Davey P, Bachmann M, Egger M, *et al*.

No. 39
Is hydrotherapy cost-effective? A randomised controlled trial of combined hydrotherapy programmes compared with physiotherapy land techniques in children with juvenile idiopathic arthritis.
By Epps H, Ginnelly L, Utley M, Southwood T, Gallivan S, Sculpher M, *et al*.

No. 40
A randomised controlled trial and cost-effectiveness study of systematic screening (targeted and total population screening) versus routine practice for the detection of atrial fibrillation in people aged 65 and over. The SAFE study.
By Hobbs FDR, Fitzmaurice DA, Mant J, Murray E, Jowett S, Bryan S, *et al*.

No. 41
Displaced intracapsular hip fractures in fit, older people: a randomised comparison of reduction and fixation, bipolar hemiarthroplasty and total hip arthroplasty.
By Keating JF, Grant A, Masson M, Scott NW, Forbes JF.

No. 42
Long-term outcome of cognitive behaviour therapy clinical trials in central Scotland.
By Durham RC, Chambers JA, Power KG, Sharp DM, Macdonald RR, Major KA, *et al*.

No. 43
The effectiveness and cost-effectiveness of dual-chamber pacemakers compared with single-chamber pacemakers for bradycardia due to atrioventricular block or sick sinus syndrome: systematic review and economic evaluation.
By Castelnuovo E, Stein K, Pitt M, Garside R, Payne E.

No. 44
Newborn screening for congenital heart defects: a systematic review and cost-effectiveness analysis.
By Knowles R, Griebsch I, Dezateux C, Brown J, Bull C, Wren C.

No. 45
The clinical and cost-effectiveness of left ventricular assist devices for end-stage heart failure: a systematic review and economic evaluation.
By Clegg AJ, Scott DA, Loveman E, Colquitt J, Hutchinson J, Royle P, *et al*.

No. 46
The effectiveness of the Heidelberg Retina Tomograph and laser diagnostic glaucoma scanning system (GDx) in detecting and monitoring glaucoma.
By Kwartz AJ, Henson DB, Harper RA, Spencer AF, McLeod D.

No. 47
Clinical and cost-effectiveness of autologous chondrocyte implantation for cartilage defects in knee joints: systematic review and economic evaluation.
By Clar C, Cummins E, McIntyre L, Thomas S, Lamb J, Bain L, *et al*.

No. 48
Systematic review of effectiveness of different treatments for childhood retinoblastoma.
By McDaid C, Hartley S, Bagnall A-M, Ritchie G, Light K, Riemsma R.

No. 49
Towards evidence-based guidelines for the prevention of venous thromboembolism: systematic reviews of mechanical methods, oral anticoagulation, dextran and regional anaesthesia as thromboprophylaxis.
By Roderick P, Ferris G, Wilson K, Halls H, Jackson D, Collins R, *et al*.

No. 50
The effectiveness and cost-effectiveness of parent training/education programmes for the treatment of conduct disorder, including oppositional defiant disorder, in children.
By Dretzke J, Frew E, Davenport C, Barlow J, Stewart-Brown S, Sandercock J, *et al*.

Volume 10, 2006

No. 1
The clinical and cost-effectiveness of donepezil, rivastigmine, galantamine and memantine for Alzheimer's disease.
By Loveman E, Green C, Kirby J, Takeda A, Picot J, Payne E, *et al*.

No. 2
FOOD: a multicentre randomised trial evaluating feeding policies in patients admitted to hospital with a recent stroke.
By Dennis M, Lewis S, Cranswick G, Forbes J.

No. 3
The clinical effectiveness and cost-effectiveness of computed tomography screening for lung cancer: systematic reviews.
By Black C, Bagust A, Boland A, Walker S, McLeod C, De Verteuil R, *et al*.

No. 4
A systematic review of the effectiveness and cost-effectiveness of neuroimaging assessments used to visualise the seizure focus in people with refractory epilepsy being considered for surgery.
By Whiting P, Gupta R, Burch J, Mujica Mota RE, Wright K, Marson A, *et al*.

No. 5
Comparison of conference abstracts and presentations with full-text articles in the health technology assessments of rapidly evolving technologies.
By Dundar Y, Dodd S, Dickson R, Walley T, Haycox A, Williamson PR.

No. 6
Systematic review and evaluation of methods of assessing urinary incontinence.
By Martin JL, Williams KS, Abrams KR, Turner DA, Sutton AJ, Chapple C, *et al*.

No. 7
The clinical effectiveness and cost-effectiveness of newer drugs for children with epilepsy. A systematic review.
By Connock M, Frew E, Evans B-W, Bryan S, Cummins C, Fry-Smith A, *et al*.

No. 8
Surveillance of Barrett's oesophagus: exploring the uncertainty through systematic review, expert workshop and economic modelling.
By Garside R, Pitt M, Somerville M, Stein K, Price A, Gilbert N.

No. 9
Topotecan, pegylated liposomal doxorubicin hydrochloride and paclitaxel for second-line or subsequent treatment of advanced ovarian cancer: a systematic review and economic evaluation.
By Main C, Bojke L, Griffin S, Norman G, Barbieri M, Mather L, *et al*.

No. 10
Evaluation of molecular techniques in prediction and diagnosis of cytomegalovirus disease in immunocompromised patients.
By Szczepura A, Westmoreland D, Vinogradova Y, Fox J, Clark M.

No. 11
Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study.
By Wu O, Robertson L, Twaddle S, Lowe GDO, Clark P, Greaves M, *et al*.

No. 12
A series of systematic reviews to inform a decision analysis for sampling and treating infected diabetic foot ulcers.
By Nelson EA, O'Meara S, Craig D, Iglesias C, Golder S, Dalton J, *et al*.

No. 13
Randomised clinical trial, observational study and assessment of cost-effectiveness of the treatment of varicose veins (REACTIV trial).
By Michaels JA, Campbell WB, Brazier JE, MacIntyre JB, Palfreyman SJ, Ratcliffe J, *et al*.

No. 14
The cost-effectiveness of screening for oral cancer in primary care.
By Speight PM, Palmer S, Moles DR, Downer MC, Smith DH, Henriksson M *et al*.

No. 15
Measurement of the clinical and cost-effectiveness of non-invasive diagnostic testing strategies for deep vein thrombosis.
By Goodacre S, Sampson F, Stevenson M, Wailoo A, Sutton A, Thomas S, *et al*.

No. 16
Systematic review of the effectiveness and cost-effectiveness of HealOzone® for the treatment of occlusal pit/fissure caries and root caries.
By Brazzelli M, McKenzie L, Fielding S, Fraser C, Clarkson J, Kilonzo M, *et al*.

No. 17
Randomised controlled trials of conventional antipsychotic versus new atypical drugs, and new atypical drugs versus clozapine, in people with schizophrenia responding poorly to, or intolerant of, current drug treatment.
By Lewis SW, Davies L, Jones PB, Barnes TRE, Murray RM, Kerwin R, *et al*.

No. 18
Diagnostic tests and algorithms used in the investigation of haematuria: systematic reviews and economic evaluation.
By Rodgers M, Nixon J, Hempel S, Aho T, Kelly J, Neal D, *et al*.

No. 19
Cognitive behavioural therapy in addition to antispasmodic therapy for irritable bowel syndrome in primary care: randomised controlled trial.
By Kennedy TM, Chalder T, McCrone P, Darnley S, Knapp M, Jones RH, *et al*.

No. 20
A systematic review of the clinical effectiveness and cost-effectiveness of enzyme replacement therapies for Fabry's disease and mucopolysaccharidosis type 1.
By Connock M, Juarez-Garcia A, Frew E, Mans A, Dretzke J, Fry-Smith A, *et al*.

No. 21
Health benefits of antiviral therapy for mild chronic hepatitis C: randomised controlled trial and economic evaluation.
By Wright M, Grieve R, Roberts J, Main J, Thomas HC on behalf of the UK Mild Hepatitis C Trial Investigators.

No. 22
Pressure relieving support surfaces: a randomised evaluation.
By Nixon J, Nelson EA, Cranny G, Iglesias CP, Hawkins K, Cullum NA, *et al*.

No. 23
A systematic review and economic model of the effectiveness and cost-effectiveness of methylphenidate, dexamfetamine and atomoxetine for the treatment of attention deficit hyperactivity disorder in children and adolescents.
By King S, Griffin S, Hodges Z, Weatherly H, Asseburg C, Richardson G, *et al*.

No. 24
The clinical effectiveness and cost-effectiveness of enzyme replacement therapy for Gaucher's disease: a systematic review.
By Connock M, Burls A, Frew E, Fry-Smith A, Juarez-Garcia A, McCabe C, *et al*.

No. 25
Effectiveness and cost-effectiveness of salicylic acid and cryotherapy for cutaneous warts. An economic decision model.
By Thomas KS, Keogh-Brown MR, Chalmers JR, Fordham RJ, Holland RC, Armstrong SJ, *et al*.

No. 26
A systematic literature review of the effectiveness of non-pharmacological interventions to prevent wandering in dementia and evaluation of the ethical implications and acceptability of their use.
By Robinson L, Hutchings D, Corner L, Beyer F, Dickinson H, Vanoli A, *et al*.

No. 27
A review of the evidence on the effects and costs of implantable cardioverter defibrillator therapy in different patient groups, and modelling of cost-effectiveness and cost–utility for these groups in a UK context.
By Buxton M, Caine N, Chase D, Connelly D, Grace A, Jackson C, *et al*.

No. 28
Adefovir dipivoxil and pegylated interferon alfa-2a for the treatment of chronic hepatitis B: a systematic review and economic evaluation.
By Shepherd J, Jones J, Takeda A, Davidson P, Price A.

No. 29
An evaluation of the clinical and cost-effectiveness of pulmonary artery catheters in patient management in intensive care: a systematic review and a randomised controlled trial.
By Harvey S, Stevens K, Harrison D, Young D, Brampton W, McCabe C, *et al*.

No. 30
Accurate, practical and cost-effective assessment of carotid stenosis in the UK.
By Wardlaw JM, Chappell FM, Stevenson M, De Nigris E, Thomas S, Gillard J, *et al*.

No. 31
Etanercept and infliximab for the treatment of psoriatic arthritis: a systematic review and economic evaluation.
By Woolacott N, Bravo Vergel Y, Hawkins N, Kainth A, Khadjesari Z, Misso K, *et al*.

No. 32
The cost-effectiveness of testing for hepatitis C in former injecting drug users.
By Castelnuovo E, Thompson-Coon J, Pitt M, Cramp M, Siebert U, Price A, *et al*.

No. 33
Computerised cognitive behaviour therapy for depression and anxiety update: a systematic review and economic evaluation.
By Kaltenthaler E, Brazier J, De Nigris E, Tumur I, Ferriter M, Beverley C, *et al*.

No. 34
Cost-effectiveness of using prognostic information to select women with breast cancer for adjuvant systemic therapy.
By Williams C, Brunskill S, Altman D, Briggs A, Campbell H, Clarke M, *et al*.

No. 35
Psychological therapies including dialectical behaviour therapy for borderline personality disorder: a systematic review and preliminary economic evaluation.
By Brazier J, Tumur I, Holmes M, Ferriter M, Parry G, Dent-Brown K, *et al*.

No. 36
Clinical effectiveness and cost-effectiveness of tests for the diagnosis and investigation of urinary tract infection in children: a systematic review and economic model.
By Whiting P, Westwood M, Bojke L, Palmer S, Richardson G, Cooper J, *et al*.

No. 37
Cognitive behavioural therapy in chronic fatigue syndrome: a randomised controlled trial of an outpatient group programme.
By O'Dowd H, Gladwell P, Rogers CA, Hollinghurst S, Gregory A.

No. 38
A comparison of the cost-effectiveness of five strategies for the prevention of non-steroidal anti-inflammatory drug-induced gastrointestinal toxicity: a systematic review with economic modelling.
By Brown TJ, Hooper L, Elliott RA, Payne K, Webb R, Roberts C, *et al*.

No. 39
The effectiveness and cost-effectiveness of computed tomography screening for coronary artery disease: systematic review.
By Waugh N, Black C, Walker S, McIntyre L, Cummins E, Hillis G.

No. 40
What are the clinical outcome and cost-effectiveness of endoscopy undertaken by nurses when compared with doctors? A Multi-Institution Nurse Endoscopy Trial (MINuET).
By Williams J, Russell I, Durai D, Cheung W-Y, Farrin A, Bloor K, *et al*.

No. 41
The clinical and cost-effectiveness of oxaliplatin and capecitabine for the adjuvant treatment of colon cancer: systematic review and economic evaluation.
By Pandor A, Eggington S, Paisley S, Tappenden P, Sutcliffe P.

No. 42
A systematic review of the effectiveness of adalimumab, etanercept and infliximab for the treatment of rheumatoid arthritis in adults and an economic evaluation of their cost-effectiveness.
By Chen Y-F, Jobanputra P, Barton P, Jowett S, Bryan S, Clark W, *et al*.

No. 43
Telemedicine in dermatology: a randomised controlled trial.
By Bowns IR, Collins K, Walters SJ, McDonagh AJG.

No. 44
Cost-effectiveness of cell salvage and alternative methods of minimising perioperative allogeneic blood transfusion: a systematic review and economic model.
By Davies L, Brown TJ, Haynes S, Payne K, Elliott RA, McCollum C.

No. 45
Clinical effectiveness and cost-effectiveness of laparoscopic surgery for colorectal cancer: systematic reviews and economic evaluation.
By Murray A, Lourenco T, de Verteuil R, Hernandez R, Fraser C, McKinley A, *et al*.

No. 46
Etanercept and efalizumab for the treatment of psoriasis: a systematic review.
By Woolacott N, Hawkins N, Mason A, Kainth A, Khadjesari Z, Bravo Vergel Y, *et al*.

No. 47
Systematic reviews of clinical decision tools for acute abdominal pain.
By Liu JLY, Wyatt JC, Deeks JJ, Clamp S, Keen J, Verde P, *et al*.

No. 48
Evaluation of the ventricular assist device programme in the UK.
By Sharples L, Buxton M, Caine N, Cafferty F, Demiris N, Dyer M, *et al*.

No. 49
A systematic review and economic model of the clinical and cost-effectiveness of immunosuppressive therapy for renal transplantation in children.
By Yao G, Albon E, Adi Y, Milford D, Bayliss S, Ready A, *et al*.

No. 50
Amniocentesis results: investigation of anxiety. The ARIA trial.
By Hewison J, Nixon J, Fountain J, Cocks K, Jones C, Mason G, *et al*.

Volume 11, 2007

No. 1
Pemetrexed disodium for the treatment of malignant pleural mesothelioma: a systematic review and economic evaluation.
By Dundar Y, Bagust A, Dickson R, Dodd S, Green J, Haycox A, *et al*.

No. 2
A systematic review and economic model of the clinical effectiveness and cost-effectiveness of docetaxel in combination with prednisone or prednisolone for the treatment of hormone-refractory metastatic prostate cancer.
By Collins R, Fenwick E, Trowman R, Perard R, Norman G, Light K, *et al*.

No. 3
A systematic review of rapid diagnostic tests for the detection of tuberculosis infection.
By Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, *et al*.

No. 4
The clinical effectiveness and cost-effectiveness of strontium ranelate for the prevention of osteoporotic fragility fractures in postmenopausal women.
By Stevenson M, Davis S, Lloyd-Jones M, Beverley C.

No. 5
A systematic review of quantitative and qualitative research on the role and effectiveness of written information available to patients about individual medicines.
By Raynor DK, Blenkinsopp A, Knapp P, Grime J, Nicolson DJ, Pollock K, *et al*.

No. 6
Oral naltrexone as a treatment for relapse prevention in formerly opioid-dependent drug users: a systematic review and economic evaluation.
By Adi Y, Juarez-Garcia A, Wang D, Jowett S, Frew E, Day E, *et al*.

No. 7
Glucocorticoid-induced osteoporosis: a systematic review and cost–utility analysis.
By Kanis JA, Stevenson M, McCloskey EV, Davis S, Lloyd-Jones M.

No. 8
Epidemiological, social, diagnostic and economic evaluation of population screening for genital chlamydial infection.
By Low N, McCarthy A, Macleod J, Salisbury C, Campbell R, Roberts TE, *et al*.

No. 9
Methadone and buprenorphine for the management of opioid dependence: a systematic review and economic evaluation.
By Connock M, Juarez-Garcia A, Jowett S, Frew E, Liu Z, Taylor RJ, *et al*.

No. 10
Exercise Evaluation Randomised Trial (EXERT): a randomised trial comparing GP referral for leisure centre-based exercise, community-based walking and advice only.
By Isaacs AJ, Critchley JA, See Tai S, Buckingham K, Westley D, Harridge SDR, *et al*.

No. 11
Interferon alfa (pegylated and non-pegylated) and ribavirin for the treatment of mild chronic hepatitis C: a systematic review and economic evaluation.
By Shepherd J, Jones J, Hartwell D, Davidson P, Price A, Waugh N.

No. 12
Systematic review and economic evaluation of bevacizumab and cetuximab for the treatment of metastatic colorectal cancer.
By Tappenden P, Jones R, Paisley S, Carroll C.

No. 13
A systematic review and economic evaluation of epoetin alfa, epoetin beta and darbepoetin alfa in anaemia associated with cancer, especially that attributable to cancer treatment.
By Wilson J, Yao GL, Raftery J, Bohlius J, Brunskill S, Sandercock J, *et al*.

No. 14
A systematic review and economic evaluation of statins for the prevention of coronary events.
By Ward S, Lloyd Jones M, Pandor A, Holmes M, Ara R, Ryan A, *et al*.

No. 15
A systematic review of the effectiveness and cost-effectiveness of different models of community-based respite care for frail older people and their carers.
By Mason A, Weatherly H, Spilsbury K, Arksey H, Golder S, Adamson J, *et al*.

No. 16
Additional therapy for young children with spastic cerebral palsy: a randomised controlled trial.
By Weindling AM, Cunningham CC, Glenn SM, Edwards RT, Reeves DJ.

No. 17
Screening for type 2 diabetes: literature review and economic modelling.
By Waugh N, Scotland G, McNamee P, Gillett M, Brennan A, Goyder E, *et al*.

No. 18
The effectiveness and cost-effectiveness of cinacalcet for secondary hyperparathyroidism in end-stage renal disease patients on dialysis: a systematic review and economic evaluation.
By Garside R, Pitt M, Anderson R, Mealing S, Roome C, Snaith A, *et al*.

No. 19
The clinical effectiveness and cost-effectiveness of gemcitabine for metastatic breast cancer: a systematic review and economic evaluation.
By Takeda AL, Jones J, Loveman E, Tan SC, Clegg AJ.

No. 20
A systematic review of duplex ultrasound, magnetic resonance angiography and computed tomography angiography for the diagnosis and assessment of symptomatic, lower limb peripheral arterial disease.
By Collins R, Cranny G, Burch J, Aguiar-Ibáñez R, Craig D, Wright K, *et al*.

No. 21
The clinical effectiveness and cost-effectiveness of treatments for children with idiopathic steroid-resistant nephrotic syndrome: a systematic review.
By Colquitt JL, Kirby J, Green C, Cooper K, Trompeter RS.

No. 22
A systematic review of the routine monitoring of growth in children of primary school age to identify growth-related conditions.
By Fayter D, Nixon J, Hartley S, Rithalia A, Butler G, Rudolf M, *et al*.

No. 23
Systematic review of the effectiveness of preventing and treating *Staphylococcus aureus* carriage in reducing peritoneal catheter-related infections.
By McCormack K, Rabindranath K, Kilonzo M, Vale L, Fraser C, McIntyre L, *et al*.

No. 24
The clinical effectiveness and cost of repetitive transcranial magnetic stimulation versus electroconvulsive therapy in severe depression: a multicentre pragmatic randomised controlled trial and economic analysis.
By McLoughlin DM, Mogg A, Eranti S, Pluck G, Purvis R, Edwards D, *et al*.

No. 25
A randomised controlled trial and economic evaluation of direct versus indirect and individual versus group modes of speech and language therapy for children with primary language impairment.
By Boyle J, McCartney E, Forbes J, O'Hare A.

No. 26
Hormonal therapies for early breast cancer: systematic review and economic evaluation.
By Hind D, Ward S, De Nigris E, Simpson E, Carroll C, Wyld L.

No. 27
Cardioprotection against the toxic effects of anthracyclines given to children with cancer: a systematic review.
By Bryant J, Picot J, Levitt G, Sullivan I, Baxter L, Clegg A.

No. 28
Adalimumab, etanercept and infliximab for the treatment of ankylosing spondylitis: a systematic review and economic evaluation.
By McLeod C, Bagust A, Boland A, Dagenais P, Dickson R, Dundar Y, *et al*.

No. 29
Prenatal screening and treatment strategies to prevent group B streptococcal and other bacterial infections in early infancy: cost-effectiveness and expected value of information analyses.
By Colbourn T, Asseburg C, Bojke L, Philips Z, Claxton K, Ades AE, *et al*.

No. 30
Clinical effectiveness and cost-effectiveness of bone morphogenetic proteins in the non-healing of fractures and spinal fusion: a systematic review.
By Garrison KR, Donell S, Ryder J, Shemilt I, Mugford M, Harvey I, *et al*.

No. 31
A randomised controlled trial of postoperative radiotherapy following breast-conserving surgery in a minimum-risk older population. The PRIME trial.
By Prescott RJ, Kunkler IH, Williams LJ, King CC, Jack W, van der Pol M, *et al*.

No. 32
Current practice, accuracy, effectiveness and cost-effectiveness of the school entry hearing screen.
By Bamford J, Fortnum H, Bristow K, Smith J, Vamvakas G, Davies L, *et al*.

No. 33
The clinical effectiveness and cost-effectiveness of inhaled insulin in diabetes mellitus: a systematic review and economic evaluation.
By Black C, Cummins E, Royle P, Philip S, Waugh N.

No. 34
Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis.
By Thompson Coon J, Rogers G, Hewson P, Wright D, Anderson R, Cramp M, *et al*.

No. 35
The Birmingham Rehabilitation Uptake Maximisation Study (BRUM). Home-based compared with hospital-based cardiac rehabilitation in a multi-ethnic population: cost-effectiveness and patient adherence.
By Jolly K, Taylor R, Lip GYH, Greenfield S, Raftery J, Mant J, *et al*.

No. 36
A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food.
By Abubakar I, Irvine L, Aldus CF, Wyatt GM, Fordham R, Schelenz S, *et al*.

Health Technology Assessment Programme

Current and past membership details of all HTA 'committees' are available from the HTA website (www.hta.ac.uk)

Diagnostic Technologies & Screening Panel

Members

Chair,
Dr Ron Zimmern, Director of the Public Health Genetics Unit, Strangeways Research Laboratories, Cambridge

Ms Norma Armston, Freelance Consumer Advocate, Bolton

Professor Max Bachmann, Professor of Health Care Interfaces, Department of Health Policy and Practice, University of East Anglia

Professor Rudy Bilous Professor of Clinical Medicine & Consultant Physician, The Academic Centre, South Tees Hospitals NHS Trust

Ms Dea Birkett, Service User Representative, London

Dr Paul Cockcroft, Consultant Medical Microbiologist and Clinical Director of Pathology, Department of Clinical Microbiology, St Mary's Hospital, Portsmouth

Professor Adrian K Dixon, Professor of Radiology, University Department of Radiology, University of Cambridge Clinical School

Dr David Elliman, Consultant in Community Child Health, Islington PCT & Great Ormond Street Hospital, London

Professor Glyn Elwyn, Research Chair, Centre for Health Sciences Research, Cardiff University, Department of General Practice, Cardiff

Professor Paul Glasziou, Director, Centre for Evidence-Based Practice, University of Oxford

Dr Jennifer J Kurinczuk, Consultant Clinical Epidemiologist, National Perinatal Epidemiology Unit, Oxford

Dr Susanne M Ludgate, Clinical Director, Medicines & Healthcare Products Regulatory Agency, London

Mr Stephen Pilling, Director, Centre for Outcomes, Research & Effectiveness, Joint Director, National Collaborating Centre for Mental Health, University College London

Mrs Una Rennard, Service User Representative, Oxford

Dr Phil Shackley, Senior Lecturer in Health Economics, Academic Vascular Unit, University of Sheffield

Dr Margaret Somerville, Director of Public Health Learning, Peninsula Medical School, University of Plymouth

Dr Graham Taylor, Scientific Director & Senior Lecturer, Regional DNA Laboratory, The Leeds Teaching Hospitals

Professor Lindsay Wilson Turnbull, Scientific Director, Centre for MR Investigations & YCR Professor of Radiology, University of Hull

Professor Martin J Whittle, Clinical Co-director, National Co-ordinating Centre for Women's and Childhealth

Dr Dennis Wright, Consultant Biochemist & Clinical Director, The North West London Hospitals NHS Trust, Middlesex

Pharmaceuticals Panel

Members

Chair,
Professor Robin Ferner, Consultant Physician and Director, West Midlands Centre for Adverse Drug Reactions, City Hospital NHS Trust, Birmingham

Ms Anne Baileff, Consultant Nurse in First Contact Care, Southampton City Primary Care Trust, University of Southampton

Professor Imti Choonara, Professor in Child Health, Academic Division of Child Health, University of Nottingham

Professor John Geddes, Professor of Epidemiological Psychiatry, University of Oxford

Mrs Barbara Greggains, Non-Executive Director, Greggains Management Ltd

Dr Bill Gutteridge, Medical Adviser, National Specialist Commissioning Advisory Group (NSCAG), London

Mrs Sharon Hart, Consultant Pharmaceutical Adviser, Reading

Dr Jonathan Karnon, Senior Research Fellow, Health Economics and Decision Science, University of Sheffield

Dr Yoon Loke, Senior Lecturer in Clinical Pharmacology, University of East Anglia

Ms Barbara Meredith, Lay Member, Epsom

Dr Andrew Prentice, Senior Lecturer and Consultant Obstetrician & Gynaecologist, Department of Obstetrics & Gynaecology, University of Cambridge

Dr Frances Rotblat, CPMP Delegate, Medicines & Healthcare Products Regulatory Agency, London

Dr Martin Shelly, General Practitioner, Leeds

Mrs Katrina Simister, Assistant Director New Medicines, National Prescribing Centre, Liverpool

Dr Richard Tiner, Medical Director, Medical Department, Association of the British Pharmaceutical Industry, London

Therapeutic Procedures Panel

Members

Chair,
Professor Bruce Campbell, Consultant Vascular and General Surgeon, Department of Surgery, Royal Devon & Exeter Hospital

Dr Mahmood Adil, Deputy Regional Director of Public Health, Department of Health, Manchester

Dr Aileen Clarke, Consultant in Public Health, Public Health Resource Unit, Oxford

Professor Matthew Cooke, Professor of Emergency Medicine, Warwick Emergency Care and Rehabilitation, University of Warwick

Mr Mark Emberton, Senior Lecturer in Oncological Urology, Institute of Urology, University College Hospital

Professor Paul Gregg, Professor of Orthopaedic Surgical Science, Department of General Practice and Primary Care, South Tees Hospital NHS Trust, Middlesbrough

Ms Maryann L Hardy, Lecturer, Division of Radiography, University of Bradford

Dr Simon de Lusignan, Senior Lecturer, Primary Care Informatics, Department of Community Health Sciences, St George's Hospital Medical School, London

Dr Peter Martin, Consultant Neurologist, Addenbrooke's Hospital, Cambridge

Professor Neil McIntosh, Edward Clark Professor of Child Life & Health, Department of Child Life & Health, University of Edinburgh

Professor Jim Neilson, Professor of Obstetrics and Gynaecology, Department of Obstetrics and Gynaecology, University of Liverpool

Dr John C Pounsford, Consultant Physician, Directorate of Medical Services, North Bristol NHS Trust

Dr Karen Roberts, Nurse Consultant, Queen Elizabeth Hospital, Gateshead

Dr Vimal Sharma, Consultant Psychiatrist/Hon. Senior Lecturer, Mental Health Resource Centre, Cheshire and Wirral Partnership NHS Trust, Wallasey

Professor Scott Weich, Professor of Psychiatry, Division of Health in the Community, University of Warwick

Disease Prevention Panel

Members

Chair,
Dr Edmund Jessop, Medical Adviser, National Specialist Commissioning Advisory Group (NSCAG), London

Mrs Sheila Clark, Chief Executive, St James's Hospital, Portsmouth

Mr Richard Copeland, Lead Pharmacist: Clinical Economy/Interface, Wansbeck General Hospital, Northumberland

Dr Elizabeth Fellow-Smith, Medical Director, West London Mental Health Trust, Middlesex

Mr Ian Flack, Director PPI Forum Support, Council of Ethnic Minority Voluntary Sector Organisations, Stratford

Dr John Jackson, General Practitioner, Newcastle upon Tyne

Mrs Veronica James, Chief Officer, Horsham District Age Concern, Horsham

Professor Mike Kelly, Director, Centre for Public Health Excellence, National Institute for Health and Clinical Excellence, London

Professor Yi Mien Koh, Director of Public Health and Medical Director, London NHS (North West London Strategic Health Authority), London

Ms Jeanett Martin, Director of Clinical Leadership & Quality, Lewisham PCT, London

Dr Chris McCall, General Practitioner, Dorset

Dr David Pencheon, Director, Eastern Region Public Health Observatory, Cambridge

Dr Ken Stein, Senior Clinical Lecturer in Public Health, Director, Peninsula Technology Assessment Group, University of Exeter, Exeter

Dr Carol Tannahill, Director, Glasgow Centre for Population Health, Glasgow

Professor Margaret Thorogood, Professor of Epidemiology, University of Warwick, Coventry

Dr Ewan Wilkinson, Consultant in Public Health, Royal Liverpool University Hospital, Liverpool

Expert Advisory Network

Members

Professor Douglas Altman, Professor of Statistics in Medicine, Centre for Statistics in Medicine, University of Oxford

Professor John Bond, Director, Centre for Health Services Research, University of Newcastle upon Tyne, School of Population & Health Sciences, Newcastle upon Tyne

Professor Andrew Bradbury, Professor of Vascular Surgery, Solihull Hospital, Birmingham

Mr Shaun Brogan, Chief Executive, Ridgeway Primary Care Group, Aylesbury

Mrs Stella Burnside OBE, Chief Executive, Regulation and Improvement Authority, Belfast

Ms Tracy Bury, Project Manager, World Confederation for Physical Therapy, London

Professor Iain T Cameron, Professor of Obstetrics and Gynaecology and Head of the School of Medicine, University of Southampton

Dr Christine Clark, Medical Writer & Consultant Pharmacist, Rossendale

Professor Collette Clifford, Professor of Nursing & Head of Research, School of Health Sciences, University of Birmingham, Edgbaston, Birmingham

Professor Barry Cookson, Director, Laboratory of Healthcare Associated Infection, Health Protection Agency, London

Dr Carl Counsell, Clinical Senior Lecturer in Neurology, Department of Medicine & Therapeutics, University of Aberdeen

Professor Howard Cuckle, Professor of Reproductive Epidemiology, Department of Paediatrics, Obstetrics & Gynaecology, University of Leeds

Dr Katherine Darton, Information Unit, MIND – The Mental Health Charity, London

Professor Carol Dezateux, Professor of Paediatric Epidemiology, London

Dr Keith Dodd, Consultant Paediatrician, Derby

Mr John Dunning, Consultant Cardiothoracic Surgeon, Cardiothoracic Surgical Unit, Papworth Hospital NHS Trust, Cambridge

Mr Jonothan Earnshaw, Consultant Vascular Surgeon, Gloucestershire Royal Hospital, Gloucester

Professor Martin Eccles, Professor of Clinical Effectiveness, Centre for Health Services Research, University of Newcastle upon Tyne

Professor Pam Enderby, Professor of Community Rehabilitation, Institute of General Practice and Primary Care, University of Sheffield

Professor Gene Feder, Professor of Primary Care Research & Development, Centre for Health Sciences, Barts & The London Queen Mary's School of Medicine & Dentistry, London

Mr Leonard R Fenwick, Chief Executive, Newcastle upon Tyne Hospitals NHS Trust

Mrs Gillian Fletcher, Antenatal Teacher & Tutor and President, National Childbirth Trust, Henfield

Professor Jayne Franklyn, Professor of Medicine, Department of Medicine, University of Birmingham, Queen Elizabeth Hospital, Edgbaston, Birmingham

Dr Neville Goodman, Consultant Anaesthetist, Southmead Hospital, Bristol

Professor Robert E Hawkins, CRC Professor and Director of Medical Oncology, Christie CRC Research Centre, Christie Hospital NHS Trust, Manchester

Professor Allen Hutchinson, Director of Public Health & Deputy Dean of ScHARR, Department of Public Health, University of Sheffield

Professor Peter Jones, Professor of Psychiatry, University of Cambridge, Cambridge

Professor Stan Kaye, Cancer Research UK Professor of Medical Oncology, Section of Medicine, Royal Marsden Hospital & Institute of Cancer Research, Surrey

Dr Duncan Keeley, General Practitioner (Dr Burch & Ptnrs), The Health Centre, Thame

Dr Donna Lamping, Research Degrees Programme Director & Reader in Psychology, Health Services Research Unit, London School of Hygiene and Tropical Medicine, London

Mr George Levvy, Chief Executive, Motor Neurone Disease Association, Northampton

Professor James Lindesay, Professor of Psychiatry for the Elderly, University of Leicester, Leicester General Hospital

Professor Julian Little, Professor of Human Genome Epidemiology, Department of Epidemiology & Community Medicine, University of Ottawa

Professor Rajan Madhok, Consultant in Public Health, South Manchester Primary Care Trust, Manchester

Professor Alexander Markham, Director, Molecular Medicine Unit, St James's University Hospital, Leeds

Professor Alistaire McGuire, Professor of Health Economics, London School of Economics

Dr Peter Moore, Freelance Science Writer, Ashtead

Dr Andrew Mortimore, Public Health Director, Southampton City Primary Care Trust, Southampton

Dr Sue Moss, Associate Director, Cancer Screening Evaluation Unit, Institute of Cancer Research, Sutton

Mrs Julietta Patnick, Director, NHS Cancer Screening Programmes, Sheffield

Professor Robert Peveler, Professor of Liaison Psychiatry, Royal South Hants Hospital, Southampton

Professor Chris Price, Visiting Professor in Clinical Biochemistry, University of Oxford

Professor William Rosenberg, Professor of Hepatology and Consultant Physician, University of Southampton, Southampton

Professor Peter Sandercock, Professor of Medical Neurology, Department of Clinical Neurosciences, University of Edinburgh

Dr Susan Schonfield, Consultant in Public Health, Hillingdon PCT, Middlesex

Dr Eamonn Sheridan, Consultant in Clinical Genetics, Genetics Department, St James's University Hospital, Leeds

Professor Sarah Stewart-Brown, Professor of Public Health, University of Warwick, Division of Health in the Community Warwick Medical School, LWMS, Coventry

Professor Ala Szczepura, Professor of Health Service Research, Centre for Health Services Studies, University of Warwick

Dr Ross Taylor, Senior Lecturer, Department of General Practice and Primary Care, University of Aberdeen

Mrs Joan Webster, Consumer member, HTA – Expert Advisory Network

Feedback

The HTA Programme and the authors would like to know your views about this report.

The Correspondence Page on the HTA website (http://www.hta.ac.uk) is a convenient way to publish your comments. If you prefer, you can send your comments to the address below, telling us whether you would like us to transfer them to the website.

We look forward to hearing from you.

The National Coordinating Centre for Health Technology Assessment,
Mailpoint 728, Boldrewood,
University of Southampton,
Southampton, SO16 7PX, UK.
Fax: +44 (0) 23 8059 5639 Email: hta@hta.ac.uk
http://www.hta.ac.uk

ISSN 1366-5278