A loop-mediated isothermal amplification test for yaws: a multi-country diagnostic accuracy evaluation

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Summary

Background To meet the WHO target of eradicating yaws by 2030, highly sensitive and specific diagnostic tools are needed. A multiplex *Treponema pallidum–Haemophilus ducreyi* loop-mediated isothermal amplification (TPHD-LAMP) test holds promise as a near-patient diagnostic tool for yaws and *H ducreyi*. We conducted a prospective evaluation in Cameroon, Côte d'Ivoire, Ghana, and the Republic of the Congo to determine the diagnostic accuracy of the TPHD-LAMP test, as well as to assess its acceptability, feasibility, and cost.

Methods Active case searching within schools and communities was used to locate participants with clinically suspicious laws-like lesions. Individuals with serologically confirmed active yaws provided paired lesion swabs between March, 2021, and April, 2023. For each participant, one swab was tested with the TPHD-LAMP at a local district laboratory and the other with reference quantitative PCR (qPCR) tests conducted at national reference laboratories. The primary outcome was TPHD-LAMP test sensitivity and specificity compared with qPCR. Laboratory technicians were interviewed using a multiple-choice survey to gauge acceptability and feasibility of the TPHD-LAMP test. Costs of each test were calculated.

Findings Of 3085 individuals with at least one suspected yaws lesion, 531 (17%) were serologically confirmed. We enrolled 493 participants with seropositive yaws and a further 32 with negative serology. The sensitivity of the TPHD-LAMP test for detecting *T pallidum* was 63% (95% CI 56–70) and the specificity was 66% (95% CI 61–71). Sensitivity and specificity for *T pallidum* improved to 73% (63–82; p=0·0065) and 75% (68–80; p=0·0003), respectively, in *H ducreyi*-negative samples. Interviews highlighted challenges in user-friendliness and practicality of the TPHD-LAMP test. The cost of the test per sample was one third of that of qPCR, although the TPHD-LAMP test entailed higher costs to establish the assay.

Interpretation This was the first multi-country diagnostic evaluation of a molecular test for yaws. The TPHD-LAMP testing, in its current form, falls short of the WHO target product profile criteria for yaws diagnostics. These findings highlight the importance of assessing new diagnostics in real-world conditions to ensure their suitability for programmatic use.

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Introduction

Yaws is a neglected tropical disease (NTD) characterised by cutaneous lesions. The disease is caused by infection with the bacterium *Treponema pallidum* subspecies *pertenue*. Transmission is via skin–skin contact with infectious lesions and primarily affects children younger than 15 years.[1,2] Yaws is known to be endemic in 15 countries,[3] with the highest burden in the Pacific and West Africa.[3] The WHO 2021–30 NTD road map set a goal of eradicating yaws by 2030,[4] primarily through mass drug administration of azithromycin to endemic communities.[5]

Effective diagnostic tools are crucial for achieving the eradication target, including confirming endemicity, supporting surveillance post mass drug administration, and enabling certification of eradication. It is important that tests can accurately differentiate between yaws and skin ulcers caused by other bacteria, particularly *Haemophilus ducreyi*.[6–8] Molecular tests are essential because only around one third of participants with seropositive yaws-like lesions are positive for *T pallidum* bacteria using nucleic-acid amplification tests.[6–8] Despite the central role of diagnostics, a survey published in 2022 highlighted major gaps in diagnostic access, with only four countries out of 14 respondents having molecular testing capacity.[9]

A WHO target product profile (TPP) for yaws diagnostics published in 2022 outlines the criteria for an ideal diagnostic test.[10] The test should be a simple and cost-effective molecular assay with a sensitivity of more than 95% and a specificity of more than 99·9%. Developing diagnostics that align with the TPP will facilitate the success of yaws eradication efforts.

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Research in context

Evidence before this study

The success of yaws eradication campaigns relies on the availability of effective diagnostic tools. A novel loop-mediated isothermal amplification (LAMP) test, designed for simultaneous detection of *Treponema pallidum* and *Haemophilus ducreyi* (the TPHD-LAMP assay), has shown promise in a high-income laboratory setting, with reported sensitivity of 84·7% for *T pallidum* and 84·8% for *H ducreyi,* and specificity of 91·6% for *T pallidum* and 95·7% for *H ducreyi.*

We searched PubMed, Embase, Scopus, and Web of Science on June 1, 2024, for relevant isothermal molecular diagnostic studies for yaws and *H ducreyi,* using the terms: (yaws OR *Treponema pallidum* OR *Treponema pallidum pertenue* OR *Haemophilus ducreyi*) AND (LAMP OR loop mediated isothermal amplification OR RPA or recombinase polymerase amplification OR isothermal). We did not impose language or date restrictions. The search generated 16 results, four of which were precursors to this study. These included a report on primer selection, development of the assay, and the high-income laboratory evaluation results. Additionally, one study assessed a lateral flow LAMP test on 63 samples from individuals with suspected yaws but had insufficient positive cases for accurate sensitivity determination. One report was a high-income laboratory evaluation of a recombinase polymerase amplification (RPA) assay. Of note, no previous diagnostic evaluations of LAMP or RPA tests for yaws have been conducted under programmatic conditions.

Added value of this study

This is the first multi-country prospective diagnostic accuracy evaluation of a novel molecular diagnostic test for yaws conducted in real-world conditions. Here, we found that the TPHD-LAMP assay did not meet the WHO target product profile for a yaws diagnostic test.

Implications of all the available evidence

The performance of the TPHD-LAMP assay in a real-world evaluation in our study was markedly reduced compared with the previous laboratory-based evaluation. Our data emphasise the need for robust, programmatic evaluations of new assays to guide their adoption. There remains an urgent need for new diagnostic tests to support the WHO 2030 target for yaws eradication.

A molecular loop-mediated isothermal amplification (LAMP) assay was previously developed to detect both *T pallidum* and *H ducreyi* concurrently (TPHD-LAMP).[11,12] It cannot distinguish between subspecies of *T pallidum* but, due to the clinical nature and geographical distribution of yaws, it is assumed that a seropositive, non-genital, cutaneous ulcer in a yaws-endemic country indicates infection with *T pallidum pertenue*. Although the TPHD-LAMP assay has previously demonstrated good sensitivity and specificity for detecting *T pallidum* in a controlled laboratory setting (84·7% and 95·7%, respectively),[12] it has not yet been evaluated in real-world conditions. We aimed to evaluate the diagnostic accuracy of a TPHD-LAMP test, including sample storage and DNA extraction, for yaws in Cameroon, Côte d'Ivoire, and Ghana, as well as in the Republic of the Congo following a protocol amendment. We also assessed the acceptability, feasibility, and costs of implementing the assay in Cameroon, Côte d'Ivoire, and Ghana.

Methods

Study design and participants

The protocol for this study has been previously published.[13] The study is reported following the STARD checklist (appendix pp 1–2).[14]

See Online for appendix

Briefly, we used active case searching within schools and communities to locate and recruit participants with clinically suspicious yaws-like lesions (ulcerative or nodular, non-genital skin lesions >1 cm in diameter) that were positive for both treponemal and non-treponemal antibodies using the SD Bioline (Abbott, Charlottesville, VA, USA) and Chembio Dual Path Positive (Chembio Diagnostics, New York, NY, USA) tests. Two lesion swab samples were collected simultaneously from the participant's largest lesion. We also randomly selected 5% of people with yaws-like lesions but with either a negative SD Bioline or Chembio Dual Path Positive test and invited them for inclusion into the study. This allowed us to sample from people with negative serology that might not have seroconverted yet—for example, if they were early in an infection. Sample collection was done between March, 2021, and April, 2023, across the four study countries (figure 1). On Feb 1, 2022, an amendment was made to the previously published protocol to extend recruitment into the Republic of the Congo, to enable us to reach the desired sample size because there were few participants in Cameroon presenting with yaws-like lesions. Identical processes were performed in the Republic of the Congo, where samples were collected but laboratory testing was performed by the core study team in Cameroon.[13]

Ethical approval for this study was obtained from the London School of Hygiene & Tropical Medicine ethics committee (21633; Aug 19, 2020) and local and national committees in each country: Cameroon National Ethics committee for Human Research (2020/12/1327/CE/CNERSH/SP; Dec 22, 2020); Côte d'Ivoire National Research Ethics Committee (133-20/MSHP/CNESVS-kp; Sept 16, 2020); Ghana Noguchi Memorial Institute for Medical Research institutional review board (019/20-21; Nov 6, 2020) and Ghana Health Service National Research Ethics Committee (GHS_ERC-005/12/20; April 29, 2021); and the Republic of the Congo Comité d'Éthique de la Recherche en Sciences

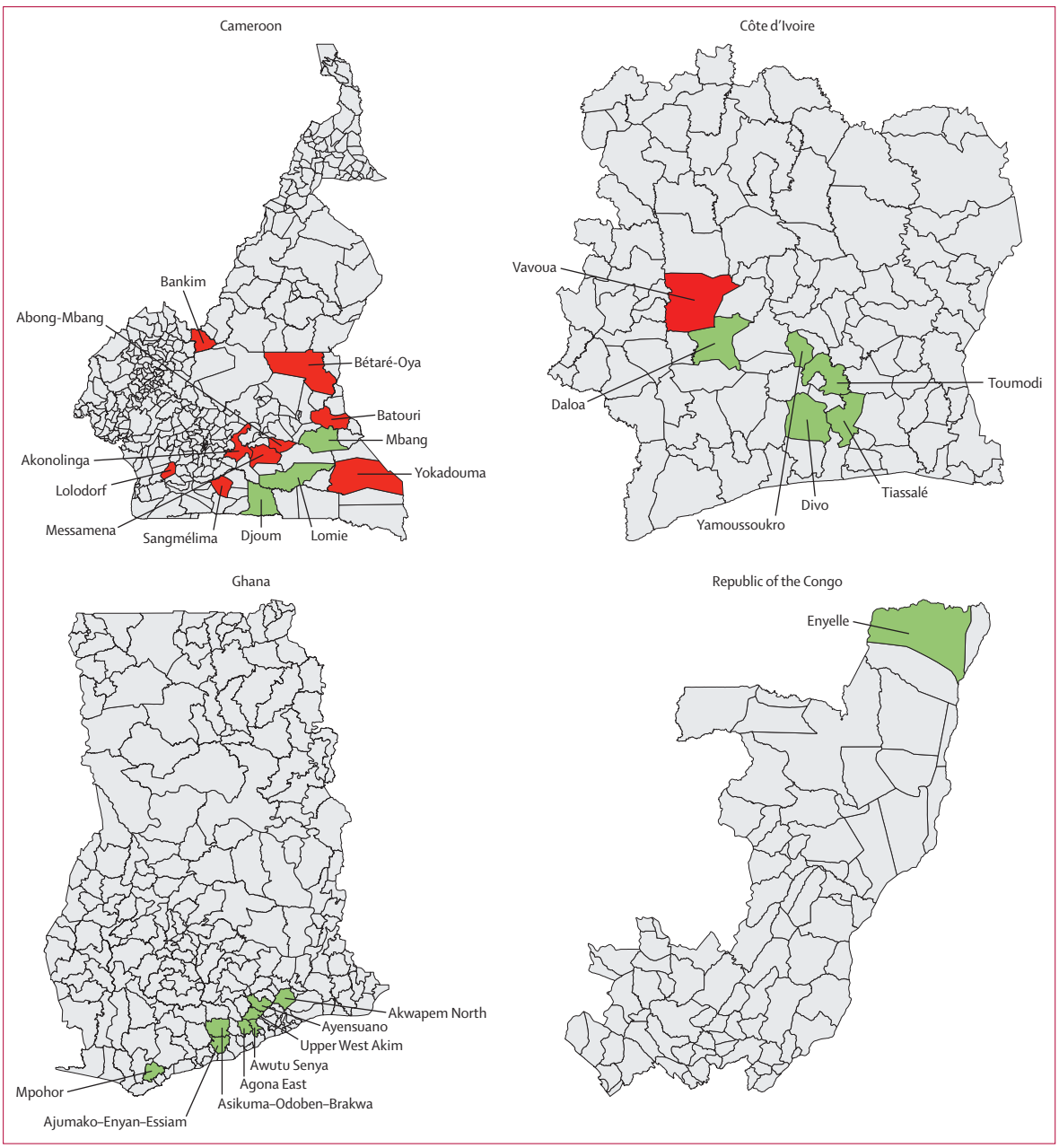

Figure 1: **Map of study recruitment**
Green indicates districts where serologically confirmed yaws cases were recruited. Red indicates districts where no serologically confirmed yaws cases were recruited.

de la Santé (0060; March 4, 2022). Before any study procedures, written informed consent was obtained from participants or from parents or guardians if the participant was under the age of consent (18 years in Côte d'Ivoire, Ghana, and the Republic of the Congo; 21 years in Cameroon). Informed consent was conducted in English or French, with the assistance of a community representative translating into the local language, if necessary. If the person was illiterate, the study information and consent procedure was explained to them by a member of the study team or a local translator. Children aged 10 years or older were also required to give verbal assent for enrolment.

The primary outcome was TPHD-LAMP test sensitivity and specificity compared with the reference standard, quantitative PCR (qPCR). To achieve 95% sensitivity with a precision of plus or minus 3% and two-sided α level of 0·05, we calculated that 203 samples positive for *T pallidum* on qPCR were needed.[15] Previous data indicate that around one third of people with yaws-like

lesions and a positive non-treponemal serological point-of-care test would have *T pallidum* detected by qPCR (with a similar number for *H ducreyi*),[6–8] so we aimed to recruit approximately 609 individuals. There was no age limit for inclusion. For acceptability and feasibility interviews, we aimed to recruit as many reference and district laboratory staff involved in the project as possible.

Sample storage and handling

On the day of collection, all samples were transferred to a refrigerator at the local district laboratory. District laboratory swabs remained in the refrigerator (2–8°C) for DNA extraction within 24 h or were transferred to a –20°C freezer until processing. The duplicate paired swabs were frozen at –20°C until they were transferred on ice to the reference laboratory for processing within 24 h or returned to a –20°C freezer for longer-term storage. Extracted DNA was stored in a refrigerator (2–8°C) for testing within 24 h or at –20°C for longer-term storage.

Laboratory tests

Although we intended the reference laboratory technicians to be masked to the results of the reference tests and for the district laboratory technicians to be masked to the results of the index tests, assistance to run the tests in the district laboratories was often needed and this was provided by national reference laboratory staff, meaning they were not always masked. The DNA extraction methodology and qPCRs used were optimised before the study (data not shown) and all qPCR assays were validated in-country before clinical study samples were tested.

For qPCR, DNA was extracted using the Qiagen QiAMP DNA mini kit (Qiagen, Hilden, Germany) and three singleplex Taqman qPCR reactions were performed on each sample.[13] The first qPCR detected the human housekeeping gene RNASe P to confirm the presence of human DNA and absence of qPCR inhibitors. If positive (Ct <38), qPCRs targeting *T pallidum* and *H ducreyi* were performed. All reference tests came from the published literature, having undergone rigorous optimisation and validation and being widely used for the detection of the three targets.[16,17] Negative controls (molecular grade water) and positive controls were included in all qPCR runs. For the RNase P assay, extracted human serum acted as the positive control and, for the *T pallidum* and *H ducreyi* assays, a plasmid containing both target gene sequences was included. Samples were run in duplicate, with a positive sample defined as both replicates displaying a Ct value of less than 38. If both replicates failed to amplify, the result was considered negative. If one of the two replicates failed to amplify, the sample underwent additional testing in triplicate, which was deemed positive if at least two of the three replicates had a Ct value of less than 38. The Ct cutoff was based on the limit of detection analysis in the previously published reference test qPCR protocols.[18]

The TPHD-LAMP molecular assay was performed as previously described,[12] except that reactions were run in a MAST ISOPLEX MD12 Tubescanner (Mast Diagnostica, Reinfeld, Germany) and samples were extracted using a MAST ISOPlex DNA/RNA magnetic bead-based extraction kit. Reactions were performed at 64°C for 60 min and fluorescence was measured every 30 s in the Cy5 and FAM channels for *H ducreyi* and *T pallidum*, respectively. Each sample was run in one replicate, and the test results were categorised as positive or negative based on a manufacturer-designed algorithm. If positive control samples failed to amplify or the no-template control was positive for either target, samples from that analysis were retested.

Acceptability and feasibility

To assess acceptability and feasibility of the TPHD-LAMP test, we designed a multiple-choice survey using a Likert scale, with the option of voice-recorded answers if the respondent selected a negative choice for specific questions. Interviews were conducted by a qualified local social scientist using the Open Data Kit application, in English or French, as appropriate. Questions were specific to the interviewed group: health-care workers, reference laboratory technicians, and district laboratory technicians (appendix pp 3–13). The questionnaire assessed various aspects of the TPHD-LAMP test, including participants' impressions, ease of use, availability of facilities and staff, the training process, and perceived challenges.

Costing analysis

For qPCR, we calculated the cost to perform DNA extraction and three singleplex reactions for RNAse P, *T pallidum*, and *H ducreyi*. For TPHD-LAMP, we estimated the cost of running the assay, including DNA extraction. To estimate price per test, we used an ingredients costing approach in which we calculated the number or volume of reagents and consumables required to perform the test and multiplied this value by the cost per item.

We estimated machine running costs by calculating how much the equipment would cost to run per day and then generated an approximate cost per sample. Because district laboratory technicians usually require training in molecular techniques, we calculated how much it would cost to implement training and monitoring in district laboratory settings. Both assays required cold-chain shipping of reagents from Europe, but we did not include these costs because we assumed it would be broadly equivalent for TPHD-LAMP and qPCR. All prices are presented in euros, calculated using an exchange rate from November, 2023.

Data analysis

Analyses were performed in Microsoft Excel version 2408 and R version 4.3.1. Sensitivity, specificity, and the negative and positive predictive values of the

TPHD-LAMP test against the singleplex qPCRs were calculated. 95% CIs were calculated using the binom.test function in R.[19] Samples for which the RNAse P failed to amplify (Ct ≥38) or which did not have paired data were excluded. A χ^2 test for proportions, conducted at the 95% confidence level, was used to determine whether performance differed between single infections and co-infections. Data on acceptability and feasibility were summarised by category of participant interviewed (health-care workers, district laboratory staff, and reference laboratory staff).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

We screened 61 350 people of all ages and detected 3085 (5%) individuals with at least one suspected yaws lesion. 531 (17%) were serologically confirmed and enrolled into the main study, alongside 32 randomly selected participants who presented with yaws-like lesions but were seronegative. After exclusions due to missing data (n=31) or samples in which RNase P failed to amplify (n=7), 525 participants (including 493 seropositive individuals and 32 seronegative individuals) were included in the final dataset (figure 2). Most participants were recruited in Ghana (n=408, 78%; table 1)

Using qPCR, 96 (18%) participants had detectable *T pallidum* only, 157 (30%) had detectable *H ducreyi* only, and 86 (16%) had a co-infection. The remaining 186 participants (35%) had no detectable level of *T pallidum* or *H ducreyi*. Overall, 36% of serologically confirmed cases of yaws were considered qPCR positive. Of the 32 seronegative participants, two (6%) were positive for *T pallidum* by qPCR.

Using the TPHD-LAMP test, 232 (44%) samples were positive for *T pallidum* alone, 143 (49%) had detectable *H ducreyi* alone, and a further 112 (21%) had a co-infection. Taking qPCR as the reference standard, the overall sensitivity of the TPHD-LAMP test was 63% (95% CI 56–70) for detecting *T pallidum* and 67% (61–73) for detecting *H ducreyi*. The specificity was 66% (95% CI 61–71) for *T pallidum* and 67% (62–73) for *H ducreyi* (table 2).

The TPHD-LAMP performed better when only a single pathogen was detected by qPCR. For *T pallidum*, the sensitivity increased to 73% (95% CI 63–82) in *H ducreyi*-negative samples, compared with 52% (41–63) in samples co-infected with *H ducreyi* (p=0·0065). The specificity for detecting *T pallidum* also improved to 75% (95% CI 68–80) compared with 55% (47–64) in co-infected samples (p=0·0003; table 2). There was no evidence of a statistical difference in the sensitivity for detecting *H ducreyi* in *T pallidum*-positive samples (67% [57–77]) compared with in *T pallidum*-negative samples (67% [59–74]; p>0·99). This was also true for the specificity, which was 74% (64–82) in *T pallidum*-positive samples and 64% (57–71) in *T pallidum*-negative samples (p=0·12; table 2). The sensitivity of the TPHD-LAMP test for correctly identifying both *T pallidum* and *H ducreyi* in samples in which both had been detected by qPCR was only 29% (20–40).

We interviewed 27 participants from Cameroon, Côte d'Ivoire, and Ghana, including 12 health-care workers, ten district laboratory staff, and five reference laboratory technicians. Interviews were conducted between September, 2021, and July, 2023. All health-care workers (12 of 12) agreed it was easy to collect swab samples from

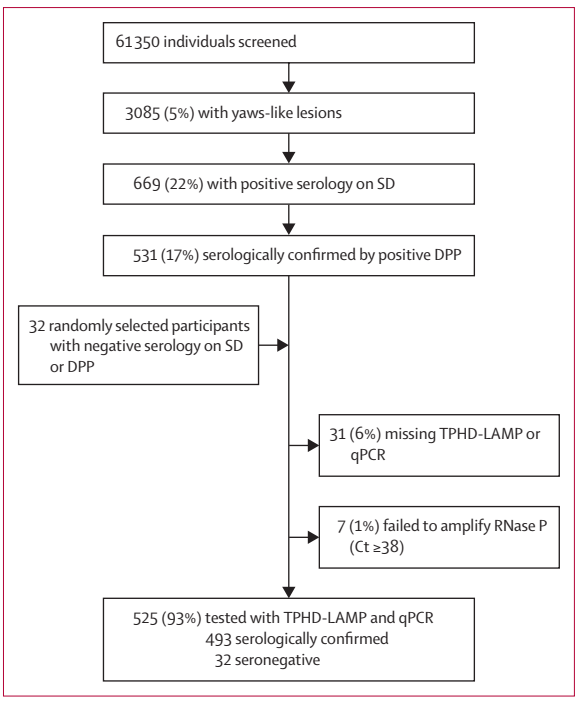

Figure 2: **Flowchart of participants**
DPP=Chembio Dual Path Positive test. SD=SD Bioline treponemal test. TPHD-LAMP=*Treponema pallidum–Haemophilus ducreyi* molecular loop-mediated isothermal amplification.

	All participants (n=525)	Cameroon (n=20)	Republic of the Congo (n=4)	Côte d'Ivoire (n=93)	Ghana (n=408)
Age group, years					
0–14	511 (97%)	20 (100%)	4 (100%)	90 (97%)	397 (97%)
≥15	14 (3%)	0	0	3 (3%)	11 (3%)
Proportion of female participants	149 (28%)	9 (45%)	1 (25%)	26 (28%)	113 (28%)
Age 0–14 years	145 (28%)	9 (45%)	1 (25%)	26 (28%)	109 (27%)
Age ≥15 years	4 (1%)	0	0	0	4 (1%)
Mean age, years (SD)	9·5 (2·3)	7·5 (3·7)	9·3 (1·3)	10·1 (2·8)	9·4 (2·7)
Age range, years	2–20	2–14	8–11	2–20	2–17

Data are n (%) unless indicated otherwise.

Table 1: **Demographic data**

	TPHD-LAMP test for detecting *T pallidum* and *H ducreyi* in all 525 samples		TPHD-LAMP test for detecting *T pallidum* in samples positive and negative for *H ducreyi*		TPHD-LAMP test for detecting *H ducreyi* in samples positive and negative for *T pallidum*	
	T pallidum positive*	*H ducreyi* positive*	*H ducreyi* positive*	*H ducreyi* negative*	*T pallidum* positive*	*T pallidum* negative*
Number of samples, n (%)	182 (35%)	243 (46%)	243 (46%)	282 (54%)	182 (35%)	343 (65%)
Sensitivity, % (95% CI)	63% (56–70)	67% (61–73)	52% (41–63)	73% (63–82)	67% (57–77)	67% (59–74)
Specificity, % (95% CI)	66% (61–71)	67% (62–73)	55% (47–63)	75% (68–80)	74% (64–82)	64% (57–71)
PPV, % (95% CI)	50% (43–56)	64% (58–70)	39% (30–49)	60% (50–69)	70% (59–80)	61% (53–69)
NPV, % (95% CI)	77% (72–82)	70% (65–76)	68% (59–76)	84% (78–89)	72% (62–80)	70% (62–76)

TPHD-LAMP=*T pallidum–H ducreyi* molecular loop-mediated isothermal amplification. *T pallidum=Treponema pallidum*. *H ducreyi=Haemophilus ducreyi*. PPV=positive predictive value. NPV=negative predictive value. *Evaluated by quantitative PCR.

Table 2: **Sensitivity and specificity estimates**

patients with lesions, and all believed community members would be willing to provide these samples.

Most district laboratory staff (seven of ten) reported that refrigerators and freezers required to store reagents and samples were reliably powered with stable power supply and adequate backup generators. However, only six of ten reported that necessary consumables, such as pipette tips and tubes, could be easily procured.

Almost all (nine of ten) district laboratory staff had no previous experience of conducting molecular assays before the study. After training, almost half (four of ten) found the extraction method extremely difficult, with two staff reporting finding the TPHD-LAMP assay somewhat difficult or extremely difficult to perform (appendix p 23). Reasons for this included finding the equipment, particularly the pipettes, difficult to use. All five reference laboratory staff interviewed had experience conducting molecular assays, and four reported that the DNA extraction and TPHD-LAMP techniques were easy to learn and perform. However, most reference laboratory staff (four of five) found these methods somewhat or extremely difficult to teach to district laboratory staff. Key challenges included keeping reagents on ice, preparing the master mix, and ensuring accurate pipetting while avoiding contamination, as well as following all the required steps in the procedure.

Two trainers also indicated that there was a lack of will of the local staff to perform the tests alongside their other laboratory duties. Generally, trainers and trainees reported feeling more confident after training and found the Tubescanner easy to use and results easy to interpret (appendix p 23). However, there were technical difficulties with the Tubescanner within the district laboratories. Seven of nine Tubescanners experienced interruptions in proper functioning during the project. Most of these machines were returned to the manufacturer in Europe, where no consistent fault was identified.

We estimated that it would cost around €2850 (appendix p 21) to train district laboratory technicians to perform the DNA extraction and TPHD-LAMP assay. In order of greatest expense, this included: per diems, transport, food and drink, accommodation, room hire, and other miscellaneous costs. We estimated a further €8764 (appendix pp 16–17) to purchase the equipment needed to run the test. Annual or biannual monitoring visits to support district laboratories were estimated at a cost of €2097 (appendix p 22) per visit.

For the Qiagen column-based DNA extraction, with three separate qPCRs on two replicates of each sample at the reference laboratory, the cost was estimated to be €24·91, excluding hands-on staff time. We estimated that the magnetic-based DNA extraction and performing TPHD-LAMP on one replicate would cost €7·83 per sample, again excluding hands-on staff time (appendix pp 14–20).

Discussion

To our knowledge, we report the first ever multi-country diagnostic evaluation of a new molecular test designed to support yaws eradication programmes. When performed under programmatic conditions, we found that the test was neither sensitive nor specific enough for use in eradication campaigns. Our analysis of the acceptability and feasibility data suggests that the DNA extraction procedure and assay are too complicated to be performed by local laboratory technicians, who generally do not have molecular laboratory experience. Although the per-sample cost of the TPHD-LAMP test was substantially lower than that of qPCR, the TPHD-LAMP test entailed higher costs to establish the assay.

This study was started before the publication of the WHO TPP for yaws, which recommends that diagnostics have a specificity of at least 99·9% and a sensitivity of at least 95% for the detection of *T pallidum pertenue*.[10] The multiplex TPHD-LAMP test falls substantially below the criteria established in the TPP. Both the sensitivity (73%) and specificity (75%) of the TPHD-LAMP test for detecting *T pallidum* were improved in *H ducreyi*-negative samples, indicating that the assay might perform better if only single infections are present, but even in this context the observed performance was below the required standard. These findings are similar to those reported in high-income laboratory evaluations of the LAMP and recombinase polymerase amplification

(RPA) assays which showed the performance of the index test was reduced in co-infected samples.[12,20] Previous novel isothermal molecular tests for yaws have only been evaluated in a controlled laboratory setting. A laboratory evaluation of a lateral flow LAMP performed in Ghana reported high sensitivities and specificities, but the sample size was low, including only 12 *T pallidum*-positive samples. The TPHD-LAMP assay tested here[11,12] and an RPA assay[20] have shown encouraging performance characteristics, but these evaluations were performed in highly standardised environments by experienced laboratory staff and are unlikely to reflect the true performance of the whole test procedure if adopted programmatically.[21,22]

A strength of this study is that we performed a first real-world evaluation of test performance. The reduced performance demonstrated here might have arisen because of challenges at each step of the testing process, including sample storage, DNA extraction, running the assay, and inadequate infrastructure in which to perform the test. We found that both the extraction method and assay were challenging for district laboratory staff. Simplifying one or both components might improve the diagnostic accuracy of the test. Additionally, more extensive training might have improved the performance of the TPHD-LAMP test. However, support was provided by reference laboratory staff when required.

Our costing analysis showed the TPHD-LAMP test was considerably cheaper than the qPCR assays. We did not include costs of cold-chain shipping, which can be substantial, because they would not have affected the comparison between tests, but it is important to note they would affect the real-world cost of implementing either test platform. A lack of infrastructure in district laboratories also meant a high initial capital cost for establishing test platforms at district laboratories. We also identified clear challenges for staff working in the district laboratories to access consumables without sourcing these from the reference laboratory. Several Tubescanner platforms failed to perform correctly during the study period when housed at the district laboratories. We could not identify any specific fault in some of the machines that were returned to the manufacturer. One machine functioned well within the national reference laboratory but had issues when used in the less climatically controlled district laboratory. We believe that the most likely explanation is that the machines had power supply issues with performance under conditions of high environmental temperatures and humidity. Collectively, these findings highlight the challenge of delivering molecular assays outside of larger reference laboratories or where testing models other than so-called plug-and-play devices (such as the Cepheid GeneXPert platform) are required.

The main limitation of our study was that we cannot conclusively identify which components of this process resulted in the poorer performance observed here compared with the previous laboratory-based evaluation.[12] Our staff interviews suggest that technical challenges in performing the assay in district laboratories account for at least some of the drop-off in performance, with the DNA extraction technique found to be too challenging by almost half of the ten district laboratory staff interviewed. Although we suspect that climatic conditions were responsible for Tubescanners not performing effectively, we were unable to show this conclusively. We also failed to reach our desired sample size. Despite screening more than 60 100 individuals, we located only 531 individuals with seropositive yaws-like lesions across four yaws-endemic countries, highlighting the challenges of evaluating diagnostics for diseases approaching eradication. Although recruitment was slightly below our preplanned sample size, the number of positive qPCR results was in line with our estimates, so it is unlikely to have impacted the overall conclusions drawn in our study.

Future research should explore alternative near-patient diagnostic tests that are both simple, with minimal handling and equipment requirements, and compatible with resource-limited settings. A simplified lyophilised TPHD-LAMP assay has been designed to eliminate the need for cold-chain shipments and simplify the testing process. A laboratory evaluation of this test is ongoing to determine whether it overcomes some of the limitations of the liquid assay evaluated in the current study. If successful, this evaluation too would need to be extended into a real-world setting before it is adopted programmatically. In addition, simpler, equipment-free DNA extraction methodologies or automated sample-to-answer systems should be explored. Any new test should be trialled in a range of endemic settings and an established external quality assurance scheme should be used to monitor the quality of the data.

Our recent survey of yaws diagnostic access[9] underscored a significant lack of molecular testing capacity in most yaws-endemic countries. Collaborative initiatives among countries are needed to improve molecular testing capacity, adopt established reference tests, and develop and implement external quality assurance schemes to overcome these challenges and ensure access to adequate diagnostics. It is only through the development of more accurate and user-friendly diagnostics, in conjunction with diagnostic capacity strengthening of yaws-endemic countries, that the WHO targets for yaws and other NTDs can be achieved.

Contributors

All coauthors contributed to the LAMP4yaws study. All authors aided with development of the protocol. MM, EMH-E, SK, and OM conceptualised the original study. BLH, ST, and CG-B trained and supervised the research teams. ST, LAB, KAH, IA, PN, ATK, DKA, PA, AS, AA, and MSK-S were responsible for participant recruitment, laboratory processing and analysis, or both. KKA, SE, SNK, ENT, and TC oversaw activities in the four study countries. MB, EL, NB, HG, TH, SF, SK, CM, SL, LB, and SB provided resources for, and supervision of, the laboratory activities. All authors had access to the raw data. BLH and MM verified the data and, with EMH-E, performed the data analysis.

BLH drafted the original version of the manuscript. All authors reviewed the final version of the manuscript. BLH and MM made the final decision to submit the manuscript for publication.

Declaration of interests

SF, MB, and EL are employees of Mast Diagnostica, which produces and sells LAMP products. A patent covering the mediator displacement probe technique, a component of the TPHD-LAMP assay described in this study, has been applied for by the University of Freiburg and Hahn-Schickard. All other authors declare no competing interests.

Data sharing

Non-identifiable results of the serological tests, qPCR reference tests, and LAMP tests are publicly available and provided within the appendix (pp 24–35). The original protocol was published previously.[13]

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Editorial note: The Lancet Group takes a neutral position with respect to territorial claims in published maps.

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