

1 **Diagnostic accuracy of a prototype rapid chlamydia and gonorrhoea recombinase**
2 **polymerase amplification assay: a multi-centre cross-sectional pre-clinical evaluation**

3

4 **Running title: Prototype CT/NG RPA assay early evaluation**

5

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51 care; performance evaluation; diagnostic accuracy

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54

55 **ABSTRACT**

56 **Objectives**

57 Rapid and accurate sexually transmitted infection diagnosis can reduce onward transmission
58 and improve treatment efficacy. We evaluated the accuracy of a 15-minute run-time
59 recombinase polymerase amplification (RPA)-based prototype point-of-care test (TwistDx) for
60 *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG).

61

62 **Methods**

63 Prospective, multi-centre study of symptomatic and asymptomatic patients attending three
64 English sexual health clinics. Research samples provided were: additional self-collected
65 vulvo-vaginal swab (SCVS) (females); first-catch urine (FCU) aliquot (females and males).
66 Samples were processed blind to the comparator (routine clinic CT/NG Nucleic Acid
67 Amplification Test (NAAT)) results. Discrepancies were resolved using Cepheid CT/NG
68 GeneXpert.

69

70 **Results**

71 Both RPA and routine clinic NAAT results were available for 392 males and 395 females. CT
72 positivity was 8.9% (35/392) (male FCU), 7.3% (29/395) (female FCU) and 7.1% (28/395)
73 (SCVS). Corresponding NG positivity was 3.1% (12/392), 0.8% (3/395) and 0.8% (3/395).

74

75 Specificity and positive predictive values (PPVs) were 100% for all sample types and both
76 organisms, except male CT FCU (99.7% specificity (95% confidence interval (CI) 98.4-100.0;
77 356/357), 97.1% PPV (95%CI 84.7-99.9; 33/34)). For CT, sensitivity was \geq 94.3% for FCU and
78 SCVS. CT sensitivity for female FCU was higher (100%, 95%CI 88.1-100; 29/29) than for
79 SCVS (96.4%, 95%CI 81.7-99.9; 27/28). NG sensitivity and negative predictive values were
80 100% in FCU (male and female).

81

82 **Conclusions**

83 This prototype test has excellent performance characteristics, comparable to currently-used
84 NAATs, and fulfils several WHO ASSURED criteria. Its rapidity without loss of performance
85 suggests that once further developed and commercialised, this test could positively impact
86 clinical practice and public health.

87

88 **INTRODUCTION**

89 *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are major contributors to the
90 burden of sexually transmitted infections (STIs) in England and elsewhere [1, 2]. They are
91 frequently asymptomatic (especially in women) [3], commonly remaining undiagnosed, and if
92 untreated can lead to serious complications [4, 5].

93

94 Currently, it can take up to two weeks to obtain CT and NG results and treatment after STI
95 testing in sexual health clinics (SHCs) in the UK [6], but delays may be considerably longer in
96 other settings [7]. During this period, sexual risk-taking may continue, including acquisition of
97 new partners [8]. Rapid and accurate CT/NG point-of-care tests (POCTs), enabling diagnosis
98 and treatment of infected patients within the same clinical visit [9] (a “test-and-treat” strategy),
99 could potentially reduce rates of inappropriate presumptive treatment, shorten time to
100 treatment, decrease rates of untreated CT and NG for patients lost to follow-up, limit onward
101 transmission, and reduce rates of sequelae [10-12].

102

103 British Association for Sexual Health and HIV (BASHH) guidelines state that CT and NG
104 detection must use nucleic acid amplification tests (NAATs) (and/or culture for NG) [5, 13]. A
105 number of rapid and POC NAAT-based tests for CT and NG are being, or have recently been,
106 developed [14]. Newer NAAT technologies that use isothermal amplification, avoiding the
107 need for thermal cycling, have potential to enable fast turnaround times from sample-to-result.
108 TwistDx Ltd. (Cambridge, UK) have developed an isothermal Recombinase Polymerase
109 Amplification (RPA) method, which can detect CT/NG infection (single NG target) in
110 approximately 15 minutes, requires no thermal cycling, can be battery-powered, and has a
111 reaction temperature of 37°C. The RPA CT/NG assay is run on the Alere™ i instrument (Alere,
112 Massachusetts, USA). The TwistDx RPA CT/NG assay is therefore an excellent candidate for
113 development as a “true” molecular CT/NG POCT, allowing for test-and-treat pathways in
114 SHCs, community and resource-poor settings [15].

115

116 We aimed to assess the diagnostic accuracy of the prototype TwistDx RPA assay for genital
117 CT and NG detection on prospectively collected clinical samples from males and females in
118 English SHCs.

119

120 **METHODS**

121 Ethical approval was granted by the London Bridge Research Ethics Committee (REC
122 Reference 13/LO/0691). This manuscript was written following Standards for Reporting
123 Diagnostic Accuracy (STARD) guidelines (see web-only Supplementary Table S1) [16].

124

125 **Sample size and recruitment**

126 This prospective multi-centre diagnostic accuracy evaluation was powered to obtain a
127 minimum of 50 CT and 20 NG positive, and 200 negative, samples for both male and female
128 participants. Assuming 92% sensitivity and 99% specificity of the RPA CT/NG assay
129 compared to standard NAATs, the 95% confidence intervals (CIs) obtained would be 81.2-
130 96.8% and 96.4-99.9%, respectively.

131

132 Assuming a CT prevalence of 8.3% (based on Genito-Urinary Medicine Clinical Activity
133 Dataset (GUMCAD) [17] data from the South London SHC), 600 individuals would lead to the
134 requisite number of CT positive and negative samples. With a lower expected NG prevalence
135 of 3%, 800 participants were needed. In order to allow sub-group analysis by gender, we
136 planned to recruit 400 males and 400 females.

137

138 **Study sites and participant selection**

139 Three SHCs located in South London, Yorkshire and on the south coast of England
140 participated. Eligible patients were recruited during routine consultations by clinic staff using
141 the following eligibility criteria: aged ≥ 16 years; attending the SHC; had not passed urine in
142 the previous two hours; provided written informed consent for the collection of research
143 samples; provided all sample types (males: first-catch urine (FCU) and meatal swabs pre- and

144 post- micturition; females: FCU and self-collected vulvo-vaginal swabs (SCVS)). Participant
145 demographic and clinical data were collected on case report forms (CRFs).

146

147 **Sample collection and processing**

148 All samples for this evaluation were self-taken by participants following collection of routine
149 clinical samples. A minimum volume of 20 ml male FCU was collected; an aliquot of 2-3 ml
150 was taken for routine clinical testing, and the remainder immediately stored at 2-8°C until
151 shipment (twice weekly) on wet ice to TwistDx for RPA CT/NG testing. In addition, male
152 participants were asked to self-collect two external penile meatal swabs, one pre-urination
153 and the second post-urination (meatal swab data will be published separately).

154

155 Females provided two SCVS, the first for the clinic's routine CT/NG NAAT, followed by an
156 FCU specimen. Female FCU was processed as per male FCU, except that no aliquots for
157 routine CT/NG NAAT testing were taken. Research SCVS samples were eluted in-clinic within
158 10 minutes of collection. Swabs were immersed and swirled in 1 ml lysis buffer for 5 seconds,
159 left to stand (in the lysis buffer) for 90 seconds, and then disposed of. 2 ml neutralisation buffer
160 was added to the lysis buffer and the tube inverted 10 times. Tubes were stored at -20°C (or
161 lower) prior to shipment (twice weekly) on dry ice to TwistDx for RPA CT/NG testing.

162

163 **Sample testing and resolution of discrepant results**

164 Routine clinical NAAT testing was performed locally on male FCU and female SCVS, as per
165 clinic standard practice (BD Viper CT/NG assay (Becton Dickinson, Oxford, UK) at the South
166 London clinic; GenProbe Aptima CT/NG test (Hologic Gen-Probe, Marlborough, USA) at the
167 other clinics).

168

169 Research sample processing and testing were in accordance with TwistDx protocols,
170 developed through internal optimisation (unpublished data). The RPA CT/NG assay was
171 performed on research samples (FCU for men, FCU and SCVS for females) by staff at

172 TwistDx, who were blinded to the routine clinic NAAT and CRF results. FCU samples were
173 processed through a Size Exclusion Chromatography device (Zeta Sep FPLC Desalting
174 Columns; Generon, Slough, UK) for the purposes of de-salting the sample before testing on
175 the Alere™ i instrument.

176

177 The comparator test for male FCU samples was the routine clinic NAAT performed on male
178 FCU; that for female SCVS and FCU was the routine clinic NAAT on female SCVS samples.
179 Data were sent to the Applied Diagnostic Research & Evaluation Unit (ADREU), St George's
180 University of London, where the routine clinic NAAT and RPA CT/NG assay results were
181 compared for each participant and sample type. We defined the reference standard [16] as
182 the routine clinic NAAT result when in agreement with the RPA CT/NG assay, and no further
183 testing was performed. Otherwise, all sample eluates from patients where a discrepant result
184 had been found were tested at the TwistDx facility using the CT/NG GeneXpert as per
185 manufacturer's instructions, blinded to sample type (FCU or swab) and initial CT or NG results.
186 In these cases, the reference standard was defined as the *resolved* result when two out of
187 three of the test results were in agreement.

188

189 **Data and statistical analysis**

190 Data were entered into a database by ADREU. Participants for whom either the RPA CT/NG
191 or routine clinic NAAT results were missing, and/or who did not provide both sample types
192 (swab and FCU) in the case of females as per the eligibility criteria, were excluded from
193 analyses. Calculation of RPA CT/NG assay diagnostic accuracy measures (sensitivity,
194 specificity, positive predictive value (PPV), and negative predictive value (NPV)) and their
195 binomial exact 95% CIs was carried against the reference standard. Comparison of
196 performance by sub-group (symptomatic vs. asymptomatic; female FCU vs. SCVS) was
197 performed using the Pearson chi-squared statistic. All analyses were conducted in Stata v12.0
198 (StataCorp, Texas, TX, USA).

199

200 **RESULTS**

201 **Overview of participants**

202 Recruitment took place May-September 2014. 414 males and 442 females provided written
203 informed consent (Figures 1 and 2). Both RPA CT/NG assay and routine clinic NAAT results
204 were available for FCU for 392/414 (94.7%) males. 395/442 (89.7%) females had both FCU
205 and SCVS results available for all tests performed (RPA CT/NG for FCU and SCVS; routine
206 clinic NAAT for SCVS). Participant characteristics are summarised in Tables 1A and 1B. Study
207 CT positivity was 35/392 (8.9%) for male FCU, 29/395 (7.3%) for female FCU and 28/395
208 (7.1%) for SCVS. Corresponding NG positivities were 12/382 (3.1%), 3/395 (0.8%) and 3/395
209 (0.8%) (Table 2A).

210

211 **CT/NG RPA assay diagnostic accuracy**

212 Table 2 summarises the RPA CT/NG assay diagnostic accuracy estimates (see web-only
213 Supplementary Table S2 for tables of agreement between the RPA CT/NG assay and
214 reference standard). In 3/392 (0.8%) FCU samples, RPA CT/NG results disagreed with routine
215 clinic NAAT results for CT only (there were no NG discrepant results) (see web-only
216 Supplementary Table S3A). Following discrepant testing, 0/3 (0%) RPA CT/NG results agreed
217 with the resolved result. Subsequently, in males, all diagnostic accuracy measures were 100%
218 for NG (12/12, 95%CI 73.5-100 for sensitivity and PPV; 380/380, 95%CI 99.0-100 for
219 specificity and NPV). For CT, specificity and NPV were $\geq 99.4\%$ (356/357, 95%CI 98.4-100,
220 and 356/358, 95%CI 98.0-99.9, respectively), PPV was 97.1% (33/34, 95%CI 84.7-99.9), and
221 sensitivity was 94.3% (33/35, 95%CI 80.8-99.3) (Table 2A).

222

223 For females, 395 FCU and 395 SCVS were tested for CT and NG by the RPA CT/NG assay
224 (Table 2A). For CT, 6/790 (0.76%; three FCU, three SCVS) results disagreed with the routine
225 clinic NAAT SCVS result (see web-only Supplementary Table S3B). Following discrepant
226 testing, the RPA CT/NG assay agreed with the resolved result for all three FCU discrepant,
227 and 2/3 SCVS discrepant. For NG, 7/790 (0.89%; three FCU, four SCVS) RPA CT/NG results

228 disagreed with the routine clinic NAAT SCVS result (see web-only Supplementary Table S3B).
229 Of these, all three FCU and 3/4 SCVS discrepant results agreed with the resolved result. Thus, in
230 females, all measures of diagnostic accuracy were 100% for FCU for both CT and NG. For
231 CT and NG in SCVS, specificity and PPV were 100%, NPV was 99.7% (367/368, 95%CI 98.5-
232 100.0), and sensitivity was 96.4% (27/28, 95%CI 81.7-100) for CT and 66.7% (2/3, 95%CI
233 9.0-100) for NG (Table 2A). No female had a discrepant result for both CT and NG.

234

235 When performance was analysed by participant-reported symptomatic status, there was no
236 evidence of a significant difference between symptomatic and asymptomatic patients
237 ($p > 0.05$). All point estimates were 100% for asymptomatic participants (Table 2C). Among
238 symptomatic participants (Table 2B), the RPA CT/NG assay's sensitivity was lower: 15/16
239 (93.8%, 95%CI 69.8-99.8) for male CT FCU and 13/14 (92.9%, 95%CI 66.1-99.8) for female
240 CT SCVS, but specificity and NPV remained high. In addition, all diagnostic accuracy
241 measures for NG detection in both male and female FCU, and female CT FCU detection, were
242 100% regardless of symptomatic status. (See web-only Supplementary Table S2 for tables of
243 agreement between the RPA CT/NG assay and reference standard).

244

245 **DISCUSSION**

246 In this diagnostic accuracy evaluation of a prototype ultra-rapid isothermal RPA assay for
247 detection of CT and NG, performance (sensitivity, specificity, PPV and NPV) against the
248 reference standard for CT was >94% for all sample types evaluated (male FCU; female FCU
249 and SCVS). Performance for NG was 100% except for SCVS sensitivity and NPV; it was
250 however not possible to assess NG sensitivity and PPV in females confidently due to low
251 numbers of positives. The RPA CT/NG also demonstrated excellent technical performance,
252 as no inhibitory results and very few RPA CT/NG assay device errors were observed.

253

254 With respect to rapidity, the RPA CT/NG assay's sample preparation, amplification and
255 detection take place <20 minutes (sample preparation and RPA CT/NG assay run-time). A

256 simple-to-use desalting device has been included in newer iterations of the assay, allowing
257 immediate (in seconds) processing of FCU samples prior to running the assay, although
258 currently it is only appropriate for research laboratory use. The test's rapidity enhances the
259 possibility of implementation as a POCT, enabling "test and treat" strategies with patients
260 diagnosed and treated in the same clinical visit, and is potentially rapid enough to be
261 incorporated into clinical practice with minimal change to clinical pathways. To date, a major
262 barrier identified for STI POCT implementation has been patient willingness to wait, even for
263 a 90 minute rapid test [11, 18], and the major changes to clinic care pathways necessary to
264 incorporate rapid tests as POCTs as part of SHC consultations [11, 12]. Consequently, CT/NG
265 GeneXpert implementation has enabled a same- or next-day results service, rather than a
266 POCT "test and treat" strategy [19-21]. The RPA CT/NG assay's rapidity, combined with its
267 high performance, therefore has the potential to revolutionise STI diagnosis and management.

268

269 Furthermore, the RPA CT/NG assay would be well-suited for use in non-laboratory conditions,
270 both in low- and high-income countries, due to its limited operational requirements. In
271 resource-limited settings, laboratory services for STIs are either not available, or are difficult
272 to access (physically and/or financially), and the development and introduction of affordable
273 STI POCTs are part of the World Health Organization (WHO)'s strategic direction [22]. The
274 RPA CT/NG assay fulfils many of the ASSURED criteria, developed by WHO as a benchmark
275 to decide if tests address disease control needs in developing countries [23], and could
276 therefore be an excellent candidate for a true CT/NG POCT in multiple settings.

277

278 BASHH guidelines for NG testing indicate a minimum PPV of 90%; below this, positives should
279 be confirmed with supplementary testing using a different nucleic acid target from the original
280 test [5]. Although our NG PPV point estimates were all 100%, the lower 95%CI were all <90%.
281 As the RPA CT/NG assay has only a single NG molecular target, it may ultimately have lower
282 specificity and PPV compared to two-target assays, especially if applied to lower prevalence
283 settings. That said, a diagnostic evaluation with large sample size of the two-target GeneXpert

284 by Gaydos *et al.* [24] also resulted in NG PPVs with lower 95% CIs <90% in all sample types
285 despite the point estimate being >90%, indicating that supplementary testing may be required
286 for both assay types in low prevalence settings.

287

288 The RPA CT/NG assay shows promise for both screening of asymptomatics and diagnosis of
289 symptomatics, as we found no significant difference in point estimates by symptomatic status,
290 in accordance with previously reported findings [24]. Furthermore, both SCVS and FCU are
291 possible sample types for females. This is interesting, as it has previously been reported that
292 urine is less sensitive than swabs, probably because of lower bacterial load [25]; we did not
293 have data on organism load to explore this finding further. It is also possible that different
294 sample storage (extracted SCVS eluate frozen versus FCU refrigerated prior to testing) could
295 have contributed to this finding. Freeze-thaw is unlikely to have impacted on results (one
296 freeze-thaw for FCU prior to discrepant testing; maximum two freeze-thaws for SCVS (the first
297 for initial testing and the second for discrepant testing)), particularly as CT DNA detection by
298 PCR is unaffected by extended (≤ 2 years) storage [26]. Our results must however be
299 interpreted with caution, as it would have been more appropriate to compare the RPA CT/NG
300 assay FCU results to clinic FCU NAAT results, had these been available, but female FCU is
301 not routinely collected in England. Due to the very high performance of the assay in this
302 evaluation, it is expected that use of the clinic FCU NAAT as the gold standard would have
303 made very little difference.

304

305 It is known that the discrepant analysis approach employed in this study can lead to biases,
306 particularly when the assay under evaluation is also part of the algorithm used to define true
307 positive and negative results [27]. However, agreement between the initial clinic NAAT and
308 RPA CT/NG assay was very high, with few samples requiring discrepant resolution. Logistical
309 and funding constraints meant an alternative study design (for example, composite gold
310 standard or Patient Infection Status (PIS) as used for FDA approval [27]), with a consistent
311 definition for all sample types, was not possible.

312

313 The results of our evaluation are promising for the further development of the RPA CT/NG
314 assay, the aims of which should be to: (a) increase CT sensitivity; (b) ensure the PPV remains
315 >90%; c) ensure usability; (d) and perform larger evaluations to achieve tighter confidence
316 intervals around point estimates, especially for NG. An important addition to this test's
317 development would be validation of extra-genital (pharyngeal and rectal) sample types. Extra-
318 genital samples are routinely collected for MSM, with the majority of NG infections in MSM
319 detected extra-genitally [28].

320

321 This prototype RPA CT/NG assay has excellent performance characteristics, comparable to
322 currently-used NAATs, and fulfils several WHO ASSURED criteria, most notably accuracy,
323 rapidity, and thermo-stability. Its rapidity without loss of performance suggests that once
324 further developed and commercialised, this test could positively impact on both clinical
325 practice and public health.

326

327 **AUTHOR CONTRIBUTIONS**

328 EMHE, MJP, MP, OP, DGB, CML and STS conceived the study. EMHE, SSF, CC, AVN, MH,
329 MP, OP, DGB, PDB, TP, CML and STS designed the study. MH, MP, MSF, RP, PH and NF
330 acquired the data. EMHE, SSF, AVN, JKD, CML and STS analysed and interpreted the data.
331 EMHE, SSF, AVN, MH, JKD, CLM and STS drafted the manuscript. All authors critically
332 appraised the manuscript and provided final approval of the version to be submitted.

333

334 **TRANSPARENCY DECLARATION**

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338

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345 employees of TwistDx. Advisory board membership is declared by EHE, SF, RP and PH for
346 Becton Dickinson, by RP for Roche, Novartis, GSK, Genoccea, and CLJC, and by PH for
347 Hologic, and Bayer Consumer Healthcare, outside the submitted work. In addition, MP has
348 a patent EP2029782 issued, a patent EP2426221 issued; and OP has a patent 7,666,598
349 issued (not owned by the author), and a patent 9,663,820 issued (not owned by the author).

350

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360 database.

361

362 Results from the study have previously been presented as a poster, with the abstract
363 published in Clarke, I. (Ed.) Proceedings: Eighth Meeting of the European Society for
364 Chlamydia Research, Oxford, UK, September 6-9, 2016. The full study protocol and datasets
365 used and/or analysed during the current study are available from the corresponding author on
366 reasonable request.

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449 **TABLES AND FIGURES**

450

451 **Fig. 1. Patient and sample flow for male participants**

452 **Fig. 2. Patient and sample flow for female participants**

453

Table 1. Participant characteristics						
	Male participants			Female participants		
Characteristic	No. of participants (%)	CT	NG	No. of participants (%)	CT	NG
		No. (%) with CT^a	No. (%) with NG^a		No. (%) with CT^a	No. (%) with NG^a
Age						
16-19	15 (3.8)	1 (6.7)	0 (0)	40 (10.1)	5 (12.5)	0 (0)
20-24	126 (32.1)	22 (17.5)	6 (4.8)	157 (39.7)	18 (11.5)	0 (0)
25-34	148 (37.8)	11 (7.4)	2 (1.4)	140 (35.4)	4 (2.9)	1 (0.7)
35-44	64 (16.3)	0 (0)	3 (4.7)	41 (10.4)	1 (2.4)	1 (2.4)
45-64	37 (9.4)	1 (2.7)	1 (2.7)	16 (4.1)	0 (0)	0 (0)
65+	2 (0.5)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)
Clinic						
1	127 (32.4)	13 (10.2)	7 (5.5)	157 (39.7)	8 (5.1)	2 (1.3)

2	186 (47.4)	18 (9.7)	4 (2.2)	161 (40.8)	16 (9.9)	1 (0.6)
3	79 (20.2)	4 (5.1)	1 (1.3)	77 (19.5)	4 (5.2)	0 (0)
Contact						
No	340 (87.4)	18 (5.3)	10 (2.9)	363 (92.8)	16 (4.4)	2 (0.6)
CT only	33 (8.5)	14 (42.4)	2 (6.1)	22 (5.6)	11 (50.0)	0 (0)
NG only	7 (1.8)	2 (28.6)	0 (0)	1 (0.3)	0 (0)	1 (100)
Both CT & NG	9 (2.3)	1 (11.1)	0 (0)	5 (1.3)	0 (0)	0 (0)
Taken CT/NG active medication since test/6 weeks before test						
No	375 (95.7)	34 (9.1)	11 (2.9)	367 (92.9)	27 (7.4)	3 (0.8)
Yes	17 (4.3)	1 (5.9)	1 (5.9)	28 (7.1)	1 (3.6)	0 (0)

Symptomatic ^b						
No	249 (63.7)	18 (7.2)	2 (0.8)	208 (52.8)	14 (6.7)	1 (0.5)
Yes	142 (36.3)	16 (11.3)	10 (7.0)	186 (47.2)	14 (7.5)	2 (1.1)
Currently menstruating						
No	N/A	N/A	N/A	368 (93.4)	28 (7.6)	3 (0.8)
Yes	N/A	N/A	N/A	26 (6.6)	0 (0)	0 (0)

454 ^a CT and NG positives defined as reference standard (positive by at least 2 of the 3 tests:
455 clinic NAAT, RPA CT/NG assay, Cepheid GeneXpert).

456 ^b Male participants considered symptomatic if they reported ≥ 1 of the following symptoms on
457 the Case Report Form: Discharge (clear or cloudy liquid from the penis); Irritation at the top
458 of the penis; Itching; Needing to pass urine more often than usual; Pain/burning when
459 urinating. Female participants considered symptomatic if they reported ≥ 1 of the following
460 symptoms on the Case Report Form: Itching; Discharge (clear or cloudy liquid from the
461 vagina); Pain/burning when urinating; Needing to pass urine more frequently; Pain during
462 sex; Bleeding after sex; Bleeding in between periods; Pelvic abdominal pain.

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Table 2. RPA CT/NG assay performance						
A: All participants						
	Males		Females			
	FCU		FCU		SCVS	
	CT	NG	CT	NG	CT	NG
No. positives ^a / total	35/392	12/392	29/395	3/395	28/395	3/395
Positivity	8.9%	3.1%	7.3%	0.8%	7.1%	0.8%
Sensitivity (% , 95%CI)	94.3 (80.8-99.3)	100 (73.5-100)	100 (88.1-100)	100 (29.2-100)	96.4 (81.7-99.9)	66.7 (9.0-100)
n/N	33/35	12/12	29/29	3/3	27/28	2/3
Specificity (% , 95%CI)	99.7 (98.4-100)	100 (99.0-100)	100 (99.0-100)	100 (99.1-100)	100 (99.0-100)	100 (99.1-100)
n/N	356/357	380/380	366/366	392/392	367/367	392/392
PPV (% , 95%CI)	97.1 (84.7-99.9)	100 (73.5-100)	100 (88.1-100)	100 (29.2-100)	100 (87.2-100)	100 (15.8-100)
n/N	33/34	12/12	29/29	3/3	27/27	2/2
NPV (% , 95%CI)	99.4 (98.0-99.9)	100 (99.0-100)	100 (99.0-100)	100 (99.1-100)	99.7 (98.5-100)	99.7 (98.6-100)

n/N	356/358	380/380	366/366	392/392	367/368	392/393
B: Symptomatic participants^b						
	Males		Females			
	FCU		FCU		SCVS	
	CT	NG	CT	NG	CT	NG
No. positives ^a / total	16/142	10/142	14/186	2/186	14/186	2/186
Positivity	11.3%	7.0%	7.5%	1.1%	7.5%	1.1%
Sensitivity (% , 95%CI)	93.8 (69.8-99.8)	100 (69.2-100)	100 (76.8-100)	100 (15.8-100)	92.9 (66.1-99.8)	50 (1.3-98.7)
n/N	15/16	10/10	14/14	2/2	13/14	1/2
Specificity (% , 95%CI)	99.2 (95.7-100)	100 (97.2-100)	100 (97.9-100)	100 (98.0-100)	100 (97.9-100)	100 (98.0-100)
n/N	125/126	132/132	172/172	184/184	172/172	184/184
PPV (% , 95%CI)	93.8 (69.8-99.8)	100 (69.2-100)	100 (76.8-100)	100 (15.8-100)	100 (75.3-100)	100 (2.5-100)
n/N	15/16	10/10	14/14	2/2	13/13	1/1
NPV (% , 95%CI)	99.2 (95.7-100)	100 (97.2-100)	100 (97.9-100)	100 (98.0-100)	99.4 (96.8-100)	99.5 (97.0-100)

n/N	125/126	132/132	172/172	184/184	172/173	184/185
C: Asymptomatic participants						
	Males		Females			
	FCU		FCU		SCVS	
	CT	NG	CT	NG	CT	NG
No. positives ^a / total	18/249	2/249	15/208	1/208	14/208	1/208
Positivity	7.2%	0.8%	7.2%	0.48%	6.7%	0.48%
Sensitivity (% , 95%CI)	100 (81.5-100)	100 (15.8-100)	100 (78.2-100)	100 (2.5-100)	100 (76.8-100)	100 (2.5-100)
n/N	18/18	2/2	15/15	1/1	14/14	1/1
Specificity (% , 95%CI)	100 (98.4-100)	100 (98.5-100)	100 (98.1-100)	100 (98.2-100)	100 (98.1-100)	100 (98.2-100)
n/N	231/231	247/247	193/193	207/207	194/194	207/207
PPV (% , 95%CI)	100 (81.5-100)	100 (15.8-100)	100 (78.2-100)	100 (2.5-100)	100 (76.8-100)	100 (2.5-100)
n/N	18/18	2/2	15/15	1/1	14/14	1/1
NPV (% , 95%CI)	100 (98.4-100)	100 (98.5-100)	100 (98.1-100)	100 (98.2-100)	100 (98.1-100)	100 (98.2-100)

n/N	231/231	247/247	193/193	207/207	194/194	207/207
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466 FCU, First-catch urine; SCVS, Self-Collected Vulvo-Vaginal Swab; CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; PPV, Positive

467 Predictive Value; NPV, Negative Predictive Value

468 ^a Positives defined as reference standard (positive by at least 2 of the 3 tests: clinic NAAT, RPA CT/NG assay, Cepheid GeneXpert)

469 ^b Male participants considered symptomatic if they reported ≥ 1 of the following symptoms on the Case Report Form: Discharge (clear or cloudy
470 liquid from the penis); Irritation at the top of the penis; Itching; Needing to pass urine more often than usual; Pain/burning when urinating.

471 Female participants considered symptomatic if they reported ≥ 1 of the following symptoms on the Case Report Form: Itching; Discharge (clear
472 or cloudy liquid from the vagina); Pain/burning when urinating; Needing to pass urine more frequently; Pain during sex; Bleeding after sex;
473 Bleeding in between periods; Pelvic abdominal pain

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