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

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

**Authors**:

Emma M. Harding-Esch (1) (2) eharding@sgul.ac.uk

Sebastian S. Fuller (1) (2) sfuller@sgul.ac.uk

S-L Christine Chow (1) christine.chow@imperial.ac.uk

Achyuta V. Nori (1) (2) (3) achyuta.nori@nhs.net

Mark A. Harrison (1) Mark.Harrison1@lshtm.ac.uk

Mathew Parker (4) m.parker@TWISTDx.co.uk

Olaf Piepenburg (4) o.piepenburg@TWISTDx.co.uk

Matthew S. Forrest (4) M.Forrest@TWISTDx.co.uk

David G. Brooks (4) d.brooks@TWISTDx.co.uk

Rajul Patel (5) prj467@gmail.com

Phillip Hay (3) phillip.hay@stgeorges.nhs.uk

Nicola Fearnley (6) nicola.fearnley@bthft.nhs.uk

Marcus J. Pond (1) marcus.pond@phe.gov.uk

J Kevin Dunbar (2) kevin.dunbar@phe.gov.uk

Philip D. Butcher (1) butcherp@sgul.ac.uk

Timothy Planche (1) (3) tim.planche@nhs.net

Catherine M. Lowndes (2) (1) cmlowndes@googlemail.com

S Tariq Sadiq (1) (2) (3)\* ssadiq@sgul.ac.uk

\* Corresponding author

Applied Diagnostic Research & Evaluation Unit (ADREU)

Institute for Infection & Immunity

St George’s University of London,

London, SW17 0RE, UK

ssadiq@sgul.ac.uk

Tel: +44 (0)20 8725 5740/3355

Fax: +44 (0)20 8725 0137

**Affiliations**:

1. Applied Diagnostic Research & Evaluation Unit (ADREU), Institute for Infection & Immunity, St George’s University of London, UK
2. HIV/STI Department, National Infection Service, Public Health England, UK
3. St George's University Hospitals NHS Foundation Trust, London, UK
4. TwistDx Limited, Cambridge, UK
5. Department of Sexual Health, University of Southampton, UK
6. Bradford Teaching Hospitals NHS Foundation Trust, UK

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**ABSTRACT**

**Objectives**

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

**Methods**

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

**Results**

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

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

**Conclusions**

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

**INTRODUCTION**

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

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

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

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

**METHODS**

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

**Sample size and recruitment**

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

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

**Study sites and participant selection**

Three SHCs located in South London, Yorkshire and on the south coast of England participated. Eligible patients were recruited during routine consultations by clinic staff using the following eligibility criteria: aged ≥16 years; attending the SHC; had not passed urine in the previous two hours; provided written informed consent for the collection of research samples; provided all sample types (males: first-catch urine (FCU) and meatal swabs pre- and post- micturition; females: FCU and self-collected vulvo-vaginal swabs (SCVS)). Participant demographic and clinical data were collected on case report forms (CRFs).

**Sample collection and processing**

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

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

**Sample testing and resolution of discrepant results**

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

Research sample processing and testing were in accordance with TwistDx protocols, developed through internal optimisation (unpublished data). The RPA CT/NG assay was performed on research samples (FCU for men, FCU and SCVS for females) by staff at TwistDx, who were blinded to the routine clinic NAAT and CRF results. FCU samples were processed through a Size Exclusion Chromatography device (Zeta Sep FPLC Desalting Columns; Generon, Slough, UK) for the purposes of de-salting the sample before testing on the Alere™ i instrument.

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

**Data and statistical analysis**

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

**RESULTS**

**Overview of participants**

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

**CT/NG RPA assay diagnostic accuracy**

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

For females, 395 FCU and 395 SCVS were tested for CT and NG by the RPA CT/NG assay (Table 2A). For CT, 6/790 (0.76%; three FCU, three SCVS) results disagreed with the routine clinic NAAT SCVS result (see web-only Supplementary Table S3B). Following discrepant testing, the RPA CT/NG assay agreed with the resolved result for all three FCU discrepants, and 2/3 SCVS discrepants. For NG, 7/790 (0.89%; three FCU, four SCVS) RPA CT/NG results disagreed with the routine clinic NAAT SCVS result (see web-only Supplementary Table S3B). Of these, all three FCU and 3/4 SCVS discrepants agreed with the resolved result. Thus, in females, all measures of diagnostic accuracy were 100% for FCU for both CT and NG. For CT and NG in SCVS, specificity and PPV were 100%, NPV was 99.7% (367/368, 95%CI 98.5-100.0), and sensitivity was 96.4% (27/28, 95%CI 81.7-100) for CT and 66.7% (2/3, 95%CI 9.0-100) for NG (Table 2A). No female had a discrepant result for both CT and NG.

When performance was analysed by participant-reported symptomatic status, there was no evidence of a significant difference between symptomatic and asymptomatic patients (p>0.05). All point estimates were 100% for asymptomatic participants (Table 2C). Among symptomatic participants (Table 2B), the RPA CT/NGassay’s sensitivity was lower: 15/16 (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 CT SCVS, but specificity and NPV remained high. In addition, all diagnostic accuracy measures for NG detection in both male and female FCU, and female CT FCU detection, were 100% regardless of symptomatic status. (See web-only Supplementary Table S2 for tables of agreement between the RPA CT/NG assay and reference standard).

**DISCUSSION**

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

With respect to rapidity, the RPA CT/NG assay’s sample preparation, amplification and detection take place <20 minutes (sample preparation and RPA CT/NG assay run-time). A simple-to-use desalting device has been included in newer iterations of the assay, allowing immediate (in seconds) processing of FCU samples prior to running the assay, although currently it is only appropriate for research laboratory use. The test’s rapidity enhances the possibility of implementation as a POCT, enabling “test and treat” strategies with patients diagnosed and treated in the same clinical visit, and is potentially rapid enough to be incorporated into clinical practice with minimal change to clinical pathways. To date, a major barrier identified for STI POCT implementation has been patient willingness to wait, even for a 90 minute rapid test [11, 18], and the major changes to clinic care pathways necessary to incorporate rapid tests as POCTs as part of SHC consultations [11, 12]. Consequently, CT/NG GeneXpert implementation has enabled a same- or next-day results service, rather than a POCT “test and treat” strategy [19-21]. The RPA CT/NG assay’s rapidity, combined with its high performance, therefore has the potential to revolutionise STI diagnosis and management.

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

BASHH guidelines for NG testing indicate a minimum PPV of 90%; below this, positives should be confirmed with supplementary testing using a different nucleic acid target from the original test [5]. Although our NG PPV point estimates were all 100%, the lower 95%CI were all <90%. As the RPA CT/NG assay has only a single NG molecular target, it may ultimately have lower specificity and PPV compared to two-target assays, especially if applied to lower prevalence settings. That said, a diagnostic evaluation with large sample size of the two-target GeneXpert by Gaydos *et al.* [24] also resulted in NG PPVs with lower 95%CIs <90% in all sample types despite the point estimate being >90%, indicating that supplementary testing may be required for both assay types in low prevalence settings.

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

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

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

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

**AUTHOR CONTRIBUTIONS**

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

**TRANSPARENCY DECLARATION**

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Dr. Sadiq reports on behalf of himself and colleagues of the SGUL Applied Diagnostic Research and Evaluation Unit (ADREU) grants from UKCRC, other from TwistDx, and RP, PH and NF declare CLRN support to conduct portfolio research during the conduct of the study. Dr. Sadiq declares the following relevant financial activities outside the submitted work: other from Atlas Genetics Ltd, other from Alere, other from Cepheid, other from SpeeDx, other from Sekisui, grants from Innovate UK, grants from NIHR. MP, OP, MSF and DGB are employees of TwistDx. Advisory board membership is declared by EHE, SF, RP and PH for Becton Dickinson, by RP for Roche, Novartis, GSK, Genoccea, and CLJC, and by PH for Hologic, and Bayer Consumer Healthcare, outside the submitted work. In addition, MP has a patent EP2029782 issued, a patent EP2426221 issued; and OP has a patent 7,666,598 issued (not owned by the author), and a patent 9,663,820 issued (not owned by the author).

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

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**TABLES AND FIGURES**

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

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

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| --- |
| **Table 1. Participant characteristics** |
|  | **Male participants** | **Female participants** |
| **Characteristic** | **No. of participants (%)** | **CT** | **NG** | **No. of participants (%)** | **CT** | **NG** |
| **No. (%) with CTa** | **No. (%) with NGa** | **No. (%) with CTa** | **No. (%) with NGa** |
| 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) |
| Symptomaticb |  |  |  |  |  |  |
| 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) |

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

b Male participants considered symptomatic if they reported ≥1 of the following symptoms on the Case Report Form: Discharge (clear or cloudy liquid from the penis); Irritation at the top of the penis; Itching; Needing to pass urine more often than usual; Pain/burning when urinating. Female participants considered symptomatic if they reported ≥1 of the following symptoms on the Case Report Form: Itching; Discharge(clear or cloudy liquid from the vagina); Pain/burning when urinating; Needing to pass urine more frequently; Pain during 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. positivesa / 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)n/N | 94.3 (80.8-99.3)33/35 | 100 (73.5-100)12/12 | 100 (88.1-100)29/29 | 100 (29.2-100)3/3 | 96.4 (81.7-99.9)27/28 | 66.7 (9.0-100)2/3 |
| Specificity (%, 95%CI)n/N | 99.7 (98.4-100)356/357 | 100 (99.0-100)380/380 | 100 (99.0-100)366/366 | 100 (99.1-100)392/392 | 100 (99.0-100)367/367 | 100 (99.1-100)392/392 |
| PPV (%, 95%CI)n/N | 97.1 (84.7-99.9)33/34 | 100 (73.5-100)12/12 | 100 (88.1-100)29/29 | 100 (29.2-100)3/3 | 100 (87.2-100)27/27 | 100 (15.8-100)2/2 |
| NPV (%, 95%CI)n/N | 99.4 (98.0-99.9)356/358 | 100 (99.0-100)380/380 | 100 (99.0-100)366/366 | 100 (99.1-100)392/392 | 99.7 (98.5-100)367/368 | 99.7 (98.6-100)392/393 |
| **B: Symptomatic participantsb** |
|  | Males | Females |
|  | FCU | FCU | SCVS |
|  | CT | NG | CT | NG | CT | NG |
| No. positivesa / 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)n/N | 93.8 (69.8-99.8)15/16 | 100 (69.2-100)10/10 | 100 (76.8-100)14/14 | 100 (15.8-100)2/2 | 92.9 (66.1-99.8)13/14 | 50 (1.3-98.7)1/2 |
| Specificity (%, 95%CI)n/N | 99.2 (95.7-100)125/126 | 100 (97.2-100)132/132 | 100 (97.9-100)172/172 | 100 (98.0-100)184/184 | 100 (97.9-100)172/172 | 100 (98.0-100)184/184 |
| PPV (%, 95%CI)n/N | 93.8 (69.8-99.8)15/16 | 100 (69.2-100)10/10 | 100 (76.8-100)14/14 | 100 (15.8-100)2/2 | 100 (75.3-100)13/13 | 100 (2.5-100)1/1 |
| NPV (%, 95%CI)n/N | 99.2 (95.7-100)125/126 | 100 (97.2-100)132/132 | 100 (97.9-100)172/172 | 100 (98.0-100)184/184 | 99.4 (96.8-100)172/173 | 99.5 (97.0-100)184/185 |
| **C: Asymptomatic participants** |
|  | Males | Females |
|  | FCU | FCU | SCVS |
|  | CT | NG | CT | NG | CT | NG |
| No. positivesa / 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)n/N | 100 (81.5-100)18/18 | 100 (15.8-100)2/2 | 100 (78.2-100)15/15 | 100 (2.5-100)1/1 | 100 (76.8-100)14/14 | 100 (2.5-100)1/1 |
| Specificity (%, 95%CI)n/N | 100 (98.4-100)231/231 | 100 (98.5-100)247/247 | 100 (98.1-100)193/193 | 100 (98.2-100)207/207 | 100 (98.1-100)194/194 | 100 (98.2-100)207/207 |
| PPV (%, 95%CI)n/N | 100 (81.5-100)18/18 | 100 (15.8-100)2/2 | 100 (78.2-100)15/15 | 100 (2.5-100)1/1 | 100 (76.8-100)14/14 | 100 (2.5-100)1/1 |
| NPV (%, 95%CI)n/N | 100 (98.4-100)231/231 | 100 (98.5-100)247/247 | 100 (98.1-100)193/193 | 100 (98.2-100)207/207 | 100 (98.1-100)194/194 | 100 (98.2-100)207/207 |

FCU, First-catch urine; SCVS, Self-Collected Vulvo-Vaginal Swab; CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*;PPV, Positive Predictive Value; NPV, Negative Predictive Value

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

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