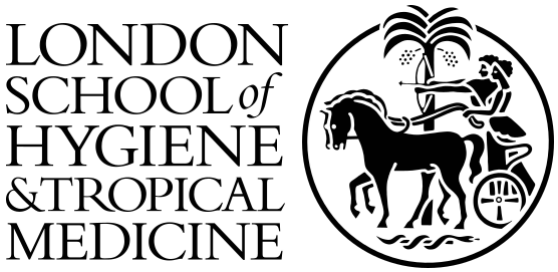

Diagnostic accuracy of a prototype rapid chlamydia and gonorrhoea recombinase polymerase amplification assay: a multi-centre cross-sectional pre-clinical evaluation

Running title: Prototype CT/NG RPA assay early evaluation

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Word count: 2664

Intended category: Original article

Key words:

Chlamydia trachomatis; *Neisseria gonorrhoeae*; Nucleic Acid Amplification Tests; Point-of-care; performance evaluation; diagnostic accuracy

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/NG assay'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

This study was funded by TwistDx, St George's, University of London, and a UK Clinical Research Collaboration, Translational Infection Research Initiative Consortium Grant (Grant number G0901608).

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).

We would like to thank all the participants for taking part, and the staff who recruited them to the study, including Jo Turpitt (Solent NHS Trust) and Paula Sharratt (Bradford Teaching Hospitals NHS Foundation Trust). We would also like to thank Anthony Nardone and Rebecca Howell-Jones from Public Health England for their input within the *e*STI^2 consortium. We thank Hamish Mohammed from Public Health England for providing national GUMCAD 2014 prevalence data, and Soazig Clifton from University College London for providing Natsal-3 weighted prevalence data. ADREU also acknowledges the support of the National Institute of Health Research Clinical Research Network (NIHR CRN). This study was included in the UKCRN ID 15136 “Patient-consented samples for STI diagnostic & biomarker evaluation” database.

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.

REFERENCES

[1] PHE, Sexually transmitted infections and chlamydia screening in England, 2015, Health Protection Report, https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/534601/hpr2216_stis.pdf, 2016, p. 8th July 2016.

[2] Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N, et al. Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. PLoS One 2015;10:e0143304.

[3] Korenromp EL, Sudaryo MK, De Vlas SJ, Gray RH, Sewankambo NK, Serwadda D, et al. What proportion of episodes of gonorrhoea and chlamydia becomes symptomatic? Int J STD AIDS 2002;13:91-101.

[4] Lee YS, Lee KS. Chlamydia and male lower urinary tract diseases. Korean J Urol 2013;54:73-7.

[5] Bignell C, Fitzgerald M. UK national guideline for the management of gonorrhoea in adults, 2011. Int J STD AIDS 2011;22:541-7.

[6] Menon-Johansson AS, Mcnaught F, Mandalia S, Sullivan AK. Texting decreases the time to treatment for genital *Chlamydia trachomatis* infection. Sex Transm Infect 2006;82:49-51.

[7] NCSP, Audit report on turnaround times, Public Health England, https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/380604/NCSP_audit_report_turnaround_times.pdf, 2014.

[8] Harding-Esch E, Sherrard-Smith E, Fuller SS, Harb A, Furegato M, Mercer C, et al. P65 Sexual behaviour in the time period between being tested for chlamydia and receiving test result and treatment. Sex Transm Infect 2015;91:A37.

[9] St John A, Price CP. Existing and Emerging Technologies for Point-of-Care Testing. Clin Biochem Rev 2014;35:155-67.

[10] Turner KM, Round J, Horner P, Macleod J, Goldenberg S, Deol A, et al. An early evaluation of clinical and economic costs and benefits of implementing point of care NAAT

394 tests for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in genitourinary medicine clinics
395 in England. Sex Transm Infect 2014;90:104-11.
396 [11] Harding-Esch EM, Nori AV, Hegazi A, Pond MJ, Okolo O, Nardone A, et al. Impact of
397 deploying multiple point-of-care tests with a 'sample first' approach on a sexual health
398 clinical care pathway. A service evaluation. Sex Transm Infect 2017;93:424-429.
399 [12] Adams EJ, Ehrlich A, Turner KM, Shah K, Macleod J, Goldenberg S, et al. Mapping
400 patient pathways and estimating resource use for point of care versus standard testing and
401 treatment of chlamydia and gonorrhoea in genitourinary medicine clinics in the UK. BMJ
402 Open 2014;4:e005322.
403 [13] Nwokolo NC, Dragovic B, Patel S, Tong CY, Barker G, Radcliffe K. 2015 UK national
404 guideline for the management of infection with *Chlamydia trachomatis*. Int J STD AIDS
405 2016;27:251-67.
406 [14] Herbst De Cortina S, Bristow CC, Joseph Davey D, Klausner JD. A Systematic Review
407 of Point of Care Testing for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and
408 *Trichomonas vaginalis*. Infect Dis Obstet Gynecol 2016;2016:4386127.
409 [15] WHO, Point-of-care tests - Target product profiles and research questions.
410 http://who.int/reproductivehealth/POTC-TPPs-2016.pdf?ua=1, 2016. (Accessed 1st
411 September 2017).
412 [16] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD
413 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ
414 2015;351:h5527.
415 [17] PHE, Genitourinary medicine clinic activity dataset (GUMCADv2),
416 https://www.gov.uk/guidance/genitourinary-medicine-clinic-activity-dataset-gumcadv2,
417 2013.).
418 [18] Atkinson LM, Vijeratnam D, Mani R, Patel R. 'The waiting game': are current chlamydia
419 and gonorrhoea near-patient/point-of-care tests acceptable to service users and will they
420 impact on treatment? Int J STD AIDS 2016;27:650-5.

[19] Wingrove I, Mcowan A, Nwokolo N, Whitlock G. Diagnostics within the clinic to test for gonorrhoea and chlamydia reduces the time to treatment: a service evaluation. Sex Transm Infect 2014;90:474.

[20] Sharman N, Sri T, Chow C, Pond MJ, Oakeshott P, Planche T, et al. Chlamydia testing: Reaching high-risk sexually active young people in the community. Int J STD AIDS 2016;27:78-9.

[21] Badman SG, Vallely LM, Toliman P, Kariwiga G, Lote B, Pomat W, et al. A novel point-of-care testing strategy for sexually transmitted infections among pregnant women in high-burden settings: results of a feasibility study in Papua New Guinea. BMC Infect Dis 2016;16:250.

[22] WHO, Global health sector strategy on Sexually Transmitted Infections 2016-2021: towards ending STIs, http://apps.who.int/iris/bitstream/10665/246296/1/WHO-RHR-16.09-eng.pdf?ua=1, 2016.

[23] Peeling RW, Holmes KK, Mabey D, Ronald A. Rapid tests for sexually transmitted infections (STIs): the way forward. Sex Transm Infect 2006;82 Suppl 5:v1-6.

[24] Gaydos CA, Van Der Pol B, Jett-Goheen M, Barnes M, Quinn N, Clark C, et al. Performance of the Cepheid CT/NG Xpert Rapid PCR Test for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. J Clin Microbiol 2013;51:1666-72.

[25] Michel CE, Sonnex C, Carne CA, White JA, Magbanua JP, Nadala EC, Jr., et al. *Chlamydia trachomatis* load at matched anatomic sites: implications for screening strategies. J Clin Microbiol 2007;45:1395-402.

[26] Van Dommelen L, Wolffs PF, Van Tiel FH, Dukers N, Herngreen SB, Bruggeman CA, et al. Influence of temperature, medium, and storage duration on *Chlamydia trachomatis* DNA detection by PCR. J Clin Microbiol 2013;51:990-2.

[27] Alonzo TA, Pepe MS. Using a combination of reference tests to assess the accuracy of a new diagnostic test. Stat Med 1999;18:2987-3003.

[28] Fairley CK, Hocking JS, Zhang L, Chow EP. Frequent Transmission of Gonorrhea in Men Who Have Sex with Men. Emerg Infect Dis 2017;23:102-104.

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)

[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.

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) 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)	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) 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)	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) 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)	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

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