AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

Laboratory Validation of Xpert *Chlamydia trachomatis/Neisseria gonorrhoeae* and *Trichomonas vaginalis* Testing as Performed by Nurses at Three Primary Health Care Facilities in South Africa

Remco P. H. Peters,[a,b,c] Lindsey de Vos,[d] Liteboho Maduna,[a] Maanda Mudau,[d] Jeffrey D. Klausner,[e,f] Marleen M. Kock,[a,g] Andrew Medina-Marino[d]

Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa[a]; Department of Medical Microbiology, CAPHRI School for Public Health and Primary Care, University of Maastricht, Maastricht, The Netherlands[b]; Anova Health Institute, Johannesburg, South Africa[c]; Research Unit, Foundation for Professional Development, Pretoria, South Africa[d]; David Geffen School of Medicine, University of California—Los Angeles, Los Angeles, California, USA[e]; Department of Epidemiology, University of California—Los Angeles, Los Angeles, California, USA[f]; National Health Laboratory Services, Tshwane Academic Division, Pretoria, South Africa[g]

KEYWORDS antenatal care, molecular diagnostics, STI screening, human immunodeficiency virus, sexually transmitted diseases

The introduction of molecular diagnostic tests provides an important step to address the burden of sexually transmitted infections (STIs), especially *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*. Recently developed Xpert CT/NG (for *C. trachomatis/N. gonorrhoeae*) and TV (for *T. vaginalis*) assays provide opportunities to detect these STIs in resource-limited settings (1). When performed by staff at primary health care (PHC) facilities, patients can be provided results and treatment within 2 h.

We implemented Xpert CT/NG and TV assay testing of HIV-infected pregnant women at three PHC facilities in Pretoria, South Africa (2), and conducted a laboratory validation of Xpert results obtained at these facilities.

Participants self-collected three vulvovaginal swabs. The first swab was immediately processed and tested using Xpert CT/NG and TV assays (Cepheid, Sunnyvale, CA) at the PHC facility per the manufacturer's instruction and as described elsewhere (3). The two other swabs were shipped to the Department of Medical Microbiology, University of Pretoria, for additional laboratory and molecular analysis.

For laboratory confirmation, DNA was extracted from the second swab using the High Pure PCR template preparation kit (Roche Diagnostics, Basel, Switzerland) and analyzed with the Presto^{Plus} CT/NG/TV assay (Microbiome, Ltd., Houten, The Netherlands) as per the manufacturer's instruction. The Presto^{Plus} assay has reported high concordance with the Roche Cobas CT/NG assay and the TIB Molbiol LightMix TV assay (4, 5). Specimens with discordant results between Xpert and Presto^{Plus} were confirmed with the Anyplex II STI-7 assay (Seegene, Seoul, South Korea) per the manufacturer's instruction (6).

The results from 50 randomly selected specimens by Xpert testing identified that 26 were *C. trachomatis* positive, 7 were *N. gonorrhoeae* positive, and 28 were *T. vaginalis* positive. Xpert and Presto^{Plus} results were concordant for 47/50 (94%) of participants (Fig. 1). Two of the three discordant results may be attributed to sampling and testing variation as suggested by the high Xpert cycle threshold (C_T) values (>38 cycles). While the initial Presto^{Plus} test also gave equivocal C_T values of >38 cycles for

Accepted manuscript posted online 11 October 2017

Citation Peters RPH, de Vos L, Maduna L, Mudau M, Klausner JD, Kock MM, Medina-Marino A. 2017. Laboratory validation of Xpert *Chlamydia trachomatis/Neisseria gonorrhoeae* and *Trichomonas vaginalis* testing as performed by nurses at three primary health care facilities in South Africa. J Clin Microbiol 55:3563–3565. https://doi.org/10.1128/JCM.01430-17.

Editor Andrew B. Onderdonk, Brigham and Women's Hospital

Address correspondence to Andrew Medina-Marino, andrewmedinamarino@gmail.com.

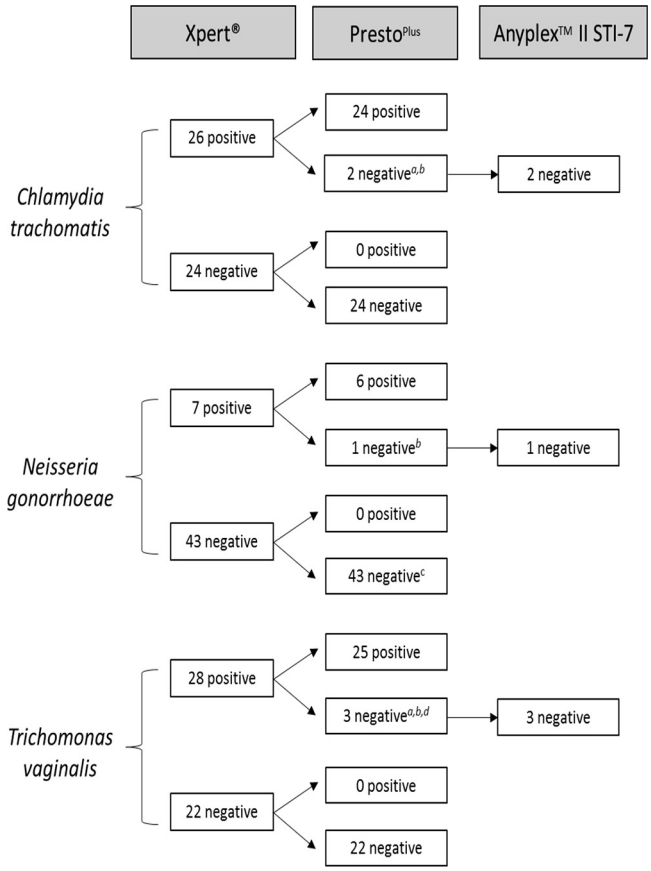

FIG 1 Results of laboratory validation of Xpert CT/NG and TV tests of self-collected vaginal swabs from 50 HIV-infected pregnant women. Footnote *a* indicates this patient had discordant results for both *Chlamydia trachomatis* (Xpert C_T value of 38.3) and *Trichomonas vaginalis* (Xpert C_T value of 39.9). Both showed amplification in the initial Presto^{Plus} test (C_T values: for *Chlamydia trachomatis*, 38.7; for *Trichomonas vaginalis*, 39.4) but were negative in the Presto^{Plus} repeat test as per the manufacturer's instruction. Footnote *b* indicates this patient had discordant results for all three microorganisms, with Xpert C_T values as follows: for *Chlamydia trachomatis*, 34; for *Neisseria gonorrhoeae*, 34.0 for NG1 probe and 35.2 for NG2 probe; for *Trichomonas vaginalis*, 37.0. Footnote *c* indicates the initial Presto^{Plus} test was low positive (C_T value of 37.6), but the specimen tested negative upon Presto^{Plus} retest. Footnote *d* indicates the C_T value of this specimen was 39.7 in the Xpert assay.

C. trachomatis and *T. vaginalis* for one of these patients, repeat Presto^{Plus} and confirmatory test results were negative. In addition, three Xpert-negative specimens had initial equivocal Presto^{Plus} results but were negative upon repeat testing. This highlights the challenges with interpretation of low-positive results in molecular tests. The third patient was positive for all three STIs by Xpert and negative for all three by Presto^{Plus} and Anyplex. We attribute this discordance to either an inadvertent specimen exchange or mislabeling.

Our study is limited by the fact that confirmation by retesting was not conducted using GeneXpert assays, as additional swabs were specifically collected for nucleic acid extraction to be used for research purposes. However, we used two established molecular detection assays that have a similar range of technical performance to Xpert (1, 2, 7, 8). Repeat Xpert testing of specimens with high C_T values was not performed, whereas equivocal results in Presto^{Plus} were retested as per the manufacturer's instruction.

In conclusion, we demonstrate that reliable STI diagnoses can be obtained from self-collected vaginal swabs through Xpert CT/NG and TV testing by nurses at PHC facilities in South Africa. This observation supports the feasibility of implementation of easy-to-use molecular tests for STI diagnosis in resource-constrained settings.

ACKNOWLEDGMENTS

Our research team received donated CT/NG and TV test cartridges from Cepheid. Cepheid has had no input in the study design, result generation, data analysis, or data interpretation of the submitted work.

REFERENCES

1. Gaydos CA. 2014. Review of use of a new rapid real-time PCR, the Cepheid GeneXpert (Xpert) assay, for Chlamydia trachomatis and Neisseria gonorrhoeae: results from patients while in a clinical setting. Expert Rev Mol Diagn 14:135–137. https://doi.org/10.1586/14737159.2014.871495.
2. Gaydos CA, van der Pol B, Jett-Goheen M, Barnes M, Quinn N, Clark C, Daniel GE, Dixon PB, Hook EW, III, CT/NG Study Group. 2013. Performance of the Cepheid CT/NG Xpert rapid PCR test for detection of Chlamydia trachomatis and Neisseria gonorrhoeae. J Clin Microbiol 51: 1666–1672. https://doi.org/10.1128/JCM.03461-12.
3. Mudau M, Peters RP, de Vos L, Olivier D, Davey DJ, Mkwanazi E, McIntyre JA, Klausner JD, Medina-Marino A. 11 August 2017. High prevalence of asymptomatic sexually transmitted infections among human immunodeficiency virus-infected pregnant women in a low-income South African community. Int J STD AIDS https://doi.org/10.1177/0956462417724908.
4. De Waaij DJ, Dubbink JH, Peters RP, Ouburg S, Morre SA. 2015. Comparison of GMT Presto assay and Roche Cobas 4800 CT/NG assay for detection of Chlamydia trachomatis and Neisseria gonorrhoeae in dry swabs. J Microbiol Methods 118:170–174. https://doi.org/10.1016/j.mimet.2015.08.020.
5. de Waaij DJ, Ouburg S, Dubbink JH, Peters RP, Morre SA. 2016. Evaluation of Presto^{Plus} assay and LightMix kit Trichomonas vaginalis assay for detection of Trichomonas vaginalis in dry vaginal swabs. J Microbiol Methods 127:102–104. https://doi.org/10.1016/j.mimet.2016.06.002.
6. Bercot B, Amarsy R, Goubard A, Aparicio C, Loeung HU, Segouin C, Gueret D, Jacquier H, Meunier F, Mougari F, Cambau E. 2015. Assessment of coinfection of sexually transmitted pathogen microbes by use of the Anyplex II STI-7 molecular kit. J Clin Microbiol 53:991–993. https://doi.org/10.1128/JCM.03370-14.
7. Tabrizi SN, Unemo M, Golparian D, Twin J, Limnios AE, Lahra M, Guy R, TTANGO Investigators. 2013. Analytical evaluation of GeneXpert CT/NG, the first genetic point-of-care assay for simultaneous detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. J Clin Microbiol 51: 1945–1947. https://doi.org/10.1128/JCM.00806-13.
8. Peuchant O, de Diego S, Le Roy C, Frantz-Blancpain S, Hecke C, Bebear C, de Barbeyrac B. 2015. Comparison of three real-time PCR assays for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in young pregnant women. Diagn Microbiol Infect Dis 83:335–337. https://doi.org/10.1016/j.diagmicrobio.2015.09.002.