Impact of specimen storage temperature and time on the implementation of GeneXpert® testing for sexually transmitted infections in resource-constraint settings

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ABSTRACT

Implementation of routine laboratory diagnostics is imperative to address the high burden of sexually transmitted infections (STI) in Sub-Saharan Africa. We demonstrate that logistical challenges of specimen storage, temperature and transport time are unlikely to impact on performance of routine STI diagnostics using the GeneXpert® platform implemented in these settings.

HIGHLIGHTS

- Xpert® CT/NG and TV assays can detect STIs in resource-constraint settings.
- Logistical challenges (temperature, transport time) may affect Xpert® performance.
- *Chlamydia trachomatis* and *Neisseria gonorrhoeae* detection is not affected.
- Small reduction of *Trichomonas vaginalis* detection occurs in urine after two weeks.
- Xpert® detection of STIs is logistically feasibly in resource-constraint settings.

MANUSCRIPT

Sexually transmitted infections (STIs) caused by *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* remain a major health challenge globally and the Sub-Saharan Africa has the highest burden of STIs with 98 million infections reported in 2012 (Newman et al., 2015). STIs in Sub-Saharan Africa are treated using a syndromic management approach which has well documented limitations such as the inability to detect asymptomatic infections, lack of drug susceptibility testing, unnecessary and inappropriate use of antibiotics (overtreatment) with the risk of antimicrobial resistance development, and lack of laboratory infrastructure for surveillance (Garrett et al., 2018, White et al., 2008). Therefore, implementation of routine molecular laboratory diagnostics is imperative in order to address the high burden of STIs in Sub-Saharan Africa.

Traditionally laboratory diagnosis of *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* from clinical specimens relies on microscopy and culture methods which are time and labour intensive and have a low sensitivity (Hobbs and Seña, 2013, Jacobsson et al., 2018). The Cepheid GeneXpert® CT/NG and Xpert® TV assays are rapid fully automated molecular assays that have excellent sensitivity and specificity for diagnosing STIs and have been used in various settings (Gaydos, 2014, Peters et al., 2017, Schwebke et al., 2018). These assays can be rolled-out as routine diagnostics in resource-constraint settings by leveraging existing infrastructure of GeneXpert® platforms already in place for the detection of *Mycobacterium tuberculosis* (Parsons et al., 2011).

However, the effectiveness of GeneXpert® assays for STI diagnosis in resource-constraint settings may be undermined by logistical challenges such as the temperature at which specimens are stored and specimen transport time to laboratory facilities (Lu et al., 2016). Our aim was to determine the effects of storage temperature and transport time on the detection of

C. trachomatis, N. gonorrhoeae and *T. vaginalis* infections using the GeneXpert® platform in a real-life setting in Pretoria, South Africa. The manufacturer recommends collection and storage of specimens for Xpert® processing using the urine specimen collection kit (for Xpert® CT/NG testing male urine is considered stable at 2-30 °C for 45 days and female urine up to 45 days at 2-15 °C and for 3 days up to 30 °C in this kit; for processing by the Xpert® TV assay urine is considered stable up to 14 days at 30 °C) and the endocervical specimen collection kit (stable at 30 °C for up to 60 days for Xpert® CT/NG as well as Xpert® TV testing). To our knowledge, there has not been an independent evaluation of these specimen collection kits when used in practice in settings in sub-Saharan Africa.

To investigate the impact of specimen storage temperature and transport time for detection of *C. trachomatis* and *N. gonorrhoeae* using the Xpert CT/NG test we created a stock solution by repeated centrifugation followed by suspension of the pellet in phosphate saline buffer (PBS) of a known *C. trachomatis*-positive urine sample (quantified using the Light mix Kit 480 HT CT/NG assay (TIB MOLBIOL, Berlin, Germany) on the LightCycler® (Roche Diagnostics, Switzerland)) that was spiked with a stock culture of *N. gonorrhoeae* ATCC® 49226 strain based on viable count. Three 10-fold dilutions of this stock solution were prepared and these were spiked to urine and PBS. Aliquots of these spiked stock dilutions were then transferred to the Xpert® urine specimen collection (6mL) and the Xpert® endocervical specimen collection (0.25 mL) kits in line with the manufacturer's instruction (Cepheid, Sunnyvale, USA). Thereafter, the transport collection kits were either stored at room temperature (27°C ± 3 °C), which is normal summer room temperature for our setting in Pretoria, South Africa, or kept in the refrigerator (4 °C± 1 °C).

One millilitre of sample from each stored Xpert® specimen collection kit was transferred to the Xpert® CT/NG reaction cartridge resulting in a final cycle threshold (Ct) value range of 25-34 for *C. trachomatis* and 10, 000 -100 colony forming units for *N. gonorrhoeae* for the 10-fold dilution series in the Xpert® test reaction. Xpert® CT/NG test was performed of two samples

from each specimen transport medium at days 0, 7, 14 and 28 for detection of presence of *C. trachomatis* and *N. gonorrhoeae* DNA. We chose a series of up to 28 days as realistically specimens should be processed within that time frame to have clinical value in routine practice. The same procedure was followed for analysis of *T. vaginalis*; filter sterilised urine (6 mL) that was spiked with a counted stock solution of *T. vaginalis* ATCC® 30001 strain was transferred to the Xpert ® specimen transport kits resulting in a concentration range of 11000-1100 organisms per millilitre in the kits. From these kits 0.5 mL was transferred to the Xpert® TV reaction cartridges corresponding to 5,500-55 protozoal cells per Xpert® test reaction. The Ct values provide a semiquantitative concentration of DNA in the sample and a change in Ct value of more than 2 cycles for the spiked samples was considered clinically relevant (Ingersoll et al., 2008). Specimens from the different time points were concurrently processed and tested in the same run.

We did not observe any clinically relevant increase in the Ct value over time between baseline (day 0) and the subsequent time points (days 7, 14, 28) for detection of *C. trachomatis* and *N. gonorrhoeae* spiked in either Xpert® specimen collection kits irrespective of the storage temperature (Figure 1). However, there was a pronounced increase in the Ct-value of *T. vaginalis* spiked in the Xpert® urine specimen collection kit stored at room temperature (3,5 (13%), 2,6 (8.5%) and 0,45 (1.3%) respectively for the highest, medium and lowest spiking concentration), but not in the refrigerator. The increase in the Ct value from the aliquots of the Xpert® urine specimen collection kit stored at room temperature correlates to a reduction in *T. vaginalis* DNA load of ~10 fold. There were no differences observed for detection of *T. vaginalis* from the endocervical collection kit.

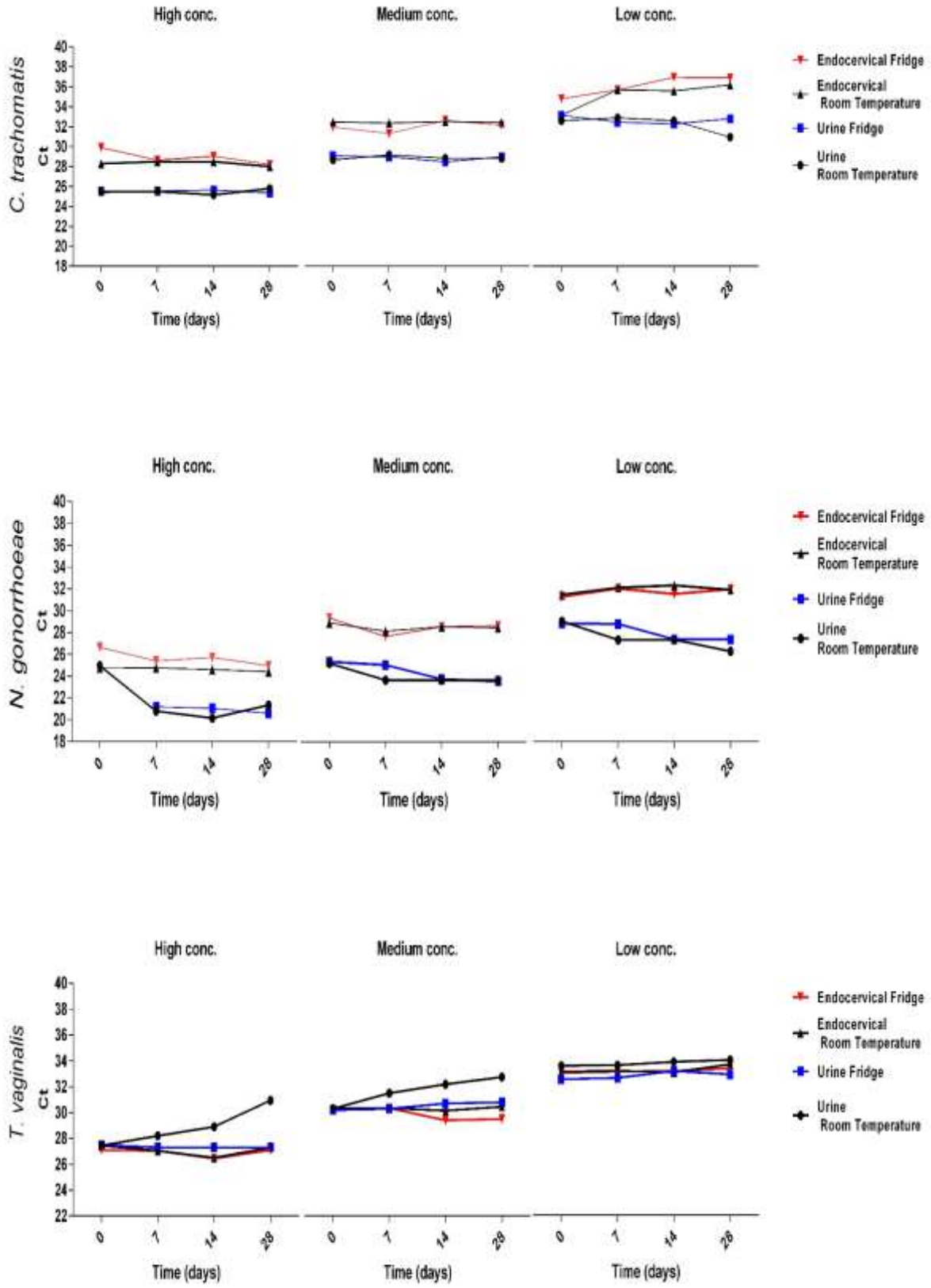

Figure 1. Mean cycle threshold values for *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis* spiked to the Xpert® endocervical and urine specimen collection kits for each time point and different storage conditions.

This study has several limitations. First, specimens were not actually transported but left at room temperature in a single venue with its temperature fluctuations during a summer month in Pretoria, South Africa. In practice, the delay in sample processing with fluctuations in temperature over time would most likely occur from storage in a single place rather than continuously for a longer period of time. As such, we do not think that this really influenced our results. To mimic what would happen in routine practice in a sub-Saharan setting, we decided to keep a realistic scenario for our setting, i.e. using the normal room temperature during summer instead of artificially modifying temperature height and fluctuations. This might have biased the results, in particular in relation to detection of *Trichomonas vaginalis* from urine, however, we do think it is important to test specimen storage conditions in a realistic manner as to inform guideline and policy design. In practice, simple solutions such as a foam/cooler box without ice could be implemented to prevent specimens being exposed to temperatures higher than recommended and evaluated in our study. We used PBS for spiking instead of endocervical fluid for spiking of the endocervical specimen collection kit. The absence of vaginal microbiota that would normally have been present in specimens and might affect DNA integrity and stability might have biased our results. Finally, we only included a single strain of each microorganism, evaluated a dual rather than single positive for *C. trachomatis* and *N. gonorrhoeae*, and used filtered sterilised urine to for spiking of microorganisms, but have no reason to think that this has influenced our results.

C. trachomatis, *N. gonorrhoeae* and *T. vaginalis* remain important STI pathogens in Sub-Saharan Africa where these infections are managed using a syndromic approach and absence of routine laboratory diagnostics. Our data show that genital specimens stored in transport medium at temperatures of up to 30°C and processed within 28 days from collection provide reliable results in Xpert® CT/NG testing. However, we observed a small decrease in detection of *T. vaginalis* DNA from the Xpert urine collection kit specimens at room temperature. This

suggests that specimens being tested for *T. vaginalis* should preferably be refrigerated to avoid a potential small reduction in test sensitivity. This observation is confirmed by Ingersoll et al (Ingersoll et al., 2008) who reported better recovery of *T. vaginalis* DNA when specimens were refrigerated. Our data confirm the specifications made by the manufacturer in the package insert, however, we are not aware of any other evaluations of these specifications. Moreover, we used an experimental set-up that translates to implementation of routine diagnostics in practice in sub-Saharan Africa, and confirm that this would be feasible using the Xpert® collection media with subsequent laboratory STI testing. A simple solution to keep the specimens at recommended temperature would be to use a foam or cooler box, without an ice requirement, to keep specimens at an appropriate temperature.

In summary, our work highlights that logistical barriers associated with specimen storage temperature at 30°C and transport time of up to 28 days may not impact the implementation of STI diagnostics using existing GeneXpert® platform in resource-constraint settings.

Acknowledgements

This work was supported, in part, by the Eunice Kennedy Shriver National Institute of Child Health & Human Development, U.S. National Institute of Health, under grant 1R21HD084274-01A1

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