To Pool or Not to Pool Samples for Sexually Transmitted Infections Detection in Men Who Have Sex With Men? An Evaluation of a New Pooling Method Using the GeneXpert Instrument in West Africa

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Background: Men who have sex with men (MSM) using preexposure prophylaxis (PrEP) are at risk for sexually transmitted infections (STIs). Therefore, PrEP services should include regular screening for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) at urethra, anorectum, and pharynx. However, financial and logistic challenges arise in low-resource settings. We assessed a new STI sample pooling method using the GeneXpert instrument among MSM initiating PrEP in West Africa.

Methods: Urine, anorectal, and pharyngeal samples were pooled per individual for analysis. In case of an invalid result only (strategy 1) or a positive result of the pool (strategy 2), samples were analyzed individually to identify the infection's biological location. The results of 2 different pooling strategies were compared against the individual results obtained by a criterion standard.

Results: We found a prevalence of 14.5% for chlamydia and 11.5% for gonorrhea, with a predominance of infections being extragenital (77.6%). The majority of infections were asymptomatic (88.2%). The pooling strategy 1, had a sensitivity, specificity and agreement for CT of 95.4%, 98.7%, and 0.93, respectively; and 92.3%, 99.2%, and 0.93 for pooling strategy 2. For NG, these figures were 88.9%, 97.7%, and 0.85 for strategy 1, and 88.9%, 96.7%, and 0.81 for strategy 2.

Conclusions: West African MSM have a high prevalence of extragenital and asymptomatic STIs. The GeneXpert method provides an opportunity to move from syndromic toward etiological STI diagnosis in low-income countries, as the platform is available in African countries for tuberculosis testing. Pooling will reduce costs of triple site testing.

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The incidence of sexually transmitted infections (STIs), including *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG), is increasing globally. Their burden is disproportionally higher in low- and middle-income countries (LMIC) and in key populations, such as men who have sex with men (MSM).[1] Fast detection and treatment of STIs is essential, as they raise serious health concerns, including increased risk of acquiring human immunodeficiency virus (HIV) infection.[2]

United Nations Programme on HIV/AIDS recommends a combination strategy of biomedical, behavioral, and structural approaches for HIV prevention.[3] The use of preexposure prophylaxis (PrEP) is an effective new biomedical HIV prevention tool, which is increasingly used among MSM in many high-resource countries.[4,5] However, PrEP may lead to a decrease in condom use and hence enhance STIs.[6] Indeed, PrEP demonstration studies among MSM in high-resource settings reported a high STI prevalence and incidence, whereby most of the STIs were of extragenital origin, that is, pharynx and anorectum. These STIs are frequently asymptomatic.[7–11] Although frequent screening of STIs among MSM in high-resource settings is currently debated,[12] African MSM often report sexual relations with women.[13] This sexual behavior may contribute to the spread of STIs to the general population. Hence, fast detection and treatment of STI infections is recommended in this population.

The World Health Organization (WHO), therefore, advocates the integration of STI testing and treatment in all PrEP services, so that populations at risk have access to both STI prevention and care.[14] Furthermore, triple-site testing is recommended in MSM.[15,16] To date, nucleic acid amplification tests (NAATs) are the recommended diagnostic methods to detect STIs due to their high sensitivity and specificity. However, this method

requires a state-of-the-art molecular laboratory and highly trained laboratory technicians.[17,18] Unfortunately, the screening of STIs using NAATs is hampered or even absent in LMIC due to the lack of adequate laboratory services and limited resources. Because of these barriers, LMIC use a syndromic approach for the diagnosis and treatment of symptomatic STIs.

The GeneXpert platform (Cepheid, Sunnyvale, CA) holds promise as a method to detect STIs in LMIC. This platform is a molecular assay which requires minimal training and yields results within 2 hours. Since 2010, the WHO has recommended the use of the GeneXpert platform for the confirmation of tuberculosis and the detection of rifampicin resistance of *Mycobacterium tuberculosis*. As a consequence, the GeneXpert platform has become widely available throughout Africa.[19] Currently, the Food and Drug Administration approved a cartridge based CT/NG assay on the GeneXpert system which can be used to test samples of genital, pharyngeal, and anorectal origin.[20] However, the high cost of the GeneXpert CT/NG cartridge hinders its utilization for the diagnosis of CT/NG in Africa.

In addition, testing 1 genital (urine) and 2 extragenital samples (anorectal and pharyngeal) for STI screening in MSM will further increase this cost. Hence, pooling of the 3 collected samples per individual offers potential cost-savings in CT/NG detection in MSM presenting for PrEP.[21] Several pooling methods are described, including 3 using the GeneXpert instrument for STI detection.[21–26] To our knowledge, none of these pooling methods have been implemented in LMIC. In addition, very few are able to determine the biological location of the infection, which could be important for treatment and surveillance purposes.

We evaluated the performance of a new pooling method using the GeneXpert platform for the detection of CT and NG among MSM initiating PrEP in 4 West African countries.

MATERIALS AND METHODS

Study Setting

The CohMSM-PrEP study is being conducted in 4 sites in West Africa: Ouagadougou, Burkina Faso; Lomé, Togo; Bamako, Mali and Abidjan, Côte d'Ivoire. Its aim is to assess the feasibility of PrEP among a cohort of approximately 500 MSM, including STI prevalence. Samples for CT/NG testing were collected from all participants at their initiation visit and transported to research laboratories (SEREFO, Bamako and Institut Pasteur, Abidjan) or to national reference laboratories for tuberculosis (Laboratoire National de Recherche sur la Tuberculose-TB, Ouagadougou and CHU-SO-LNR-TB, Lomé) where the GeneXpert instrument was available (hereafter called local STI laboratory).

The study has been approved by all applicable ethics committees, and all participants provided written informed consent.

Quality Control

The STI reference laboratory of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, ensured the reliability and quality of the results of the local STI laboratories, including compliance with Good Clinical and Laboratory Practice Standards. This laboratory provided hands-on training in sample collection, processing, and storage at study initiation in each site. An external quality control (EQC) panel was tested at study initiation and quarterly during the study. If DNA contamination was suspected, an environmental control of the laminar flow, bench, pipettes and the surface of the GeneXpert instrument was done.

Specimen Collection

Participants provided first-void urine and a physician took 2 pharyngeal and 2 anorectal samples (Eswab 1 mL, Copan Diagnostics, Brescia, Italy). After collection, samples were stored refrigerated (2–8°C) or frozen (−20°C) on site, depending on the time of transport to the local STI laboratory (2–8°C < 48 hours < −20°C). Transport of samples was performed under temperature-monitored conditions using a cool box and cooling elements. Upon sample receipt in the local STI laboratory, 1 aliquot (1 mL) of urine, 1 anorectal, and 1 pharyngeal Eswab (both randomly chosen from the duplicates) were immediately frozen (−20°C) until shipment on dry ice to ITM for reference testing. Samples for local testing were stored refrigerated (2–8°C) if analysis was performed within 72 hours of collection, otherwise, samples were stored frozen (−20°C).

Laboratory Methods

To provide a direct comparison of the pooling method on the GeneXpert platform in the 4 West African countries with standard molecular testing and to avoid site-specific biases, we compared the results obtained with the GeneXpert platform with the results obtained using a criterion standard performed in a controlled laboratory environment.

The original text mentioned using a gold standard. Criterion standard is however used throughout the text now, so in case this term is used, please keep it like this.

Pooling Method

At the local STI laboratory, a volume of 400 μL of the 3 samples (urine, anorectal, and pharyngeal sample) per participant was transferred in a microtube (hereafter called pool). After vortexing, 1 mL of the pool was transferred into the CT/NG Xpert cartridge. When the result of the pool was negative, all samples were considered negative and were not individually tested. When the pool was positive or invalid, individual samples were tested as follows: 400 μL of 1 sample was added to 800 μL diluted phosphate-buffered saline (hereafter called unpooling). After vortexing, 1 mL was transferred into the cartridge and analyzed. Sample processing was performed in a laminar flow cabinet.

The CT/NG Xpert assay is not labeled for use on pooled samples nor is it licensed to be used with samples collected with Eswabs. The use of Eswabs on the CT/NG Xpert assay was validated prior to the study, and no decrease in assay performance was detected (Supplemental Digital Content (SDC) 1, http://links.lww.com/OLQ/A497).

Criterion Standard Test Algorithm

All duplicate samples were tested individually at ITM according to the following test algorithm in place: CT/NG was detected using the Abbott RealTi*m*e (RT) CT/NG assay (Abbott Molecular, Des Plaines, IL) according to the manufacturer's instructions. DNA extracts of positive samples were tested by in-house RT-PCR assays for CT and/or NG, both based on previously published primer sets.[27,28] The in-house RT-PCR for CT is able to differentiate L-type from non-L types. A sample was considered positive when positive in both the Abbott and the in-house RT-PCR. An initial positive Abbott assay result followed by a negative confirmatory NAAT result was defined as "not confirmed." Inhibition according to the Abbott assay was defined as "inhibition." The Abbott RT CT/NG assay and the in-house RT-PCRs were validated for the use of extragenital samples using Eswabs and no decrease in sensitivity was found (SDC 1, http://links.lww.com/OLQ/A497).

To exclude for sampling errors and confirm the quality of the sample, the presence of human material in the duplicate sample

tested at ITM was assessed using a human Endogenous Retrovirus-3 PCR on anorectal and pharyngeal samples that were solely positive on site.[29]

Identification and Validation of the 2 Pooling Strategies

Two different on-site pooling strategies were evaluated. Strategy 1 consisted of triple-site pooling and testing, and unpooling only when the pooled sample result was invalid. Strategy 2 consisted of triple-site pooling and testing, and unpooling when the pooled sample result was invalid or positive for either CT or NG.

The result of the 2 strategies was compared with the infection status according to the criterion standard. A participant was defined as not infected when his 3 samples were all negative according to the criterion standard. A participant was defined as infected if at least 1 sample was positive. In the event that 1 or more sampling site(s) were not confirmed and the other sampling site(s) were negative, the participant infection status was defined as not confirmed.

Statistical Analysis

The sensitivity, specificity, positive predictive value, negative predictive value, with 95% confidence intervals were calculated for strategies 1 and 2, excluding inhibited and not confirmed infection status. In addition, agreement of both strategies with the criterion standard test algorithm was assessed by the Cohen's kappa statistic.

All analyses were performed in STATA V15.0.

Cost Analysis

The costs of the 2 different screening strategies were compared against triple-site testing using the GeneXpert CT/NG assay. The obtained prevalence of CT/NG in this study was used to simulate the costs of STI testing in a population of 500 MSM. An invalid rate of 4% was assumed.

RESULTS

Patient Characteristics, Test Results, and Prevalence of CT/NG

The ITM received baseline samples from 503 CohMSM-PrEP study participants. However, because the pooling method was not performed on 6 participants' samples, samples from 497 participants were included in the analysis.

All participants were MSM, with a median age of 24 years (interquartile range, 22–28).

Prevalence of STIs According to the Criterion Standard Algorithm

According to the criterion standard test algorithm performed at ITM, the study population had a prevalence of 14.5% CT (72 of 497), 11.5% NG (57 of 497), and 22.1% CT or NG (110 of 497). The anorectal site was the most commonly infected with CT or NG (n = 76; 60.8%); followed by the urethra (n = 28; 22.4%) and pharynx (n = 21; 16.8%). Two participants were positive in 2 biological sites for CT and 11 for NG. All confirmed CT-positive samples were non-L genotypes.

Of the 110 infected participants, 97 (88.2%) of them reported no symptoms of STI.

Test Results at the Study Sites

Using pooled samples 131 participants were positive for CT or NG: 21 had a mixed CT/NG infection; 71 were solely CT-infected; and 39 solely NG-infected. A total of 353 participants tested negative and were not further investigated. An invalid result was obtained in 13 participants.

Using strategy 1, 4 additional NG infected participants were detected by unpooling the pools with an invalid result.

Using strategy 2, individual testing of the pooled samples with invalid or positive results decreased the number of infected participants with CT or NG to 128: 26 with a dual CT/NG infection, 60 with CT only and 42 with NG only.

The CT/NG results obtained on site (pooled and unpooled samples) and the results obtained at ITM are available in the SDC 2 Figure 1, http://links.lww.com/OLQ/A498.

Test and Sample Quality

The study sites participated in a quarterly EQC: 1 NG-positive sample was missed; no false-positive results were reported.

The number of samples positive for CT/NG, whatever the biological site, at the study sites was systematically higher as compared with the numbers found at ITM (SDC 2 Fig. 1, http://links.lww.com/OLQ/A498). An environmental check revealed the contamination of the GeneXpert instrument's surface with CT at 1 site. The contamination is probably the cause of overreporting CT in this site. Another site had a large number of falsely detected NG; however, contamination with NG was not detected during the quarterly EQC assessments and the environmental check.

The presence of human DNA was assessed in 43 of 46 individual extragenital samples and in 11 (9 anorectal and 2 pharyngeal) (25.6%) samples, human DNA was not detected.

Performance of the 2 Pooling Strategies Using the GeneXpert Method

Samples from the CT contaminated site collected after August 31, 2018, were excluded from statistical analyses, which limited the number of participants to 448. The SDC 2, http://links.lww.com/OLQ/A498 documents all discordant cases (SDC 2 Tables 1 and 2, http://links.lww.com/OLQ/A498).

The evaluation of the 2 pooling strategies using the GeneXpert against the criterion standard for CT and NG is presented in Tables 1 and 2.

Chlamydia trachomatis

According to strategy 1, 3 CT were missed, resulting in a sensitivity of 95.4%. The Abbott delta cycle values (difference in cycle numbers between the cutoff control and the sample cycle number) of the individual specimens from 2 discordant pools were low, which correlates with a low target concentration. *Chlamydia trachomatis* was falsely detected on site in 5 participants (specificity, 98.7%). Applying strategy 2, the sensitivity decreased to 92.3% and the specificity increased to 99.2%.

Neisseria gonorrhoeae

Six NG infections were missed with strategy 1, yielding a sensitivity of 88.9%. The Abbott delta cycle values for the individual specimens included in the 6 pools indicated a high bacterial load.

Using strategy 1, 9 samples were positive but not confirmed by the criterion standard algorithm (specificity, 97.7%). When applying the second strategy, 1 sample was actually negative, however, 5 additional tests were positive, almost all from 1

TABLE 1. Comparison of the 2 Test Strategies to Detect CT and NG

Organism				Criterion Standard Test Algorithm			
	Strategy 1	GeneXpert		Positive	Negative	Not confirmed	Total
CT			Positive	62	**5**	3	70
			Negative	**3**	372	3	378
			Total	65	377	6	448
	Strategy 2	GeneXpert	Positive	60	**3**	3	66
			Negative	**5**	374	3	382
			Total	65	377	6	448
NG	Strategy 1	GeneXpert	Positive	48	**9**	3	60
			Negative	**6**	381	1	388
			Total	54	390	4	448
	Strategy 2	GeneXpert	Positive	48	**13**	3	64
			Negative	**6**	377	1	384
			Total	54	390	4	448

Strategy 1 will only test the samples individually when the pooled sample result was invalid. Strategy 2 will test the samples individually when the pooled sample result was invalid or positive for either CT or NG. The discordant samples are explained in detail in the Supplementary material.

study site, suggesting a possible DNA contamination (specificity, of 96.7%).

Cost Analysis

A prevalence of 22% CT/NG, as found in this study, was assumed. Compared with triple testing, a 56% decrease in costs was noted with strategy 1 and 30% with strategy 2 (Table 3).

DISCUSSION

We are among the first to report on the prevalence of chlamydia and gonorrhea in MSM initiating PrEP in West Africa. The data indicate a high prevalence of chlamydia (14.5%) and gonorrhea (11.5%), mainly in asymptomatic (88.2%) individuals. These asymptomatic infections would not have been treated according to the syndromic approach, which is currently the standard of care in LMIC. In addition, 77.6% of infections were extragenital. These findings reinforce the recommendation that STI services, including triple-site testing, should be integrated in PrEP programs in LMIC. Therefore, we aimed to implement an STI screening strategy using the GeneXpert instrument as its availability throughout Africa will facilitate STI testing. The Xpert CT/NG assay is now put forward as a potential point-of-care assay for STI detection in remote health care settings as it is easy, robust, and has very high analytical performance.[30–32] Previous studies showed that this technology is acceptable in identifying STIs among Sub-Saharan African young women, however, to date, no study has been performed among African MSM.[32–37]

We used a new pooling method with the GeneXpert platform to screen for STIs in genital and extragenital samples among MSM. Although the pooling method was designed as such to identify the origin of infection, we showed that there is no clinical utility. First of all, we did not detect a single case of Lymphogranuloma venereum (LGV) in our study population. LGV is frequently detected in European MSM and requires a 3-week treatment with doxycycline versus 1 week in the event of a regular chlamydia infection. Secondly, all antimicrobials recommended nowadays for the treatment of gonorrhea are equally effective in the 3 biological sites.,[19,20,38] Furthermore, the performance of the pooling method did not improve when unpooling positive samples. Nevertheless, we favor keeping the possibility to test the individual samples in case of an invalid pooled sample result to avoid additional sample collection and subsequent delay in result reporting. Using this strategy would decrease the testing cost with 56% compared with triple-site testing and would further decrease the burden on the laboratory's workload.

Applying strategy 1 resulted in 9 participants not receiving treatment (9 [2.0%] of 448), and 14 participants receiving unnecessary treatment (14 [3.1%] of 448). The number of false positives increased to 16 for the individually tested samples, mainly caused by a probable contamination of NG at one of the sites. On the other hand, the Xpert CT/NG assay can detect as little as 10 NG genome copies per reaction.[32] We cannot exclude the idea that some of the positive NG results solely obtained with the GeneXpert were truly low positive results not detectable by the criterion standard.

However, we also report on a CT DNA contamination of the GeneXpert instrument's surface at another site. The GeneXpert method is a closed system that reduces contamination to an absolute minimum, yet, due to its very low lower limit of detection, the assay is more prone to target contamination caused by the presence of genetic targets in the work environment or by sample cross contamination.

Although the GeneXpert CT/NG assay can be integrated into remote health care settings, this apparent risk of contamination may lead to erroneous results, which may cause emotional

TABLE 2. Evaluation of the 2 Pooling Strategies Against the Criterion Standard Algorithm for Either CT or NG

		Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	% Agreement	K-coeff
Chlamydia	Strategy 1 (unpooling of INV)	95.4% (87.1–99.0)	98.7% (96.9–99.6)	92.5% (83.8–96.7)	99.2% (97.6–99.7)	98.2%	0.93
	Strategy 2 (unpooling of INV and POS)	92.3% (83.0–97.5)	99.2% (97.7–99.8)	95.2% (86.7–99.0)	98.7% (97.0–99.6)	98.2%	0.93
Gonorrhea	Strategy 1 (unpooling of INV)	88.9% (77.4–95.8)	97.7% (95.7–98.9)	84.2% (73.5–91.1)	98.5% (96.8–99.3)	96.6%	0.85
	Strategy 2 (unpooling of INV and POS)	88.9% (77.4–95.8)	96.7% (94.4–98.2)	78.7% (66.2–86.4)	98.4% (96.7–99.3)	95.7%	0.81

INV, invalid; POS, positive; PPV, positive predictive value; NPV, negative predictive value; K-coeff, kappa-coefficient; CI, confidence interval.

TABLE 3. Number of Tests Required According Different Strategies and Cost Simulation Using a Combined Prevalence of CT/NG of 22%

STI Screening Strategies	No. Participants	Calculation of No. Tests Required	Total No. Tests	Assay Cost (US $19 Per Test)	Cost Per Infection
Triple site testing	500	Every site of sampling = 500 × 3	1500	US $ 28,500	US $ 220
Pooling strategy 1	500	500 pooled samples = 500 Individual testing of 20 invalid results = 20 × 3	560	US $ 10,640	US $ 97
Pooling strategy 2	500	500 pooled samples = 500 Individual testing of 20 invalid results = 20 × 3 Individual testing of 110 positive results = 110 × 3	890	US $ 16,910	US $ 154

distress, stigma and unnecessary antibiotic pressure. Therefore, we strongly recommend that detection of CT/NG using the GeneXpert instrument is performed under the supervision of qualified laboratory personnel and regular environmental control is integrated into a quality assurance program.

This is, to our knowledge, the first study reporting on a sample pooling method among a large number of African MSM initiating PrEP. Other pooling methods in MSM have been published, including methods using the GeneXpert assay.[21–26] Our results are in accordance with previously described studies that reported a reduced sensitivity for CT (90–94%) and/or NG (89.7–91.7%). Using our pooling method, sensitivity for CT and NG also decreased (95.4% and 88.9% respectively). A possible explanation for these reduced sensitivities is that pooling of samples will additionally dilute samples with low CT or NG bacterial load. To decrease the risk of over dilution, we opted to work with simple urine containers and Eswabs (1 mL transport medium) whereas most pooling methods elute the swabs in a transport medium volume of over 1 mL.

Furthermore, using Eswabs, our pooling method can tackle one of the most important global health priorities set forward by the WHO, namely the surveillance for antimicrobial resistance of NG.[39] Future research will need to show if surveillance for AMR of NG using Eswabs can be implemented in LMIC.

Our study design did not include a direct comparison of pooled and individual sample testing using the GeneXpert assay. Therefore, negative pools were not retested. In addition, the reduced sensitivities may be further explained by the use of Eswabs which are not licensed for use in both molecular techniques, however the use of Eswabs was evaluated on both assays prior to study start. Furthermore, samples tested with the criterion standard algorithm underwent an additional freeze-thawing cycle which may have impaired the DNA in low concentration samples. Finally, even though physicians were trained in sample collection to avoid sampling errors, the present study showed that human DNA was lacking in one quarter of the anorectal and pharyngeal samples which were positive solely using GeneXpert. This error may be due to the fact that 2 samples of each anatomical site were requested. This finding can further clarify the discordant results found in the study.

In conclusion, we showed that MSM initiating PrEP in Africa have high prevalence rates of extragenital and asymptomatic STIs and that African countries can perform an etiological diagnosis of STIs without implementing specialized NAATs.

The availability of PrEP in LMIC is a unique opportunity to strengthen STI services in high risk populations. The momentum is now to move to efficient STI screening and to limit onward transmission. In this new PrEP-era, the WHO, ministries of health and stakeholders at a global level will need to ensure that STI management is integrated in PrEP services, and negotiations with companies to provide the tests at affordable prices are, therefore, essential.

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