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Diagnostic accuracy of Xpert CT/NG for point-of-care STI testing amongst young women in South Africa

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SHORT REPORT

Title: Diagnostic accuracy of Xpert CT/NG for point-of-care STI testing amongst young women in South Africa

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Keywords: Sexually transmitted infections, Point-of-care testing, Xpert CT/NG, South Africa

Running title: Diagnostic accuracy of POC Xpert CT/NG in South Africa

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Abstract

Objectives: Syndromic management of sexually transmitted infections (STIs) omits asymptomatic infections, particularly among women. Accurate point-of-care (POC) assays may improve STI care in low- and middle-income countries (LMICs). We aimed to evaluate the diagnostic performance of the Xpert *Chlamydia trachomatis*/*Neisseria gonorrhoeae* (CT/NG) as part of a STI care model for young women in South Africa.

Methods: We recruited 267 young women attending a public STI clinic into a cohort study (CAPRISA 083). We evaluated two POC tests, the Xpert CT/NG as well as the OSOM Trichomonas (TV) Rapid, against the laboratory-based Anyplex™ II STI-7 Detection, a multiplex real-time PCR assay. All discordant results were further tested on a third assay, the FTD STD9.

Results: We obtained vaginal swabs from 247 women and found 96.4% (238/247) concordance between Xpert and Anyplex for CT and 99.6% (246/247) for NG. All nine discrepant CT results were positive on Xpert, but negative on Anyplex. FTD STD9 confirmed three positive and six negative results, giving a confirmed prevalence of CT 15.0% (95% CI 10.5-19.4), NG 4.9% (2.2-7.5) and TV 3.2% (1.0-5.4). Sensitivity and specificity of Xpert CT/NG were 100% (100-100) and 97.1% (94.9-99.4) for CT and 100% (100-100) and 99.6% (98.7-100) for NG. The sensitivity and specificity of OSOM TV was 75.0% (45.0-100) and 100% (100-100).

Conclusion: The Xpert CT/NG showed high accuracy among young South African women in this high HIV/STI burden setting. Further implementation and cost-effectiveness studies are needed to assess the potential role of this assay for diagnostic STI testing in LMICs.

Study registration: www.clinicaltrials.gov under study number NCT03407586

Strength and limitation of this study

- This is the first evaluation of the Xpert CT/NG point-of-care (POC) assay to detect chlamydia and gonorrhoea from a low- and middle-income country that we are aware of.
- Study participants were young South African women, who are at highest risk of sexually transmitted infections (STIs) and HIV in Africa, and have been prioritized for diagnostic STI testing and treatment by the World Health Organization.
- The limitations of our study were that it was conducted at a single site, only among women, and had a relatively small sample size (N=247).

Introduction

The World Health Organization (WHO) estimates that 357 million new cases of four curable sexually transmitted infections (STIs), *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV) and *Treponema pallidum* occur annually among people aged 15-49 years, with 63 million of them in Africa (1). These STIs are responsible for foetal and neonatal deaths, pelvic inflammatory disease resulting in ectopic pregnancies, chronic pelvic pain and infertility (2); and are major risk factors for HIV infection, increasing transmission risk by 2-3 fold (3). In addition, among women, up to 80% of STIs are asymptomatic (4), and therefore remain undiagnosed by the standard syndromic management approach adopted by many low- and middle-income countries (LMICs). Recent WHO and South African guidelines recommend the introduction of diagnostic testing for high-risk populations (1, 5). However, the best diagnostic assays to use in LMIC settings like South Africa are unknown.

In high-income countries, nucleic acid amplification tests (NAAT) are recommended and widely used for the detection of CT and NG. Cheaper and faster diagnostic technologies are being developed and provide an opportunity to design diagnostic STI care models for LMICs. One of these assays is the point-of-care (POC) Xpert CT/NG performed on the GeneXpert System (Cepheid, Sunnydale, California, US), a real-time PCR test for the rapid detection of CT and NG, which was US FDA-cleared in 2012 and received the European CE mark in 2016. This assay may be particularly relevant to the South African setting, because more than 4000 GeneXpert modules have already been placed in public healthcare settings for the rapid diagnosis of Tuberculosis (6). Potentially, this existing infrastructure could be expanded to form pilot sites for diagnostic STI care serving high-risk groups, such as young or pregnant women, sex workers and men who have sex with men. However, while some studies have evaluated the performance of Xpert CT/NG in high-income countries (7, 8), we are not aware of any studies from LMICs, where the need is greatest.

Therefore, the aim of this study was to evaluate the POC Xpert CT/NG assay in young women presenting to an urban primary healthcare clinic in South Africa.

Methods

Study design, setting and population

The CAPRISA 083 prospective cohort study was conducted at a large public healthcare clinic in Durban, South Africa between May 2016 and January 2017 and was previously described in detail (9). Briefly, the study evaluated a clinic-based STI care model comprising of POC STI testing, immediate treatment, and expedited partner therapy for young women at high HIV risk. Non-pregnant, HIV-negative women, aged 18–40 years, attending for sexual and reproductive services were eligible, and once consented, were enrolled consecutively into the study. Ethical approval was granted by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal in Durban, South Africa (BFC410/15).

Evaluation of POC STI assays

At enrolment, a nurse with experience in sexual health collected two vaginal swabs for POC testing on the Xpert CT/NG and the OSOM TV assays (Sekisui, Lexington, MA, US), and one Eswab™ (Copan, Brescia, Italy) specimen, which was sent to the regional National Health Laboratory Services reference laboratory for parallel testing on the Anyplex II STI-7 Detection assay (Seegene, Seoul, Korea). All POC tests were processed by laboratory technologists with experience using the GeneXpert platform at the clinic laboratory, but no access to participant clinical data. Reference laboratory staff were blinded to the POC test results and had no access to participant clinical data. Any discordant results comparing the Xpert CT/NG and OSOM TV against the Anyplex II STI-7 assay were retested on a third multiplex real-time PCR assay, the FTD STD9 (Fast Track Diagnostics, Silema, Malta). Positive result cut-offs for all assays were pre-specified by the manufacturers.

Data analysis

Clinic laboratory data were collected and managed using REDCap electronic data capture tools (Vanderbilt University, Nashville, TN, US), checked for internal validity and analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC, US). Reference laboratory results were imported and analysed at the end of the CAPRISA 083 study. Diagnostic accuracy of the assays was measured by calculating sensitivity, specificity, positive and negative predictive values (PPV, NPV) and 95% confidence intervals using the Wald method.

Patient and Public Involvement

The syndromic STI management approach in South Africa often leaves women untreated and return to clinics with recurrent symptoms. Furthermore, partner notification and treatment services are inadequate. These experiences by patients led to the study design, and the implementation of POC testing and expedited partner therapy in the clinic. Patients

were encouraged to participate in focus group discussions to explore the intervention, and to provide feedback to the study team (9).

Results

A total of 267 women with median age 23 years (interquartile range 21–26) enrolled into the study, and 23.6% (63/267) were diagnosed with at least one of the three STIs (CT, NG or TV) using Xpert CT/NG and OSOM TV POC testing at the clinic. We obtained vaginal Eswab™ specimen from 247/267 women for the evaluation at the reference laboratory. The 20 women not included in the evaluation either did not provide consent for sample storage (N=11), were menstruating (N=6), had an invalid Xpert CT/NG result (N=2) or did not have a test processed in the reference laboratory (N=1). The confirmed prevalence among the 247 women evaluated was 15.0% (95%CI 10.5–19.4) for CT, 4.9% (2.2–7.5) for NG and 3.2% (1.0–5.4) for TV. The sensitivity, specificity, PPV and NPV of Xpert CT/NG and OSOM TV assays are shown in Table 1. Overall we found 96.4% (238/247) concordance between the Xpert and Anyplex for CT and 99.6% (246/247) for NG. All nine discrepant CT results were positive on Xpert, but negative on Anyplex. Testing on FTD STD9 confirmed three positive and six negative results. The one discordant case for NG, was positive on Xpert testing but negative on Anyplex and FTD STD9. The concordance between OSOM TV and Anyplex was 99.2% (245/247) with two discordant cases undetected on the OSOM TV assay, but positive on confirmatory testing. Most participants (265/267, 99.3%) received their POC results on the day of sampling, and were offered immediate treatment, if indicated.

Table 1: Evaluation of the Xpert CT/NG and OSOM TV against the Anyplex II STI-7 Detection and FTD STD9 assays (N=247)

POC assay		Anyplex II STI-7 Detection +/- FTD STD9		Accuracy with 95% confidence interval (95% CI)
		Positive	Negative	
Xpert CT	Positive	37	6	Sensitivity = 100% (100 - 100) Specificity = 97.1% (94.9 - 99.4) PPV = 86.1% (75.7 - 96.4) NPV = 100% (100 - 100)
	Negative	0	204	
Xpert NG	Positive	12	1	Sensitivity = 100% (100 - 100) Specificity = 99.6% (98.7 - 100) PPV = 92.3% (77.8 - 100) NPV = 100% (100 - 100)
	Negative	0	234	
OSOM TV	Positive	6	0	Sensitivity = 75.0% (45.0 - 100) Specificity = 100% (100 - 100) PPV = 100% (100 - 100) NPV = 99.2% (98.0 - 100)
	Negative	2	239	

Discussion

The aim of the study was to evaluate the performance of the Xpert CT/NG, as well as the OSOM TV, within a clinic-based diagnostic care model to rapidly detect CT, NG and TV in a high STI/HIV burden setting in South Africa. The Xpert CT/NG performed well with a high sensitivity and specificity to diagnose CT and NG, while the OSOM TV was limited by a moderate sensitivity, as shown in previous studies. Taken together, these assays could allow diagnosis and management of STIs in one clinical visit.

Our findings are consistent with a study of 1722 women and 1387 men from the US, which found a consistently high diagnostic performance of the Xpert CT/NG testing cervical and vaginal swabs from women, and urine from men and women (7). Sensitivity and specificity of the assay using vaginal swabs was 98.7% and 99.4% for CT and 100% and 99.9% for NG, which was only marginally superior to urine testing in women (97.6%, 99.8% for CT and 95.6%, 99.9% for NG). Similar to our evaluation, this study found a lower PPV of the assay for both CT (88.6%) and NG (91.7%) when using vaginal swabs in their population, while PPVs for urine testing were higher (96.4% and 95.6%). Taken together, these results indicate that urine samples may be adequate for Xpert CT/NG testing in women, and may prevent unnecessary treatment, especially in populations with lower CT prevalence.

Recently, the POC Xpert CT/NG was evaluated as part of a large cluster randomized study in remote community health services in Australia. In keeping with our findings, the assay demonstrated a high sensitivity and specificity when performed by nurses and community health workers compared to conventional NAATs for both CT (98.6%, 99.5%) and NG (100%, 99.9%) using either urine samples or vaginal swabs. The authors conclude that this POC STI assay may be particularly suitable for LMICs, where resources are limited and infrastructure is often poor (8).

We decided to complement the Xpert CT/NG testing with the OSOM TV antigen detection assay in order to provide the participants with a comprehensive 2-hour STI testing alternative to syndromic management (9). As previously reported, the OSOM TV assay showed lower sensitivity, but good specificity to detect TV. The advantage of this assay is that it is relatively cheap, has a rapid processing time, and higher accuracy than wet-prep microscopy, especially in women (10).

The limitations of our study were that it was conducted at one site, only among young women, and had a relatively low sample size. Nevertheless, to the best of our knowledge, we report the first clinic evaluation of the Xpert CT/NG assay from a LMIC. Furthermore, we

decided to focus on young women, because this population group has been prioritized for diagnostic STI care in WHO and South African guidelines (1, 5), and remains at greatest risk of HIV acquisition in LMICs.

In conclusion, we found the Xpert CT/NG to be accurate when used at the point of care in a LMIC clinic. Larger implementation studies are required to assess whether the introduction of POC STI testing could be cost-effective, and eventually replace the syndromic management approach in South Africa. In the meantime, it seems prudent to prioritize diagnostic STI care for high-risk populations as part of HIV prevention efforts.

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Contributors: NG, AR and AM are the co-principal investigators, conceived the study and wrote the study protocol. NG, JD, HN recruited the cohort. NG, NM, JN, RS and KM conducted the laboratory evaluation. NG, FO, JD, AM performed the statistical analysis. All authors contributed to the manuscript and consented to final publication.

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Competing interests: Cepheid Inc loaned two 4-module GeneXpert machines to the study team free-of-charge, but had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data sharing statement: Extra data is available by emailing the authors at nigel.garrett@caprisa.org. The study protocol is accessible at https://journals.plos.org/plosone/article/file?type=supplementary&id=info:doi/10.1371/journal.pone.0196209.s004

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Section & Topic	No	Item	Reported on page #
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	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
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	3	Scientific and clinical background, including the intended use and clinical role of the index test	3
	4	Study objectives and hypotheses	3
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	8	Where and when potentially eligible participants were identified (setting, location and dates)	4
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	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	4
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DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	7, 8
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	30	Sources of funding and other support; role of funders	Manuscript and online submission system

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

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Diagnostic accuracy of the Xpert CT/NG and OSOM Trichomonas Rapid assays for point-of-care STI testing among young women in South Africa: a cross-sectional study

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SHORT REPORT

Title: Diagnostic accuracy of the Xpert CT/NG and OSOM Trichomonas Rapid assays for point-of-care STI testing among young women in South Africa: a cross-sectional study

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Keywords: Sexually transmitted infections, Point-of-care testing, Xpert CT/NG, South Africa

Running title: Diagnostic accuracy of POC Xpert CT/NG in South Africa

Word count: Abstract 293; Text 2197; 1 Table; 1 Figure

Abstract

Objectives: Syndromic management of sexually transmitted infections (STIs) omits asymptomatic infections, particularly among women. Accurate point-of-care (POC) assays may improve STI care in low- and middle-income countries (LMICs). We aimed to evaluate the diagnostic performance of the Xpert *Chlamydia trachomatis*/*Neisseria gonorrhoeae* (CT/NG) and OSOM Trichomonas (TV) Test as part of a STI care model for young women in South Africa.

Design: Diagnostic evaluation conducted as part of a prospective cohort study (CAPRISA 083) between May 2016 and January 2017.

Setting: One large public health care facility in central Durban, KwaZulu-Natal, South Africa

Participants: 247 women, aged 18–40 years, attending for sexual and reproductive services to the clinic. Pregnant and HIV-positive women were excluded.

Outcomes: Diagnostic performance of the Xpert CT/NG and OSOM TV assays against the laboratory-based Anyplex™ II STI-7 Detection. All discordant results were further tested on the Fast Track Diagnostics (FTD) STD9 assay.

Results: We obtained vaginal swabs from 247 women and found 96.8% (239/247) concordance between Xpert and Anyplex for CT and 100% (247/247) for NG. All eight discrepant CT results were positive on Xpert, but negative on Anyplex. FTD STD9 confirmed three positive and five negative results, giving a confirmed prevalence of CT 15.0% (95% CI 10.5-19.4), NG 4.9% (2.2-7.5) and TV 3.2% (1.0-5.4). Sensitivity and specificity of Xpert CT/NG were 100% (100-100) and 97.6% (95.6-99.7) for CT and 100% (100-100) and 100% (100-100) for NG. The sensitivity and specificity of OSOM TV were 75.0% (45.0-100) and 100% (100-100).

Conclusion: The Xpert CT/NG showed high accuracy among young South African women and combined with the OSOM TV proved a useful tool in this high HIV/STI burden setting. Further implementation and cost-effectiveness studies are needed to assess the potential role of this assay for diagnostic STI testing in LMICs.

Study registration: www.clinicaltrials.gov under study number NCT03407586

Strength and limitation of this study

- This is the first evaluation of the diagnostic performance of the Xpert CT/NG point-of-care (POC) assay to detect chlamydia and gonorrhoea from a low- and middle-income country.
- Study participants were young South African women, who are at highest risk of sexually transmitted infections (STIs) and HIV in Africa, and have been prioritized for diagnostic STI testing and treatment by the World Health Organization.
- The limitations of our study were that it was conducted at a single site, only among women, and had a relatively small sample size (N=247).

Introduction

The World Health Organization (WHO) estimates that 357 million new cases of four curable sexually transmitted infections (STIs), *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV) and *Treponema pallidum* occur annually among people aged 15-49 years, with 63 million of them in Africa (1). These STIs are responsible for foetal and neonatal deaths, pelvic inflammatory disease resulting in ectopic pregnancies, chronic pelvic pain and infertility (2); and are major risk factors for HIV infection, increasing transmission risk by 2-3 fold (3). In addition, among women, up to 80% of STIs are asymptomatic (4), and therefore remain undiagnosed by the standard syndromic management approach adopted by many low- and middle-income countries (LMICs). Recent WHO and South African guidelines recommend the introduction of diagnostic testing for high-risk populations (1, 5). However, the best diagnostic assays to use in LMIC settings like South Africa are unknown.

In high-income countries, nucleic acid amplification tests (NAAT) are recommended and widely used for the detection of CT and NG. Cheaper and faster diagnostic technologies are being developed and provide an opportunity to design diagnostic STI care models for LMICs. One of these assays is the point-of-care (POC) Xpert CT/NG performed on the GeneXpert System (Cepheid, Sunnydale, California, US), a real-time PCR test for the rapid detection of CT and NG, which was US FDA-cleared in 2012 and received the European CE mark in 2016. This assay may be particularly relevant to the South African setting, because more than 4000 GeneXpert modules have already been placed in public healthcare settings for the rapid diagnosis of Tuberculosis (TB) (6). Multi-disease testing for HIV viral load monitoring, early infant diagnosis and TB using the GeneXpert platform was found to be feasible in rural Zimbabwe (7). Potentially, the existing infrastructure could be expanded to form pilot sites for diagnostic STI care serving high-risk groups, such as young or pregnant women, sex workers and men who have sex with men. However, while some studies have evaluated the diagnostic performance of Xpert CT/NG in high-income countries (8, 9), we are not aware of any studies from LMICs, where the need is greatest.

Considering the relatively high cost of the Xpert *Trichomonas vaginalis* (TV) cartridge (~ USD 19.00), we decided to complement the Xpert CT/NG with the OSOM TV antigen detection assay (Sekisui, Lexington, MA, US) and Gram stain microscopy, in order to offer the participants a comprehensive 2-hour STI testing alternative to syndromic management (10). The advantage of the OSOM TV assay is that it is relatively cheap (~ USD 8.00), has a rapid processing time (~ 10 minutes), and has shown higher accuracy than wet mount microscopy, especially in women (11, 12).

Therefore, the aim of this study was to evaluate the POC Xpert CT/NG and OSOM TV assays in young women presenting to an urban primary healthcare clinic in South Africa.

Methods

Study design, setting and population

The CAPRISA 083 prospective cohort study was conducted at a large public healthcare clinic in Durban, South Africa between May 2016 and January 2017 and was previously described in detail (10). Briefly, the study evaluated a clinic-based STI care model comprising of POC STI testing, immediate treatment, and expedited partner therapy (EPT) for young women at high HIV risk. Non-pregnant, HIV-negative women, aged 18–40 years, attending for sexual and reproductive services were eligible, and once consented, were enrolled consecutively into the study. Women diagnosed with CT, NG or TV based on POC testing were offered immediate supervised treatment with single dose antibiotics on the same visit. Treatment regimens followed international guidelines, and were compatible with national guidelines: Ceftriaxone 250mg intramuscular and Azithromycin 1g oral for NG, Azithromycin 1g oral for CT, and Metronidazole 2g oral for TV (13, 14). Ethical approval was granted by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal in Durban, South Africa (BFC410/15).

Evaluation of POC STI assays

At enrolment, a nurse with experience in sexual health collected two vaginal swabs for POC testing on the Xpert CT/NG and the OSOM TV assays, and one Eswab™ (Copan, Brescia, Italy) specimen, which was sent to the regional National Health Laboratory Services reference laboratory for DNA extraction and parallel testing on the Anyplex II STI-7 Detection assay (Seegene, Seoul, Korea) within 24 hours of sample collection, according to Clinical and Laboratory Standards Institute (CLSI) requirements. Considering that all participants received their results during the same visit and the tests were performed in the clinic, we used the term '*point-of-care' for both assays, in line with the following consensus definition: a 'point-of-care test…is a test to support clinical decision making, which is performed by a qualified…staff nearby the patient…during or very close to the time of consultation, to help the patient and physician to decide upon the best suited approach, and of which the results should be known at the time of the clinical decision making'* (15). All POC tests were processed according to manufacturers' specification (www.sekisuidiagnostics.com/products/130-osom-trichomonas-test and www.cepheid.com/us/cepheid-solutions/clinical-ivd-tests/sexual-health/xpert-ct-ng) by laboratory technologists with experience using the GeneXpert platform at the clinic laboratory, but no access to participant clinical data. Reference laboratory staff were blinded to the POC test results and had no access to participant clinical data. Any discordant results comparing the Xpert CT/NG and OSOM TV against the Anyplex II STI-7 assay were retested on a third multiplex real-time PCR assay, the FTD STD9 (Fast Track Diagnostics, Silema,

Malta). The Anyplex STI7 and FTD STD9 were chosen as confirmatory tests, because they are both CE marked and are commercially available in South Africa. For epidemiological purposes, these assays also provided the opportunity to determine the prevalence of sexually transmitted organisms not routinely screened for in surveillance studies. Positive result cut-offs for all assays were pre-specified by the manufacturers.

Data analysis

Clinic laboratory data were collected and managed using REDCap electronic data capture tools (Vanderbilt University, Nashville, TN, US), checked for internal validity and analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC, US). The sample size was pre-determined to assess the primary outcome of the CAPRISA 083 cohort study, which assessed the reduction in genital tract pro-inflammatory cytokines after POC testing, immediate treatment and EPT among women diagnosed with STIs. Reference laboratory results were imported and analysed at the end of the CAPRISA 083 study. Diagnostic accuracy of the assays was measured by calculating sensitivity, specificity, positive and negative predictive values (PPV, NPV) and 95% confidence intervals using the Wald method.

Patient and Public Involvement

Patients and the public were not involved in the design of and recruitment to the study. However, the syndromic STI management approach in South Africa often leaves women untreated and return to clinics with recurrent symptoms and partner notification and treatment services are inadequate. These experiences by patients led to the study design, and the implementation of POC testing and EPT in the clinic. Patients took part in focus group discussions and were able to provide feedback to the study team on their experiences with the POC STI testing model (9).

Results

A total of 267 women with median age 23 years (interquartile range 21–26) enrolled into the CAPRISA 083 study, and 23.6% (63/267) were diagnosed with at least one of the three STIs (CT, NG or TV) using Xpert CT/NG and OSOM TV POC testing at the clinic. We obtained vaginal Eswab™ specimen from 247/267 (92.5%) women for the diagnostic evaluation at the reference laboratory. The 20 women not included in the evaluation either did not provide consent for sample storage (N=11), were menstruating (N=6), had an invalid Xpert CT/NG result (N=2) or did not have a test processed in the reference laboratory (N=1). The study flow is illustrated in Figure 1.

The confirmed prevalence among the 247 women evaluated was 15.0% (95%CI 10.5–19.4) for CT, 4.9% (2.2–7.5) for NG and 3.2% (1.0–5.4) for TV. In addition, Anyplex testing revealed a 4.9% (2.2-7.5) prevalence of *Mycoplasma genitalium*, 33.6% (27.7-39.5) Mycoplasma hominis, 51.8% *Ureaplasma parvum* and 19.0% (14.1-23.9) *Ureaplasma urealyticum*. The sensitivity, specificity, PPV and NPV of Xpert CT/NG and OSOM TV assays are shown in Table 1. Overall we found 96.8% (239/247) concordance between the Xpert and Anyplex for CT and 100% (247/247) concordance for NG. All eight discrepant CT results were positive on Xpert, but negative on Anyplex. Testing on FTD STD9 confirmed three positive and five negative results. The Xpert cycle thresholds of the five discordant results reached 26.3 to 38.6 cycles, with two values greater than 38 cycles, indicating potential sampling or testing variation. The concordance between OSOM TV and Anyplex was 99.2% (245/247) with two discordant cases undetected on the OSOM TV assay, but positive on confirmatory testing. Most participants (265/267, 99.3%) received their POC results on the day of sampling, and were offered immediate treatment, if indicated.

Table 1: Evaluation of the Xpert CT/NG and OSOM TV against the Anyplex II STI-7 Detection and FTD STD9 assays (N=247)

POC assay		Anyplex II STI-7 Detection +/- FTD STD9		Accuracy with 95% confidence interval (95% CI)
		Positive	Negative	
Xpert CT	Positive	37	5	Sensitivity = 100% (100 - 100) Specificity = 97.6% (95.6 - 99.7) PPV = 88.1% (78.3 - 97.9) NPV = 100% (100 - 100)
	Negative	0	205	
Xpert NG	Positive	12	0	Sensitivity = 100% (100 - 100) Specificity = 100% (100 - 100) PPV = 100% (100 - 100) NPV = 100% (100 - 100)
	Negative	0	235	
OSOM TV	Positive	6	0	Sensitivity = 75.0% (45.0 - 100) Specificity = 100% (100 - 100) PPV = 100% (100 - 100) NPV = 99.2% (98.0 - 100)
	Negative	2	239	

Discussion

The aim of the study was to evaluate the performance of the Xpert CT/NG, as well as the OSOM TV, within a clinic-based diagnostic care model to rapidly detect CT, NG and TV in a high STI/HIV burden setting in South Africa. The Xpert CT/NG performed well with a high sensitivity and specificity to diagnose CT and NG, while the OSOM TV showed lower sensitivity, but high specificity. Taken together, these assays could allow diagnosis and management of STIs in one clinical visit.

Our findings are consistent with a study of 1722 women and 1387 men from the US, which found a consistently high diagnostic performance of the Xpert CT/NG testing cervical and vaginal swabs from women, and urine from men and women (8). Sensitivity and specificity of the assay using vaginal swabs was 98.7% and 99.4% for CT and 100% and 99.9% for NG, which was only marginally superior to urine testing in women (97.6%, 99.8% for CT and 95.6%, 99.9% for NG). Similar to our evaluation, this study found a lower PPV of the assay for both CT (88.6%) and NG (91.7%) when using vaginal swabs in their population, while PPVs for urine testing were higher (96.4% and 95.6%). This could indicate that urine samples may be adequate for Xpert CT/NG testing in women, and may prevent unnecessary treatment, especially in populations with lower CT prevalence.

In our study, two out of five 'false positive' Xpert CT results had a high cycle threshold above 38 cycles. A validation of 50 randomly selected vulvovaginal samples from a study of pregnant women in Pretoria, South Africa (16) also found two positive Xpert CT/NG results with high cycle thresholds that were confirmed negative after testing on the Presto^{Plus} CT/NG/TV (Microbiome, Ltd., Houten, The Netherlands) and Anyplex assays. This highlights that some caution is needed when interpreting high cycle threshold results, and that retesting may have to be considered.

Recently, the POC Xpert CT/NG was evaluated as part of a large cluster randomized study in remote community health services in Australia. In keeping with our findings, the assay demonstrated a high sensitivity and specificity when performed by nurses and community health workers compared to conventional NAATs for both CT (98.6%, 99.5%) and NG (100%, 99.9%) using either urine samples or vaginal swabs. The authors concluded that this POC STI assay may be particularly suitable for LMICs, where resources are limited and infrastructure is often poor (9).

We decided to complement the Xpert CT/NG testing with the OSOM TV antigen detection assay in order to provide the participants with a comprehensive 2-hour STI testing

alternative to syndromic management (10). Previous evaluations (11, 12) of the OSOM TV concur with our finding of a slightly lower sensitivity (92 – 98%) than PCR technology, but a consistently high specificity to detect TV (99%). The advantage of this assay is that it is relatively cheap, has a rapid processing time, and higher accuracy than wet-prep microscopy, especially in women (12).

The limitations of our study were that it was conducted at one site, only among young women, and had a relatively low sample size. We excluded pregnant women from the CAPRISA 083 cohort study, because they were referred for antenatal care, and were not an appropriate population to pilot the EPT intervention in. However, pregnant women may be an important population to offer POC testing to in the future (16, 17), and perhaps combine the testing model with a POC syphilis assay (18). A further limitation of our study was that specimens were not available to repeat discordant results on the Xpert CT/NG platform. Nevertheless, to the best of our knowledge, we report the first clinic evaluation of the diagnostic performance of the Xpert CT/NG assay from a LMIC. Furthermore, we decided to focus on young women, because this population group has been prioritized for diagnostic STI care in WHO and South African guidelines (1, 5) , and remains at highest risk of HIV acquisition in LMICs.

In conclusion, we found the Xpert CT/NG to be accurate when used at the point of care in a LMIC clinic, and it was complemented well by the OSOM TV assay. Larger implementation studies are required to assess whether the introduction of POC STI testing could be cost-effective, and eventually replace the syndromic management approach in South Africa. In the meantime, it seems prudent to prioritize diagnostic STI care for high-risk populations as part of HIV prevention efforts.

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Contributors: NG, AR and AM are the co-principal investigators, conceived the study and wrote the study protocol. NG, JD, HN recruited the cohort. NG, NM, JN, RS and KM conducted the laboratory evaluation. NG, FO, JD, AM performed the statistical analysis. All authors contributed to the manuscript and consented to final publication.

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Competing interests: Cepheid Inc loaned two 4-module GeneXpert machines to the study team free-of-charge, but had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data sharing statement: Extra data is available by emailing the authors at nigel.garrett@caprisa.org. The study protocol is accessible at https://journals.plos.org/plosone/article/file?type=supplementary&id=info:doi/10.1371/journal.pone.0196209.s004

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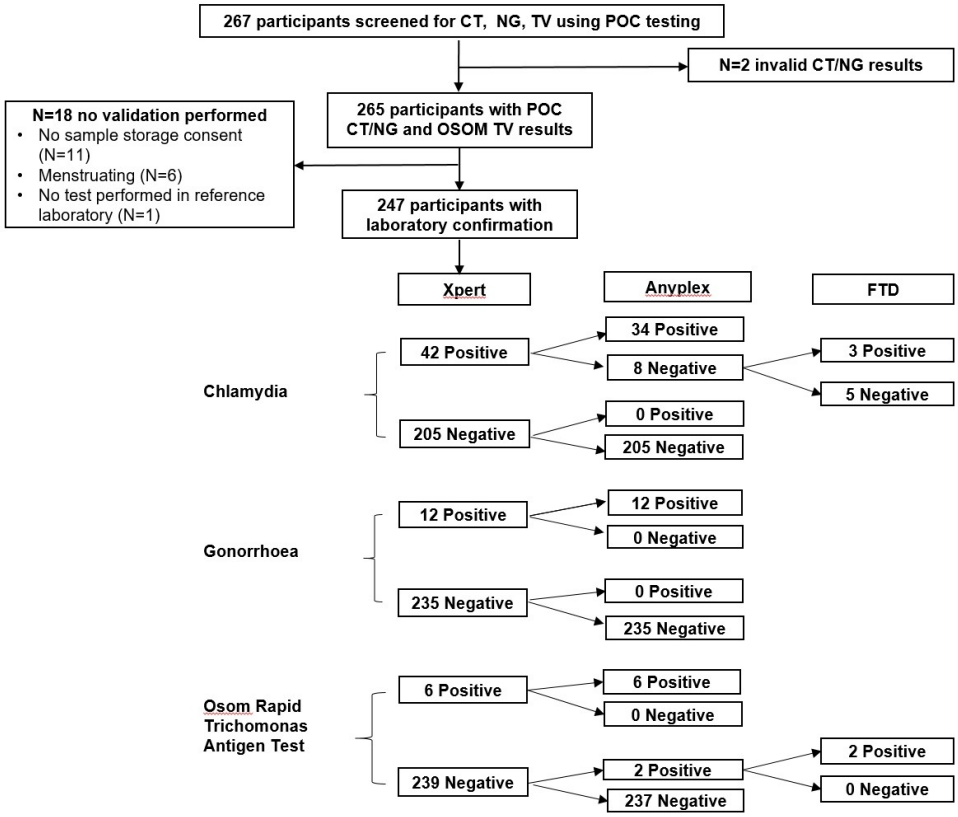

Figure 1: Study flow and results of the diagnostic evaluation of the Xpert CT/NG and Osom TV assays

183x152mm (168 x 168 DPI)

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4
	4	Study objectives and hypotheses	5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6
Participants	**6**	Eligibility criteria	6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	6
	9	Whether participants formed a consecutive, random or convenience series	6
Test methods	**10a**	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6

	13b	Whether clinical information and index test results were available to the assessors of the reference standard	6
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	7
	15	How indeterminate index test or reference standard results were handled	7
	16	How missing data on the index test and reference standard were handled	7
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	7
	18	Intended sample size and how it was determined	7
RESULTS			
Participants	**19**	Flow of participants, using a diagram	8, Figure 1
	20	Baseline demographic and clinical characteristics of participants	8
	21a	Distribution of severity of disease in those with the target condition	8
	21b	Distribution of alternative diagnoses in those without the target condition	8
	22	Time interval and any clinical interventions between index test and reference standard	8
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	9
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	9
	25	Any adverse events from performing the index test or the reference standard	8
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	11
	27	Implications for practice, including the intended use and clinical role of the index test	11
OTHER INFORMATION			
	28	Registration number and name of registry	Online submission system
	29	Where the full study protocol can be accessed	http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0196209
	30	Sources of funding and other support; role of funders	Manuscript and online submission system

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

BMJ Open

Diagnostic accuracy of the Xpert CT/NG and OSOM Trichomonas Rapid assays for point-of-care STI testing among young women in South Africa: a cross-sectional study

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Secondary Subject Heading:	HIV/AIDS, Public health, Global health, Infectious diseases
Keywords:	sexually transmitted infections, Point-of-care testing, Xpert CT/NG, South Africa, Molecular diagnostics < INFECTIOUS DISEASES

SHORT REPORT

Title: Diagnostic accuracy of the Xpert CT/NG and OSOM Trichomonas Rapid assays for point-of-care STI testing among young women in South Africa: a cross-sectional study

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Keywords: Sexually transmitted infections, Point-of-care testing, Xpert CT/NG, South Africa

Running title: Diagnostic accuracy of POC Xpert CT/NG in South Africa

Word count: Abstract 293; Text 2293; 1 Table; 1 Figure

Abstract

Objectives: Syndromic management of sexually transmitted infections (STIs) omits asymptomatic infections, particularly among women. Accurate point-of-care (POC) assays may improve STI care in low- and middle-income countries (LMICs). We aimed to evaluate the diagnostic performance of the Xpert *Chlamydia trachomatis*/*Neisseria gonorrhoeae* (CT/NG) and OSOM Trichomonas (TV) Test as part of a STI care model for young women in South Africa.

Design: Diagnostic evaluation conducted as part of a prospective cohort study (CAPRISA 083) between May 2016 and January 2017.

Setting: One large public health care facility in central Durban, KwaZulu-Natal, South Africa

Participants: 247 women, aged 18–40 years, attending for sexual and reproductive services to the clinic. Pregnant and HIV-positive women were excluded.

Outcomes: Diagnostic performance of the Xpert CT/NG and OSOM TV assays against the laboratory-based Anyplex™ II STI-7 Detection. All discordant results were further tested on the Fast Track Diagnostics (FTD) STD9 assay.

Results: We obtained vaginal swabs from 247 women and found 96.8% (239/247) concordance between Xpert and Anyplex for CT and 100% (247/247) for NG. All eight discrepant CT results were positive on Xpert, but negative on Anyplex. FTD STD9 confirmed three positive and five negative results, giving a confirmed prevalence of CT 15.0% (95% CI 10.5-19.4), NG 4.9% (2.2-7.5) and TV 3.2% (1.0-5.4). Sensitivity and specificity of Xpert CT/NG were 100% (100-100) and 97.6% (95.6-99.7) for CT and 100% (100-100) and 100% (100-100) for NG. The sensitivity and specificity of OSOM TV were 75.0% (45.0-100) and 100% (100-100).

Conclusion: The Xpert CT/NG showed high accuracy among young South African women and combined with the OSOM TV proved a useful tool in this high HIV/STI burden setting. Further implementation and cost-effectiveness studies are needed to assess the potential role of this assay for diagnostic STI testing in LMICs.

Study registration: www.clinicaltrials.gov under study number NCT03407586

Strength and limitation of this study

- This is the first evaluation of the diagnostic performance of the Xpert CT/NG point-of-care (POC) assay to detect chlamydia and gonorrhoea from a low- and middle-income country.
- Study participants were young South African women, who are at highest risk of sexually transmitted infections (STIs) and HIV in Africa, and have been prioritized for diagnostic STI testing and treatment by the World Health Organization.
- The limitations of our study were that it was conducted at a single site, only among women, and had a relatively small sample size (N=247).

Introduction

The World Health Organization (WHO) estimates that 357 million new cases of four curable sexually transmitted infections (STIs), *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV) and *Treponema pallidum* occur annually among people aged 15-49 years, with 63 million of them in Africa (1). These STIs are responsible for foetal and neonatal deaths, pelvic inflammatory disease resulting in ectopic pregnancies, chronic pelvic pain and infertility (2); and are major risk factors for HIV infection, increasing transmission risk by 2-3 fold (3). In addition, among women, up to 80% of STIs are asymptomatic (4), and therefore remain undiagnosed by the standard syndromic management approach adopted by many low- and middle-income countries (LMICs). Recent WHO and South African guidelines recommend the introduction of diagnostic testing for high-risk populations (1, 5). However, the best diagnostic assays to use in LMIC settings like South Africa are unknown.

In high-income countries, nucleic acid amplification tests (NAAT) are recommended and widely used for the detection of CT and NG. Cheaper and faster diagnostic technologies are being developed and provide an opportunity to design diagnostic STI care models for LMICs. One of these assays is the point-of-care (POC) Xpert CT/NG performed on the GeneXpert System (Cepheid, Sunnydale, California, US), a real-time PCR test for the rapid detection of CT and NG, which was US FDA-cleared in 2012 and received the European CE mark in 2016. This assay may be particularly relevant to the South African setting, because more than 4000 GeneXpert modules have already been placed in public healthcare settings for the rapid diagnosis of Tuberculosis (TB) (6). Multi-disease testing for HIV viral load monitoring, early infant diagnosis and TB using the GeneXpert platform was found to be feasible in rural Zimbabwe (7). Potentially, the existing infrastructure could be expanded to form pilot sites for diagnostic STI care serving high-risk groups, such as young or pregnant women, sex workers and men who have sex with men. However, while some studies have evaluated the diagnostic performance of Xpert CT/NG in high-income countries (8, 9), we are not aware of any studies from LMICs, where the need is greatest.

Considering the relatively high cost of the Xpert *Trichomonas vaginalis* (TV) cartridge (~ USD 19.00), we decided to complement the Xpert CT/NG with the OSOM TV antigen detection assay (Sekisui, Lexington, MA, US) and Gram stain microscopy, in order to offer the participants a comprehensive 2-hour STI testing alternative to syndromic management (10). The advantage of the OSOM TV assay is that it is relatively cheap (~ USD 8.00), has a rapid processing time (~ 10 minutes), and has shown higher accuracy than wet mount microscopy, especially in women (11, 12).

Therefore, the aim of this study was to evaluate the POC Xpert CT/NG and OSOM TV assays in young women presenting to an urban primary healthcare clinic in South Africa.

Methods

Study design, setting and population

The CAPRISA 083 prospective cohort study was conducted at a large public healthcare clinic in Durban, South Africa between May 2016 and January 2017 and was previously described in detail (10). Briefly, the study evaluated a clinic-based STI care model comprising of POC STI testing, immediate treatment, and expedited partner therapy (EPT) for young women at high HIV risk. Non-pregnant, HIV-negative women, aged 18–40 years, attending for sexual and reproductive services were eligible, and once consented, were enrolled consecutively into the study. Women diagnosed with CT, NG or TV based on POC testing were offered immediate supervised treatment with single dose antibiotics on the same visit. Treatment regimens followed international guidelines, and were compatible with national guidelines: Ceftriaxone 250mg intramuscular and Azithromycin 1g oral for NG, Azithromycin 1g oral for CT, and Metronidazole 2g oral for TV (13, 14). Ethical approval was granted by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal in Durban, South Africa (BFC410/15).

Evaluation of POC STI assays

At enrolment, a nurse with experience in sexual health collected two vaginal swabs for POC testing on the Xpert CT/NG and the OSOM TV assays, and one Eswab™ (Copan, Brescia, Italy) specimen, which was sent to the regional National Health Laboratory Services reference laboratory for DNA extraction and parallel testing on the Anyplex II STI-7 Detection assay (Seegene, Seoul, Korea) within 24 hours of sample collection, according to Clinical and Laboratory Standards Institute (CLSI) requirements. Considering that all participants received their results during the same visit and the tests were performed in the clinic, we used the term '*point-of-care' for both assays, in line with the following consensus definition: a 'point-of-care test…is a test to support clinical decision making, which is performed by a qualified…staff nearby the patient…during or very close to the time of consultation, to help the patient and physician to decide upon the best suited approach, and of which the results should be known at the time of the clinical decision making'* (15). All POC tests were processed according to manufacturers' specification (www.sekisuidiagnostics.com/products/130-osom-trichomonas-test and www.cepheid.com/us/cepheid-solutions/clinical-ivd-tests/sexual-health/xpert-ct-ng) by laboratory technologists with experience using the GeneXpert platform at the clinic laboratory, but no access to participant clinical data. Reference laboratory staff were blinded to the POC test results and had no access to participant clinical data. Any discordant results comparing the Xpert CT/NG and OSOM TV against the Anyplex II STI-7 assay were retested on a third multiplex real-time PCR assay, the FTD STD9 (Fast Track Diagnostics, Silema,

Malta). The Anyplex STI7 and FTD STD9 were chosen as confirmatory tests, because they are both CE marked and are commercially available in South Africa. For epidemiological purposes, these assays also provided the opportunity to determine the prevalence of sexually transmitted organisms not routinely screened for in surveillance studies. Positive result cut-offs for all assays were pre-specified by the manufacturers.

Data analysis

Clinic laboratory data were collected and managed using REDCap electronic data capture tools (Vanderbilt University, Nashville, TN, US), checked for internal validity and analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC, US). The sample size was pre-determined to assess the primary outcome of the CAPRISA 083 cohort study, which assessed the reduction in genital tract pro-inflammatory cytokines after POC testing, immediate treatment and EPT among women diagnosed with STIs. Reference laboratory results were imported and analysed at the end of the CAPRISA 083 study. Diagnostic accuracy of the assays was measured by calculating sensitivity, specificity, positive and negative predictive values (PPV, NPV) and 95% confidence intervals using the Wald method.

Patient and Public Involvement

Patients and the public were not involved in the design of and recruitment to the study. However, the syndromic STI management approach in South Africa often leaves women untreated and return to clinics with recurrent symptoms and partner notification and treatment services are inadequate. These experiences by patients led to the study design, and the implementation of POC testing and EPT in the clinic. Patients took part in focus group discussions and were able to provide feedback to the study team on their experiences with the POC STI testing model (9).

Results

A total of 267 women with median age 23 years (interquartile range 21–26) enrolled into the CAPRISA 083 study, and 23.6% (63/267) were diagnosed with at least one of the three STIs (CT, NG or TV) using Xpert CT/NG and OSOM TV POC testing at the clinic. We obtained vaginal Eswab™ specimen from 247/267 (92.5%) women for the diagnostic evaluation at the reference laboratory. The 20 women not included in the evaluation either did not provide consent for sample storage (N=11), were menstruating (N=6), had an invalid Xpert CT/NG result (N=2) or did not have a test processed in the reference laboratory (N=1). The study flow is illustrated in Figure 1.

The confirmed prevalence among the 247 women evaluated was 15.0% (95%CI 10.5–19.4) for CT, 4.9% (2.2–7.5) for NG and 3.2% (1.0–5.4) for TV. In addition, Anyplex testing revealed a 4.9% (2.2-7.5) prevalence of *Mycoplasma genitalium*, 33.6% (27.7-39.5) Mycoplasma hominis, 51.8% *Ureaplasma parvum* and 19.0% (14.1-23.9) *Ureaplasma urealyticum*. The sensitivity, specificity, PPV and NPV of Xpert CT/NG and OSOM TV assays are shown in Table 1. Overall we found 96.8% (239/247) concordance between the Xpert and Anyplex for CT and 100% (247/247) concordance for NG. All eight discrepant CT results were positive on Xpert, but negative on Anyplex. Testing on FTD STD9 confirmed three positive and five negative results. The Xpert cycle thresholds of the five discordant results reached 26.3 to 38.6 cycles, with two values greater than 38 cycles, indicating potential sampling or testing variation. The concordance between OSOM TV and Anyplex was 99.2% (245/247) with two discordant cases undetected on the OSOM TV assay, but positive on confirmatory testing. Most participants (265/267, 99.3%) received their POC results on the day of sampling, and were offered immediate treatment, if indicated.

Table 1: Evaluation of the Xpert CT/NG and OSOM TV against the Anyplex II STI-7 Detection and FTD STD9 assays (N=247)

POC assay		Anyplex II STI-7 Detection +/- FTD STD9		Accuracy with 95% confidence interval (95% CI)
		Positive	Negative	
Xpert CT	Positive	37	5	Sensitivity = 100% (100 - 100) Specificity = 97.6% (95.6 - 99.7) PPV = 88.1% (78.3 - 97.9) NPV = 100% (100 - 100)
	Negative	0	205	
Xpert NG	Positive	12	0	Sensitivity = 100% (100 - 100) Specificity = 100% (100 - 100) PPV = 100% (100 - 100) NPV = 100% (100 - 100)
	Negative	0	235	
OSOM TV	Positive	6	0	Sensitivity = 75.0% (45.0 - 100) Specificity = 100% (100 - 100) PPV = 100% (100 - 100) NPV = 99.2% (98.0 - 100)
	Negative	2	239	

Discussion

The aim of the study was to evaluate the performance of the Xpert CT/NG, as well as the OSOM TV, within a clinic-based diagnostic care model to rapidly detect CT, NG and TV in a high STI/HIV burden setting in South Africa. The Xpert CT/NG performed well with a high sensitivity and specificity to diagnose CT and NG, while the OSOM TV showed lower sensitivity, but high specificity. Taken together, these assays could allow diagnosis and management of STIs in one clinical visit.

Our findings are consistent with a study of 1722 women and 1387 men from the US, which found a consistently high diagnostic performance of the Xpert CT/NG testing cervical and vaginal swabs from women, and urine from men and women (8). Sensitivity and specificity of the assay using vaginal swabs was 98.7% and 99.4% for CT and 100% and 99.9% for NG, which was only marginally superior to urine testing in women (97.6%, 99.8% for CT and 95.6%, 99.9% for NG). Similar to our evaluation, this study found a lower PPV of the assay for both CT (88.6%) and NG (91.7%) when using vaginal swabs in their population, while PPVs for urine testing were higher (96.4% and 95.6%). This could indicate that urine samples may be adequate for Xpert CT/NG testing in women, and may prevent unnecessary treatment, especially in populations with lower CT prevalence.

In our study, two out of five 'false positive' Xpert CT results had a high cycle threshold above 38 cycles. A validation of 50 randomly selected vulvovaginal samples from a study of pregnant women in Pretoria, South Africa (16) also found two positive Xpert CT/NG results with high cycle thresholds that were confirmed negative after testing on the Presto^{Plus} CT/NG/TV (Microbiome, Ltd., Houten, The Netherlands) and Anyplex assays. This highlights that some caution is needed when interpreting high cycle threshold results, and while retesting may be considered, it also highlights the limitations of comparing two or more highly accurate molecular based assays, that challenge the threshold limits of each other.

Recently, the POC Xpert CT/NG was evaluated as part of a large cluster randomized study in remote community health services in Australia. In keeping with our findings, the assay demonstrated a high sensitivity and specificity when performed by nurses and community health workers compared to conventional NAATs for both CT (98.6%, 99.5%) and NG (100%, 99.9%) using either urine samples or vaginal swabs. The authors concluded that this POC STI assay may be particularly suitable for LMICs, where resources are limited and infrastructure is often poor (9).

We decided to complement the Xpert CT/NG testing with the OSOM TV antigen detection assay in order to provide the participants with a comprehensive 2-hour STI testing alternative to syndromic management (10). Previous evaluations (11, 12) of the OSOM TV concur with our finding of a slightly lower sensitivity (92 – 98%) than PCR technology, but a consistently high specificity to detect TV (99%). The advantage of this assay is that it is relatively cheap, has a rapid processing time, and higher accuracy than wet-prep microscopy, especially in women (12). It is important to note that the decision to use the OSOM TV rather than the Xpert TV was driven by the direct cost of the test. However, the relative costs of scaling up a STI screening program, including the costs and logistics of a quality assurance and training framework may make the Xpert TV assay still a reasonable addition in the future, even more so, if the company Cepheid decided to launch a CT/NG/TV multiplex cartridge.

The limitations of our study were that it was conducted at one site, only among young women, and had a relatively low sample size. We excluded pregnant women from the CAPRISA 083 cohort study, because they were referred for antenatal care, and were not an appropriate population to pilot the EPT intervention in. However, pregnant women may be an important population to offer POC testing to in the future (16, 17), and perhaps combine the testing model with a POC syphilis assay (18). A further limitation of our study was that specimens were not available to repeat discordant results on the Xpert CT/NG platform. Nevertheless, to the best of our knowledge, we report the first clinic evaluation of the diagnostic performance of the Xpert CT/NG assay from a LMIC. Furthermore, we decided to focus on young women, because this population group has been prioritized for diagnostic STI care in WHO and South African guidelines (1, 5) , and remains at highest risk of HIV acquisition in LMICs.

In conclusion, we found the Xpert CT/NG to be accurate when used at the point of care in a LMIC clinic, and it was complemented well by the OSOM TV assay. Larger implementation studies are required to assess whether the introduction of POC STI testing could be cost-effective, and eventually replace the syndromic management approach in South Africa. In the meantime, it seems prudent to prioritize diagnostic STI care for high-risk populations as part of HIV prevention efforts.

Figure 1: Study flow and results of the diagnostic evaluation of the Xpert CT/NG and Osom TV assays

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Contributors: NG, AR and AM are the co-principal investigators, conceived the study and wrote the study protocol. NG, JD, HN recruited the cohort. NG, NM, JN, RS and KM conducted the laboratory evaluation. NG, FO, JD, AM performed the statistical analysis. All authors contributed to the manuscript and consented to final publication.

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Competing interests: Cepheid Inc loaned two 4-module GeneXpert machines to the study team free-of-charge, but had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data sharing statement: De-identified participant data is available upon request by emailing the authors at nigel.garrett@caprisa.org.

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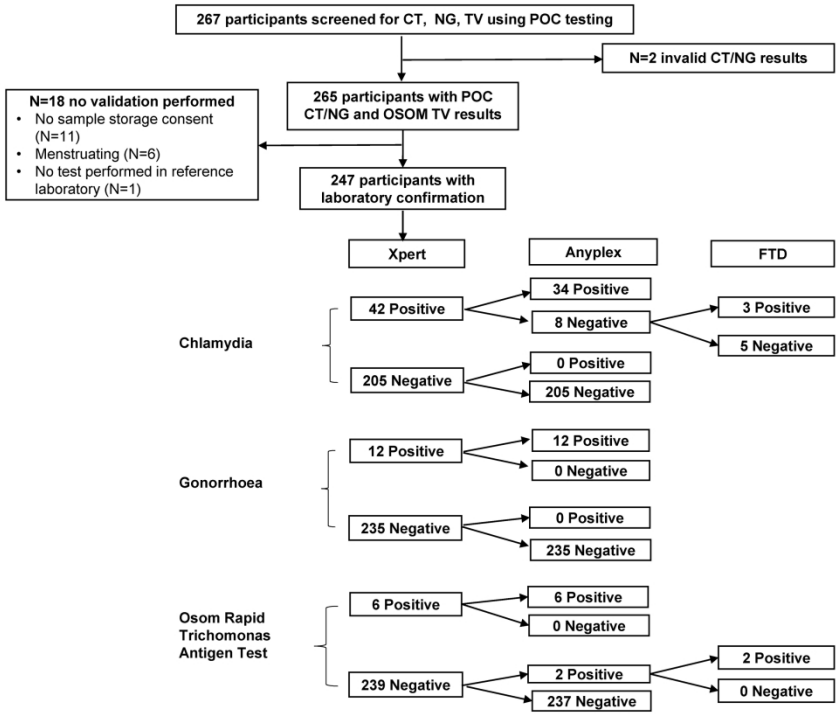

Figure 1: Study flow and results of the diagnostic evaluation of the Xpert CT/NG and Osom TV assays

254x190mm (300 x 300 DPI)

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4
	4	Study objectives and hypotheses	5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6
Participants	**6**	Eligibility criteria	6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	6
	9	Whether participants formed a consecutive, random or convenience series	6
Test methods	**10a**	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6

	13b	Whether clinical information and index test results were available to the assessors of the reference standard	6
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	7
	15	How indeterminate index test or reference standard results were handled	7
	16	How missing data on the index test and reference standard were handled	7
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	7
	18	Intended sample size and how it was determined	7
RESULTS			
Participants	**19**	Flow of participants, using a diagram	8, Figure 1
	20	Baseline demographic and clinical characteristics of participants	8
	21a	Distribution of severity of disease in those with the target condition	8
	21b	Distribution of alternative diagnoses in those without the target condition	8
	22	Time interval and any clinical interventions between index test and reference standard	8
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	9
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	9
	25	Any adverse events from performing the index test or the reference standard	8
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	11
	27	Implications for practice, including the intended use and clinical role of the index test	11
OTHER INFORMATION			
	28	Registration number and name of registry	Online submission system
	29	Where the full study protocol can be accessed	http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0196209
	30	Sources of funding and other support; role of funders	Manuscript and online submission system

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.