

## Research Article

# Validation of the GeneXpert® CT/NG Assay for use with Male Pharyngeal and Rectal Swabs

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## Abstract

**Objectives:** The GeneXpert® CT/NG (Cepheid, Sunnyvale, CA) assay is a point-of-care (POC) molecular diagnostic assay designed to rapidly test for the presence of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC). However, the test is only approved for vaginal swabs, urine, and endocervical swabs. Here, we performed an evaluation of the GeneXpert® CT/NG assay to detect the presence of CT and GC on male pharyngeal and rectal swabs.

**Methods:** Men who have sex with men participating in an HIV and Sexually Transmitted Infection (STI) screening program providing consent were enrolled into the study. Participants were asked to self-collect two pharyngeal and two rectal swabs. One set was tested on site using GeneXpert® and the other was sent to a reference lab for molecular testing using the APTIMA® system (Hologic, San Diego, CA).

**Results:** A total of 570 swabs were collected from 144 patients. GeneXpert® detected 13/15 rectal swabs testing CT positive by the APTIMA® assay (relative sensitivity=88.2%), 1/2 pharyngeal swabs testing CT positive (relative sensitivity=50%), and 7/9 pharyngeal swabs testing NG positive (relative sensitivity =77.8%). No discordance was observed for rectal NG swabs.

**Conclusions:** Although less sensitive than the APTIMA® assay for the molecular detection of NG and CT, GeneXpert®'s potential as a rapid POC diagnostic still make it a viable diagnostic test for STI screening. Molecular POC diagnostics, such as this, will allow more thorough screening of at risk individuals, and enhance the ability of clinics to provide same-day diagnosis and treatment.

**Keywords:** Diagnosis; Men; Gonorrhea; DNA amplification; Extra-genital; Chlamydia

## Introduction

While the rate of gonorrhea infections in the United States has remained relatively constant since the 1990s, in San Diego County rates have increased by ~15%. Most of this increase is due to infections in men, and rates of infection are 50% higher in men than women [1]. Of note, in men who have sex with men (MSM), gonorrheal infection is often located in non-urethral sites, thus up to 80% of MSM with Chlamydia and gonorrhea infection may remain undiagnosed if only urethral screening is performed [2]. While culture is the standard approach to screening non-urethral sites for gonorrhea, it is significantly less sensitive than molecular diagnostics [3]. The Centers for Disease Control recommends nucleic acid testing for non-urethral sites, but no test is currently approved by the US Food and Drug Administration. Sensitive Point-of-Care (POC) diagnostic tests that are able to rapidly identify infected individuals are needed, allowing diagnosis and treatment to occur in one visit to prevent further infections.

The GeneXpert® CT/NG (Cepheid, Sunnyvale, CA) assay is a molecular diagnostic test that can be used at the POC, and which rapidly identifies the presence of *Chlamydia Trachomatis* (CT) and *Neisseria Gonorrhoeae* (NG) giving results in ninety minutes or less [4]. The platform has been validated and approved for female sample

collection from vaginal swabs, urine, and endocervical swabs, but only for male urine samples [5], limiting the potential of this rapid molecular assay to identify infection in non-urethral sites in males. Here we examined the relative sensitivity and specificity of this (POC) assay for screening pharyngeal and rectal swabs from male patients.

Here, we performed a prospective cross-sectional study of high risk MSM to evaluate the GeneXpert® CT/NG assay for use with pharyngeal and rectal swabs. Eligible participants included a convenience sample of HIV uninfected MSM (seronegative for HIV) participating in an HIV and Sexually Transmitted Infection (STI) screening program through the University of California San Diego (between April 29, 2015 and July 31, 2015), who had unprotected anal sex at least once, had not been treated for any STI in the three months prior to testing, and provided informed consent. In order to demonstrate non-inferiority of the GeneXpert® CT/NG assay for the pooled analysis (<15% difference in sensitivity), 144 participants were screened and enrolled in a consecutive manner. Once enrolled, participants were instructed on how to self-collect two pharyngeal and two rectal swabs. One set of the collected swabs was tested on site using the GeneXpert® system and the other was stored at -4 °C until shipment to ARUP Laboratories (Salt Lake City, UT) for molecular testing using the APTIMA® system (Hologic, San Diego, CA).

## Methods

The first set of swabs (one rectal and one pharyngeal) was placed into a Cepheid “Swab Transport Reagent Tube”, and after gentle mixing 1-1.5 mL of this reagent was transferred into the sample chamber of the GeneXpert® CT/NG cartridge. The cartridge was then inserted into the GeneXpert® instrument for analysis by the Dx system computer software. The second set of swabs was collected using the APTIMA® Unisex Swab Specimen Collection kit and placed in a “Swab Specimen Transport Tube”. These samples were refrigerated and transported to ARUP Laboratories for testing on the same day of collection. No follow-up confirmatory test was performed if the APTIMA® diagnostic test result was positive. This assay was chosen as a reference given the superior sensitivity of molecular testing over culture based methods [3], and because this assay had been validated by ARUP for rectal and pharyngeal swabs. Clinical information and index test results were not provided to ARUP. Relative diagnostic accuracy (sensitivity and specificity) of GeneXpert® results were determined in relation to the APTIMA® results.

## Results

A total of 570 swabs were collected from 144 participants, including 144 paired pharyngeal and 141 paired rectal swabs. Sensitivities and specificities of the GeneXpert® CT/NG assay were calculated using the APTIMA® assay as a gold standard. Only 2/144 (1.4%) pharyngeal swabs tested positive for *C. trachomatis* by APTIMA®, of which only one tested positive by the GeneXpert® test, resulting in a relative sensitivity of 50% (CI: 9.5-90.6%) for detection of oral CT (negative predictive value (NPV) = 99.3%). The GeneXpert® did not detect two of fifteen rectal CT swabs testing positive by the APTIMA® assay, giving a relative sensitivity of 88.2% (CI: 62.1-96.2%) for rectal CT (NPV = 98.4% [CI: ]). The GeneXpert® detected seven of then in pharyngeal swabs found to be NG positive by the APTIMA® assay, giving a relative sensitivity for oral NG of 77.8% (CI: 45.3-93.7%, NPV = 98.5%). No discordance was observed in the rectal NG assays, giving a relative sensitivity for rectal NG 100% (CI:51-100%, NPV = 100%). All swabs that were negative for both *C. trachomatis* and *N. gonorrhoeae* by the APTIMA® test were also reported as negative by GeneXpert®, making the relative specificity of the GeneXpert® for CT/NG assay 100% (positive predictive value (PPV) for all assays = 100%). A comparison of the results from the two assays can be found in Table 1.

## Discussion

While the GeneXpert® test was less sensitive than the APTIMA® assay for the molecular detection of NG and CT, its potential as a rapid POC diagnostic still make it a viable test for use with pharyngeal and rectal swabs from male patients. Keys to reducing STI include both an improvement in detection, and a reduction in time from diagnosis to treatment. While molecular testing has been shown to be more sensitive than standard culture for NG (86.2% vs. 98.7%), and much simpler than culture for CT, its biggest advantage is the rapidly generated result [3]. The ability to perform molecular testing is not always feasible in STI clinics or other outpatient facilities, but Cepheid’s GeneXpert® and newer diagnostic systems are designed for point of care use, with simple set up and results generated within 90 minutes [4]. The ability to expand GeneXpert® testing beyond its

**Table 1:** Comparison between GeneXpert and APTIMA assays for molecular detection of NG and CT. Relative sensitivity of the GeneXpert in relation to the APTIMA assay is reported.

Organism/swab origin	APTIMA result	GeneXpert result	n	Sensitivity	Specificity
<i>C. trachomatis</i> /oral	+	positive	1	50%	
		negative	1		
	-	positive	0		100%
		negative	142		
<i>C. trachomatis</i> /rectal	+	positive	15	88.2%	
		negative	2		
	-	positive	0		100%
		negative	124		
<i>N. gonorrhoeae</i> /oral	+	positive	7	77.8%	
		negative	2		
	-	positive	0		100%
		negative	135		
<i>N. gonorrhoeae</i> /rectal	+	positive	4	100%	
		negative	0		
	-	positive	0		100%
		negative	137		

current scope of urine and endocervical swabs will allow for more thorough screening of at risk individuals, enhancing the ability of clinics to provide same-day diagnosis and treatment. The small number of tests evaluated limits our ability to make strong conclusions about which sites are particularly problematic using the Cepheid assay. Future directions for Cepheid and other manufacturers of POC molecular tests will be the design of swabs and buffers to optimize the sensitivity of detection of CT and NG from pharyngeal or rectal locations.

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## Competing Interests

D. M. S. has received grant funding from ViiV Healthcare and has served as a consultant for Genprobe and Testing Talent Services. S. J. L. reported grant funding from Gilead Sciences, Inc. All other authors report no potential conflicts.

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