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Comparison of the Cepheid GeneXpert CT/NG Assay to the Hologic Aptima Combo 2 Assay for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in self-collected rectal swabs

Laura Dize, MS¹, Barbara Silver, BS, MBA, MT (ASCP)¹, and Charlotte Gaydos, MS, MPH, DrPH¹

¹International Sexually Transmitted Diseases Research Laboratory, Division of Infectious Diseases, Johns Hopkins University, Baltimore, MD, USA

Abstract

Self-collected rectal-swabs were tested for CT and NG on GeneXpert CT/NG as compared to APTIMA Combo2. Of 448 rectal-swabs, 22 were positive for CT; 7 for NG on both assays; two were discordant. Sensitivity and specificity of GeneXpert was 95.5% and 99.7% for chlamydia, respectively; for gonorrhea both were 100%.

Introduction

In 2014 the Centers for Disease Control and Prevention (CDC) reported an increase in chlamydia and gonorrhea prevalence from previous years with 1,441,789 cases of *Chlamydia trachomatis* (CT) and 350,062 cases of *Neisseria gonorrhoeae* (NG) reported. (CDC Surveillance 2015) Although, there are no Nucleic Acid Amplification Tests (NAATs) currently cleared by the U.S. Food and Drug Administration (FDA) for use with extragenital specimens, culture is approved for NG screening and the CDC recommends sexually transmitted infection (STI) screening by NAATs for men and women who report engaging in receptive anal intercourse (Cosentino 2012; Papp 2014, Geiger 2016). Previous data shows that around 30% of women and approximately 40% of men report ever having engaged in anal intercourse for various reasons ranging from curiosity to a method of contraception, (Benson 2016; Hess 2016). Men who have sex with men (MSM) are at particular risk for contracting rectal STIs, and gonorrhea infections are often diagnosed in extragenital sites which can increase the chance for HIV infection (Geiger 2016; Levy 2012; Tao 2016). There have been many research studies evaluating the performance of rectal swab specimens for detecting CT and NG by NAATs, and these studies have demonstrated rectal swabs are an acceptable specimen type when detecting STIs and actually detect more CT and NG infections in the rectum than culture. (Moncada 2009; Cosentino 2012; Levy 2012) We compared the performance of the GeneXpert CT/NG Assay (Gaydos 2013) to the AC2 Assay, using prospectively self-collected rectal swabs from men and women.

Methods

Male and female participants recruited through an internet-based screening program, www.iwantthekit.org, could order a rectal swab collection kit as part of a home collection program for STI screening. (Gaydos 2009, Ladd 2014) Participants were instructed to insert a Copan flocced swab 1.5 inches into the rectum to collect the specimen. Each participant collected one rectal swab which was transported to the laboratory by U.S. Postal Services in a dry state to the laboratory for testing. (Gaydos 2012) Upon arrival, samples were stored at 4°C and tested within four days. Rectal swabs were expressed in 800 µL of Tris-EDTA buffer prior to testing. A 200 µL aliquot was transferred to a GeneXpert vaginal transport tube (Cepheid, Sunnyvale, CA) for GeneXpert testing; a second 200 µL aliquot was transferred to a unisex Aptima transport tube for testing by Aptima Combo2 (AC2) (Hologic, San Diego, CA). All samples were tested for CT and NG on the AC2 Assay and were de-identified prior to testing on GeneXpert. Samples which reported ERROR results on initial GeneXpert testing were retested using sample from the original GeneXpert transport tube. Samples that had INVALID results were repeated using a new 200 µL TE buffer aliquot, which was freeze-thawed. AC2 was used as the reference test because the method used had been previously validated by the laboratory for use with self-collected rectal swabs. (Ladd 2014)

Results

A total of 448 rectal swab (225 female and 223 male) samples were tested on both assays. Upon initial testing by GeneXpert there was an assay success rate of 83% (372/448); 8 samples had ERRORS and 68 specimens returned INVALID results. Of the 68 INVALID results, 66 were due to sample adequacy control for human DNA (SAC) failures; indicating no human DNA was in the sample. One was due to sample processing control (SPC) failure and 1 sample had both SAC and SPC failures. Upon repeat testing from the original Cepheid transport tube all 8 ERRORS resolved while only 21/68 (38.8%) INVALID results were resolved, all of which were negative for CT/NG. The overall assay success rate for GeneXpert testing rectal swabs was 89.5% (401/448). Only sample pairs that had valid results by both assays were included in the statistical analysis (N=401). There were 21 (5.2%) samples positive for CT and 7 (1.7%) samples positive for NG on both assays. Two sample pairs were discrepant for CT; one sample was AC2+/GeneXpert-, and one was AC2-/GeneXpert+. Sensitivity and specificity of GeneXpert for NG were both 100%, with 95% confidence intervals (95% CI) of (59.0, 100%), and (99.1, 100%) respectively. For CT, GeneXpert had a sensitivity of 95.5% (95% CI, 77.2 – 99.9) and specificity of 99.7% (95% CI, 98.5 – 100). (Table 1). Of the 21 CT positives by both assays only 8 had a genital CT infection; while only 1/7 of the GC positives also had genital infection. (Table 2)

Discussion

The Cepheid GeneXpert CT/NG Assay appeared to be an acceptable assay for detecting CT and NG in rectal swabs as compared to the APTIMA Combo 2 assay. Although there appears to be a high amount of SAC failures for human DNA by the GeneXpert assay this could be due to where samples were collected. Rectal swabs were collected by participants at home; the sample adequacy control (SAC) detects a single-copy of human gene, if

participants did not actually collect the sample this could have caused the SAC failures. (Goldenberg 2012) Sample adequacy control failures could have also been caused by potential interference of the TE buffer with the GeneXpert assay or potential inhibition of fecal/rectal material, which seemed to be resolved with the freeze-thaw cycle of the primary aliquot. Two prior studies also showed resolution of INVALID results in cerebral spinal fluid (CSF) samples on GeneXpert Assays by utilizing a free-thaw method (Seme 2008; Seters 2009). As seen in table 2 had only genital samples been analyzed and rectal sites not been screened 13 cases of CT infection and 6 cases of GC infection would have gone undiagnosed and subsequently untreated increasing transmission of these infections. Previous studies show more than half of all CT infections diagnosed were in non-urethral sites in MSM; also asymptomatic infections in extra-genital sites can go undiagnosed and increase partner transmission. (Edouard 2017, Tao 2016 and Cosentino 2012) This data was suggestive of the need for an FDA cleared assay for rectal-swab specimens for the diagnoses of CT/NG infections. Further research instructing participants to collect 2 rectal swabs which would be transported dry and placed into respective assay medium directly; Unisex transport tube for AC2 or swab transport tube for GeneXpert testing; in a randomized fashion could potentially eliminate the dilution effect of only using 200 ul in the assays. Although the low number of positive results generated by our study limits conclusions, we believe GeneXpert to be an acceptable method for detecting CT and NG infections from self-collected rectal swab specimens. GeneXpert also returns results in approximately 90 min, which could improve turnaround time and potentially expedite patient treatment in a clinic setting which has laboratory support.

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Results of Cepheid GeneXpert CT/NG (C) compared to Reference Aptima Combo2 (R) Assay for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in self-collected rectal swabs.

Table 1

STI	R+/C+	R-/C-	R+/C-	R-/C+	%Sensitivity (CI)	%Specificity (CI)	Kappa (CI)
Chlamydia	21	378	1	1	95.5 (77.2 - 99.9)	99.7 (98.5 - 100)	0.952 (.885 - 1.0)
Gonorrhea	7	394	0	0	100 (59 - 100)	100 (99.1 - 100)	1.0 (1.0 - 1.0)

Table 2

Results of individuals who had a genital (vaginal or penile) chlamydia or gonorrhea infection in addition to rectal infection.

STI	Rectal Infection	Genital Infection
Chlamydia	21	8
Gonorrhea	7	1