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Evaluation of pooled ocular and vaginal swabs by the Cepheid GeneXpert CT/NG assay for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* compared to the GenProbe Aptima Combo 2 Assay

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Abstract

Ocular swabs from Tanzania were tested for *Chlamydia trachomatis* (CT), and self-collected vaginal swabs collected through a home collection program, iwantthekit.org, were tested for *Neisseria gonorrhoeae* and CT to evaluate Cepheid GeneXpert for the use of pooling multiple specimens before testing. GeneXpert shows to be a promising test for pooling.

Keywords

Trachoma; *Chlamydia trachomatis*; *Neisseria gonorrhoeae*; Pooling

Trachoma is the most common infectious cause of blindness worldwide and is caused by repeated ocular *Chlamydia trachomatis* (CT) infections. Trachoma is endemic in 55 countries resulting in approximately 3.8 million cases of blindness and 5.3 million cases of impaired vision throughout Africa and Southeast Asia (Goodhew et al., 2012; WHO, 2007). Similarly, in 2012, in the US alone, there were 1.42 million reported genital CT infections as well as 334,826 *Neisseria gonorrhoeae* (NG) genital infections (CDC, 2013). Sexually transmitted infections including CT and NG cost the US health care system about 19 billion dollars annually (Owusu-Edusei et al., 2013).

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Nucleic acid amplification tests (NAAT) assays are recommended by the Centers for Disease Control and Prevention (CDC) for the detection of CT and NG (Papp et al., 2014). NAAT assays currently used for detecting ocular and genital infections can be costly at $10 to $15/test, plus labor charges (Goodhew et al., 2012; See et al., 2011). Pooling specimens before testing by NAATs for the detection of CT and NG can potentially reduce the cost associated with estimating the prevalence of ocular CT infections in the field of trachoma-endemic countries (Dize et al., 2013; Jenson et al., 2013) as well as for diagnosing CT and NG genital infections in clinical settings. Previously, high sensitivity and specificity as well as large cost savings have been demonstrated when testing pools of 4 or 5 genital or ocular samples by NAATs compared to individual testing (Dize et al., 2013; Kacena et al., 1998; Shipitsyna et al., 2007). This study evaluated the efficacy of pooling ocular and vaginal specimens for testing by the Cepheid GeneXpert CT/NG (GeneXpert) rapid real-time PCR test as compared to the APTIMA Combo2 (AC2) NAAT assay, as a reference standard (Gaydos et al., 2013a).

Ocular swab specimens collected in Tanzania were shipped dry to the International Sexually Transmitted Diseases Research Laboratory at Johns Hopkins University (Baltimore, MD, USA) for the detection of CT. Ocular swabs were pooled and analyzed for CT by AC2 (Hologic/GenProbe, San Diego, CA, USA), as previously described (Dize et al., 2013). Positive pools only were deconstructed for individual specimen analysis.

Vaginal swabs collected through the “I Want the Kit” (IWTK) program were tested for CT and NG using AC2 (Gaydos et al., 2013b). Upon arrival, dry-shipped vaginal swabs were expressed into 800 μL of 1X TE buffer pH 7.4 and split into 2 aliquots, 1 containing 200 μL and 1 containing 400 μL, then 200 μL was transferred into a vaginal APTIMA collection tube for testing (Gaydos et al., 2012; Masek et al., 2009). Vaginal swabs were tested individually by AC2 and later pooled for analysis by GeneXpert. We prospectively analyzed 25 ocular pools, 23 containing 4 specimens each and 2 containing 5 specimens each for a total of 102 ocular specimens. For vaginal swab analysis, there were 25 pools each containing 4 specimens, for a total of 100 prospective vaginal specimens.

GeneXpert pools were created by spiking a GeneXpert Vaginal Transport tube with 200 μL each of rehydrated ocular specimen or vaginal specimen. The transport tube was shaken vigorously for 20 seconds, and 260 μL of media from either 4 or 5 GeneXpert transport tubes was combined into a 2-mL tube. Then 1 mL of the pool was added to a GeneXpert CT/NG cartridge for testing. Positive pools were deconstructed to determine the individual positives within those pools. For deconstruction, the individual GeneXpert Vaginal transport tubes from positive pools were tested according to manufacturer’s instructions. Negative pools were not deconstructed for further analysis.

Of 25 ocular pools, 7 pools were positive for CT by AC2 and GeneXpert, each containing 1 positive specimen. Compared to AC2, GeneXpert had sensitivity of 7/7 (100%), specificity of 18/18 (100%), positive predictive value (PPV) of 7/7 (100%), and negative predictive value (NPV) of 18/18 (100%) (Table 1).

Of 25 vaginal pools, 14 were positive: 10 for CT only, 3 for NG only, and 1 for both CT and NG by GeneXpert. Upon deconstruction, there were 13 individual CT positives and 4 individual NG positives by GeneXpert; vaginal swabs were initially tested individually on AC2 and had 12 CT positives and 4 NG positives. There was 1 discrepant pool of AC2 negative/GeneXpert CT positive. Pooling vaginal swabs, GeneXpert had a sensitivity of 13/13 (100%), specificity of 11/12 (91.6%), a PPV of 13/14 (92.8%), and an NPV of 11/11 (100%) (Table 2).

Compared to AC2, GeneXpert shows promise as a rapid, near patient PCR test, demonstrating high sensitivity and specificity for pooled ocular and vaginal swabs. GeneXpert is considered a rapid test in that it produces a result for both CT and NG in just 90 minutes. Many sexually transmitted disease (STD) clinics in the Unites States receive funding for their programs from the CDC and local governments and are currently facing budget crisis; due to budget cuts, many clinics now offer “express visits” for asymptomatic patients; these patients often do not initially see a clinician (Golden and Kerndt, 2010; Hogben et al., 2013). Many studies have been performed on pooling various genital specimen types and demonstrate high sensitivities and cost savings of up to 80% on various NAAT assays, dependent on the size of the pool and the prevalence of the population being tested (Currie et al., 2004; Kapala et al., 2000; Morre et al., 2000). Pooling vaginal swab specimens for the detection of CT and NG in clinics can significantly save scarce program funds. The limited amount of “hands-on” time and the ease of use of the GeneXpert make this assay ideal in clinics with “express visits” as well as for use in developing countries that lack sophisticated laboratory equipment and infrastructure for specialized NAAT testing where trachoma is endemic. Although GeneXpert is a NAAT PCR assay, the test platform is relatively simple and was previously evaluated for ocular swab testing in the field at the Kongwa Trachoma Project in Tanzania and resulted in a positive experience suggesting that pooling on GeneXpert could also be performed in the field (Jenson et al., 2013).

Cost savings for these ocular samples would be seen by using 25 assay cartridges for pools and 28 cartridges for deconstructed tests for a total of 53, instead of 102 cartridges; for vaginal samples, 25 cartridges for pools and extra 56 cartridges for individual tests for a total of 81 instead of 100 individual tests. Cost savings depends on the prevalence of infection in the population being tested; as prevalence decreases, pool size can increase, resulting in higher cost savings (Diamant et al., 2001; Kacena et al., 1998; Peeling et al., 1998). In a previous study comparing pooling of ocular swabs for the detection of CT on 2 NAAT assays, an overall cost savings of 62.2% was observed, a cost-savings analysis for the GeneXpert would also be needed to determine more accurate cost savings (Dize et al., 2013).

This study shows the GeneXpert assay to be a good candidate for pooling both vaginal and ocular specimens. Future studies are planned to evaluate pooling by GeneXpert on different sample types including male swabs and urine. For trachoma, districts and programs could have an expedited and more accurate assessment of the risk of infection compared to the clinical assessment of trachoma. Having an accurate near-patient test in STD clinic settings, which can provide results for immediate treatment, can also potentially be viewed as better patient care and prevent costly chlamydial sequelae such as pelvic inflammatory disease for

genital infections since many patients are often lost to follow-up or not treated expeditiously (Huang et al., 2013).

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Table 1

Ocular swab pooling results on the Cepheid GeneXpert CT/NG Assay as compared to GenProbe AC2 for the detection of CT.

		No. of pools with indicated result							
Test	**No. Of pools tested**	**R+/C+**	**R−/C−**	**R+/C−**	**R−/C+**	**% Sensitivity (95% CI)**	**% Specificity (95% CI)**	**NPV (%)**	**PPV (%)**
Gene Xpert	25	7	18	0	0	100 (56–100)	100 (78–100)	100	100

R = reference method result (AC2); C = comparative method result (GeneXpert); 95% CI = 95% confidence intervals.

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Table 2

Vaginal swab pooling results on the Cepheid GeneXpert CT/NG Assay as compared to GenProbe AC2 for the detection of CT and NG.

		No. of pools with indicated result							
Test	**No. of pools tested**	**R+/C+**	**R−/C−**	**R+/C−**	**R−/C+**	**% Sensitivity (95% CI)**	**% Specificity (95% CI)**	**NPV (%)**	**PPV (%)**
Gene Xpert	25	13	11	0	1	100 (71.6–100)	91.6 (59.7–99.5)	100	92.8

R = reference method result (AC2); C = comparative method result (GeneXpert); 95% CI = 95% confidence intervals.

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