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Comparison of the Abbott *m*2000 RealTi*me* CT Assay and the Cepheid GeneXpert CT/NG Assay to the Roche Amplicor CT Assay for Detection of *Chlamydia trachomatis* in Ocular Samples from Tanzania

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The GeneXpert CT/NG assay (GeneXpert) and the Abbott *m*2000 RealTi*me* CT (*m*2000) assay were compared to Amplicor for detecting ocular *Chlamydia trachomatis*. Discordant specimens were tested by the Aptima CT assay. The *m*2000 assay sensitivity was 100% (95% confidence interval [CI], 90% to 100%), and specificity was 98.46% (95% CI, 95.2% to 99.2%); GeneXpert sensitivity was 100% (95% CI, 90% to 100%), and specificity was 100% (95% CI, 98.1% to 100%). The *m*2000 and GeneXpert assays appear to perform as well as the Amplicor assay.

The leading infectious cause of preventable blindness worldwide is trachoma, which occurs in resource-limited countries, including sub-Saharan Africa, and is caused by repetitive and untreated ocular *Chlamydia trachomatis* infections (1, 2, 3). The current reservoir of active disease and infection is in children; in villages where such infections are hyperendemic, *C. trachomatis* infections have been found in children who have been treated at least once though mass drug administration (MDA) (1, 4). PCR is considered to be the current gold standard test, although there is no defined gold standard test for ocular *C. trachomatis* infections (5, 6). The Roche Amplicor CT PCR assay (Roche Diagnostics, Indianapolis, IN) has been used in major trials to monitor infection following MDA (7). Testing for *C. trachomatis* infection is often difficult in regions associated with trachoma; the laboratory infrastructure is often deficient or nonexistent, and there is often a lack of trained personnel, equipment, funding, and cleanliness of testing areas. The Cepheid GeneXpert CT/NG Research Use Only (RUO) assay (GeneXpert) (Cepheid Inc., Sunnyvale, CA) is a rapid test designed to produce results relatively quickly, with little hands-on time required, and could be useful in areas where trachoma occurs. We evaluated the Abbott *m*2000 RealTi*me* CT (*m*2000) assay (Abbott Molecular Diagnostics, Des Plaines, IL) for detection of *C. trachomatis* in ocular specimens from Tanzania as a means to reduce turnaround time for results and increase sample throughput. A first-generation GeneXpert CT/NG assay (GeneXpert) was subsequently evaluated as a potential method for testing ocular specimens in the field to expedite provision of immediate treatment of *C. trachomatis* ocular infections, pending demonstration of its performance in a laboratory setting.

Duplicate ocular swab specimens ($n = 304$) from the same eye were collected from children in Tanzania for the detection of *C. trachomatis* infection; collection was performed as previously described (8). Samples were shipped frozen in a dry state to the Johns Hopkins University (JHU) Research Laboratory in Baltimore, MD, and stored at −80°C until testing. Swabs were rehydrated with 1 ml of sterile molecular analysis-grade diethylpyrocarbonate (DEPC) water (Quality Biological, Inc., Gaithersburg, MD). One set of the duplicate specimens was tested by Amplicor (Roche Diagnostics) and GeneXpert (Cepheid) and, for discordance testing, by the GenProbe-Aptima CT (ACT) assay (Gen-Probe Hologic, Inc., San Diego, CA) at JHU; a duplicate set was sent to Indiana University in Indianapolis, IN, for *m*2000 analysis. Targets for the assays differ. For Amplicor, the target is a sequence 207 nucleotides long within the cryptic plasmid DNA of *C. trachomatis*; *m*2000 targets two different regions of the cryptic plasmid, GeneXpert targets a conserved chromosomal genomic DNA sequence, and ACT targets rRNA from *C. trachomatis*.

DNA extraction performed on the Roche MagNA Pure LC extraction robot with 200 μl of sample resulted in 100 μl of elute using a MagNA Pure LC DNA isolation kit I (Roche Diagnostics). PCR was performed using 50 μl of elute with a Roche CT/NG

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TABLE 1 Comparison of two molecular NAATs to Roche Amplicor PCR for the detection of *Chlamydia trachomatis* in ocular swabs[a]

Test	No. of specimens tested	No. of specimens with indicated result				% sensitivity (CI)	% specificity (CI)	% NPV	% PPV	Kappa (CI [%])
		R+/C+	R−/C−	R+/C−	R−/C+					
M2000	304	44	251	0	9	100 (90–100)	96.53 (93–98)	100	83.01	0.8898 (81.9–96)
GeneXpert	304	44	257	0	3	100 (90–100)	98.84 (96–99.7)	100	93.61	0.962 (91.2–100)

[a] R, reference method result; C, comparative method result; +, positive result; −, negative result; CI, 95% confidence interval for overall agreement of the reference method and the comparative method; NPV, negative predictive value; PPV, positive predictive value.

TABLE 2 Sensitivity and specificity of M2000 and GeneXpert for ocular swabs after resolution testing of discordant specimens by GenProbe ACT assay[a]

Test	% sensitivity (CI)	% specificity (CI)	% NPV (CI)	% PPV (CI)	Kappa
M2000	100 (90–100)	98.46 (95.2–99.2)	100 (98.1–100)	90.56 (78.5–96.5)	0.9529
GeneXpert	100 (90–100)	100 (98.1–100)	100 (98.1–100)	100 (90.5–100)	1

[a] CI, 95% confidence interval.

amplification kit, including positive and negative controls. *C. trachomatis* detection was performed using the Amplicor CT assay according to the manufacturer's instructions. If equivocal results occurred, the sample was retested in duplicate. If neither duplicate gave a positive result, the specimen was considered negative by Amplicor. Specimen volume was increased to 1 ml using DEPC water after Amplicor testing. Specimens were subjected to a vortex procedure for 30 s; 800 μl of sample was added to the GeneXpert cartridge and tested according to the manufacturer's instructions. Positive specimens produced a cycle threshold (C_T) value; negative specimens did not produce a C_T value. For *m*2000 analysis, 400 μl of specimen was transferred into a dry tube, placed on the *m*2000, and tested according to the package insert instructions. Specimens with a cycle number less than or equal to the assay cutoff were interpreted as a positive result; an absence of amplification was interpreted as negative; samples with a cycle number beyond the cutoff were interpreted as equivocal. Equivocal specimens were retested by spiking 100 μl of sample into an Abbott multi-Collect tube containing 1.2 ml of transport medium and tested according to the package insert instructions. Specimens with results that were discordant by either *m*2000 or GeneXpert compared to Amplicor were tested by ACT. A 200-μl volume of sample was placed into an Aptima unisex specimen transport tube, inverted for 10 s, and tested by ACT according to the manufacturer's instructions.

The sensitivity of *m*2000 compared to PCR was 100%, the specificity was 96.53%, the negative predictive value (NPV) was 100%, and the positive predictive value (PPV) was 83.01%. GeneXpert demonstrated a sensitivity of 100%, a specificity of 98.84%, an NPV of 100%, and a PPV of 93.61% (Table 1). The kappa score for *m*2000 was 0.8898 (95% confidence interval [CI], 81.9% to 96%) and for GeneXpert was 0.962 (95% CI, 91.2% to 100%) (Table 1). Four of nine discordant *m*2000 specimens (*m*2000 positive/Amplicor PCR negative) tested by ACT were confirmed positive. All 3 samples positive by GeneXpert and negative by Amplicor were ACT positive. After discordance analysis, the specificities for *m*2000 and GeneXpert increased to 98.46% and 100%, respectively (Table 2).

GeneXpert and *m*2000 were evaluated and compared to Amplicor PCR to determine if new assay methods could be used to detect *C. trachomatis* in ocular samples. The *m*2000 assay was evaluated as a new option for detecting *C. trachomatis* in ocular specimens to increase the throughput of samples tested while reducing hands-on time, potentially decreasing the overall cost of evaluating these specimens. Compared to Amplicor, *m*2000 demonstrated excellent sensitivity and specificity. The results for *m*2000 compared to Amplicor suggest the next step of a cost analysis to determine if the use of *m*2000 would in fact decrease cost. GeneXpert was evaluated because its simple design for specimen addition, reagent addition, and cartridge insertion into the testing module as well as the reduced risk of cross contamination due to the design show the possibilities of its being used as a field test. The GeneXpert had excellent sensitivity and specificity compared to Amplicor, with a kappa score of 0.9612, showing almost perfect agreement. After confirmation testing by ACT of the three discordant specimens, the specificity and PPV of the GeneXpert increased to 100%. Discordance testing also increased the kappa score to 1, demonstrating excellent performance of the GeneXpert in the laboratory. Future studies are under way in the Kongwa region of Tanzania to determine if GeneXpert performs as well when assays are carried out under field conditions in developing countries as it did in the laboratory setting. Limitations to this study included the fact that the swabs that were collected and shipped from Tanzania to the test site were not placed into manufacturers' transport media directly but were shipped in a dry state. No manufacturer has sought FDA clearance with respect to detecting *C. trachomatis* in ocular samples. However, past experience has indicated that nucleic acid amplification tests (NAATs) perform very well when analyzing ocular samples, especially NAATs designed to detect rRNA (9). This study demonstrated that *m*2000 and GeneXpert performed with great accuracy when detecting *C. trachomatis* in ocular samples, indicating that either assay could be utilized for future trachoma studies.

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