Comparison of the Abbott m2000 RealTime 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 m2000 RealTime CT (m2000) assay were compared to Amplicor for detecting ocular Chlamydia trachomatis. Discordant specimens were tested by the Aptima CT assay. The m2000 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 m2000 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 outcome 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 m2000 RealTime CT (m2000) 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-Probelogic, Inc., San Diego, CA) at JHU; a duplicate set was sent to Indiana University in Indianapolis, IN, for m2000 analysis. Targets for the assays differ. For Amplicor, the target is a sequence 207 nucleotides long within the cryptic plasmid DNA of C. trachomatis; m2000 targets two different regions of the cryptic plasmid, GeneXpert targets a conserved chromosomal genomic DNA sequence, and ACT targets RNA 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

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

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

aR, 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.

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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<sub>T</sub>) value; negative specimens did not produce a C<sub>T</sub> value; and 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 m2000 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 m2000 specimens (m2000 positive/Amplicor 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 m2000 and GeneXpert increased to 98.46% and 100%, respectively (Table 2).

GeneXpert and m2000 were evaluated and compared to Amplicor CT to determine if new assay methods could be used to detect *C. trachomatis* in ocular samples. The m2000 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, m2000 demonstrated excellent sensitivity and specificity. The results for m2000 compared to Amplicor suggest the next step of a cost analysis to determine if the use of m2000 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 m2000 and GeneXpert performed with great accuracy when detecting *C. trachomatis* in ocular samples, indicating that either assay could be utilized for future trachoma studies.

### ACKNOWLEDGMENTS
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Testing kits were provided for the GeneXpert assay by Cepheid. Parents or guardians gave written informed consent for their children to participate in the study.

The study protocol was approved by the IRB (NA_00018439).

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### REFERENCES

### TABLE 2 Sensitivity and specificity of M2000 and GeneXpert for ocular swabs after resolution testing of discordant specimens by GenProbe ACT assay<sup>a</sup>

<table>
<thead>
<tr>
<th>Test</th>
<th>% sensitivity (CI)</th>
<th>% specificity (CI)</th>
<th>% NPV (CI)</th>
<th>% PPV (CI)</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2000</td>
<td>100 (90–100)</td>
<td>98.46 (95.2–99.2)</td>
<td>100 (98.1–100)</td>
<td>90.56 (78.5–96.5)</td>
<td>0.9529</td>
</tr>
<tr>
<td>GeneXpert</td>
<td>100 (90–100)</td>
<td>100 (98.1–100)</td>
<td>100 (90.5–100)</td>
<td>100 (90.5–100)</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> CI, 95% confidence interval.

