Automated Extraction and Amplification for Direct Detection of Mycobacterium tuberculosis Complex in Various Clinical Samples

With the incidence of culture-positive tuberculosis (TB) cases at 25.3 per 100,000 and a 25% rate of TB/HIV coinfec-
tion, the TB incidence in French Guiana is the highest of all
French regions (3, 5). In this context, there is an urgent need
for simple, automated systems for molecular diagnosis of TB
that can be adapted to small laboratories. Introduction of a
nuclear amplification test in a routine clinical laboratory is an
additional expense, and its cost-effectiveness and clinical utility
need to be evaluated and seem to be optimized when used for
diagnosis in patients with an intermediate-to-high likelihood of
TB (8, 14).

With these objectives, our study aimed to evaluate a mo-
olecular assay developed for the detection of Mycobacterium
tuberculosis complex (MTC) on the robotic workstation
easyMAG/easyQ (EMEQ; bioMérieux, France), in comparison
with culture, the gold standard. Following extraction
based on the method described by Boom et al. (1), nucleic
acid sequence-based amplification (NASBA) with real-time
molecular beacon detection was performed on the extract
(9, 12).

The specificity of the EMEQ assay was initially evaluated
with 33 mycobacterial isolates (16 M. tuberculosis, 2 Myco-
bacterium bovis, and 15 nontuberculous mycobacterium iso-
lates). Subsequently, detection of MTC was performed on
request by specialized physicians during a 1-year prospective
study of hospitalized patients (90 clinical specimens split
into 25 respiratory and 65 nonrespiratory samples). Samples
were processed for acid-fast bacillus (AFB) smear micros-
copy and cultured, and isolates were identified using bio-
chemical tests in conjunction with restriction fragment
length polymorphism of the hsp65 gene (7) and the Geno-
Type mycobacterium assay (Hain Lifescience GmbH, Ger-
many).

A total of 52 specimens were culture positive, and 44 were
identified as MTC and detected by EMEQ. The 8 remaining
culture-positive specimens were atypical mycobacteria (6
Mycobacterium avium and 2 Mycobacterium fortuitum specimens)
and found to be negative with EMEQ. No inhibition in the
specimens included was detected. Out of 38 specimens that did
not grow, 35 were found to be negative with EMEQ (Table 1).
Analysis of culture-negative but EMEQ-positive samples re-
solved 2 out of 3 discrepancies, as follows: (i) for a gastric
aspirate specimen, a second aspirate specimen received the
same day cultured positive for M. tuberculosis, and (ii) for a
urine specimen, another urinary specimen collected 1 month
later cultured positive for M. tuberculosis using the Bactec
MGIT 960 liquid culture system in another laboratory.
Nonetheless, for the single remaining specimen of ascitic
fluid, which tested positive by the EMEQ assay in 2 different
runs, no further samples could be collected. The patient who
provided the remaining specimen, whose ascites was first
clinically labeled as being of pancreatic origin, remained
“out of sight” despite subsequent calls for specimen collec-
tion.

In spite of the reported lack of sensitivity of molecular
methods for nonrespiratory and paucibacillary specimens (2, 11, 13), the sensitivity in our study was 100% (Table 2); it
was affected by neither the type of specimen nor the AFB
counts (9 AFB-negative specimens were EMEQ positive).
No false-negative result was observed. The specificity was
100% for laboratory isolates as well as respiratory clinical
specimens and 97.1% for nonrespiratory specimens.

The negative predictive value (NPV) of a diagnostic test for
ruberculosis is of great importance in deciding isolation release
(6, 14), but predictive values have to be adjusted to the pre-
valence of the infection in the population tested (4). With a
positive predictive value of 97.9% and a NPV of 100% (Table
2), EMEQ appears to be useful as a diagnostic test and to rule
out TB in the group studied for intermediate-to-high clinical
suspicion.

In addition, with 160 min needed for automatic extraction
and amplification, incorporation of an internal control en-
abling the detection of samples inhibitors without duplicate
testing of a seeded tube (10), and risk of contamination being
minimized by amplification in closed tubes, the EMEQ assay
seems to be a reliable method for use in routine clinical labo-
ratories. These preliminary results will need to be confirmed by
further studies.

TABLE 1. Results of AFB staining, culture, identification of MTC, and EMEQ for clinical specimens tested.

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>No. of specimens testing positive by</th>
<th>AFB staining</th>
<th>Culture</th>
<th>MTC identification</th>
<th>EMEQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td>18</td>
<td>11</td>
<td>15</td>
<td>12</td>
<td>12</td>
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<tr>
<td>Bronchoalveolar fluid</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Bronchial aspirate</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Nonrespiratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric aspirate</td>
<td>29</td>
<td>22</td>
<td>24</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Biopsy</td>
<td>15</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>4</td>
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<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Urine</td>
<td>3</td>
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<td>0</td>
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<tr>
<td>Blood</td>
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<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
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<tr>
<td>Nodule aspirate</td>
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<td>0</td>
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<td>Cerebrospinal fluid</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Ascites</td>
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<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Culture supernatant</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total: 90 45 52 44 47

* Before resolution of discrepancies.
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REFERENCES


TABLE 2. Correlation of EMEQ and MTC by culture

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>No. of specimens with indicated results (EMEQ/MTC by culture)</th>
<th>Sensitivity/specificity (%)</th>
<th>Predictive value (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Positive/positive</td>
<td>Positive/negative</td>
<td>Negative/positive</td>
</tr>
<tr>
<td>Respiratory</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nonrespiratory</td>
<td>30</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

a After resolution of discrepancies.