Correspondence

Evaluation of COBAS TaqMan real time PCR assay for the diagnosis of *Mycobacterium tuberculosis*

Sir,

The laboratory diagnosis of tuberculosis has long been based on microscopy and culture methods\(^1,2\) which had multiple limitations based on technical expertise and infrastructure limiting its use\(^3,4\). Difficulties in identification and detection are addressed by nucleic acid amplification-detection methods which have been recently introduced\(^5,7\). We compared the performance of the COBAS TaqMan MTB assay (Roche Molecular Systems, NJ, USA) against conventional Lowenstein-Jensen (L-J) culture and Mycobacterial growth indicator tube (MGIT) 960 (Beckton Dickinson, Maryland, USA) an automated culture system for diagnosis of *Mycobacterium tuberculosis*. Clinical specimens were collected from suspected TB adult and paediatric patients from a Revised National Tuberculosis Control Programme (RNTCP), Emmaus Swiss Hospital-Palamaner, Andhra Pradesh between May and October 2006. Ethical clearance for the study was obtained from institutional ethical review board of St John’s National Academy of Health Sciences, Bangalore.

A total of 72 sputum samples obtained from patients suspected to have pulmonary tuberculosis were decontaminated by N-acetylcysteine sodium hydroxide method\(^8\). Decontaminated samples were inoculated onto Lowenstein-Jensen (L-J) agar and MGIT 960 tubes\(^9\). The L-J cultures were incubated at 37°C in 5 per cent CO\(_2\) for 10 wk, and were periodically examined for growth according to existing standards\(^10,11\). Specimen processing and inoculation for the MGIT 960 was done as per manufacturer’s instructions\(^12\).

A further aliquot of decontaminated sputum was processed for PCR. The COBAS TaqMan assay was performed as per the manufacturer’s instructions employing a primer set specific for the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*) 16S r RNA gene. PCR amplification was used to amplify and taqman probe chemistry was employed to detect target DNA and internal control DNA (added to the sample prior to the DNA extraction step) using dual fluorescent dye-labelled oligonucleotide probes\(^11\). Additionally this test utilizes negative and positive sample controls in addition to the internal control. Internal controls are especially important when using sputum samples where enzyme inhibitors may be present in abundance. Amplification product was detected using TaqMan probe chemistry and AMPLILINK software version 3.2\(^11\).

Of the 72 specimens tested, 35 were positive and 37 were negative by L-J culture. All isolates identified as *M. tuberculosis* were confirmed by Genotype MTBC assay (Hain Life Sciences, Germany). The performance of the COBAS TaqMan assay was assessed against culture using L-J and MGIT\(^12,13\). Figures were compiled and values were calculated according by standard methods (Table)\(^14,15\). Higurashi et al\(^16\) evaluated COBAS TaqMan 48 with an assay sensitivity of 58.8 per cent. Broccolo et al\(^17\) reported the use of Taqman chemistry for the quantitative identification IS 6110 gene segment and on *senX3-regX3* intergenic region with 98, 97 and 94, 100 per cent of sensitivity and specificity respectively\(^17\).

The analytical specificity of the COBAS TaqMan MTB test was further evaluated by using *M. tuberculosis* H37Rv ATCC 27294, *M. bovis* BCG strain, ATCC *M. fortuitum* 6841, and ATCC *M. kansasii* 12478, and was found to be good. The efficiency of the COBAS TaqMan MTB test was established by determining the minimum number of *M. tuberculosis* colony-forming units (cfu) which would give a positive signal. The broad limit of
detection determined by us for the COBAS TaqMan MTB test was $5 \times 10^2$ cfu/ml.

The performance of the COBAS TaqMan MTB test was comparable with the L-J culture and MGIT 960 in its performance as a diagnostic test in our study. The COBAS TaqMan MTB assay is highly specific and rapid for routine use in a clinical diagnostic laboratory. The easy processing, fast reporting and relative lack of contamination issues (which were intrinsic to earlier assay systems) with the COBAS TaqMan MTB assay make it worthy of consideration as a possible replacement to culture techniques, a rapid screen or even a screen used to select specimens for culture which is more laborious. However, further evaluation is required with a larger and varied sample for its appropriate use in clinical practice.

Acknowledgment

Authors thank Roche Molecular Systems for supplying the COBAS TaqMan MTB kits, and SETRI Mycobacteriology Laboratory for providing clinical samples.

Conflicts of interest: Authors declare that there is no conflicts of interest.

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Table. Comparison of direct smear, MGIT culture and COBAS TaqMan MTB assay with L-J culture

<table>
<thead>
<tr>
<th></th>
<th>L-J culture positive (n=35)</th>
<th>L-J culture negative (n=37)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear positive</td>
<td>28</td>
<td>5</td>
<td>77.7</td>
<td>86.1</td>
<td>84.9</td>
<td>79.4</td>
</tr>
<tr>
<td>Smear negative</td>
<td>5</td>
<td>31</td>
<td></td>
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<tr>
<td>MGIT positive</td>
<td>32</td>
<td>2</td>
<td>94.1</td>
<td>92.1</td>
<td>94.1</td>
<td>94.6</td>
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<tr>
<td>MGIT negative</td>
<td>3</td>
<td>35</td>
<td></td>
<td></td>
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<tr>
<td>COBAS Taqman positive</td>
<td>35</td>
<td>1</td>
<td>97.2</td>
<td>100</td>
<td>100</td>
<td>97.2</td>
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<tr>
<td>COBAS TM negative</td>
<td>0</td>
<td>36</td>
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</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value; L-J, Lowenstein-Jensen


