Evaluation of the Capilia TB assay for culture confirmation of Mycobacterium tuberculosis in Zambia and South Africa

Running title: Capilia TB Assay evaluation

*Monde Muyoyeta¹, Petra E. W. de Haas¹,², Dirk H. Mueller², Paul D. van Helden³, Lawrence Mwenge¹, Ab Schaap¹,², Clarissa Kruger³, Nicolaas C. Gey van Pittius³, Katherine Lawrence⁴, Nulda Beyers⁴, Peter Godfrey-Faussett², Helen Ayles¹,²

¹Zambia AIDS Related Tuberculosis (ZAMBART), University of Zambia School of Medicine, Lusaka Zambia
²London School of Hygiene and Tropical Medicine, University of London, London, UK
³DST/NRF Centre of Excellence for Biomedical TB Research/ US/MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Health Sciences - Stellenbosch University, Tygerberg, South Africa
⁴Desmond Tutu TB Centre, Stellenbosch University, Tygerberg, South Africa

*ZAMBART Project,
Ridgeway Campus,
University of Zambia
P.O. Box 50697
Lusaka, Zambia
Tel: +260 21 1254710
Fax: +260 21 1257215
Email: monde@zambart.org.zm

Word Count (Main body Excl references, acknowledgements) 1,271

Key words: Capilia, MPB64, Niacin, Mycobacteria culture, MGIT, cost, Zambia, South Africa
Abstract

The performance and cost of the Capilia TB assay was evaluated when used in a resource limited setting. The sensitivity and specificity were 98.7% and 99.5%, respectively. The incremental costs of the Capilia test were estimated to be USD 1.46 and 1.84 when added to liquid and solid culture processes, respectively. These findings suggest that the Capilia TB assay represents a rapid, simple and inexpensive MTB identification test, which can be used in resource limited settings.
There is a push for better diagnostic tools for tuberculosis (TB) to be made available in resource limited settings. The use of culture for routine diagnosis of tuberculosis is being encouraged. (19) In response to this, studies have been funded to evaluate new laboratory techniques, which are quicker and more sensitive for detection of TB from clinical samples. (13) Laboratory mycobacterial culture isolates have to be further identified as *Mycobacterium tuberculosis* (*Mtbt*) or non tuberculous mycobacteria (NTM) by amongst others, phenotypic, biochemical and molecular techniques. Molecular techniques are less practical for use in resource limited settings because they are expensive and technologically complex needing specialised equipment, good quality control practices and specially trained personnel. Phenotypic and biochemical tests are slow to yield results as they sometimes require setting up of subcultures, which take weeks to grow and also require experienced staff to interpret the results. The Capilia TB assay is a lateral flow immunochromatographic assay that detects MPB64 antigen in *Mtbt* culture isolates. This assay does not require specialised equipment, is quick to yield results and has been shown to be highly sensitive and specific for identification of *Mtbt* from culture isolates. (1, 6, 12, 16, 18) The Capilia TB assay was evaluated and compared to the Genotype Mycobacterium CM (GTM-CM) and niacin test in resource limited settings. The GTM-CM assay is a DNA strip assay for rapid identification of mycobacterial culture isolates.

Samples used for this evaluation were collected as part of a community based TB prevalence survey in Zambia and South Africa. (3) Samples were processed for culture according to standard laboratory procedures (9, 14) and were inoculated on Mycobacterium Growth Indicator Tube (MGIT™) (BD) and Lowenstein Jensen (LJ) media (BD). Once growth was detected and confirmed to have acid-fast bacilli (AFB) present by Ziehl-Neelsen stain (ZN), an aliquot of the culture was archived in 7H10 broth with 20% glycerol at -20°C. A subculture was set up for Niacin strip test (Remel™). The Genotype Mycobacterium CM (GTM-CM) (Hain lifescience, Nehren Germany) was done on subcultures of all primary archived cultures confirmed to have AFB present. The Capilia TB assay (Capilia® TB-TAUNS Laboratories, Inc) was done on live primary cultures, repeated on archives samples and subcultures. An aliquot of 100uL from each MGIT was placed in the sample well of the Capilia assay strip and read after 15 minutes according to the manufacturer’s recommendations. To isolate DNA, a 100uL aliquot of culture isolate from the MGIT was heat killed at 95% for 20 minutes. Before use, the sample was centrifuged and finally 5uL of the supernatant was used for PCR. The
GTM-CM was done according to the manufacturer’s standards. The economic costs and cost effectiveness were estimated as part of a separate study in Zambia. Costs were established primarily by expenditure reviews and observations of culture processing procedures. The incremental costs per test as well as the incremental costs per correct test result for Niacin strip test and the Capilia TB assay were compared. Sequencing of the Capilia TB assay false negative isolate was performed using the following primers: U30 (5’-GTCAGGCATCGTCGTCAGC), U404 (TCCACCGAAGAAGCCCCCTAC), L433 (5’TGGTATGTGGCCGAGGTGA) and L891 (5-CAGTGGCGCACCGAACAC). Sequencing reactions were performed and analysed using an ABI Prism 3100 capillary sequencer (Applied Biosystems, Forster City, CA, USA).

Compared to the GTM-CM assay, the sensitivity and specificity of the Capilia TB assay was 98.5% and 99.6% respectively. On the other hand, the Niacin strip test had a sensitivity of 88.1% and a specificity of 89.2%. Irrespective of the culture technique used, cultures were less costly when results were confirmed by the Capilia than by Niacin tests (Table 2). The overall costs per culture varied between USD28 and 32 depending on the culture method used, translating into approximately USD 155 and 274 per positive culture. The incremental cost per test of Niacin strip test showed costs of USD 7.26 and 7.35 for LJ and MGIT respectively. The incremental cost per test of the Capilia TB assay was calculated to be USD 1.84 and 1.46 per test for LJ and MGIT cultures, respectively, based on prices of Capilia negotiated by FIND for resource poor settings. If the current list price is applied, the incremental cost per Capilia TB assay (USD 3.85 and 3.47, respectively) is approximately half of the cost per Niacin strip test.

As the role of culture becomes increasingly important for TB control in resource limited settings, so does the need for a rapid, simple and inexpensive identification test for the mycobacterial culture isolates. The Capilia TB assay may be such a test. The MGIT culture system is a sensitive method for isolation of mycobacteria and shortens the time to detection of growth of mycobacteria compared to traditional solid media. (2, 4, 5, 8, 11, 15, 17) These systems have the potential to reduce the delay in diagnosis of TB, as well as identification of drug resistance cases of TB. However, without a rapid, accurate test for identification of culture isolates the purpose of introducing these systems in resource limited settings will be defeated.
The Capilia TB assay is a quick and easy to use test to differentiate between Mtb complex and NTM culture isolates,(1, 6, 18) and can be performed directly on isolates or on stored isolates. Our evaluation confirms what has been shown elsewhere, with the sensitivity and specificity all approaching 100%. The Niacin strip test showed low sensitivity and specificity in our hands. The Niacin test is not recommended to be used as a stand-alone test because some strains of NTM species are known to give false positive results.(9) The Niacin test can also give false negative results in cases of mixed cultures.(1) These factors all point to the need to use the Niacin test in conjunction with other tests, but in reality, Niacin may be the only test available in many resource limited settings. Using microscopic morphology may improve the sensitivity of mycobacterial culture isolates identification tests (16), but this requires well trained and experienced personnel to interpret microscopic features and labour costs will be high. However, evaluated under the same conditions, the Capilia TB assay performs better than the Niacin test and the results of this test are comparable to evaluations done in more sophisticated laboratories. The Capilia TB assay also proved to be less costly than the Niacin strip test when added to either solid (LJ) or liquid (MGIT) culture systems with a difference of at least USD5.42 per test, comparable to findings by Ngamlert a et al. (12) This difference is to a great extent due to staff time and the amount of consumables needed to subculture before a Niacin strip test can be performed.

In our study, the Capilia TB assay gave a false negative result for three one Mtb. complex culture isolate when using the Genotype Mycobacterium CM assay as gold standard. This isolate had a CG-insertion in the coding region of the mpb64 gene resulting in a frame shift mutation and consequently a truncated protein (Table 1). Mutations of the mpb64 gene that result in false negative results have been described before. (6, 7) Two false negative isolates were not sequenced. Two NTM isolates gave false positive results for the Capilia TB assay. Although it was not detected in the Genotype assay, it is possible that these isolates had low levels of Mtb present in the isolate which was outgrown by the NTM during culture, but still enough to produce an mpb64 reaction.(7)

The use of liquid culture systems together with a rapid, simple and inexpensive identification test has potential to contribute towards reducing diagnostic delay, and most importantly quicker identification of drug resistant TB cases. The roll-out of these techniques should be done with investment in laboratory infrastructure and human resource training with safety measures taken into consideration for handling mycobacterial culture suspensions.
Table 1: Comparison of results obtained by Capilia TB assay, Niacin strip test and GTM-CM (Zambia & South Africa)

<table>
<thead>
<tr>
<th>NTM Identified</th>
<th>GTM-CM</th>
<th>Capilia</th>
<th>Niacin</th>
<th>No. of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Mtb. complex 2246</td>
<td>223</td>
<td>1</td>
<td>193</td>
<td>26</td>
</tr>
<tr>
<td>M. intracellular 177</td>
<td>-</td>
<td>177</td>
<td>6</td>
<td>132</td>
</tr>
<tr>
<td>M. scrofulaceum 35</td>
<td>-</td>
<td>35</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>M. fortuitum 35</td>
<td>-</td>
<td>35</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>M. gordonae 11</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>M. interjectum 7</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>M. peregrinum 5</td>
<td>-</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>M. kansasi 2</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>M. malmoense 1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>M. avium 3</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>M. abscessus 1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Unidentified NTM 115</td>
<td>1</td>
<td>114</td>
<td>18</td>
<td>79</td>
</tr>
</tbody>
</table>

Table 2: Incremental cost for Niacin strip test and Capilia TB assay (USD) when added to LJ and automated MGIT (MGIT 960), Zambia Only

<table>
<thead>
<tr>
<th></th>
<th>LJ (BD)</th>
<th>MGIT 960 (BD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cost per test</td>
<td>cost per correct identification</td>
</tr>
<tr>
<td>Capilia</td>
<td>1.84</td>
<td>1.85</td>
</tr>
<tr>
<td>Niacin</td>
<td>7.26</td>
<td>9.56</td>
</tr>
</tbody>
</table>

Acknowledgements

This study was supported by the Foundation for Innovative New Diagnostics (FIND). Further support was received through a subcontract from Johns Hopkins University with funds provided by Grant NO. 19790.01 from the Bill and Melinda Gates Foundation. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of FIND or the Bill and Melinda Gates Foundation. We are grateful to the Zambian Ministry of Health and the staff of CDL for their support.
The authors would also like to acknowledge Winnie Mwanza and Pike Mwamba for the laboratory work. We also thank Dr. Hillemann from the TB research Centre in Borstal for the Mpb64 sequence work done.

Conflict of interest statement

We declare that we have no conflict of interest.

Role of the funding source

Funding was received from the source acknowledged above. None of the funding sources had an influence on the design, analysis and content of the presented material.
Reference:


