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Evaluation of the Capilia TB Assay for Culture Confirmation of *Mycobacterium tuberculosis* Infections in Zambia and South Africa▽

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The performance and cost of the Capilia TB assay were evaluated for use in a resource-limited setting. The sensitivity and specificity were 99.6% and 99.5%, respectively. The incremental costs of the Capilia test were estimated to be $1.46 and $1.84 when the test was added to liquid and solid culture processes, respectively. These findings suggest that the Capilia TB assay represents a rapid, simple, and inexpensive *Mycobacterium tuberculosis* identification test that 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 that 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* or nontuberculous mycobacteria (NTM) by, among others, phenotypic, biochemical, and molecular techniques. Molecular techniques are less practical for use in resource-limited settings because these techniques are expensive and technologically complex, needing specialized equipment, good quality control practices, and specially trained personnel. Phenotypic and biochemical tests are slow to yield results, as these tests 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 the MPB64 antigen in *M. tuberculosis* culture isolates. This assay does not require specialized equipment, is quick to yield results, and has been shown to be highly sensitive and specific for identification of *M. tuberculosis* from culture isolates (1, 6, 12, 16, 18). The Capilia TB assay was evaluated and compared to the GenoType Mycobacterium CM (GTM-CM) assay and the 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 tubes (MGIT) (BD) and Lowenstein-Jensen (LJ) medium (BD). Once growth was detected and the culture confirmed to have acid-fast bacilli (AFB) present by Ziehl-Neelsen (ZN) staining, an aliquot of the culture was archived in 7H10 broth with 20% glycerol at −20°C. A subculture was set up for the niacin strip test (Remel). The GenoType Mycobacterium CM (GTM-CM) assay (Hain Lifescience, Nehren, Germany) was done on subcultures of all primary archived cultures confirmed to have AFB present. The Capilia TB assay (TAUNS Laboratories, Inc.) was done on live primary cultures and repeated on archived samples and subcultures. An aliquot of 100 μl from each MGIT was placed in the sample well of the Capilia assay strip and read after 15 min according to the manufacturer's recommendations. To isolate DNA, a 100-μl aliquot of the culture isolate from the MGIT was heat killed at 95°C for 20 min. Before use, the sample was centrifuged, and finally, 5 μl of the supernatant was used for PCR. The GTM-CM assay was done according to the manufacturer's standards. The economic costs and cost-effectiveness were estimated as part of a separate study in Zambia (10). 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 the niacin strip test and the Capilia TB assay were compared. Sequencing of the isolate for which the Capilia TB assay gave a false-negative result was performed using the following primers: U30 (5′-GTCAGGC ATCGTCGTCAGC), U404 (5′-TCCACGCGAAGAAGCCC CCTAC), L433 (5′-TGGTATGTGGCCGAGGTGA), and L891 (5′-CAGTGGCGCACCGAACAC). Sequencing reac-

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TABLE 1. Incremental costs for the niacin strip test and the Capilia TB assay with the use of LJ medium and an automated MGIT (MGIT 960) for Zambia only

Test	Cost ($) for use of test with:			
	LJ medium (BD)		MGIT 960 (BD)	
	Per test	Per correct identification	Per test	Per correct identification
Capilia TB assay	1.84	1.85	1.46	1.47
Niacin strip test	7.26	9.56	7.35	9.68

tions were performed and analyzed using an ABI Prism 3100 capillary sequencer (Applied Biosystems, Foster City, CA).

Compared to those of the GTM-CM assay, the sensitivity and specificity of the Capilia TB assay were 99.6% and 99.5%, 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 the results were confirmed by the Capilia TB assay than when they were confirmed by niacin tests (Table 1). The overall costs per culture varied between $28 and $32, depending on the culture method used (10), translating into approximately $155 and $274 per positive culture. The incremental costs per test of the niacin strip test were $7.26 and $7.35 for LJ and MGIT cultures, respectively. The incremental costs per test of the Capilia TB assay were calculated to be $1.84 and $1.46 per test for LJ and MGIT cultures, respectively, based on the prices of Capilia as negotiated by FIND for resource-poor settings. If the current list price is applied, the incremental costs per Capilia TB assay ($3.85 and $3.47 for LJ and MGIT cultures, respectively) are approximately half of the costs 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 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 required for detection of growth of mycobacteria in comparison to the time required with the use of 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 the delay in identification of drug-resistant 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 for differentiation between the *M. tuberculosis* 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 each approaching 100%. The niacin strip test showed low sensitivity and specificity in our hands. The niacin test is not recommended for use 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, the niacin test may be the only test available in many resource-limited settings. Using microscopic morphology may improve the sensitivity of mycobacterial culture isolate identification tests (16), but this requires well-trained and experienced personnel to interpret microscopic features, and labor costs will be high. However, when evaluated under the same conditions, the Capilia TB assay was found to perform better than the niacin test, and the results of this test were comparable to those obtained from 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 $5.42 per

TABLE 2. Comparison of results obtained by the Capilia TB assay, the niacin strip test, and the GTM-CM assay for Zambia and South Africa

Mycobacterial species (no. of isolates identified by the GTM-CM assay)	No. of isolates				
	Capilia TB assay		Niacin strip test		
	Positive	Negative	Positive	Negative	No growth
M. tuberculosis complex (224)	223	1	193	26	4
M. intracellulare (177)		177	6	132	36
M. scrofulaceum (35)		35	2	28	5
M. fortuitum (35)		35	6	26	3
M. gordonae (11)	1	10	1	10	
M. interjectum (7)		7		5	2
M. peregrinum (5)		5	2	3	
M. kansasii (2)		2		2	
M. malmoense (1)		1		1	
M. avium (3)		3		2	1
M. abscessus (1)		1		1	
Unidentified NTM (115)[a]	1	114	18	79	18
Mixed cultures					
M. tuberculosis and *M. intracellulare* (1)	1			1	
M. fortuitum and unidentified NTM (5)		5	1	4	
M. peregrinum and unidentified NTM (1)		1	1		

[a] NTM groups identified by the GTM-CM: *Mycobacterium avium*, *M. chelonae*, *M. abscessus*, *M. fortuitum*, *M. gordonae*, *M. intracellulare*, *M. scrofulaceum*, *M. kansasii*, *M. malmoense*, *M. marinum*, the *M. tuberculosis* complex, *M. peregrinum*, *M. xenopi*, and *M. interjectum*.

test, comparable to findings by Ngamlert et al. (12). This difference is, to a great extent, due to staff time and the amount of consumables needed for subculture before a niacin strip test can be performed.

In our study, the Capilia TB assay was found to give a false-negative result for one *M. tuberculosis* complex culture isolate when the GenoType Mycobacterium CM assay was used as the gold standard. This isolate had a CG insertion in the coding region of the *mpb64* gene, resulting in a frameshift mutation and consequently a truncated protein (Table 2). Mutations of the *mpb64* gene that result in false-negative results have been described before (6, 7). Two NTM isolates gave false-positive results by the Capilia TB assay. Although *M. tuberculosis* was not detected in these isolates in the GenoType assay, it is possible that these isolates had low levels of *M. tuberculosis* present in the isolate which were exceeded by the NTM levels during culture but still high enough to produce an *mpb64* reaction (1).

The use of liquid culture systems together with a rapid, simple, and inexpensive identification test has the potential to contribute toward reduction of 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.

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