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Diagnostic performance and costs of Capilia TB for *Mycobacterium tuberculosis* complex identification from broth-based culture in Bangkok, Thailand

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Summary

OBJECTIVES Broth-based culture (BBC) systems are increasingly being used to detect *Mycobacterium tuberculosis* complex (MTBC) in resource-limited. We evaluated the performance, time to detection and cost of the Capilia TB identification test from broth cultures positive for acid-fast bacilli (AFB) in Thailand.

METHODS From October–December 2007, broth cultures that grew AFB from specimens submitted by district TB clinics to the Bangkok city laboratory were tested for MTBC using Capilia TB and standard biochemical tests. Isolates that were identified as MTBC by biochemical tests but not by Capilia TB underwent repeat testing using Capilia TB, Accuprobe (Gen-Probe, San Diego, CA, USA) and sequencing. Costs of time, labour, infrastructure and consumables for all procedures were measured.

RESULTS Of 247 isolates evaluated, the sensitivity of Capilia TB was 97% and its true specificity 100% compared with biochemical testing. The median time from specimen receipt to confirmed MTBC identification was 20 days (range 7–53 days) for Capilia TB and 45 days (range 35–79 days) for biochemical testing ($P < 0.01$). Six isolates that were Capilia TB negative but positive by biochemical testing were confirmed as MTBC and mutations in the *mpb64* gene were detected in all. The unit cost of using Capilia TB was 2.67 USD that of biochemical testing was 8.78 USD.

CONCLUSIONS In Thailand, Capilia TB had acceptable sensitivity and specificity, was lower in cost and had shorter turn-around times. Laboratories investing in BBC should consider Capilia TB for identification of MTBC, after validation of performance in their setting.

keywords tuberculosis, Thailand, culture, identification, costs, diagnosis

Background

The global tuberculosis (TB) epidemic and recent emergence of multidrug (MDR) and extensively drug resistant (XDR) strains has accentuated the need for mycobacterial culture in resource-limited, high TB burden settings (WHO 2007). In these settings, most TB patients are diagnosed based on the results of sputum acid-fast bacilli (AFB) microscopy. In facilities that can perform culture, classic egg-based methods (Ogawa slants and/or Lowenstein–Jensen (LJ) slants) are usually used, but laboratories are increasingly implementing rapid broth-based culture (BBC) systems, facilitated by a recent World Health Organization endorsement and a reduction in the costs of equipment and supplies (Stop TB 2007). BBC systems are faster and more sensitive than egg-based media for detecting *Mycobacterium tuberculosis* complex (MTBC) and potentially pathogenic non-tuberculous mycobacteria (NTM) (Palacios *et al.* 1999; Chien *et al.* 2000; Srisuwanvilai *et al.* 2008).

Although BBC systems increase the speed and recovery rate of mycobacteria, laboratories are unable to report results to clinicians and unable to proceed to drug-susceptibility testing (DST) until the isolate is identified as MTBC. Rapid identification methods, such as DNA probes and nucleic acid amplification (NAA) are effective, but prices for commercial tests remain prohibitively expensive for routine use in resource-limited settings (Pai *et al.* 2006; Perkins & Cunningham 2007). As a result, most laboratories in resource-limited settings use biochemical tests to identify MTBC, which requires subculturing of mycobacteria from

broth onto solid media and several days or weeks of incubation until sufficient growth is observed. This process increases the turn-around-time for reporting positive results, potentially negating the speed of broth-based culture.

The Capilia TB Test Kit (TAUNS, Numazu, Japan) is a rapid, low technology method for differentiating MTBC from NTM. This immunochromatographic assay uses a monoclonal antibody to detect MPB64, one of the predominant proteins secreted by MTBC strains during culture (Nagai *et al.* 1991; Abe *et al.* 1999; Hasegawa *et al.* 2002). The test is performed directly from positive cultures (from broth or from re-suspended colonies from solid media) and, unlike NAA, does not require additional equipment or laboratory bench space. Test results are available 15 min after inoculation of the test cartridge, with a positive result indicated by a purple–reddish line in reading areas of the cartridge. Capilia TB is a promising test, because it provides rapid confirmation of MTBC, which decreases the turn-around-time for biochemical identification with minimal additional human or laboratory resources.

In this study, we evaluated the test performance and costs of Capilia TB in Thailand. Although Capilia TB has been validated in several laboratories, false negative results due to unique mutations in the *mpb64* gene have been reported, which has necessitated validation in diverse settings (Hasegawa *et al.* 2002, 2003; Hirano *et al.* 2004; Hillemann *et al.* 2005; Wang *et al.* 2007). Reagent costs are presumed to be low for both biochemical testing and Capilia TB, but the cost for the two methods have not been compared directly, an essential step in convincing laboratory personnel to change existing practice. Validation studies should also be conducted in public TB laboratories to compare the real-world performance. In the Bangkok Metropolitan Administration Health Division Laboratory we assessed sensitivity, specificity, time to detection and costs of Capilia TB compared with biochemical identification.

Methods

Mycobacterial culture and identification

From October–December 2007, we evaluated sputum specimens that were submitted by district TB clinics to the Bangkok Metropolitan Administration Health Division Laboratory for routine mycobacterial culture and susceptibility testing. Specimens were homogenized and decontaminated by the Nalc-4%NaOH-2.9% citrate method (final concentration of NaOH 1%). After vortexing and incubation at room temperature for 15 min, specimens were concentrated at 3000 ***g*** for 15 min, decanted completely and re-suspended using 2 ml (pH 6.8) phosphate buffer. Approximately 100 µl of the suspension was used to inoculate two LJ slants; one *Mycobacterium* growth indicator tube (MGIT) (BACTEC MGIT 960, Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) was inoculated using 500 µl; and a concentrated smear was prepared. LJ slants were evaluated weekly for 8 weeks; broth cultures were incubated using the MGIT 960 instrument for 6 weeks.

All broth cultures flagged by the M6IT960 as positive were removed and a portion stained for the presence of AFB using the Ziehl–Neelsen method. Once confirmed as positive for AFB, broth cultures were subcultured onto two LJ slants for niacin accumulation and nitrate reduction testing and onto one LJ slant containing para-nitrobenzoic acid (PNB). These techniques were performed using methods previously described (Kent & Kubica 1985; WHO 1998). The Capilia TB assay was performed by placing 100 µl from a broth culture onto the specimen placement area of the Capilia TB cartridge, allowing a maximum of 15 min incubation and observing a purple–reddish colour change in the test area. Because this study involved evaluating Capilia TB during routine laboratory workflow, consecutively positive broth cultures were used. Results of biochemical testing were reported to clinicians according to routine practice, but not results of Capilia TB.

Evaluation of discordant results

Discordant identification results were investigated by performing the Accuprobe–*M.tuberculosis* complex culture confirmation test (Gen-Probe) on growth from positive broth cultures. In addition, we used frozen aliquots from processed clinical specimens that yielded the discrepant results to re-inoculate MGIT and LJ for repeat Capilia TB testing.

We sequenced the *mpb64* gene of all isolates that were MTBC positive by biochemical methods and Capilia TB negative at the Centers for Disease Control and Prevention (Atlanta, USA). A DNA fragment including the open reading frame of the *mpb64* gene was PCR amplified using the primers AMS-50-F (5′-TCGATCTGCTAGCTTGAGTCTGGT-3′) and AMS-51-R (5′-ACCACCGCACCAAGGCTGTCTA -3′). Sequencing reactions were performed using the same primer set and analysed using the Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). DNA sequences of the *mpb64* gene from each MTBC isolate were compared with that of the laboratory strain H37Rv using EditSeq and SeqMan from the DNASTAR Lasergene 7.2 software package (DNASTAR, Madison, WI, USA). After sequencing the *mpb64* gene, and identifying a common mutation, we used

spoligotyping and mycobacterial interspersed repetitue unit-variable-number tandem repeat analysis (MIRU-VNTR) to rule out the possibility of cross-contamination of the processed specimen, using methods previously described (Cowan *et al.* 2004, 2005).

Economic evaluation

Indirect and direct costs associated with TB case finding using primary culture (LJ and MGIT) and biochemical identification methods were collected at the Bangkok Metropolitan Administration Health Division Laboratory from November 2007–December 2007. Unit costs for all diagnostic methods evaluated were estimated based on the routine diagnostic scheme implemented at the site, and all economic costs associated with each system were analysed using a health services perspective (Drummond *et al.* 1997; TanTan-Torres *et al.* 2003). Usage of capital assets, e.g., building space and equipment, and staff were quantified as minutes used per square metre of space and minutes worked, and were based on overall capacity, expressed as the number of specimens processed during the time observed. Laboratory consumables and chemicals were quantified based on relevant units (e.g. units, pieces, metres, grams, millilitres). This analysis did not include staff time between tests, wastage of consumables, or unused equipment capacity. All capital and recurrent costs were measured from the time a specimen arrived at the laboratory until the time that test results were obtained; therefore, costs associated with the laboratory only were measured. Unit costs were calculated using an ingredients approach, which involves multiplying the quantity of inputs used by price. Capital costs, including laboratory space, building and equipment, were annualized based on their estimated expected life–years. Overhead costs were calculated by allocating overall laboratory expenditures for each test, based on the number of staff and staff time and, for those costs related to physical infrastructure, the building space utilized by each diagnostic system.

We expressed all prices in US dollars. Prices available in the local currency were converted into the US dollar based on the average local currency exchange rate in 2007. Prices were obtained directly from the laboratory's procurement logs or, when necessary, a company catalogue. For our analysis, we used the estimated unit price of Capilia TB based on deep discounts negotiated by the Foundation for Innovative New Diagnostics (FIND), as the actual market unit price is not yet established for this product. To estimate the unit cost of using Capilia TB from solid cultures, we estimated timings and resource usage as suggested by the manufacturer and via comparative assessment of actual timings and resource usage of similar preparatory procedures (e.g. preparation of bacterial suspension for drug susceptibility testing).

Ethical review

This project underwent formal ethical review at CDC and the Bangkok Metropolitan Administration. It was determined to represent a public health programme evaluation, not requiring individual informed consent.

Results

Diagnostic performance

Of 247 isolates from BBC evaluated, Capilia TB correctly identified 226 isolates as MTBC, and 14 isolates as NTM (97%), using the results of biochemical testing as the gold standard (Table 1). The overall sensitivity and specificity of Capilia TB for MTBC identification was 97% and 93%, respectively. Of 232 isolates identified as MTBC by biochemical testing, 226 (97%) were positive by Capilia TB. Of the 15 isolates identified as NTM by biochemical testing, one (7%) was positive by Capilia TB. Further investigation by Accuprobe and re-culture identified this as a mixed NTM and MTBC culture, which means that the true specificity of Capilia TB was 100% (Table 2).

The median time from specimen receipt in the laboratory and identification of MTBC was 20 days (range 7–53 days) for Capilia TB compared with 45 days (range 35–79 days) for the complete battery of biochemical tests, 33 days (range 18–67) for niacin/nitrate alone and 45 days (range 35–79) for PNB alone. Comparing Capilia TB with all methods (niacin/nitrate or PNB alone or all biochemical test), the time to confirmed identification of MTBC was statistically significantly shorter ($P < 0.01$).

Discordant results

Six isolates tested negative for MTBC by Capilia TB but positive by biochemical testing. These isolates had other

Table 1 Identification of *Mycobacterium tuberculosis* complex (MTBC) from acid-fast bacilli positive isolates from broth-based culture using biochemical methods and the Capilia TB assay

Capilia TB result	Biochemical result		
	MTBC	NTM	Total
Positive	226	1*	227
Negative	6	14	20
Total	232	15	247

*Isolate was identified as MTBC by Accuprobe.

Table 2 Evaluation of isolates with discordant results on Capilia TB and biochemical testing by nucleic acid hybridization, repeat culture and Capilia TB testing, and genetic sequencing

No.	Initial biochemical testing	Initial Capilia TB result	Accuprobe	Repeat Capilia TB from re-inoculated MGIT	Repeat Capilia TB from LJ subculture	Mutation type and position	Spoligotype	MIRU-VNTR
50	MTBC	Negative	MTBC	Negative	Negative	2-bp insertion at 436	000000000003771	223325173543
125	MTBC	Negative	MTBC	Negative	Positive*	63-bp deletion at 196	777777757760731	224125113322
217	NTM	Positive	MTBC	Positive	Positive	N/A	N/A	N/A
288	MTBC	Negative	MTBC	Negative	Positive*	63-bp deletion at 196	777777777760771	2x5125113222
315	MTBC	Negative	MTBC	Negative	Negative	63-bp deletion at 196	777777777760771	224115113423
341	MTBC	Negative	MTBC	Negative	Negative	63-bp deletion at 196	777777777760731	224125113322
355	MTBC	Negative	MTBC	Negative	Positive*	63-bp deletion at 196	777777777760731	225125113322

*Weakly positive. MTBC, *Mycobacterium tuberculosis* complex.

classic features of MTBC including cord formation on AFB smear from MGIT and a rough, buff colony morphology. The discordant results were re-tested by performing Accuprobe, repeating Capilia TB from newly inoculated positive MGIT cultures and repeating Capilia TB from colonies taken from solid media (Table 2). All six isolates were confirmed as MTBC by Accuprobe. Repeat Capilia TB testing from positive MGIT cultures yielded negative results for all six; repeat Capilia TB testing from the subcultures, however, yielded three weakly positive results of the six subcultures tested. Mutations in the *mpb64* gene were detected by molecular sequencing for all six isolates. A 63bp deletion in the *mpb64* open reading frame, resulting in deletion of 21 amino acids (amino acids 66–86) from the MPB64 protein was observed in five isolates; the sixth isolate contained a 2-bp insertion of 'TC' at bp 436, resulting in a frame-shift mutation and subsequent truncation of the protein. To exclude the possibility of cross-contamination or false positive culture results, spoligotyping and MIRU-VNTR testing were also performed. Each isolate had a unique genotype.

Economic analysis

The total unit cost of performing Capilia TB on one MGIT positive specimen was 2.67 USD, of which 0.67 USD was associated with the use of laboratory infrastructure, building space, staff time and other consumables (Table 3). In comparison, the total unit cost of performing Capilia TB on one solid culture specimen was 3.46 USD, the increase being attributable to preparation of a bacterial suspension. This figure does not include the actual costs of the kit required to prepare the bacterial suspension, because this is currently not sold on the open market. The average unit cost for conventional biochemical assays for species identification including PNB inoculation was 8.78 USD per test, almost three times the cost of the Capilia TB method.

Table 3 Average unit cost of identification methods by biochemical testing (PNB, niacin, nitrate), Capilia TB performed on MGIT and Capilia TB performed on LJ

	Identification method		
Input	Biochemical assay Niacin + Nitrate + PNB	Capilia TB on MGIT culture	Capilia TB on LJ culture
Overhead	1.69	0.14	0.39
Building	0.10	0.01	0.06
Equipment	0.99	0.12	0.24
Staff	0.93	0.06	0.15
Reagent and chemical	2.09	2.00	2.00
Consumables	2.98	0.34	0.62
Total	8.78	2.67	3.46

MGIT, *Mycobacterium* growth indicator tube; PNB, para-nitrobenzoic acid, LJ, Lowenstein–Jensen.

Table 4 shows the overall case finding laboratory costs for LJ culture and MGIT culture with biochemical identification and MGIT culture with Capilia TB identification. Although the cost of performing MGIT culture and biochemical identification was approximately 8.00 USD higher than the cost of LJ culture and biochemical identification, the cost of MGIT culture with Capilia TB was more cost effective than both approaches.

Discussion

Consistent with previous reports, we found that the Capilia TB test for identification of MTBC from broth culture performs with acceptable sensitivity and specificity, accelerates time to MTBC confirmation and saves money

Table 4 Average unit cost per case found using LJ culture with biochemical identification, MGIT culture with biochemical identification and MGIT culture with Capilia TB identification

Input	Case-finding cost using various diagnostic scheme		
	Solid culture with biochemical identification	MGIT with biochemical identification	MGIT with Capilia TB identification
Prepare and decontaminate sputum	1.61	1.61	1.61
Prepare LJ	1.40	1.40	–
Microscopy (ZN)	1.16	1.16	1.16
Subculture to LJ	–	2.05	–
Identification	8.78	8.78	2.67
Culture	3.92	9.53	9.72
Total	16.87	24.38	15.09

MGIT, *Mycobacterium* growth indicator tube; LJ, Lowenstein–Jensen.

compared with biochemical identification in routine public health testing in Thailand. Capilia TB accurately identified one isolate from broth culture, which we would have missed as this isolate was mixed, and was therefore identified as NTM by biochemical methods. While the ability to detect MTBC from mixed broth cultures is a significant advantage of using this method, performing drug susceptibilities on such cultures may lead to the reporting of false resistance. Therefore, isolation of MTBC from mixed cultures is required before proceeding to susceptibility testing.

Interestingly, Capilia TB was not able to identify six positive MGIT cultures that were confirmed as positive for MTBC by biochemical testing and DNA probe. Sequencing identified a 21 amino acid deletion within MPB64 for five of the six discordant isolates. We hypothesize that the mutation disrupted protein folding, impairing detection by the MPB64 monoclonal antibody used in Capilia TB. Three of the five isolates found to contain the 21 amino acid deletion in MPB64 were weakly positive upon repeat testing from solid media. It is possible that the increased biomass from solid media may have overcome the decreased antibody affinity, resulting in a weakly positive signal.

Based on the common mutation identified, we performed spoligotyping and MIRU-VNTR to rule out the possibility of cross contamination between isolates. While we were able to demonstrate that the six isolates had unique genotypes, a relatedness was observed between strains. To determine if the 21 amino acid deletion is correlated with any specific genotype or spoligotype family however, additional studies are needed.

Hirano *et al.* (2004) reported five mutations in the *mpb64* gene, which lead to false negative Capilia TB results. These mutations included a IS*6110* insertion mutation at nucleotide position 501, a single base pair deletion at nucleotide position 266 resulting in a frameshift and creation of a stop codon at nucleotides 278–280 (TGA), and a 63 bp deletion from nucleotides 196–258 (similar to our findings). A point mutation at position 402 (G–A), which resulted in a stop codon at nucleotides 400–402 (TGA), and a deletion of 176 bp from nucleotides 512–687, which resulted in a deletion of C-terminal 58 amino acids from amino acid positions 147–205, were also reported (Hirano *et al.* 2004). Hillemann *et al.* (2005) also reported false negative Capilia TB results due to mutations in the *mpb64* gene. The mutations reported from their setting include a C insertion at position 287, a point mutation at position 388 (A–T), and a disruption of the *mpb64* gene sequence due to an insertion of IS*6110* at position 177 (Hillemann *et al.* 2005). These findings demonstrate the importance of thoroughly evaluating novel TB diagnostic tests in diverse geographic regions, as genotypic variation in strains may impact test performance.

We found that Capilia TB saved both time and costs compared with biochemical identification. On average, results were available for this test within 20 days of specimen receipt, 25 days shorter than for completing a battery of biochemical tests. Because our goal was to compare performance of identification methods, not primary culture methods, we did not disaggregate our results according to smear result. Based on previous experience in our laboratory, time to confirmed identification would be even more rapid for smear-positive specimens (Srisuwanvilai *et al.* 2008). Testing in our project was also batched. The time elapsed between specimen receipt and MTBC identification could be further shortened by performing Capilia TB each day that AFB positive broth cultures are confirmed. Factoring time, labour, infrastructure and consumables, we also found that Capilia TB was more cost effective than biochemical identification, primarily because there was no need to perform LJ subcultures. For laboratories with capacity to perform solid culture only, Capilia TB will also result in a substantial savings (5.32 USD per test) compared with biochemical identification.

One limitation of our study is that we did not evaluate costs associated with other methods of MTBC identification. For example, experienced microbiologists may be able to identify MTBC based on observation of colony morphology and/or cord formation. We chose not to

evaluate this approach, because standard laboratory practice and international guidelines recommend the use of confirmatory tests beyond visual inspection (WHO 1998). In addition, our analysis did not include the cost of performing biochemical tests as a back-up method on Capilia TB negative isolates. This cost should be taken into consideration, given the occurrence of false negative results because of mutations in the *mpb64* gene.

In conclusion, laboratories investing in BBC should consider using Capilia TB for routine MTBC identification, after validation of test performance in their specific country. In Thailand, the test allowed immediate confirmation of MTBC from AFB positive broth cultures, detected MTBC from a mixed broth culture, reduced turn around time to a confirmed result by 3 weeks and proved inexpensive.

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