Evaluating Auto-MODS Assay: A Novel Tool for Tuberculosis Diagnosis for Use in Resource-Limited Settings

Linwei Wang1*, Sohaib H. Mohammad1*, Boonchai Chaiyasirinroje2**, Qiaozhi Li1,
Somsak Rienthong3, Dhanida Rienthong3, Supalert Nedsuwan3, Surakameth Mahasirimongkol2, Yutaka Yasui1

Affiliation: 1- School of Public Health, University of Alberta, Alberta, Canada
2- TB/HIV Research Foundation, Chiang Rai, Thailand
3- Supra-National Tuberculosis Reference Laboratory, Thailand
4- Chiang Rai Regional Hospital, Chiang Rai, Thailand

* These authors contributed equally to this work
+ The corresponding author

Address for Correspondence:
Boonchai Chaiyasirinroje, B.Sc.
1050/1 Satarnpayabarn Rd., Muang District
Chiang Rai 57000, Thailand
Tel: +66-53-713135
Fax: +66-53-752448
Email: boonchai@tbhiv.org

Address for other authors:
Linwei Wang, M.Sc. Candidate
Edmonton Clinic Health Academy 4-051
11405 87 Avenue, Edmonton, Alberta, Canada, T6G 1C9
Email: linwei1@ualberta.ca

Sohaib H. Mohammad, M.Sc. Candidate
Edmonton Clinic Health Academy 4-051
11405 87 Avenue, Edmonton, Alberta, Canada, T6G 1C9
Email: sohaib@ualberta.ca

Qiaozhi Li, PhD
South Academic Building 3-57H
ABSTRACT

Background: There is an urgent need for simple, rapid, and affordable diagnostic tests for tuberculosis (TB) to combat the great burden of the disease in developing countries. The Microscopic Observation Drug Susceptibility (MODS) assay is a promising tool to fill this need, but is not widely used due to concerns regarding its biosafety and efficiency. This study evaluates the Automated-MODS (Auto-MODS), which operates on similar principles as MODS but with several key modifications, making it an appealing alternative to MODS in resource-limited settings.

Methods: In the operational setting of Chiang Rai, Thailand, we compared the performance of Auto-MODS with the gold-standard liquid culture method in Thailand, Mycobacteria Growth Indicator Tube (MGIT) 960, plus SD Bioline TB Ag MPT64 test, in terms of accuracy and efficiency in differentiating TB and non-TB samples as well as distinguishing TB and MDR-TB samples. Sputum samples from both clinically-diagnosed TB and non-TB subjects across 17 hospitals in Chiang Rai were consecutively collected from May 2011 to September 2012.

Findings: A total of 360 samples were available for evaluation, of which 221 (61.4%) were positive and 139 (38.6%) were negative for mycobacteria cultures according to MGIT 960. Of the 221 “true-positive” samples, Auto-MODS reported 212 positive and 9 negative (Sensitivity: 95.9%, 95% confidence interval (CI): 92.4%-98.1%). Of the 139 “true-negative” samples, Auto-MODS reported 135 negative and 4 positive (Specificity: 97.1%, 95% CI: 92.8%-99.2%). The median time to culture positivity was 10 days with an inter-quartile-range of 8-13 days for Auto-MODS.
Interpretation: The Auto-MODS is an effective and cost-sensitive alternative diagnostic tool for TB diagnosis in resource-limited settings.

Funding: Alberta Innovates Centre for Machine Learning (AICML), Canada

Key words: Tuberculosis, multi-drug resistant tuberculosis, diagnosis accuracy, automated-microscopic observation drug susceptibility assay, sensitivity, specificity
INTRODUCTION

Tuberculosis (TB) is an infectious disease of major concern worldwide, with the majority burden borne by developing countries (1). Multi-drug resistant tuberculosis (MDR-TB) is a variant strain of TB that is resistant to at least rifampicin (RIF) and isoniazid (INH), the two most common TB treatments. Thailand is among the highest TB-burdened countries with an estimated 110,000 TB cases (159 cases/100,000 population) in 2012 (1). Thailand also has one of the highest burdens of MDR-TB among Southeast Asian countries (1, 2). Mycobacterial laboratories in Thailand are faced with challenges of meeting the growing demands of the TB and MDR-TB epidemics, and simple, rapid, and affordable tests are pressingly needed.

In the province of Chiang Rai as well as the other provinces in Thailand, Acid-Fast Bacilli (AFB) sputum-smear test is used in combination with chest X-Ray and clinical symptoms to diagnose TB in the hospital setting. Patients diagnosed in this way will be registered as TB patients. This will then be followed by solid mycobacterial culture which requires a long waiting period (approximately one month) to make a more conclusive diagnosis. TB and Nontuberculous Mycobacteria (NTM) are not distinguished in the current practice. Liquid mycobacterial culture, which is faster but much more expensive, is only used for highly suspected TB cases in order to obtain a more definite diagnosis or for drug-susceptibility testing (DST). Mycobacteria Growth Indicator Tube (MGIT) 960 is an industry gold-standard liquid mycobacterial culture method, but due to its higher operational cost, it has limited use in resource-limited settings such as Chiang Rai, Thailand (3).
The microscopic observation drug susceptibility (MODS) assay is a rapid, economical, and highly sensitive/specific method for the detection of *Mycobacterium Tuberculosis* (*M.tb*) and DST directly from a sputum sample which was developed by a research team in Lima, Peru (4). The test uses 24-well plates with four wells for a single patient specimen: two wells are drug free, while the other two wells contain RIF and INH, respectively. After inoculation, the plates are inserted and sealed in zip-lock bags and then incubated. *M.tb* from sputum is rapidly grown in a liquid medium and a diagnosis is made using morphological characterization patterns specific to *M.tb* following inoculation under an inverted light microscope (4). MODS assay has also been recommended by the WHO as an affordable and highly effective alternative to existing gold-standard liquid mycobacterial culture methods for testing sputum samples of TB-suspected individuals (5).

Despite this, MODS is not widely used in resource-limited settings due to concerns regarding biosafety and efficiency for handling large numbers of samples (6). With respect to biosafety, the MODS assay uses liquid wells; manipulation of these liquids for inoculation carries the risk of aerosolization, spillage, cross-contamination, and occupational infection, though sealed plates may minimize this risk (6, 7). MODS also requires manual reading of each individual well by trained laboratory professionals which requires both time and human resources given that a large number of sputum samples may need to be tested in high TB-burdened settings. Moreover, there is also concern about MODS’ ability to differentiate *M.tb* and NTM (7).

In this study, we evaluated the Automated-MODS (Auto-MODS) which was developed by TB/HIV Research Foundation (THRF) in Chiang Rai, Thailand, in collaboration with
University of Alberta, Canada, to address these concerns. We compared the performance of Auto-MODS for TB diagnosis and DST in reference to the gold-standard method used in Thailand: MGIT 960 plus SD Bioline TB Ag MPT64 (SD-Bioline) test, in the real clinical setting of Chiang Rai, Thailand.

**METHODS**

**Ethics**

The protocol of this study has been approved by the Chiang Rai Regional Hospital Ethics Committee, Thailand, and University of Alberta’s Health Research Ethics Board, Canada. The study was approved by the Department of Disease Control, Ministry of Public Health, Thailand.

**Study Patients and Sample Collection**

In the current clinical practice of Chiang Rai, Thailand, patients are diagnosed and registered as TB patients based on the AFB sputum-smear test, chest X-Ray and clinical presentations. Since the diagnosis is made by physicians without mycobacterial culture results, we will refer to it as “clinically diagnosed” as to indicate that clinically diagnosed is not necessarily the true TB status (which is later defined by the gold-standard: MGIT 960 plus SD-Bioline).

Since 1996, clinically diagnosed TB patients in all the 17 hospitals across Chiang Rai, Thailand, are registered and required to send sputum samples to the mycobacterial laboratory of Chiang Rai Regional Hospital. Sputum samples routinely received from May 2011 to March 2012 were consecutively recruited into our study. Additionally, we
collected samples from individuals who visited the same 17 hospitals between June 2012 to September 2012 and who were clinically diagnosed as non-TB patients.

The following inclusion criteria were used: (i) incident TB patients, defined by a clinical diagnosis; (ii) clinically diagnosed non-TB subjects with negative AFB smear test results; (iii) age 18 years and older at clinic visit; and (iv) sputum samples must have been collected prior to, or up to 2 weeks after, the treatment initiation. Sputum samples with an amount less than 1ml, or that arrived at the laboratory without proper packaging, or that were cultured more than one week after arrival, or that from extra pulmonary TB patients were excluded from the study.

**Laboratory Methods**

*Specimen Processing*

To enhance the reliability of sampling and to obtain the most biologically active samples, each patient was asked for 3 sputum samples at three different times within one week. Only one sample was kept for culture in our study: if all 3 sputum samples were AFB smear negative, then the most active AFB smear negative sputum sample (usually the most recently collected one) was kept. Otherwise, the most active AFB smear positive sputum sample was chosen.

Sputum samples were immediately and safely stored after sampling in a refrigerator between 2°C to 8°C and were cultured within 7 days. All sputum samples were decontaminated and inoculated using the sodium-hydroxide-N-Acetyl-L-Cysteine method (8), and were subsequently split into four aliquots, 500 μl, 500 μl, 300 μl, and 100 μl, to
be used for MGIT 960 culture, Auto-MODS culture, Ogawa culture and AFB smear slide, respectively. Detailed sample processing procedure is shown in Supplementary Figure 1.

MGIT 960 Culture and SD-Bioline Test Techniques

MGIT 960, which is endorsed in 2007 by the World Health Organization (WHO) (9), served as the gold-standard reference method in this study in reporting the culture positive samples (can be either TB or NTM) and drug-resistant TB. The SD-Bioline test, an immuno-chromatographic assay using mouse monoclonal antibodies to detect MPT64 antigen/protein which is specific for M. tuberculosis complex was used in combination with the MGIT 960 to distinguish TB and NTM in the National Tuberculosis Reference Laboratory (NTRL) as a routine reference method (10). Our goal was to evaluate the performance of Auto-MODS against MGIT 960 plus the SD-Bioline test.

Mycobacteria culture by MGIT 960 was performed at the mycobacterial laboratory of the Chiang Rai Regional Hospital. Culture positive samples were sub-cultured to get mycobacteria isolates and were then sent to the NTRL in Thailand, where the SD-Bioline TB test was used to distinguish TB and NTM, and the DST by MGIT 960 was conducted to identify drug-resistant TB cases. This is a routine process in Thailand as mycobacteria culture can be done in mycobacterial laboratories of regional hospitals, while DST can only be done in NTRL to ensure biosafety. The MGIT 960 culture testing and DST adhered strictly to the guidelines outlined in the official MGIT 960 manual (11).

A culture result was determined by the MGIT 960 instrument and then confirmed by visual observation of cord formation followed by an AFB smear test. A culture positive
result was reported only if the MGIT 960 instrument and AFB smear test both showed a positive result. A culture negative result was reported if a positive culture result was not observed for 42 days. Contaminated culture results were confirmed by visual observation of rapid overgrowth and clouding of the MGIT 960 tube.

Auto-MODS Culture Techniques

The techniques of the original MODS assay have been described previously (4, 12). Briefly, broth cultures will be prepared in a 24 well-plate apparatus with 4 wells per sputum sample (2 well with culture, 1 well with INH, 1 well with RFP). To minimize cross-contamination and occupational exposure, plates are recommended to be permanently sealed inside plastic zip-lock bags after inoculation. The cultures in each well will be examined under an inverted light microscope.

Four modifications to the original MODS assay (4) were made for Auto-MODS.

Modification One: To reduce the risk of occupational exposure to and cross-contamination between samples, 1.5mL screw-capped tubes were used instead of the well-plates.

Modification Two: To differentiate TB and NTM, a PNB-containing tube was used. The growth of \(M. tb\) is inhibited by PNB, whereas, NTM (including species such as \(M. chelonae, M. kansasii,\) the \(M. avium\) complex and \(M. marinum\)) are resistant (4, 13, 14).

Auto-MODS, thus, uses 5 tubes for each sputum sample (2 tubes with culture, 1 tube with INH, 1 tube with RFP, and 1 tube with PNB) (Figure 1).

“Figure 1. The 5-tube Design of Auto-MODS”
Modification Three: To enhance biosafety and to reduce the human-resource need for frequent reading, a computer-assisted digital camera to automatically and consecutively take images of each tube was used in Auto-MODS (Figure 2). Images taken by the digital camera once per day were automatically transferred to the computer and available for daily visual reading by the laboratory technician directly on the computer screen.

“Figure 2. The automatic image capturing system

Modification Four: To enhance the digital image quality by reducing background noise, a low-speed centrifuge is applied to remove large particles in the liquid before inoculating each sample into the Auto-MODS tube (supplementary Figure 1).

The identification of TB and testing of drug-susceptibility were performed simultaneously using Auto-MODS. The Middlebrook 7H9 Broth culture was used (15). The concentration of PNB, INH, and RFP used in each tube were 0.5mg/ml, 0.1μg/ml, and 1ug/ml, respectively. Tubes were incubated at 37°C in an incubator and images were captured by a computer-assisted digital camera daily from each tube automatically. Both the incubator and the digital camera were integrated into the Auto-MODS system.

The same interpretation criteria of results were used in Auto-MODS as those of MODS (15) except one change: if one of the two control tubes was positive and the other was contaminated, the result was interpreted as positive. The remaining three tubes were only examined if the culture result was found to be positive. In addition to the culture positive result, a negative PNB (no growth of TB) was interpreted as TB positive and a positive PNB was interpreted as NTM. Growth in a drug-containing tube indicated drug-resistant against the corresponding drug.
For the purpose of quality control, three positive control tubes were used for each run of Auto-MODS: 1) M.tb, H37Rv strain; 2) INH resistant strain; 3) RFP resistant strain. In addition, we used a tube with NTM as a non-TB control in each run.

Ogawa Solid Culture

The Ogawa solid culture is a highly sensitive and specific solid culture method and is routinely used in Chiang Rai, Thailand for the majority of TB sputum samples due to a relatively low cost. The Ogawa solid culture was also performed in this study for the purpose of evaluating the speed of Auto-MODS.

Results Reading

All readings of the Auto-MODS cultures and MGIT 960 cultures conducted in the mycobacterial laboratory of the Chiang Rai Regional Hospital were performed by the same technician directly on the computer screen after a digital image of the sample tube was taken. The technician, BC, is a certified medical technologist and the laboratory coordinator for the TB/HIV Foundation. He has over 20 years of experience working as a laboratory technician and worked with the original MODS for over 6 months and handled over 300 samples during that time. He was blinded to the culture results of one test when he read the results of the other test. For Auto-MODS, only the captured digital images, but not the actual tubes, were read.

Statistical Analysis

Sensitivity, specificity, and positive and negative likelihood ratios were calculated to evaluate the performance of Auto-MODS in reference to MGIT 960. The proportion of
contamination and median time to culture positivity of both Auto-MODS and MGIT 960 were calculated. McNemar’s Test was used to compare the contamination rate of Auto-MODS and MGIT 960. For samples that were cultured positive by both Auto-MODS and MGIT 960, the time to culture positivity between the Auto-MODS and MGIT 960 was compared using Wilcoxon Signed-Rank Test. A p-value less than 0.05 was used to indicate statistical significance. The 95% exact confidence intervals (CIs) were calculated under the binomial assumption. Stata 12.0 software was used for statistical analysis.

RESULTS

A total of 380 sputum samples from clinically diagnosed TB patients and 138 from clinically diagnosed non-TB subjects were collected. Among clinically diagnosed TB-patients, 39 were excluded due to non-incident cases; 7 were excluded due to age under 18; 53 were excluded because they had already been receiving treatment for more than two weeks; 18 were excluded because samples were cultured more than one week after collection; and 6 were excluded because the site of TB was extra pulmonary. Among clinically diagnosed non-TB subjects, 3 were excluded due to age under 18. Therefore, a total of 257 clinically diagnosed TB patients and 135 clinically diagnosed non-TB subjects were included in the study. The characteristics of these 392 patients are shown in Table 1.

“Table 1: Characteristics of 392 patients at sample collection”

A total of 392 sputum samples were cultured by both MGIT 960 and Auto-MODS. Among these, 6 sputum samples were contaminated during both the MGIT 960 and Auto-MODS culture process; 11 samples were contaminated during the MGIT 960 culture
process only; and 12 samples were contaminated during the Auto-MODS culture process only. There was no statistically significant difference in the contamination rate: 4.3% for MGIT 960 and 4.6% for Auto-MODS (p=0.83). Both culture methods conducted in the Mycobacterial Laboratory in Chiang Rai Regional Hospital meet the contamination rate criteria of lower than 8% (11).

In addition, three clinically diagnosed TB samples were excluded from the analysis because the image taken by the Auto-MODS digital camera was too dark to lead to a confirmed result. In total, this left us with 360 sputum samples for comparative analysis. The recruitment of subjects and culture results has been shown in a flowchart (Figure 3).

“Figure 3. STARD flowchart showing recruitment of subjects and culture results”

Auto-MODS Performance in Reporting Culture Results

The validity and speed of the Auto-MODS in reporting the culture positivity was evaluated in reference to MGIT 960 culture results: that is, we took MGIT 960 culture results as the truth in distinguishing culture positive samples (can be either TB or NTM) from culture negative samples (non-TB).

Validity

Of the 360 samples, 221 (61.4%) were positive and 139 (38.6%) were negative by MGIT 960 for mycobacteria cultures. Of the 221 true positive samples, Auto-MODS reported 212 positives and 9 negatives, yielding a sensitivity of 95.9% (95% CI: 92.4%-98.1%). Of the 139 true negative samples, Auto-MODS reported 135 negatives and 4 positives, yielding specificity of 97.1% (95% CI: 92.8%-99.2%). The positive and negative
likelihood ratios of Auto-MODS in reference to MGIT 960 were 33.3 (95% CI: 12.7-87.6) and 0.04 (95% CI: 0.02-0.08), respectively. Sensitivity analysis by splitting the samples into 0-3 days culture delay and 4-7 days culture delays did not affect the reported results (supplementary Tables 1 and 2). The culture results, overall analysis results, as well as the sensitivity and specificity estimates based on AFB smear positive and AFB smear negative samples separately are shown in Table 2 and Table 3.

"Table 2. Culture results of Auto-MODS in reference to MGIT 960"

"Table 3. Performance measurements of Auto-MODS in reference to MGIT 960"

Speed

Of the 221 true culture positive samples, the median time to culture positivity was 10 days (Inter Quartile Range (IQR): 8-13 days) for Auto-MODS. Ninety percent of those samples were cultured positive within three weeks. This is statistically and clinically-meaningfully faster than the Ogawa solid culture method which has a median culture positive time of 30 days (IQR: 23-40 days) (p<0.0001). Auto-MODS was statistically slower than the gold-standard method MGIT 960: median of 6 days (IQR: 5-9 days) (p<0.0001) (Figure 4).

“Figure 4. Cumulative probability of culture positivity of Auto-MODS, MGIT 960 and Ogawa by days since the start of assay”

Reporting NTM and Drug-Susceptibility Testing (DST)

Performance of distinguishing TB and NTM, and drug-susceptibility testing was evaluated based on the 212 samples that were cultured positive by both MGIT 960 and
Auto-MODS. Of the 212 positive culture samples, 188 remained for further analysis: 8 samples were excluded due to contamination by Auto-MODS in either the PNB tube or drug containing tubes; and 16 samples were excluded because of the limitation of conducting DST by MGIT 960 (the sub-culturing process resulted in contaminated mycobacteria isolates or it did not yield sufficient mycobacteria isolates).

Among these 188 samples, both SD-Bioline test and Auto-MODS identified the same 183 TB samples and the same 5 NTM samples. DST results of the 183 TB samples are shown between MGIT 960 and Auto-MODS (Table 4).

“Table 4: Drug-susceptibility results from Auto-MODS compared to MGIT 960”

Of the 3 Rifampicin-resistant TB samples and 13 Isoniazid-resistant TB samples, Auto-MODS reported 3 (100%) and 12 (92.3%), respectively. Among these, there were 3 MDR-TB (both Rifampicin and Isoniazid resistant), all of which were reported by Auto-MODS (100%). The sensitivity is not reported here due to the limitation of small sample size of drug-resistant TB. The specificity values of Auto-MODS to detect Rifampicin-susceptible TB, Isoniazid-susceptible TB, and non MDR-TB were 100% (95% CI: 98.0%-100%), 98.2% (95% CI: 94.9%-99.6%), and 100% (95% CI: 97.9%-100%). Thus, Auto-MODS is a highly specific test for DST.

DISCUSSION

Missed and misdiagnosed TB cases are key concerns preventing effective control of TB; 2.9 million missed TB cases occurred worldwide in 2012 (1). Given the high disease burden and constrained financial and technical resources in developing countries, conventional tools for rapid TB detection and DST, such as the gold-standard MGIT 960,
are simply not practical because of high operational costs and advanced laboratory requirements.

The MODS is a non-commercial laboratory method which is feasible for use in any laboratory setting that is equipped with an incubator, centrifuge and an inverted light microscope (4, 12). Numerous studies have demonstrated MODS to be a valid and cost-effective alternative tool for TB diagnosis in resource-limited settings (4, 6, 12).

However, the MODS requires a greater workforce and has biosafety concerns which restricts its use in hospital settings (12).

The samples used in this study came from actual patients who were seeking a TB diagnosis in the highly TB-burdened and resource-limited setting, Chiang Rai, Thailand. Sputum sample collection, transportation, storage, and processing were conducted through the routine system currently used in Chiang Rai, Thailand. Thus, the strength of our study is rooted in its clinically-relevant, real-world design. One limitation of our study is that no back-up samples were stored so we failed to report the results of approximately 5% of the samples that were contaminated. Another limitation is that the reference methods, MGIT 960 and SD-Bioline TB test may not fully correspond to the truth, but the potential degree of misclassification should be minimal.

We found Auto-MODS to be both a highly sensitive and specific test for TB detection. When a culture positive result is reported by Auto-MODS, we can effectively rule in the diagnosis of TB. When a culture negative result is reported by Auto-MODS, we can effectively rule out the diagnosis of TB. This means that Auto-MODS is at a low risk of missing TB cases which qualifies it as a highly sensitive screening assay for an infectious
disease like TB. Also, the first priority in the 2013 WHO global TB report for combatting
TB is to reach the missed cases (1). In practice, if the culture results by Auto-MODS are
to be used in combination with AFB smear results to draw a diagnosis conclusion,
patients with negative AFB smear results should be diagnosed as TB-patients if the Auto-
MODS culture result is positive.

The high specificity of Auto-MODS in performing DST means that once a positive drug-
resistant result is reported by Auto-MODS, we have confidence in diagnosing a patient as
a drug-resistant TB patient. However, due to the small sample size of MDR-TB in our
study, the evaluation of the sensitivity of Auto-MODS in performing DST requires
further study.

The Auto-MODS presents several potential advantages for its use in hospitals: (i) all
samples are cultured in screw-capped tubes reducing the biohazard risk to laboratory
operators; this is expected to also reduce cross-contamination after inoculation (although
it is possible that cross-contamination may already have occurred prior to inoculation). In
our study, among 392 cultured samples, there were 4 samples that were culture positive
in one of the control tubes, and contaminated in another. Three of them were truly
positive according to the gold-standard MGIT 960. Thus, only one sample might be cross
contaminated; (ii) the automatic image capturing system contributes to reduced human
effort and increased biosafety; (iii) digital copies of the culture results by an assisted
computer enable sharing and review of the results, even remotely at any time; (iv) the
“five-tube” design simplifies the culture and DST into a one-step process; and (v) a PNB
tube was used to differentiate between TB and NTM. The use of PNB in combination
with liquid culture methods such as MGIT 960 and MODS to distinguish TB and NTM
has been reported by other studies (13, 16). However, it is important to note that PNB may not be able to inhibit the growth of rapid growers other than NTM that have a cord factor. This is a limitation of both MGIT 960 and Auto-MODS.

Compared to MGIT, Auto-MODS might have a lower biohazard risk because tubes were not opened during the culturing process. Meanwhile, MGIT is opened when there is a high TB count in the culture, smeared on slides, and subsequently re-inoculated for DST; these processes carry significant biosafety concerns. However, caution should be exercised by technicians because both MGIT and Auto-MODS require decontamination of sputum samples which also involves substantial biohazard risk.

The time to report DST results is the same as the time to report culture positivity in the Auto-MODS, based on the rationale of our design. However, in addition to the time of reporting TB cases, approximately another 7 days are needed to report drug-susceptibility results by MGIT 960, given that DST process is performed after the mycobacteria culture in MGIT 960 and subculture on solid media. Also, in Chiang Rai, Thailand, DST is routinely performed at the NTRL which requires an additional sub-culture and waiting time to receive results; this also increases the likelihood of contamination.

However, compared to the gold-standard MGIT 960 which is able to perform tests for different sample types (i.e., urine, cerebrospinal fluid), the Auto-MODS is currently limited to sputum samples in pulmonary TB patients. Also, the automatic image-capturing system had two drawbacks: (i) compared to MODS, a slightly longer time to culture positivity was observed (4, 12); and (ii) of the 257 clinically-diagnosed sputum samples, Auto-MODS failed to report the result of 3 samples because the captured
images were too dark at the start and it was not possible to observe cord formation.

Future work to fine-tune the automatic image capturing system should consider this point.

Recent work using an autofocus algorithm for automatic diagnosis through pattern recognition has already shown promise for TB detection in resource-limited settings, further reducing the need for manual labor (17, 18). Our team is also near completion of an image analysis algorithm for fully automated pattern recognition (19).

These modifications came at a slightly increased cost for initially setting up Auto-MODS ($7000) compared to MODS ($5000 ($3000 for machine and $2000 for incubator)). But the consuming costs are the same for Auto-MODS and MODS in terms of TB detection and two-drug DST at approximately $2.00 per sample. As for labor costs, although they were not evaluated in this study, it is reasonable to expect lower labor costs of Auto-MODS given the automated image-capturing system. Compared to the gold-standard MGIT 960, Auto-MODS is cost effective both for initial set-up ($7000 vs. $85,000) and for individual sample cost ($2.00 vs. $20.00). These costs are estimates made by experienced medical technicians in the mycobacterial laboratory of Chiang Rai Regional Hospital and the NTRL.

As evidenced by this study, Auto-MODS holds significant potential as a cost-effective tool for TB diagnosis. Future work to evaluate the Auto-MODS’s ability in DST is needed to solidify its promise for use in resource-limited setting.

Acknowledgements

The development of the Auto-MODS and this study to evaluate it would not have been possible without the generous support of the AICML.
REFERENCES


2. World Health Organization. 2012. Tuberculosis control in the South-East Asia region. World Health Organization, Regional Office for South-East Asia, India.


Figure 1. The 5-tube Design of Auto-MODS
Figure 2. The automatic image capturing system
Table 1. Characteristics of 392 patients at sample collection

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Clinically Diagnosed TB Number (N=257)</th>
<th>Percentage</th>
<th>Clinically Diagnosed non-TB Number (N=135)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>177</td>
<td>68.9</td>
<td>80</td>
<td>59.3</td>
</tr>
<tr>
<td>Female</td>
<td>80</td>
<td>31.1</td>
<td>55</td>
<td>40.7</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>48 (36-62)</td>
<td>---</td>
<td>54 (39-64)</td>
<td>---</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thai</td>
<td>169</td>
<td>65.8</td>
<td>101</td>
<td>74.8</td>
</tr>
<tr>
<td>Non-Thai</td>
<td>37</td>
<td>14.4</td>
<td>7</td>
<td>5.2</td>
</tr>
<tr>
<td>Hill Tribe</td>
<td>33</td>
<td>12.8</td>
<td>27</td>
<td>20.0</td>
</tr>
<tr>
<td>Unknown</td>
<td>18</td>
<td>7.0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Patient Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New</td>
<td>245</td>
<td>95.3</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Relapse</td>
<td>12</td>
<td>4.7</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>AFB Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>212</td>
<td>82.5</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Negative</td>
<td>45</td>
<td>17.5</td>
<td>135</td>
<td>100</td>
</tr>
<tr>
<td>HIV Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>195</td>
<td>75.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>45</td>
<td>17.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>17</td>
<td>6.6</td>
<td>135</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 3. STARD flowchart showing recruitment of subjects and culture results.
Table 2. Culture results of Auto-MODS in reference to MGIT 960

<table>
<thead>
<tr>
<th></th>
<th>MGIT 960</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>AFB+</td>
<td>AFB-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Auto-MODS</td>
<td>+</td>
<td>212</td>
<td>4</td>
<td>187</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>9</td>
<td>135</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>221</td>
<td>139</td>
<td>190</td>
<td>6</td>
<td>31</td>
<td>133</td>
</tr>
</tbody>
</table>
Table 3. Performance measurements of Auto-MODS in reference to MGIT 960

<table>
<thead>
<tr>
<th>Performance Measure</th>
<th>Overall % (95% CI)</th>
<th>AFB+</th>
<th>AFB-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>95.9 (92.4-98.1)</td>
<td>98.4 (95.5, 99.7)</td>
<td>80.6 (62.5, 92.5)</td>
</tr>
<tr>
<td>Specificity</td>
<td>97.1 (92.8-99.2)</td>
<td>50.0 (11.8, 88.2)</td>
<td>99.2 (95.9, 100)</td>
</tr>
<tr>
<td>LR+</td>
<td>33.3 (12.7-87.6)</td>
<td>1.97 (0.88, 4.38)</td>
<td>107.3 (15.1, 760)</td>
</tr>
<tr>
<td>LR-</td>
<td>0.04 (0.02-0.08)</td>
<td>0.03 (0.01, 0.13)</td>
<td>0.20 (0.10, 0.40)</td>
</tr>
</tbody>
</table>

Note: LR+, positive likelihood ratio; LR-, negative likelihood ratio.
Figure 4. Cumulative probability of culture positivity of Auto-MODS, MGIT 960 and Ogawa by days since the start of assay.
Table 4: Drug-susceptibility results from Auto-MODS compared to MGIT 960

<table>
<thead>
<tr>
<th></th>
<th>Rifampicin</th>
<th>Isoniazid</th>
<th>MDR-TB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant (+)</td>
<td>Susceptible (-)</td>
<td>Resistant (+)</td>
</tr>
<tr>
<td>MGIT</td>
<td>3</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Auto-MODS</td>
<td>0</td>
<td>180</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>180</td>
<td>13</td>
</tr>
</tbody>
</table>