Microscopic-observation drug susceptibility assay provides rapid and reliable identification of MDR-TB

G. S. Ejigu,*† Y. Woldeamanuel,† N. S. Shah,§ M. Gebyehu,‡ A. Selassie,¶ E. Lemma‡

* Medical Faculty, College of Health Sciences, Hawassa University, Awassa, † Medical Faculty, Addis Ababa University, Addis Ababa, ‡ Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia; § Albert Einstein College of Medicine, Bronx, New York, New York, USA; ¶ St Peter’s TB Specialised Hospital, Addis Ababa, Ethiopia

SUMMARY

BACKGROUND: Liquid culture systems are more rapid and sensitive for both the detection and drug susceptibility testing (DST) of Mycobacterium tuberculosis.

SETTING: St Peter’s TB Specialised Hospital and public health laboratory, Addis Ababa.

OBJECTIVE: To compare the microscopic-observation drug susceptibility (MODS) assay with the BACTEC-MGIT 960 system for isoniazid and rifampicin DST (i.e., multidrug-resistant tuberculosis [MDR-TB] identification) of M. tuberculosis.

DESIGN: The evaluation was based on 58 smear- and culture-positive sputum samples from patients diagnosed in Addis Ababa, Ethiopia. BACTEC-MGIT was used as the reference standard.

RESULTS: For the detection of MDR-TB, MODS has a sensitivity, specificity and accuracy rate of respectively 95%, 100% and 98.3% (κ 0.981, concordance 98.3%). Concurrent culture detection and DST results are obtained in a median of 9 days with MODS, while indirect DST results with BACTEC-MGIT are obtained in a median of 8 days (this does not include time to primary isolate).

CONCLUSION: MODS is an accurate, rapid and relatively inexpensive method for the identification of MDR-TB.

KEY WORDS: MODS; multidrug-resistant; M. tuberculosis; Ethiopia

IN 2005, there were 8.8 million new cases of tuberculosis (TB) worldwide.¹ Although TB incidence is stable or declining in most parts of the world, it is rising in the African region, fuelled largely by the human immunodeficiency virus/acquired immune-deficiency syndrome (HIV/AIDS) pandemic.²–⁴ Growing rates of TB drug resistance are further complicating the diagnosis, prevention and treatment of TB-HIV co-infected patients. Multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid (INH) and rifampicin (RMP), has been documented in nearly 90 countries and regions worldwide,³ with an estimated 424 203 MDR-TB cases occurring in 2004, or 4.3% of all new and previously treated TB cases.⁶

Globally, it is estimated that only 45% of TB cases are currently being detected, largely because of the lack of effective diagnostic tools and inadequate health care infrastructure.⁷ Sputum smear, the cornerstone of TB diagnosis, fails to detect nearly half of all incident cases.⁸ Sputum culture is more sensitive, but currently available methods are suboptimal for resource-limited settings. Diagnosis of drug-resistant TB requires not only sputum culture, but also further drug susceptibility testing (DST). The recent description of the global emergence of extensively drug-resistant TB (XDR-TB) (defined as MDR-TB with further resistance to one fluoroquinolone and at least one of the injectable second-line drugs) has highlighted the importance of accurate diagnosis of drug resistance to inform TB programmes and treatment.⁹ The alarmingly high rates of MDR-TB and XDR-TB in KwaZulu-Natal, South Africa, coupled with a rapid and high death rate among HIV co-infected patients,¹⁰ further underscores the urgent need for a rapid, reliable diagnostic test. Prompt diagnosis and treatment of drug-resistant TB is also crucial for halting transmission by reducing the time that an infectious patient receives incorrect treatment and allowing for timely implementation of infection control measures.

Among culture technologies, liquid culture systems are more rapid and more sensitive than solid culture.¹¹ Liquid culture systems include the automated BACTEC method,¹²–¹⁴ colorimetric assays,¹⁵–²¹ and the microscopic-observation drug susceptibility (MODS) assay.²²–²⁶ Given the differing characteristics of these assays and their potential for widespread application in high-burden TB settings with limited resources, there is a need to describe the comparative merits of these...
tests to inform decisions. The purpose of this study therefore was: 1) to perform DST for INH and RMP using MODS directly on sputum specimens and to compare these results with the indirect DST using the automated BACTEC mycobacterial growth indicator tube (MGIT) 960 system, and 2) to determine the time elapsed between sputum processing to culture detection and DST result by MODS compared to indirect DST results using BACTEC MGIT-960.

**MATERIAL AND METHODS**

**Study patients and settings**
The study was conducted at St Peter’s TB Specialised Hospital in Addis Ababa, Ethiopia, from July to November 2005. St Peter’s is a government-owned national referral hospital for diagnosis and treatment of TB patients. We enrolled a convenience sample of patients considered at high risk for drug-resistant TB (i.e., previously treated patients or contacts of drug-resistant TB patients). Patients were eligible for inclusion in the study if they were registered as sputum smear-positive cases (new or previously treated) according to the World Health Organization/International Union Against Tuberculosis and Lung Disease definition.27

All patients (35 new and 23 previously treated) who consented to participate in the study submitted an additional early morning sputum sample. After collection, sputum samples were refrigerated and transported in an ice-box to the National TB Reference Laboratory (Ethiopian Health and Nutrition Research Institute [EHNRI]), located approximately 15 min away by car.

This study was part of a MODS evaluation project that was reviewed and approved by the Faculty Research and Publication Committee of the Faculty of Medicine, Addis Ababa University and the Research and Ethical Clearance Committee of the EHNRI.

**Sputum digestion and decontamination**
All sputum specimens were digested and decontaminated using the modified Petroff method, which is used routinely in the National TB Reference Laboratory.26

**M. tuberculosis growth detection and direct DST using MODS**
Laboratory procedures for MODS and interpretation of final results were performed as described previously.24,26,28

**DST using BACTEC MGIT 960**
With primary isolates obtained from Löwenstein-Jensen (LJ) media, DST was performed using BACTEC MGIT 960 SIRE Kits, 7 ml BACTEC MGIT tubes (Becton Dickinson, Sparks, MD, USA), the AST Set Carrier and the BACTEC MGIT machine. The DST laboratory protocol as provided by the manufacturer was strictly adhered to and an automated result was provided by the BACTEC-MGIT machine. The reference strain H37Rv MTB (ATCC 27294) was also included in each test batch as a quality control. Final critical concentrations of 0.1 and 0.4 µg/ml for INH and 1 µg/ml for RMP were applied.

MODS and BACTEC-MGIT results were each interpreted blind, with the readers being unaware of the results of the other test.

**Statistical analysis**
The MODS turnaround time (TAT) was defined as the time elapsed from the date of inoculation (sample processing) to the date of a positive result. Detection of *M. tuberculosis* and drug susceptibility were concurrent with MODS. As primary isolates obtained from growth in LJ media were used, the BACTEC MGIT DST test was indirect. The time elapsed from the date of loading of processed samples into the BACTEC-MGIT machine to the availability of DST results was considered the TAT for BACTEC-MGIT (recorded from the unloaded AST Set Report of the machine). Time to growth of primary isolate in LJ (3–4 weeks) was not included in the TAT for the BACTEC-MGIT DST results.

Data were analysed using SPSS version 11 (SPSS, Chicago, IL, USA) for Windows (Microsoft Corp., Redmond, WA, USA). Descriptive statistics were analysed using SPSS, and 2 × 2 contingency tables were used for the calculation of diagnostic parameters such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and test efficiency. Kappa (κ) statistic was applied to test agreement between the DST results of MODS and BACTEC-MGIT.

**RESULTS**

**Patients and samples**
We collected sputum samples from 58 smear-positive TB patients, of whom 35 (60%) were new TB cases and 23 (40%) were retreatment cases. The median age of the patients participating in the study was 24 years (range 12–55); 25 (43%) were female. The minimum volume of sputum collected was 3 ml per patient and respectively 55%, 35% and 10% of patients had an acid-fast bacilli grade of 0, 1+, 2+ and 3+. Sputum was collected before the start of anti-tuberculosis treatment.

**Time to DST result**
The median TAT for DST results by MODS was 9 days (range 5–21), while the median TAT for BACTEC-MGIT, using indirect DST, was 8 days (range 5–13) after obtaining a primary isolate from LJ culture.

*0 = 0 acid-fast bacilli (AFB); exact no. seen = 1–9 AFB/100 fields; 1+ = 10–99 AFB/100 fields; 2+ = 1–10 AFB/field; 3+ = >10 AFB/field.*
Table 1 Comparison of DST results for *M. tuberculosis* isolates as determined by MODS and BACTEC-MGIT

<table>
<thead>
<tr>
<th>Drug*</th>
<th>INH L</th>
<th>INH H</th>
<th>RMP</th>
<th>MDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains tested</td>
<td>58</td>
<td>57</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Types of results obtained</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both susceptible</td>
<td>23 (39.7)</td>
<td>24 (42.1)</td>
<td>38 (65.5)</td>
<td>38 (65.5)</td>
</tr>
<tr>
<td>MODS-resistant</td>
<td>1 (1.7)</td>
<td>2 (3.5)</td>
<td>1 (1.7)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>MGIT-resistant</td>
<td>2 (3.4)</td>
<td>1 (1.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Both resistant</td>
<td>32 (55.2)</td>
<td>30 (52.6)</td>
<td>19 (32.8)</td>
<td>19 (32.8)</td>
</tr>
</tbody>
</table>

Agreement:
- Concordant: 55 (94.5%); 54 (94.7%); 57 (98.3%); 57 (98.3%)
- Discordant: 3 (5.2%); 3 (5.3%); 1 (1.7%); 1 (1.7%)

*P < 0.001

 Comparison of MODS with the reference BACTEC-MGIT

Of 58 isolates tested for INH susceptibility at low concentration (0.1 μg/ml), results obtained by MODS and BACTEC-MGIT were in agreement for 55 isolates (94.5%; 23 susceptible, 32 resistant; χ² = 0.894) (Table 1). Among discordant samples, one strain tested susceptible with MODS but resistant with MGIT, and two strains tested resistant with MODS and susceptible with MGIT. Of 57 isolates tested for INH susceptibility at high concentration (0.4 μg/ml), the results were in agreement for 54 (94.7%) isolates (24 susceptible, 30 resistant; χ² = 0.894). Among discordant samples, two strains tested susceptible with MODS and resistant with MGIT, and one strain tested resistant with MODS and susceptible with MGIT. Of 58 isolates tested for RMP susceptibility, complete agreement between MODS and MGIT results was found for 57 strains (98.3%; 38 susceptible, 19 resistant; χ² = 0.961). The one discordant isolate tested susceptible with MODS but resistant with MGIT. For the detection of MDR-TB, the results of the two methods agreed strongly for 57 strains (98.3%; 19 MDR and 38 not MDR; χ² = 0.961).

The false-resistant and false-susceptible rates for MODS compared with the BACTEC-MGIT are shown in Table 2. The specificity was 92% and 96% for INH at low and high concentrations, respectively, and 100% for both RMP resistance and MDR-TB detection. Sensitivity was respectively 97.0% and 93.8% for INH at low and high concentrations, and 95% for both RMP resistance and MDR-TB detection.

**DISCUSSION**

Drug-resistant TB poses a serious threat to progress made in global TB control. With the recent emergence of XDR-TB among HIV-infected patients in South Africa and the associated high mortality, HIV/AIDS care and treatment programmes are also in peril. Sputum smear microscopy, the central diagnostic tool in the DOTS strategy, is insufficient to address this emerging problem in TB control. Recently, there has been some headway in this area, and new tools for identification of TB drug resistance are in the pipeline. Conventional culture using solid media takes 3–4 weeks for results and has a sensitivity of 76–84%23,24 while this is far better than sputum smear microscopy, it still fails to detect a large proportion of TB cases. The currently recommended culture and DST method of choice is liquid media, due to its better speed and sensitivity compared to solid culture. A number of liquid systems, such as the BACTEC-MGIT, MODS and colorimetric methods, are available, but their comparative performance in a particular setting needs to be based on factors such as bio-safety, cost,

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cases</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>NPV</th>
<th>PPV</th>
<th>Efficiency</th>
</tr>
</thead>
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<tr>
<td>INH</td>
<td>New</td>
<td>35</td>
<td>89.5%</td>
<td>93.8%</td>
<td>94.4%</td>
<td>88.2%</td>
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<tr>
<td></td>
<td>Previously treated</td>
<td>23</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>58</td>
<td>92.0%</td>
<td>97.0%</td>
<td>95.8%</td>
<td>94.1%</td>
</tr>
<tr>
<td>INH</td>
<td>New</td>
<td>34</td>
<td>94.7%</td>
<td>93.3%</td>
<td>94.7%</td>
<td>93.3%</td>
</tr>
<tr>
<td></td>
<td>Previously treated</td>
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<td>100.0%</td>
<td>94.1%</td>
<td>85.7%</td>
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<tr>
<td></td>
<td>Combined</td>
<td>57</td>
<td>96.0%</td>
<td>93.8%</td>
<td>92.3%</td>
<td>96.8%</td>
</tr>
<tr>
<td>RMP</td>
<td>New</td>
<td>35</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
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<tr>
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<td>Combined</td>
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<td>100.0%</td>
<td>95.0%</td>
<td>97.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td>MDR</td>
<td>New</td>
<td>35</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Previously treated</td>
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<tr>
<td></td>
<td>Combined</td>
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<td>100.0%</td>
<td>95.0%</td>
<td>97.4%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

MODS = microscopic-observation drug susceptibility assay; MGIT = mycobacterial growth indicator tube; NPV = negative predictive value; PPV = positive predictive value; INH L = isoniazid at low concentration (0.1 μg/ml); INH H = INH at high concentration (0.4 μg/ml); RMP = rifampicin; MDR = multidrug-resistant.
ease of performance and interpretation, time to availability of results, typeability, reproducibility and test accuracy.

The MODS assay is a direct method for simultaneous culture detection and DST of M. tuberculosis. Results are obtained significantly more quickly than with conventional solid or liquid media culture. In this study, the median TAT for MODS was 9 days, and for indirect DST with BACTEC-MGIT it was 8 days after primary isolates were obtained on LJ (an additional 3–4 weeks). Bacterial DST in most cases is done indirectly, which assures the use of a primary pure colony from the outset and allows for easy determination of the standard McFarland dilution. BACTEC-MGIT and colorimetric tests depend on ultraviolet light and a colour indicator for the detection of bacterial growth, respectively. A major limitation of these systems is that a positive result may be obtained for any organism grown in the media which consumes oxygen. If DST is performed directly on sputum specimens, checking for purity of growth (i.e., through staining in each of the drug and control tubes and/or use of general purpose media in parallel) may require additional time and labour to ensure reliable results. With the MODS assay, DST is direct and the problems outlined above are overcome because the results are interpreted following microscopic observation, which allows the reader to ensure the purity of the colony in the media when interpreting results. Direct DST using the BACTEC-MGIT system has been described elsewhere in the literature.

However, the manufacturer currently recommends only indirect DST, which requires a primary isolate of M. tuberculosis. This may take 3–4 weeks in LJ media or, at best, 1–2 weeks in BACTEC-MGIT. Likewise, promising results have also been reported in the direct colorimetric test, although ruling out growth of organisms other than M. tuberculosis is unreliable.

Possible biohazard risk is also an important factor for the adoption of new tests such as MODS in a given setting. DST and culture amplification for the MODS assay were performed in sealed 24-well plates. Up to four samples are handled in a single plate, results are obtained quite rapidly, and culture materials are subsequently discarded much faster than with traditional methods. MODS may also be safer than indirect DST methods, which also require the manipulation of an amplified culture of M. tuberculosis. Sputum decontamination and concentration at the outset is currently common to all culture-based tests. Another potential limitation of the MODS assay is that as it is a closed system, obtaining isolates for further testing may be difficult. Identification of M. tuberculosis in both MODS and BACTEC-MGIT is based on cord formation properties of the organism, and results are therefore presumptive. A routine back-up of isolates using parallel inoculation of samples in solid media may help laboratories to have additional isolates if further testing for species identification, molecular typing and even storage of strains are required. Such an approach is recommended for its cost-effectiveness in modern mycobacteriology laboratories using liquid systems. Another practical limitation of the MODS assay is that daily microscopic observation may require an increase in labour. With training, however, even novice technicians can read a well in one minute, which is much faster than the time it takes to read a sputum smear or malaria film. Another option is for microscopic observation to be performed at intervals that suit laboratory workloads (e.g., on days 5, 7, 10 and 15), with the knowledge that, if it is possible, daily observation would yield the fastest result. The overall running cost for MODS is minimal and comparable to the currently available inexpensive tests.

In our study of the MODS assay in an urban setting in Ethiopia, the combined results for INH and RMP (i.e., MDR-TB detection) had excellent agreement and high sensitivity and specificity compared with DST results from the BACTEC-MGIT system. These findings are similar to results from other studies in Peru and Ethiopia. This study was based on small sample size, and contamination rates were minimal for both MODS and the reference tests. It may therefore be difficult to compare the contamination rates of the two methods. In a previous study done in the same laboratory as the current study, significantly higher culture detection with lower contamination rates were obtained with MODS compared to solid culture using LJ. The MODS assay has also been shown to have excellent culture detection rates among sputum smear-negative and or paucibacillary TB patients. These findings, together with the further corroboration of reliable results from the MODS assay, strongly suggest that MODS has the potential to improve the proportion of microbiologically confirmed TB and drug resistance, especially in areas with high rates of smear-negative and or paucibacillary TB disease. The results of this and previous studies support serious consideration of the MODS assay for wide-scale use in resource-limited settings to improve concurrent case detection and identification of drug resistance (for INH and RMP), and thereby improve the management of individuals with TB/drug-resistant TB and the communities that are exposed to the risks of undiagnosed, untreated patients. Plans are currently underway in Ethiopia for decentralising and improving the laboratory diagnosis of TB and MDR-TB, including use of MODS after further field testing.

Acknowledgements

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References

CONTEXTE : Les systèmes de culture sur milieu liquide sont plus rapides et plus sensibles à la fois pour la détection et pour les tests de sensibilité aux médicaments de *Mycobacterium tuberculosis*.

CADRE : Hôpital St Peter spécialisé pour la tuberculose (TB) et le laboratoire de santé publique.

OBJECTIF : Comparer le test d’observation microscopique de la sensibilité de *M. tuberculosis* aux médicaments (MODS) avec le système MGIT 960 de BACTEC pour les tests de sensibilité à l’isoniazide et à la rifampicine (c à d pour l’identification de TB-MDR).

SCHEMA : L’évaluation est basée sur 58 échantillons de crachats positifs à la bacilloscopie et à la culture et provenant de patients diagnostiqués à Addis-Ababa, Ethiopie. On a utilisé comme standard de référence le BACTEC-MGIT.

RÉSULTATS : Pour la détection de la TB-MDR, les taux de sensibilité, de spécificité et de précision du MODS sont respectivement de 95%, 100% et 98,3% (valeur $\kappa$ 0,981 ; concordance 98,3%). Les résultats simultanés de la détection de la culture et de la sensibilité aux médicaments sont obtenus après une durée médiane de 9 jours avec le MODS, alors que les résultats indirects de sensibilité aux médicaments par le BACTEC-MGIT sont obtenus après une durée médiane de 8 jours (qui n’inclut pas la durée nécessaire à l’isolement primaire).

CONCLUSION : Le MODS est une méthode précise, rapide et relativement peu coûteuse d’identification de la TB-MDR.

MARCO DE REFERENCIA : Los sistemas de cultivo en medio líquido son más rápidos y más sensibles para las pruebas de detección de *Mycobacterium tuberculosis* y de su sensibilidad a los antituberculosos. El presente estudio se llevó a cabo en el hospital St Peter, especializado en tuberculosis (TB) y en un laboratorio de salud pública.

OBJETIVO : Comparar la prueba de sensibilidad a los medicamentos por observación microscópica directa (MODS) con el sistema BACTEC-MGIT 960, en el estudio de la sensibilidad de *M. tuberculosis* a isoniazida y rifampicina (es decir, la detección de TB multidrogorresistente [MDR]).

MÉTODO : Se estudiaron 58 muestras de esputo con bacilloscopía y cultivo positivos, provenientes de pacientes diagnosticados en Addis Abeba, Etiopía. El método de referencia utilizado fue el sistema BACTEC-MGIT.

RESULTADOS : La MODS tuvo una sensibilidad de 95%, una especificidad de 100% y un índice de precisión de 98,3% en la detección de TB-MDR (índice $\kappa$ 0,981 ; concordancia 98,3%). Se obtuvieron simultáneamente resultados de detección en cultivo y de sensibilidad a los medicamentos en un lapso mediano de 9 días con la MODS ; la mediana del lapso hasta obtener resultados indirectos de sensibilidad a los medicamentos por el método BACTEC-MGIT fue 8 días (sin tener en cuenta el tiempo necesario hasta obtener el aislado primario).

CONCLUSIÓN : La MODS representa un método preciso, rápido y relativamente poco costoso de detección de la TB-MDR.