

# Evaluation of the Microscopic-Observation Drug-Susceptibility Assay Drugs Concentration for Detection of Multidrug-Resistant Tuberculosis

Anita, Sari Septiani Tangke, Rusdina Bte Ladju, Nasrum Massi

**Abstract**—New diagnostic tools are urgently needed to interrupt the transmission of tuberculosis and multidrug-resistant tuberculosis. The microscopic-observation drug-susceptibility (MODS) assay is a rapid, accurate and simple liquid culture method to detect *multidrug-resistant tuberculosis (MDR-TB)*. MODS were evaluated to determine a lower and same concentration of isoniazid and rifampin for detection of MDR-TB. Direct drug-susceptibility testing was performed with the use of the MODS assay. Drug-sensitive control strains were tested daily. The drug concentrations that used for both isoniazid and rifampin were at the same concentration: 0.16, 0.08 and 0.04µg per milliliter. We tested 56 M. tuberculosis clinical isolates and the control strains M. tuberculosis H37RV. All concentration showed same result. Of 53 M. tuberculosis clinical isolates, 14 were MDR-TB, 38 were susceptible with isoniazid and rifampin, 1 was resistant with isoniazid only. Drug-susceptibility testing was performed with the use of the proportion method using Mycobacteria Growth Indicator Tube (MGIT) system as reference. The result of MODS assay using lower concentration was significance ( $P < 0.001$ ) compare with the reference methods.

A lower and same concentration of isoniazid and rifampin can be used to detect MDR-TB. Operational cost and application can be more efficient and easier in resource-limited environments. However, additional studies evaluating the MODS using lower and same concentration of isoniazid and rifampin must be conducted with a larger number of clinical isolates.

**Keywords**—Isoniazid, MODS assay, MDR-TB, Rifampin.

## I. INTRODUCTION

THERE is an urgent need for low-cost methods for the detection of Multidrug-resistant Tuberculosis (MDR-TB). MDR-TB threatens the success of global TB control. Detection of drug resistance is important, but widely used drug susceptibility tests on Lowenstein-Jensen slants or agar-containing plates are slow [1]-[4]. Drug susceptibility tests were performed by automated liquid culture systems are more rapid but expensive and therefore not widely used in resource-limited settings [2], [5]. Therefore, an accurate, rapid, inexpensive and technically simple method for *M. tuberculosis* drug susceptibility testing is urgently needed priority for use in resource-poor settings and for areas with high rates of MDR TB.

Rusdina Bte Ladju is with the Medical Faculty of Hasanuddin University, Jl. Perintis Kemerdekaan km.10, 90245 Makassar, Indonesia (Corresponding Author; phone: 62-815-24420838; fax: 62-586-297; e-mail: rusdinaadam@yahoo.co.id).

Anita, Sari Septiani Tangke, and Nasrum Massi are with the Biology Faculty of Hasanuddin University (e-mail: nita\_uh@yahoo.co.id, sari.atte@gmail.com, nasrumm2000@yahoo.com).

Microscopic-observation drug-susceptibility assay (MODS), a noncommercial TB culture and DST method, is recommended by World Health Organization (WHO) [6], [7]. It is cheaper and has shorter turn-around time than conventional gold standard methods [8]. MODS assay has been validated in Peru and evaluated in some developing countries [9]-[14]. Previous studies showed that MODS assay had 92%–97.5% sensitivity [5], [8], [13]. MODS can detected drug resistance within 1–2 weeks even with low establishment cost and technical needs. It can be a reasonable solution for strengthening the TB diagnostic in developing countries [15].

The MODS assay is a liquid culture method based on microscopic detection of characteristic *M. tuberculosis* morphology. The microscopic detection of mycobacterial by MODS is technically similar to microscopic examination of a smear. These are features that make MODS suitable for use in resource-poor settings [16]. MODS assay uses enriched Middlebrook 7H-9 liquid medium and relies on microscopic detection of cording growth that is the characteristic of *Mycobacterium tuberculosis*. Potential advantages of the MODS assay are relatively rapid mycobacterial growth and reliance on microscopy skills similar to those used for smear microscopy [13].

Given the challenges of the evaluation of techniques for the diagnosis of MDR-TB, our study was conducted to determine a lower and same concentration of isoniazid and rifampin for MDR-TB detection in more simple and cheaper application.

## II. MATERIAL AND METHOD

### A. Sample Collection

A total of 53 M. tuberculosis clinical isolates were selected from the patients with suspected tuberculosis at the Infection Center of Wahidin Sudirohusodo Hospital, Makassar. The isoniazid-susceptible and rifampicin-susceptible strain M. tuberculosis H37Rv was used as controls in the susceptibility test for isoniazid and rifampin in lower concentrations.

### B. Detection of *Mycobacterium tuberculosis*

Sputum samples were decontaminated according to the sodium hydroxide–N-acetyl-L-cysteine method [17]. An aliquot was used for microscopical examination of Ziehl-Neelsen stained sputum smears, and the remainder was used for parallel Lowenstein–Jensen culture, Mycobacteria Growth Indicator Tube (MGIT) culture, and MODS culture. Lowenstein–Jensen culture and MGIT culture were used as reference culture to confirm the detection of *Mycobacterium*

*tuberculosis*. They were selected because they were reference methods commonly used in developing and industrialized countries, respectively. After inoculation of 5µl of decontaminant specimen, Lowenstein-Jensen slants were incubated at 37°C and examined twice weekly from day 7 through day 60 [17]. MGIT tubes were inoculated with 500 µl of decontaminant specimen, and cultures were monitored continuously every day after 2 days of inoculation using manual ultra violet machine. MGIT media were contain of middlebrook 7H9, 500µl of oxalic acid, albumin, dextrose, and catalase (OADC) and 100 µl of polymyxin, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA) antibiotics. Samples were negative culture if there were no *Mycobacterium* growth after monitored until 42 days.

The MODS cultures were prepared in 24-well tissue-culture plates, each well containing 720µl of decontaminant specimen, Middlebrook 7H9 broth, oxalic acid, albumin, dextrose, and catalase (OADC), and polymyxin, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA). Antibiotic stock solutions were added to give the following final critical drug concentrations of INH and RIF: 0.16 µg/ml, 0.08 µg/ml and 0.04 µg/ml. The cultures were examined under an inverted light microscope at a magnification of 40× every day, from day 4 to day 15, on alternate days from day 16 to day 25, and twice weekly from day 26 to day 40. To minimize cross-contamination and occupational exposure, plates were permanently sealed inside plastic zip lock bags after inoculation and were subsequently examined within the bag. Positive cultures were identified by cord formation, characteristic of *M. tuberculosis* growth, in liquid medium in drug-free control wells, as described previously. Non-tuberculous mycobacteria were recognized by their lack of cording or, for *M. chelonae* (which is the only non-tuberculous mycobacteria that does form cords), by rapid overgrowth by day 5. Fungal or bacterial contamination was recognized by rapid over growth and clouding in wells. If contamination was detected, the original sample was cultured again after being decontaminated once more.

### C. Drug-Susceptibility Testing

Direct drug-susceptibility testing was performed with the use of the MODS assay, as previously described [12], [15]. Growth in drug-free control wells but not in drug-containing wells indicated susceptibility. The drug concentrations that used for both isoniazid and rifampin were at the same concentration: 0.16µg/ml, 0.08µg/ml and 0.04µg/ml. These drugs concentration were lower than were used in previous study. MGIT System drug-susceptibility testing was performed as a reference. Drug-sensitive control strains were tested daily using MODS assay and MGIT.

### D. Statistical Analysis

Data were analyzed with the use of IBM SPSS Statistical version 20 with the samples as the units of analysis. The data was analyzed using chi-square test for the result significances. The data also calculated for the sensitivity and specificity of

MODS assay for drug susceptibility test. By determining the growth of *Mycobacterium tuberculosis* using MODS assay.

## III. RESULTS

All of 56 *M. tuberculosis* clinical isolates and controls have positive results for acid fast bacilli test. All of the positive acid fast bacilli samples that have been cultured with MODS assay give identical results with reference method (LJ culture and MGIT culture) for the bacterial culture (Table I). Drug susceptibility test were also performed directly with MODS assay using lower drugs concentrations: 0.16µg/ml, 0.08µg/ml and 0.04µg/ml for both Isoniazid and Rifampin. From 56 *M. tuberculosis* clinical isolates and controls that have been tested with MODS assay, 14 samples were MDR-TB, 41 samples were susceptible with both of isoniazid and rifampin, and 1 samples was resistant with isoniazid only. There was no sample that resistant with rifampin only or susceptible with rifampin only (Tables I and II). Out of 56 *M. tuberculosis* clinical isolates that have been tested with MODS assay, 35 samples (86.4%) gave identical results (susceptible) with reference method for the three drugs concentration tested. 13 samples (86.7%) were resistant for both of MODS assay and MGIT System. There were 8 samples that have been tested with MODS assay have discrepancies with MGIT System results (Table III). 6 (14.6%) were susceptible with MODS but resistant with MGIT System. 2 (13.3%) were resistant with MODS but susceptible with MGIT System. But the results were significance with P value < 0.001 (chi-square). The MODS assay sensitivity result was 94.6% and the specificity result was 68.4%.



Fig. 1 Cord formation of MODS assay

TABLE I  
CULTURE PERFORMANCE RESULT OF MODS ASSAY IN REFERENCE TO LJ  
AND MGIT CULTURE

	MODS	LJ	MGIT
	culture	culture	Culture
H37RV n=3	Positive	Positive	Positive
AFB + n=53	Positive	Positive	Positive

TABLE II  
MODS ASSAY RESULTS OBTAINED WITH LOWER CONCENTRATION OF  
ISONIAZID

Drug conc. (µg/ml)	No. of isolates			
	Susceptible		Resistant	
	Susceptible	MDR	with INH only	with INH only
0.04	38	14	0	1
0.08	38	14	0	1
0.16	38	14	0	1

TABLE III  
MODS ASSAY RESULTS OBTAINED WITH LOWER CONCENTRATION OF  
RIFAMPIN

Drug conc. (µg/ml)	No. of isolates			
	Susceptible		Resistant	
	Susceptible	MDR	with RIF only	with RIF only
0.04	38	14	1	0
0.08	38	14	1	0
0.16	38	14	1	0

TABLE IV  
DST PERFORMANCE RESULT OF MODS ASSAY IN REFERENCE TO MGIT  
SYSTEM

MODS	MGIT			
	Susceptible	MDR	INH resistant only	RIF resistant Only
Susceptible	35	3	3	0
MDR	1	12	1	0
INH resistant only	1	0	0	0
RIF resistant only	0	0	0	0

TABEL V  
DST PERFORMANCE RESULT OF MODS ASSAY IN REFERENCE TO MGIT  
SYSTEM

MODS	MGIT		P
	Susceptible	Resistant	
Susceptible	35(86,4%)	6(14,6%)	P<0.001*
Resistant	2(13,3%)	13(86,7%)	

\*Chi square

#### IV. DISCUSSION

MODS assay provides low-cost, low technology culture method, safe [15] and more rapid time to positivity [18] compared with the reference method for isoniazid and rifampin drug susceptibility testing with concurrent highly accurate identification of multidrug-resistant (MDR) strains in resource-limited settings with high tuberculosis burden [16], [19]. Our study defines strengths of MODS assay in detecting MDR-TB with lower and same concentration, and should enable the development of a streamlined for clinically useful method. In performing drug susceptibility test for detecting MDR-TB using MODS assay, previous studies were used drug concentration for isoniazid: 0.1µg/ml and 0.4µg/ml; rifampin: 1µg/ml and 2µg/ml. The used of lower concentration for isoniazid and rifampin: 0.16µg/ml, 0.08µg/ml and 0.04µg/ml in our study reduces the operational costs >100%. There were no discrepancies among the MODS assay result of the three concentrations that have been tested to detect MDR-TB. Reducing the drugs concentration did not affect the performance of the MODS assay compare with the references

method (P<0.001) in detecting MDR-TB. By reducing the amount of the isoniazid and rifampin, we can detect a large number of MDR-TB samples. Using the same concentration for both of the antibiotics give us an easily workflow on antibiotic dilution which is more efficient and effective. In this case, any laboratory that has incubator, centrifuge, and microscope and is adequately biologically secured can safely perform MODS assay because all of the materials are available from major laboratory supplier.

In conclusion, based on our results, MODS assay using lower and same concentration of isoniazid and rifampin can be performed to detect MDR-TB. Operational cost can be managed efficiently in resource-limited environments because of more simple and cheaper application of the MODS assay for drug susceptibility testing. However, additional studies evaluating the MODS assay using lower and same concentration of isoniazid and rifampin must be conducted with a larger number of clinical isolates and several more types of drugs.

#### REFERENCES

- [1] J. Crofton, P. Chaulet, and D. Maher. Guidelines for the Management of Drug-Resistant Tuberculosis. World Health Organization, Geneva, Switzerland. 1997.
- [2] C. B. Inderlied, and M. Salfinger. Antimycobacterial Agents and Susceptibility Tests. In Manual of Clinical Microbiology, 7<sup>th</sup> ed. P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover, Ed. American Society for Microbiology, Washington, D.C. 1999, pp. 1601–1623.
- [3] P. T. Kent and G. P. Kubica. Public Health Mycobacteriology—A Guide for the Level III Laboratory. Centers for Disease Control, U.S. Department of Health and Human Services, Atlanta, Georgia. 1985.
- [4] S. H. Siddiqi, J. E. Hawkins, and A. Laszlo. Interlaboratory drug susceptibility testing of *Mycobacterium tuberculosis* by a radiometric procedure and two conventional methods. *J. Clin. Microbiol.* 1985, 22:919–923.
- [5] Caviedes L, Lee TS, Gilman RH, et al. Rapid, efficient detection and drug susceptibility testing of *Mycobacterium tuberculosis* in sputum by microscopic observation of broth cultures. The Tuberculosis Working Group in Peru. *J Clin Microbiol* 2000; 38:1203–8.
- [6] Bwanga F, Hoffner S, Haile M, Joloba M. Direct susceptibility testing for multi drug resistant tuberculosis: a meta-analysis. *BMC Infect Dis.* 2009;9:67.
- [7] Moore DA, Mendoza D, Gilman RH, et al. Microscopic observation drug susceptibility assay, a rapid, reliable diagnostic test for multidrug resistant tuberculosis suitable for use in resource-poor settings. *J Clin Microbiol.* 2004;42(10):4432–4437.
- [8] Moore DA, Evans CA, Gilman RH, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. *N Engl J Med* 2006; 355:1539–50.
- [9] Shiferaw G, Woldeamanuel Y, Gebeyehu M, Girmachew F, Demessie D, Lemma E. Evaluation of microscopic observation drug susceptibility assay for detection of multidrug-resistant *Mycobacterium tuberculosis*. *J Clin Microbiol.* 2007; 45(4):1093–1097.
- [10] Michael JS, Daley P, Armstrong L, et al. Prospective evaluation of microscopic observation drug-susceptibility (MODS) assay for the diagnosis of active tuberculosis (TB) in India – preliminary analysis. *Int J Infect Dis.* 2008; 12(Suppl 1):e324.
- [11] Mello FCQ, Arias MS, Rosales S, et al. Clinical evaluation of the microscopic observation drug susceptibility assay for detection of *Mycobacterium tuberculosis* resistance to isoniazid or rifampin. *J Clin Microbiol.* 2007;45(10):3387–3389.
- [12] Ejigu GS, Woldeamanuel Y, Shah NS, Gebeyehu M, Selassie A, Lemma E. Microscopic-observation drug susceptibility assay provides rapid and reliable identification of MDR-TB. *Int J Tuberc Lung Dis.* 2008; 12(3):332–337.

- [13] Arias M, Mello FC, Pavón A, et al. Clinical evaluation of the Microscopic observation drug-susceptibility assay for detection of tuberculosis. *Clin Infect Dis*. 2007;44(5):674–680.
- [14] Limaye K, Kanade S, Nataraj G, Mehta P. Utility of microscopic observation of drug susceptibility (MODS) assay for *Mycobacterium tuberculosis* in resource constrained settings. *Indian J Tuberc*. 2010; 57(4):207–212.
- [15] Boonchai C, Myo Nyein A, Saiyud M, Yuthichai K, et al. Prospective evaluation of simply modified MODS assay: an effective tool for TB diagnosis and detection of MDR-TB. *Dove Press Journal: Infection and Drug Resistance*. 2012:79-86
- [16] Walter P, William B, Richard C, et al. Performance of the Microscopic Observation Drug Susceptibility Assay in Drug Susceptibility Testing for *Mycobacterium tuberculosis*. *Journal of Clinical Microbiology*. Vol. 40, No 12. 2002;4750-4752
- [17] Laboratory services in TB control.Parts I, II, and III. Geneva: World Health Organization, 1998. (Publication no.WHO/tb/98.258.)
- [18] Enrico T, Marta B, Alessandra F, and M. Tullia S. Evaluation of Automated BACTEC MGIT 960 System for Testing Susceptibility of *Mycobacterium tuberculosis* to Four Major Antituberculous Drugs: Comparison with the Radiometric BACTEC 460TB Method and the Agar Plate Method of Proportion. *Journal of Clinical Microbiology*. Vol. 40, No 2. 2002;607-610
- [19] Enrico Tortoli, and C. Piersimoni. Molecular mechanisms of drug resistance in *Mycobacterium tuberculosis*. *Alpe Adria Microbiol. Journal*. 1998; 7:275–283