

MODS culture for primary diagnosis of tuberculous meningitis and HIV-associated pulmonary tuberculosis in Indonesia

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ABSTRACT

Aims: To compare the microscopic observation drug susceptibility (MODS) culture with microscopy and solid culture for diagnosis of TB meningitis and HIV-associated pulmonary tuberculosis (TB).

Study design: Comparative study

Place and Duration of Study: Department of Clinical Pathology, Hasan Sadikin Hospital, Bandung, Indonesia, between 2010 and 2012.

Methodology: Two groups of patients were included. The first group consisted of 167 consecutive HIV-infected patients presenting with suspected pulmonary TB. The second group consisted of 88 patients with clinical suspicion of TB meningitis. Sputum samples from HIV-associated pulmonary TB patients and cerebrospinal fluid (CSF) from patients with TB meningitis were analyzed using microscopy of Ziehl-Neelsen (ZN) stained smears, culture on solid medium (Ogawa), and MODS culture.

Results: MODS showed the highest detection rate in both patient groups. Among HIV-associated pulmonary TB patients, positivity of MODS was 31.2% compared with 26.9% for Ogawa and 20.6% for ZN. Among TB meningitis patients, positivity of MODS was 41.2% compared with 38.8% for Ogawa and 8.3% for ZN. The median time to culture positivity was significantly shorter for MODS compared to Ogawa, both for sputum (median 11 vs 21 days) and CSF (14 vs 33 days). In 14 days, MODS detected significantly more cases compared with Ogawa in both patients group (79.2% vs 2.4% and 68.6% vs 0%, respectively). Laboratory staff readily used MODS after two weeks of training.

Conclusion: We were able to implement MODS culture as a robust, sensitive, and rapid method for diagnosis of HIV-associated pulmonary TB and TB meningitis in a hospital setting in Indonesia. Further studies may be needed to assess the feasibility of MODS culture in other settings and assess its impact on case detection and timely treatment of both forms of TB.

Keywords: MODS culture, primary diagnosis, HIV-associated pulmonary TB, TB meningitis, Indonesia.

1. INTRODUCTION

Diagnosis of tuberculosis (TB) is often difficult, especially among HIV-infected patients [1] and among patients with extrapulmonary TB [2]. Microscopy is insensitive and conventional culture is more sensitive but slow. Commercial liquid culture systems, which can shorten the time to detect growth of *Mycobacterium tuberculosis* require sophisticated instrumentation and are more costly [1]. The World Health Organization has recently endorsed the Xpert MTB/RIF which is a nucleic acid amplification-based test. The Xpert test is a rapid and simple method for TB diagnosis and detection of rifampicin resistance. However, it is less sensitive for diagnosis of paucibacillary disease [2], it is relatively expensive, and cannot evaluate treatment response. In addition, culture is still needed to confirm the results of drug susceptibility testing (DST). Therefore, there is a need for rapid, efficient, and inexpensive culture-based diagnostic tools.

One of several recently developed methods for diagnosis and DST of TB is the microscopic observation drug susceptibility (MODS) culture. MODS uses broth culture and an inverted light microscope to rapidly detect specific growth of *M. tuberculosis*. MODS can be used for primary diagnosis as well as DST. With regard to primary diagnosis, a few studies have examined MODS for diagnosis of pulmonary TB [1, 3, 4], and one study has evaluated MODS for diagnosis of TB meningitis [5]. These studies suggest that MODS culture, with its high sensitivity, speed and low price, and its potential to add DST, may be a suitable alternative for diagnosis of paucibacillary forms of TB, and for use in low-resource settings.

However, for successful implementation more studies are needed, in different patient groups and settings. We compared MODS culture with microscopy and solid culture for diagnosis of TB meningitis and HIV-associated pulmonary TB in Indonesia, which has a high burden of TB and a rapidly growing HIV epidemic. Like in other settings, bacteriological confirmation of HIV-associated pulmonary TB and TB meningitis is difficult, and mortality is very high [6, 7]. More rapid diagnosis would help timely treatment to improve patient outcome and reduce TB transmission.

2. MATERIAL AND METHODS

This study was conducted between February 2010 and January 2012 at Dr. Hasan Sadikin Hospital, the referral hospital for West Java Province, Indonesia. Written informed consent was obtained from all participants and the study was approved by the ethical committee of the Faculty of Medicine, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia.

We included two groups of patients with suspected TB. The first group consisted of HIV-infected patients, either from the outpatient clinic or the HIV clinic in the hospital, who had symptoms or chest X-ray (CXR) abnormalities suggesting pulmonary TB. From this group one to three consecutive sputum samples were collected per patient prior to initiation of TB treatment. Direct smears were prepared from each sputum specimen for Ziehl-Neelsen (ZN) staining. The remaining sputum specimens were decontaminated by a standard N-acetyl-L-cystein (NaLC)-NaOH method and concentrated by centrifugation at 3000 x g for 15 minutes. For each patient, one sputum culture was made. Sputum specimens from patients who had provided ≥ 1 specimen were mixed before decontamination. The resulting

75 sediments were then resuspended and inoculated into MODS medium as described
76 previously [8, 9] and onto two slants of Ogawa solid medium.

77 The second group consisted of adult patients (≥ 18 years old) with suspected TB meningitis.
78 A clinical diagnosis of meningitis was based on clinical findings, CSF criteria or both [10].
79 Five to ten milliliters of CSF was obtained and divided into two tubes. The first tube (0.5–1
80 mL) was used for CSF cells, protein and glucose. The second tube (4–10 mL) was
81 concentrated by centrifugation at 3000 x g for 15 minutes. The CSF sediment was used to
82 prepare smears for ZN-staining and was inoculated into MODS medium [5] and onto two
83 slants of Ogawa solid medium.

84 For this study, MODS was not performed on a daily basis to simplify the technical work,
85 reduce the high work load in our laboratory, and to standardize the time for microscopic
86 observation. After preparation of smears, specimens were stored in 4°C before they were
87 processed for culture. Positive MODS cultures due to cross-contamination seem very
88 unlikely, as previous studies have shown that cross-contamination is extremely rare in
89 MODS [11]. To further reduce the possibility of cross-contamination and to improve
90 biosafety, MODS plates were sealed in ziplock bags after inoculation.

91 ZN-staining and microscopic reading was done according to standard guidelines. Ogawa
92 cultures, considered positive when mycobacterial growth of > 1 colony forming unit (CFU)
93 was observed were read twice weekly until 8 weeks after incubation. Mycobacterial growth in
94 MODS plates was examined twice weekly under an inverted light microscope at 40x
95 magnification from day 4 until day 40 after inoculation. Positive MODS cultures were
96 identified by cord formation which is characteristic of *M. tuberculosis* growth.

97 For quality control of microscopy positive (*M. tuberculosis* H37Rv) and negative (sterile
98 distilled water) controls are included daily. Our laboratory also participates and qualifies for
99 the external quality assurance (EQA) program for smear microscopy provided by the WHO.
100 For mycobacterial culture, we monitor the contamination rate on a monthly basis, as a
101 quality control for the decontamination process. For MODS, negative controls (sample-free
102 liquid medium) were included in each plate, and positive controls (H37Rv) for each run. To
103 minimize the risk of cross-contamination, the positive controls were set up in a separate
104 plate, after all plates with patients samples had been sealed.

105 The positivity rate of microscopy and mycobacterial culture using Ogawa and MODS was
106 expressed as percentage. For HIV-infected individuals suspected of having pulmonary TB,
107 more than one sputum sample was often available. However, analysis in this group was
108 done per patient, not per individual sample. Time to culture positivity was expressed as
109 median days (interquartile range, IQR). Differences between groups were compared using
110 Chi-square test for proportions with $P < .05$ considered statistically significant.

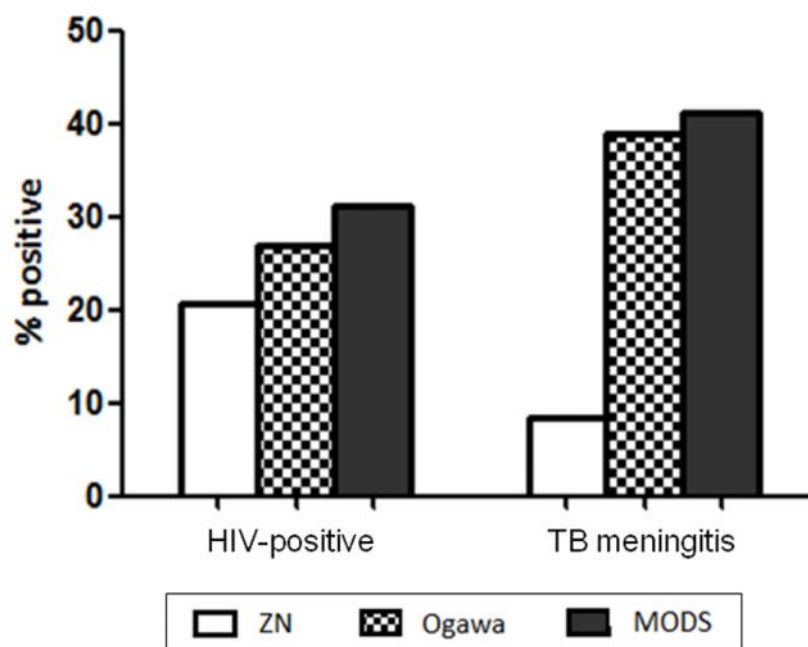
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112 This study was approved by the ethical committee of the Faculty of Medicine, Padjadjaran
113 University/Hasan Sadikin Hospital, Bandung, Indonesia. Written informed consent was
114 obtained from each patient.

115 116 3. RESULTS

117
118 We included 167 HIV-infected patients screened for pulmonary TB. All patients were
119 antiretroviral therapy (ART)-naïve at the time they were screened for TB. Most patients came
120 with advanced HIV infection, with a median CD4 cell count of 51/ μ L (IQR 10–252). As a
121 second group, we included 88 patients with suspected TB meningitis. Most patients
122 presented with lowered consciousness and focal neurological signs, and with typical CSF

findings of moderate pleiocytosis, elevated protein and lowered glucose, 9 patients (11%) were HIV-infected.

MODS culture had the highest detection rate among both groups (Figure 1). In HIV-infected patients with suspected pulmonary TB, MODS culture of 7/167 patients (4.2%) were contaminated and were excluded from the calculation of positivity rate. Of the remaining 160 patients, MODS culture was found positive in 50 patients (31.2%) compared with 43 patients (26.9%) for Ogawa culture and 33 patients (20.6%) for ZN staining and microscopy; the difference between MODS culture and ZN was statistically significant ($P = .02$). None of the patients whose MODS cultures were contaminated had a positive Ogawa culture. Among 127 ZN smear-negative patients, MODS was positive in 20 patients (15.8%) compared with 15 (11.8%) using Ogawa. Among patients with suspected TB meningitis, MODS cultures of 3 patients (3.4%) were contaminated and were excluded from the analysis. Of the remaining 85 patients, MODS culture also gave the highest detection rate; positive in 35 patients (41.2%) compared with 33 patients (38.8%) for Ogawa and 7 patients (8.3%) for ZN. Both MODS and Ogawa culture were significantly more sensitive compared to ZN ($P < .001$). Of patients whose MODS cultures were contaminated, one patient was positive for the Ogawa culture. Among 77 smear-negative patients, MODS was positive in 30 (39.0%) and Ogawa in 27 (35.1%) patients. Ten patients in both patient groups were MODS-positive/Ogawa-negative, and 3 and 8 patients, respectively, were MODS-negative/Ogawa-positive. This was thought to be due to the low numbers and clumping of mycobacteria which makes that bacilli are not always uniformly distributed in sample aliquots.



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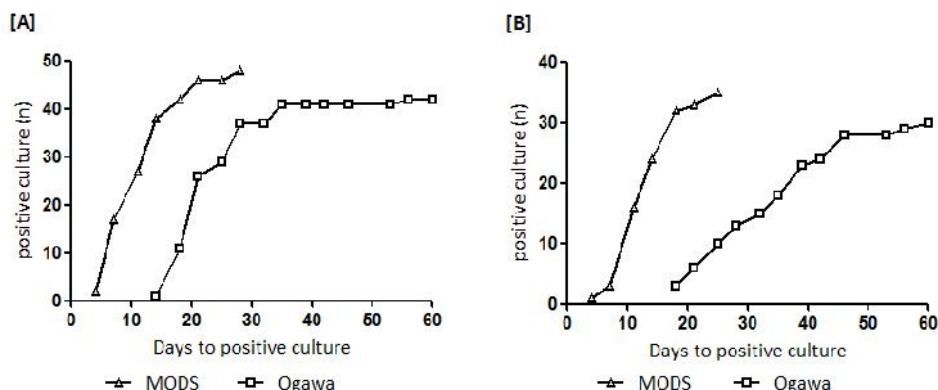
Figure 1. Positivity rate of microscopy of Ziehl-Neelsen-stained smears, Ogawa solid culture, and MODS culture for suspected pulmonary TB among 160 HIV-infected patients and suspected TB meningitis among 85 patients. Seven patients in HIV-infected pulmonary group and three patients in TB meningitis group were excluded due to contamination in MODS cultures.

The median time to culture positivity was shorter for MODS culture than for Ogawa culture in both groups of patients (figure 2). Using sputum from HIV-infected patients, positive results

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for MODS were obtained after a median 11 days (IQR 7-14 days) compared with 21 days (IQR 18-28 days; $P < .001$) for Ogawa. Within 14 days, MODS detected 79.2% of positive samples, compared to only 2.4% for Ogawa ($P < .001$). Among patients with suspected TB meningitis, the median time to culture positivity of CSF samples was 14 days (IQR 11-18 days) for MODS, compared to 33 days (IQR 23-39 days, $P < .001$) for Ogawa. In this group, MODS detected 68.6% of positive cultures within 14 days, while all Ogawa cultures were still negative by this time.

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161 Figure 2 Positive culture results (n) in time for MODS (Δ) and Ogawa ($\square$) among patients
162 with suspected HIV-associated pulmonary TB [A] and suspected TB meningitis [B]
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164 4. DISCUSSION

165

166 Bacteriological confirmation of certain paucibacillary forms of TB, like TB meningitis, is
167 problematic. We examined the performance of MODS as a rapid culture-based method for
168 diagnosis of TB meningitis and HIV-associated pulmonary TB in a hospital setting in
169 Indonesia. As comparative tests we used solid culture and microscopy of Ziehl-Neelsen
170 stained smears, the two most widely used methods for TB diagnosis in low-resource
171 settings. For both study groups, MODS culture had the highest positivity rate. In addition,
172 MODS culture was much quicker compared with Ogawa solid culture.

173 The high positivity rate of MODS culture, which is due to the use of liquid medium, was in
174 line with previous studies [8, 12, 13]. One previous study reported that MODS was more
175 sensitive than automatic mycobacterial culture for diagnosis of pulmonary TB [3], but this
176 was not confirmed in another study [1]. To our knowledge, only one study evaluated MODS
177 for suspected TB meningitis [5]. This study from Vietnam showed that MODS was a
178 sensitive and simple culture-based method for diagnosis of TB meningitis. Mycobacterial
179 Growth Indicator Tube (MGIT, Becton Dickinson) showed a slightly higher sensitivity in the
180 Vietnam study, but the difference was not significant. A smaller volume of deposit was
181 inoculated into the MODS culture than the MGIT culture, possibly contributing to the slight
182 difference in sensitivity.

183 In our study MODS culture significantly reduced the time to both positive and negative
184 results. After two weeks, 79.2% of HIV-associated pulmonary TB had been diagnosed with
185 MODS, and 68.6% of TB meningitis, compared with 2.4% and 0%, respectively, using
186 Ogawa solid culture. These findings are in line with previous studies in HIV-associated
187 pulmonary TB [1, 3] and TB meningitis patients [5]. With the high sensitivity and rapidity,

188 MODS culture is suitable for the TB screening of HIV-infected patients, as shown by a study
189 in Peru [4].

190 Among HIV-infected patients examined for pulmonary TB, the positivity rate of ZN was
191 relatively high compared to culture. This is probably caused by the fact that ZN microscopy
192 was performed on all available sputum samples for each patient, while only single Ogawa
193 and MODS culture were performed for every patient.

194 The contamination rate of MODS culture was around 3-4%. A contamination rate of 2%-3%
195 is acceptable for fresh specimens, but it may be as high as 5%-10% for stored samples [14].

196 MODS culture is a simple test. Sample preparation for MODS is similar to that required for
197 other culture methods. The microscopic detection of mycobacteria by MODS is technically
198 similar to that of a smear. Our laboratory technicians were able to perform the MODS assay
199 and accurately read the results after two weeks of training. The cord formation, which is
200 characteristic of *M. tuberculosis* in liquid culture, is easy to recognize using inverted
201 microscopy. Certain species of non-tuberculous mycobacteria (NTM), for example *M.*
202 *chelonae*, may also form cording in liquid culture but this can be recognized by rapid
203 overgrowth by day 5 [3]. Of note, genotyping of > 800 cultures isolated in our laboratory has
204 only revealed a very low number of NTM (<3%)[15].

205 A possible limitation of this study is that we did not include automated liquid culture (MGIT),
206 which is recommended as diagnostic standard for culture, as the comparative test. The few
207 studies comparing MODS culture with MGIT showed slightly different results [1, 3, 5].
208 Furthermore, our study was performed in a single laboratory. However, as this is a busy
209 hospital laboratory of a large hospital, our results suggest that MODS can be implemented in
210 high-burden settings. Finally, we did not evaluate MODS for DST. Therefore, further studies
211 may be needed to assess the feasibility of MODS culture in other settings, and assess its
212 impact on case detection and timely treatment of paucibacillary forms of TB like TB
213 meningitis.

214

215 **5. CONCLUSION**

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217 MODS culture showed a high sensitivity and rapidity (compared with solid culture), simplicity,
218 and potential low cost (compared with automated liquid systems) for diagnosis of HIV-
219 associated pulmonary TB and TB meningitis in a hospital setting in Indonesia. As such, this
220 seems a reliable method to help implement *M. tuberculosis* culture more widely and speed
221 up TB diagnosis in TB endemic settings.

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225

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Lidya Chaidir designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Jessi Annisa managed the specimens and data collection in laboratory. Sofiati Dian and Ahmad Rizal Ganiem managed the inclusion of TB meningitis patients in the hospital as well as the patient information. David A. J. Moore reviewed the manuscript before submission. Soni Muhsinin and Tina Kusumaningrum performed the study in the laboratory. Ida Parwati coordinated the laboratory work. Merry Lestari managed the inclusion of HIV-infected pulmonary TB in the hospital as well as the patient information. Bacht Alisjahbana and Reinout van Crevel designed the study and reviewed the data analysis and the manuscript. All authors read and approved the final manuscript."

CONSENT

All authors declare that written informed consent was obtained from the patients.

ETHICAL APPROVAL

This study was approved by the ethical committee of the Faculty of Medicine, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia.

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