

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 ~~drug resistance testing~~ 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-cysteine (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

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

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

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

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

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

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

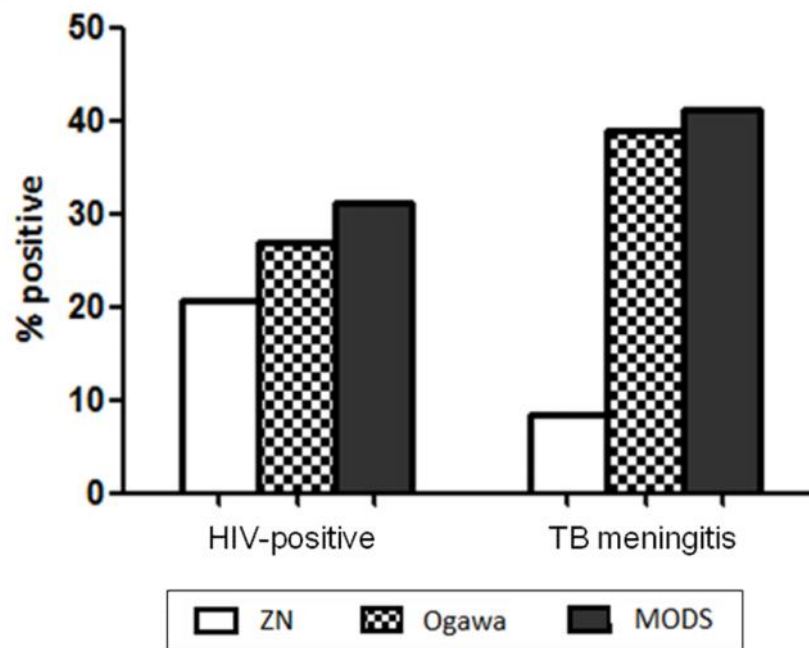
This study was approved by the ethical committee of the Faculty of Medicine, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia. Written informed consent was obtained from each patient.

3. RESULTS

We included 167 HIV-infected patients screened for pulmonary TB. All patients were antiretroviral therapy (ART)-naïve at the time they were screened for TB. Most patients came with advanced HIV infection, with a median CD4 cell count of 51/ μ L (IQR 10–252). As a second group, we included 88 patients with suspected TB meningitis. Most patients 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 suspects, MODS culture of 7/167 patients (4.2%) were contaminated and were excluded from the calculation of positivity rate. Of the remaining 160 patients left, 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 suspects, MODS cultures of 3 patients (3.4%) were contaminated and were excluded from the analysis. Of the remaining 85 patients left, 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.



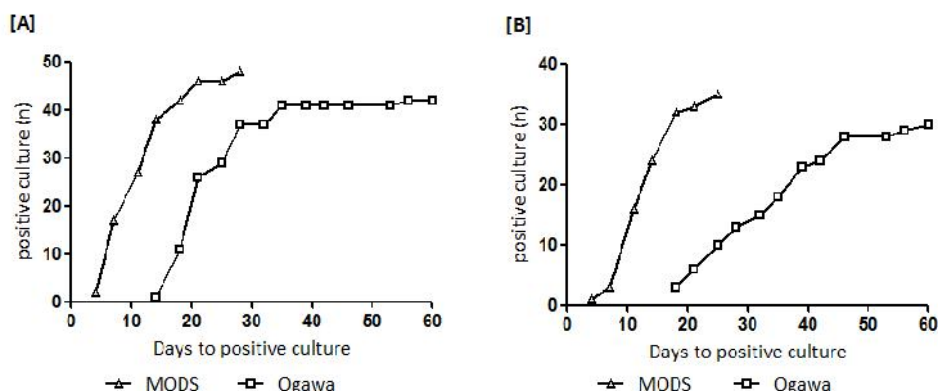
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Figure 1. Positivity rate of microscopy of Ziehl-Neelsen-stained smears, Ogawa solid culture, and MODS culture for suspected pulmonary TB among 160 HIV-infected patients and suspected TB meningitis among 85 patients. Seven patients in HIV-infected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

151

152 The median time to culture positivity was shorter for MODS culture than for Ogawa culture in
 153 both groups of patients (figure 2). Using sputum from HIV-infected patients, positive results
 154 for MODS were obtained after a median 11 days (IQR 7-14 days) compared with 21 days
 155 (IQR 18-28 days; $P < .001$) for Ogawa. Within 14 days, MODS detected 79.2% of positive
 156 samples, compared to only 2.4% for Ogawa ($P < .001$). Among patients with suspected TB
 157 meningitis patients, the median time to culture positivity of CSF samples was 14 days (IQR
 158 11-18 days) for MODS, compared to 33 days (IQR 23-39 days, $P < .001$) for Ogawa. In this
 159 group, MODS detected 68.6% of positive cultures within 14 days, while all Ogawa cultures
 160 were still negative by this time.

161



162

163 Figure 2 Positive culture results (n) in time for MODS (Δ) and Ogawa ($\square$) among patients
 164 with suspected HIV-associated pulmonary TB [A] and suspected TB meningitis [B]
 165

166 4. DISCUSSION

167

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

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

185 In our study MODS culture significantly reduced the time to both positive and negative
 186 results. After two weeks, 79.2% of HIV-associated pulmonary TB had been diagnosed with
 187 MODS, and 68.6% of TB meningitis, compared with 2.4% and 0%, respectively, using
 188 Ogawa solid culture. These findings are in line with previous studies in HIV-associated

189 pulmonary TB [1, 3] and TB meningitis patients [5]. With the high sensitivity and rapidity,
190 MODS culture is suitable for the TB screening of HIV-infected patients, as shown by a study
191 in Peru [4].

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

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

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

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

216 217 **5. CONCLUSION**

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

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227
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232 help in sample collection.
233

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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