

# MODS ASSAY FOR RAPID DIAGNOSIS OF TUBERCULOSIS AMONG HIV TB CO INFECTED INDIVIDUALS IN A TERTIARY CARE HOSPITAL, ANDHRA PRADESH.

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## ABSTRACT

**BACKGROUND:** Rapid, reliable, economical methods are required for diagnosis of tuberculosis. The Microscopic Observation of Drug Susceptibility (MODS) assay is a relatively low-cost and simple liquid culture method. The objective of this study is to determine the sensitivity and specificity of MODS test in comparison to the Lowenstein-Jensen medium to diagnose tuberculosis among HIV seropositive individuals in GSL Medical College.

**METHODS:** Sputum specimens were evaluated using smear microscopy, culture on Lowenstein-Jensen medium and MODS assay. A study subject is considered to have tuberculosis if at least 1 culture on Lowenstein-Jensen medium or MODS technique showed growth for *M. tuberculosis*.

**RESULTS:** Spot Morning sputum samples were obtained from 873 HIV seropositive individuals. Two hundred and ninety seven (34%) [95% CI=30.8 – 37.2] patients were culture positive by MODS and 277 (32%) [95% CI=28.7 – 34.9] were culture positive on LJ slopes ( $P < 0.001$ ). MODS sensitivity was 99.3% and specificity was 96.3%. Mean times for TB detection were 21 days (range 15 – 25 days) and 12 days (range 7- 15 days) for culture on Lowenstein-Jensen medium and MODS (including drug susceptible testing) respectively ( $P < 0.001$ ). Culture contamination was low in MODS assay than culture on Lowenstein-Jensen medium (1.35 vs. 15.6%;  $P < 0.001$ ). Drug resistance was 12.6% for both RIF and INH, 12.6 % for RIF and 15% for INH.

**CONCLUSIONS:** The MODS assay is a relatively simple test whose good performance for detection of pulmonary tuberculosis in HIV patients may make it suitable for resource-limited environments.

**KEY WORDS:** Tuberculosis, Sputum smear, HIV, MOD

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## Introduction:

In India, ~ 5% of tuberculosis (TB) patients registered under Revised National tuberculosis Control Program (RNTCP) are co-infected with HIV.<sup>1</sup> The existence of HIV and TB together, greatly amplifies harmful effects of each other at individual level and contribute substantially to mortality among patients living with HIV (PLHIV).<sup>2</sup> The risk of developing TB is estimated to be between 20-37 times greater in PLHIV than among those without HIV infection.<sup>3</sup> Inadequate treatment, default behavior further result in Drug Resistance (DR) TB, HIV is one of the main predisposing factors.

Well equipped clinical laboratories can detect Myco-

bacterium tuberculosis (MTB) within 7-14 days, using sophisticated liquid culture systems such as BACTEC and Mycobacterium Growth Indicator Tubes (MGITs).<sup>3,4</sup> In most of the developing countries TB laboratories lack sophisticated, costly equipment and skilled technicians. Though ZN staining is a rapid test, sensitivity is relatively low, require about ten thousand bacilli per ml of the specimen.<sup>5,6</sup> In half of the HIV TB patients sputum smears are negative for Acid Fast Bacilli (AFB) by ZN staining.<sup>7,8</sup> This is the major limitation of ZN staining in PLHIV.

Most of the laboratories in developing countries use solid media such as Lowenstein Jensen (LJ). Under optimum conditions TB diagnosis takes 4 weeks and drug

susceptibility test (DST) takes additional 3 to 4 weeks using LJ medium.<sup>9</sup> To detect the TB rapid molecular tests like line probe assay have been developed.<sup>10, 11</sup> These molecular diagnostic tests are rapid and highly accurate. But cost, expertise and infrastructure are the major obstacles to offer these tests. Mycobacterium growth is more rapid in liquid medium as strings and tangles.<sup>12</sup> Based on this a new, rapid, reliable, inexpensive method<sup>13-16</sup> namely Microscopic Observation of Drug Susceptibility (MODS) is devised, which permits MTB detection and drug susceptibility in less than 2 weeks.

Hence in the current study the diagnosis and DST of TB in HIV patients is done by MODS and culture results are compared with gold standard LJ.

## MATERIAL AND METHODS

The study was conducted from March 2009 to December 2013, in the department of Microbiology, GSL Medical College, Rajahmundry. Study was approved by the Institutional Research and Ethics committee. Study included PLHIV with clinical suspicion suggestive of TB. Children with HIV aged below 14 years, individuals who refused to give two consecutive samples of sputum and HIV sero negative individuals were excluded from the study. An informed written consent in the presence of witness was taken from all the volunteers who participated in the study. Sputum samples were collected from HIV sero-positive individuals by Spot Morning (SM) scheme.<sup>18</sup>

All the volunteers were explained regarding the importance of submission of good quality sputum sample. The visual difference between the sputum and saliva and the procedure for production of good quality sputum sample was demonstrated.<sup>17</sup> After collection of spot sample, the individuals were provided with pre labeled sample containers for collection of morning samples. Immediately after collection, sputum smears were prepared on new glass slide and were stained by ZN technique as per RNTCP guidelines.<sup>18</sup>

After smear preparation, decontamination and concentration of the sputum samples was done by standard N-acetyl-L-cysteine- Sodium hydroxide method.<sup>19</sup> Specimen with equal parts of N-acetyl-L-cysteine- Sodium hydroxide solution, mixed for 15 seconds on vortex mixture. Then enough phosphate buffer saline was added to reach within 1cm of the top of the test tube, cap was closed tightly and inverted to mix the solution. Then it was centrifuged at 3600xg for 15minutes. The supernatant was decanted and sediment was suspended in to 2ml of Middlebrook 7H9 broth.

New, sterile flat bottom 24 well microtitre plates

were used to test three sputum samples (eight wells per sample) by MODS technique. Five hundred and forty ml of sputum medium solution was placed into each of four micro wells. Sixty ml of each drug solutions were added. The final drug concentrations are as follows:

- a. Isoniazid (INH): 0.4& 0.1 mg/ml
- b. Rifampicin (RIF): 1 & 0.5 mg/ml

In the remaining four wells first two were filled with sputum media mixture, acts as drug free control. In the other two wells, one was filled with media, this act as sterilization control. The last well was filled with known drug sensitive strain of MTB, by standardizing the turbidity with 0.5 Mc Farlands standard. Plates were sealed with scotch polyethylene tape, incubated at 37°C. Every day (except on Sundays and public holidays) wells were examined for the presence of MTB under an inverted light microscope under 40X objective.

A drop of processed sputum sample was also inoculated in blood agar and another drop was inoculated in Sabourauds Dextrose Agar. These were incubated at 37°C. If any bacteria or fungal growth was observed, another sample was collected from patient. Simultaneously samples were inoculated in LJ slopes, incubated at 37°C for 3 – 4 weeks. Regular decontamination of incubators was followed to avoid contamination of cultures.

Presence of pink colored bacilli in the ZN staining indicates sputum sample is positive for AFB. Presence of growth in microtiter well containing sputum media mixture was considered as TB positive. Individual was considered as non TB, if growth is absent in media sample containing well. Presence of growth in all the four drug containing wells (two RIF wells and two INH wells) along with media sample wells indicates DR. Presence of growth in drug free wells and absence in drug containing wells indicates that the clinical sample is TB positive and drug sensitive. LJ Media was home made.

## Statistical methods:

Data were analyzed using of SPSS v. 16 (SPSS Inc., Chicago, IL, USA), with the patient as the unit of analysis. The Wilcoxon signed-rank test was used to compare the times to each end point among the two methods. A P value of less than 0.05 was used to indicate statistical significance. The concordance of susceptibility results was determined with the use of the sensitivity, specificity, and positive and negative predictive values for the detection of resistance (with 95% confidence intervals [CIs]). For sensitivity and speci-

ficity of detection and predictive-value calculations for each of the two methods, a positive reference result was defined as a positive culture according to at least one method for which cross-contamination had been conclusively ruled out. A negative reference result was defined as any sample in which both the culture methods yielded negative results. McNemar's  $\chi^2$  test was used to compare the sensitivities of detection of the two methods.

## RESULTS

During the study period a total of 873 patient's sputum samples were processed by both MODS and LJ slant techniques. Two hundred and ninety seven (34%) [95% CI= 30.8 – 37.2] patients were culture positive by MODS and 277 (32%) [95% CI= 28.7 – 34.9] were culture positive on LJ slopes ( $P<0.001$ ) (Table 1). MODS sensitivity was 99.3% and specificity was 96.3% compared to the LJ slope as standard technique ( $P<0.001$ ). Mean times for TB detection were 21 days (range 15–25 days) and 12 days (range 7- 15 days) for culture on LJ medium and MODS (including drug susceptible testing) respectively ( $P<0.001$ ) (Table 1). The percentage of contaminated cultures was lower for the MODS assay than culture on LJ medium (1.35 vs. 15.6;  $P<0.001$ ) (Table 2). Out of 297 MODS positivity, DR was 12.6% for both RIF & INH, 12.6% and 15% were resistant to RIF and INH respectively (Table 2). The recurring expenditures were Rs: 340/-, 120/- and 30/- respectively for

MODS, LJ and ZN staining.

## DISCUSSION:

In spite of the disadvantages like inability to detect DR, limited utility to diagnose TB in HIV individuals, sputum smear microscopy (ssm) is the only diagnostic method in the developing countries like India.<sup>20</sup> In a study by Ingrid V et al<sup>21</sup> the sputum smear positivity was 9% among HIV seropositive individuals. Whereas in the current study 8.6% of HIV patients sputum smears were positive for TB. Simon walusimbi et al in the meta analysis study on MODS stated that substantial proportion (35%) of TB cases were smear negative.<sup>22</sup>

Currently culture on solid media is the only feasible method for confirmed diagnosis of TB in developing countries. But MTB growth is rapid in liquid media than solid media. In liquid media MTB growth was observed in 7 to 15 days. On solid media MTB growth was observed in 15 to 27 days. As per the available literature, the average time period required to declare MODS results were 10 -12 days, in the current study it is 12 days. During this time period 30% of sputum samples only showed positive results on LJ slants and DST require additional time. This helps in large reduction of time, space and infrastructure in the laboratory. Early initiation of treatment is the major advantage of this method.

Table1: Sensitivity and specificity of MODS for TB diagnosis

MODS culture	LJ culture			
		Positive	Negative	Total
	Positive	275	22	297
	Negative	2	574	576
	Total	277	296	873

Sensitivity – 99.3%; 95% CI=0.989-0.996

Specificity – 96.3%; 95% CI=0.957-0.968

Positive Predictive Value (PPV) – 92.6%; 95% CI=0.915-0.936

Negative Predictive Value (NPV) – 99.7%; 95% CI=0.994-0.998

Table 2: Culture positivity cum contaminations

Variable	MODS				LJ-Medium				P
	Pos	Tot	Prop	CI	Pos	Tot	Prop	CI	
Culture	297	873	34.0	30.8 - 7.2	277	873	31.7	30.8 -37.2	0.001
Contamination	4	873	0.5	0.02-0.82	43	873	4.9	3.47 – 6.33	0.001
Median time to detection	12 (Inter Quartile Range=11.5 to 13.00); Standard Deviation-2.99				21 (Inter Quartile Range=18 -31) Standard Deviation-11				0.001

Pos: Positive

Cont: Contamination

Tot: Total

CI: Confidence Interval

Prop:Proportion

TAT: Turnaround Time

As per the Moore et al study,<sup>15</sup> the diagnostic yield of single sputum sample among the patients suspected with TB was 37%, 80% and 89% respectively for smear microscopy, LJ & MODS and with second sample the additional use is 11.6%, 7.5% and 8.2% respectively. In HIV patients, with single sputum sample diagnostic yield of PT was 42.9%, 78.6% and 92.9% and the incremental yield was 3.6%, 3.6% and 3.6% with second sputum sample respectively for sputum smear, LJ and MODS. In the current study the diagnostic yield of ssm was 7.9%, 8.6% respectively for spot and morning samples. Due to limited resources single sputum sample was only processed for culture.

In a meta analysis study by Jessica Minion et al,<sup>23</sup> DST of MODS has a sensitivity of 98% (95% CI 94.5 - 99.3), specificity 99.4% (95.7 - 99.9) for RIF resistance. For INH resistance, pooled sensitivity was 97.7% (94.4 - 99.1) and pooled specificity was 95.8% (88.1 - 98.6). In the current study, due to limited resources DST was not performed on LJ media. This could be the limitation of the current study.

In a study by Lazarnu<sup>24</sup> the authors reported that MODS is 94.12% sensitive and 89.39% specific when compared with LJ media and the concordance with DST by the proportion method on LJ media to RIF & INH was 91.5% & 90.8% respectively. In one of the south Indian studies on MODS assay for detecting TB among HIV individuals, the overall sensitivity and specificity was 89.1% & 99.1%. In the diagnosis of DR TB, MODS was 84.2% sensitive and the authors also reported that MODS has 87% and 100% sensitivity for INH and RIF mono resistant.<sup>25</sup>

In another south Indian study MODS was reported to be 78.9% sensitive and 96.7% specific and the authors also coated that the true positivity in 4/6 reference culture negative MODS positives.<sup>26</sup> Kashmira Limaye et al studied MODS on sputum smear positive TB cases and the investigators declared that culture positivity was 100% for both MODS assay as well as LJ medium.<sup>27</sup> In this study among sputum smear positive cases the sensitivity is 100% for MODS as well as LJ media.

In a study by Reddy et al<sup>28</sup> MODS test had 100% sensitivity to detect TB among HIV patients. In the current study MODS sensitivity is 99%. So MODS could be used as a diagnostic test to detect TB in HIV sero positive patients.

Culture contamination was low in MODS assay, compared to LJ. In the current study contamination rate was 1.35 vs 15.6% for MODS and LJ media respectively. In a study by Reddy et al<sup>28</sup> culture contamination was 7.3% vs 22% respectively for MODS and LJ.

Cord formation in MODS can be recognized more easily and rapidly than a ZN smear. With 2 weeks training, one can read MODS cultures easily. But DST on LJ may take several months of training. The other available rapid culture systems require computer attached incubators, in addition to the standard equipment. But MODS culture requires just an inverted microscope.

The recurring expenditure for MODS technique (both culture & DST) is 3 times more compared to LJ culture and 10 times high compared to ZN smears. So MODS is relatively expensive than the present routine techniques under the RNTCP conditions; Early detection and treatment would prevent spread of infection which is estimated to be 10 - 15 individuals per year per open case.<sup>29</sup> Due to misdiagnosis spread of PT can occur, for which national TB control programmes (NTPs) have to spend significant amount of money for anti TB treatment in the form of DOTS / DOTS plus. When compared to this, the expenditure on MODS is negligible.

Limitation of this study is that due to limited resources only one set of LJ media was used which deviation from the RNTCP rule. Another point is high contamination rate of LJ media but this is possibly due to long incubation period of this media.

## CONCLUSION:

To conclude MODS test is rapid, economical, require minimum infrastructure, less contamination and the cord formation is read very easily than ZN smear. Like molecular techniques, MODS do not require any sophisticated equipment or skilled person, except bio-hazard safety cabinet and an inverted microscope. So, MODS is suggested as alternative method for the diagnosis of TB and DST in HIV patients.

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