1.2 Rapid culture and direct drug sensitivity testing of *Mycobacterium tuberculosis* to Isoniazid and Rifampicin using liquid culture media.

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OBJECTIVES

1. To Standardize and evaluate rapid method for culture and sensitivity of *M. tuberculosis.*
2. To support RNTCP with culture of *Mycobacterium tuberculosis* from sputum samples.

PROGRESS

The new method was used by us for 471sputum samples received from KN Chest Hospital and Distt. TB Clinic Jodhpur. All these samples were processed with Modified Petroff Method and were inoculated on LJ Medium, prepared locally at DMRC and the results of culture on LJ Medium are given in Table 1.

Table 1. Results of inoculation of sputum samples on LJ medium

S. No.	Result of inoculation of sputum samples on LJ medium	No. of sputum samples
1.	Culture Negative	129
2.	Culture positive	258
3.	Contamination	66
4.	LJ Culture not done	18
	TOTAL	471

Out of 258 positive cultures, seven showed mycobacterial colonies on LJ slants containing PNB and these 7 also showed negative niacin test and negative CARD Test. These were therefore taken as non-tubercular mycobacteria (NTM) or mycobacteria other than tuberculosis (MOTT). All these 7 samples showed resistance to rifampicin as well as isoniazid on proportion method. These were excluded from analysis.

Out of 251 sputum samples with sputum culture positive for Mtb on LJ, drug sensitivity testing (DST) by 'Proportion Method' (Gold Standard) was carried out on 237 samples. Three of these showed contaminations on DST, therefore results of DST by 'Proportion Method' were available for 234 samples.

Table 2. Observations of Day '0' smear of CVB and observations of Day '7' smear of CVBR

Smear on Day 0	Smear on Day 7 in CVBR (Vial with RIF)						TOTAL
	Negative	Scanty	No Clumps	Small Clumps	Large Clumps	Micro-colonies	
Negative	108	6	2	3	3	2	**124**
Scanty	33	12	5	4	3	1	**58**
No Clumps	13	15	11	14	5	4	**62**
Small Clumps	15	20	17	30	15	12	**109**
Large Clumps	8	3	4	12	31	21	**79**
Microcolonies	3	1	2	5	8	20	**39**
TOTAL	180	57	41	68	65	60	471

The proposed method of rapid culture and DST by liquid culture medium i.e. Rapid Microscopic Detection of Sensitivity (RMDS) described under methodology was carried out for all 471 samples and observations of Day '0' smear of CVB and observations of Day '7' smear of CVBR were available for all 471 samples (Table 2), but results of DST to Rifampicin by 'Proportion Method' for comparison were available for only 234 samples. Since during early months of project during standardization of the method, only CVB and CVBR were inoculated results of DST to Isoniazid by Proportion method as well as by RMDS method were available for only 182 samples.

Table 3. Results of RMDS for rifampicin as compared to proportion method (Gold Standard)

Results of RMDS for Rifampicin	Result of Proportion Method for Rifampicin		TOTAL
	Resistant	Sensitive	
Resistant	22	42	64
Sensitive	23	108	131
Indeterminate	4	35	39
TOTAL	49	185	234

As shown in Table 3, out of 185 sputum samples found sensitive to rifampicin by proportion method, the results of RMDS were indeterminate for 35 samples. Of remaining 150 samples, 108 were found to be sensitive to rifampicin by RMDS method indicating specificity of 72.0 per cent (95% CI 64.8 to 79.2%). Out of 49 sputum samples found resistant to rifampicin by proportion method, the results of RMDS were indeterminate for 4 samples. Of remaining 45 samples, 22 were found to be sensitive to rifampicin by RMDS method indicating sensitivity of 48.9 per cent (95% CI 34.3 to 63.5%). Out of 131 sputum samples found sensitive to rifampicin by RMDS 108 were sensitive to rifampicin by proportion method (True Negatives) indicating Negative Predictive value of 82.4 per cenet. Out of 64 sputum samples found resistant to rifampicin by RMDS 22 were resistant to rifampicin by proportion method (True positives) indicating Positive Predictive value of 34.3 per cent.

Table 4. Results of RMDS for Isoniazid as compared to proportion method (Gold Standard)

Results of RMDS for Isoniazid	Result of Proportion Method for Isoniazid		TOTAL
	Resistant	Sensitive	
Resistant	25	25	50
Sensitive	23	86	109
Indeterminate	5	18	23
TOTAL	53	129	182

As shown in Table 4, out of 129 sputum samples found sensitive to Isoniazid by proportion method, the results of RMDS were indeterminate for 18 samples. Of remaining 111 samples, 86 were found to be sensitive to Isoniazid by RMDS method indicating specificity of 77.5 per cent (95% CI 69.7 to 85.3%). Out of 53 sputum samples found resistant to Isoniazid by proportion method, the results of RMDS were indeterminate for 5 samples. Of remaining 48 samples, 25 were found to be resistant to Isoniazid by RMDS method indicating sensitivity of 52.1 per cent (95% CI 37.9 to 66.2%). Out of 109 sputum samples found sensitive to Isoniazid by RMDS 86 were sensitive to Isoniazid by proportion method (True Negatives) indicating Negative Predictive value of 78.9 per cent. Out of 50 sputum samples found resistant to Isoniazid by RMDS 25 were resistant to Isoniazid by proportion method (True positives) indicating Positive Predictive value of 50.0 per cent.

The proposed method of rapid culture and DST by liquid culture medium i.e. Rapid Microscopic Detection of Sensitivity (RMDS) described under methodology was standardized and evaluated against Proportion Method of drug sensitivity testing (Gold Standard). For DST to rifampicin, RMDS showed specificity of 72.0 per cent (95% CI 64.8 to 79.2%) and Negative Predictive value of 82.4 per cent. However, sensitivity of RMDS for the same was 48.9 per cent (95% CI 34.3 to 63.5%) and Positive Predictive value was only 34.3 per cent, which were not in acceptable limits. Results were not encouraging, therefore, external validation of method was not attempted.