COMMUNICABLE DISEASES

1.9 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

First version of the new method was used by us for 11 sputum samples which were also examined by RNTCP using LPA. It was decided to refine the method after getting the results of this method as compared to results of LPA/cartridge based nucleic acid amplification testing (CBNAAT). It was not a formal evaluation of the method. Our method was followed blindly and results were available on 7th day for 11 samples. Results of our method as compared to LPA are shown in Table 1. Results of LPA were available for these samples after two months. LPA showed 2 as resistant to rifampicin, of which one was also detected as resistant by our method; LPA showed 6 as sensitive to rifampicin, of which 5 were also detected as sensitive by our method, giving sensitivity of 83.3%. The method needs to be more refined.

Table 1: Results of new method as compared to results of LPA (Line Probe Assay)

Results of our method			Results of LPA	
	Negative	Resistant	Sensitive	Total
AFB Negative	3	0	1	4
Resistant	0	1	0	1
Sensitive	0	1	5	6
Total	**3**	**2**	**6**	**11**

First version of this new method was also used for 54 sputum samples of suspects of MDR-TB, sent to us by DTC Jodhpur. DTC Jodhpur had examined these samples with CBNAAT (Cartridge Based Nucleic Acid Amplification Testing) The results of our metod as compared to CBNAAT (Cartridge

Based Nucleic Acid Amplification Testing) are shown in Table 2.

CBNAAT showed 5 as resistant to rifampicin, of which four were also detected as resistant by our method, giving sensitivity of 80.0% ; LPA showed 28 as sensitive to rifampicin, of which 19 were also detected as sensitive by our method, giving sensitivity of 76.0%. The method needs to be more refined. For two samples, results of first day smear and seventh day smear were same and therefore any conclusion could not be drawn (Indeterminate).

Table 2. Results of new method as compared to results of CBNAAT (Cartridge Based Nucleic Acid Amplification Testing)

Results of our method	Results of CBNAAT					
	Negative	Contamination	Error	Resistant	Sensitive	Total
Indeterminate	0	0	0	0	2	2
AFB Negative	18	1	0	0	6	25
Resistant	0	0	0	4	1	5
Sensitive	0	1	1	1	19	22
Total	**18**	**2**	**1**	**5**	**28**	**54**

The new method needs to be further refined and compared with standard methods. Since liquid medium was mixed with sputum and then smear of day 0 was prepared, it got diluted and often showed scanty AFBs. The method has now been changed, highly concentrated liquid medium is being prepared, of which 0.1 ml will be needed to be added to 1 ml of sputum, this step is likely to improve the method and better results are likely to be obtained. Many times clumps or microcolonies are very few in the smear of day 7 and are missed on microscopic examination of ZN stained smear. It is proposed to use fluorescent staining and LED microscopy to improve the sensitivity of the method. We were not able to procure Rifampicin from Sigma, the Rifampicin of CDH, which we are using does not mention potency factor or date of manufacture or expiry. The potency of this rifampicin might be low, which might be a factor responsible for detection of false resistance. NTI Bangalore has been contacted to provide solution of this problem, which will be implemented. After making these modifications, this method is likely to perform better.

We have applied for accreditation of our laboratory by RNTCP for drug sensitivity testing of *M. tuberculosis.* Consequently, our lab was visited by team of RNTCP. At our laboratory, we have standardized Proportion method using LJ medium for drug sensitivity testing of *M. tuberculosis*. We have carried out drug sensitivity testing of *M. tuberculosis,* using proportion method from 75 sputum samples. We are continuing the same as report of 100 such samples from our laboratory is required for accreditation.