COMMUNICABLE DISEASES

1.8 Standardization of a rapid method for direct drug sensitivity testing of *Mycobacterium tuberculosis* from sputum samples.

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OBJECTIVE

To standardize safe, simple, economic, closed method of direct drug sensitivity testing of *M. tuberculosis* from sputum samples in seven days.

PROGRESS

First version of the new method was used for 111 sputum samples. 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. We collected sputum samples from 111 patients admitted at KN Chest hospital with suspicion of MDR-TB. Our method was followed blindly and results were available on 7th day for 103 samples. Out of the 111 samples, results of LPA were available for 45 samples, as shown in Table 1. Out of these, 22 were AFB negative, 28 were labeled as resistant to rifampicin, 47 as sensitive to rifampicin and six showed contamination. Results of LPA were available for 49 of these samples after two months. LPA showed 8 as resistant to rifampicin, of which six were also detected as resistant by our method, giving sensitivity of 75.0% ; LPA showed 25 as sensitive to rifampicin, of which 18 were also detected as sensitive by our method, giving sensitivity of 72.0%. The method needs to be more refined. For eight samples, results of first day smear and seventh day smear were same and therefore any conclusion could not be drawn.

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

Results of our method	Results of LPA					
	Negative	Contamination	NTM	Resistant	Sensitive	Total
Indeterminate	0	0	1	0	2	3
Negative	7	0	0	0	2	9
Contamination	0	1	0	0	1	2
Resistant	0	0	0	6	4	10
Sensitive	1	0	0	2	18	21
Total	**8**	**1**	**1**	**8**	**27**	**45**

First version of this new method was also used for 41 sputum samples of suspects of MDR-TB, sent to us by DTC Jodhpur. The results are shown in Table 2.

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	1	1	3
Negative	15	1	0	1	6	22
Contamination	0	0	0	0	0	0
Resistant	0	0	0	3	0	3
Sensitive	0	1	1	0	11	13
Total	**15**	**2**	**1**	**5**	**18**	**41**

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.