

**Original Research Article****Comparison between the conventional method & molecular line probe assay for identification & drug sensitivity of mycobacteria tuberculosis from clinical specimens**Ritu Kansal<sup>1</sup>, Molly Madan<sup>2</sup>, Richa Kansal<sup>3\*</sup>, Vivek Agwan<sup>4</sup>, Isha Bansal<sup>5</sup>, Nivesh Agrawal<sup>6</sup><sup>1</sup>Demonstrator, Department of Microbiology, Subharti Medical College, Meerut (U.P.), India<sup>2</sup>Professor, Department of Microbiology, Subharti Medical College, Meerut (U.P.), India<sup>3</sup>Asso. Prof, BPS Govt. Medical college for women, Khanpur Kalan, Sonapat, Haryana, India<sup>4</sup>Professor, Department of Microbiology, Subharti Medical College, Meerut (U.P.), India<sup>5</sup>Lecturer, Department of Surgery, BPS Govt. Medical college for women, Khanpur Kalan, Sonapat, Haryana, India<sup>6</sup>Prof., Department of Surgery, BPS Govt. Medical college for women, Khanpur Kalan, Sonapat, Haryana, India**ARTICLE INFO:****Article history:**

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Rapid susceptibility testing of *Mycobacterium tuberculosis* strains is imperative for therapy selection but traditional drug susceptibility tests take weeks or are expensive. Classical drug susceptibility (DST) may take up to 2 to 4 months. The line probe assay is a commercially available line-probe assay that rapidly detects *Mycobacterium tuberculosis* (MTB) complex, as well as the most common mutations associated with rifampicin and isoniazid. In this study we assessed the sensitivity and specificity of the rapid molecular method in comparison with the conventional method.

**Introduction**

Tuberculosis (TB) remains a major health problem in India accounting for more than 20 percent of the global incident cases[1]. In 2008, the annual incidence was 1.9 million cases, of which 0.8 million were infectious new smear positive pulmonary TB cases [PTB] cases[2]. Prevalence of MDRTB in new cases and previously treated cases is reported as 3% and 12-17% respectively [3]. Pulmonary TB can be diagnosed by its symptoms, chest radiography, sputum smear microscopy and by cultivation of *M.tuberculosis*, which is considered as the gold standard. On a global scale laboratory diagnosis of TB principally relies on identification of acid-fast bacilli (AFBs) by sputum smear microscopy, which is simple, rapid, and economic. Culture of clinical specimens is more sensitive than smear microscopy, as only 10-100 viable organisms will result in a positive culture while a minimum of 5000-10,000 acid-fast bacilli (AFB) per ml are required for detection by smear. Identification of *M.tuberculosis* is done by performing

several further biochemical tests [4]. In addition isolates obtained from cultures can be used for mycobacterial species identification, determination of drug susceptibility and molecular epidemiology. Although the use of culture has been advocated for routine diagnosis, it requires 3-4 weeks to detect mycobacterial growth, plus an additional period for identification of mycobacterium tuberculosis. Recent advances in molecular biology and molecular epidemiology, and a better understanding of the molecular basis of drug resistance in TB, have provided new tools for rapid diagnosis; however, the high cost of most of these techniques, and their requirement for sophisticated equipment and skilled personnel have precluded their implementation on a routine basis, especially in low-income countries.

A combination of solid and liquid media is currently regarded as "gold standard" for primary isolation of mycobacteria, and

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the turnaround times not exceeding 21 to 30 days after specimen collection are recommended for MTB identification and drug susceptibility testing .

There is an urgent need to identify methods that would rapidly detect both the presence of *Mycobacterium tuberculosis* [MTB] and drug resistant strains. Culture based methods are considered as the gold standard. However, these methods require moderate to prolonged incubation time or expensive equipment and reagents. Molecular tools perform robustly for both detection and drug resistant status determination, in a very less time. So this study was conducted to see the concordance between the slow conventional and rapid molecular method, if rapid method can be adopted in a routine basis.

Drug susceptibility testing (DST) methods include the proportion method, the absolute concentration method, and the resistance method .

The GenoType® MTBDR*plus* assay (Hain Lifescience, Nehren, Germany) is a commercially available assay that

## Material and methods

**Strains:** In this study a total of 342 clinical specimens, both respiratory and extrapulmonary, were consecutively received for mycobacterial culture in the Department of Medical Microbiology, Subharti Medical College, Meerut. Total 147 (42.98%) were mycobacteria positive, of which 125 (85%) were *M. tuberculosis* and 22 (14.96%) were MOTT (mycobacteria other than tuberculosis). MOTT were not further characterized/tested. Blood specimens were excluded from the study. Investigated specimens included sputa, bronchial washings, urine, normally sterile fluids (pleural, pericardial, synovial, and cerebrospinal fluids and ascites), biopsies, and miscellaneous samples such as pus and gastric aspirate specimens.

Isolation and identification of *mycobacteria*: Sputum samples from each patient was decontaminated, and inoculated on to two Lowenstein-Jensen tubes and incubated at 37°C and read weekly for eight weeks. No growth after eight week of incubation was treated as negative. Growth of *M. tuberculosis* was typed by colony appearance, niacin production, catalase activity at 68°C and pH 7 and susceptibility to p-nitrobenzoic acid[6].

Specimens collected from contaminated sites were liquefied and decontaminated by the standard NALC procedure (11). After decontamination, all specimens were neutralized with phosphate-buffered saline (0.067 M, pH6.8) and centrifuged at 3,500xg for 20 minutes. The pellet was used for smear preparation (Ziehl-Neelsen staining), culturing and DNA extraction, Specimens collected from sterile sites were concentrated by centrifugation without prior decontamination. For mycobacterial culture, each processed sample (0.5 ml) was randomly inoculated into solid media. After inoculation,

combines detection of MTB complex with prediction of resistance to rifampicin and isoniazid. The assay combines detection of MTB complex with detection of mutation in the 81-bp hotspot region of *rpoB*, at codon 315 of the *katG* gene and in the *inhA* promoter region. It was found to have high sensitivity and specificity for rifampicin and isoniazid resistance and performs well when applied directly to AFB-positive sputum specimens.

The spread of multiple drug resistant strains of *Mycobacterium tuberculosis* has become a major public health concern in both developed and developing countries[5]. Factors contributing to recent outbreak and continued spread of multi drug resistant tuberculosis (MDR-TB) includes upsurge of human immunodeficiency virus (HIV) infection/acquired immune deficiency syndrome (AIDS), insufficient control procedures and laboratory delays in identification and susceptibility testing of *M. tuberculosis* isolates,.

LJ slants were kept for a week with the caps loose, in order to enhance sample evaporation, and later tightened. LJ cultures were examined visually weekly for up to 8 weeks, according to standard recommendations. DST for isoniazid (0.2 µg/ml), streptomycin (4 µg/ml), ethambutol (2 µg/ml), and rifampicin (40 µg/ml) was performed using LJ media following the proportion method.

**Microscopy:** Smear grading was performed using WHO recommendations[7]. Smears were stained by the Ziehl-Neelsen method to detect mycobacteria.

Conventional DST on solid media was a standard technology.

**Proportion method:** Drug susceptibility of all positive culture isolates to isoniazid (INH), rifampicin (RIF) was performed by standard method[8]. Briefly, LJ media with drug incorporated in various concentrations and plain LJ medium for control were prepared. The growth from a 3-4 weeks old culture was scraped with a loop and bacterial suspension was made in sterile distilled water, vortexed and matched with McFarland opacity tube No. 1. Dilutions of 10<sup>-2</sup> and 10<sup>-3</sup> were made and inoculated on both the control and drug containing media and incubated at 37°C. The first reading was taken after 28 days of incubation and the second on 40<sup>th</sup> day.

They are based on the estimation of growth or no growth of an *M.tuberculosis* strain in the presence of a single critical concentration of one drug. The critical concentration of an antitubercular drug represents the lowest concentration of the drug in the medium that indicates clinically relevant resistance if growth is observed. Susceptible wild-type strains are inhibited by this concentration. Resistance is defined if over 1% of the bacterial population of a strain is able to grow.

**GenoType® MTBDR*plus* assay** – Assay was performed on smear positive pulmonary samples and culture positive extra-

pulmonary samples according to the manufacturer's instructions.

## Result and Discussion

### Concordance between conventional DST and GenoType® MTBDRplus assay

Based on phenotypic DST, 48 strains were MDR-TB, 20 strains were rifampicin mono-resistant and 44 strains were susceptible to all first-line drugs. Considering the phenotypic DST method as gold standard, the Line probe assay correctly identified 47 of 48 MDR-TB strains (97.9%); 66 of 68

rifampicin resistant strains (95.58%); 62 of 66 INH resistant strains (93.9%). The specificity for detecting MDR-TB was 100%. The overall concordance of the GenoType® MTBDRplus test and phenotypic test was 91.2% (114/125). Sensitivities, specificities and predictive values are listed in Table 1 and 2

One hundred forty seven mycobacterial isolates grew from 342 specimens. Of these 342 isolates, 140 (40.9%) were smear positive and 202 (59%) were smear negative. The percentage of all specimens testing positive for any mycobacteria was 42.98%, whereas the MTB isolation rate was 85%.

**Table 1: Summary of Results of Multidrug Resistance by GenoType MTBDRplus compared with Conventional drug susceptibility results**

GenoType® MTBDRplus				Conventional DST			
MDR	RIF monoresistance	INH monoresistance	RIF and INH sensitive	MDR	RIF monoresistance	INH monoresistance	RIF and INH susceptible
47	19	15	44	48	20	18	39

**Table 2: Performance of GenoType® MTBDRplus assay in detection of RIF, INH and MDR resistance in sputum & positive culture specimens (N=149)**

	RIF	INH	MDR	Pan susceptible
Sensitivity	95.58%	93.9%	97.9%	94.87%
Specificity	98.2%	99.4%	100%	91.86%
PPV (%)	98.48%	98.38%	100%	84.09%
NPV (%)	94.9%	92.06%	98.7%	97.5%



Figure 1: The specificity for detecting MDR-TB was 100 %.

## Discussion

Cultures on solid media is labor-intensive and it may take several weeks for colonies to become detectable; even then, the process may require further subculture for definitive identification. Multi-drug resistant (MDR) *Mycobacterium tuberculosis* isolates may be transmitted within communities due to dense population and poor hygiene conditions. In general, molecular methods offer several advantages over conventional techniques for the rapid detection and identification of *M.tuberculosis*, such as the turn around time

for results, reliability, reproducibility and the possibility to improve patient management.

With the 100% specificity of the GenoType® MTBDRplus assay to detect MDR in *M.tuberculosis* isolates, no patient would be inappropriately treated with category 4 (MDR-TB) treatment if this test would be used in routine for rapid MDR-TB diagnosis. On the other hand 2% of patients would not receive appropriate category 4 treatment (which is based on detection of rifampicin resistance) if identification of MDR-TB patients is done using only the GenoType® MTBDRplus test. These performance characteristics suggest that the assay

is equivalent to conventional Lowenstein-Jensen medium-based DST performed in quality-assured reference laboratories. Considering that test performs well on the specimens that subsequently are contaminated on culture, its overall performance for detection of MDR TB is superior to conventional methods. None of the molecular tests established targets all possible genes or mechanisms (some are not identified yet) involved in resistance, and thus, a variable proportion of resistant strains will not be detected. 10% mutant DNA in a mixture of wild-type and mutant DNA. If the proportion of resistant cells in an isolate is less than that

### Conclusion

Definitive laboratory diagnosis of tuberculosis still revolves around the smear examination and culture confirmation for *M.tuberculosis*. Conventional culture confirmation using LJ method has the inherent disadvantage of time required to observe the growth. This, on many occasions, can delay the start of treatment, facilitating spread of the disease, or lead to unnecessary treatment of non-specific pulmonary infections. Nevertheless, LJ medium remains the gold standard for culture and sensitivity testing of MTB. It is useful for (1) early confirmation of viable MTB, (2) monitoring response to therapy, and (3) monitoring treatment response in multi drug resistant tuberculosis. Overall the GenoType® MTBDR*plus* test is a reliable, rapid and easy to perform for the simultaneous detection of RMP and INH resistance in *M. tuberculosis*. With high sensitivity for detection of rifampicin resistance and high specificity for MDR, we conclude that this test strongly facilitates adequate treatment of MDR-TB patients, long before the results of conventional DST are available. Substantial reduction in the time to diagnose drug-resistant TB, the earlier commencement of appropriate therapy and the potential to prevent transmission of drug-resistant strains constitutes the major advantages of these methods. Because discordance still exists between the conventional and molecular approach of DST and susceptibility of bacteria to drugs is defined as inhibition of growth, we recommend that the GenoType® MTBDR*plus* test should serve as an early guidance of therapy, which should be followed by a phenotypic DST confirmation for all suspected MDR-TB patients.

### Acknowledgement

amount, it can hardly be detected by molecular methods, whereas classical susceptibility testing might give a more sensitive test result in these cases. Nevertheless, the MTBDR culture assay appears to be a valuable tool that allows the detection of resistant *M.tuberculosis* isolates within one working day and easily be included in routine workflow. Considering the high rates of resistant and MDR isolates in several parts of the world, such a test has the potential to complement and accelerate the variety of different measures in laboratory diagnostics that are necessary for improved tuberculosis control in the future.

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### Conflict of interest statement

We declare that we have no conflict of interest.

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