

DIAGNOSIS OF TUBERCULOSIS BY PHAGE AMPLIFICATION TECHNIQUE: A SIMPLE BUT RAPID & RELIABLE ALTERNATIVEC. P. Bhattacharya¹, T. K. Bhattacharya², Late. Reba Chatterjee³**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: In view of the grim scenario that exists in India as far as tuberculosis is concerned, despite various plans and programmes like RNTCP, 'Early diagnosis' of the disease particularly of sputum-positive cases and their treatments are of paramount importance to contain the disease. Sputum microscopy – the mainstay of diagnosis proves inadequate because of its wide variation in sensitivity. There has been more concern in this era of MDR-TB and HIV co-infection. To address these problems the present study has been taken to detect the tubercle bacilli by phage amplification technique (FAST plaque TB™) with comparison to BACTEC 460 TB culture system in 12B vial. 42 smear-positive and 62 smear- negative samples were selected. In 42 smear-positive cases, radiometric culture positivity was seen in 40(95%) and plaque positivity in 35(83%). In 62 smear-negative cases, culture positive cases were 30(48%), but plaque positivity was seen in only 10(16%). Overall culture positivity was 67% (total no. 70) and plaque positivity being 43% (Total no. 46); whereas smear microscopy showed only 24.1% positivity. So phage amplification showed greater sensitivity than smear microscopy; higher specificity (Detects only live bacilli of *M. tuberculosis*). This is rapid, simple, and no need for specialized technical skill and no expensive equipment is required.

KEYWORDS: Mycobacterium tuberculosis, FAST plaque TB™, Phage amplification technique, radiometric culture, BACTEC 460 TB culture.

INTRODUCTION: It is estimated that, in 2013, there were 9 million new cases of tuberculosis, of which 1.1 million were HIV positive. An estimated 1.5 million people died of tuberculosis in the same year.^[1] The estimated global burden of *M. tuberculosis* infection is about one-third of total population of whom 5-10% will develop clinical disease during their life time.^[2]

India is the highest tuberculosis-burden country in the world and accounts for almost 20% of global cases.^[3] Incidence of tuberculosis is approximately 1.8 million cases per year and there were 2.5 million people living with HIV and AIDS at the end of 2007.^[4] In India, tuberculosis kills more adults in the most reproductive age group (15-54 years) than any other infections disease. In India two persons die of TB every three minutes, more than 1000 people die every day and almost 0.4 million die every year.^[5] Drug resistance surveillance in several zones in India has indicated that 2.4% of new patients have multi-drug resistant (MDR) tuberculosis.^[6]

Mycobacterium tuberculosis complex forms a tight, discrete group of organisms and display more than 95% DNA – DNA homology and consists of *M. Tuberculosis*, *M. Bovis*, *M. Africanum*, *M. Microti*, *M. Canettii*, *M. Pinnipedii*.^[7] *M. tuberculosis* has an extremely low level of genetic variation, suggesting that the entire population of *M. tuberculosis* organisms resulted from clonal expansion after an evolutionary bottleneck some 35,000 years ago.^[8] For developing countries, the smear microscopy of sputum to detect Acid Fast Bacilli (AFB) by ZN staining, fluorescent staining is cost effective for diagnosing and to monitor treatment outcome. The specificity of stains for AFB is 99% or more, and the sensitivity ranges from 25% - 75%.^[9]

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However, sputum microscopy requires skilled and dedicated staffs and strict supervision. A minimum of 300 fields should be examined under magnification of x1000 before the smear is reported as negative.^[10] For sputum microscopy positivity at least 10,000 microorganisms per ml of sputum should be present. The sputum smear positivity rate in TB/HIV patients is less in severely immune compromised state.^[11]

Although culture remains the gold standard for diagnosis of M. tuberculosis infection it requires elaborate infrastructures and good amount of investments. Conventional method of growth in egg-based media requires 4-6 weeks. To determine drug sensitivity would require further 4 weeks. These difficulties have led to the development of some rapid techniques. Phage amplification assay is a promising one in this direction.

Bacteriophages are viruses that infect bacteria. Mycobacteriophages are double-stranded phages with a G + C content similar to that of the host bacterium. Most of them possess a hexagonal or oval head and a long noncontractile tail.^[12] Gardner and Weise discovered the first mycobacteriophage in 1947, and since that time over 250 of these viruses have been identified.^[13]

Phages exhibit two different types of life cycles – lytic and lysogenic. In lytic cycle intracellular multiplication of phage culminates in the lysis of the host bacterium. When a phage is applied on the culture of a susceptible bacterium, areas of clearing occur after incubation. These zones of lysis are called plaques. Since under optimal conditions a single phage particle is capable of producing one plaque, plaque assay can be employed for titrating the number of viable phages in a preparation.^[14]

MATERIALS AND METHODS: All the samples of sputum were collected from the patients, attending a tuberculosis speciality centre from Kolkata, who were all getting treatment for pulmonary tuberculosis. Total number of sputum specimens were 104. The study period was from 1999 – 2001.

The collected sputum samples were processed immediately after the collection. They were decontaminated and concentrated by NaCl – NaOH (4%). The samples were centrifuged at 3500g for 30 minutes. The supernatant was discarded and the pellet was resuspended in phosphate buffered saline (PBS).^[15] Smears were made in duplicate and stained with ZN staining.

FAST – Plaque – TB assay: Mycobacteriophages infect specific viable M. tuberculosis in the specimen. Phages that have not infected a viable target cell remain extracellular and are inactivated using a virucide. Phages that infect cells (i.e. M. tuberculosis) are not affected and continue to replicate and lyse to release progeny bacteriophage.

Following virucide neutralization, high numbers of rapid growing non-pathogenic organisms (M. Smegmatis – which can also be infected by the same phage) are added. One plated in an agar mixture, a lawn of M. Smegmatis growth will develop overnight and plaques or zones of clearance will form. One plaque represents one bacterium in the sputum. If the number of plaques is 20 or more, the test is said to be FAST-Plaque – TB™ positive and between 0-19 plaques are considered FAST-Plaque-TB negative. All specimens were processed according to the manufacturer's instructions (Biotec Laboratories Ltd., Ipswich, UK).

Radiometric Method: The processed specimens were also inoculated in 12B vial of BACTEC 460 TB for radiometric culture. (Becton Dickinson, Sparks, MD, USA).

RESULTS: Comparative evaluation of detection of M. Tuberculosis by radiometric and phage-amplification technique has been shown in Table I.

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Total number of sputum specimens was 104. 42 smear-positive and 62 smear-negative samples were selected for the study. All the isolates were confirmed by using NAP.

Of the 42 smear-positive specimens 35 were both plaque and radiometric culture positive; one specimen was plaque positive but culture negative; 5 specimens were plaque negative but culture positive whereas only one was neither plaque nor culture positive. Therefore, overall plaque positivity was 35 out of 42 (83%).

Of the 62 smear - negative specimens, 10 were both plaque and radiometric culture positive; 20 were plaque negative but culture positive and remaining 32 were neither plaque nor culture positive. Therefore, overall plaque positivity was 10 out of 62(16%) and culture positive was 30 out of 62(48%).

Considering both smear - positive and smear - negative specimens (104 specimens), there were 45 both plaque and culture positive (43%); one was plaque positive but culture negative (<1%); 25 were plaque negative but culture positive (24%); 33 were both plaque and culture negative (32%). Overall culture positivity was found 70(67%) and plaque positivity in 46(43%).

Individual isolation rates by smear microscopy, fast plaque and radiometric culture were 24.1%, 43%, respectively.

Isolation time for FAST - Plaque - TB was within 48 hrs. In radiometric culture, the isolation time for smear positive cases was 9 days on an average and for smear - negative cases 13.5 days.

Direct smear	Number	P+C+	P+C-	P-C+	P-C-	%C+	%P+
Positive	42	35	01	05	01	95%	83%
Negative	62	10	0	20	32	48%	16%
Total	104	45	01	25	33	67%	43%

Table 1: Comparative evaluation of detection of M. tb by radiometric & phage-amplification technique

Culture done on Radiometric system (BACTEC 460 TB)

P+ = Plaque positive.

C+ = Culture positive.

P- = Plaque negative.

C- = Culture negative.

DISCUSSION: Phage amplification technology shows greater sensitivity than smear microscopy. It has obviously higher specificity because this method detect only live bacilli of M. tuberculosis as well as it can exclude the cases of mycobacteria other than tuberculosis (MOTT). It is simple to perform. There is no need for specialized technical skill. Compared to radiometric method, there is no requirement of expensive equipment. Fast plaque technology does not involve culture of M. tuberculosis, thereby, can be done in less equipped laboratory.

As tuberculosis rates continue to increase in HIV endemic region, improved diagnostic techniques should be implemented for tuberculosis control strategies. New diagnostic tools which involve expensive technologies may be beyond the reach of developing countries like ours affected by dual tuberculosis (particularly MDR-TB) and HIV epidemic. Such high-tech tools are unlikely to make an impact in these countries even if they are accurate.

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With the above view point, the present preliminary report shows that FAST – plaque – TB may meet the long – standing need of rapid, simple, low cost alternate to old, time – consuming, conventional methods of culture as well as newer high cost rapid methods.

It is worth mentioning that recently there has been an important finding where FAST – Plaque – TB has been able to detect *M. tuberculosis* in 50-65% of smear – negative specimens with a specificity of 98% and that a combination with smear microscopy confirmed the presence of *M. tuberculosis* in 80 – 90% of culture positive specimens.^[16] There is a provision of FAST – plaque – TB – MDRI Kit which is designed to detect rifampicin resistance in culture isolates and FAST – plaque – TB – response of direct use of clinical specimens.^[17]

REFERENCES:

1. World Health Organization. Global Tuberculosis report, 2014 (Executive summary).
2. World Health Organization. Weekly Epidemiological Record: World Health Organization, 23rd Jan 2004, No. 4.
3. Government of India, TB India 2006, RNTCP status Report, DOTS for All – All for DOTS, Ministry of Health and Family Welfare, New Delhi, 2006.
4. Swaminathan S, Narendran G HIV and tuberculosis in India, *J Biosci* 2008; 33: 527-37.
5. K Park. Park's Textbook of Preventive and Social Medicine. 19th ed. 2007; 150.
6. Government of India Annual Report 2003-2004, Ministry of Health and Family Welfare, New Delhi 2004.
7. Gaby E Pfyffer. *Mycobacterium: General Characteristics, Laboratory Detection, and Staining Procedures*. In: *Manual of Clinical Microbiology*, 9th ed. Editor in chief: P R Murray. (ASM Press) 543-567.
8. C Gutierrez et al, *Abstr. 26th Annu. Congr. Eur. Soc. Mycobacteriol*, abstr. L-3, 2005.
9. G L Woods. *Mycobacteria*. In: *Clinical Diagnosis and Management by Laboratory Methods*, edited By J B Henry, 20th ed. 2001; (W. B. Saunders Company) 1144-1157.
10. Lipsky et al. Factors affecting the clinical value of microscopy for acid – fast bacilli. *Rev infect Dis* 1984; 6: 214-222.
11. K Park. Park's Text book of Preventive and Social Medicine. 19th ed. 2007: 155.
12. *Zinsser Microbiology*: edited by Wolfgang K Joklik et al. 20th ed. 1002; 500-501.
13. G E Pfyffer, V Vincent: *Mycobacterium Tuberculosis complex*. In: *Topley and Wilson's Microbiology and Microbial Infection*, 10th ed. 2005; (Hodder Arnold). 1189.
14. *Zinsser Microbiolog*: edited by Wolfgang K Joklik et al. 20th ed. 1992; 906-907.
15. G E Pfyffer. *Mycobacterium: General Characteristics, Laboratory Detection and Staining Procedures*. In: *Manual of Clinical Microbiology*: Editor in chief: P R Murray. 9th ed. (ASM Press) Appendix I, 563.
16. Takiff H, Heifets L. In search of rapid diagnosis and drug – resistance detection tools: is the FAST Plaque TB test the answer? *Int J Tuberc Lung Dis*. 2002; 6: 560-561.
17. Pai M, Kalantri S, Dheda K. New Tools and emerging technologies for the diagnosis of tuberculosis: Part II. Active tuberculosis and any resistance. *Expert Rev Mol Diagn*. 2006: 6: 423 – 32.

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