REVIEW ARTICLE

BACTERIOPHAGE BASED ASSAY TO DETECT MYCOBACTERIA IN THE SPUTUM OF TUBERCULOSIS PATIENTS

***Baye Gelaw*[1]**

ABSTRACT

Early, efficient, and inexpensive methods for the detection of pulmonary tuberculosis (TB) are urgently needed for effective patient management as well as to interrupt transmission. The major factors influencing the clinical outcome and control of the transmission of TB from patients includes the low sensitivity of the conventional Ziehl-Neelsen method and the long time taken to obtain drug susceptibility data. One of the most promising approaches is the use of mycobacteriophage D29 to demonstrate the viable mycobacterium cells. The aim of this review was to document the role of mycobacteriophages, particularly mycobacteriophage D29 to detect M. tuberculosis in sputum samples from TB patients. The review was organized by collecting different published information and after core laboratory skills were acquired. Fast Plaque™ is a rapid test which utilizes bacteriophage amplification technology for the detection of viable M. tuberculosis in clinical specimens. Taking BACTEC 460 TB culture as the gold standard, the sensitivity of Fast Plaque™ test reported 87.5% that Ziehl-Neelsen microscopy can detect when the sample has > 10^4 bacilli/ml of sputum, but the Fast Plaque™ test can detect 100-300 bacilli/ml of sputum. The diagnostic performance of the Fast Plaque™ test is reported to be comparable with the GeneXepert test, especially on smear negative specimens (sensitivity of 65% versus 72.5%, respectively). Moreover, the cost is by far less than that of the other available tests, like culture or polymerase chain reaction (PCR). In conclusion, the Fast Plaque™ test has a good potential for rapid diagnosis of M. tuberculosis. Thus it can be useful in the diagnosis of tuberculosis as an adjunct to sputum microscopy particularly in TB endemic and resource poor countries. In addition, Fast Plaque™-RIF™ offered a performance comparable to the gold standard proportion methods of rifampicin susceptibility testing.

Key words: *M. tuberculosis, Bacteriophage, Mycobacteriophage D29, FastPlaque assay*

INTRODUCTION

TB is a disease of antiquity caused by *M. tuberculosis* complex. It is a major public health problem with around 9 million new cases and 2 million deaths estimated to occur each year globally (1). Geographically, the burden of TB is highest in Asia and Africa. India and China together account for almost 40% and the African region for 24% of the world's TB cases. The highest prevalence of TB infection and its estimated annual risk are in Sub-Saharan Africa and Southeast Asia. In Africa, by the year 2002, TB prevalence per a population of 100,000 was 757 for Somalia, 695 for Mali, 598 for Rwanda, 588 for Zambia and 508 for Ethiopia. TB death rate per 100,000 people by the same year was 117 for Somalia, 77 for Mali, 66 for Rwanda, 68 for Zambia and 60 for Ethiopia (3). The 2012 WHO report documented the highest prevalence (620/100,000) of TB in Nigeria and (570/100,000) in DR Congo (2). The WHO global TB report (2006) showed that Ethiopia had more than 267,000 TB cases in 2004, with an estimated incidence rate of 353 cases per 100,000 people. In 2012, WHO reported the prevalence of TB in Ethiopia as 240/100,000 and the country ranked seventh among the worlds 22 countries with a high TB burden (2). Recently Ethiopia conducted a population based national TB study for 2010/2011, and the prevalence of bacteriologically confirmed (smear + culture positive) pulmonary TB infection was reported as 277/100,000.

In controlling TB, early diagnosis of the disease caused by *M. tuberculosis* complex (MTBC) is vital. One of the biggest challenges to TB control programs is the poor performance of TB diagnostic techniques used in identifying TB in resource constrained settings where more than 98% of the cases occur. In most cases, diagnosis is limited to only smear microscopy and clinical work up which identifies less than 50% of TB cases (4). In many countries the diagnosis of TB is performed by microscopic examinations of a stained smears of sputum or other body fluids by

[1]*University of Gondar, College of Medicine and Health Sciences, School of Biomedical and Laboratory Sciences, Department of Medical Microbiology, Gondar, Ethiopia. P.O.box 196; Mobile +251918703723; e-mail:tedybayegelaw@gmail.com.*

the Ziehl-Neelsen (ZN) method. Although easy to perform and specific, the ZN method lacks sensitivity, requiring 5,000 to 10, 000 bacilli per milliliter of sputum to become positive (5).

The advantages of acid –fast bacilli (AFB) microscopy are well known: it is inexpensive to perform, very specific in high prevalence settings, and detects the most infectious subset of patients. However, the shortcomings of AFB microscopy seriously limit both the extent and quality of its application and ultimately impact on TB control. This problem is made more critical by the rising incidence of the smear-negative disease in countries where the Human Immunodeficiency Virus (HIV) infection is prevalent, such as Sub-Saharan Africa (6).

Conventional culture – based techniques for *M. tuberculosis* detection take weeks from the time of receipt of a clinical specimen (7). The gold standard for TB diagnosis is the cultivation of *M. tuberculosis*. It can be performed on a variety of specimens, such as sputum and bronchial washings and other non-pulmonary samples. It can detect 100 bacilli per milliliter of sputum in comparison with 5,000 to 10,000 bacilli per milliliter needed for microscopy (8). Conventional methods of culture have relied on egg-based and agar-based media, such as Lowenstein-Jensen (LJ) medium and Middle brook agar or broth (9).

Traditional solid culture methods, for example, LJ slants have sensitivities of around 76.9% (10). Previous guidelines recommend that at least one solid medium should be inoculated along with an automated liquid medium (11).The major limitations of the use of both solid and liquid medias simultaneously include the high cost and prolonged result time.

The introduction of the BACTEC radiometric system (BACTEC TB-460, Becton Dickinson, sparks, MD, USA) in the 1980s was a breakthrough since it allowed the detection of *M. tuberculosis* in a few days compared with weeks in the conventional culture media methods (12). However, the use of radioisotopes and lack of the equipment precluded its use on routine basis, except in reference laboratories predominantly in developed countries. A few years ago, Becton Dickinson proposed another system based on fluorescence detection of mycobacterium growth. The mycobacterium growth indicator tube (MGIT) system which is based on a glass tube containing a modified Middlebrook broth together with fluorescence quenching-based oxygen sensor embedded at the bottom of the tube (13).
Results using automated systems also take an average of 10-21 days (14). Liquid culture analysis with a semi-automated liquid BACTEC 460 instrument and automated liquid culture systems, such as the MB/BacT system (Organon Teknika, Cambridge, United Kingdom) have significantly reduced turn around times but require expensive monitoring equipment and costly reagents (12). Other diagnostic modalities used in the diagnosis of TB include molecular and serological methods. Molecular methods are expensive (15). Serological methods are reported to have low sensitivities (60 to 75%) and specificities (85 to 95%) (16).

Although new rapid molecular techniques have been developed, their expense plus the requirement for specialist skills and equipment have prohibited their adoption in many diagnostic laboratories. As for laboratory services beyond microscopy, little is available in most disease endemic countries outside referral centers. There is a need for the development of novel, low-cost, rapid, sensitive techniques that are appropriate for routine use, particularly in developing countries. The aim of this review was to document the principle and knowledge of the recently developed technology, the mycobacteriophage based method of detecting *M. tuberculosis* in the sputum of tuberculosis patients.

METHODS OF REVIEW

This review has been written after core skills had been acquired and knowledge learned at the University of Leicester, UK, to run the mycobacteriophge assay to detect *M. tuberculosis* and other mycobacteria from sputum and other body fluids. Both previous and recent works concerning bacteriophage based assay were reviewed. The review documented some of the important achievements regarding bacteriophages and the roles of the Mycobacteriophage D29 as a diagnostic virus used to detect the tubercle bacilli in the sputum by the help of *M. smegmatis* and plaque formation.

THE BACTERIOPHAGES

Bacteriophage, a term formed from "bacteria" and the Greek phagin, "to eat", which is commonly used in its shortened form, "phage", is a virus that infects and replicates in bacteria. Lytic bacteriophages kill the host cell whereas so called temperate phages can establish a stable relationship in which the bacteriophage genome is stably maintained within the host. Bacteriophages were discovered by Felix d'Herelle and Frederick Tworte in the 1910s. In the 1940s, Max Delbruck encouraged the phage group to concentrate on seven specific bacteriophages (T1 to T7) (17). Typically, bacteriophages consist of an outer protein enclosing the genetic material. The genetic material can be ssRNA, dsRNA, ssDNA or dsDNA between 5 to 500 killo base pairs long with either circular or liner arrangements (Figure 1). Phages are estimated to be the most widely distributed and diverse entities in the biosphere. The dsDNA tailed phages, or Caudovirals, account for 95% of all phages reported in scientific literature, and possibly make up the majority of phages on the planate (18). However, there are other phages that occur abundantly in the biosphere.

Bacteriophage may have a lytic cycle or a lysogenic cycle, but a few viruses are capable of carrying out both. With lytic phages such as the T4 phage, bacterial cells are broken open (lysed) and destroyed after the immediate replication of the virion. As soon as the cell is destroyed, the new bacteriophage viruses can find new hosts. Lytic phages are the kind suitable for phage therapy. In contrast, the lysogenic cycle does not result in immediate lysing of the host cell (18).

Phages able to undergo lysogeny are known as temperate phages. Their viral genome will integrate with the host DNA and replicates along with it fairly harmlessly, or may even become established as a plasmid (19).

Figure 1: *The structure of a typical bacteriophage (20)*

To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins or even flagella (figure 2). This specifically means that a bacteriophage can only infect certain bacteria-bearing receptors that can bind to it, which in turn determines the phage's host range (21). Within minutes, bacterial ribosomes start translating viral mRNA into proteins. For RNA-based phage, RNA replicase is synthesized early in the process. Proteins modify the bacterial RNA polymerase so that it preferentially transcribes viral mRNA. The host's normal synthesis of proteins and the nucleic acids are disrupted, and it is forced to manufacture viral products. These products go on to become part of the new virions within the cell (22).

In the case of the T4 phage, the construction of new virus (assembly) particles involves the assistance of helper proteins. The base plate is assembled first, with the tails being built upon them afterwards. Phages may be released via cell lysis or by host cell secretion; in just over 20 minutes after injection, upwards of 300 phages will be released via lysis within a certain time scale (22).

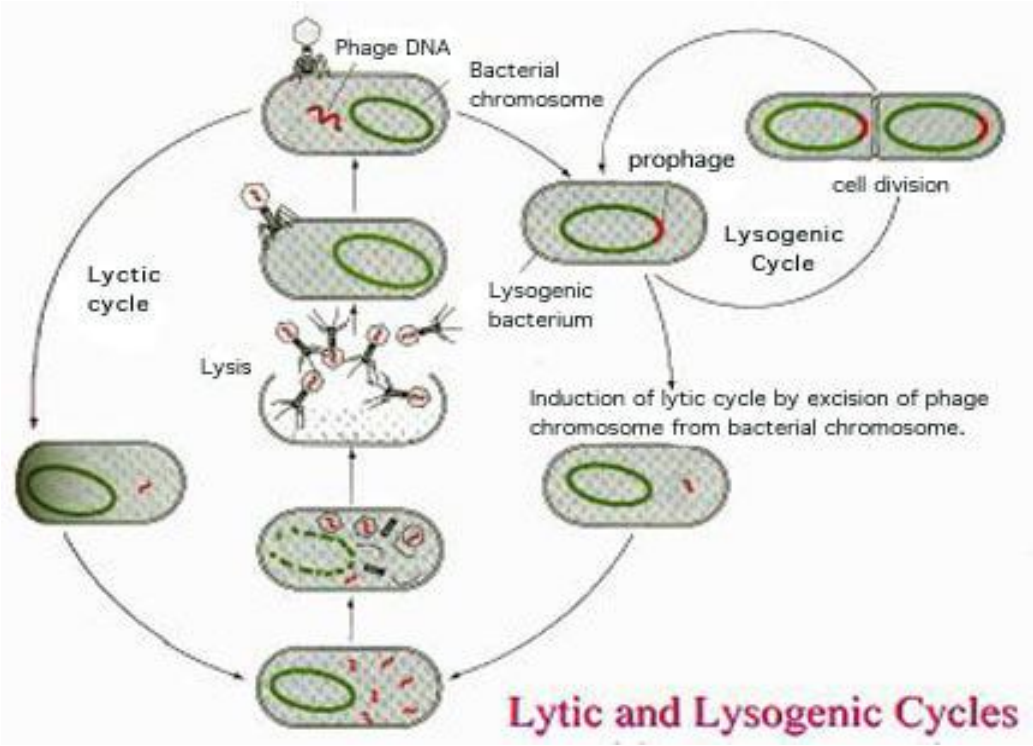

Figure 2: *Lytic and lysogenic cycles of bacteriophages (23)*

Phage genetics

The transfer of genetic elements from one bacterium to another by a bacteriophage is termed as transduction. Transduction can be generalized or specialized. The generalized transduction is seen in lytic cycle where segments of bacterial DNA are packaged inside phage capsid instead of phage DNA. When such phages infect new bacterial cells, the bacterial DNA is injected inside. This piece of DNA may then transfer genes to the host chromosome by recombination. Any bacterial gene may be transferred in a generalized transduction. Generalized transduction is usually seen in temperate phages that undergolytic cycle. Only those genes that are adjacent to the prophage are transferred in a specialized transduction (23).

The mycobacteriophage D29

Mycobacteriophage D29 was isolated from soil by Forman in 1954 and infects a broad range of *Mycobacterium* species including *M. smegmatis* and *M. tuberculosis*. It has also been shown to adsorb to *Mycobacterium leprae* cells. Previous studies demonstrated that D29 efficiently adsorbs to *M. smegmatis* and initiates DNA replication soon after infection, with a progeny phage appearing about 90 minutes after infection (24).

Mycobacteriophage D29 is a lytic phage that infects both fast and slow-growing *Mycobacterium* species (25).The features of a completely sequenced mycobacteriophage D29 show Guanine-cytosine (GC) percent (63.5%), size in base pairs (bp) (49136) , number of open reading frames (ORFs) (77), number of tRNA (5) and no mRNAs (26). A genomic analysis of D29 demonstrates that it is a close relative of the temperate mycobacteriophage L5, and is presumably a non-temperate derivative of a temperate region (27). The similarities of the morphologies of L5 and D29 , the close correlation of L5 and D29 virion proteins, as well as the observation that D29 is the subject to L5 supper infection immunity (i.e., it does not infect an L5 lysogen) suggests that L5 and D29 are closely related bacteriophages. Although L5 and D29 genomes have quite distinct restriction maps, they strongly cross hybridize to

each other. It is therefore not surprising that DNA sequencing of the D29 genome reveals it to be a very close relative of L5 (27).

D29 forms clear plaques on Lawns of *M. smegmatis* and *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) in which a very high proportion of infected cells are killed (28). The bacteriophage plaque is a clear zone in an otherwise confluent growth of bacteria on an agar surface resulting from bacterial lysis by bacterial viruses.

The principle of the D29 phage assay

The ability of a mycobacteriophage to multiply in *M. tuberculosis* and other mycobacteria is the basis of the diagnostic assay developed recently. Mycobacteriophage D29 multiplies within mycobacteria and is detected as plaques using a separate indicator *Mycobacterium* species (29). After phage infection, a virucidal solution (FAS) destroys all phages that have not infected the tubercle bacilli. The remaining phages in the infected bacilli replicate until new progeny phages are released as the cell lyses. The new phages are amplified by the addition of a non-pathogenic rapid-growing mycobacterial host, *M. smegmatis*, which is also able to support phage replication. Phages can be visualized as clear areas or plaques in a lawn of host cells. The number of plaques visualized from a given sample is related to the number of viable tubercle bacilli in the original sample.

Detection of M. tuberculosis using in-house prepared phage assay

Sputum specimens can be decontaminated and digested using standard decontaminants such as 4% NaOH or N-acetyl-L-cysteine (NALC**)**-NaOH followed by centrifugation at 3000 xg for 15 minutes to concentrate the TB bacilli. After the supernatant is removed, the pellet is suspended with a phosphate buffer and re-centrifuged after which the supernatant is decanted. Then, sediment is vortex mixed and allowed to stand for 3 minutes to allow aerosols to settle.

The sediment is re-suspended in Middlebrook 7H9 broth with Oleic acid dextrose catalase (OADC), glycerol and calcium chloride and incubated at 37^{o}C. To allow the mycobacterium to recuperate after the harsh decontamination treatment, specimen is cultured for at least 24 hours prior testing. Mycobacteriophage D29, 0.5 ml at a concentration of 10^{8} plaque forming unit per milliliter (pfu/ml) is added, mixed and incubated at 37^{o}C for 90 minutes. Molten 1.5% Middlebrook 7H9 agar is prepared and cooled to approximately 50 ^{o}C in a water bath. Inactivation of exogenous D29 phage is achieved by the addition of 0.3 ml of 50 mM ferrous ammonium sulphate (FAS) to **e**ach preparation followed by vortex mixed.

The preparation is incubated at room temperature for 5 minutes. One ml of stationary phase *M. smegmatis* mc^{2}, 1 ml of sample and 9 ml of molten Middlebrook 7H9 agar is mixed in a test tube and after mixing by inversion, it is poured into a 90 mm sterile Petri dish. After allowing the agar to solidify, the preparation is incubated at 37 ^{o}C overnight. Both negative and positive controls, (1ml of Middlebrook 7H9 broth and 1 ml of *M. smegmatis* grown in Middlebrook 7H9-OADC respectively) are run simultaneously with the test assay.

Prior to phage infection, 3 replica of 20 µl of the preparation is taken and inoculated separately on Middle brook 7H10 agar and incubated at 37 ^{o}C for 2 to 4 weeks to evaluate the colony forming unit (CFU) of the test. The colonies at the 3 inocula are counted and the average is used to determine the colony forming unit per ml (CFU/ml) of the sample.

Interpretation of in-house prepared phage assay results

The phage assay is interpreted on the basis of the reference values as presented below. To calculate the plaque forming unit/ml (PFU/ml) the following are considered. The volume of sample tested, usually 1 ml; total volume of phage reaction before plating (1 ml sample + 0.5 ml D29 + 0.3 ml FAS), 1.8 ml. Therefore, the PFU/ ml are equal to PFU/ plate x1.8. The colonies for each replica of the 20 µl drop are counted and the average is taken. CFU/ml is the average colony counted x 50 (to scale up to 1ml) x dilution factor. The recovery efficiency (%) is calculated by dividing the PFU/ml to CFU/ ml multiplied by 100.

The negative control (Middle brook 7H9 broth) should result in less than 10 plaques. The positive control, consisting of *M. smegmatis* infected phage and treated with a virucide (FAS), should have resulted in 20 to 300 plaques. The result is considered valid only if the positive and negative control values are within the specified range. A positive result revealed at least 20 plaques. A complete lysis is the lysis of the lawn of *M. smegmatis* cells indicat-

ing greater than 10^5 *M. smegemasis (M. tuberculosis* or other mycobacteria in the sample). Confluent lysis is the lysis of 80 to 90% of the lawn *M. smegmatis* cells indicating 10^4 to 10^5 mycobacteria in the sample. Too numerous to count (TNTC) is the presence of 10^3 to 10^4 (M. *smegmatis*) *M. tuberculosis* plaques per plate with some merging of individual plaques (Figure 4).

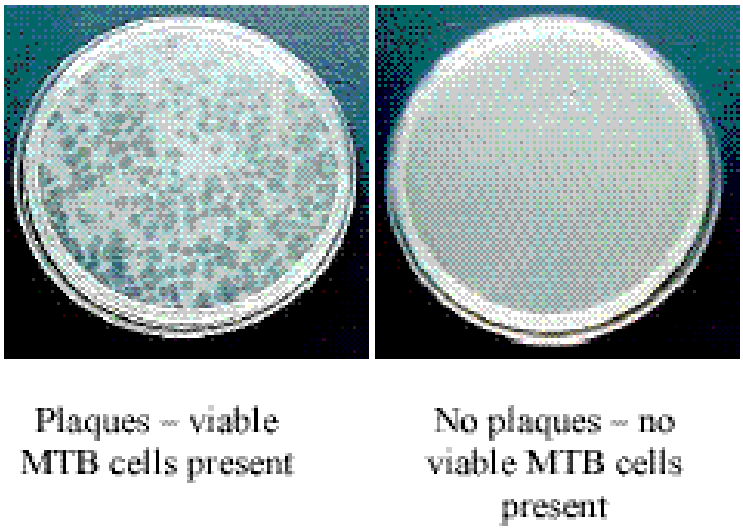

Figure 3: *Mycobacteriophage D29 assay results to detect M. tuberculosis from sputum samples (30)*

Plaque forming unit (PFU): For dilutions that show few plaques, count each, but for dilutions that show many plaques, divide the plate into four; count the plaques in the four quadrants and take the average. The plaque forming unit (PFU/plate) is calculated by multiplying the number of plaques counted by the total volume used (0.5 ml+1ml+0.3 ml=1.8 ml).

Colony forming unit (CFU): Count the colonies at 3 inocula of each dilution and take the average.

THE FAST PLAQUE ™ TEST

The new Fast Plaque ™ system, developed and manufactured by BIOTEC laboratories, Ltd, Ipswich, UK and FIND (Foundation for Innovative New Diagnostics) is a novel application of phage amplification technology which allows the rapid detection of TB within 24 hours of sample preparation (31). Fast plaque TB™ is a rapid manual bacteriophage based test used to detect *M. tuberculosis* in clinical specimens. The technique uses a mycobacteriophage which is able to infect and replicate in slow growing pathogenic strains, example, *M. tuberculosis*, *M. ulcerans* and also in some rapidly growing strains such as *M. smegmatis*. Mycobacteriophages have the potential to become useful tools in the diagnosis of TB, as they are specific for mycobacteria and only replicate in and hence detect viable cells. Phage based techniques involve simple manual manipulation and yield results rapidly (32).

Phage amplification technology is simple, rapid and requires 2 days for results. No expensive delicate equipment or specialized facilities are required and results are read by a naked eye. Therefore, it is well suited for use in countries which have a high prevalence of TB (33).

Principles of the Fast plaque ™ test

The Fast Plaque™ assay is based on phage amplification technology in which bacteriophage D29 is used as an indicator to detect the presence of viable tubercle bacilli in sputum samples (34). *M. tuberculosis* present in decontaminated sputum is infected with the phage (Actiphage) (figure 4). After infection, addition of a virucide (Virusol) results in the destruction of the residual extra-cellular phage. The phage replication in an infected cell results in cell lysis and release of progeny. The addition of a rapid growing *Mycobacterium* strain, *M. smegmatis* (Sensor cells), which is also sensitive to the phage, results in the amplification of the progeny which can be visualized as zones of clearing (plaques) in a lawn of sensor cells and results are available in 48 hours (35).

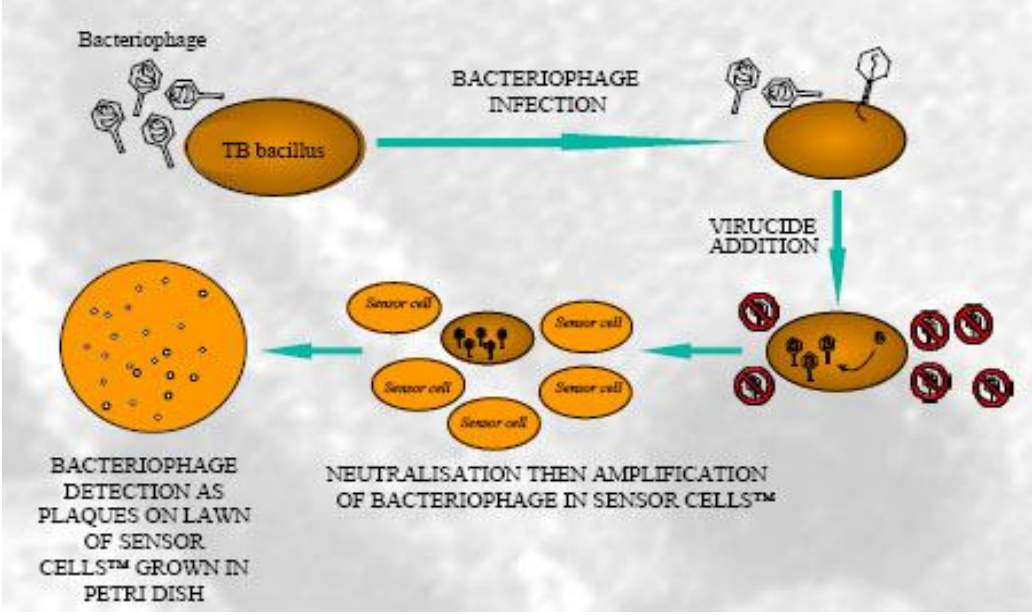

Figure 4: *Summery of the Fast plaque TB ™ test principle (31)*

Procedure of the Fast Plaque TB ™ test

Sputum samples collected are decontaminated using suitable decontaminants, such as N-acetyl-L-Cysteine-NAOH or 4% NAOH and concentrated by centrifugation at 3000 xg for 15 minutes. After the supernatant is removed, the sediment is re-suspended in 15 ml Fast plaque TB medium plus and incubated at 37°C overnight to enrich viable TB bacilli present in the sample. After enrichment, Actiphage™ solution is added and incubated for further one hour to allow infection to take place. Then virusol™ solution is added for destruction of all bacteriophages which have not infected host cells and then incubated at room temperature for 15 minutes. Fast Plaque TB medium plus is again added to neutralize excess of virucide, followed by Sensor™ cells. Five ml of Fast PlaqueTB (FPTB) molten agar is poured to pre-label the Petri-dish and to it is added the above solution. The plates are mixed well and allowed to set at room temperature. Then they are incubated at 37°C overnight. Next day, results are recorded as plaque formation. Plaque formation is indicated by the presence of viable bacilli in the original sample. Results are interpreted as positive if ≥20 plaques are present and the presence of 0-9 plaque (≤ 10 plaques) is classified as signified negative result (31).

Sensitivity, specificity and predictive values of the Fast Plaque ™ test

In one study, the sensitivity of the phage test with respect to AFB smear positivity was found to be 94.34% and specificity as 93.88%. The positive predictive value was 94.34% and the negative predictive value 93.88% (32). Fast plaque TB ™ test has very good sensitivity (86.2%) when compared to culture and specificity of 96.42%. It has a high positive predictive value of 93.75% and negative predictive value of 93% (Table1 and 2) (36).

Table 1: Comparison of Fast plaque TB ™ test with culture on LJ media and smear (36)

	Smear		**Culture**		**Fast Plaque**	
	Pos	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**
Smear						
Positive	--------	--------	13	11	13	11
Negative	--------	--------	4	17	4	20
Culture						
Positive	13	11	-----	--------	15	2
Negative	4	17	-----	--------	1	27
Fast Plaque						
Positive	13	11	15	2	------	-------
Negative	4	20	1	27	------	-------

Pos=positive; Neg=negative

Table 2: Comparison of sensitivity and specificity of the Fast plaque TB ™ test with culture and smear (36).

Performance	FPTB ™ test versus LJ	FPTB ™ test versus smear versus LJ	Smear/LJ
Sensitivity	86.23	54.16	54.16
Specificity	96.42	83.33	80.95
Pp value	93.75	76.47	76.47
Np value	93.1	64.51	60.71

Pp= Positive predictive value; Np= Negative predictive value; FPTB ™=Fast Plaque TB test; LJ=Lowensetin Jensen.

Albert et al (29) compared the Fast plaque TB assay with the Auramine smear microscopy and sputum culture on Lowenstein-Jensen medium, detecting TB in 75% of culture-confirmed cases and 70% of cases with a clinical diagnosis of TB with specificities and sensitivity of 98.7% and 99.0%, respectively. Criteria defining a good test to improve the differential diagnosis of AFB smear-negative cases of pulmonary TB compared to culture are that it should have 95%-99% specificity and 80% sensitivity.

ADVANTAGES OF FAST PLAQUE TB ™ ASSAY

The benefits of the Fast Plaque TB ™ assay used to detect mycobacteria are that it is so rapid that assay results are available within 24 hours of sample preparation; it detects patient's positive samples missed by smear; no advanced instrumentation is required; it is safe because no culturing of pathogen is required; it detects only live bacilli that reduce the possibility of false positives; the technology can be extended for antibiotic susceptibility testing.

BACTERIOPHAGE ASSAYS TO DETECT RIFAMPICIN RESISTANCE

The emergence of drug resistant strain *M. tuberculosis* is a growing concern, and a multi-drug resistant disease (MDR-TB) has been reported from all regions of the world (37). Traditional phenotypic methods of detecting drug resistant TB bacilli are slow due to the protracted growth rate of *M. tuberculosis*, with results often taking weeks to obtain. Rapid molecular methodologies that detect the mutation of the β-subunit of RNA polymerase (*rpoB*) to demonstrate rifampicin resistance have been developed. In some settings, resistance to rifampicin is highly predictive of MDR-TB (38). However, the new molecular technologies have not been implemented in resource limited settings due to their high cost. The recently reported relatively low cost and rapid method for investigating the susceptibility of *M. tuberculosis* to refampicin using mycobacteriophage D29 is given a considerable attention (39). In this technology mycobacteriophages are allowed to infect the bacteria, successful replication and production of progeny phage being indicative of the presence of viable mycobacteria. Rifampicin disrupts phage replication by preventing the synthesis of bacterial mRNA, and when critical concentrations of this drug are present progeny phage will be observed in only those strains resistant to the drug (40). A microwell plate version of this technology which allows a high-throughput screening of *M. tuberculosis* isolates has been developed (41).

COMPARISON OF GENEXPERT AND LINE PROBE ASSAY WITH FASTPLAQUE™ TEST FOR THE DIAGNOSIS OF MDR-TB

In 2010, WHO endorsed GeneXpert® MTB/RIF, an automated, bench-top device that tests for TB and rifampicin resistance. GeneXpert is a TB-specific automated, cartridge-based nucleic acid amplification assay, with a fully integrated and automated sample preparation, amplification and detection using a real-time PCR and providing results within 100 minutes. Clinical validation trials done in four distinctly diverse settings showed that 92.2 per cent of culture-positive patients were detected by a single direct Xpert MTB/RIF test (in comparison with the sensitivity of a single direct smear of 59.5%) (42).

The sensitivity of a single Xpert MTB/RIF test in smear-negative/culture-positive patients was 72.5 per cent and specificity was 99 per cent. HIV co-infection substantially decreased the sensitivity of microscopy (to 47%) but did not significantly affect Xpert MTB/RIF performance (43). The mean time to detection was <1 day for Xpert MTB/ RIF, 1 day for microscopy, 17 days for liquid culture and >30 days for solid culture (42, 43).

Thus this test seems to have the potential to complement the current reference standard of TB diagnostics and increase its overall sensitivity and speed. Molecular line probe assays (LPA), endorsed by WHO in 2008, apply principles of nucleic acid amplification to detect both TB bacteria and mutations that indicate drug resistance. They can be used with sputum samples and culture isolates, and when used with the former can deliver results within days, rather than weeks.

However, they require specialized facilities, can detect only well characterized drug resistance alleles, and are best utilized on smear positive TB cases (thereby limiting their utility in people living with HIV and in children). Phage -based TB diagnostics use mycobacteriophage-based approaches to detect TB cells in sputum or from culture. Bacteriophage-based assays have been used for TB diagnostics, and the FAST Plaque TB assay can detect mycobacteria in 50-65 per cent of smear negative specimens with a specificity of 98 per cent (44). Although there are no reports that compared the Bacteriophage-based assay with the GeneXepert MTB/RIF test, the diagnostic performance of the Fast Plaque TB assay together with that of the GeneXepert MTB/RIF test, particularly on smear negative specimens was comparable as documented earlier.

CONCLUSION AND RECOMMENDATIONS

Phage assay is a rapid, reliable, and cost-effective method to detect *M. tuberculosis* from sputum samples of pulmonary TB patients. Since it gives results within 2 days, it hastens the diagnosis of the disease, thereby helping in the treatment of the disease. Moreover, the phage assay can be used for the screening of isolates for resistance to refampicin with high sensitivity and specificity when using a drug concentration of 4µg/ml. The technique is easily transferable to low-income countries. A number of published works suggest that the test may be useful in resource limited laboratories as a diagnostic tool and for a rapid screening of rifampicin resistance and potentially MDR-TB cases as rifampicin resistance is a marker for MDR-TB.

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