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# Evaluation of *FAST Plaque* TB Assay for the Direct Detection of *Mycobacterium tuberculosis* in Sputum Specimens

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## Introduction:

The laboratory diagnosis of tuberculosis in Pakistan depends mainly on microscopic examination of direct smears for the presence of acid fast bacilli (AFB). Infections by non-tuberculous mycobacteria are uncommon and specificity of smear is high. But the method lacks sensitivity, requiring $>10^3$-$10^4$ organisms per ml of specimen. This causes problems in the diagnosis of smear-negative disease where culture may require several weeks to months to obtain positive results. Although considered to be less infectious than smear positives, smear negative patients have been shown to transmit disease. Thus there is a great need for a rapid test which can detect smear-negative tuberculosis cases.

The aim of this study was to evaluate the usefulness of the *FASTPlaque*TB (FPTB) assay for rapid and specific detection of *M.tuberculosis* in sputum samples. This assay detects the presence of viable MTB complex in sputum samples. Results are available in 48 hours. The results of the assay were compared with conventional culture, which was used as the 'gold standard'.

## Materials and Methods:

Sputum samples were collected from 585 patients coming to different out patient clinics of the Ojha Institute of Chest Diseases, Karachi between June 2000 to February 2001. Sputa were decontaminated and digested by the standard NALC-NaOH treatment. Concentrated smears were stained by the Ziehl-Neelson method. LJ slopes in duplicate were inoculated for culture isolation.

All positive cultures were identified by conventional methods and confirmed as *M. tuberculosis* by *p*-NBA testing and a PCR assay specific for MTB complex. FPTB was performed essentially according to manufacturer instructions. Initially, 241 specimens were tested according to the instructions accompanying the kit. For the next 182 samples, test was performed with the addition of Penicillin (50µg /ml final concentration) both in the assay and LJ growth medium to inhibit bacterial contamination. Another 102 specimens were tested with the addition of Microclens, an anti-microbial agent provided by the manufacturer.

### *FASTPlaque*TB assay:

1 Bacteriophages specific for *M. tuberculosis* are added to decontaminated sputum.
2. The phages infect viable TB bacilli present in the sample.
3. Residual phages are destroyed by the addition of a potent virucide.
4. The virucide is neutralized and non-pathogenic, rapid growing mycobacterium, *M. smegmatis* cells (also susceptible to phage) are added.
5. Replication of phage in the infected bacilli results in cell lysis and release of progeny phages.
6. Once plated in an agar mixture, a lawn of bacterial growth develops overnight, and plaques will form.
7. Plaques indicate the presence of viable TB bacilli in the original specimen.

**4) Comparison of smear results with culture – All specimens**

| | Culture +ve | Culture -ve | Total |
|---|---|---|---|
| Smear +ve | 160 | 39 | 199 |
| Smear -ve | 65 | 261 | 326 |
| Total | 225 | 300 | 525 |

**5) Comparison of combined smear and FPTB results with culture**

| | Culture +ve | Culture -ve | Total |
|---|---|---|---|
| FPTB and/or smear +ve | 204 | 45 | 249 |
| FPTB and smear -ve | 21 | 255 | 276 |
| Total | 225 | 300 | 525 |

## Results:

A total of 585 sputum samples were tested. Of these 60 specimens were lost due to the over-growth of contaminating microorganisms either on assay plates or on culture slants. Complete results were available for 525 specimens (table 1). There were 18 specimens that were smear and MTB culture positive, but FPTB negative. When assay was repeated with culture it was positive confirming the phage host range. Since majority of these specimens had sufficient number of AFB (2+ to 4+ on smear), low positivity of specimens was not an explanation for these results. These samples were encountered throughout the study.

Of the 37 smear positive samples that were FPTB and culture negative, 24 were on anti-tuberculous therapy, 6 were new patients, and for 7 patients, clinical histories were not available.

There was no adverse effect of anti-microbial addition on assay performance, and sensitivity and specificity values (79% and 100%, without anti-microbial, and 87% and 96%) were comparable. The addition of anti-microbials, the number of samples lost due to contamination went down considerably from 17% to 7%). No contamination of gram positive bacteria was seen, remaining contaminants were NaOH resistant gram-negatives.

**1) Comparison of FPTB results with culture – All specimens**

| | Culture +ve | Culture -ve | Total |
|---|---|---|---|
| FPTB+ve | 186 | 09 | 195 |
| FPTB-ve | 39 | 291 | 330 |
| Total | 225 | 300 | 525 |

**2) Comparison of FPTB results with culture – Smear positive**

| | Culture +ve | Culture -ve | Total |
|---|---|---|---|
| FPTB+ve | 142 | 02 | 144 |
| FPTB-ve | 18 | 37 | 55 |
| Total | 160 | 39 | 199 |

**3) Comparison of FPTB results with culture – Smear negative**

| | Culture +ve | Culture -ve | Total |
|---|---|---|---|
| FPTB+ve | 44 | 06 | 51 |
| FPTB-ve | 21 | 255 | 275 |
| Total | 65 | 261 | 326 |

## Discussion:

The ability to rapidly detect *M. tuberculosis* in clinical specimens has important implications in the treatment of TB. In Pakistan, laboratory diagnosis of TB is mostly based on smear-microscopy results. Thus a significant number of cases are likely to be missed if the number of bacilli present are less than that required for a positive smear. In this study, 62% of the samples tested were negative on smear. Our results demonstrate that *FASTPlaque*TB has good sensitivity (68%), and specificity (98%) in the diagnosis of smear negative TB. These results also show that *FASTPlaque*TB is a better predictor of culture results (PPV 0.99) than smear microscopy (PPV 0.80).

## Conclusions:

The purpose of this study was to determine the utility of FP TB assay in rapid diagnosis of pulmonary tuberculosis.

- Assay has good sensitivity for detecting smear negative TB
- The assay has an excellent PPV, a positive result indicates true positive
- Can be used to confirm smear positive results
- Results are available in 48 hours
- Does not require specialized equipment

| 6) Performance summary | FPTB All specimens | FPTB Smear positive | FPTB Smear negative | Smear Alone | Combined Smear and FPTB |
|---|---|---|---|---|---|
| Sensitivity (%) | 83 | 89 | 68 | 71 | 91 |
| Specificity (%) | 97 | 95 | 98 | 87 | 85 |
| PPV | 0.95 | 0.99 | 0.86 | 0.80 | 0.82 |
| NPV | 0.88 | 0.67 | 0.93 | 0.80 | 0.92 |

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