Performance and feasibility of FASTPlaqueTB™ to diagnose tuberculosis in smear-negative patients

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BACKGROUND

- **Developing countries**
  - Most patients are living in areas with access to direct smear microscopy only to confirm TB
  - Culture available only in national/regional TB laboratory
- **High prevalence of TB and HIV co-infected patients**
  - Lower sensitivity of direct smear microscopy (50%)
  - Risk of under and late TB diagnosis
  - Urgent need for better diagnostic test for smear negative patients

RATIONALE

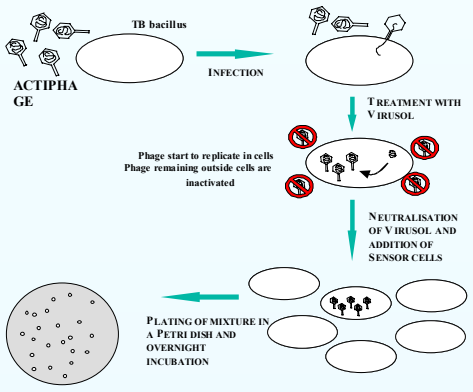
Reasons for selecting FASTPlaqueTB™ test for evaluation

- 2 days test
- According to literature, detects 50 to 67% smear-negative culture-positive cases
- Presented by the Manufacturer as potentially suitable for district laboratory
- No multiplication of *Mycobacterium tuberculosis* bacilli
- To evaluate the feasibility of FASTPlaqueTBTM test in a laboratory performing in routine only direct smear-microscopy

FASTPlaqueTB™ test principle

Based on Phage amplification and utilises Mycobacteriophage to reflect the presence of viable *Mycobacterium tuberculosis* in sputum specimens

Figure 1: *FASTPlaque*™ test principle.



Source: www.biotec.com

Uncontaminated plate (Negative result) Contaminated plate (Unreadable result)

Uncontaminated plate (Positive result)

OBJECTIVES

- To evaluate the performance of FASTPlaqueTB™ test to detect tuberculosis in smear-negative patients in a peripheral setting and high HIV prevalence context
- To evaluate the feasibility of FASTPlaqueTB™ test in a laboratory performing only direct smear-microscopy

METHODS

- **Prospective study**
- **Urban primary health care setting, Mathare, Nairobi city, Kenya**
- **Inclusion criteria**
  - ≥ 15 years old
  - Cough > 2 weeks
  - 3 negative smear microscopy results
  - No response to one week amoxicillin course
  - Abnormal chest X-ray
  - Informed consent
- **Consecutive sampling**
- **Voluntary Counselling HIV Test**

- **Laboratory procedure**
  - Collection of 1 spot sputum specimen
  - Decontamination: NALC/NaOH followed by neutralization with Phosphate buffer
  - Half of specimen tested locally with FASTPlaqueTB™ according to the manufacturer's instructions
  - Half of specimen referred for culture on Lovenstein Jensen medium
- **Outcomes**
  - Sensitivity, specificity and predictive values
  - Inter reader reliability

    Very good agreement if Kappa test >0.80
  - Feasibility criteria
    - Culture and FASTPlaqueTB™ contamination rates
    - Facility, equipment, human resources requirements
    - Workload assessed by the duration of the test procedures
    - Time between specimen collection and result

RESULTS OF PILOT STUDY

- **High contamination rate**
  - FASTPlaqueTB™ 95.6% (44/46)
  - Culture 21.7% (10/46)
- **Modifications before to starting inclusions**
  - Retraining of laboratory technologists in:
    - Aseptic techniques
    - Autoclave use
    - Working with a Laminar Flow Cabinet (LFC)
  - Increase in autoclave time to compensate for local altitude and volumes of liquid autoclaved
  - Move of LFC to a separate room with restricted access
  - Maintenance of LFC by technician from South Africa (expertise not available locally) and change of the HEPA filter after 3 months of use

Recruitment stopped early due to the high rate of FASTPlaqueTBTM unreadable

- **201 patients included**

  HIV status
  - 35 (17.4%) negative
  - 115 (57,2%) positive
  - 51 (25.4%) not done
- **FASTPlaqueTB™ results**
  - 10 (5.0%) positive
  - 98 (48.8%) negative
  - **93 (46.3%) unreadable**
- **Preliminary culture results***
  - Contaminated: 10/198 (5.1%)
  - Positive: 32/188 (16.5%)

  ** 3 cultures still under process*

Performance of FASTPlaqueTB™

- **N= 101 after exclusion of contaminated cultures and unreadable FASTPlaqueTB™**

	n/N	%	95% Confidence interval
Sensitivity	5/16	**31.2**	12.1-58.5
Specificity	80/85	**94.9**	86.8-98.1
Positive Predictive Value	5/10	**50.0**	18.7-81.3
Negative Predictive Value	80/91	**87.9**	79.4-93.8

Investigation of the source of contamination

- **Evaluation of procedures and working of the LFC**
  - Sterile water aseptically poured into a sterile conical tube and processed as a specimen
  - No contamination on the resulting plate
    - **LFC was working efficiently**
    - **Aseptic techniques were good**

The vast majority of contaminants were Gram positive bacilli

Investigation of the source of contamination

- **Specimen collection**
  - Sterile water poured aseptically into a sterile conical tube and exposed to the air where specimens were collected
  - Tube processed as a specimen
  - Contamination of plate with gram positive bacilli

The spores were probably introduced during the collection process

Bacterial and fungal spores are very common in dust, and are extremely difficult to control

The decontamination process could kill all the vegetative forms of bacteria that could have been introduced by the dusty air, but failed to kill the spores

Feasibility

- **Inter-reader reliability**

  Kappa [95% CI] = 0.81 [0.76 - 0.84]
- **Test duration**
  - Weekly containers sterilisation: median 2.8h ((IQR* 2.5-3.1)
  - Weekly reagent preparation: median 2.6h (IQR 2.3-3.1)
  - Test procedure
    - Decontamination (1st day): median 2.4 hours (IQR 2.1-2.5)
    - 2nd day procedure: median 2.5 hours (IQR 2.3-3)
- **Time between sputum collection and result**

  2 to 9 days because tests were performed only once a week to prevent wasting of tests and reagents (kits of 10 tests)
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- **Human resource**

  Intensive training of technician with no experience of working in aseptic conditions and under a LFC
- **Cost within the study context**
  - The test costs 7€/ patient, 60% being extra-cost to the cost the FASTPLaqueTB™ test
  - Upgrading the laboratory, equipement and maintenance cost 19,800€

DISCUSSION

- **Main findings**
  - 40% unreadable results due to contamination
  - 10 tests kit might not be adapted for settings with low activity when used only in smear-negative patients
  - Requirement of culture level laboratory to perform FASTPlaqueTB™
  - Difficult and costly to upgrade peripheral laboratory to perform FASTPlaqueTB™
    - Human resource ability to work under aseptic conditions
    - Two rooms laboratory with a separate room for the LCF
    - Expensive and fragile equipment
    - 24h electrical power required
    - Maintenance not available locally
- **Perspectives**
  - Modified FASTPlaqueTB™ with expected lower contamination currently under evaluation by the Manufacturer
  - FASTPlaqueTB™ remains still a potentially interesting test considering the 2 days results but requires culture level laboratory
  - Upgrading of peripheral laboratory to perform culture level test might only be feasible in very few settings
  - More R&D on new tests suitable for peripheral setting is a top priority