Performance and feasibility of FASTPlaqueTM 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 FASTPlaqueTM 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 FASTPlaqueTB test in a laboratory performing in routine only direct smear-microscopy

FASTPlaqueTM test principle

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

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™ 99.6% (44/44)
  – 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 FASTPlaqueTB™ test unprocessable

  201 patients included
  – FASTPlaqueTB™ results
    – Sensitivity: 92/105 (87.9%)
    – Specificity: 89/96 (92.7%)
    – Positive Predictive Value: 80/91 (88.1%)
    – Negative Predictive Value: 90/110 (81.8%)

• Preliminary culture results
  – Contaminated: 10/95 (10.5%)
  – Positive: 32/188 (16.5%)

• 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)

• Test duration
  – Weekly containers sterilisation: median 2.8h (IQR 2.3-3.1)
  – Weekly reagent preparation: median 2.6h (IQR 2.3-3.1)

• Time between sputum collection and result
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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 70’000 francs, 60% being extra cost to the cost the FASTPlaqueTB™ test
  – Upgrading the laboratory; equipment and maintenance cost: 19’800 francs

• Inter reader-reliability
  – Kappa [95% CI] = 0.81 [0.76 - 0.84]

• Evaluation of procedures and working of the LFC
  – Sterile water aseptically poured 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

• 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
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• 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

• Investigation of the source of contamination
  – The vast majority of contaminants were Gram positive bacilli

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• Perspectives
  – Modifications 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

DISCUSSION

High contamination rate

Main findings

40% unprocessable 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 LFC
– Expensive and fragile equipment
– 24h electrical power required
– Maintenance not available locally

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