Evaluation of the performance and feasibility of the fluorescein diacetate (FDA) vital staining method for follow up of Tuberculosis (TB) treatment.

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Background

To evaluate the performance and feasibility of the FDA vital staining method compared to MTB culture to follow up of Tuberculosis (TB) treatment.

Sputum: Patients aged ≥15 years

Objective

To evaluate the performance and feasibility of the FDA stain vital staining method compared to MTB culture to differentiate live from dead bacilli in ZN smear positive patients during treatment follow up in a smear microscopy laboratory setting.

Methods

Study Design Field evaluation of a TB diagnostic test

Study site Outpatient chest clinic, Mae Sot, Thailand

Case inclusion criteria: Patients ≥15 years 

Under TB treatment (ear or cat 1) since 2 months

≥ 2 of 1 to 2 ZN smear positive (≥ 1 fast acid-fast bacilli (AFB)/100 high power fields (HPF))

Preliminary Results

Sample size and analysis: 218 ZN smear +ve cases

Sensitivity, Specificity, positive and negative predictive values (PPV, NPV):

Cut-off of 1 AFB/100 HPF

Sensitivity = 99% / specificity = 82% compared to MTB culture when used on fresh sputum specimens of TB failures

Sample size and analysis: 218 ZN smear +ve cases

28th November 2007 - September 2008 MTB culture and FDA results available for:

98.2% new cases

46.4% end of intensive phase

20.5% prolonged intensive phase

28.6% continuation phase

6.3% end of treatment

48% cases with 2 ZN smear +ve smears

6.6% ZN smear "false-positive"

FDA performance:

PPV: Culture + Culture -

NPV: Culture + Culture -

Sensitivity: Culture + Culture -

Specificity: Culture + Culture -

FDA - Culture +

FDA + Culture -

FDA - Culture -

PPV (23.5 – 47.6)

NPV (8.5 – 97.0)

Sensitivity (57.7 – 90.0)

Specificity (58.7 – 75.3)

FDA staining/reading

The stage of TB treatment: ZN-failures versus others

FDA staining: Negative (0.5 mg/ml FDA)

30 minutes in 37°C incubator

Staining:

3. Rinse with water

4. Acid alcohol 3 minutes (de-colorisation)

5. Rinse with water

6. 10 minutes 5% w/v Cerebrum (paraffin oil)

7. Rinse with water

8. Dry smear (30 min -1 hour)

Prescribe: FDA incubator (maybe required)

FDA freezer (storage of FDA stock solution: 25 mg/ml FDA in Aceton)

Low FDA performance: possible reasons

Low Sensitivity: 78% of specimens were ZN scanty (versus 26% Bangladesh study)

- 67% of false negative FDA were on scanty ZN smear

- Study population was not TB failures: Culture positivity 19% vs 66% in the Bangladesh study

Further investigation of FDA performance according to:

- the stage of TB treatment: ZN-failures versus others

- Consider AFB cut-off for definition of FDA positive (specificity)

- Possibilities for improvement:

- Further standardization/simulation of the method

- Assessment of the method on concentrated specimen

- Double staining (fluorescent markers for "live" versus "dead" in the same specimen)- feasible?

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