

PS-101089-15 Evaluation of direct and concentrated smear microscopy for TB diagnosis in HIV prevalence setting

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Introduction: Direct and concentrated smear of sputum samples for AFB microscopy have been the most commonly used tools for diagnosis of TB. However, the sensitivity of the direct smear method, which is mainly used in the resource-poor countries is presently reported low especially in HIV-infected individuals. This study was therefore carried out to compare the sensitivity of the direct and concentrated smear methods for diagnosis of TB in both HIV-positive and negative individuals.

Method: The study population comprised of 480 patients drawn from the ARV and DOTS clinics in NIMR Lagos between 2008 and 2009. Two hundred and twenty-four (46.7%) of these patients were positive for HIV, 73 (15.2%) were negative while 183 (38.1%) had unknown status. Direct and concentrated smears of sputum samples obtained from the patients were stained by ZN and examined microscopically for AFB. Data was analyzed statistically using Prism version 5.03 computer software programme.

Results: AFB detection rates were 27.7% and 57.5% for HIV-positive and negative patients. In the HIV-positive patients, the AFB detection rates were 32 (14.3%) by direct smear and 75 (33.5%) by concentrated smear. In HIV-negative patients, 24 (32.9) were diagnosed by direct smear and 25 (34.2%) were diagnosed by concentrated smear. The rate of diagnosis by concentrated method was significantly higher than the direct method in the HIV-positive ($P < 0.05$) while there was no significant difference in these rates in the HIV-negatives.

Conclusion: Data showed a higher sensitivity in TB case detection by the concentrated smear method in the HIV-positive patients. About 43 patients amongst the group would have been missed if direct smear alone was carried out. The observation in this study highlighted the urgent need to develop capacity for concentrated smear in developing countries to be able to detect more TB amongst HIV patients.

PS-101120-15 Impact of three years EQA for AFB microscopy on the TB laboratories network performance in Mali

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Background: From 2006 to 2008 level of health centers increased from 2 to 3 and the number of

microscopy centers from 66 to 77. Considering that DOTS strategy requires high quality TB laboratories network an EQA system was implemented in 2006 that relies on Blinded Rechecking and Supervisory visits coordinated by the NRL in Bamako.

Objective: To evaluate the performance of microscopy centers during a three years EQA process in order to come up with new recommendations and interventions for scaling up laboratory services.

Methods: Blinded Rechecking was implemented according to international guidelines and sampling by LQAS method. Detection proportional to controllers and percentage of true positives among all positives were calculated. Blinded Rechecking was supplemented with Supervisory visits conducted by NRL and District Hospitals.

Results: Blinded rechecking covered 65 of 66 microscopy centers in 2006, 68 of 70 in 2007 and 73 of 77 in 2008. Overall detection proportional to controllers increased from 0.89 in 2006 to 0.96 in 2007 and 2008 and percentage of true positives among all positives from 95% in 2006 and 2007 to 98% in 2008. The percentage of centers doing poorly decreased from 30.8% in 2006 to 13.2% in 2007 and 12.3% in 2008. Analysis by region was discussed. Supervisory visits allowed us to investigate problems. The most frequent of them was staff transfer to other health areas.

Conclusions: EQA by Blinded Rechecking over a period of 3 years resulted in an increase of detection relative to controllers at either national or regional level and a diminution of microscopy centers doing poorly. In this setting this improvement was likely due to training sessions and staff motivation linked to Blinded Rechecking. In addition, we conclude that more often the Supervisory visits were done more respect of national guidelines were observed. Staff transfer hampered the reliable functioning of the TB laboratories network. Recommendations were proposed.

PS-101181-15 Auramine microscopy diagnoses specific groups of TB patients who are not diagnosed by ZN microscopy

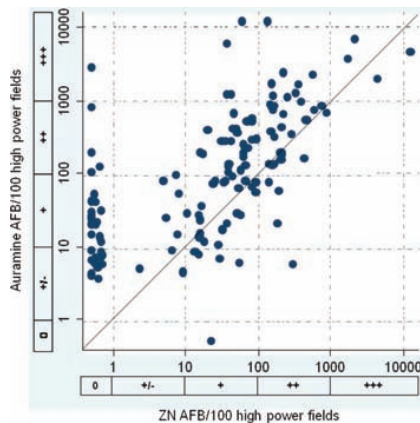
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Background: Fluorescence microscopy of auramine-stained sputum smears is quicker and more sensitive than Ziehl-Neelsen (ZN) microscopy for the detection of acid-fast bacilli (AFB). We quantitatively compared these techniques.

Method: Duplicate smears were made from 496 sputum samples. One was stained with ZN and read by

light microscopy, the other was stained with auramine and read by fluorescence microscopy on the same microscope. All smears were examined at $\times 1000$ magnification, the number of AFB in 100 fields counted and geometric means calculated.

Results: Of 138 sputum samples positive by either method, the sensitivity of auramine was 99% (1 false-negative) vs. 73% for ZN (37 false-negatives, $P < 0.001$). For samples positive by both methods, auramine detected an average of 2 more AFB per 100 fields than ZN ($P < 0.001$). Thus sputum samples with low auramine AFB counts were often false-negative with ZN. However some ZN false-negatives had high auramine counts (Figure). For ZN false-negatives, auramine detected an average of 40 more AFB per 100 fields than ZN ($P < 0.001$), suggesting that factors other than low bacillary load may explain some ZN false-negatives. In logistic regression, ZN false-negatives were independently more likely during treatment (OR 6.1, $P = 0.0001$), from patients with isoniazid-susceptible TB (OR 8.3, $P = 0.05$) and for paucibacillary '+/-' sputum (OR 9.0, $P < 0.001$). Similarly in linear regression, excess AFB count in auramine increased with days of TB treatment ($P = 0.01$), increased as the AFB concentration decreased ($P = 0.03$) and tended to be greater for isoniazid susceptible samples. ZN false-negatives were not associated with rifampicin or multidrug resistance, sputum storage or viscosity or culture results.



Conclusion: Auramine microscopy is more sensitive than ZN for paucibacillary sputum. In addition, sputum from patients with isoniazid-susceptible TB or collected during TB treatment are more likely to have TB detected by auramine microscopy than by the ZN technique.

PS-101264-15 Comparison of microscopy techniques in diagnosis of TB among smear-negative HIV patients in Uganda

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Introduction: There is an urgent need to develop low-cost diagnostic tools to enhance the accurate diagnosis of TB in patients with HIV/AIDS. TB incidence in Uganda is 369 per 100 000 per pop/per year with approximately 50% of identified TB cases co-infected with HIV. Sputum smear microscopy has a low sensitivity with a higher rate of smear negative pulmonary tuberculosis (PTB) in patients co-infected with HIV/AIDS. Indirect fluorescence microscopy (IFM) and analysis of concentrated sputum smears are credited with increased sensitivity and higher detection rates, but there is concern that specificity may be lower.

Methods: Prospective study carried out September 2008–July 2009. Sputum samples were obtained from patients with HIV/AIDS with suspected PTB on the basis of WHO clinical criteria. Initial smear microscopy by direct ZN at sample site were negative. Patients submitted two further sputum samples which were decontaminated according to the sodium hydroxide–N-acetyl-L-cysteine method and concentrated. A repeat Ziehl-Neelsen, IFM and MGIT were performed on the concentrated decontaminated samples. MGIT was the gold standard.

Results: 762 specimens were received from 381 patients. See the Table for comparison of the techniques against MGIT. Sensitivity of concentrated ZN method on previously 'sputum-negative' samples was 22.1% (9.7–39.6%) with specificity of 96.3% (93.7–98%). Sensitivity of IFM was 22.1% (9.7–39.6) with specificity of 96.6% (94–98.2).

Conclusions: Although ZN smears on concentrated sputum and indirect fluorescent microscopy perform better than traditional ZN microscopy, their sensitivity remains low.

Recommendations: IFM and concentrated ZN smears can be utilised in a low-income high TB prevalence setting to further aid rapid diagnosis of TB.

Table Results of concentrated ZN and indirect fluorescent microscopy compared to MGIT

	Mycobacterial growth indicator tube (MGIT)		Total
	Negative	Positive	
Concentrated ZN			
Negative	668	53	721
Positive	26	15	41
Total	694	68	762
Indirect fluorescent microscopy			
Negative	670	53	723
Positive	24	15	39
Total	694	68	762