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# CASE-DETECTION RATE OF DIRECT SPUTUM SMEAR MICROSCOPY FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS IN ABIA STATE, NIGERIA

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**ABSTRACT**

**The accuracy of sputum smear microscopy, the tuberculosis case-finding method in the Abia State TB Control Programme has never been assessed due to lack of culture facilities. To assess the accuracy of sputum smear microscopy in routine control programme conditions in Abia State, sputum samples from patients undergoing investigation for tuberculosis were analyzed using Ziehl-Neelsen staining technique for sputum smear microscopy and culture on Löwenstein-Jensen medium as reference standard. Out of 150 participants tested, 51 were smear –positive for acid fast bacilli (positivity rate, 34.0 %, 51/150) while 79 were culture positive for *Mycobacterium tuberculosis* complex and 12 for non-tuberculous mycobacteria (NTM). Thirty-seven of the 79 culture positive for *M. tuberculosis* were smear positive giving a ratio of smear to culture positivity of 46.84%. Forty-two (42.4%) of the 99 smear negative cases were culture positive for *M. tuberculosis*. The sensitivity of smear microscopy was 50.0% (95%CI=39.0-61.0) and specificity was 92.3% (95% CI=86.4-98.2). The prevalence of HIV/TB coinfection among the study participants was 48% (12/25). Although the case- detection rate of smear microscopy was moderate in this study, the large proportion of TB patients missed by smear microscopy is a cause for concern and requires concerted effort to improve the sensitivity of smear microscopy. Introduction of more sensitive diagnostic methods like culture also need to be considered.**

# TAUX DE DETECTION DIRECTE FROTTIS POUR LE DIAGNOSTIC DE TUBERCULOSE PULMONAIRE EN ÉTAT D'ABIA, NIGERIA

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**RÉSUMÉ**

**La précision des frottis, la méthode la recherche de cas de tuberculose dans le programme de lutte contre la tuberculose dans l'Etat d'Abia n'a jamais été évalué en raison du manque d'installations de culture. Pour évaluer la précision des frottis dans les conditions du programme de contrôle de routine dans cet Etat , les expectorations de patients subissant une enquête de la tuberculose ont été analysées à l'aide de technique de coloration de Ziehl-Neelsen pour la microscopie des frottis de crachats et de la culture sur milieu de Löwenstein- Jensen en tant que norme de référence . Sur les 150 participants testés, 51 étaient à frottis positif pour les bacilles acido rapide (taux de positivité, 34,0%, 51/150), tandis que 79 étaient positifs à la culture pour complexe *Mycobacterium tuberculosis* et 12 pour les mycobactéries non tuberculeuses (NTM). Trente-sept de la culture 79 positive pour *M. tuberculosis* étaient à frottis positif donnant un ratio de frottis de positivité de la culture de 46,84 %. Quarante-deux (42,4%) des 99 cas à frottis négatifs étaient positifs à la culture pour *M. tuberculosis*. La sensibilité de la microscopie des frottis était de 50,0 % (IC à 95% = 39,0 à 61,0) et la spécificité était de 92,3 % (IC à 95% = 86,4 à 98,2). La prévalence du VIH / TB coïnfection parmi les participants à l'étude était de 48% (12 /25). Bien que le taux de microscopique des frottis de dépistage des cas ait été modéré dans cette étude, la forte proportion de patients atteints de tuberculose manqués par examen microscopique des frottis est un sujet préoccupant et exige un effort concerté pour améliorer la sensibilité de la microscopie des frottis. La présentation des méthodes de diagnostic plus sensibles comme la culture doivent aussi être pris en considération.**

## INTRODUCTION

In most of the high-prevalence countries, rapid laboratory diagnosis of TB relies on the microscopic examination of direct sputum smear stained by the Ziehl Neelsen technique (1, 2). It is considered the most appropriate method available for case-finding in TB Control Programmes in resource-limited countries as it is good in detecting the most infectious cases excreting large number of bacilli (3). Culture is a more sensitive method than smear microscopy and is regarded as the gold standard for definitive diagnosis of TB but due to technical constraints, culture of *M. tuberculosis* is not done routinely in developing countries.

The sensitivity of Sputum smear microscopy (SSM) may be low and variable depending on several factors, both technical and epidemiological, which include the proficiency and diligence of the laboratory personnel, the quality of reagents, condition of microscope, workload of the laboratory, the disease stage at presentation as well as the prevalence of HIV/AIDS in the population of TB suspects (4, 5). To minimize the effects of these factors, the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) have produced guidelines which recommend quality control of SSM as an essential part of an effective national TB control Programme (6). The goal of quality control of SSM is to optimize the technical and operational components of the test so as to minimize diagnostic errors such as false positives and false negatives (7).

Assessing the case-detection rate of SSM in a particular TB Control Programme is of interest as it can indicate how effective the quality control measures have been observed as well as the proportion and the category of TB patients the laboratory is detecting. The ratio of smear to culture positivity is commonly used as a measure of the overall performance of SSM. In developed countries with well functioning laboratories and lower prevalence of smear positive cases, the ratio of smear to culture positivity on the same specimen was estimated to be around 40% to 60% (8, 9). In high-prevalence countries such as South Africa, Kenya or India, maximum values of 57% to 75% were recorded (10). In routine laboratory diagnosis in African laboratories, the sensitivity of the direct sputum smear microscopy ranged from 8.8%-46.6% (11). More recently, Mfinanga *et al*., (12), reported a sensitivity of 36.9% among the peripheral diagnostic centres in Dar es Salam, Tanzania.

The assessment of the case-detection rate of SSM has never been done in the Abia State TB Control Programme. The availability of such data will inform the Programme managers on the current status and the appropriate course of action to improve the diagnostic performance of the programme. The aim of the study reported here was therefore to assess the case detection rate of direct sputum smear microscopy for diagnosis of TB in the Abia State TB Control Programme.

## MATERIALS AND METHODS

**Study Participants.** Study participants consisted of 150 patients with high clinical index of suspicion for tuberculosis, defined as patients with cough of ≥3 weeks' duration. The patients were undergoing investigation for TB at the Leprosy and Tuberculosis Referral Hospital, Uzuakoli, Bende Local Government Area (LGA) and the Sputum Smear Microscopy Centre, Aba South LGA Health Office, Aba, all in Abia State, between November 2008 and February 2010. Eligible participants were individuals ≥ 15 years of age newly referred to the study centres to undergo SSM examination for acid-fast bacilli (AFB). Those already on anti-tuberculosis treatment at the time of recruitment were excluded from the study. Sputum collection was done according to routine procedure using spot-morning-spot (SMS) scheme. Informed consent was obtained from the study participants and the study protocol was approved by the research Ethical Committee of the Federal Medical Centre, Umuahia, Abia State.

### Acid-fast staining

Direct smear was made from each sputum specimen, stained by the ZN method, and read at the study centres by experienced laboratory technicians. A subject was diagnosed as a smear-positive TB patient if at least one of the three smears was positive for AFB.

### Sputum culture

The sputum culture was performed on the morning sputum specimen, or if not available, on a spot specimen from each study participantat the Microbiology Laboratory, Michael Okpara University of Agriculture, Umudike (MOUAU). The specimens were decontaminated and concentrated using modified Petroff's alkali method and inoculated unto slants of Lowenstein-Jensen (LJ) medium. The inoculated LJ slants were incubated at 37OC and examined for growth daily for the first 1 week and once weekly thereafter up to 8 weeks. Cultures that showed no growth after 8 weeks were recorded as negative. A patient was defined as true TB patient if the culture produced *Mycobacterium tuberculosis* and as a non-TB case if the culture showed no growth. The mycobacterial isolates were identified according to

criteria based on the rate of growth, colonial characteristics such as roughness and pigment production and nitrate reductase test. Representative suspected NTM isolates were sent to a reference laboratory for further identification.

**HIV Screening**

The study participants were screened for HIV using two rapid HIV tests according to the National HIV Screening guidelines (13). A patient was screened first with DetermineTM HIV 1& 2 (manufactured by Abbot Japan Co., Ltd for Inverness Medical, Japan Co., Ltd). If positive, the specimen was retested with DoublecheckGoldTM Ultra HIV 1& 2 (Orgenics Ltd, Israel) for confirmation. The two tests must be positive for a patient to be regarded as HIV positive.

**RESULTS**

A total of 150 participants were enrolled in the study. The demographic and clinical characteristics of the study participants are presented in Table 1. There were 67 (44.7%) males and 83 (55.33%) females, median age was 37 years (range, 17-85 years) and the mean age was 41.66 years. All the study participants submitted the first sputum specimens, 136 (90.7%) submitted 3 complete sputum specimens, 2 submitted two specimens and 12 (8%) submitted one sputum specimen each. The age bracket of 25 to 34 years contained the highest number of study participants and 42.6% (20/47) of them were positive for AFB.

**TABLE 1 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE STUDY PARTICIPANTS**

| | Overall | Male | Female |
|---|---|---|---|
| **No. of study participants** | **150** | **67** | **84** |
| **Age group (yr)** | | | |
| **15-24** | **19** | **4** | **15** |
| **25-34** | **47** | **18** | **29** |
| **35-44** | **26** | **16** | **10** |
| **45-54** | **21** | **11** | **10** |
| **55-64** | **15** | **8** | **7** |
| **>65** | **22** | **10** | **12** |
| **Age range (Min.-Max.)** | **17-85** | **17-84** | **17-85** |
| **Median age(yr)** | **37.00** | **34.00** | **33.00** |
| **Mean age(yr)** | **41.66** | **44.31** | **39.52** |
| **Sputum smear microscopy** | | | |
| **No. smear Positive** | **51** | **29** | **22** |
| **No. smear Negative** | **99** | **38** | **61** |
| **Sputum culture** | | | |
| **No. culture positive (Mtb)** | **79** | **36** | **43** |
| **No. culture negative** | **41** | **17** | **24** |
| **No. culture positive (NTM)** | **12** | **7** | **5** |
| **No. Contaminated(CTN)** | **18** | **7** | **11** |
| **HIV status** | | | |
| **No. tested** | **140** | **64** | **76** |
| **No. positive** | **25** | **11** | **14** |
| **No. negative** | **115** | **53** | **62** |
| **HIV/TB coinfection** | **12** | **6** | **6** |

**Mtb :** *Mycobacterium tuberculosis* **complex; NTM: Non-tuberculous mycobacteria**

Human immunodeficiency virus (HIV) screening was done in 140(93.3%) of the study participants who gave consent, 25 (17.9%) were sero-positive for HIV and 48% (12/25) were co-infected with TB. The detection rates of pulmonary tuberculosis (PTB) by SSM and culture among the study population is presented in

Table 2. The sputum smear positivity rate was 34.0% (51/150).Of the 51 smear positive subjects, 44 (86.27%) had all the three specimens positive for AFB, 6(11.76%) had two positive specimens and 1(1.96%) had one positive specimen. A total of 91 participants were culture positive for mycobacteria, 79(86.8%) for *Mycobacterium tuberculosis* complex and 12(13.19 %) for non-tuberculous mycobacteria (NTM). Eighteen (18) [12%] of the 150 sputum cultures were contaminated. The culture positivity rate for *M. tuberculosis* was 52.67% (79/150).

The smear positive cases and pattern of culture positivity according to the AFB grading is presented in Table 3. Thirty-seven (46.84%) of the 79 cases culture positive for *M. tuberculosis* were smear positive. However, culture revealed that 8 of the smear positive cases were due to NTM. Forty- two (95.45%) of the 44 subjects with all three specimens positive for AFB were culture positive; the remaining two were contaminated. Forty-two (42.4%) of the 99 smear negative cases were culture positive for *M. tuberculosis*. In 136 subjects who gave three sputum specimens, 47(34.56%) were smear positive for AFB while 80 (58.82%) were culture positive for mycobacteria. The sensitivity of SSM with reference to culture was 50.0% (95%CI=39.0-61.0) and the specificity was 92.3% (95% CI=86.4-98.2).

## DISCUSSION

Sputum microscopy remains the sole diagnostic test used for diagnosis of TB in most areas where TB is endemic because there is no alternative method that is technically feasible and can be implemented affordably (14, 1). In this study, the case detection rate of sputum microscopy was assessed using culture as the reference standard. The sensitivity and specificity of SSM in this study was 50 % (95% CI: 39.0%-61.0%) and 92.3% (95% CI: 86.4% - 98.2%) respectively. This suggests that the proficiency of our laboratory system was moderate and falls within the acceptable range of diagnostic performance of smear microscopy commonly reported (9, 10). In a study among peripheral diagnostic centres in Dar es Salaam, Tanzania, a sensitivity of 36.9% was reported for SSM (12).

**TABLE 2 DETECTION RATES OF PTB IN THE STUDY POPULATION BY SSM AND CULTURE**

| Sex | № (%) tested | №.(%) Positive for AFB № (%) aCx +ve (Mtb) | № (%) bCx+ve (NTM) |
|---|---|---|---|
| **Male** | **67** | **29(43.28)** | **36(53.73)** | **7(10.45)** |
| **Female** | **83** | **22 (26.51)** | **43(51.81)** | **5(6.02)** |
| **Total** | **150** | **51 (34.00)** | **79(52.67)*** | **12(8.00)** |

*** Not excluding contaminated cultures (n=18); aCx Mtb- Culture positive** *Mycobacterium tuberculosis* **complex bCx NTM- Nontuberculous mycobacteria**

Our study also showed a high level of agreement between the culture and SSM results among the patients with high bacillary load (3+ and 2+ categories). The discrepancy was highest among the paucibacillary patients (1+ and scanty categories) and AFB smear negative patients. This indicates that the performance of the laboratory was acceptable as per the diagnostic objective of DOTS which is to detect the most infectious cases with high bacillary load. However, a sensitivity of 50% implies that SSM failed to detect half of true PTB cases in this setting. On the basis of microscopy results alone, the 42 culture positive but smear negative PTB cases would have been missed for treatment. Majority of these cases would continue to transmit the disease in the community and delay in detection would result in increased risk of greater morbidity and mortality. This emphasizes the inadequacy of SSM as the case-finding method in the long term and overall success of controlling TB.

**TABLE 3 DIAGNOSTIC PERFORMANCE OF SSM IN ABIA STATE IN RELATION TO CULTURE**

| Sputum smear microscopy | | Culture | | | |
|---|---|---|---|---|---|
| AFB+ve Grading | № of cases AFB positive | № culture positive Mtb (% agreement) | № culture Positive NTM | № culture Negative | № contaminated |
| 3+ | 15 | 12 (80.0) | 3 | 0 | 0 |
| 2+ | 16 | 15 (93.8) | 0 | 0 | 1 |
| 1+ | 14 | 8 (57.1) | 2 | 3 | 1 |
| Sct | 6 | 2 (33.3) | 3 | 1 | 0 |
| AFB+ve | 51 | 37 (72.5) | 8 | 4 | 2 |
| AFB-ve | 99 | 42 (42.4) | 4 | 37 | 16 |

**a 1-9 AFB/100HPF (High power field); b 10-99 AFB/100HPF; c 1-10 AFB/HPF; d >10 AFB/HPF; Mtb:** *M. tuberculosis* **complex**

To our knowledge, this study is the first ever attempt to assess the diagnostic performance of SSM against culture in the Abia State TB Control Programme. The study has thrown light on important aspects of laboratory diagnosis of TB in the Abia State TB Control Programme. First, although the sensitivity of SSM during the period of this study was moderate, it is clear that a high proportion of smear negative TB patients were not being detected by the routine smear microscopy in use. Furthermore, the use of culture also revealed that 12(13.19%) of the subjects were infected with NTM.

Culture of *M. tuberculosis* from clinical specimens is more sensitive and specific than smear microscopy and is considered the gold standard for definitive diagnosis and bacteriological confirmation of tuberculosis. However, culture has a major disadvantage of being too slow for immediate clinical utility. The growth of mycobacteria may take from 4-8 weeks on conventional solid media. In addition, culture requires high technical skill, it is labour intensive and there is need for expensive biosafety system. Despite these shortcomings and challenges associated with utilizing culture, culture is indispensable for addressing the increasing incidence of sputum smear negative TB, particularly in Human Immunodeficiency Virus (HIV) associated TB in Africa (15). Culture is also needed for drug susceptibility testing (DST) in the face of emerging multidrug-resistant TB (MDR-TB). Although culture cannot replace sputum smear microscopy as a routine laboratory diagnostic method in developing countries, international authorities and experts are of the view that culture and DST capacity need to be strengthened at country level to address smear – negative TB and multidrug resistant TB (16, 17).

The specificity of SSM for detection of AFB is widely believed to be very high (4, 17,). The specificity of SSM of 92.3 % (95% CI: 86.4%-98.2%) found in this study is in agreement with this view. However, the inclusion of nontuberculous mycobacteria (NTM) isolates in the analysis reduced the specificity to 77.36% and the sensitivity was reduced to 46.8%. The significance of this is that in the absence of culture, all the acid fast bacilli (AFB) detected by smear microscopy would be treated as *M. tuberculosis* infections. The 12 patients with NTM would have been put on anti-TB treatment wrongly on the basis of the SSM results. This fact and the low sensitivity of SSM underscore the necessity to implement culture for diagnosis of pulmonary tuberculosis in developing countries despite the great technical challenges (18).

The incidence of NTM in patients undergoing investigation for TB has not been fully investigated in developing countries. This may be due to the low level of research involving culture of mycobacteria in developing countries. The incidence of NTM in this study was 12% which appears to be high but it is comparable with the prevalence rates reported by others. In a study in Plateau State in the north central part of Nigeria, a prevalence rate of 23.08% was reported (Mavak *et al* (19). Petersen *et al* (20) reported a prevalence rate of 20% for *M. avium-M-intracellulare* complex and 15% for other mycobacteria and Srisuwanvilai *et al*., (21) reported a prevalence of 11%.

The rate of contamination of classical LJ falls within 2-5%. The contamination rate in this study was well above this. This is a reflection of the technical difficulties encountered in the course of establishing the culture facility used in this study. The LJ medium was prepared locally and did not include a cocktail of antibiotic supplement commonly included in commercial mycobacterial culture media. Furthermore, we employed the Petroff decontamination and digestion method which might not have been as effective as the N-acetyl-L-cysteine-NaOH (NALC-NaOH) employed in most advanced laboratories conducting culture. However, contamination figures of 10.1%, 11% and 14.7% have been reported by others even in well established laboratories (21, 22, 23).

Sputum smear negative TB is a real challenge to laboratory based diagnosis of TB. About 5,000 to 10,000 AFB per milliliter of sputum is required for AFB to be detected in a sputum specimen (24). In early disease, disease outside the lungs, in the elderly and in children and HIV associated TB, the extent of cavitations is minimal and as a result, lower number of bacilli are present in the airways (25).This often results in undetectable number of bacilli in the specimen leading to false negative smear result. Other factors that may lead to false negative smear results include inadequate specimen quality, inadequate number of specimens, heavy workload, poorly prepared smears and poor staining technique (25). To ensure the reliability of laboratory results, external quality assurance (EQA) systems for smear microscopy should be implemented (3, 26). Adequate training and supervision of the laboratory personnel are also important in optimizing the performance of sputum smear microscopy (10, 12). Recent innovations such as the use of bleach for sputum concentration (27, 28) and the use of light emitting diode (LED) fluorescence microscopy (29, 30, 31) have potential to improve the sensitivity of sputum smear microscopy.

About half of the HIV-positive subjects were coinfected with *M. tuberculosis* but non with NTM. This may mean that NTM is not a common cause of TB in our setting but this should be taken with caution in few of the small number of HIV-positive cases.

In conclusion, the proficiency of our laboratory system in detecting PTB cases with high bacillary load was acceptable but SSM is inadequate as the method for case-detection of PTB for long term success of our TB Control Programme. There is a need to implement a more sensitive method like sputum culture for diagnosis of sputum smear negative pulmonary tuberculosis in our TB Control Programme.

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