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QUALITY ASSESSMENT OF SPUTUM SMEAR MICROSCOPY FOR DETECTION OF ACID FAST BACILLI IN PERIPHERAL HEALTH CARE FACILITIES IN DAR ES SALAAM, TANZANIA
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D. BASRA, M.I.N. MATEE and R. McNERNEY

ABSTRACT

Background: **There is no published information regarding the quality of sputum smear microscopy in Tanzania.**

Objective: **To evaluate technical quality and results of smear microscopy for acid-fast bacilli (AFB) in peripheral health care facilities in Kinondoni and Ilala Districts in Dar es Salaam, Tanzania.**

Design: **Cross-sectional study.**

Setting: **All tuberculosis diagnostic centres in Dar es Salaam, Tanzania.**

Results: **The proportion of well prepared smears was 86.2% and that of well stained smears was 81.2%. The overall average agreement in reading was (89.2%). The overall sensitivity was *88.5%* and specificity was 100%. High false negatives (HFN) were the major errors found in this study and Low false negative (LFN) and quantification errors (QE) were the minor errors found. There were no false positive errors. Minor errors occurred more frequently in hospitals than dispensaries, while major errors occurred more frequently in dispensaries than in hospitals.**

Conclusions: **The types of errors found in this survey, HFN, LFN and QE, suggest a systematic under-reading of smears in all the surveyed health facilities, probably due to a number of technical factors (quality of smears, poor stains, bad microscopes, or inadequate training) and other factors such as overwork and lack of motivation which need to be addressed.**

Recommendations: **Regular supervision using the new WHO quality assurance guidelines should be conducted countrywide. We do recommend that blind re-checking as the most efficient means of making the first broad assessment of sputum smear microscopy in Tanzania.**

INTRODUCTION

In many countries with a high prevalence of tuberculosis (TB), direct sputum smear microscopy remains the most cost effective tool for diagnosing patients with infectious tuberculosis and for monitoring their progress on treatment (1). The World Health Organisation strategy for tuberculosis control (DOTS) relies on a network of laboratories that provides acid fast bacilli (AFB) sputum smear microscopy. TB control will be most effective (and efficient) in countries that have a network of laboratories providing a reliable service within the framework of the National Tuberculosis Programme. Microscopy errors are likely to result in failure to detect persons with infectious TB who will then continue to spread infection in the community, or unnecessary treatment for "non-cases." Likewise,

errors in reading follow up smears can result in patients being placed on prolonged treatment or re-treatment, or in-treatment being discontinued prematurely (2). It is important to understand the limitations of smear microscopy in the detection of TB. When done properly, approximately 60 to 70% of all adults with pulmonary tuberculosis can be identified with the current direct microscopy test using Ziehl-Neelsen staining (ZN). In practice, however, this proportion is around 40 to 60% at best (3). This reduced sensitivity is related to problems associated with the stringent requirements of the test (4). Therefore, quality assurance of AFB sputum smear microscopy, is essential for any TB control programme (5). As defined by both the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD), a quality assurance programme for smear microscopy include: quality assurance (QA), external quality assessment (EQA) and quality improvement (QI). EQA includes on-site evaluation of the laboratory to review QC and should include on-site re-reading of smears as well as panel testing (5,6). The recommended approach is to use blinded re-checking of a sample of slides selected randomly from the laboratory register. The previously used approach of re-checking 100% of positives and 10% of negatives is no longer recommended since it is a burden for high-volume laboratories and inadequate for low-volume laboratories. Furthermore, prior knowledge of slide results provides basis for bias reading (7). Finally, re-checking programmes are intended to assess the overall performance and not to confirm any individual patient's diagnosis. Thus, the emphasis of checking all positive slides has been discontinued and replaced with a method that samples a representative collection of all slides, both positive and negative (8).

In Tanzania most of the TB diagnosis is done in peripheral diagnostic facilities by microscopists with minimum training. There are no published reports regarding the quality assessment of sputum smear microscopy. With the increasing incidence and prevalence of tuberculosis in the country, there is a compelling need to evaluate the quality of sputum smear microscopy for the diagnosis of TB. About 20% of all TB cases in Tanzania are found in Dar es Salaam, the capital city of Tanzania (9). The aim of this study was to perform external quality control of the sputum smears for the detection of acid fast bacilli in randomly selected peripheral health care facilities located in two of the three administrative districts in Dar es Salaam, Tanzania using the new World Health Organisation (WHO) guidelines (8).

MATERIALS AND METHODS

Settings: This study was conducted in Tandale, Sinza, Mnazi Mmoja and Tabata dispensaries and two municipal hospitals located in two of the three administrative districts in Dar es Salaam, namely Ilala and Kinondoni.

Study design: External quality assurance of sputum smears by blind re-checking of randomly selected slides from laboratory register for quality (size, thickness, quality of staining, and colour of the bacilli) and verification of peripheral smear results conducted during the third quarter of the year 2002 according to the WHO guidelines for external quality assessment for AFB smear microscopy. The slides were read by a second and third readers, without sharing results. Results of the second and third readers were then compared and discordances, when found, were resolved by re-examination of all the slides with discrepant results. The agreed result of reader two and three were regarded as a reference and were compared with those of a peripheral reader.

Sample size: The number of slides required was calculated based on the annual number of smears, positivity rates, the expected performance (sensitivity) compared to the controller of 80% set by the Tanzania National TB and Leprosy Programme and acceptance number (d), maximum number of false negative errors of zero. The sensitivity, as defined here, is the detection of all positives, including low positives (1-9 AFB/100).

Slide selection and re-checking process: Slides were randomly selected from laboratory registers by an independent supervisor (the district tuberculosis and leprosy coordinator) from a list of specimens processed during the third quarter of 2002. Slides were first re-evaluated for technical quality (size of spread and thickness as well as quality of stain, and colour of bacilli) and then blindly re-examined under the microscope for acid fast bacilli after re-staining using the Ziehl-Neelsen hot staining method, which is the technique used for examination of smears in all TB laboratories in Tanzania. The same number

of 100 microscopic fields and grading system (negative, 1-9 AFB/ 100 fields, 1+, 2+ and 3+) used in peripheral was used in the re-checking process.

Reading and interpretation of results: Discordances between the initial results and the results of the controller were compared. Errors were classified as major or minor. Major errors were sub-classified as (i) high false positive (HFP) — if a negative smear was misread as 1+ to 3+ positive and (ii) high false negative (HFN) — if a 1+ to 3+ positive smear was misread as negative. Minor errors included (i) quantification error — when there is a difference of more than one grade in reading a positive slide between examinee and controller, (ii) low false positive (LFP) — when a negative smear was misread as low (1 -9 AFB/I00 fields) positive and (iii) low false negative (LEN) — when a low (1-9 AFB/I00 fields) was read as negative.

RESULTS

Results of this study are presented in Tables 1 and 2. The proportion of well prepared smears (nicely spread and with appropriate thickness) was 86.2%, being higher among dispensaries (89.7%) than hospitals (84.0%), and higher in Ilala district (91.7%) than Kinondoni district (82.0%). The proportion of well stained smears was 81.2%, with no differences between dispensaries (80.7%) and hospitals (82.0%), but being higher in Ilala district (85.7%) than Kinondoni district (76.1%). The overall average agreement in reading was (89.2%), being higher in dispensaries (92%) than hospitals (89.6%), and higher in Ilala district (91%%) than Kinondoni district (87.3%). The overall sensitivity was 88.5%, being higher among dispensaries (91.5%) than hospitals (85.7%) and higher in Ilala district (91.0%) than Kinondoni district (86.2%). The level of specificity was 100% and was similar for all the peripheral health facilities. High false negatives were the major errors found in this study and were seen more frequently in Kinondoni district than in Ilala district, but occurred equally in dispensaries and hospitals. Low false negative errors occurred more frequently than in dispensaries than hospitals but occurred equally in both districts. Quantification errors occurred more frequently in hospitals than dispensaries and slightly more in Kinondoni district than Ilala district. The quantification errors involved underscoring by peripheral readers as compared with controllers (Table 2). In both hospitals and dispensaries total minor errors occurred twice more frequently compared with total major errors, and slightly more in Ilala district than Kinondoni district. Total major errors occurred more frequently in Kinondoni district more than Ilala district.

DISCUSSION

Blinded re-checking or re-reading of a sample of routine smears from the peripheral sites and intermediate laboratories by controllers at a higher level is considered to be the best method for evaluating performance and providing motivation to staff for improvement. According to recent technical guidelines published by the IUATLD in blind re-checking performance is based on the number and type of errors exceeding a predetermined threshold, rather than calculating a percentage of errors. Logically, a re-checking programme will start by focusing on major errors and laboratories with large number of errors. It is important to realise that one or more errors will be found even in laboratories performing at or above the expected level. This is an important concept for the National Reference Laboratory and the NTP to realise when providing feedback to peripheral laboratories.

The major error found in this survey was high false negatives (HFN), while minor errors were low false negatives and quantification errors which were mainly due to under-quantification by peripheral readers (Tables 1 and 2). The occurrence of these errors by health facilities showed considerable variations, with major errors occurring more frequently among dispensaries rather than hospitals and vice versa (Table 1). This difference cannot be explained by the quality of smear or staining, which were in-fact, better in dispensaries than hospitals (Table 1). The high rates of HEN seen in the dispensaries is likely to be inadequate training, overwork (being involved in diagnosis of a number of many other infections), gross neglect and overall lack of motivation leading significant under-reading. We found two dispensaries with defective microscopes, with lenses being infested with fungi.

On the other hand the LFN errors which were found more frequently in hospitals seem also to indicate superficial or inadequate microscopy possibly associated with reading of fewer than the

Table 1

External evaluation report of selected peripheral health care facilities in Dar es Salaam

Parameter	Peripheral health facilities				
	Dispensaries	Hospitals	Kinondoni	Ilala	Total
Annual number of slides	17015	9868	19053	9561	28614
Positivity rate	5.0	10	5.0	16.0	7.5
Required sample size	208	104	213	69	144
Slides examined	300	300	300	300	600
Percentage of good quality smears	89.7	84.0	82.0	91.7	86.2
Percentage well stained smears	80.3	82.0	76.7	85.7	81.2
Percentage agreement of results#	92	89.6	87.3	91	89.2
Sensitivity	91.5	85.7	86.2	91.0	88.5
Specificity	100	100	100	100	100
High false negative (HFN)	10	9	17[a]	7	24
High false positive (HPN)	0	0	0	0	0
Low false negative (LFN)	15[b]	5	7	8	15
Low false positive (LFP)	0	0	0	0	0
Quantification errors (QE)	9	17[b]	14	12	23
Total minor errors (LFN + LFP + QE)	24	22	21	20	38
Total major errors (HFN+HFP)	10	9	17[a]	7	24

Agreement between initial reader and the controller

[a]Significant differences between Ilala and Kinondoni districts ($X^2 = 22.1$ df = 1, $P<0.01$)

[b]Significant differences between dispensaries and hospitals (($X^2 = 17.3$ df = 1, $P < 0.01$)

Table 2

Comparison of peripheral reading with controller reading

Peripheral reading	Controller reading		
	1+2+3+	1-9/100	Negative
Positive			
1+2+3+	258	9	0
1-9/100	17	16	0
Negative	24	15	261
Total	299	40	261

recommended number of microscopic fields. This may, at least in part, explain the numerous quantification errors, which were mainly under-reading by peripheral readers (Table 2).

The general under reading of smears seen in this study has also been reported in a Kenyan study; in which about 25% of results were falsely negative (10). Apart from resulting in delayed treatment and prolonged transmission and patient suffering, such results may exaggerate the true magnitude of smear negative tuberculosis reported to be on the rapid raise in HIV-endemic areas and may also affect case notification rate leading to underestimation of the disease and does pose tremendous challenge to the concept of TB prophylaxis, which requires exclusion of active TB (11). Urgent measures are required to reduce workload of microscopists by either dedicating them to sputum smear microscopy or increase the work force. There is also a need for regular maintenance of microscopes, provision of disposable wooden stick applicators and re-training of staff on procedures in assessing quality of

specimens, preparation of smears and stains and staining techniques, reading of slides as well as the highlighting the limitations of smear microscopy. This should be coupled with regular and systematic quality assurance programme involving on-site visits, proficiency testing as well as blind re-checking of slides using a standard checklist to be performed countrywide, which has been shown to be effective elsewhere (12).

In conclusion, although a few errors will be found in many laboratories, the authors emphasise that the findings in Dar es Salaam are alarming. In 39 (13%) out of 300 smears reported negative by the peripheral laboratory a positive smear (24) or a smear with 1-9 bacilli per 100 fields (15) was missed. We do recommend panel testing as the most efficient means of making the first broad assessment of sputum smear microscopy in Tanzania.

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