Application of lot sampling of sputum AFB smears for the assessment of microscopy centres

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SUMMARY

SETTING: Designated microscopy centres (DMC) and additional microscopy centres (AMC) performing sputum acid-fast bacilli (AFB) microscopy, the District TB Centre (DTC) and a reference laboratory (RL).

OBJECTIVES: To ascertain the feasibility of adopting lot sampling of AFB smears and to assess the performance of MCs employing Senior Tuberculosis Laboratory Supervisors (STLS) with no knowledge about the principles of quality assurance of AFB microscopy and RL-based laboratory technicians with training on quality assurance for blinded checking of AFB smears.

METHODS: Slides from MCs were transported to the DTC and the RL; 20 smears per month per MC were selected systematically; 1547 slides from DMCs and 726 from AMCsc were checked, respectively, by STLSs at the DTC and by RL laboratory technicians. Discrepancies were resolved by referee.

RESULTS: The discrepancy between MC laboratory technicians and STLSs at the DTC was 4.7%, compared to 1% at the RL. The STLSs and RL-based laboratory technicians had 70 and 2 errors, respectively.

CONCLUSIONS: Lot sampling of AFB smears is feasible under field conditions. Assessment of MCs was more valid with RL-based technicians trained in principles of quality assurance of sputum AFB microscopy than with STLSs with no such training and working in the field.

KEY WORDS: pulmonary tuberculosis; lot sampling; sputum smears; AFB; quality control

EFFECTIVE CONTROL of tuberculosis (TB) is dependent on a network of local laboratories that provide accurate and reliable acid-fast bacilli (AFB) microscopy testing for diagnosis, treatment and monitoring. Direct sputum smear microscopy remains the main tool used by national TB control programmes (NTP) in low-income, high TB prevalence countries.1 It is imperative that NTPs ensure the quality of the AFB sputum smear microscopy services provided by these laboratories through appropriate quality control measures. The present study documents the experiences gained using lot sampling of sputum AFB smears in a tuberculosis unit in Tiruvallur district, Tamil Nadu state, India, during the period May 2002 to April 2003.

MATERIALS AND METHODS

Organisation and microscopy centres

Under India’s Revised National TB Control Programme (RNTCP), the district of Tiruvallur has been subdivided into six sub-districts, otherwise known as tuberculosis units (TUs). Each TU serves a population of approximately 500 000. For administrative reasons Velliyur TU, where this study was carried out, has 12 microscopy centres (MC): seven RNTCP designated microscopy centres (DMCs) and five additional microscopy centres (AMCs), to serve a population of 580 000. One trained laboratory technician (LT) is responsible for sputum AFB microscopy in each MC, and one trained Senior Tuberculosis Laboratory Supervisor (STLS) is responsible for coordinating all the sputum AFB microscopy services within the TU. All the centres implement the DOTS strategy to treat TB patients under the RNTCP.

Training of LTs and STLSs

Using the standardised RNTCP training modules, all the 12 LTs in the study TU and the six STLSs of the district were trained in sputum AFB microscopy at the reference laboratory (RL) situated at the Tuberculosis Research Centre, Chennai, before the RNTCP was implemented in Tiruvallur district.
Sputum smears and examination
For diagnosis, three sputum samples—two spot and one early morning—were collected from each patient with cough of \( \geq 3 \) weeks. The samples were labelled A, B, and C, preceded by the serial number allotted to each patient. For follow-up, two samples were collected and labelled A and B, along with the patient’s number. A direct sputum smear was made from each sample, stained, examined, and reported as described in the RNTCP laboratory manual.\(^1\) All the slides were arranged in serial order in slide boxes and stored in cupboards until the STLS visited the respective centre for supervision.

Transportation of smears
Every month, as per the instruction of the District Tuberculosis Officer (DTO), the A, B, or C slides from the seven DMCs and five AMCs from the previous month were transported to the District TB Centre (DTC) and the RL, respectively, for blinded rechecking. The smear results of the DMCs and AMCs were given to the DTC and the RL in sealed envelopes.

Selection of slides
A laboratory technician under the supervision of the DTO at the DTC and a trained senior technician under the supervision of a mycobacteriologist at the RL systematically selected 20 slides per month per MC for checking as per RNTCP guidelines.\(^2\) The gap between first reading at the local MCs and cross-checking at the DTC and RL did not exceed 50 days.

Checking and discrepancy solving
The slides from the DMCs and the AMCs were checked by the STLSs at the DTC and the laboratory technicians at the RL. The smear results were matched and verified by the DTO at the DTC and by a mycobacteriologist at the RL. Discrepant results were resolved by referee reading by a second STLS at the DTC and by a senior laboratory technician at the RL. The referees were provided with the results of the peripheral laboratory technician and the first STLS, but the identity of the readers was concealed. The results of the referees were taken as final.

Validity of checking
The validity of checking was reviewed by comparing the type and number of errors for the first checkers (STLS at the DTC and laboratory technician at the RL) using the referees’ results as the gold standard.

Definitions
The definitions of errors as described in recent international guidelines\(^3\) were followed (Table 1). The slide positivity rate (SPR) is the percentage of positive slides of the total number of slides examined annually at an MC. The negative slide volume is the total number of slides, excluding positive slides, examined in a year in an MC.

RESULTS
The direct sputum smears prepared from sputum samples collected during the period April 2002–March 2003 were included in the study. The details of SPR, total slides and negative slide volume for each MC are given in Table 2. The average SPR and negative slide volume were 11% (range 7.3–17.6%) and 1480 (range 286–7814), respectively.

Table 1 Description of errors\(^3\)

<table>
<thead>
<tr>
<th>Results by technician</th>
<th>Negative</th>
<th>Scanty</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative*</td>
<td>C</td>
<td>LFN</td>
<td>HFN</td>
<td>HFN</td>
<td>HFN</td>
</tr>
<tr>
<td>Scanty</td>
<td>LFP</td>
<td>C</td>
<td>C</td>
<td>QE</td>
<td>QE</td>
</tr>
<tr>
<td>1+</td>
<td>HFP</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>2+</td>
<td>HFP</td>
<td>QE</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>3+</td>
<td>HFP</td>
<td>QE</td>
<td>QE</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

* Negative = no AFB/100 fields; scanty = 1–9 AFB/100 fields; 1+ = 10–99 AFB/100 fields; 2+ = 1–9 AFB/field in at least 50 fields; 3+ = >10 AFB/field in at least 20 fields.

C = correct result; HFP = high false-positive; HFN = high false-negative; LFP = low false-positive; LFN = low false-negative; QE = quantification error.

Table 2 Quality assurance report on the microscopy centres

<table>
<thead>
<tr>
<th>Designated microscopy centre</th>
<th>Additional microscopy centre</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>L</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Total slides</td>
<td>8715</td>
</tr>
<tr>
<td>Negative slide volume</td>
<td>7814</td>
</tr>
<tr>
<td>Slide positive rate (%)</td>
<td>10.3</td>
</tr>
<tr>
<td>Type of error, n*</td>
<td>HFP</td>
</tr>
<tr>
<td></td>
<td>HFN</td>
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<tr>
<td></td>
<td>LFP</td>
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<tr>
<td></td>
<td>LFN</td>
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<tr>
<td></td>
<td>QE</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
</tr>
</tbody>
</table>

* See Table 1 for explanation.
DISCUSSION

Guidelines for external quality assurance (EQA) of sputum AFB microscopy are periodically reviewed and modified. Different guidelines have their own merits and faults. The lot quality assurance sampling (LQAS) methodology, developed in industry for quality control of medical records, is increasingly adopted for use in public health service settings, for example in monitoring immunisation coverage, malaria transmission surveys and quality control of medical records. Recently published international guidelines on external quality assurance of AFB smear microscopy have recommended adopting LQAS for sampling sputum AFB smears under the blinded rechecking of routine slides component for EQA of AFB smear microscopy services. In the present study, the criteria adopted to select the sample of 20 slides/month/MC match those given as an example in the guidelines on EQA: sensitivity 85% (identification of positive smears relative to checker); specificity 100%; acceptance number (d) 1; slide positivity rate (SPR) 10%; negative smear volume per centre 1000. The performance of MCs was interpreted and determined based on the above.

The quality of sputum microscopy in these MCs was previously monitored by checking all positive and 10% of negative slides in an unblinded fashion during the monthly supervisory visits by the STLS. The number of slides checked each quarter by the STLS was very high (range 540–1240, including positive and negative slides) and agreement was falsely extremely high. In the present study, a fixed number of 240 slides/annum/MC was rechecked in a blinded fashion, thus minimising the STLS workload.

The EQA report on the performance of the MCs is relatively simple to complete and interpret. Recording the number and type of errors in the EQA table makes the identification of faulty MCs easy and promotes action taking by supervisory staff.

In quality assurance, it is also important for trends in MC performance to be monitored, to initiate appropriate corrective actions. If there are inconsistencies in the performance of the first checkers, however, reliable information about MC performance will not be available to inform the corrective measures to be taken. In the present study, the assessment of the DMCs at the DTC could be inaccurate, as the first checkers, i.e., the STLSs, committed a number of errors themselves. Assessment of the AMCs at the RL seemed to be more valid, as fewer errors were made by the RL laboratory technicians.

The STLSs visit the 4–6 microscopy centres in their respective TUs routinely for supervision. Blinded rechecking of routine slides at the DTC is in addition to the other responsibilities of the STLS. Moreover, STLSs were not trained in the various aspects of quality control of sputum AFB microscopy contained in the 2001 RNTCP quality assurance document. Lack of motivation or appropriate training in EQA of sputum AFB microscopy might have contributed to the poor performance by the STLSs at the DTC. These observations suggest that the laboratory supervisors working in the field need to be trained specifically in EQA before they can be assigned the responsibility of rechecking routine smears.

The LTs at the RL, on the other hand, are provided with good laboratory facilities and are highly motivated. In addition, they were trained in EQA before being assigned the fixed responsibility of rechecking smears. These factors may have contributed to the greater validity of rechecking in this group.

CONCLUSIONS

It is feasible, under field conditions, to adopt lot sampling of sputum AFB smears for the assessment of sputum AFB microscopy centres. The quality of check-
References


Acknowledgements

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CONTEXTE : Centres affectés à la microscopie (DMC) et centres de microscope complémentaire (AMC) pratiquant l’examen microscopique des expectorations pour bacilles acido-alcoolo-résistants (BAAR), le Centre de TB du District (DTC) et un laboratoire de référence (RL).

OBJECTIFS : Évaluer la faisabilité de l’adoption d’un échantillonnage de lots de frottis BAAR et évaluer les performances des MC faisant appel à des Superviseurs Seniors de Laboratoire de Tuberculose (STLS) sans connaissance des principes du contrôle de qualité de la microscopie pour BAAR ainsi que celles des techniciens de laboratoire basés sur la RL formés sur le contrôle de qualité pour l’examen aveugle des frottis BAAR.

MÉTHODES : Les lames provenant des MC ont été transférées au DTC/RL ; 20 frottis par mois ont été sélectionnées systématiquement par MC ; 1 547 lames provenant des DMC et 726 des AMC ont été contrôlées respectivement par les STLS au DTC et par les techniciens de laboratoire au RL. Les discordances ont été résolues par arbitrage.

RÉSULTATS : La discordance entre les techniciens de laboratoire MC et les STLS au DTC a été de 4,7% alors qu’elle était de 1% au RL. Il y a eu 70 erreurs chez les STLS au DTC et 2 erreurs chez les techniciens au RL.

CONCLUSIONS : L’échantillonnage de lots de frottis pour BAAR est réalisable dans les conditions du terrain. L’évaluation des MC est plus valable lorsqu’elle est assurée par les techniciens au RL entraînés aux principes du contrôle de qualité de la microscope des expectorations pour BAAR que par les STLS sans cet entraînement et travaillant sur le terrain.