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WORKSHOP REPORT

Critical appraisal of current recommendations and practices for tuberculosis sputum smear microscopy

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SUMMARY

This report is the consensus-based, agreed position of the participants in a workshop for experts in sputum smear microscopy organised in August 2005 by the International Union Against Tuberculosis and Lung Disease (The Union), and as such reflects their views, but not necessarily those of their respective organisations.

The group reviewed current practices in sputum smear microscopy for tuberculosis (TB) and suggests considering certain changes in standard guidelines for the sputum collection strategy and the definition of sputum smear-positive TB. The Ziehl-Neelsen staining guidelines need to incorporate a wider error margin for widespread application under field conditions. Quality assurance is of utmost importance, and needs more commitment from National Tuberculosis Programmes and other health authorities. In particular, allocation of sufficient resources for rechecking and integration of laboratory supervision must be ensured. Countries must make better investments in the purchase of high quality microscopes and laboratory supplies. To address the human resource crisis, personnel without specific laboratory schooling can, in principle, be trained to respond to immediate needs for TB diagnostic microscopy services. Periodic reporting on acid-fast smear examinations is highly desirable for regular monitoring and a more balanced provision of supplies.

KEY WORDS: acid-fast smear; diagnosis; microscopy; laboratory services; tuberculosis

SPUTUM SMEAR MICROSCOPY for acid-fast bacilli (AFB) is of vital clinical and epidemiological importance in the diagnostic process for tuberculosis (TB) in both affluent low-incidence countries[1] and high-burden countries with limited resources.[2] The detection of AFB in pulmonary secretions identifies those patients with the greatest potential for transmission of *Mycobacterium tuberculosis*.[3–5] Sputum smear microscopy offers the triple advantages of speed, simplicity and low cost.[6] In many countries with a high burden of TB, it is the most appropriate and most accessible diagnostic tool.[7] An effective mechanism for quality assurance (QA) is required to maintain the national TB laboratory network in optimal condition, providing accurate, reliable and timely results, so as to generate ongoing confidence in the service.[8]

SPECIMEN COLLECTION, TRANSPORT AND CONCENTRATION

The ideal specimen is a good quality early morning sputum. In many high-prevalence settings, cases frequently present with advanced TB and a high sensitivity of sputum smear examination: in a study in Madras, less than 20% of culture-positive cases were missed by a single microscopic specimen.[9] In Bangalore, the efficiency of two microscopic examinations was almost identical to that of a culture from a single specimen.[7] Examination of three early morning sputum specimens collected on consecutive days thus approaches the sensitivity of a single sputum specimen submitted for culture. To reduce the number of visits

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required among out-patients, the spot-morning-spot strategy was introduced. Various studies have shown that the incremental gain from the third specimen is low, commonly adding only 1–4% additional smear-positive cases.[10–13] In settings with excessive workloads, examination quality may be compromised. Reducing the number of required sputum specimens examined to two, with at least one morning specimen, has thus been suggested for some high-prevalence settings.[14–16]

Among human immunodeficiency virus (HIV) positive patients there is concern about the sensitivity of sputum microscopy.[17] The main diagnostic dilemma in HIV-infected TB patients is posed by disseminated TB.[18] Among those with pulmonary TB, studies using various sputum collection and staining protocols found that high yields can be obtained.[19–22]

Health centres collecting but not examining sputum specimens must forward either the specimen or a prepared smear to a higher level laboratory. The latter approach requires additional training, sustained supervision and an adequate supply of materials to the peripheral centres. While prolonged exposure to ambient temperature does not adversely affect the yield of AFB,[10,23] delays in diagnosis with such a practice are almost unavoidable.

Using bleach (sodium hypochlorite) to increase diagnostic sensitivity has given variable results, for reasons that are not entirely understood.[24–26] Possible factors include insufficient standardisation and differences in baseline sensitivity of microscopy without concentration. Additional qualitatively well-designed efficacy and effectiveness studies are required before it can be recommended as a routine procedure.[26]

DEFINITION OF A SMEAR-POSITIVE CASE

Current case definitions require two sputum specimens positive on smear microscopy or at least one positive on culture before a person can be registered as a new sputum smear-positive ('definite') case, or as a relapse.[27] The most likely explanation for the requirement of two positive smears is concern about clerical errors. Clerical errors can occur with both smear and culture examinations, yet such errors were found to be exceedingly rare in a large study in Dar es Salaam (Tanzania), Kampala (Uganda) and Nairobi (Kenya).[28] In high-prevalence settings, sputum smear examination is also likely to be more specific than culture,[7] because the latter has the added risk of cross-contamination.[29,30] Importantly, absence of a confirmatory positive microscopic examination does not preclude treatment or influence the choice of treatment regimen.[27]

Positive sputum smear microscopy results define the classification of TB for surveillance purposes. The United States surveillance definition of a bacteriologically confirmed TB case includes those with AFB demonstrated in a clinical specimen when a culture is unavailable without requiring more than one specimen.[31] In high-burden settings, where both the specificity of AFB for TB and the prevalence of cases among suspects are high, it seems justifiable to define a sputum smear-positive case as a suspect with a single AFB-positive sputum specimen. With a functional external QA system for microscopy, the definition could justifiably be further simplified by accepting a single AFB in one specimen among at least two examined.[32]

DEFINITION OF TREATMENT FAILURE

Disease extent at diagnosis is the major determinant of the time until sputum smear conversion.[33,34] After 2 months of treatment, around 30% of patients with severe advanced pulmonary disease are still likely to be smear-positive, decreasing to 10% at 3, and to 3% at 4 months.[35] In particular, scanty (1–9 bacilli/100 oil immersion fields)[32,36] smear-positive follow-up specimens are likely to be culture-negative at later points in the course of treatment, suggesting that such AFB are non-viable and thus a false indication of bacteriological failure.[33,37] Because non-viable bacilli are generally fewer in number, the specificity of the definition of failure could be increased by examining a single (early morning) specimen rather than two specimens on each occasion. To further increase the specificity, the threshold for action might be set to at least 1+, requiring confirmation of a scanty result by a second scanty or higher positive sputum smear examined after an appropriate interval of at least 2 weeks.

LABORATORY SAFETY

Using a correct technique, direct smear microscopy is unlikely to produce any appreciable aerosols containing *M. tuberculosis* compared with the potential of encountering aerosols when educating patients about specimen collection (which should be done outdoors). Sputum smear microscopy alone carries no TB hazard.[38] Biological safety cabinets (BSCs) offer little additional protection for laboratory staff who prepare smears only in comparison to good laboratory practice with adequate natural ventilation. Furthermore, BSCs in national reference laboratories in high TB prevalence countries are frequently incorrectly installed and poorly maintained.[39] They thus might increase rather than reduce the hazard while conveying a false sense of safety. Efficient natural ventilation is thus of the utmost importance. If BSCs are used in laboratories that perform only sputum smear microscopy, they should allow complete extraction of air to the outdoors rather than be fitted with filters, which tend to block quickly.

STAINING

Basic fuchsin and phenol are the key components of carbol fuchsin. Basic fuchsin is a mixture of four dyes

in different proportions.[40] There are substantial differences between manufacturers and batches with respect to composition, quality and solubility.[41] National Tuberculosis Programmes (NTPs) should purchase only basic fuchsin that is certified by the Biological Stain Commission. The original Ziehl-Neelsen (ZN) method used a concentration close to 1% of basic fuchsin in the carbol fuchsin,[42] commonly referred to as 'strong carbol fuchsin'. It is historically not clear what led to the current reduced concentration of 0.3% recommended by the World Health Organization (WHO), The Union, and other organisations.[6,43,44] Crucial steps in the ZN method are: 1) heating the carbol fuchsin to steaming, and 2) allowing exposure of the smear to the heated carbol fuchsin for a minimum of 5 min.[43] Prolonged exposure, e.g., for 10 min, is more practical than repeated heating within only 3–5 min. A 1.0% carbol fuchsin solution provides more consistently strongly stained AFB and introduces a safety margin.[44–47] More operational research is required to compare a 0.3% versus a 1% carbol fuchsin concentration. As it seems not to have a negative effect, countries currently using a concentration of >0.3% should not reduce it. A standardised system for use by NTPs to ascertain the quality of basic fuchsin dyes or ready-made solutions should be developed. The phenol for the stain preparation should be of analytical grade. Visual inspection of phenol (clear, colourless crystals) might suffice to ascertain its quality.

Acid-alcohol produces a cleaner smear than watery dilutions of acid, but prolonged exposure to it may result in over-decolourisation. Different types of aqueous acid solutions yield very similar results. The use of hydrochloric acid in water may be more economical and environmentally acceptable, but it needs field evaluation; resources permitting, the water may be replaced by technical (denatured grade) ethanol or methanol.

The longer the smear is exposed to methylene blue, the more likely it is that the AFB will become obscured. The exposure time should thus not exceed 30 seconds with a 0.3% stain. As the aim of a smear is a strong red against a weak blue background, a lower concentration (0.1%) for a similar time may be preferable.

FLUORESCENCE MICROSCOPY

Fluorescence microscopy can be useful in well-defined settings. The reduced examination time and the frequently increased sensitivity form the rationale for the current recommendations.[48] It is frequently recommended to confirm fluorescence microscopy positive results with the ZN technique.[6] This is cumbersome, and defeats the advantage of the superior sensitivity. With sufficient experience, examining the same field in case of doubt at a higher magnification under fluorescence is adequate. Systematic false-positive errors will be readily identified with external quality assessment. In some settings, fluorescence microscopy has been difficult to introduce or sustain. More operational research is therefore needed to determine why this occurs and to better define the settings where fluorescence microscopy is appropriate.

QUALITY ASSURANCE

Reliable microscopy services require an effective QA system that incorporates internal quality control measures, external quality assessment and quality improvement.[8,49] Motivation of laboratory staff is of the highest importance for AFB microscopy quality, and should be promoted through both internal and external QA measures.

Quality control needs more emphasis, not least as a motivator for laboratory staff by promoting confidence in the quality of their results, using standard operating procedures, quality control of stains, using positive and negative control smears and monitoring essential indicators from the laboratory register.[8,50,51]

Blinded slide rechecking combined with problem-solving supervisory visits is the preferred system for improving laboratory quality, applicable everywhere. Excellent results have been achieved in a number of settings using the currently recommended system.[52–54] The NTP must be strongly committed and allocate sufficient resources. The sample size must be kept at a minimum using the 'lot quality assurance sampling' (LQAS) system. In highly decentralised services, LQAS fails to reduce workload and refinements such as targeting sub-groups with a higher prevalence may have to envisaged. The implementation of fluorescence microscopy requires the development of its own external quality assessment system.

Determining the proficiency of microscopists through panel testing may be useful in the absence of a blinded rechecking programme. Smear panels must be highly consistent and challenging, requiring modification of the existing protocols, a difficult and challenging task. A procurement source for high quality ready-made panels would be desirable.

Supervision by laboratory specialists should focus on problem microscopy centres, with routine visits to well-performing laboratories only once a year. With training in external quality assessment and laboratory essentials, non-laboratory NTP supervisors are expected to collect samples of smears, provide feedback and act as coordinators. Supervision checklists are not a substitute for a good understanding of the subject and a supportive attitude towards laboratory staff. The development of practical guidelines for problem-oriented laboratory supervision, including identification of laboratory quality indicators, is urgently needed. The laboratory register should be better exploited to extract such indicators.

The AFB laboratory network should be integrated into the NTP and the general laboratory services.

Individual laboratories need good communication within their own setting, such as via representation in a hospital management team.

SUPPLIES

Stocks of laboratory products should consist of a 1-year running requirement plus a 1-year buffer stock maintained at the national level.[55] Staining solutions are best consumed within 1 year of manufacture. A supply system based on the number of smears examined and the balance of remaining products is the most accurate and efficient approach. Procurement for critical equipment and supplies (microscopes, basic fuchsin, phenol, immersion oil) by programmes must be centralised and formulated only in consultation with laboratory experts. Stain procurement and solution preparation in peripheral diagnostic units causes more frequent problems.

MICROSCOPES

Procuring quality microscopes requires purchase from reputable manufactures, and is not the cheapest solution. A list of recommended models would be useful to guide NTPs and donor organisations.[56] Each microscopy unit should have spare bulbs, while other essential spares are kept at the intermediate level. In humid climates, a cabinet with a lightbulb (25 W) is a practical way of preventing fungal damage to microscopes. Newer anti-fungal devices need to be field-tested. Care and maintenance of microscopes relies on correct manipulation, setup and cleaning. In contrast to others,[6] this group recommends that xylene should never be used to clean any part of a microscope,[57] and it should not even be made available to peripheral laboratories. Non-drying synthetic oil of medium viscosity (refractive index >1.5) is essential for the long life of an objective.[58] Cedarwood oil or xylene-diluted oils should never be used.[56,58] Gently wiping the objective at the end of each reading session with soft tissue paper or lens cleaning paper is adequate for removing excess oil. For more thorough cleaning, manufacturer-recommended fluids or a mixture of ethyl ether and alcohol (80/20) should be used.

TRAINING

Many countries have a shortage of qualified health care personnel in general,[59,60] and of technicians in particular. Government regulations and professional bodies permitting, training personnel without formal laboratory qualifications in basic laboratory techniques is an effective emergency solution, obviously requiring regular supervision.

Different levels of service will need appropriate training, with assessment of proficiency. Refresher courses have little impact if other causes of poor performance have not been addressed first. Workshops provide an opportunity for the exchange of experience and feedback on external quality assessment. For implementation of blinded rechecking, training of NTP supervisory staff at intermediary level is critical.

REPORTING LABORATORY ACTIVITIES

A quarterly laboratory activity report is needed for close monitoring (epidemiological, laboratory workload and quality, supplies). It should include the number of negative, positive and scanty results by type of specimen (suspect vs. follow-up), and stocks of essential laboratory supplies remaining at the end of the quarter.

CONCLUSIONS

The performance of currently available tests is unsatisfactory.[17] While new diagnostic tests are desperately needed, it is imperative to exploit the currently available tools to their fullest potential.

Successful sputum smear microscopy requires correct execution of every step, from sputum collection to reporting the result. Any error or deficiency in any of the steps potentially creates false results. TB-HIV co-infection has created an enormous workload for many NTPs and laboratory services, provoking a substandard approach and compromising work quality.

The performance of microscopy is also greatly affected by external factors, including:

- international guidelines on sputum collection, case definition, staining solutions and techniques that must be appropriate, efficient and evidence based;
- the quality of the microscope, reagents and consumables, often purchased without the necessary input from the laboratory service;
- a workload exacerbated by the human resource crisis in many settings;
- the high motivation required that is frequently contrasted by the low esteem in which the technicians are held, compounded by poor salaries;
- the absence of any external quality assurance programme or site visits to support laboratory performance.

The Working Group has specifically examined technical aspects that can and need to be addressed to improve the efficiency of sputum smear microscopy. Some of the group's findings have a sufficient evidence base to be implemented swiftly, while others need to be pursued further through appropriate field research. The Group urges that the pivotal role of sputum smear microscopy be given due recognition. Complacency about the potential of improvements in any step of the procedures is detrimental to global TB control.

Fortunately, current available funding usually allows the necessary improvements to be made in laboratory

infrastructure, equipment and supplies. However, the availability of funds has made it even clearer that commitment from the international community and individual governments towards optimal microscopy services remains essential.

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RÉSUMÉ

Ce rapport est une position acceptée sur base d'un consensus par les participants à un groupe de travail pour experts dans le domaine de la microscopie organisé en août 2005 par l'Union Internationale Contre la Tuberculose et les Maladies Respiratoires (L'Union), et à ce titre reflète leurs vues mais pas nécessairement celles de leurs organisations respectives.

Ce groupe a passé en revue les pratiques actuelles de l'examen microscopique des frottis de crachats pour la tuberculose (TB) et suggère de prendre en considération quelques modifications des directives standard concernant la stratégie de collecte des crachats et la définition d'une TB à bacilloscopie positive des frottis de crachats. Les directives pour la coloration de Ziehl-Neelsen doivent incorporer une plus large marge d'erreurs pour obtenir une application élargie dans les conditions du terrain. L'assurance de qualité est d'une importance majeure et nécessite plus d'implications des Programmes Nationaux de Tuberculose et d'autres autorités de santé. Il faut en particulier assurer une allocation suffisante de ressources pour le recontrôle et pour l'intégration de la supervision des laboratoires. Les pays doivent faire de meilleurs investissements dans l'acquisition de microscopes et de produits de laboratoire d'excellente qualité. Pour répondre à la crise en ressources humaines, le personnel sans formation spécifique en matière de laboratoire peut en principe être formé pour répondre aux besoins immédiats des services de microscopie pour le diagnostic de la TB. Des rapports périodiques sur les examens des frottis pour recherche de bacilles acido-résistants sont fortement souhaitables pour le suivi régulier et pour l'attribution plus équilibrée des fournitures.

RESUMEN

El presente informe representa la posición unánime acordada por los participantes en un taller de expertos en la materia de la baciloscopia, organizado en agosto de 2005 por la Unión Internacional Contra la Tuberculosis y las Enfermedades Respiratorias (La Unión), y en consecuencia refleja sus puntos de vista, mas no necesariamente la posición de sus respectivas organizaciones.

El grupo estudió las prácticas vigentes del examen microscópico del esputo para tuberculosis (TB) y propuso que se consideren algunas modificaciones en las recomendaciones habituales, con respecto a la estrategia de recogida del esputo y a la definición de TB con baciloscopia positiva. Es necesario incorporar un margen de error más amplio a las normas de la tinción de Ziehl-Neelsen destinada a una amplia aplicación sobre el terreno. La garantía de la calidad constituye un aspecto primordial y exige un mayor compromiso por parte del Programa Nacional de Tuberculosis y demás autoridades de salud. En particular, es preciso obtener una asignación de recursos suficientes a la reverificación y a la integración de la supervisión de laboratorios. Los países deben optimizar las inversiones de compra de microscopios y de suministros de laboratorio de alta calidad. Con el propósito de solucionar la crisis de recursos humanos, es posible en principio capacitar personal sin una formación específica de laboratorio, a fin de responder a la demanda inmediata de servicios de microscopia en el diagnóstico de la TB. Se recomienda vivamente la presentación de informes periódicos de las baciloscopias realizadas, con miras a una supervisión periódica y a una provisión más equilibrada de los suministros.