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COUNTERPOINT

Tuberculosis bacteriology—priorities and indications in high prevalence countries: position of the technical staff of the Tuberculosis Division of the International Union Against Tuberculosis and Lung Disease

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SUMMARY

Smear microscopy for acid-fast bacilli (AFB) remains the first priority for national tuberculosis programmes (NTPs) in high-prevalence countries. No other established technique offers the same advantages of accuracy, speed, appropriateness and accessibility. Its sensitivity may be reduced in HIV-positive cases or because of technical deficiencies, and it lacks specificity for viable bacilli in follow-up examinations. Its main problem is that it is tedious, necessitating an effective external quality assessment (EQA) system following international guidelines. Operational research is needed to optimise the staining technique, to define the place of sputum concentration and fluorescence microscopy, to challenge difficult and obsolete strategies and to streamline procedures.

Culture is much more difficult to set up and is usually impossible to decentralise. Because of its lower yield and higher costs, its efficiency for case detection in NTPs will lag well behind that in industrialised countries. The technique should only be used as a preliminary to drug susceptibility testing (DST).

DST should not be developed to the detriment of the AFB microscopy network and its EQA. It should be used mainly for monitoring drug resistance. Continuous monitoring of resistance in a representative sample of isolates from first-line failure and relapse cases may be more efficient and more accurate than periodic surveys among new cases, and can be used to identify MDR-TB, whose treatment should be standardised, because of considerable risk of error in the laboratory.

A specialist service offering molecular techniques may be useful for exceptional cases, but it has no place in the routine work of NTPs.

KEY WORDS: tuberculosis; microscopy; culture; quality assurance; drug resistance surveillance

BECAUSE IT HAS RECEIVED insufficient emphasis during training and supervision, and insufficient resource allocation within programmes, tuberculosis (TB) bacteriology has been a weak element of TB control in high-prevalence areas. It has recently received more attention.[1–4] For example:

- Guidelines for external quality assessment (EQA) of smear microscopy have been published.[5]
- The Global Project on Anti-Tuberculosis Drug Resistance Surveillance has published its third report,[6] including the results of proficiency testing.[7]
- A sub-group for laboratory strengthening has been added to the DOTS Expansion Working Group.
- The Special Programme for Research and Training in Tropical Diseases (TDR) is facilitating the search for appropriate diagnostics.
- Laboratories are engaged in clinical trials of TB treatment.

There is a trend towards devaluing smear microscopy for acid-fast bacilli (AFB), and special initiatives put a strain on the capacity of laboratory networks. For these reasons, the technical staff employed within the TB Division of the International Union Against Tuberculosis and Lung Disease (The Union) considers it important to report their position on bacteriological services for TB control in low-income countries. These views are those of the individuals working within the Division and reflects the experience of the authors in field work in low-income countries throughout the world, and the discussions held by the staff in their regular meetings. The opinions expressed were

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derived from these discussions and a consensus reached through an iterative process among the staff members. They are, consequently, neither the official position of the organisation nor are they views held by the entire membership.

The priorities for laboratories in high-prevalence countries, in order of importance, should be:

1 improvement of the AFB microscopy network and organisation of its EQA;
2 drug resistance surveillance (DRS), especially where the risk of drug resistance is high;
3 drug susceptibility testing (DST) to document multidrug-resistant tuberculosis (MDR-TB, defined as resistance to at least isoniazid [INH] and rifampicin [RMP]) for the purpose of standardised treatment;
4 support of operational research on subjects identified by the national tuberculosis programme (NTP).

These priorities have been ordered mainly by technique and cost benefit ratio, but evidently the choices made will need to be linked with the whole system of TB laboratory services in the country, ranging from infrastructure to supervision.

SMEAR MICROSCOPY FOR AFB

For diagnosis

Smear microscopy remains the cornerstone for the diagnosis of pulmonary TB in adults because it identifies the most powerful sources of transmission of TB, can be performed quickly and has high specificity in high-prevalence countries.[8–10] Unlike cultures,[11] its specificity is rarely affected by technical deficiencies such as cross-contamination between specimens.[12,13] While it has a relatively low sensitivity in identifying all cases of pulmonary TB,[14] correctly applied, it detected the transmitters of 83% of infections in San Francisco and 91% in British Columbia and Saskatchewan.[15,16] In low-income but high TB prevalence countries this proportion might be much higher, because of the far higher proportion of smear-positives of all culture-positives presenting spontaneously in such settings.[14] Such smear-positive patients are more likely to die and are less likely to be cured without specific treatment.[17–20]

Microscopy is inexpensive and simple to perform. Field workers can carry it out in the peripheral health unit, using a multi-purpose instrument that is widely available, close to the dwelling of the patient but sufficiently centralised to ensure monitoring of performance. It is a strategic error to replace microscopy by a technique with similar characteristics but higher cost, requiring the patient to pay for it. Reduced accessibility would offset any expected gain in case detection even if the new tool were more sensitive. Deficient case detection is more often due to poor access to services and to poor proficiency than to lower sensitivity of the test. Inexpensive tests (other than smear microscopy) with good performance, applicable under field conditions, are not currently available.[21,22]

There are limitations to microscopy. It can fail because of technical deficiencies and with paucibacillary specimens, a problem in human immunodeficiency virus (HIV) prevalent areas.[23] It is tedious, requiring motivated staff, with increasing risk of false-negative error when large numbers of specimens are examined.

For treatment monitoring

Smear microscopy cannot distinguish live from dead bacilli, reducing its utility for treatment monitoring. It should be continued because it is the only technique widely available in the field, is more accurate than chest radiography and more rapid than culture.[24,25] Where the treatment regimen does not contain RMP throughout, the intensive phase must be prolonged for an additional month if AFB are seen on smear examination at the end of the initial intensive phase of treatment, even in small numbers and even in a single sputum specimen. There is a higher frequency of failure or relapse for late smear or culture converters,[26,27] and the frequency of failures is significantly reduced by prolongation (unpublished data presented at TSRU meeting, Bagamoyo 2003). Prolongation of the intensive phase may not be necessary in regimens employing RMP throughout.[28,29]

The definition of treatment failure based on a positive smear at 5 months may be unreliable, especially in patients with high initial bacillary loads and when careful microscopy detects low numbers of AFB. Two positive smears are required to declare a case a failure.[30–32] Should the second smear be negative, treatment can be continued as planned, and cure declared on condition that smears at the end of treatment remain negative.

Development and research priorities

Smear microscopy is the first priority for laboratory networks within the NTP, making EQA, following international guidelines, vital. The national reference laboratory (NRL), while not carrying out this work alone, must set up the screening system, organise the training, maintain microscopes, and improve stain preparation and distribution.

Operational research is vital to improve smear microscopy. Key topics for research include:

- The value of sputum concentration techniques. Many report greatly increased yield, but a few note little or no gain, with some false-positives.[33–37] This discrepancy might be due to variation in performance, or differences in bacillary content of the specimens.[38] Studies are needed, comparing the results of optimally performed direct AFB microscopy with those on concentrated sputum, especially where HIV is prevalent.

- The role of fluorescence microscopy. Although it is superior to ordinary bright-field technique, its cost and technical complexity may affect its feasibility and reduce the gain expected.
- Recommendations for fuchsin concentration. For a long time, a number of NTPs have continued to use the original Ziehl-Neelsen concentration of 1% rather than the recommended 0.3%. A higher yield of positives, but also more false positives, has been reported using basic fuchsin 1% instead of 0.3%.[39] Although the different sensitivity was probably due to the reduced phenol concentration used in the study,[40] the ideal concentration under field conditions should be determined.
- The method of staining. Hot staining is recommended,[41] although cold staining may perform comparably under optimal conditions.[42,43] The latter does have more reported deficiencies,[44–46] and is more sensitive to error under field conditions.
- Methods to streamline and optimise procedures, aiming at a balance between yield and workload, as well as improved user-friendliness. The third diagnostic specimen has a lower incremental yield,[47,48] and the first spot specimen is too often negative because of its poor quality. Two good morning specimens may be superior[49] and should always be the practice for hospitalised patients. The requirement of a second positive specimen to confirm a diagnosis reduces false diagnoses by a negligible fraction compared to those accepted on the basis of chest radiography. A good quality assurance system may obviate the requirement altogether. More research is required to weigh this small gain in accuracy against the number of delayed or missed treatment opportunities the practice might cause.
- The optimal cut-off used to declare a smear examination positive. The recommended threshold of 10 AFB per 100 fields is high for diagnosis, and may be unnecessary in NTPs with efficient quality assurance.[50] A higher threshold may be more appropriate for follow-up because of the observation of dead bacilli.[51]

A series of studies into these issues is crucial to improving recommendations.

CULTURE FOR MYCOBACTERIA

Diagnostic services

Routine culture for diagnosis of TB in high-prevalence countries is not currently recommended. Although culture clearly has a higher sensitivity, its superiority may be reduced in high-prevalence settings because higher bacillary loads are readily detectable by microscopy in the majority of patients. Technical problems may reduce the efficiency of culture.[14,52] In HIV-positive patients, the sensitivity of culture for diagnosis is lower.[38] Moreover, culture on classical media does not yield results in a timely fashion, and physicians often disregard the results when they arrive long after the patient has been started on treatment. In high-prevalence countries, cultures may not add much to the yield or accuracy of case detection as compared with smear microscopy plus chest radiography.[53] Emphasising culture may result in simultaneous neglect of the microscopy network.

Modern commercial systems have faster reporting of results, but they are inappropriate because of high recurrent costs of the media. In smear-negative patients they still require 2 to 3 weeks, too long for many physicians to delay a diagnosis. Moreover, they tend to have a higher yield of environmental mycobacteria,[54,55] adding to the costs of rapid identification tests. In practice, these systems tend to be used only in the main cities and for patients who can afford to pay for the tests.

Due to lack of infrastructure and staff, it is difficult to maintain a decentralised network of culture facilities, and running costs should not be underestimated.

Drug susceptibility testing

Culture is necessary to carry out DST. If needed, the viability of TB bacilli can be maintained for several weeks during transport from peripheral centres at ambient temperature, using an appropriate medium such as cetylpyridinium chloride (or bromide).[56] Alternatively, a simplified decontamination procedure followed by inoculation on centrally prepared acidified egg media can yield excellent results when used in peripheral laboratories.[57,58] At present, this seems to be the main indication for routine use of culture in high-prevalence areas.

DST is used for epidemiological monitoring and evaluation of NTP performance, as well as for identifying patients carrying resistant strains. However, it is not indispensable for TB control, and NTP managers should be aware of its limitations and resource requirements. The use of DST should not be detrimental to the provision of high quality AFB microscopy, which is the higher priority. NTPs may not be able to implement both simultaneously.

It is not easy to set up reliable DST nor to maintain proficiency. Discordant results are observed even between the most renowned laboratories and irrespective of the method used (unpublished reports on the supranational reference laboratory network [SRLN] quality assurance Rounds 6 to 10). This is especially true for ethambutol (EMB) and streptomycin,[7] and even more so for cycloserine and ethionamide.[59,60] Such errors may not fully explain a poor correlation between DST result and clinical outcome, as is the case with EMB[61] or INH[62] resistance. Low-level INH resistance is frequent, even in MDR strains,[63] and controversy on its inclusion in regimens for such cases remains.[64] The same is true for low-level RMP resistance, which may not be as rare as is generally assumed.

The observed lack of response to standard retreatment[65] in cases with apparently fully susceptible or INH-resistant strains might be considered an indication of the need for further investigations into the subject of undetected but clinically significant borderline RMP resistance.

The use of DST for individualising treatment regimens has inherent limitations and may be dangerous. Any cases of MDR-TB detected by DST should be offered an appropriate standardised treatment regimen based on second-line drugs. Surveillance data from chronic patients on those drugs for which DST is more reliable, such as kanamycin and fluoroquinolones, should be used, when available, to determine this regimen. In their absence, the probability of resistance to the main second-line drugs must be estimated by the extent of their previous (mis)use.

Drug resistance surveillance

Drug resistance surveillance (DRS) for monitoring epidemiology and programme performance should focus on evaluating trends. This is hampered by inaccuracies in the laboratory, in the classification of patients, and by variations in sampling of the study population.[66] This is a fortiori the case for strains isolated from new cases, as in this group the levels of resistance, especially to RMP, are usually quite low. Large numbers of strains must be tested to demonstrate statistically significant differences, and serious bias will result from even minor levels of technical imprecision or patient misclassification. Under favourable conditions, primary resistance may take decades to decline.[67] Under unfavourable conditions, rapid rises of resistance levels may occur, but this will first be evident among strains isolated from retreatment cases.[68]

The current recommendations for surveys at 5-year intervals among new cases may not be the most efficient approach. Trends are more easily detected using strains isolated before starting retreatment of first-line failure and relapse cases,[69–71] as these represent a combination of primary resistance and resistance acquired during treatment.[72,73] The two are not distinguishable when only the retreatment isolate is available, but for the purpose of DRS and programme performance monitoring this may not be important. In the past, routine DST of strains from retreatment cases, especially failures and relapses, was recommended. When done systematically, this could constitute the main thrust for drug resistance monitoring, providing at the same time the identification of MDR cases in need of second-line treatment within a DOTS-Plus programme, as stipulated by the Green Light Committee of the Stop TB Partnership.

In practice, it is seldom possible to obtain a sample from all incident relapse and failure cases because of inaccessibility or limited laboratory capacity. Use of sentinel centres might offer a pragmatic solution. Proficiency would be easier to guarantee, and the probability of resistance is higher in the larger centres. The main requirement is that the sample is representative of all retreatment cases detected in the centre, best assured by systematic sampling.

Continuous monitoring of drug resistance levels of retreatment strains can be complemented by periodic drug resistance surveys comprising all types of cases from the same population. This demonstrates the level of combined resistance,[74] which is not influenced by errors in patient classification, and reflects the total reservoir of drug-resistant strains.[20] When sentinel centres are used for monitoring drug resistance among relapse and failure cases, the same centres should provide the survey with a sufficiently large sample of all registered patients. This will avert the risk of including different populations in the various surveys as is the case with cluster sampling. It will also indicate the magnitude of true acquired resistance among relapses and failures. Other programme performance monitoring indicators, such as treatment outcome reports and registration of relapses, can give some indication on trends of drug resistance in other than sentinel centres.[20]

A sentinel strategy cannot provide epidemiological information reflective of the whole community. To obtain this, the total patient population must be eligible for sampling. This is a different objective, requiring a different sampling technique. It is unlikely that these surveys can be used to monitor trends of drug resistance or to assess programme performance within a reasonably short time frame.

DST and DRS are difficult and costly and are not indispensable to TB control. They should be given priority only in specific circumstances. Well-performing NTPs with high levels of drug resistance resulting from poor past performance may use DST and DRS to focus on these problems and at the same time identify MDR-TB cases for second-line treatment. It may be particularly important to monitor drug resistance levels where NTPs use potentially dangerous first-line regimens, such as intermittent regimens with RMP throughout, where strict directly observed treatment is vital.[75]

In most instances, only the national reference laboratory should perform DST, although some large countries may require more than one laboratory. The quality of the tests at every level must be augmented by participation in proficiency testing using panels of strains and by retesting selected strains at a higher-level laboratory. If the quality of the results cannot be guaranteed, these should never be used for individual patient management. NTPs that cannot rely on a national laboratory for their DST needs, or where the development of this capacity is not a current priority, can make use of external services; the best option is to link up with one of the supranational laboratories.

MOLECULAR TECHNIQUES

Molecular techniques can provide diagnosis of TB at a level of sensitivity and specificity comparable to that of culture, but much faster. The tests are less influenced by sample transport delay as they do not require viable bacilli. However, they are not routinely used in most high-prevalence communities.[76] Lack of infrastructure and of qualified personnel and high recurrent costs prevent the development of a sufficiently decentralised service based on these techniques.

National research institutes or supranational reference laboratories could offer these services to the NTP for specific purposes, such as the rapid determination of RMP resistance and species identification for MDR-TB treatment. For determination of RMP resistance, one large supranational laboratory could offer molecular detection services to a number of countries.[77]

In high-prevalence situations, routine use of molecular fingerprinting techniques by the NTP for epidemiological evaluation or for contact investigations is not usually appropriate. Such techniques have a place in research, such as investigations into nosocomial transmission of TB, especially MDR-TB. Differentiation of acquired resistance from reinfection through fingerprinting is another important function in scientific investigations.

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RÉSUMÉ

Dans les pays à haute prévalence, l'examen des frottis à la recherche de bacilles acido-résistants (BAAR) reste la première priorité pour les programmes nationaux antituberculeux (PNT). Aucune autre technique bien établie n'offre les même avantages de précision, rapidité, applicabilité et accessibilité. Sa sensibilité peut être réduite dans les cas séropositifs pour le VIH ou en raison de déficiences techniques ; lors des examens de suivi, elle manque de spécificité concernant les bacilles viables. Son problème principal réside dans son caractère fastidieux qui impose un système effectif externe d'évaluation de la qualité (EQA) répondant aux directives internationales. Des recherches opérationnelles s'imposent pour optimiser la technique de coloration, pour définir la place de l'homogénéisation des expectorations et de l'examen microscopique par fluorescence, pour répondre au défi des stratégies difficiles et dépassées et pour rationaliser les procédures.

La culture est beaucoup plus difficile à mettre en route et elle ne peut généralement pas être décentralisée. En raison de son rendement plus faible et de ses coûts plus élevés, son efficience dans la détection des cas se situera bien en dessous de celle observée dans les pays industrialisés. La technique ne devrait être utilisée que préalablement aux tests de sensibilité aux médicaments (DST).

Les tests de sensibilité ne devraient pas être développés aux dépens du réseau de microscopie pour BAAR et de son EQA. Ils devraient être utilisés principalement pour la surveillance de la résistance aux médicaments. Une surveillance continue de la résistance dans un échantillon représentatif d'isolats provenant des échecs de première ligne et des cas de rechute peut être plus efficiente et plus précise que les enquêtes périodiques parmi les nouveaux cas. Les DST peuvent être utilisés pour identifier la TB-MR dont le traitement devrait être standardisé en raison du risque considérable d'erreurs de laboratoire.

Un service spécialisé utilisant les techniques moléculaires peut être utile pour des cas exceptionnels, mais n'a pas sa place dans le travail de routine des PNT.

RESUMEN

La baciloscopia directa sigue siendo la primera prioridad de los Programas Nacionales de Tuberculosis (PNT) en países con alta prevalencia. Ninguna otra técnica establecida ofrece las mismas ventajas de precisión, rapidez, adecuación y accesibilidad. La sensibilidad de la baciloscopia puede encontrarse reducida en pacientes seropositivos para el VIH o a causa de deficiencias técnicas y carece de especificidad para los bacilos viables en los exámenes de seguimiento. La baciloscopia es un examen tedioso y requiere un sistema externo de evaluación de calidad (EQA) que cumpla con las recomendaciones internacionales. Se precisa una investigación operativa para optimizar la técnica de coloración, definir la importancia de la concentración del esputo y de la microscopia de fluorescencia, para cuestionar las estrategias difíciles y obsoletas y para racionalizar los métodos.

El cultivo es mucho más difícil de establecer y su descentralización es por lo general imposible. Dado su menor rendimiento y mayores costes, la eficacia real del cultivo en la detección de casos será siempre muy inferior al observado en los países industrializados. Esta técnica debería utilizarse sólo previamente a las pruebas de sensibilidad a los medicamentos (DST).

Las DST no deben establecerse en detrimento de la red de baciloscopia y de su EQA. Deben utilizarse principalmente para vigilar la farmacorresistencia. La vigilancia continua de la resistencia, en una muestra representativa de aislados clínicos provenientes de casos de fracaso y recaída del tratamiento de primera línea, puede ser más eficaz y más precisa que los estudios periódicos de casos nuevos, dado el riesgo considerable de error en el laboratorio y puede utilizarse para identificar la MDR-TB, cuyo tratamiento debe estandarizarse.

Un servicio especializado que ofrezca técnicas de biología molecular puede ser útil para casos excepcionales, pero no está indicado como parte de las actividades ordinarias de un PNT.