CHAPTER

72 Laboratory systems and strategies for tuberculosis

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INTRODUCTION

Laboratories play a critical role in diagnosing, monitoring and treating TB – all significant in an effective TB control programme. A positive TB diagnosis is considered certain only if the diagnosis is laboratory confirmed, and patient classifications and treatment management have depended largely, and often solely, on bacteriology.[1,2] However, in many high-prevalence and resource-limited countries, reliance on smear microscopy as the most effective and practical tool for diagnosis and control of TB has helped contribute to a growing disparity in the quality and availability of laboratory testing compared with countries with sufficient resources. Industrialized countries, especially in Europe and North America, have embraced and supported new technologies that provide rapid detection, identification, and drug-susceptibility testing of *Mycobacterium tuberculosis*.[3] These enhanced diagnostic capabilities have helped control TB, when combined with good treatment programmes and effective public health disease control strategies.[4,5]

In contrast, many countries with high TB prevalence, but few resources for diagnosing, treating and controlling the disease, still struggle to provide high-quality microscopy.[6] Moreover, culture and drug-susceptibility testing (DST) are rarely available outside the TB national reference laboratory and are mainly used only for epidemiological monitoring of drug resistance. Poorly managed TB programmes and laboratory systems have hindered progress towards more consistent testing and treatment world-wide, and progress is now further hampered by the additional burden of the human immunodeficiency virus (HIV) epidemic and multidrug-resistant (MDR) TB. Although strengthening laboratory services and technology has been given higher priority on the TB agenda, for example introducing programmes such as the new Stop TB Strategy,[7,8] this reprioritization has not yet resulted in increased allocation of resources for TB testing in many countries. More global guidance, research and programmes focused on capacity building are needed to provide access to and use of existing diagnostics, and to develop and implement new technologies.

A different situation exists in industrialized countries. Industrialized countries have implemented technologies such as fluorescence microscopy, liquid cultures for isolation and DST, and have developed molecular amplification methods for detecting TB and any possible drug resistance. These diagnostic methods, although effective, are expensive, labour-intensive and relatively slow in producing test results, in comparison with technological developments in other areas of microbiology such as direct detection of organisms with polymerase chain reaction. These developments have sparked renewed interest in developing new diagnostics and examining successful models for implementing modern diagnostic methods in low-income countries. The Foundation for Innovative and New Diagnostics (FIND) is supporting a world-wide initiative to apply a systematic approach to research and development of new diagnostics.[9] One danger in only addressing diagnostic methods and funding for supplies is that system requirements could be neglected. These include needs for well-trained staff, quality management systems and other organizational structures that support higher standards of practice found in industrialized countries. In settings with sufficient resources, the clinician expects and advocates for certain diagnostic tests. The pressure to provide newer technologies is accompanied by expectations for quality that have not only medical care but legal implications (i.e. false-positive test results have led to law suits). By contrast, in many low-income countries, the clinician is aware of deficiencies in the laboratory system and may treat empirically (i.e. physician treats for TB based on recognized symptoms as opposed to actual test results) due to a lack of trust in laboratory results.[10] Unfortunately, this reduces the incentive to upgrade technologies and services, and to implement quality standards that strengthen the laboratory system.

Problems associated with MDR-TB and recent outbreaks of extensively drug-resistant (XDR) TB have highlighted the need to increase capacity for culture and DST.[11] Culture and DST, however, are much more complex than microscopy; thus, countries and international organizations are being forced to examine the critical elements needed to significantly scale up laboratory capacity. There is also a corresponding interest in determining how national TB programmes (NTPs) can work with other programmes and agencies to build effective laboratory networks that integrate services and leverage scarce resources in the midst of a growing private sector. Scaling up services for culture and DST, and optimizing microscopy might succeed if the technology and system requirements of TB laboratory services are integrated into a general initiative to upgrade the country's laboratory network.[12] Many countries and international organizations recognize that improving the quality of TB laboratory networks is required to continue progress in TB control. This chapter will focus on some of the critical technical and organizational challenges with microscopy, culture, DST and corresponding safety issues. Successful laboratory services will also be examined with a focus on both historical and new perspectives in laboratory strategy, human resources considerations, research, quality management systems and the structure of the laboratory network. All are necessary components for strengthening laboratory capacity.

LABORATORY METHODS: STRENGTHS AND WEAKNESSES

MICROSCOPY

In most countries, especially those with the highest burden of disease, the Ziehl–Neelsen (ZN) smear remains the first test given and the only one accessible to a large part of the population for years to come. Reaching the best possible sensitivity is essential and requires diligence and appropriate technique. Numerous examples exist where the testing sensitivity is low because of neglecting to adhere to details in the guidelines and lack of regular laboratory onsite evaluation, even in the absence of HIV coinfection. Concerns that the ZN smear has lower sensitivity when used on HIV-infected patients have stimulated interest in practical methods for improving microscopy.[13,14] Industrialized countries use centrifugation-concentrated smears and fluorescent microscopy, a combination that could provide higher sensitivity also in low-income, high-HIV-prevalent countries, but requires more resources.[15] A strategy using simple bleach digestion and concentration, not requiring high-force centrifugation, has been intensively studied for more than 10 years. However, doubts about the correct use of and indications for this approach remain, requiring further operational research.[16–18] Also, the use of fluorescence microscopy is still not widespread, but for other reasons. There is no doubt about fluorescence microscopy's greater efficiency and increased sensitivity.[19] That said, for a wide variety of reasons, of which poor acceptance may be most important, this expensive equipment is rarely used in low-income countries, even in clearly overloaded laboratories where its advantages are obvious. The new light-emitting diode (LED) lamp fluorescence systems are simpler and less costly, and are a potential catalyst to the final breakthrough of this technology in countries with a high prevalence of HIV.

Considering the tedious nature of acid-fast bacilli (AFB) microscopy, internal and external quality assessment (EQA) programmes are necessary to monitor smear preparation, staining and interpretation, and to ensure that all microscopy centres achieve an acceptable level of performance. The implementation of EQA for microscopy also strengthens laboratory networks and contributes to quality improvement.[20] Systematic review by a laboratory expert is a critical component to strengthening network management, but can be limited by funding and staff requirements (i.e. transportation and supervision). In integrated health systems, one solution is to broaden the scope of onsite supervision to include reviewing and monitoring other testing services (e.g. HIV, malaria, chemistry and haematology). An integrated onsite evaluation of laboratory services could inevitably lead to a reduced focus on TB, but is more efficient and cost-effective, considering limited human resources. Integrated review is also the standard for laboratory accreditation in industrialized countries.

CULTURE AND DST

The use of culture and DST is standard diagnostic practice in industrialized countries, but at the other extreme most low-resource countries still cannot provide culture for priorities that include drug resistance surveillance (DRS), extrapulmonary and childhood TB and suspicion of MDR-TB. There is continued debate on whether providing culture for routine detection of TB cases in the high-prevalence setting is cost-effective and feasible.[21] Enhanced culture capacity would benefit both patients and the TB control effort with higher sensitivity than the now widely used harsh decontamination methods and inoculation on egg media. These controversies aside, many countries with high TB prevalence have not yet developed capacity for accurate and reliable culture for DRS and diagnosis of MDR-TB. The scarcity of data on drug resistance in most of the high-prevalence countries demonstrates that countries have not sufficiently addressed the priorities of surveillance and diagnosing drug resistance, and so are unable to even consider using limited culture services for routine diagnosis of TB.[22] NTPs must first adopt and enforce policies for appropriate use of limited culture capacity so that priority requests are met and TB laboratory services are extended throughout the country, as opposed to in selected urban areas.[23]

In the past it has proven extremely difficult and time-consuming to introduce efficient culture in laboratories at the national level and the challenges go much farther than scarcity of equipment and adequate budgets for supplies. Infrastructure and staffing constitute enormous barriers, as do problems with continuity and other factors that have become common among poor and sometimes politically unstable countries, making a quick transfer of technology impossible. Resources also remain extremely scarce to provide technical assistance internationally. Only within the past few years has a handful of experts been employed full-time by the major TB organizations, leaving much work to be done by the remaining number of volunteer temporary consultants. One large body of work is a huge backlog of policy documents to be developed, such as guidelines, standard operating procedures, equipment specifications and training modules. The update of guidelines and procedures started very recently, and has so far only been completed for part of the microscopy-related materials. It is alarming that, although laboratory services have been identified as the main stumbling block for major areas of the expanded directly observed treatment, short-course (DOTS) strategy, adequate funding for guidance at the international level is still not available.

NUCLEIC ACID AMPLIFICATION TESTS (NAATs)

Using NAATs for drug resistance holds immediate promise for scaling up laboratory capabilities. The use of direct specimen NAAT testing for rifampin resistance provides a rapid diagnosis of TB that needs to be treated with second-line drugs, and avoids the difficulty of accomplishing rapid and accurate DST with culture methods. Testing services can also be centralized with direct specimen testing with NAATs without the transportation *cold chain* systems required for culture.[24] Although NAAT testing has inspired great interest and promise for detecting drug resistance and cost and determining which high-risk patients will benefit most from early detection, there are still issues, especially in areas of low MDR-TB prevalence.[25] Quality issues must be addressed for any laboratory test, and false-positive cultures as well as NAATs are well documented in the literature.[26–28] There should be additional support, in terms of training, EQA programmes, and guidelines for standard practices to ensure accurate results that will encourage programmes to allocate resources to expand services for DST and NAATs.

PROGRAMME STRATEGIES AND REQUIREMENTS

BACKGROUND

In low-income countries, emphasis is appropriately put on smear-positive patients to prevent and control TB transmission.[29] This emphasis, however, often leads to excessively repressive attitudes regarding diagnosis of other forms of the disease. Since their initial development by Dr Karel Styblo, modern TB programmes have aimed to control the disease by means of detection and cure in microscopy-positive cases, without creating drug resistance.[30] It has taken many years to expand the DOTS strategy to most countries and to increase the number of TB patients cured. Making the use of microscopy and identification of smear-positive cases a priority has its roots also in the limited funding and high drug prices of recent times, and in the difficult conditions in the sub-Saharan African countries from where most of the early programmes derived.[31] For these reasons more demanding bacteriological tests, such as culture, were slow to be adopted. Moreover, drug resistance was not initially tested during individual patient management for several reasons:

- The successively employed standard regimens for new and retreatment cases would cure all patients except those with MDR-TB (TB bacilli resistant to isoniazid and rifampicin).
- Using thioacetazone, and later ethambutol, rather than rifampicin as a companion drug to isoniazid in the continuation phase of the standard regimen for new cases made it possible to avoid creating new resistance to the main drugs to a very large extent.
- Steering therapy based on drug resistance test results was tried initially, but proved to be unrealistic and subsequently unnecessary because the outcome of individualized treatment was not improved and effective treatment of MDR-TB was considered impossible.[32]

Emphasizing prevention, but not diagnosis or treatment of serious drug resistance, proved correct as shown by continuously low or decreasing MDR-TB levels in programmes following this strategy.[33] If not for the HIV epidemic, control of transmission would have perhaps succeeded. However, in most countries this strategy also led to neglect of laboratory services and non-support of microscopy network activities, such as training and supervision.

Many factors have changed since the initial development of TB control strategies and these changes exacerbated the acute inadequacy in laboratory services. Fortuitously, TB has since been declared a global emergency, which led to an exponential increase in funding. This funding was used to scale up activities and led clinicians to focus on target populations for case detection but not exclusively detection of smear-positive cases. In addition, the HIV epidemic has dramatically increased the demand for laboratory services, particularly in Africa. The relatively frequent paucibacillary disease found in HIV-infected persons has generated a need for highly sensitive microscopy and culture.

From a treatment perspective, establishing parity in drug pricing and promising quick results has led to an almost complete replacement of other drugs by rifampicin-throughout regimens that have a higher risk for acquired drug resistance.[34,35] Frighteningly high levels of MDR-TB exist in several hot spots.[22] A fast rise to at least moderately high prevalence is expected soon in many other areas where MDR-TB's effective treatment is even more difficult.[36,37] With the advent of the fluoroquinolones, effective MDR-TB treatment has indeed become possible, but it is accompanied by the danger of misuse of second-line drugs. Recent reports on extensive drug resistance demonstrate the impact of misusing second-line drugs (i.e. XDR-TB, MDR-TB also resistant to the injectable anti-TB drugs and fluoroquinolones).[38]

NEW STRATEGIES

Tuberculosis control programmes in poor countries continue to emphasize bacteriological detection and diagnosis, at least for pulmonary TB. Algorithms generally prescribe a series of three sputum specimens for microscopy, which can still allow rapid diagnosis for the majority of spontaneously presenting cases in populations with little or no HIV coinfection. However, the expanded DOTS strategy foresees rapid expansion of culture services, particularly for improved diagnosis of TB in countries with a high prevalence of HIV, TB in children and extrapulmonary TB.[23,39] Feasibility and effectiveness of this expanded strategy still need to be shown.[21] Another controversial point is the collection strategy. The yield of the third sputum is too low to justify this strategy wherever it has been examined,[40] and reliance on high-quality morning sputa, as opposed to spot sputa, might be more rewarding than modifications of technique to increase sensitivity of microscopy and culture (e.g. in the HIV/TB coinfected patient). New World Health Organization (WHO) recommendations for the use of two sputum specimens, when quality measures are in place and there is a high laboratory workload, allow programmes the flexibility to address country and regional needs.[41]

In industrialized countries, DST is often performed on all isolates from TB patients for epidemiological monitoring, individual case management and legal back-up. This approach is neither feasible nor affordable elsewhere. Also, because of the scarcity of rapid and accurate DST, this approach might not be useful or desirable for individual patients, except in a few countries with a serious MDR-TB problem. Although there is a call for expansion of DST to all cases, in the average TB control programme DST is still exclusively used for epidemiological investigation and increasingly also for MDR-TB diagnosis. Diagnosis for MDR-TB involves screening of high-risk groups such as treatment failures, relapses while on the retreatment regimen and cases of known MDR-TB.[42] Systematic screening of other groups, such as first-line treatment failures and relapses, should be decided upon in the local context and will depend mainly on the prevalence of MDR-TB within these groups. MDR-TB patients are rare, particularly in the poorest countries, where testing is most difficult to perform correctly and with good coverage. Such difficulties lead unavoidably to poor predictive values of resistant results in the absence of accurate patient selection. The same is true for second-line drugs in countries where these drugs have hardly been misused. Resistance is then very rare, and thus testing for individual patient management may be unrewarding. In fact, until methods and performance become more accurate, it might even be undesirable because of the unavoidably low predictive value of a resistant result.[43] Deciding not to test for individual patient management could be a considerable operational advantage, provided that standardized second-line treatment regimens for MDR-TB are identified. Unfortunately, research in this field has been badly neglected for far too long, possibly because of unrealistically high expectations on further development of laboratory services.

Although recommended since the early days of TB testing and treatment programmes, systematic follow-up of drug resistance among failure and relapse cases has rarely been implemented. This approach could nevertheless be the most efficient strategy for early detection and monitoring of MDR-TB and tracking the TB control programme's impact on drug resistance trends.[44] Instead, more difficult random surveys among new cases have been emphasized by the Global Project for Drug-Resistance Surveillance.[45] As a result, available epidemiological data mainly concern point prevalence among new cases, and reliable data on trends from low-income countries hardly exist. Although such information certainly has value in estimating the extent of the drug resistance problem, it responds insufficiently to current priorities (i.e. avoidance of drug resistance under programme conditions and MDR-TB treatment). The choice of drugs tested should also be considered. Typically, isoniazid, rifampicin, ethambutol and streptomycin are all tested, but with a 6-month rifampicin primary treatment regimen, virtually only resistance to this drug is decisive for treatment outcome. Besides monitoring of rifampicin resistance, the expansion of MDR-TB treatment calls for the testing of its key drugs, fluoroquinolones and injectables as well.

QUALITY MANAGEMENT SYSTEM AND NETWORK REQUIREMENTS

HUMAN RESOURCES

Highly skilled laboratory scientists are needed to manage microscopy networks and referral laboratories for culture and DST. However, there is a paucity of them and those who are skilled are often employed by private organizations and research institutions which tend to pay far better than publicly funded positions. This is true particularly in African countries where the human resources crisis has been fuelled by the HIV epidemic and the more highly skilled laboratory staff are in search of greater opportunities to relocate to more industrialized countries. At the same time that there is competition for skilled laboratory scientists, governments and worker unions are increasingly restricting laboratory work to certified technicians, which only makes the shortage worse. Moreover, in many cultures there is still serious fear and stigma associated with contracting TB, and working with sputum is almost universally disliked. This is also a service that, unlike many others, has remained free of charge to the patient so revenues to support staff are low. Recruitment of fully qualified TB laboratory workers is, thus, a challenge. If culture and DST for TB are further expanded, it seems likely that little technician time will be devoted to AFB microscopy. At the peripheral level, the shortage of trained laboratory technicians has already led countries to train and employ a new cadre of individuals who have little formal laboratory education. After brief on-the-job training, these people can perform on the level of formally trained laboratory technicians when administering simple, but vitally important tests such as AFB microscopy and HIV rapid tests, provided these individuals work under proper supervision and are supported by training programmes and routine EQA.[46,47] Such cadres could be ideal for easy-to-learn but challenging tasks, such as AFB microscopy; however, the highly educated persons might be more attracted to tasks they consider closer to their intellectual level.

Lack of human resources is a serious problem that often also exists at an intermediate level (i.e. regional or provincial laboratories). This is the essential level for implementing labour-intensive parts of laboratory network management, such as training, EQA and supervision. However, most often such tasks are usually left to often overburdened technicians of the regional hospital laboratory. Experience from laboratories in many countries trying to accomplish the difficult job of rechecking smears, without appointing the additional staff needed, has shown that it cannot work without some form of compensation (e.g. pay, reduction of routine work by introduction of fluorescence microscopy, a vehicle for supervision). Since allowances for supervision are often the only incentive available, sometimes the time spent on supervision exceeds the time spent doing the routine important testing tasks. Training good laboratory supervisors, who not only know the guidelines verbatim but are capable of providing supportive supervision, problem identification and problem solving, is badly needed even for simple tests like AFB microscopy. To achieve better integration and motivation of laboratory staff who often work in isolation and under very difficult conditions, managers of TB control programmes should make an effort to educate their general supervisors on the basics of AFB microscopy. They should also have them include a visit to the clinic microscopy laboratory as a standard element of their supervision.

Training and education of laboratory technicians (or technologists) varies by country, with some programmes offering a 2- to 3-year diploma, as opposed to a formal undergraduate degree. Scaling up culture and DST will require that new competencies and policies be added to the curriculum of laboratory technology to ensure that graduates have the skills to do increasingly specialized work. One of the greatest gaps that occurs in both high- and low-resource countries, but is more pronounced in low-resource countries, is the lack of training and education programmes for laboratory managers and leaders. Whereas in many high-resource countries doctorate degrees are required to direct a laboratory, in many African countries it is rare to find TB laboratory staff at the national level with graduate degrees. Doctorate degrees granted within the country are usually focused on research (e.g. PhD, EdD), with little or no training in laboratory management and no orientation on career opportunities in diagnostic laboratory services. This is in contrast to medical degrees that provide training and curriculum designed entirely around patient care and healthcare delivery. Laboratory management and network management are just beginning to be addressed through new mentoring and training programmes to provide the leaders who will be responsible for scaling up new technologies and programmes.

LABORATORY NETWORK

Healthcare systems in many countries are either evolving or are under pressure to evolve to include private providers, integrated services and new organizational models. Expanding and strengthening TB laboratory networks will require taking into account the evolution of health systems and the reluctance to invest in new systems that do not represent integrated service delivery. Most high-resource countries have a large private healthcare system, including laboratories that provide high-quality care and services. Many issues concerning quality of care can be attributed to laboratory quality standards and regulations, mandatory reporting of TB and other infectious diseases, and private/public initiatives, in addition to the availability of resources.[48,49] In contrast, many countries with high TB prevalence still struggle to monitor quality in government-provided healthcare and the expanding availability of private laboratories. If funding is available, testing the poor in the

private sector becomes possible, although often at greater expense. However, supporting and maintaining AFB microscopy is even less popular with private providers. So unless the programme is successful at truly changing the habits of private physicians, access to private sector testing will remain temporary. Tuberculosis cultures, and particularly rapid automated culture systems, are developed in the private sector more often than in the public sector. Provided long-term prospects make the investment worthwhile, the NTPs and national reference laboratories (NRLs) must develop strategies to enrol private laboratories in EQA programmes and require reporting and referral of TB cases. The NTPs and NRLs are unlikely to establish and sustain programmes that only monitor TB testing in private laboratories. The NTPs will probably have to work in partnership with other disease control and health programmes to lobby for national laboratory standards and organizational resources to implement regulations.

Interest is growing in defining and striving for the optimum alignment of laboratories to effectively provide service. This requires examining the organization of TB laboratory services in relation to the NTPs and the general health services. Some countries may have the NRLs report directly to the NTPs, even though the NRLs could be located at a separate hospital or facility. In the past, the NRLs have typically reported directly to the NTPs to ensure that laboratory activities and services were focused on the needs of the programme. One of the disadvantages of having TB NRLs structurally and organizationally separate from other laboratories is that frequently the NRLs are relatively small in terms of staff and service support. Locating the TB NRLs in a larger integrated NRL facility or organization provides the advantages of shared support services such as staff, equipment, supplies and larger facilities. This arrangement also provides more interaction between laboratory peers who share various ranges of technical expertise. Integrated NRLs can also benefit from sharing quality assurance, information technology and specimen transportation functions. For many countries the intermediate-level laboratories must be integrated because there is insufficient staff and infrastructure to justify separately managed and supported facilities for different testing services. Many countries cannot accumulate the human and other resources required for quality assurance and other tasks at this level when focusing on a single programme. The major drawback of locating a TB section in an integrated NRL is ensuring that laboratory activities are focused on supporting NTP needs and goals. In some situations, the laboratory requirements for NTPs could greatly exceed those of the general services or other programmes. This need leads to difficulties in integrating services and meeting programme needs. All sectors of the healthcare system should be involved in a process to decide on the best structure for expanding and improving laboratory services.[50]

To be efficient, a microscopy network must be sufficiently decentralized to be readily accessible, but not so much that it becomes uncontrollable (e.g. for supplies and quality assurance). The rule of thumb is a population of 50,000–150,000 per microscopy laboratory, but this must be seen as highly flexible. A far larger population can be adequately and efficiently served by one large-volume laboratory in a densely populated urban area, particularly when using fluorescence microscopy, whereas only a few thousand people may need a laboratory in forest or semi-desert areas. Poor quality or broken microscopes are a frequent problem of microscopy networks, together with bad-quality immersion oil, which leads to microscope damage.[51] Even if periodic rechecking is not possible, surveying all AFB-smear laboratories with panels, during a visit or sent by postal mail, followed by correction of microscope problems, will go a long way to improving quality.

Low-income countries have minimal experience with culture networks because culture has almost exclusively been used to obtain an isolate for DST in the context of drug resistance surveys. However, reports from middle-income countries, such as Peru and Cuba, with fairly advanced-to-extensive culture networks, show that these cultures contribute only between 5% and 10% to case detection. In large part, this must be because the requirements for high yield of culture were not being fulfilled (i.e. exclusive use of solid media of unknown quality, harsh decontamination methods necessary because of delayed processing, lack of internal quality control). Although this does not affect the yield of microscopy, a major limiting factor for culture is transport delay of specimens, which is an additional factor to be considered when deciding on the density of a culture network. The expanded Stop TB Plan calls for one culture facility per 5 million people by 2015. In most high-prevalence countries only urban populations would be effectively covered geographically. However, considering the high demands of culture facilities on infrastructure, utilities and skilled staff, this would already be a remarkable achievement, serving a priority population in terms of TB and HIV prevalence, intensity of transmission and drug resistance.

In high-prevalence areas, DST can remain more limited, with increased diagnosis of MDR-TB and better surveillance. This does not require a dense network of DST laboratories. Even very large countries can be served by a few very large facilities. Considering the difficulty of continuously providing high-quality DST results, centralized, well-equipped, -staffed and -controlled DST services are, in fact, preferred. For surveillance, it is always possible to isolate strains in the periphery, for instance on acidified media, and to forward these strains for DST.[52] There are high hopes that rapid testing for MDR-TB will prove to be best using transport-insensitive NAATs at an advanced facility in the capital, and even abroad.[53]

LABORATORY SAFETY

Safety regulations are very strict in TB laboratories in industrialized countries, requiring a biosafety level 3 (BSL3) designated facility for work with strains. This is consequently a growing concern for laboratory staff in high-prevalence countries where such facilities are rarely affordable. Those who work with culture and DST, and increasingly those only involved with AFB microscopy, request safety measures that are difficult and costly to provide. The NRLs and NTPs are responsible for addressing safety concerns through a combination of training and education to help promote risk assessment and safe practices. Experience has shown that laboratory staff often lack basic knowledge of transmission routes and a differentiation of practices taking into account assessment of risk levels. Reasonable and rational safety improvements in equipment, supplies and facilities should be supported, but there are still serious questions and concerns in this area. Microscopy carries a negligible risk if direct smears are prepared with careful technique in well-ventilated areas.[54,55] Rather than purchase biological safety cabinets (BSCs) that are very difficult to maintain, microscopy centres may want to purchase simple air extraction cabinets or fan boxes. These are relatively inexpensive and efficiently exhaust air without filters, provided they have sufficient extraction power.[56] When properly installed, these devices provide a level of protection that also reassures technical staff. In fact, there is no reason why they could not

be used also for simple culture. Aerosol creation is minimal, except in the case of accidents with grown cultures. This type of cabinet, equipped with a backflow stop mechanism and exhausted above the upper floor, gives more guarantee of negative pressure and complete expulsion of infectious particles than the sophisticated class II BSCs. As with class I cabinets, the challenge is to conduct proper and regular maintenance to ensure perfect functionality of filters that are replaced before they get completely clogged; thus, these machines become hazards rather than sources of protection. As countries expand culture capacity, there will be a need for guidance and policy decisions on minimum safety standards that are affordable and sustainable.

Sophisticated class II BSCs, which protect decontaminated products, are needed for laboratories performing identification and DST. They are also considered to be one of several prerequisites for successful introduction of liquid cultures. However, a liquid DST system has been used successfully for rapid diagnosis of MDR-TB from fresh smear-positive sputa, containing contamination by use of antibiotic cocktails, as recommended by Mitchison et al.,[57] despite manipulations in a class I type cabinet.[58] Moreover, this system used virtually unbreakable universal containers, which were heated before opening, thus reducing the risk for technicians to a very low level. These factors should also be considered for determining the chosen method among the multitude of rapid, liquid media-based DST methods described.[59,60]

QUALITY ASSURANCE, QUALITY CONTROL AND EXTERNAL QUALITY ASSESSMENT

Poor quality assurance of testing services remains a major problem, not only for culture, DST and NAAT methods, but first and foremost for microscopy. In addition to good internal controls, effective EQA is necessary to ensure accurate testing and particularly to improve test sensitivity. Until further evidence is obtained on the appropriateness of more complicated methods such as fluorescence microscopy and concentration techniques and until new diagnostics are tested, this will be the only way to enhance case detection by microscopy for years to come.[61] Internally, quality assurance must first of all be set up for ZN or fluorescence microscopy staining solutions which are increasingly identified as major sources of error. The issue is further compounded by older WHO and International Union against Tuberculosis and Lung Disease (IUATLD) technical guidelines, which require practical improvements and currently leave no margin for error.[62–64] Additionally, there is an increasing reliance on locally manufactured, ready-made staining solutions of unknown quality.

The international guidelines for effective EQA programmes were issued only a few years ago,[65] and their implementation has been slow in most countries. The major obstacle is lack of staff; EQA requires dedicated staff for onsite supervisory visits in addition to rechecking a relatively large workload of smears at higher levels.[28,66] Progress is also hampered by poor understanding and comprehension of the guidelines, even among laboratory consultants. Many countries have not fully or correctly implemented rechecking: and some regions continue to use older methods of unblinded rechecking that have been demonstrated to be ineffective and misleading.[67,68] Panel tests (sending out smears with known results prepared by a central laboratory) are considered less efficient because they correlate well with capacity, but not always with routine performance, and are less reliable. However, they will still be more rewarding than rechecking in countries not able or willing to invest what is needed for the latter (i.e. sufficient manpower and meticulous execution).

Proven programmes that measure performance of microscopy and DST do exist.[69] Culture performance is often more difficult to measure. Existing EQA programmes do not necessarily measure the sensitivity of performance. Universal guidelines for quality assurance of culture are still under development. Mainly, internal controls (i.e. of medium sterility and growth-supporting quality) and monitoring (i.e. contamination and false-negative rates) must be used. EQA of decontamination and culture itself might not be feasible because of extreme lability of samples during transport. The low yield of culture in some settings is clearly shown by surveillance for drug resistance where laboratories might have difficulties isolating *M. tuberculosis* from smear-positive specimens.

It is only possible to guess at the quality of DST without EQA, but so far few DST laboratories or even NRL in high-prevalence countries participate in panel testing, originating from the Center for Disease Control and Prevention in Atlanta or the (WHO/IUATLD) Global Project for TB Drug Resistance Surveillance. This activity and linking national TB reference laboratories to a Supra-National TB Reference Laboratory (SRL) for technical assistance and rechecking of routine DST is hampered by lack of funding for the SRL. So far, rechecking DST has almost exclusively been done in the context of surveys, and it is not always clear how the results have been used.[70] A minimal requirement would be rechecking all resistant strains (at least those resistant to rifampicin or isoniazid), plus at least about 100 susceptible strains.[45]

Quality problems cannot be solved by EQA alone and one must consider the total quality management systems that include all the components of documents, records, personnel, standards, facilities and quality control. One critical difference in many industrialized countries is the presence of laboratory regulations or accreditation programmes.[71,72] Until such time that countries regulate performance against national laboratory standards there should be consideration in the TB community to develop an accreditation process for NRLs.

RESEARCH

Tuberculosis laboratories play a key role in research, especially operational research, supporting evidence-based decisions to guide not only laboratory practice but also areas of fundamental importance to TB programmes, such as the efficacy of standard treatment regimens. To be meaningful for programme applications, research should be performed in the field in low-resource settings so there are conditions of utilities, equipment, supplies and staffing replicating the situation in most high-burden countries. Research performed in academic research centres with human and material resources that differ from public sector services in low-resource countries are useful initially for providing proof of principle, but results might not always be reproducible under programme conditions. Operational research is still needed to improve diagnostic methods and techniques, even the most basic ones such as smear microscopy. Because of the potentially huge impact, research might be needed even more urgently to test the appropriateness of existing programme guidelines, or to simplify procedures after uprooting longstanding dogma. Many NRLs are interested in research although few also have the capacity to carry out the more demanding research of large international projects without compromising their daily routine activities of NTP service delivery

and network guidance. A downside to taking on research is that the research agenda often takes precedence over routine tasks because of prestige and financial incentives. Hence, a major concern is to ensure that the NRL and other institutions balance research activities with NTP priority initiatives to monitor and support the laboratory network.

CONCLUSION

In most resource-poor countries, expanding and strengthening laboratory systems for quality-assured microscopy, culture and DST services must and can be done – but not overnight, and probably not all at the same time. Great care should thus be taken not to overstretch the scarcest resources (i.e. the staff responsible for implementation of these changes). Priorities must be set, and, although introduction of diagnostic culture and detection of MDR-TB cannot wait until microscopy reaches perfection, their expansion should be gradual and not at the cost of deterioration of important processes such as microscopy EQA. Gradual expansion logically means that first the NRL should be proficient in basic techniques, before starting to train and supervise others. To avoid wasting donor money and limited country resources there should be guidance in purchasing suitable laboratory equipment. Whatever the targets set, the process is going to be lengthy. It would be better to be done slowly, but correctly, now that global funds and other resources are finally available at the country level. A fast gain, however, may be possible, introducing improved microscopy techniques (i.e. appropriate, user-friendly and robust fluorescence microscopy systems). The Global Plan to Stop TB calls for 800 new culture and DST facilities at an estimated cost of $700 million to reach the year 2015 goals and the Global Fund for TB, HIV, and Malaria can help with these costs.[7] For phasing in culture and drug resistance testing, techniques and patients will have to be carefully selected for individual diagnosis; use for epidemiological monitoring must be optimized starting with the international guidelines for drug resistance surveillance. Liquid cultures are highly desirable but remain controversial if they are performed using automated machines rather than manually. They also carry very high infrastructure and system requirements, including safety. For these reasons their introduction best be delayed until culture on solid media has been firmly established. Susceptibility testing should focus on the main first-line drugs, such as rifampicin and possibly isoniazid, and mainly for epidemiological monitoring. This is also the case for the main second-line drugs (i.e. the fluoroquinolones and injectables). At the same time, NAATs for rapid detection of rifampicin resistance might be within reach for countries because the commercial formats can be easily implemented by any laboratory proficient in molecular techniques, and because their speed and ease of transport allow strong, centralized testing. In fact, contracted NAAT testing for limited numbers of well-selected patients by a laboratory external to the NTP and NRL could be an excellent solution, at least in the short term.

Laboratories are not defined just by technologies, equipment and buildings, but rather they are composed of people and systems that manage the processes and standards required for accurate and timely results. Successful implementation of new diagnostic tests will probably require well-functioning networks of laboratories with trained and motivated staff, quality management systems and safe working environments. The experience of laboratory networks in many countries indicates that there must also be a corresponding requirement for attention and investment in people, organizations and systems to successfully expand services. Although there are increasing resources for commodities, there are currently little or no resources or efforts to address human resources for EQA, guidance and processes to establish and enforce quality systems, practical and reasonable safety standards, and appropriate steps for determining optimum organizational structures and requirements for TB laboratory services. Rather than waiting for technological developments as the only solution for improving diagnosis, international organizations and countries should work immediately to strengthen laboratory leadership and systems through shared guidance at the global level. The solutions, in the form of technical guidance, effective quality assurance, systems and capacity building are all attainable. However, these solutions will require a new focus on the laboratory as a system and coordination and support from organizations and countries in the Stop TB Partnership to develop the people and networks in tandem with improvements in facilities, equipment and methods.

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