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Recommended Standards for Modern Tuberculosis Laboratory Services in Europe.

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Abstract

The principles underpinning these standards are that any tuberculosis (TB) laboratory diagnostic procedure should be performed by appropriately trained staff, working to standardized operating procedures in appropriately equipped and safe laboratories against clear national and international proficiency and quality standards. Quality should be the pre-eminent criteria, not cheapness.

The standards are technologically feasible but may not be within the financial capacity of all laboratories initially. There is a requirement for government and international donors to adequately fund an appropriate safe infrastructure.for staff to deliver accurate and timely results at whatever level of activity they are performing. There is a need for national reference laboratories to train a new cadre of mycobacterial laboratory experts which will require the funding of appropriate individuals at these centres to train and then assist in the implementation of good practice and laboratory evaluation in the field to build sustainable capacity. Further operational research is needed to determine the optimal configuration of new technologies to determine isoniazid, rifampicin and second-line drug susceptibility in mycobacterial cultures but increasingly directly on specimens as well. Better integration of laboratory medicine as a core part of all TB programmes is needed to achieve and maximize the potential of new developments. 200 words

INTRODUCTION

There were 9 million new tuberculosis (TB) cases and approximately 2 million TB deaths in 2004 of which 3.9 million (62/100 000) were smear-positive and 741 000 were in adults infected with the human immunodeficiency virus (HIV) [1]. In the most recent WHO report on Global Tuberculosis Control a decade of progress to achieving the targets proposed by the World Health Assembly (WHA) and the Millenium Development Goals (MDGs) were summarised . The WHO targets were to detect, by 2005, 70% of new sputum smear-positive cases and to successfully treat 85% of these cases. MDG target 8 (of 18) is to have halted and begun to reverse the TB incidence rate by 2015. The Stop TB Partnership endorsed additional targets of halving 1990 prevalence and deaths rates by 2015.

WHO EURO LABORATORY TASK FORCE

The WHO Laboratory Strengthening Task Force for Tuberculosis Control for the WHO European Region (LSTF) was established in 2005 at the recommendation of the WHO European Technical Advisory Group (TAG). The main function of the LSTF is to provide guidance to WHO and TAG on strategic and technical aspects of tuberculosis (TB) laboratory capacities within the WHO European region. The LSTF acts as a technical advisory board, assisting in situational analyses of laboratory services in priority countries, reviews relevant international laboratory documents and guidelines, assists WHO in estimating budgets needed for laboratory capacity strengthening, and helps to identify and train national and international experts to assist countries in strengthening their national tuberculosis (TB) services.

The need for such a group was driven by the increasing incidence and prevalence of TB in Europe as well as globally, particularly in those countries with the highest burdens of TB, and the low rates of laboratory case detection and confirmation of TB cases. The case detection rate was 53% globally in 2004, and would probably exceed 60% in 2005, falling short of the 70% target. Treatment success was 82% in the 2003 cohort of 1.7 million patients, approaching the 85% target [1]. Implementation of the new Global Plan for TB is expected to reverse the rise in incidence globally by 2015, as specified in the MDGs, and to halve the 1990 prevalence and death rates globally and in most regions by 2015, although this is not expected to occur in Africa and eastern Europe. In the former region this is mainly due to the association with HIV infection but in Eastern Europe [1,2] the major factor is the high level of drug resistance and particularly multiple drug resistant TB [2-6].

This has been due in large part to the failure to recognize laboratories as a corner-stone of TB control policy. With this in mind, this report is aimed at those responsible for managing and implementing TB control programmes as well as heads of laboratories.

This document is the first papers from the LSTF. It describes the principal standards that a competent TB laboratory, at whatever level of activity, should aspire to in Europe. Some countries will be in a position to implement these recommended standards sooner than others but consideration has been given to studies published in the scientific literature and all recommendations are technically feasible. Nevertheless it is not intended to describe in detail how to meet these standards here or to present detailed standard

operating procedures. It complements existing WHO and other international recommendations and guidelines [7-23].

KEY PRINCIPLES AND OVERARCHING RECOMMENDATIONS:

The underlying principles underpinning this document are that any TB related procedure (microscopy, bacterial culture, identification, drug susceptibility testing (DST), molecular diagnosis) should be performed by appropriately trained staff, working to standardized operating procedures in appropriately equipped and safe laboratories against clear national and international proficiency and quality standards. Quality should be the pre-eminent criteria, not cheapness.

ACCREDITATION: All laboratories should conduct a thorough internal quality control (IQC) programme. Analyses and diagnostic services should be accredited wherever such a scheme exists and laboratories must participate in relevant proficiency schemes. Laboratories should not conduct procedures in which they have failed to demonstrate proficiency through a quality control scheme. Similarly where a licensing system exists, laboratories should be licensed to perform TB-related microbiological activity.

BIOSAFETY AND INFRASTRUCTURE: Laboratory staff are entitled to work in a safe environment. Staff working with patient specimens and live mycobacterial cultures must operate under appropriate biosafety conditions with adequate infection control measures, including staff health checks, in place. There are several documents available which can be used as the basis for development of appropriate safety standards [3,24-27]

Many laboratories do not meet reasonable safety standards and have too few staff to complete their tasks adequately. As infrastructure and biosafety standards are improved an inevitable consequence is that fewer laboratories should perform complex procedures such as DST or molecular diagnosis than currently.

NATIONAL REFERENCE LABORATORY: The LSTF supports the recommendations of the WHO Euro Technical Advisory Group that there should be one national designated reference laboratory (NRL) in each country with a designated head. There must be official recognition by the Ministry of Health (or equivalent body) of the NRL, its leadership and the remaining laboratory structure. The NRL head should participate in meetings with the National TB Control Programme (NTP) manager (or the person fulfilling the equivalent role) and should have sufficient funding for the tasks required of the NRL. Smaller countries with little or no TB may determine that there is no need for their own national reference laboratory in which case the country should establish links with a national (or supranational) reference laboratory in another country. Conversely in larger countries with a significant TB case load large regional centers will be needed to amplify and extend the role of the national reference center.

FUNDING: Just as it is appropriate to ask laboratories to meet the standards described below, it is appropriate for government and TB control programmes to provide sufficient funding for laboratories to perform their activities. In particular, the Ministry of Health (or equivalent body) must take direct responsibility for ensuring that there is an adequate NRL budget for all its designated functions.

Funding should ensure that there is:

(a) safe and functioning infrastructure with appropriate and well-maintained equipment including access to sufficient spare parts to maintain activity [23, 24-27]. The most important items are the presence of appropriately maintained class 1, 2 or 3 biological safety cabinets (BSC) and where concentration techniques are applied, an effective centrifuge in which biological material is contained preferably within sealed buckets or at least within a 'windshield-type' enclosure. High quality binocular light or fluorescence microscopes, incubators/rooms and refrigerators freezers capable of maintaining an appropriate temperature are also essential items.

(b) sufficient trained staff to perform the expected workload

(c) sufficient budgetary control at the laboratory level to ensure service continuity and development by those best placed to achieve it

(d) sufficient laboratory consumables (key items include disposable plasticware, single-use glass slides ideally with frosted ends, high quality smear-microscopy reagents and pure antibiotic drug reagents for drug susceptibility testing.

HUMAN RESOURCES

Arguably one of the most neglected areas relates to the need for sufficient adequately trained staff to perform TB laboratory procedures, with an understanding of the staff needed to perform the actual workload. Staff numbers for all laboratories including the national reference laboratory should be calculated on the basis of TB incidence, number of tests conducted, and quality control and other specialized work that the laboratories

conduct. Staff should have clear job descriptions and training to perform their work correctly.

There is a need for national reference laboratories to have sufficient human resources to provide national as well as international training, to replace staff who have retired or left laboratory medicine and to increase the number of available qualified laboratory staff available to support TB control internationally. In reality in many areas of Europe, the staff age structure has meant that whole laboratories are frequently staffed by too few individuals, many of whom are too close to retirement age. In other industrialised countries such as the USA, a high proportion of experienced technical staff are also approaching retirement age [28] and similar problems have been reported by many high TB burden countries [29].

COMMUNICATION.

Good communication is essential both within the laboratory and between the laboratory and others interacting with it including reporting of results to the relevant clinical staff, others involved in case management or contact tracing/outbreak investigations, and the national TB programme and surveillance system as defined in each country. Delays in referring specimens and reporting information can lead to delays in diagnosis, treatment, implementation of infection control [30].

Clinicians must inform the laboratory of relevant patient information. Laboratories should provide information on the performance of their analyses and support the taking

of appropriate high quality specimens to improve the sensitivity of analyses. Clinicians should be informed of results as soon as possible.

LABORATORY PROCEDURES

It is recommended that microscopy and culture for diagnosis of new cases is performed prior to the institution of treatment using a limited number of high quality specimens.

Microscopy

Smear microscopy should be performed in one working day from the arrival of the specimen in the laboratory. Although tests might be performed quickly, laboratories should also identify and address delays in reporting systems. Positive results should be reported immediately by telephone, fax or other electronic means, as soon as they are available, for diagnostic and infection control purposes. Although fluorescent microscopy appears to be slightly more sensitive than conventional Ziehl-Neelsen acid-fast staining [31] its principal purpose is to allow greater specimen throughput per microscopist by reducing the time taken to examine each slide.

Ideally, specimens should be transported to the laboratories as quickly as possible and not longer than 4 days before processing the specimens should be refrigerated if transportation will take more than 48 hrs.

The presence of acid-fast bacilli in a single sputum specimen (‘smear-positive’) may be considered as presumptive diagnosis of TB but awaits definitive identification as TB

Other key extra-pulmonary specimens such as cerebro-spinal fluid should be examined during one working day as well. Resources should be redeployed where they are currently used for an activity of minimal benefit eg there is little or no value in performing microscopic examination of urine samples (except where there is a strong suspicion of renal TB) and this practice should be discontinued.

Although diagnostic sensitivity may be improved by using concentration methods including centrifugation, this is not necessarily the case . Centrifugation needs to be with sufficient g-force in appropriately calibrated and enclosed biosafe centrifuges. Smear microscopy using 'direct smears' only is appropriate and sufficient for assessment of patient infectivity.

Bacterial culture

For patients with a clinical suspicion of TB (symptoms, signs, x-ray) specimens should be taken for microscopic examination and bacterial culture, for diagnosis, prior to the initiation of treatment. In practice we do not recommend more than three sputum specimens for the diagnosis of pulmonary TB and two high-quality specimens may be sufficient. Urine samples should only be cultured where there is a clear suspicion of renal TB.

A combination of liquid (eg Middlebrook 7H9, Kirchner) and solid culture (eg Löwenstein-Jensen, Middlebrook 7H10, 7H11) gives the most optimal rates of mycobacterial recovery for diagnosis. Where resources permit, automated liquid culture

systems can be used. Solid culture media will remain the backbone of culture in many countries for the foreseeable future [32-37].

Cultures for treatment monitoring (and treatment failure) should be limited in number and not performed more frequently (both in frequency and number of specimens) than indicated in international guidelines.

Identification

Positive cultures should be identified as *Mycobacterium tuberculosis* within 1-2 working days of receipt (ideally with the concurrent identification of rifampicin resistance, see below). Laboratory systems should aim to culture and identify *M. tuberculosis* from sputum within 21 days from receiving the patient specimen (and in 30 days for any pulmonary specimen) in at least 90% of cases. In practice this will mean employing liquid culture (which may be manual or automated) and/or novel molecular diagnostic systems.

The laboratory as well as clinicians should recognize the risk of false positive cultures and should assess the results in connection with clinical findings and laboratory quality assurance data..

Drug susceptibility testing (DST)

The real level of drug resistance in the world today is unknown although national and regional studies and anecdotal evidence indicates that it has been increasing in recent years [1,3, 38-39] . Most Western and Central European countries do not have a significant problem with drug resistance in newly diagnosed cases, reporting

approximately 5-10% isoniazid resistance and 1-2% MDRTB (resistance to at least rifampicin and isoniazid resistance) [1,3,39,40]. Countries in eastern Europe, however, have reported significantly higher rates of MDRTB particularly in countries of the former Soviet Union reporting rates of approximately 10-20% in new patients [4-6,41-46]. Previously treated patients, especially in eastern Europe, show high rates of isoniazid resistance and MDRTB.

Modern four-drug treatment regimens (i.e isoniazid, rifampicin, pyrazinamide, and ethambutol (or streptomycin)) are designed to successfully treat drug sensitive TB (and isoniazid-resistant TB (during the 2 month intensive phase of treatment) [47-50].
These points form the basis of the recommendations given below.

DST is recommended for all new cases for first line drugs with specimens taken before initiating treatment, if the patient continues to be culture positive after 2-3 months and if there is a history of prior TB treatment (a major risk factor for drug resistance). Individual circumstances may dictate additional testing. Accuracy is more important that speed and DST results should come from a small number of well-equipped, experienced laboratories who participate and perform well in an international DST quality control scheme. The WHO Supranational Laboratory Quality Control Network offers the greatest global coverage assessing participating laboratories in their ability to identify isonaizid, rifampicin, ethambutol and streptomycin resistance correctly [3] .

The absolute concentration, resistance ratio, and proportion methods can all give accurate results provided they are carefully quality controlled and standardized [3]; the

basic principles of these methods for first-line drugs are given in references [51-52]. As a minimum, laboratories supplying DST data to clinicians, government and the WHO, and for surveys or surveillance, should correctly identify resistance to isoniazid and rifampicin in over 90% of quality control samples in two out of the last three quality control rounds.

For Europe, the early identification of mycobacterial growth as *M. tuberculosis* complex (principally *M. tuberculosis* and *M.bovis)* and the identification of rifampicin resistance should be the first priority as rifampicin resistance invalidates standard 6 month short-course chemotherapy and is a useful marker in most countries for MDR-TB. Laboratories should aim to identify isolates as *M. tuberculosis* complex and perform rifampicin resistance in 90% of isolates within 1-2 working days. This is technologically feasible [53-67].

The early identification of isoniazid resistance in *M. tuberculosis* isolates is also of importance although less critical than rifampicin resistance. Modern molecular techniques permit the successful identification of isoniazid resistance in at least 75% of *M. tuberculosis* complex isolates within 1-2 working days and are useful preliminary screens for isoniazid resistance.

Laboratories should aim to identify *M. tuberculosis* and rifampicin resistance in over 90% of cases from smear-positive sputum directly where resources are available for this (this will require an investment in new methodological techniques). This is

technologically feasible but challenging: in a recent analysis of a routine national non-trial service, 1,997 primary clinical specimens, including 658 non-respiratory specimens were analysed [67]. The overall adjusted concordance, sensitivity, specificity, positive predictive value, and negative predictive value for detecting MTBC were 91.2%, 85.2%, 96.2%, 95.7%, and 86.7%, respectively (unadjusted, 86.7%, 85.2%, 88.2%, 86.9%, and 86.7%), when false-positive samples from patients (n =83) with a known microbiological diagnosis of *Mycobacterium tuberculosis* complex or patients receiving current or recent antituberculous treatment were excluded [67].

The non-respiratory specimen types with the highest sensitivity rates were vertebral aspirates biopsy specimens (n = 30, sensitivity 83.3%), gastric aspirates (n = 18, sensitivity 80.0%), and lymph node aspirates/ biopsy specimens (n = 144, sensitivity 72.5%). Sensitivities are significantly lower for pleural, ascitic or cerebro-spinal fluid. Fluid. The parameters for detecting rifampicin resistance were 99.1%, 95.0%, 99.6%, 92.7%, and 99.7%, respectively [67].

Further operational research is needed to develop methods which can identify TB, rifampicin and isoniazid resistance in all sputum specimens with the same sensitivity as the best bacterial culture methods but within 1-2 working days.
For patients with MDRTB or who are genuinely unable to tolerate first-line therapy, second line drug therapy should be instituted. There remains a need to standardize second line drug resistance testing and such testing should only be performed at the national reference laboratory in Western and Central Europe countries due to the

relatively small number of cases and the concomitant difficulty of maintaining testing proficiency if multiple centers perform this activity. It is proposed that a center performs second line testing only if it is performing such analyses on at least 50 new patients or 200 specimens per annum to maintain expertise. This argument holds for smaller eastern European countries such as the Baltic states where the overall case numbers are small but additional qualified centers will be needed for the largest countries such as Russia, Ukraine and Turkey. There is a need to further develop international QA for second-line drug resistance analysis.

Standardized second-line treatment programmes based on surveys or individualized treatment will produce higher treatment cure rates than no therapy or first-line drug therapy alone although treatment must be prolonged. Although individualized treatment strategies will produce the highest cure rates, this will be dependent on the continuous availability of appropriate drugs and the adherence of the patient to the regimen. It is the responsibility of the laboratory in consultation with the clinician to determine the spectrum of drugs to be tested within the laboratory rather than the other way round. The frequency of repeat testing of known MDRTB cases should be limited. Whilst it is true that new resistances can emerge reasonably quickly, a greater priority is often to ensure that the reasons for a patient acquiring MDRTB or developing MDRTB therapy on treatment is identified and corrected before new drugs are administered. Clinicians should identify where they are simply determining treatment progress (i.e they only need to establish that the patient is still smear/culture positive) so that DST is not repeated unnecessarily. In practice, patients with established MDRTB do not need to have the

DST repeated more than every 2 months and in most cases every 6 months will be sufficient.

CONCLUSIONS

The above standards are technologically feasible but may not be within the financial capacity of all laboratories initially. There is a requirement for government and international donors to adequately fund an appropriate safe infrastructure in which well-trained staff working to clear SOPs can deliver accurate and timely results at whatever level of activity they are performing (microscopy, culture, DST etc). There remains a need for national reference laboratories to train a new cadre of mycobacterial laboratory experts which will require the funding of appropriate individuals at these centres to train and then assist in the implementation of good practice and laboratory evaluation in the field to build sustainable capacity. Further operational research is needed to determine the optimal configuration of new technologies to determine isoniazid, rifampicin and second-line drug susceptibility in mycobacterial cultures but increasingly directly on specimens as well. Despite the pessimistic statements found in many textbooks it is currently feasible to diagnose nearly all patients with infectious pulmonary TB and rifampicin resistance, and most with isoniazid resistance, within 1-2 working days. The better integration of laboratory medicine as a core part of all TB programmes is needed to achieve and maximize the potential of new technological developments.

3091 words

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