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In most resource-restricted settings, individual patient diagnosis and being addressed in many regions. In bacterial sepsis, or second-line combination therapy for AIDS, unaffordable, antimicrobials such as broad-spectrum antimicrobials to antimicrobials in many infections and the need for newer, possibly of endemic and epidemic infections all require laboratory support. Laboratories have an increasing role in infection control in health care. Infections by pathogens that are resistant to multiple antimicrobials are common in many tropical countries where there is widespread availability of over-the-counter antimicrobials. Accurate disease surveillance requires a laboratory network for the first time. Tuberculosis can be diagnosed in many patients with a Ziehl–Neelsen-stained smear of sputum, but to extend the diagnosis in those who are acid-fast bacilli (AFB) negative or have multi-drug–resistant (MDR) disease requires more developed laboratory support. Furthermore, laboratories have an increasing role in infection control in health care settings and congregate facilities and in the prevention of health care–associated infections. Accurate disease surveillance requires a laboratory network and is vital to inform public health policy concerning allocation of resources and disease prevention. Laboratories can help to
define clinical problems by sampling surveys. For example, determining the antimicrobial susceptibilities of bacterial pathogens such as *S. aureus*, *S. pneumoniae*, or *S. enterica* for a selection of isolates can inform the appropriate empiric therapy in a particular area. An understanding of the burden of disease in an area—drug-resistant typhoid in an urban slum, for example—could lead to public health measures such as a vaccination program. Laboratory surveillance programs may produce the clue to the possibility of new organisms emerging, including both bacteria and viruses, most commonly at the animal–human interface.

At an international level, under revised International Health Regulations, countries are now committed to reporting events that could have health implications beyond their borders. Many countries do not have the capacity to do this. The World Health Organization (WHO), with other partners, have been working to address this issue by creating an international network of laboratories known as the *Emerging and Dangerous Pathogens Laboratory Network for Response and Readiness*. International organizations such as the American Society of Microbiology, the Centers for Disease Control, the Fleming Fund, and global academic institutions are actively contributing to training and mentoring of developing-country laboratory networks.¹

**WHAT ARE THE PROBLEMS FOR LABORATORY SERVICES?**

For many health care staff working in resource-restricted areas, the major problem is simply a lack of laboratory services. Hospital laboratories may be absent, or, if they are available, only offer a limited repertoire of tests. In other areas, particularly in Asia, a wide range of alternative services is offered by private diagnostic laboratories, typically outside the front gate of the hospital but with uncertain quality. Even when the tests are available, they may not be used or the results ignored. Lack of use may stem from a poor perception of the laboratory, and tests may not be available because the costs are prohibitive.

Even when laboratories are present, they face the many challenges that are familiar to all areas of the health care sector. Inadequate facilities are common, with laboratories that lack space and a secure supply of electricity and water. Appropriate equipment may be unavailable or poorly maintained. Even basic equipment required for a functioning laboratory can be in disrepair because of the absence of regular care and servicing. A functioning microscope is a key piece of equipment for a basic microbiology laboratory but is frequently found in poor condition. In a survey of 90 microscopes in laboratories in nine districts in Malawi, only 50% were in good condition.¹ There were 1.1 functioning microscopes per 100,000 population, and even microscopes in need of full servicing were still in daily use. The 90 microscopes were from 16 different manufacturers, illustrating the lack of standardization of laboratory equipment so frequently seen. The provision of biological safety cabinets is another area where equipment from multiple manufacturers and lack of spare parts and maintenance are common, and in this case may lead to unsafe and hazardous conditions for laboratory workers. Standardization of equipment and consumables with central ordering, maintenance contracts, and supplies of spare parts would seem a sensible response to this issue but is rarely seen. Tests may also be unavailable because of an inadequate supply route for consumables. This is another area where standardization of tests and central ordering and supply can lead not only to more reliable supply of quality-assured consumables but also to potential cost savings for the country.

The laboratory can generate results, but the quality may be poor. Standard operating procedures may be absent and quality control of routine procedures non-existent. The absence of national or regional laboratory guidelines or programs of external quality assurance (QA) by the laboratory network is common. Communications between different levels within the laboratory network may be rudimentary so that specimens referred to the next level are not transported in a timely manner and results do not return in a time period that will influence clinical management. It is standard practice in tuberculosis programs that patients who fail treatment should have a sample cultured for tuberculosis so that susceptibility tests can be performed. In a study of the transport of such specimens to the central reference laboratory in Malawi, only 40% of specimens arrived in the reference laboratory and only 36% of those samples received were successfully cultured for susceptibility testing.⁴

The shortage of staff with appropriate education and training is a further problem. Many laboratory workers have no formal training and are simply trained at the bench. At the peripheral level, there may be only one laboratory assistant, with no more than secondary school education. At the district level, there may be assistants and technicians (formally educated in laboratory medicine for 3 years). At the central level, technicians may work alongside technologists (with 2 years specialist post-technician training) and scientists (university science graduates). Regardless of qualifications, laboratory workers often have a lowly status within the health sector, and the attrition of health care personnel out of government service results in low morale among those who remain. Private or research laboratories may attract the best technicians from the government sector. Diagnostic laboratories frequently have no representation at the local, provincial, or national level, or, if they do, it is only as part of the support services. In many countries, the voice of the laboratory is rarely heard.

These many problems contribute to a poor biosafety situation in laboratories. The lack of equipment, knowledge, and training means that laboratory workers are processing samples with hazardous pathogens in an unsafe manner. In a study of tuberculosis laboratories in Korea, before safety conditions had been upgraded, the relative risk of being diagnosed with tuberculosis by the technicians performing drug susceptibility tests was 21.5 (95% CI 4.5–102.5) compared with non-laboratory workers.⁶ The true magnitude of this problem in laboratory workers is difficult to gauge because surveillance of infection in laboratory workers is rarely performed or reported.

**WHAT CAN BE DONE TO IMPROVE LABORATORY SERVICES?**

At a national level, the important contribution of laboratories needs to be appreciated within the ministry of health, by national and local health care managers, and by funding organizations. A representative of the laboratory services should be present in the key decision-making committees. Support is also needed from clinicians, who often have disproportionate influence within the system. A plan for the laboratory network should become part of the overall health care development plan. There needs to be a priority list of core and essential services provided in a quality-assured manner. The laboratory plan should include the provision for a tiered laboratory network at the primary, district, regional/provincial, and national levels. The plans should be realistic, affordable, and sustainable.

At the Level I or primary level, perhaps in a health post or health center serving outpatients, microscopy for malaria and tuberculosis and testing for HIV with a same-day service would be essential. These laboratories can serve as a collection point for samples that need referral to the next level. The Level II facility in the local district hospital would have a dedicated laboratory space and a broader repertoire of tests serving inpatients and outpatients. The tests offered would depend on the spectrum of local diseases and resources available, and may be limited to microscopy, simple biochemistry and serology, and blood transfusion, or may include bacterial culture facilities. Laboratories can act as a hub for the primary-level laboratories, providing them with support, supplies of reagents, and QA activities. At the Level III,
provincial or regional level, laboratories will be located in larger referral hospitals. Laboratories at this level should be performing a more sophisticated range of tests with higher throughput. For example, facilities for tuberculosis culture might be available, together with molecular techniques for specific diseases and the ability to investigate disease outbreaks. Support for the Level II laboratories would be an important function, including periodic visits and laboratory assessment as part of a QA program.

National reference laboratories at Level IV are likely to be located in the capital and serve specialized public health functions that may be linked to specific disease control programs such as the central reference laboratory for the National Tuberculosis Programme. It is important that laboratories at the national level have links to regional supranational reference laboratories for advice and quality assurance. Level III and IV laboratories would conduct surveillance and monitoring of infections using laboratory data collected throughout the network, establish standard operating procedures and protocols, conduct training and quality improvement, and plan for equipment needs and maintenance throughout the network.

THE IMPORTANCE OF BIOSAFETY

Biosafety is an essential consideration at all levels of the laboratory network and depends on three principles. Good laboratory practice and technique are fundamental and require established standard operating procedures and appropriate induction and training of staff. Safety equipment provides a primary barrier, and this includes appropriate, properly maintained and used equipment (e.g., centrifuges, biological safety cabinets) and personal protective equipment (e.g., gloves, respirators). Finally, facility design and construction are a secondary barrier providing, for example, appropriate workflows (from clean to dirty areas) and directional airflows and containment if required.

Microorganisms are categorized into four hazard groups according to their risk to individuals and society and the availability of treatment and preventive measures (Table 21.1). Diagnostic laboratories are further categorized into biosafety levels (BSL) so that the facilities available are matched to the pathogens handled. A standard diagnostic laboratory would be at BSL2, and the basic requirements for such a laboratory are outlined in Table 21.2 and Box 21.1. More specialized laboratories such as tuberculosis reference laboratories where culture and susceptibility testing are performed require BSL3 facilities. BSL3 laboratories have particular design features to reduce the hazard of airborne transmission and incorporate directional airflows and the use of biological safety cabinets. They are particularly appropriate for laboratories handling pathogens such as tuberculosis and influenza. However, BSL3 facilities are very expensive and difficult to build and maintain. The WHO has recently indicated that in some circumstances, slightly less rigorous guidelines, so-called BSL2+ as outlined in Table 21.2, may be appropriate for selected laboratories, for example, processing samples for tuberculosis culture.

WHAT TESTS SHOULD BE AVAILABLE?

Health care staff working at the district hospital level may be asked to advise on what would constitute an appropriate laboratory service for the hospital and district. The provision of an extensive range of tests is likely to be unaffordable and impractical. In a study evaluating the role of the laboratory in a district hospital in Malawi, the services considered essential were blood transfusion (including blood grouping and compatibility testing and screening for HIV, hepatitis B, and syphilis), hemoglobin estimation, and the microscopic diagnosis of malaria and tuberculosis. This list will vary in different areas, and the services of the laboratory should be orientated to the requirements of the district and the available resources. Other tests that require relatively little investment and can be done where there are limited resources include microscopy of urine and stool samples for ova, cysts, and parasites; Gram stain and cell count in cerebrospinal fluid and other sterile fluids; and Gram stains of pus samples. The microscopic appearance of some typical bacterial pathogens is shown in Fig. 21.1A–F. Guidelines for standard laboratory methods appropriate for resource-restricted areas are available. A checklist of issues that should be considered when evaluating a diagnostic laboratory is in Box 21.2.

| TABLE 21.1 Classification of Microorganisms on the Basis of Hazard |
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| Class | Description of Microorganism | Biosafety Level | Laboratory Type |
| 1 | Unlikely to cause human disease | Level 1 | Basic teaching or research laboratory |
| 2 | May cause human disease; might be a hazard to laboratory workers; unlikely to spread in the community; laboratory exposure rarely causes infection; effective prophylaxis and therapy | Level 2 | Routine diagnostic laboratory |
| 3 | May cause serious human disease; may be serious hazard to laboratory workers; may spread in the community; effective prophylaxis and therapy | Level 3 | Special diagnostic laboratory (e.g., tuberculosis reference laboratory) |
| 4 | Causes serious human disease; serious threat to laboratory workers; high risk of spread in the community; no effective prophylaxis or therapy | Level 4 | Supranational dangerous pathogen laboratories |

Modified from World Health Organization (WHO). Laboratory Biosafety Manual (3rd ed.). Geneva: WHO; 2004.

| BOX 21.1 Basic Requirements for a BSL2 Laboratory |
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| • Access should be restricted to essential personnel, not including the general public; biohazard signs should be on the entry door. |
| • A laboratory coat should be worn in the laboratory area. Laboratory coats should be side or back fastening and made of strong material. |
| • Gloves should be worn for contact or potential contact with infectious material. |
| • All work should be performed using good microbiologic technique. |
| • Hands should be washed after handling infectious material before leaving the laboratory area. |
| • Eating, drinking, smoking, and applying make-up are not allowed. |
| • No materials should be placed in the mouth, including no pipetting by mouth. |
| • The laboratory bench surfaces should be easy to clean. |
| • There should be clear protocols for cleaning, decontaminating, and dealing with spillages of potentially infected materials. |
| • Potentially infected material should be decontaminated (by autoclaving, incineration, or disinfection) before disposal. |
The diagnosis of infection depends on detection of the pathogen or the host response to the pathogen. Direct pathogen detection is traditionally performed by light microscopy, although antigen detection and nucleic acid amplification tests (such as polymerase chain reaction [PCR]) are increasingly used. Pathogen detection may also be carried out by isolation of the microorganism by culture of relevant clinical samples, and this allows susceptibility testing to be performed. Methods based on detecting the immune response mainly rely on detecting pathogen-specific IgM or IgG antibodies. Technological advances in the design of testing methods have simplified antigen and antibody detection to the point that simple point-of-care test kits are now widely available. The rapid kits for HIV antibody detection have an established place in the voluntary counseling and testing framework being established in many countries. Rapid malaria detection tests have been recommended as a replacement for malaria microscopy in some guidelines and need to be positive before antimalarial treatment is given.

In recent years, organizations such as the UNICEF/United Nations Development Programme/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), and the Foundation for Innovative New Diagnostics (FIND) have played an important role in developing and evaluating new diagnostic tests for many tropical diseases. The WHO Sexually Transmitted Diagnostics Initiative has developed an approach to the characteristics of an ideal diagnostic test in the developing-country context. “ASSURED” tests should be affordable by those

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**Fig. 21.1** Microscopic appearance of selected microorganisms. (A) Gram stain of *Staphylococcus aureus*. (B) Gram stain of *Streptococcus pyogenes*. (C) Gram stain of *Streptococcus pneumoniae*. (D) Gram stain of *Enterobacteriaceae* (*E.* coli, *Salmonella*, *Shigella*). (E) Gram stain of *Neisseria meningitidis*. (F) Gram stain of a yeast.
There has been increased attention on the problem of antimicrobial resistance for many important pathogens and the critical role that the laboratory plays in the management of this. Initiatives have focused on methods and systems of surveillance of antimicrobial resistance in bacterial infections that countries can readily implement. The WHO guideline has recommended a focus on eight priority pathogens as described in the Global Antimicrobial Resistance Surveillance System manual (E. coli, Klebsiella pneumoniae, Acinetobacter baumannii, S. aureus, S. pneumoniae, Salmonella spp., Shigella spp., and Neisseria gonorrhoeae), as well as other pathogens of local or national importance.

There have also been considerable advances in the format and ease of use of molecular tests. This is exemplified by the increasing use in tuberculosis laboratories of nucleic acid amplification tests directly from AFB smear-positive sputum, or from culture isolates. Line probe assays (LPAs) use a multiplex PCR amplification followed by reverse hybridization to identify Mycobacterium tuberculosis complex and mutations in the genes associated with
rifampicin and isoniazid resistance. LPA can be performed with results in 1 to 2 days, which is considerably quicker than the weeks required for traditional culture methods, and the overall agreement for the diagnosis of MDR between these tests and conventional methods is 99%. The format of these tests is being simplified so that the feasibility of their routine use in tuberculosis reference laboratories in developing countries is becoming a reality. These methods are an important component of the roll-out of the programmatic management of MDR tuberculosis globally.

Quality assurance is defined as “planned and systematic activities to provide adequate confidence that requirements for quality will be met.” The QA system is the basis for a guaranteed result. If this system is not followed, patients may get the wrong results, with important consequences for their health—such as receiving inadequate treatment. A program of QA in diagnostic laboratories involves not only internal quality control and external QA but also attention to appropriate staffing, training and supervision, and maintenance of equipment and facilities. International guidelines are now available and increasingly implemented for QA in many areas of laboratory practice such as AFB smear microscopy and HIV testing.

CONCLUSION

Accurate clinical diagnosis in resource-restricted settings relies strongly on the laboratory service. The increasing recognition of the need to support the development of a quality-assured laboratory service in such settings is therefore welcome. In many regions, international organizations are actively working with local providers to improve laboratory services. The development of laboratory services will contribute to improved health for the local population and ensure better use of scarce health care resources.

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