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REVIEW

Establishing molecular microbiology facilities in developing countries

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Summary Microbiology laboratories play an important role in epidemiology and infection control programs. Within microbiology laboratories, molecular microbiology techniques have revolutionized the identification and surveillance of infectious diseases. The combination of excellent sensitivity, specificity, low contamination levels and speed has made molecular techniques appealing methods for the diagnosis of many infectious diseases. In a well-equipped microbiology laboratory, the facility designated for molecular techniques remains indiscrete. However, in most developing countries, poor infrastructure and laboratory mismanagement have precipitated hazardous consequences. The establishment of a molecular microbiology facility within a microbiology laboratory remains fragmented. A high-quality laboratory should include both conventional microbiology methods and molecular microbiology techniques for exceptional performance. Furthermore, it should include appropriate laboratory administration, a well-designed facility, laboratory procedure standardization, a waste management system, a code of practice, equipment installation and laboratory personnel training. This manuscript lays out fundamental issues that need to be addressed when establishing a molecular microbiology facility in developing countries.

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Introduction

Microbiology laboratories play an important role in the management of infectious diseases in terms of national or global epidemiology. In the twenty-first century, molecular microbiology techniques for epidemiology and infection control programs have become more demanding. Molecular techniques in high-quality clinical microbiological laboratories are routinely used to make clinical decisions on the basis of how and when to treat patients. Molecular techniques are also useful for monitoring the effectiveness of therapeutic regimes and identifying potential resistant strains that may impact a long term treatment program. The management and control of infectious diseases requires accurate diagnosis and knowledge of pathogen distributions. The inclusion of molecular typing techniques for almost all infectious disease agents within a clinical microbiology laboratory provides a detailed assessment of any given strain's interrelationships, and thus can identify the source of the organism [1]. Almost all epidemiological studies depend on molecular techniques for the identification, characterization and genetic typing of microbes. Nevertheless, the lack of infrastructure for infectious disease management in developing countries has been blamed for the uncontrollable rates of infection and mortality [2,3]. In some resource-limited countries, sentinel hospitals lack even basic microbiology laboratory facilities [4]. In other regions, laboratories have all of the requisite reagents and instruments but lack skilled staff. Other laboratories can amplify DNA but are unable to report the necessary results in time. Furthermore, many laboratories neglect accreditation, quality assurance and quality control programs. For these reasons, infectious diseases in most developing countries remain unknown and uncharacterized [5]. The Centers for Disease Control (CDC) has initiated various strategies in developing countries that require clinical microbiology laboratories to provide accountable data as part of an active surveillance of infectious diseases. However, clinical microbiology laboratories have not been recognized as a priority by government bodies in developing countries. As mentioned above, part of the problem is associated with a lack of skilled laboratory personnel and prohibitive laboratory maintenance costs where resources are available but inappropriately used [6]. This manuscript discusses the role of molecular techniques in clinical microbiology and epidemiology and the various issues required to establish molecular laboratories in developing countries.

The role of molecular techniques in clinical microbiology and infection control programs

Conventionally, the function of a clinical microbiology laboratory is to identify various pathogens using direct examination of microbes and culture-based methods. At present, nucleic acid amplification and DNA probes are useful for the characterization of microbes because cultured-based methods are insufficient, expensive and tedious. Typically, nonamplified DNA probe-based methods are suitable for in situ hybridization of organisms when the location and distribution are ascertained, including slow growing microbes such as *Neisseria gonorrhoeae* and *Mycobacterium tuberculosis*. Pathogenic dimorphic fungi have also been detected radiometrically. Many DNA probe methods are commercially available for the identification of various microbial species, such as *Chlamydia trachomatis*, *Legionella pneumophila*, *Trichomonas vaginalis*, *Candida* species, and human papillomavirus (HPV). In addition to the benefit of the confirmation of culture methods, DNA probes are useful for identifying enterotoxin-producing strains of *Escherichia coli* because these strains are biochemically similar to other non-enterotoxigenic *E. coli*. Although traditional DNA probe methods have a lower sensitivity than nucleic acid amplification techniques, DNA probes are useful for the direct identification of clinical specimens in certain situations. For example, DNA probes are used to identify group A streptococci in strep throat infections and *C. trachomatis* or HPV in cervical infections. Furthermore, the high level sensitivity of nucleic acid amplification makes it an ideal choice for the direct detection of specimens. However, expensive molecular procedures should not replace cost-effective conventional methods that are rapid, sensitive and candid. For instance, the use of shell vials and monoclonal antibodies for the diagnosis of cytomegalovirus viremia in an immunosuppressed bone marrow transplant recipient is an excellent method with high specificity and sensitivity and results that are available within 16 hours. Thus, it would be unfavorable to disregard this method and replace it with costlier nucleic acid amplification methods without additional documentation of the substantial benefits of the latter methods.

When considering the correct molecular method to be used in the laboratory, careful and firm decision-making should be directed toward the potential benefits of modern methodology. Decisions should not be made based solely on the cost-effectiveness of the molecular method. Instead, the impact of the method on the clinical practice and patient management should also be considered. For instance, direct detection of methicillin-resistant *Staphylococcus aureus* and *M. tuberculosis* in a patient's specimens would result in quicker patient isolation, better targeted therapy and overall cost savings. In other situations, microbes can be extremely fastidious, difficult to culture and hazardous to laboratory staff. These microbial samples are often sent to referral centers; however, fragile microbes could lose their viability, become overgrown or even become contaminated during the transferal process. Often, a sample with a limited volume submitted to the laboratory becomes impossible to culture. In such cases, accurate usage of the sample is needed to culture all pathogens. In many resource limited countries, biosafety level 3 laboratories may not be available or may be economically inappropriate to culture pathogenic microbes [7]. Molecular techniques have resolved many of these problems by identifying microbes more rapidly and accurately than conventional techniques. For instance, more than 100 genotypes of HPV have been found using DNA sequence heterogeneity as the basis for investigation.

It is important to remember that despite their sensitivity, accuracy and speed, nucleic acid amplification methods cannot totally replace conventional culture and serologic methods because nucleic acid amplification and conventional methods produce different results. Nucleic acid amplification methods detect the presence of DNA or RNA in a particular specimen, but they cannot demonstrate the viability of an organism or determine whether the organism is part of the infectious process. Furthermore, nucleic acid amplification methods are prone to errors, and large amounts of amplified products can lead to momentous cross-contamination and cause false positive results. Conventional methods such as culture-based techniques validate the viability of an organism, and quantification of the antibody titer strongly suggests its involvement as part of the infectious process. Commercial DNA probes have brought molecular techniques into the field of clinical microbiology and have been used for several years. Today, most of the procedures have been standardized and are frequently simpler than target amplification assays. Additionally, the advent of the synthetic short oligonucleotide DNA probe has reduced the time required for hybridization assays. The niche for DNA probe technology in clinical microbiology appears to be in situ hybridization for tissue specimens and culture conformation of

slow-growing microbes. Although DNA probes are more expensive than other biochemical tests, their rapid turnaround time can be translated into overall savings by providing a timely diagnosis. Using standardized methodology, DNA probes can broaden the various types of pathogens that a microbiology laboratory can routinely identify. The three different formats commonly used today are solution-phase hybridization, in situ hybridization and solid-phase hybridization. At present, the nucleic acid amplification technique is routinely used in numerous microbiology laboratories. This technique improves the sensitivity of assays based on nucleic acids and can simplify assays by automating and incorporating non-radioactive detection formats. The technology behind the nucleic acid amplification method is diverse and in constant flux. There are three different categories: (i) target amplification by PCR, which relies on self-sustaining sequence replication or strand disruption amplification; (ii) probe amplification including a ligase chain reaction; and (iii) signal amplification. All of these techniques are currently used to varying degrees, and many have been cleared by the American Food and Drug Administration (FDA). A detailed account of all of the molecular methods used in clinical microbiology laboratories is beyond the scope of this manuscript. However, some commercially available molecular assays for various infectious agents are discussed in Table 1.

Molecular typing techniques have greatly facilitated epidemiologists and infection control specialists. The main purpose of an infection control program is to identify possible infection problems and monitor infection trends. Infection control programs depend on cooperation from microbiology laboratories, which must provide easy access to high-quality data and guide the use of resources for epidemiological purposes. Furthermore, laboratory findings are used to support epidemiologic evidence of the spread of common microbes and determine the modes of transmission [8]. Molecular typing techniques have been successfully used in microbiology laboratories for the epidemic surveillance of various pathogens [9]. Several molecular typing methods have been proposed, such as pulse field gel electrophoresis (PFGE), ribotyping and nucleic acid amplification-based typing schemes. Currently, pulse field gel electrophoresis is widely used for the molecular typing of various bacterial species. This technique allows investigators to separate larger pieces of DNA using restriction enzymes and has been used to characterize bacterial species from different geographical regions. This technique has better discriminating power than other typing schemes, such as ribotyping and multi locus sequence typing based on DNA amplification reactions. The results can be visualized with a dendrogram generated by matrix pair differences between the electrophoretic types. Despite PFGE being considered by analogy as a 'gold standard' by many investigators, many strains cannot be typed due to degradation of the DNA in the gel. Moreover, the apparatus and the specialized chemicals are expensive, time consuming and require skilled technicians. PFGE has a lower resolution power than multi locus sequence typing (MLST), which is a nucleic acid amplification-based method, and as such it is widely accepted as 'the typing success of the decade' because it can be applied to bacteria and other microbes. This technique can be used to study the evolutionary relationships between bacteria due to its high discriminatory power. MLST is portable, and unlike PFGE its results can be usefully shared between laboratories. A huge number of microbes have been studied under these two schemes [10]. Moreover, MLST requires a normal thermocycler for amplification that can also be used for many other direct detection assays and for the identification of various genes, while MLST requires an expensive nucleic acid sequencing apparatus. However, post-amplification products can also be sequenced using commercially available sequencing apparatuses by paying for just the cost of the reaction. To date, various molecular typing techniques have been used to map various epidemiological traits. However, the selection of these techniques depends on the aim and objectives of the particular epidemiological study. This is especially true in developing countries or regions where resources are limited and skilled personnel are in demand.

New molecular technologies

Currently, the application of nucleic acid amplification and other molecular platforms is limited to large laboratories and referral centers. The ability to use these techniques efficiently is another important factor that determines how large a role these techniques will play in an average microbiology laboratory. There is a growing need for newer technologies that are rapid, cost effective and suitable for low resource countries.

Real-time droplet PCR

Because PCR requires thermal cycling, it is typically performed in a bench top unit. This process is slow, expensive, error-prone due to contamination and

Table 1 FDA approved commercially available molecular assays.

Organism	Specimen	Assay	Method	Target	Sensitivity (%)	Specificity (%)
Human papilloma virus (HPV)	Cervical cytology specimen	Digene HC2 HPV DNA test	Hybridization protection assay using microplate chemiluminesence hybrid capture of DNA—RNA hybrid.	RNA probe cocktail for 13 h types	93.0	61.1
Chlamydia trachomatis	Endocervical and vaginal swabs (female); urethral (males) swabs	APTIMA Assay for *Chlamydia trachomatis*	Transcription mediated amplification	23S rRNA	95.6	98.8
Neisseria gonorrhoeae	Cervical and vaginal swabs (female); urethral (males) swabs	AMPLICOR CT-NG for NG	PCR	*M.NgoPII* putative methyl transferase gene of NG	95.9—96.5	98.7—97.3
Human immuno-deficiency virus-1 (HIV-1)	Plasma separated from blood collected using EDTA	COBAS AmpliPrep/COBAS TaqMan HIV-1 test and COBAS AmpliPrep/COBAS TaqMan	Real-time reverse transcriptase-PCR	*Gag* gene	48—10,000,000 copies/mL (HIV-1 group M)	
Herpes simplex virus (HSV)	Vaginal lesion swab	MultiCode RTx HSV 1&2 Kit	Real-time PCR using isoC:isoG synthetic DNA base pair technology	*Glycoprotein* gene segment of HSV-1 and HSV-2	HSV-1, 92.4 HSV-2, 95.2	HSV-1, 98.3 HSV-2, 93.6
MRSA/SA (screening, surveillance)	Lesion swab from skin/soft tissue	Xpert MRSA/SA and GeneXpert System	Real-time PCR	Staphylococcal protein A (*spa*), the gene for *MecA*-mediated oxacillin resistance (*mecA*), and SCC*mec* inserted in the SA chromosomal *attB* site	MRSA positive with culture 93.8 SA positive with culture 95.7	MRSA negative with culture 97.3 SA negative with culture 89.5
Clostridium difficile	Stool sample	Xpert *C. difficile* and GeneXpert	Real-time PCR	*C. difficile tcd*B	93.5	94.0

Table 1 (*Continued*)

Organism	Specimen	Assay	Method	Target	Sensitivity (%)	Specificity (%)
Enterococcus	Rectal swabs	Xpert *vanA* and GeneXpert System	Real-time PCR	Gene sequences for *VanA*-encoded resistance to vancomycin-teicoplanin	98.0	81.1—90.0
Organisms causing sepsis: yeast	Smears made directly from yeast-positive blood cultures	*Candida albicans* PNA FISH	PNA FISH species-specific probes	16S rRNA of *C. albicans*	100	100
Group A *Streptococcus*	Throat swabs	GAS Direct Test	Hybridization protection assay	rRNA	91.7	99.3
Mycobacteria	Growth from solid media or broth	AccuProbe *M. avium* AccuProbe *M.intracellula-re*	Hybridization protection assay	rRNA	99.3 100	100 100
Respiratory virus panel: influenza A and B and RSV	Nasopharyngeal swab, cultured clinical specimens	Verigene Respiratory Virus Nucleic Acid Test	Multiplex RT-PCR	Influenza A *matrix* gene Influenza B *NS* and *matrix* genes *L* and *F* genes of RSV	Influenza A, 99.2 Influenza B, 96.8 RSV, 89.8	Influenza A, 90.1 Influenza B, 98.5 RSV, 91.5
Enterovirus	CSF	Xpert EV and GeneXpert System	Real-time PCR	Consensus region of enterovirus, 5′ UTR between nucleotides 452 and 596	96.3—100	97.0—97.2
Mycobacterium tuberculosis	Sputum and bronchial specimens	AMPLIFIED MTD Test	Transcription-mediated amplification, hybridization protection assay	Mycobacterial 16S rRNA	96.9	100
Hepatitis B Virus quantitative	EDTA plasma or serum	COBAS TaqMan HBV Test	Real-time PCR	Core—precore region of the HBV genome; primer pairs to genotypes A—G of HBV and the precore mutation	20—170,000,000 IU/mL	

requires a large amount of sample to perform the reactions. Utilizing the concept of both spatial and temporal microfluidic PCR, droplet-based microfluidic PCR formats are emerging as alternatives to single-phase PCR. Droplet PCR formats have many advantages, such as low reagent usage, faster thermal cycling and limited interactions of DNA and polymerase with the channel walls. Carryover contamination can be reduced using these formats, resulting in high throughput and sensitivity. Droplets are discrete small volumes that can be adapted for parallel amplification in small concentrations. There are a number of pressure-driven flow systems that use microdroplets, nanodroplets, and picodroplets to perform both PCR and reverse transcriptase PCR. Many commercially available products based on real-time droplet PCR have various applications, such as absolute quantification, rare gene identification, genomic alterations, and single cell analysis. A few studies have reported the use of droplet PCR for the diagnosis of infectious disease agents such as *C. trachomatis* (73.3% sensitivity) and influenza subtypes (96% sensitivity and 100% specificity). This technique can be adopted in small clinics and average laboratories [11,12], especially in resource limited countries.

Mass spectrometry

Mass spectrometry (MS) was recently introduced as a new diagnostic technique in clinical microbiology with the first successful application in detection and sequence-based identification of PCR products. Based on MS, matrix-assisted laser desorption/ionization (MALDI) coupled with time of light (TOF) allows the analysis of various biomolecules in microbes. This method is very effective and requires less intense sample preparation because the matrix is less susceptible to interference from salts and detergents. MALDI-TOF is an extremely useful technique for the identification of many microbial species because the major mass profile is generated from ribosomal proteins that align perfectly with current taxonomic classifications. For epidemiological typing studies of bacterial isolates, mass peaks that are specific to a given strain can easily be identified and used to develop a binary typing system. Furthermore, substantial resistance mechanisms can also be identified. Cellular modifications under the influence of antibiotics can also be detected, as was recently demonstrated by the interaction between *Candida albicans* cells and fluconazole and *Candida* and *Aspergillus* species and caspofungin. Additionally, MS can be used to identify PCR products derived from resistance genes. The iPLEX MassArray developed by Sequenom is a very good example of this extensible technology. However, there are many opposing issue with this diagnostic MST. The assays typically require 10^4–10^5 cells, which are infrequently detected in patient specimens except urinary tract infections. Moreover, the equipment is very expensive and needs expensive maintenance [13].

Nucleic acid sequencing

DNA sequencing is already used in many diagnostic, typing and research studies and is particularly useful for bacterial identification based on 16S rRNA sequences. Previously, this method was employed to identify short nucleotide sequences of PCR-amplified products. However, with advances in technology it now is able to identify complex genome mixtures of bacteria in a process called 'metagenomics' and can generate complete catalogues of genomic components of the bacterial species present in a variety of clinical specimens. Although at present, it remains unclear whether this technology is suitable for routine usage in microbiology laboratories, metagenomics is essential for understanding the effects of antibiotic treatment of the human microbiome as a whole. Furthermore, next-generation sequence analysis can facilitate testing of host susceptibility toward infection and colonization if the high cost of the apparatus is considered to be a subsidiary issue for the circumstances of each individual case. This current basic research development will become more common in diagnostic laboratories in coming years [14].

Microbial volatile organic compound detection

Microbial volatile organic compounds (MVOCs) are defined as a variety of compounds formed during metabolism in bacteria and fungi. To date, more than 200 different MVOCs have been identified, and the diagnostic relevance of MVOCs has been described in the literature. However, difficulties in the reproducibility of diagnostic assays and the concentrations of MVOCs cannot be overlooked [15]. The most widely used MVOC detection technique is the micro-weighing technique using vibration methods, gas chromatography and metal chip electrical conductivity. This technology has been developed based on the design of a system that allows the kinetic measurement of MVOC production by growing bacteria; indeed, several bacterial species are identifiable on the basis of their MVOC production

profile. For instance, a study was conducted in which the diagnostic specificities varied from 100% for *C. difficile* to 67% for *Enterobacter cloacae* [16]. This finding suggests that the diagnosis capability of this technique can be developed in the future for use by clinical microbiology laboratories.

Raman spectroscopic analysis

The most evident contribution of spectroscopy to clinical microbiology has been in microbial epidemiology. In recent years, Raman spectroscopic analysis (RSA) has been validated for the bacterial typing of different species. Although RSA has been proposed to be capable of distinguishing virtually all bacterial species, definitive data are missing. RSA is a label-free, optical technology based on the non-elastic scattering of light by the molecules. The change in wavelength is molecule-specific and can be displayed in a Raman spectrum. These changes can be seen as spectroscopic fingerprints and can be used to visualize the comprehensive molecular composition. Several studies concluded decisively that the typing of different strains belonging to a single species can be performed appropriately. The molecular typing of various species such as *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus*, and *Klebsiella pneumoniae* produced similar results that were equal in quality to other gold standard techniques [17].

Nanoparticles

A variety of methods have been introduced into clinical microbiology over the course of the last decade. These methods clearly meet some diagnostic standards and development is continuing. Moreover, many new technologies are making their way into diagnostic microbiology, such as cell-based biosensors in the area of 'nanodiagnostics'. This technology uses cells as biochemical detectors with high sensitivity and specificity. Furthermore, there has been an emergence of 'theranostics', which appropriately combines attributes of diagnostics and therapy to fight against infection. Recently, silver nanoparticles (a noble metal known to exhibit strong toxic effects against microbes and less toxicity to humans) have been tested. A recent study [18] using electron microscopy revealed 'pits' in the cell walls of *E. coli* and other microbes that were caused by the accumulation of silver nanoparticles. These 'pits' resulted in the alteration of membrane morphology and increased membrane permeability, leading to cell death. Thus, this study clearly exemplifies the role of nanoparticles in circumventing the forthcoming threat of bacterial infections.

Although there are numerous benefits of molecular techniques, the advantages of many conventional methods should not be overlooked. Currently, a well-equipped microbiology laboratory should have both reliable conventional methods and new molecular techniques, and staff should focus on issues such as cost, performance, durability and maintenance.

Laboratory facilities in developing countries

The quality of the laboratory service depends upon various fundamental strategies, including adequately trained laboratory staff, management of the physical laboratory infrastructure, good laboratory practices, strict quality control, quality assurance programs, and investment into new and improved technology [19]. However, in many developing countries there is scarcity of basic policies required for a quality functional laboratory. Laboratory settings without proper infrastructure pose the greatest challenge for adequate performance. A lack of clean water and inadequate supply of electricity and cold storage immensely limit the quality of laboratory procedures [20].

Laboratory management

The management of a high-quality clinical microbiology laboratory is crucial for the control of hospital and community acquired infections. Due to the lack of expertise in managing clinical microbiology laboratories in developing countries, there should be international guidance or training for the staff; if possible, training programs should be conducted by high-quality laboratories from developed countries. Careful review of the available resources, foundation and facilities would expedite the available support required. Furthermore, visiting various established laboratories that perform identical work can provide important information [20]. Evaluation and accreditation of the laboratory is also important; however, this process is dependent on the overall cost and available funding. Laboratory administration should take care of their internal staff and external communication systems. The staff should receive medical evaluation in case of emergency, and medical records maintenance should be performed appropriately [7]. Effective communication between the laboratory and health care providers should be supported, especially with the physician. Because the shared

information is both qualitative and analytical, adequate provision for bidirectional interactions should be maintained. Telephone, email, facsimile and central laboratory information systems are useful for documentation [21].

Laboratory networking systems

Networking between laboratories may increase infection surveillance within a huge geographical region. The WHO supports the establishment of national networks for the regular exchange of information and proper support for the laboratory. However, only a few countries have national and international laboratory networks [22]. The establishment of an integrated laboratory system is very important to combat many infectious diseases. However, integrated systems are financially neglected in developing countries. Strengthening laboratory systems in developing countries requires effective strategic plans involving (a) legal and policy framework, (b) institutional and management framework, (c) human resources, (d) laboratory quality management, (e) evaluation and monitoring, (f) maintenance of equipment, and (g) laboratory structure and design [23].

Laboratory biosafety and risk assessment

Clinical microbiology laboratories expose their staff and the public to various clinical samples, which may include various biohazards such as infectious diseases. Therefore, it is important to develop a biosafety management program or operational plan for the laboratory with regular training schemes, thereby ensuring the use of standard practices and procedures. Laboratory facilities are designed to meet certain biosafety levels that have designated constructional designs, containment accessibilities, equipment, operations and procedures that are employed for risky biological agents. Sample collection, transportation, handling, labeling and identification should be performed according to WHO recommendations [24]. A laboratory biosafety plan should be maintained to reduce any accidental release of biohazardous material. The comprehensive plan should include working guidelines, primary care designs such as protective equipment and biosafety cabinets, and secondary care designs including laboratory facilities. Furthermore, the biosafety plan should promote the prevention of laboratory-associated infections and environmental protection. A biosafety committee should be established to gauge the success of the biosafety plan by implementing and enforcing laboratory protocols and encouraging strict adherence to safety guidelines. Because the identity of infectious agents remains unknown during the initiation of microbial investigations, biosafety level-2 is recommended for clinical microbiology laboratories [25,26].

Laboratory accreditation

Medical laboratories are regulated by government bodies and strengthened by the standards validated by other professional organizations. Accreditation is an assessment process performed by an authorized body to ensure quality control and the assurance of the laboratories. This process provides formal recognition that the laboratory can perform quality experiments. Laboratories testing human samples for the diagnosis, prevention or treatment of diseases are within the scope of the assessment [27]. All of the accreditation regulations are developed through consensus, collaboration and consultation of health care professionals and health care authorities. The laboratory standards are monitored by on-site inspection, management requirements for patient testing, and follow-up actions. Although there are differences in accreditation service procedures and programs, the overall assessment remains the same [28]. The international organization for standardization (ISO) is a global organization for national standards that is comprised of 140 members across the globe. The International Accreditation Forum (IAF) and the International Laboratory Accreditation Cooperation (ILAC) deal specifically with laboratories. The ILAC commissions tasks local bodies such as the Asia Pacific Laboratory Accreditation Cooperation (APLAC) in Asia using ISO standards [29]. The standard guidelines for regulating quality laboratory management are included in ISO 15189:2007, which can be obtained from the ISO website (http://www.iso.org/iso/catalogue_detail?csnumber=42641).

Laboratory design

The establishment of a clinical microbiology laboratory in low-resource countries requires the following strategies: (a) requirement for physical infrastructure, (b) various assays, (c) supplementation of reagents, (d) provision of equipment and maintenance, and (e) dedicated working

stations. In developing countries, these key points are usually overlooked due to other issues. The main requirement for designing a microbiology laboratory is a clean and well-controlled environment [30]. Surfaces contaminated with dust and aerosols and humid areas will be hazardous for the microbiological procedures. Mechanical ventilation systems providing inward flow of air are recommended to provide containment zones with increased negative pressure. The reliable supply and maintenance of gas and a standby electricity generator are preferred. Special attention should be paid to the huge volume of microbes, overcrowding equipment, infestation and unauthorized entry. Other laboratory features must also be addressed, such as dead space areas with no airflow and low ceilings that prevent the exclusion of contamination during microbiology procedures [26]. Waste pipes, drainage, and ventilation systems managing contaminated material should be maintained efficiently. Even building material, such as a leaking roof that causes moisture, could be sources of contamination. The location of heavy equipment should facilitate the cleaning of the laboratory walls and floors or may be placed on mobile benches [31]. Illumination should be appropriate for the safe conduct of laboratory work. Storage should be appropriate for all supplies, reagents, hazardous chemicals and radioactive materials. A holding zone for laboratory garments such as aprons and lab coats should be placed outside or possibly at the entrance of the laboratory. Hand hygiene basins should be provided at the entry and exit points with alcohol rubs. Eating and drinking should not be permitted within the laboratory because these activities may serve as the main source of contamination. If possible, every room in the laboratory should have hand glove stations and medical waste collection bags. Doors should have vision panels, be self-closing and have fire ratings [32]. Although there is no special guideline for the manner in which the space in a laboratory is used, an area of 350 ft/person within the laboratory would enable worker safety [26]. The high risk of specimen—specimen contact when performing PCR-based assays requires specialized set-up. Four different physically separated working areas are recommended, including an area for reagent preparation, PCR master mix preparation, nucleic acid processing and an amplification area. All of these working spaces should be kept free of all other patient specimens [2]. Post-amplification and ultraviolet (UV) photography should be performed in specialized rooms with a UV containment area. The use of UV aprons should be made mandatory within this area. The equipment used within UV area should not be used outside of the area.

Laboratory equipment

In resource-limited settings, there is lack of decentralized molecular diagnostic testing and sparse access to centralized medical facilities. Because many molecular technique apparatuses are expensive to install in average microbiology laboratories, cost-effective equipment can be used. Portable molecular technologies especially designed for developing countries are available; for example, portable nucleic acid thermocyclers are battery powered and cheaper than bench top units. These portable thermocyclers can perform PCR, reverse transcriptase-PCR and many formats of isothermal amplification, including nucleic acid sequencing-based amplification, strand displacement amplification, rolling circle amplification, loop-mediated isothermal amplification, and helicase-dependent amplification. There are valuable applications of these portable thermocyclers in diagnostic microbiology and biothreat detection. Various infectious agents have been identified using these portable apparatuses, such as HPV, *N. gonorrhoeae*, *C. trachomatis*, *Treponema pallidum* and *T. vaginalis* [33]. Several varieties of portable apparatuses are commercially available; important evaluation criteria for each technology include maturity, power requirements, cost, sensitivity, speed, and manufacturability. Ultimately, the needs of a particular market will lead to user requirements that drive the decision between available technologies.

There is a serious issue with instrument maintenance in developing countries because the lack of functional equipment will decrease the efficacy and quality of the laboratory procedures [22]. For quality assurance, the facility should have documented procedures for the maintenance, calibration, verification and validation of all equipment. Various successful infection control programs have come to a halt in a number of developing countries due to the lack of maintenance and service of the laboratory equipment. Equipment failure and breakdown delays laboratory output; thus, equipment maintenance should be given priority. Selection of equivalent brands is important and depends on the service provided by the local dealer [19]. Sometimes it is wiser to buy simple instrumentation that is user friendly and less expensive rather than opting for automated instruments. Sophisticated electric equipment must also be avoided

in developing countries unless it is essential due to the irregular supply of electricity and the high cost.

Furthermore, the laboratory staff should receive on-site training for equipment by the manufacturer [34]. Thermocyclers are important instruments for molecular microbiology laboratories; any change in the performance of thermocyclers will directly affect the precision of the tests. Determination and documentation of cycling time or any errors should be recorded; fluctuations in the cycling times should serve as warnings and be adjusted. Additionally, chiller, heater and block temperature adjustments should be performed according to the manufacturer's recommendations [35]. Pipettes and micro-pipettes are used in almost every step of routine procedures; therefore, special attention should be paid to their usage because they are the prime source of errors. Pipette calibrations should be performed regularly using recommended guidelines. Special care should be given to the PFGE apparatus, with proper cleaning of the tank performed before and after every run and the electrodes changed regularly. Most molecular instruments are expensive. Recently, a reagent rental agreement program has been introduced as a means to acquire molecular instruments; however, the availability of this program depends upon the manufacturer [36].

Laboratory practice

General laboratory practice requires careful attention, including regular cleaning and strict monitoring of all methods performed. The practice code is a list of the most essential procedures used in basic good microbiology practices. It is important to develop written laboratory practices and methods for safe laboratory operations. International biohazard signs must be displayed on the laboratory door if the laboratory is working with isolates from risk group 2 or above. Only authorized personnel should be given access, and laboratory doors should always be closed. All of the surface areas should be cleaned before and after usage. Working stations, tables, refrigerators and reagent containers should be regularly swabbed with 70% ethyl alcohol [37]. The use of disinfectants recommended by the environmental protection agency should be promoted. Cleaning should be performed on all surfaces, benches, racks, tiles, rockers and wherever a spill occurs. For molecular procedures (especially PCR and PFGE), each working area should contain dedicated reagents, materials and pipetting devices. Reagents should be prepared in small aliquots to avoid contamination [3].

Laboratory personnel requirement

Increasing the number of well-trained and skilled personnel is a major limitation in developing countries. Although there is a steady demand for appropriate and adequate workers, governmental policies restrict the flow of skills. Laboratory personnel should be trained with all safety measures. Updating staff about the control measures for laboratory biohazards is a key to preventing laboratory-acquired infections. An effective laboratory program should be introduced by integrating safe laboratory practices into the basic training of the personnel [38]. Training should include all safety measures for handling hazardous materials, such as aerosol inhalation, ingestion of specimens, percutaneous exposure to sharp instruments, and handling and disposal of hazardous material. The skills required for molecular techniques are more precise and include micro-pipetting, DNA handling and disposal, software manipulation during analysis of the results and reporting [26]. Personal protective equipment should always be used as a first line of safety:

a) Protective laboratory coats must be worn at all times inside the laboratory.
b) Hand protection should be followed by wearing disposable gloves made of nitrile or chloroprene.
c) Eye and face protection should be made compulsory whenever a splash or spill occurs.

Waste management

In most low-income regions and especially in Asian countries the management of medical waste is a severe problem; only a handful of centers can process medical waste efficiently. Waste generated by microbiology laboratories in the form of specimen samples, culture plates and tubes all carry a high potential risk of infection and need to be managed effectively. Most of the biomedical waste produced should be decontaminated, discarded or incinerated [39]. Non-infectious materials such as glassware are recyclable and can be sterilized. Decontamination should be performed by steam autoclaving. Contaminated sharp wastes, such as hypodermic needles, disposable knives, and syringes, should be discarded in a puncture-proof bag [32].

Prospective instructions

Due to the lack of financial resources in developing countries, partnerships between public, private and commercial sectors should be maintained in order to build a well-equipped laboratory. Funding bodies should address the financial inequity, and an appropriate balance should be encouraged that emphasizes laboratory infrastructure and management. There should be a focus on accurate identification and the use of highly reproducible methods. The funding bodies should target long-term training and education of future laboratory personnel. There should be improved communication between the laboratory and clinicians, which could affect the accuracy of disease treatment. Policy makers and health care personnel must understand that accurate and rapid identification are important for the prevention and treatment of disease. It is very important to maintain a well-maintained clinical microbiology laboratory using current knowledge to control infections. Building a molecular microbiology laboratory facility to provide rapid, accurate and reliable tests will help health care staff to deliver more effective treatment regimens, thereby reducing mortality, overall expenditure of health care resources and improving the quality of health care services in developing countries.

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