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Molecular Diagnosis in Resource-Limited Settings

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Abstract

The advantages of molecular testing for accurate diagnosis and optimal therapeutic management of patients infected with microorganisms is well established, yet many test methods are not feasible in underdeveloped or resource-limited settings. The disparity in testing methods is controversial from ethical, financial, and scientific perspectives. Over the years, philanthropic funding for early and accurate diagnosis has helped curtail the spread of disease and improved overall survival, yet infections caused by microorganisms are still the leading cause of death in the resource-limited parts of the world. Fortunately, there are promising new tests that are inexpensive, portable, easy-to-use, and practical at even small, rural, and remote health centers. This review describes the potential problems encountered in the routine implementation and performance of molecular assays in resource-poor settings and discusses the adaptations and alternative methodologies that are routinely used in most microbiology laboratories in such settings.

Introduction

There are several advantages to making a specific diagnosis of an infectious disease. In addition to better patient management with appropriate therapy, preventive measures can be initiated in a timely manner to make health care more cost-effective. Early and accurate diagnosis of infectious disease is important, not only for prompt treatment, but also to limit the spread of disease and avoid the waste of resources on ineffective treatments. Over the years, the molecular diagnosis of infectious diseases has become the standard of care for the identification and diagnosis of viral infections and several bacterial infections. The advantages of molecular testing for accurate diagnosis and optimal therapeutic management of patients infected with microorganisms is well established, yet many test methods are not feasible in underdeveloped or resource-limited settings. The disparity in testing methods is controversial from ethical, financial, and scientific perspectives [1]. In these setting, clinicians are often forced to rely on clinical diagnosis rather than diagnostic testing [2], which is not always an option for diseases such as meningitis [3] and ocular disease [4], for HIV viral-load monitoring [3,5], or for many other situations. Over the years, philanthropic funding for early and accurate diagnosis has helped curtail the spread of disease and has impacted overall survival [6-8], yet infections caused by microorganisms are still the leading cause of death in the resource-limited world [1,8].

On most continents, molecular testing for infectious agents has benefited from the accelerated pace of technology that has driven the field. The application of nucleic-acid-based technologies for amplification of targets and signals using specific primers and probes has revolutionized the identification and quantification of microorganisms with rapid, robust, and reliable assays that are routinely used in clinical microbiology laboratories. Molecular tests represent a powerful strategy for screening, early and accurate identification of pathogens, and monitoring the efficacy of intervention.

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For example, screening tests for human papillomavirus (HPV) continue to gain utility in the identification of women at risk for cervical cancer [9], and the recent need for remote Ebola virus testing was addressed by a molecular assay [10], as well as commercial multiplex assays [11-13]. The quantification of viruses, or "viral load," changed the management of transplant recipients with molecular assays that quantify cytomegalovirus CMV [14]. Patients infected with viruses such as HIV and hepatitis B virus (HBV) also benefit from viral-load testing, which drives their therapy [15-19]. From a screening perspective, rapid HIV testing is now the standard of care in most countries, and point-of-care testing for HIV is one of the few assays that have become accessible even in rural areas [15-18]. Concurrent identification of drug resistance genes continues to show promise, particularly the identification of mutations that are responsible for drug resistance in bacteria such as *Mycobacterium tuberculosis* [6,20-23] and drug resistance mutations in HIV [17].

Challenges for Assay Performance in Resource-Limited Settings

Facilities in developing countries frequently lack access to equipment and innovative technologies for rapid, reliable, and reproducible test results. Consequently, resource-poor countries are limited in the type of molecular assays that can be practically implemented as standards of care for patients. Unfortunately, it is these resource-poor settings where infectious diseases predominate and form the nucleus for potential global threats. These challenges have encouraged laboratorians to perform molecular laboratory testing for infectious diseases in a practical manner suitable to the financial, environmental, and health care needs of all countries.

Cost-prohibitive testing

While the applications of molecular assays in diagnosing infectious diseases have increased exponentially, the cost of performing these assays has not declined in a proportional manner; therefore, testing can be cost-prohibitive in some resource-limited settings. Some of the factors that are responsible for the high costs of performing these assays are reagents, including costly enzymes and fluorescent dyes; sophisticated instrumentation that needs to be housed and maintained in controlled settings; properly engineered testing facilities; and trained personnel to perform the assays. These major and other, minor issues have restricted the performance of such molecular assays in the parts of the globe that are economically constrained. Moreover, creating and maintaining infrastructure to adequately support supply chains, storage needs (temperature, humidity, etc.), sales, and service networks are quite challenging in some settings. Some suppliers provide larger discounts to global, resource-limited laboratories.

Specimen collection and transport

Pre-analytical aspects of microbiology, such as specimen collection and transport, are among the first vital steps in any laboratory test method, and maintaining pre-analytical variables can be a significant challenge in resource-poor settings. While it is important to collect the specimen using sterile equipment (such as swabs) and containers to protect the sample from contamination by extraneous environmental sources and normal flora, even sterile supplies can be limited or unavailable. Nucleic acid-based tests are extremely sensitive and are able to detect microorganisms that may be present in a non-sterile or improperly sterilized container.

Specimen transport is a major concern. The specimens used for molecular testing for infectious diseases range from whole blood to plasma to body fluids, swabs, and occasionally tissues. Transportation of the specimen in a timely manner and in an appropriate transport medium is important to prevent degradation of nucleic acids or overgrowth by normal flora that may actually be contaminants.

Since regions of the world where infectious diseases are more rampant generally have a tropical climate with higher than average heat and humidity, transportation of specimens to testing sites may pose a challenge. Temperatures during transportation are crucial to maintain the integrity of nucleic acids, especially those from RNA viruses, which tend to be more unstable in transport than DNA molecules. When using whole blood as a specimen for the detection or quantification of viruses, the plasma should be separated within 4 to 6 hours to avoid degradation of RNA that can be caused by the RNases normally found in whole blood.

Transport media recommended for molecular testing are often designed to control the overgrowth of potential contaminants. In resource-poor settings, it is still important to collect a separate tube of blood for molecular testing. Problems can be averted by using several localized collection sites where specimens that are collected by trained personnel are transported regularly using a courier service. Likewise, local sites that can separate plasma or serum from whole blood prior to transport can lengthen the shelf life of the specimens. Sputum transport is undergoing assessment to optimize specimen transport for molecular methods in tuberculosis (TB) testing [24,25]. Self-collection of samples is also a common strategy for patients in remote areas, where medical staff are limited [26]. Finally, the PAXGene Blood DNA tube (PreAnalytiX GmbH) is a novel blood collection tube for whole blood specimens to extend the stability of nucleic acids. It is used for the collection, anti-coagulation, stabilization, transport, and storage of venous whole blood for preparation of high-quality DNA for use with molecular diagnostic test methods.

Personnel

Technologist training and certification is generally country specific, with guidelines and regulations set by clinical laboratory organizations that regulate clinical testing. Due to the complexity of molecular testing, it is recommended that technologists undergo training and certification, particularly in specific laboratory practices to avoid and monitor amplicon contamination and in handling of minute amounts of reagents/materials. Extensive training and competency is required, and much of this training in resource-limited settings must be achieved through online webinars and courses in cooperation with regulatory and governmental organizations. Over the past decade, global organizations that actively organize such educational efforts have improved the functionality of resource-limited laboratories; however, this is still a very challenging task [27,28].