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Diagnostics: Conventional Versus Modern Methods

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Authors' contributions

This work was carried out in collaboration among authors TWB and ARC. Both authors contributed equally to the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

There are many research have been done on the development of rapid and effective diagnostic methods. New modern diagnostic test is widely accepted because of the quick and effective test results, with in a short time duration which will help physicians to correctly diagnose the disease for treatment purposes, however cost of the test will increase because of specific equipment, expensive reagents and qualified staff to run the test. Some pathology labs still uses traditional methods because of cost effectiveness. The objective of an article is to compare conventional and modern diagnostic test to understand the demand of the community for the successful acceptance of modern diagnostic methods. Most considerable factors to decide suitable diagnostic methods are commonly cost effectiveness, specificity, sensitivity as well as the availability of qualified staffs. Currently, nucleic acid manipulation and testing are common test used in the scientific community. Promising approach involves advances in nanotechnology field that provide new directions to simplify assay procedures to make it user-friendly and enhance the detection sensitivity of the assay.

Keywords: Diagnostic methods; conventional methods; modern methods.

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1. INTRODUCTION

Infectious diseases are major cause of death despite of dramatic progress in their treatment and prevention. According to WHO, there is a reduction from one million in 2000 to 781,000 in 2009 in the estimated number of deaths for malaria cases. However, the number of people living with HIV worldwide continues to grow, reaching an estimated 33.3 million people in 2009 [1]. The main challenges to diagnose infectious diseases are the increasing demand and changing disease patterns which is the challenge for healthcare to diagnose the disease accurately.

An infectious disease can be caused by any microorganism including bacteria, virus, fungi or parasites. Recently, many diagnostic methods were developed to identify the causal pathogen in infectious diseases [2]. Interestingly, it was also reported that most often, infectious diseases is originated from animals [3].

Diagnostic methods have to be carefully performed in the shortest possible time, at the lowest possible cost with accurate specificity and sensitivity. Conventional diagnostic methods are mainly focus on the detection of pathogens (at a particular stage of lifecycle) with the help of a microscope. However, molecular diagnostic methods are slowly replacing or complementing the conventional methods [4]. Pathogens were identified, isolated and characterized to permit the implementation of optimal therapies and accomplished under the demands of limited time. Many infectious diseases are usually nonspecific in which they cannot be clinically distinguished from other infections. Thus, a definite laboratory confirmation is required. Specially, whenever there are cross-symptomatic infections, the importance of differential diagnosis is increased [5].

2. OVERVIEW OF CONVENTIONAL AND MODERN DIAGNOSTICS

The laboratory diagnosis of infection usually requires the demonstration, either direct or indirect of viral, bacterial, fungal or parasitic agents in tissues, fluids, or excreta of the host. Traditionally, detection of pathogenic agents mainly depends on microscopic analysis, the growth of the microorganisms and staining [6]. Identification of pathogen is mostly based on the phenotypic characteristics such as fermentation profiles of bacteria, cytopathic effects in the tissue culture for viral agents, and microscopic morphology of fungi and parasites [7]. These techniques are reliable but time-consuming. The trend of using genotypic-based tests such as the use of nucleic acid probes or rapid real-time PCR based assays are more common in laboratories [8]. PCR based assays have the advantage as small quantity of the specimen is sufficient to be analyzed and instant results will be produced by this technique. The precise handling of very small quantity combined with sensitive detection methods like electrochemical microarrays, predispose these technologies for point-of-care (POC) and lab-on-a-chip (LOC) diagnostics. This provides rapid and affordable diagnostics without the need of sophisticated and expensive laboratory equipment [9]. Diagrammatical representation of an overview of conventional and modern diagnostic methods is shown in Fig. 1.

3. CONVENTIONAL DIAGNOSTIC METHODS

Conventional diagnostic methods have battery of test to identify the causal organism. Conventional diagnostic methods are broadly categorized into four steps: first and the ‘gold standard’ test is the macroscopic and microscopic identification of the pathogen using stain to identify the basic morphology of an organism under microscope. Different staining procedures are used, such as Gram stain, fluorochrome stains, and immuno fluorescent stains, which help to narrow down the identification of an organism. The identification and characterization of the pathogen still needs further microbiological identification of an organism using culture and growth of pathogens on appropriate media. To culture bacterial or viral pathogens, an appropriate sample must be placed into the appropriate medium for growth or amplification. Thirdly, the biochemical methods are used to differentiate types of microorganisms based on their characteristics. Fourthly, immunological assays are used as a confirmatory test as based on the ability of an antibody to bind with high specificity to one or a very limited group of pathogens [6].

The phenotypic methods for the detection of pathogens are well tried, relatively affordable and available in the most hospital and private pathology laboratories. However, there are some limitations to these methods. Direct visualization or culture comparatively grows slower and has a lower sensitivity and specificity of the test. Cost constraints often limits the identification of an

organism by conventional methods to those thought to be of the greatest clinical significance. Traditionally, battery of biochemical tests used to identify an organisms often requires 24 hours. Performing rapid tests like staphylococcal and streptococcal latex agglutination tests, oxidase, and catalase can be time-consuming [10]. Staff reading plates manually takes more time and constantly interrupt the flow of the further biochemical tests, thus extend the process.

Furthermore, phenotypic variation can occur during a pathogen's lifecycle, for example, eggs, larvae and adult forms of a species may alter depending on the stage of development, the associated host or vector and whether the organism is free-living. Thus, antibodies or isoenzyme techniques have the limitation for the detection purposes and dependent on the stages of the life cycle. Host immune responses can be delayed, or remain persistent even after resolution of a previous infection.

Phenotypic methods can be used to discriminate between isolates, genera and species but when it comes to distinguish differences within the species, these approaches are less effective. Recently, the emergence of new pathogens, or the concern about bioterrorism; has brought an added urgency to the development of more efficient and rapid methods to detect the pathogens and predict their potential virulence [11]. For many non-life-threatening conditions/chronic diseases, this model has logistic, operational and economic merits, but when infectious diseases are considered, the conventional methods have serious flaws in terms of speed, timeliness, and communication of the results [12].

4. MODERN DIAGNOSTIC

Modern diagnostic methods have an advantage in those cases where pathogen cannot be identified by microscopic methods and

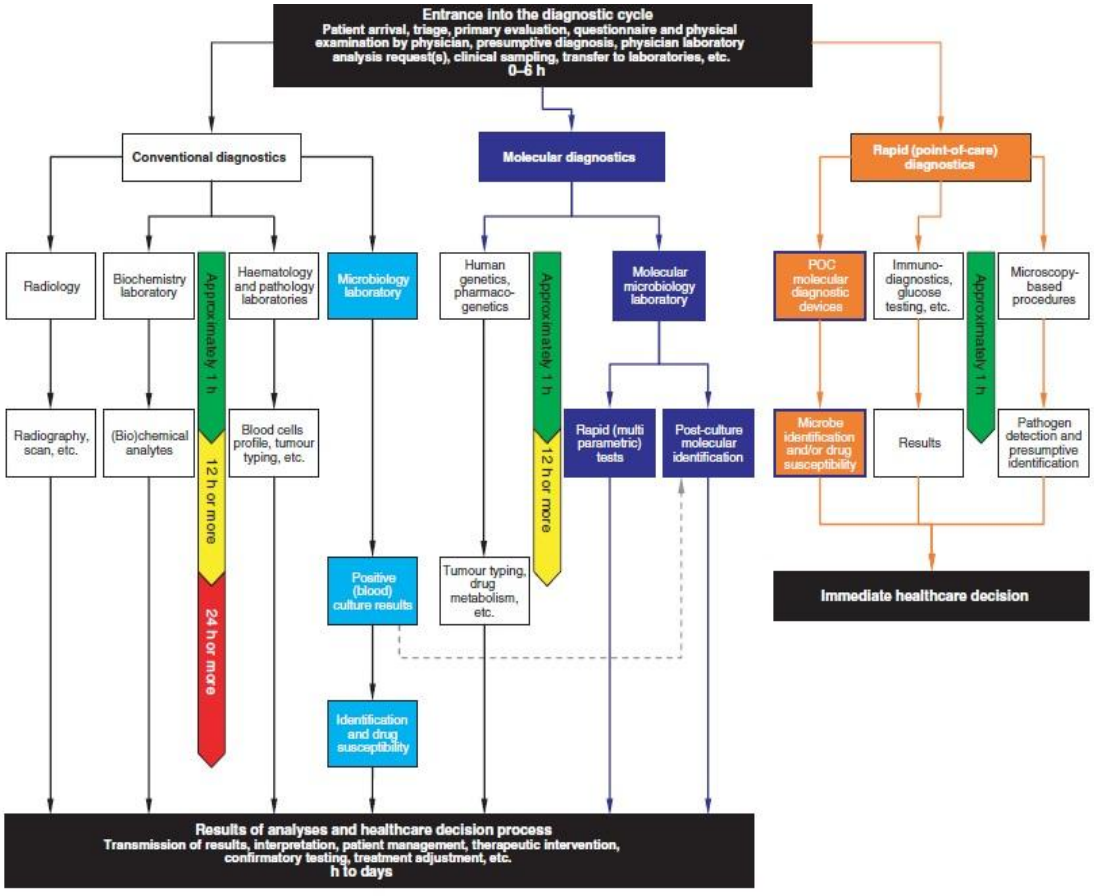

Fig. 1. Overview of conventional and modern diagnostic methods

pathogens cannot grow outside their hosts. Thus, molecular diagnosis, which is comparatively more sensitive and specific, has become the method of choice to identify certain pathogens that could not be detected by phenotypic-based methods [13,14]. Current assay systems have the necessary reliability, accuracy and sensitivity to allow wider applications such as outpatient monitoring, large screening programs in developing countries and point-of-care service. As a result of these continual improvements, nucleic acid-based assays are poised to serve as the new gold standard for the detection, identification, and characterization of microbial pathogens because conventional gold standard techniques such as culture are too slow and comparatively less sensitive. Nucleic acid-based techniques are also extended to detect drug resistance, the presence of virulence factors and molecular typing.

Assays approved by the U.S. Food and Drug Administration (FDA) are available to detect fastidious organisms like *Neisseria* gonorrhea and *Chlamydia trachomatis* or even methicillin-resistant *Staphylococcus aureus* [15]. The use of these modern technology is often affected by certain factors such as the availability of high quality, commercial diagnostic kits and the cost of the kits performed. Moreover, another emerging trend is the point of care (POC) diagnostics of infectious disease. The point of care testing is currently the fastest growing segment of the market of clinical laboratory testing [16].

4.1 Signal Amplification Assays

The signal amplification methods combines with type of nucleic acid probe with the generation of a signal often through an enzymatic reaction. A signal from fluorescence *in situ* hybridization (FISH) may be directly observed microscopically following the hybridization of a fluorescent labeled probe to its complementary nucleic acid target. Signal amplification technologies have several advantages over nucleic acid target amplification methods [17]. These procedures are far less likely to produce false-positive results secondary to contamination compared with traditional nucleic acid amplification assays. The types of signal amplification methods include nucleic acid probes, Hybrid Capture, branched chain DNA, and *in situ* hybridization [18].

4.2 Microarray

This technique has been used efficiently in clinical diagnostics for the identification of disease-related genes with the help of its biomarkers. Device detect a large number of signals simultaneously and used to detect deoxyribonucleic acid and ribonucleic acid (DNA or RNA [19]. These microarrays generate either fluorescent or electrical signals during identification and characterization of microorganisms and genetic polymorphisms associated with increased susceptibilities of humans to infectious diseases. Different types of microarrays have been developed based on their target material like cDNA, mRNA, protein etc. [20]. The primary limitations of using microarrays are cost, and for large microarrays, data management and interpretation may be difficult.

4.3 Nucleic Acid Testing and Sequencing

The most common sequencing method used currently is the Sanger-based sequencing technology. This method is accurate, user-friendly and large data can be generated in a short time. State-of-the-art systems can sequence up to 20 million nucleotides in 4 hr [21]. Rapid results are an advantage for diagnostic applications where time-to-result is essential, such as in the cases of sepsis/meningitis. However, due to a large amount of data generated, powerful interpretation of data is required by the software, stress the need of an equally advance progress in bioinformatics [18].

4.4 Nucleic Acid Amplification Tests (NAT)

One of the examples is real-time polymerase chain reaction (RT-PCR). The two FDA-approved real-time PCR kits are used for the rapid detection of the nasal colonization by methicillin-resistant *S.aureus* (MRSA) and the vaginal or rectal colonization of pregnant women by Group B *Streptococcus* [22]. The use of real-time PCR affords a more rapid time to detect and avoid the need for subculture and traditional identification of suspect isolates present in the complex mixture of normal flora [23].

4.5 Point-of-care Diagnostic Kits

The current POC test is dominated by glucose, cardiac and pregnancy test [12]. These test kits allow patients to be instantly diagnosed at home and there is no need to go to the hospital.

However, POC diagnosis of infectious diseases is currently only limited to rapid microscopy or immuno-chromatographic tests. There is no DNA-based test has yet achieved POC status.

5. ISSUES IN ASSAY DEVELOPMENT AND VALIDATION

Development of nucleic acid-based assays is subjected to quantitative and qualitative aspects [24]. Key among the former is sensitivity, specificity, and positive & negative predictive values. The assay is better if it has a higher sensitivity and specificity, as well as higher the positive predictive and negative predictive values. Furthermore, qualitative metrics affects an assay includes the ease of operation, training required, sensitivity to contaminants, the range of specimens that can be analyzed and most importantly, evaluation against accepted standard methods [25]. As a part of the development process, assays must be optimized to achieve an acceptable levels of performance based on these metrics [26].

The principle of beneficence requires that researchers maximize the potential benefits to the participants and minimize the potential risk of harm [27]. On the other hand, the principle of non-maleficence advises researchers to prevent predictable injury either through act of omission or commission. Besides these, the scientific merit of the study is itself an ethical issue. Laboratory personnel should be qualified and capable of conducting the appropriate diagnostic methods [28]. Furthermore, the patients those are involved should be respected in terms of the welfare, perceptions, rights, and customs. This can be done through informed consent that conveys different options that are available for patients to choose. In order to choose, they must be informed of their options which includes the possible risks and benefits of those options. Moreover, privacy and confidentiality play and important role in the protection and promotion of human dignity and a person's mental or psychological well-being. Steps must be taken into consideration to ensure that patients are protected from any harm that might be caused as the result of access to their personal information [29]. Last but not the least, justice should be practiced to avoid imposing an unfair burden on a particular group. Vulnerable patients are to be responded as quickly as possible without neglect or discrimination.

6. CONCLUSION

When deciding on which diagnostic methods to be used, lab personnel should consider following factors: cost effectiveness, specificity and sensitivity as well as the availability of qualified staffs. Currently, nucleic acid manipulation and testing are common in the scientific community and central to all future health care. There is still a need for further improvements in such assays. One promising approach involves advances in nanotechnology that provide new directions for simplifying assay procedures and enhancing detection sensitivity. Better diagnostic tests are definitely needed to improve the quality of healthcare and in the meantime, the FDA has to develop new rules to accommodate the innovation to benefit more patients.

7. FUTURE PERSPECTIVES

Among the emerging technologies, microminiaturized devices such as microchips and nano-scale devices like nanoparticles, carbon nanotubes are providing new ways to detect nucleic acids. The overall analytical process can be simplified not only in clinical laboratories but also in the more demanding point-of-care environment. Applications of micro-miniaturization and microchip devices include DNA enrichment, DNA extraction from the cells, capillary electrophoretic-based mutation detection, continuous flow PCR and reverse transcriptase PCR [6]. Examples of this technology are the lab-on-a-chip and microfluidics. The advantages of new techniques include lowering contamination, reagent consumption and time of detection while enhancing rapidity, performance and automation ability. On the other hand, nano-biotechnology is an emergent topic that need to be addressed and improved. A brand new range of electronic devices and biosensor nano-platforms has emerged as a consequence of the inherently small size and unusual properties of nanoparticles [30]. Nanotechnologies that can be used in molecular diagnostics include nanoparticle bio-labels, nanotechnology-based microarrays, nano-biosensors and nanoscale optics [31]. These technologies hold a lot of promise in the design of future detection systems.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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