

Title: Diagnostics: Conventional versus Modern methods

Abstract: Researches have been done on the development of rapid and effective diagnostic methods. Many of new modern diagnostic test are widely accepted. The objective of writing an article on comparison of conventional and modern diagnostic test is to understand the demand of community for the successful acceptance of modern diagnostic methods. When deciding on which diagnostic methods to use, one should consider factors such as the cost effectiveness, specificity and sensitivity as well as the availability of qualified staffs. A compromise of these factors should be reached to provide the most accurate and rapid results. 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 to make it more user-friendly and enhancing detection sensitivity.

Key words: Diagnostic methods, conventional methods, modern methods

INTRODUCTION

Infectious diseases remain a major cause of death and debility despite decades of dramatic progress in their treatment and prevention. According to WHO, there is a reduction from 1 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 of infectious diseases are the increasing demand and changing disease patterns which challenge the physician's diagnostic skill and differential diagnoses

25 An infectious disease can be caused by any bacteria, virus, fungi or parasites.
 26 Diagnostic methods have evolved throughout the years to identify the causal pathogen in
 27 infectious diseases [2]. Most often, infectious disease is originated from animals [3]. Once in
 28 or on a host, most pathogens must anchor themselves to a tissue, tissue factor or enters the
 29 bloodstream directly and multiply before causing a full-blown infection and disease. It is
 30 thus important to detect the pathogens early to enhance the rate of cure.

31

32 Diagnostic methods have to be carefully performed in the shortest possible time, at
 33 the lowest possible cost equipped with accurate specificity and sensitivity. Conventional
 34 diagnostics methods focus on detecting pathogens at a particular lifecycle with the help of a
 35 microscope. However, molecular diagnostic methods and point of care diagnosis are slowly
 36 replacing or complementing the conventional methods [4]. Not only must pathogens be
 37 identified, isolated and characterized to permit the implementation of optimal therapies, but
 38 these tasks often must be accomplished under the demands of limited time, organisms that are
 39 challenging to isolate and characterize, and the increasing need to define the functionality of
 40 a pathogen in terms of drug resistance, special virulence attributes and epidemiological
 41 markers. Many infectious diseases are usually nonspecific in which they cannot be clinically
 42 distinguished from other infections. Thus, a definite laboratory confirmation is required.
 43 Whenever there are cross-symptomatic infections, the importance of differential diagnosis is
 44 even greater [5]. This review article aims to provide an overview of the comparison of
 45 conventional and modern diagnostic methods.

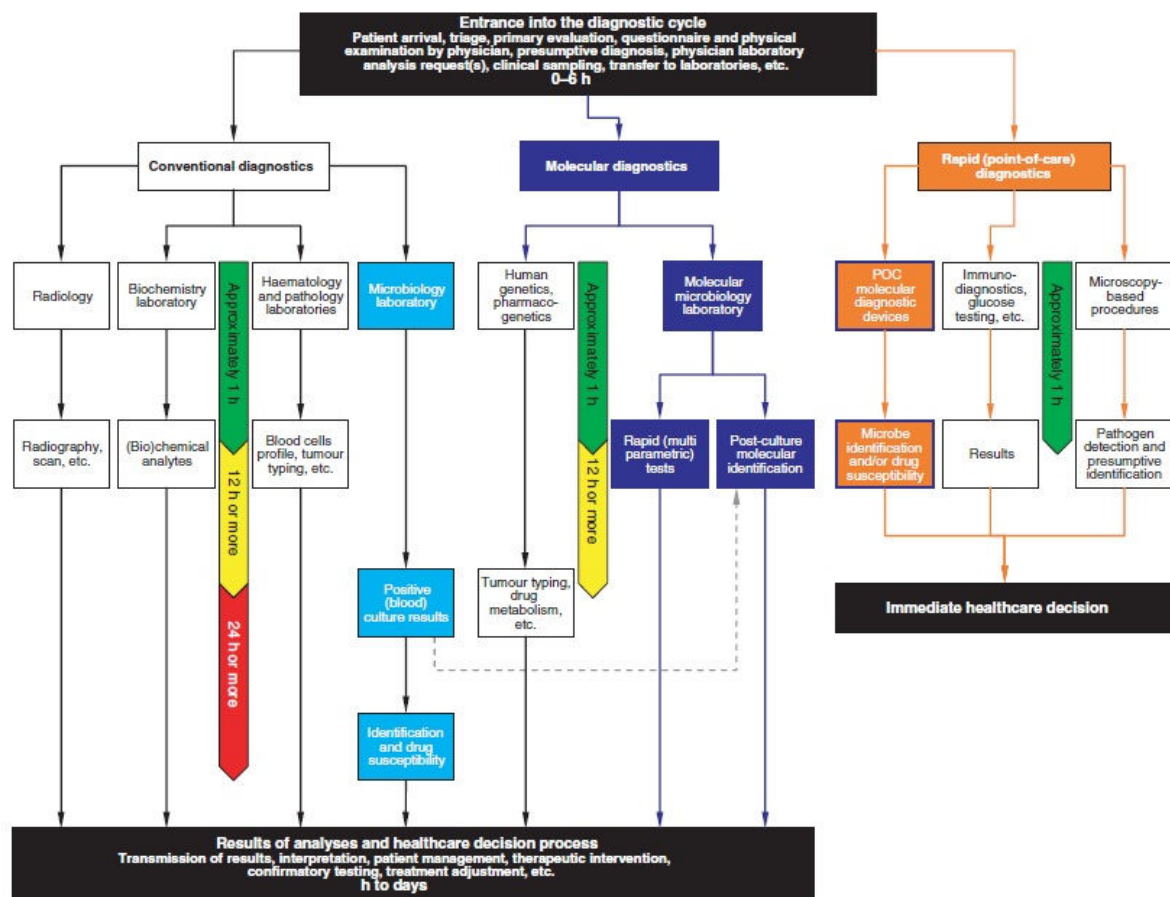
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47 **Overview of conventional and modern diagnostics**

48 The laboratory diagnosis of infection usually requires the demonstration, either direct
 49 or indirect of viral, bacterial, fungal or parasitic agents in tissues, fluids, or excreta of the

50 host. Traditionally, detection of pathogenic agents depends largely on microscopic analysis,
 51 the growth or culture of the microorganisms and staining [6]. Identification is mostly based
 52 on phenotypic characteristics such as fermentation profiles for bacteria, cytopathic effects in
 53 tissue culture for viral agents, and microscopic morphology of fungi and parasites [7]. These
 54 techniques are reliable but are often time-consuming. But the trend of using genotypic-based
 55 tests such as the use of nucleic acid probes or rapid real-time PCR based assays are becoming
 56 more common in today's laboratories [8]. Small amounts of the specimen are sufficient to be
 57 analyzed and results are often produced instantly. The precise handling of very small amounts
 58 combined with sensitive detection methods like electrochemical microarrays, predispose
 59 these technologies for point-of-care (POC) and lab-on-a-chip (LOC) diagnostics. This
 60 provides rapid and affordable diagnostics without the need for sophisticated and expensive
 61 laboratory equipment [9]. Diagrammatical representation of an overview of conventional and
 62 modern diagnostic methods is shown in Figure 1.

63



64

65 Figure 1. Overview of conventional and modern diagnostic methods

66

67 Conventional diagnostic methods

68 Conventional diagnostics can be broadly categorized into 4 methods. The first and the
 69 'gold standard' test is the staining and visual identification under naked eye or microscope.
 70 Many different staining procedures are available such as Gram stain, fluorochrome stains,
 71 and immune fluorescent stains provide a first rough classification of the detected organism.
 72 The characterization and identification still rely on the second method which is the culture
 73 and growth of pathogens on appropriate media. To culture bacterial, mycotic, or viral
 74 pathogens, an appropriate sample must be placed into the proper medium for growth or

75 amplification. Thirdly, the biochemical methods are used to differentiate types of
 76 microorganisms based on their characteristics. Fourthly, immunological assays are used
 77 based on the ability of an antibody to bind with high specificity to one or a very limited group
 78 of pathogens [6].

79

80 The phenotypic methods for the detection of pathogens are well tried, relatively
 81 affordable and available in the most hospital and private pathology laboratories as shown in
 82 Table 1.

83 However, there are various limitations to these methods. Direct visualization or
 84 culture is slow and has a poor diagnostic sensitivity and specificity. Cost constraints often
 85 limit identification of organisms by conventional methods to those thought to be of the
 86 greatest clinical significance. Traditional biochemical methods used to identify organisms
 87 often requires a further 24 hours. Performing rapid tests like staphylococcal and streptococcal
 88 latex agglutination tests, oxidase, and catalase can be time-consuming [10]. Staff reading
 89 plates are constantly interrupting the flow of plate reading to perform the biochemical tests,
 90 thus extending the process well into the afternoon.

91

92 Table 1: Phenotypic methods for the detection of pathogens

Stages	Mechanism
1	A microbe that is present in animals but that has not been detected in humans under natural conditions. Examples: most malarial plasmodia, which tend to be specific to one host species or to a closely related group of host species.
2	A pathogen of animals that, under natural conditions, has been transmitted from animals to humans but has not been transmitted between humans. Examples: anthrax and tularemia bacilli, and Nipah, rabies and West Nile viruses.
3	Animal pathogens that can undergo only a few cycles of secondary

	transmission between humans, so that occasional human outbreaks triggered by a primary infection soon die out. Examples: Ebola, Marburg and monkeypox viruses.
4	A disease that exists in animals, and that has a natural cycle of infecting humans by primary transmission from the animal host, but that also undergoes long sequences of secondary transmission between humans without the involvement of animal hosts. Examples: Chagas' disease, influenza A, West African sleeping sickness
5	A pathogen exclusive to humans. These pathogens could have become confined to humans in either of two ways. Examples: the agents causing <i>falciparum</i> malaria, measles, mumps, rubella, small pox and syphilis.

93

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

100

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

109

110 **Modern diagnostic**

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

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

129

130 **(I) Molecular biology based technologies**

131 **(i) Signal amplification assays**

132 The signal amplification methods combine some type of nucleic acid probe with the
 133 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 fluorescently 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].

(ii) Microarray

This technique has been used efficiently in clinical diagnostics for identifying disease-related genes with the help of its biomarkers. These devices detect a large number of signals simultaneously and used to detect DNA or RNA [19]. These microarrays may 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 or small molecules which make them different from each other [20]. The primary limitations of using microarrays are cost, and for large microarrays, data management and interpretation may be difficult.

(iii) 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 sepsis or meningitis. However, due to a large amount of generated data, powerful

interpretation software is required, stressing the need for an equally advancing progress in bioinformatics [18].

(iv) 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 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 detection and avoids the need for subculture and traditional identification of suspect isolates present in the complex mixture of normal flora [23].

(v) Point-of-care diagnostic kits

The current POC testing is dominated by glucose, cardiac and pregnancy testing [12]. These testing 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 immune-chromatography tests, no DNA-based test has yet achieved POC status.

Issues in assay development and validation

Development of nucleic acid-based assays is subjected to quantitative and qualitative [24]. Key among the former is sensitivity, specificity, and positive and negative predictive values. The assay is better if it is more sensitive and specific, as well as higher the positive predictive and negative predictive values. Furthermore, several qualitative metrics affect 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 part of the development process, assays must be optimized to achieve acceptable levels of performance based on these metrics. Once in use, finished assays must also be monitored continually for false positive and false negative results [26].

A fundamental moral commitment and to advance the knowledge and understanding is essential to the individuals involved in research. Firstly, 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 acts of omission or commission. Secondly, 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 that are involved should be respected in terms of welfare, perceptions, rights, and customs. This can be done through informed consent that conveys different options that are available for patients to choose the best option. In order to choose, they must be informed of their options which include the possible risks and benefits of those options. Moreover, privacy and confidentiality play an important role in the protection and promotion of human dignity and a person's mental or psychological well-being. Steps must be taken to ensure that patients are protected from any harm that might be caused as the result of access to their personal information [29]. Lastly, justice should be practiced to avoid imposing on a particular group an unfair burden. Vulnerable patients are to be responded as quickly as possible without neglect or discrimination.

CONCLUSION

When deciding on which diagnostic methods to use, one should consider factors such as the cost effectiveness, specificity and sensitivity as well as the availability of qualified

staffs. A compromise of these factors should be reached to provide the most accurate and rapid results. 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 for accommodating the innovation to benefit more patients.

FUTURE PERSPECTIVES

Among the emerging technologies, microminiaturized devices such as microchips and nanoscale 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 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 include lowering contamination, reagent consumption and time of detection while enhancing rapidity, performance and automation ability. On the other hand, nanobiotechnology is another emergent topic that needs to be addressed and improved. A brand new range of electronic devices and biosensor nanoplatfroms 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 biolabels, nanotechnology-based microarrays, nano-biosensors and nanoscale optics [31]. These technologies hold a lot of promise in the design of future detection systems.

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