

Title: **Diagnostics: Conventional versus Modern methods**

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

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

24 demand and changing disease patterns which is the challenge for healthcare to diagnose the
25 disease accurately.

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

30

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

41

42 **Overview of conventional and modern diagnostics**

43 The laboratory diagnosis of infection usually requires the demonstration, either direct
44 or indirect of viral, bacterial, fungal or parasitic agents in tissues, fluids, or excreta of the
45 host. Traditionally, detection of pathogenic agents mainly depends on microscopic analysis,
46 the growth of the microorganisms and staining [6]. Identification of pathogen is mostly based
47 on the phenotypic characteristics such as fermentation profiles of bacteria, cytopathic effects
48 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 Figure 1.

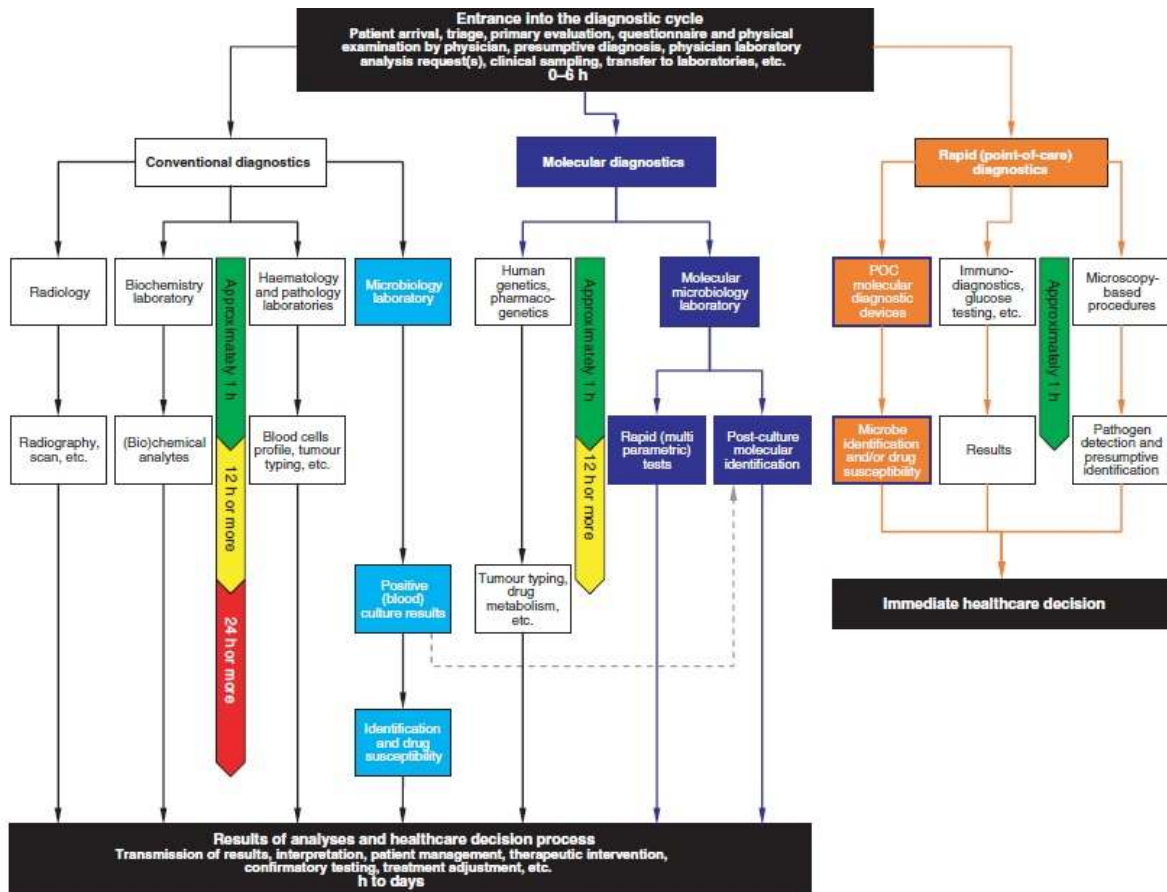


Figure 1. Overview of conventional and modern diagnostic methods

61 **Conventional diagnostic methods**

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

75

76 The phenotypic methods for the detection of pathogens are well tried, relatively
77 affordable and available in the most hospital and private pathology laboratories. However,
78 there are some limitations to these methods. Direct visualization or culture comparatively
79 grows slower and has a lower sensitivity and specificity of the test. Cost constraints often
80 limits the identification of an organism by conventional methods to those thought to be of the
81 greatest clinical significance. Traditionally, battery of biochemical tests used to identify an
82 organisms often requires 24 hours. Performing rapid tests like staphylococcal and
83 streptococcal latex agglutination tests, oxidase, and catalase can be time-consuming [10].
84 Staff reading plates manually takes more time and constantly interrupt the flow of the further
85 biochemical tests, thus extend the process.

86

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

93

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

102

103 **Modern diagnostic**

104 Modern diagnostic methods have an advantage in those cases where pathogen cannot
105 be identified by microscopic methods and pathogens cannot grow outside their hosts. Thus,
106 molecular diagnosis, which is comparatively more sensitive and specific, has become the
107 method of choice to identify certain pathogens that could not be detected by phenotypic-
108 based methods [13, 14]. Current assay systems have the necessary reliability, accuracy and
109 sensitivity to allow wider applications such as outpatient monitoring, large screening
110 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].

(i) 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].

(ii) 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.

(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 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].

(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 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

160 to detect and avoid the need for subculture and traditional identification of suspect isolates
161 present in the complex mixture of normal flora [23].

162 163 (v) Point-of-care diagnostic kits

164 The current POC test is dominated by glucose, cardiac and pregnancy test [12]. These
165 test kits allow patients to be instantly diagnosed at home and there is no need to go to the
166 hospital. However, POC diagnosis of infectious diseases is currently only limited to rapid
167 microscopy or immuno-chromatographic tests. There is no DNA-based test has yet achieved
168 POC status.

169 170 Issues in assay development and validation

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

179
180 The principle of beneficence requires that researchers maximize the potential benefits
181 to the participants and minimize the potential risk of harm [27]. On the other hand, the
182 principle of non-maleficence advises researchers to prevent predictable injury either through
183 act of omission or commission. Besides these, the scientific merit of the study is itself an
184 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 an 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.

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.

FUTURE PERSPECTIVES

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

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