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An Overview on the Current and Futuristic Rapid Diagnostic Assays

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Abstract

There is now a huge need to innovate, design and use novel and practicable rapid diagnostic assays (RDA) to combat the grave challenge of emerging life-threatening and multiple drug resistant bacteria (MDR) bacterial pathogens. The conventional methods of diagnosing bacterial pathogens are well enough to give good specificity. However, the sensitivity of assays in terms of short time is still not well settled. The increased population of the world along with availability of global transport means resulted in the emergence of critical health situations that the current time-consuming means of diagnosis cannot cope with the timely management of diseases caused by serious human pathogens.

Introduction

The limitations of the conventional procedures for diagnosing bacteria are various depending on the type of the assay. For cultures, they are labor intensive, need special media, prolonged period of time to culture, some organisms are uncultivable on artificial media, and have potential health hazards. Other assays are considered semi-slow such as antigen detection and serology assays. For antigen detection assays, negative tests require confirmation and usually effected by poor specimen collection. Serological assays are unhelpful during early stage of infection and are not quite useful in immunocompromised patients [(1)].

Therefore, rapid diagnostic assays (RDA) have become a genuine need. The typical qualities of RDA are high sensitivity and specificity, high accuracy compared to gold standard, simple to perform, results in minutes to 1-2 hours, and cost effective. RDA without cultures are especially needed for microorganisms that grow poorly, cannot be cultured e.g. T. B., need for speed, there is critical case management (life threatening), and there is therapy failure (e.g Antibiotics resistance) [(2)].

Most pathogenic microorganisms need RDA for proper and rapid diagnosis. However, there are some pathogens that need RDA more than others. Those pathogens are usually fastidious bacteria e.g. C. trachomatis, Gonococci, pertussis, TB, M. genitalium, T. whipplei, C. burnetti, and B. henselae. Moreover, rapid Bacterial diagnosis is essential in life-threatening systemic infections e.g. N. meningitidis, S. pneumoniae, and H. influenza. Also, MDR bacteria are in urgent need for rapid diagnosis as most their infections need prompt intervention such as MRSA, multi-drug resistant TB [(3)].

Methods of RDA

The main currently used or developed methods of RDA are antigen detection assays, antibody detection (serology) assays, molecular detection

assays, and bacteriophage-based RDA. For antigen detection assays, they detect bacterial, viral or parasite antigen (surface antigen, soluble antigen) or toxin in biological fluids (CSF, blood, urine). The primary techniques are direct agglutination: slides, cards, latex agglutination: slides, cards, Immunochromatography: dipsticks and latex agglutination test. For serological assays, the main techniques are direct agglutination (antigen, latex + antigen), agglutination inhibition, immunodot, and immunochromatography. For molecular detection assays, the conventional and real-time PCR, hybridization probes, and DNA chip microarrays. PCR assays are most widely used because of their high sensitivity and specificity [(2)].

The advantages of molecular RDA are numerous including rapid, accurate, specific, sensitive, have powerful amplification potential useful for detecting traces of target pathogens nucleic acids. Moreover, molecular RDA does not require viable microorganisms and quantitative assessments are highly accurate [(4)]. One of the currently focused techniques using the molecular RDA is using microarrays in blood pathogens or those pathogens detected by cultures. Such microarrays are all-in-one kits able to detect specifically certain pathogens along with identifying their genotypes, antibiotic-resistant genes, virulent genetic makeup, mutated pathogens, and also able to quantitate the microbial load specifically. These all-in-one kits are capable to perform all these tasks in several hours rather than days or weeks as used to be before by using the conventional methods. However, there are some disadvantages for using molecular RDA including cost, false positives caused by amplification of contaminants, only sample from normally sterile sites should be considered for broad-range PCR, specimen is required to be refrigerated or stored in alcohol before processing, need training and highly qualified personnel, no antimicrobial sensitivity is available, and cannot differentiate viable versus dead microbes [(5)].

Phage-based RDT for Bacteria

Phages or bacteriophages = bacterial viruses. Phages are initially discovered in 1915 by Twort and independetly in 1917 by d'Herelle. It is estimated that every 2 days 50% of the world's population is destroyed by bacteriophages. Unfortunately, during Antibiotic era, phages were considered "non-conventional" medicine in spite of the continued use of phages in rapid diagnosis and in therapy in the former USSR: Eliave Institute in Tbilisi, Georgia (http://www.evergreen.edu/phage/home.htm).

There are numerous prospects for the Phage-based RDA (PBRDA) including phage-based rapid (1-60 minutes) detection methods were developed recently as both qualitative and quantitative assessments for several human pathogens such as *E. coli*, *E. coli O157:H7*, Methicillin-resistant staphylococcus aureus (MRSA), and other MDR bacteria. PBRDA are rapid, highly specific (up to species and strain) and unlike PCR, PBRDA can differentiate between viable and dead microbes. PBRDA are extremely sensitive, amplification effect is capable by using phage-based amplification means by which can detect up to 1-10 bacterial cells per sample. Also PBRA are good quantitative assays and there is possibility to develop phage lysins from used phages; these phage lysins are able to perform extra rapid assays, within seconds to 1 minute [(6-8)].

Phage lysins are conceived to be the ultimate goal for human hopes for a completely successful and resistance hassle-free bacterial therapy and extra-rapid diagnostic assays for human and animal pathogens. Phage lysins are cationic enzymes that have been designated using various names including phage-lysozyme, endolysin, lysozyme, lysin, phage lysine which are able to hydrolyze specific bonds in the murrain or peptidoglycan layer of the cell wall. Phage lysins are known to kill the target bacteria in few seconds. The interesting thing that their host range is wider than the corresponding phage particles which sometimes host range of phage lysins reach to the species level [(7,8)].

One of the PBRDA invented by us is the Lumulus amoebocyte lysate (LAL) phage-based rapid detection assay for pathogenic *E. coli*. LAL assay is able to detect traces of endotoxin by certain enzymatic reaction resulting in visible coloration measured by any spectrophotometer. It is traditionally used to measure any endotoxin contamination in food and pharmaceutical preparations. Specific designed phages lyse target Gram negative bacteria can lead to specific lysis which in turn leads to liberation of endotoxin. Hence, combing the use of specific phages with LAL resulted in inventing novel highly specific phage-based LAL rapid diagnostic assay for *E. coli*. Actually this novel approach can be simulated for any bacterial pathogen. The total assay time is 40-50 minutes only. It is specific assay at strain/ species level and can use a portable handy spectrophotometer. This assay proved to be successful for Gram negative bacteria (E. coli, other enterobacteriacae) and is a revolutionary step for rapid diagnosis at strain level [6-8].

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