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Review Article

Role of Molecular Biology as Diagnostic tools of Infectious disease-an update

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Introduction

The last ten years of the twentieth century allowed for an exponential increase in the knowledge of techniques in molecular biology, following the cellular and protein era of the 1970s and 1980s. This explosion of technologies of molecular biology has had major consequences and allowed for significant developments in life sciences, including microbiology [1]. The gaps in our knowledge of genetic diversity of microorganisms have been overcome to a great extent by advanced technology. Unraveling the mystery of the genes has opened a Pandora's box of evolutionary changes, microbes use to survive in adverse conditions [2].

Diagnosis of several other communicable diseases has come to rely heavily on molecular diagnostic tests. An important case is the use of molecular kits for the detection of MDR TB. Keeping in mind the steady rise in cases with drug-resistant TB, and limitations of drug susceptibility testing, rapid molecular tests appear promising. An increasing number of laboratories are adopting the techniques, especially in developing countries with a heavy burden of the disease. It has been reported that mutations outside the region of a specific gene may not be detected by some assays designed to detect drug resistance. These strains could still be resistant, with the risk of the patient being subjected to ineffective treatment with the drug. Another scenario is the highly mutable Human Immunodeficiency Virus (HIV). Rapid mutations in a drug-resistant strain can impact the potential for detection [3]. Another major drawback is that the mere presence of resistance genes may not correlate with expressed resistance and vice versa. The absence of the gene does not rule out resistance, which could be by another mechanism [4]. Similarly, resistance genes may be present but unexpressed due to mutational or regulatory silencing. As much as it is necessary to use rapid molecular tests, it is also important to be vigilant towards emergence of mutants which might escape the detection limit. Awareness and vigilance are required to avoid this pitfall in addition to looking out for new and emerging mutants [5].

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Identification from culture-negative specimens

Pasteur and Koch were ardent exponents of bacteriological culture and the affinity between the bacteriologist and laboratory culture has remained strong for the past 100 years. Indeed, it may be argued that the historical success of bacteriology has been a direct result of bacteriological culture, as well as its widespread adoption throughout the world. Today the ability to culture bacteria in vitro remains the cornerstone of this issue. However, there are several situations where molecular approaches should be considered when conventional culture fails to identify the causative agent due to one or more of the following reasons including (i) prior antibiotic therapy, (ii) where the organism is fastidious in nature, such as the HACEK group of organisms in the case of endocarditis, (iii) where the organism is slow growing, e.g. Mycobacterium spp. (iv) where specialized cell culture techniques are required, e.g. Chlamydia spp. and Coxiella burnetti [6].

Specific Polymerase chain reaction (PCR)

Specific PCR is the simplest PCR approach of which is designed for detecting specific target microbes. In specific PCR, primers are designed complimentary to a known DNA target and specific for the microbe being assayed. This is a key point for specific PCR so that the primers should be so-designed so that they are strictly specific for the targeted microorganisms [7]. As the result is specific for the detection of target microbes, this method can be used as a direct detection and identification method. This is the most widely used method in the detection of anti-dsDNA antibody, particularly recognizes the hybridization product, resulting from the reaction between target DNA and a DNA probe.

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR is the technique of synthesis of cDNA from RNA by reverse transcription (RT) firstly, which is then followed with amplification of a specific cDNA by PCR. This is the most useful and sensitive technique for mRNA detection and quantitation that is currently available [8]. RT-PCR is mostly used to detect viruses and the viability of microbial cells through examination of microbial mRNA.

Real-time PCR

This method is based on using fluorescent labeled probes to detect, confirm, and quantify the PCR products as they are being generated in real time. In recent years, some commercial automated real-time PCR systems have been available (Light Cycler & TaqMan). These systems perform the real-time fluorescence monitoring by using fluorescent dyes which binds non-specifically to double-stranded DNA generated during the PCR amplification [9]. The real-time PCR systems not only reduce the detection time but results can be ready in less than on drying process. Therefore it is important to give careful consideration to what one wishes to achieve from a molecular assay and thus careful emphasis must be placed on the target gene locus [10].

Gene targets

Traditionally the majority of diagnostic assays in medical bacteriology have been based around the amplification of DNA in a target gene, as opposed to mRNA or other nucleic acid signal. This may be performed as DNA is an extremely stable molecule, as opposed to mRNA which has a short half-life. Generally molecular diagnostics in clinical bacteriology is not concerned with regard to the viable status of an organism being detected, but is concerned with the qualitative detection of an organism in a symptomatic patient with the relative clinical presentation, e.g., the detection of meningococcal DNA in the CSF of a pediatric patient with suspected meningitis. The scenario is completely different where medical bacteriology interfaces with food/public health microbiology, where simple qualitative detection of DNA from pathogenic food borne bacteria is insufficient or can indeed be misleading. In this situation, other more defined molecular viability assays are required, e.g. the molecular detection of viable versus non-viable Salmonella sp. in a sample of dried milk powder suspected of causing food-poisoning [11].

Clinical microbiologists are engaged in the field of diagnostic microbiology to determine whether pathogenic microorganisms are present in clinical specimens collected from patients with suspected infections. If microorganisms are found, these are identified and susceptibility profiles are determined [12]. During the past two decades, technical advances in the field of diagnostic microbiology have made constant and enormous progress. The diagnostic capabilities of modern clinical microbiology laboratories have improved rapidly and have expanded greatly due to a technological revolution in molecular aspects of microbiology and immunology [13]. In particular, rapid techniques for nucleic acid amplification and characterization combined with automation and user-friendly software have significantly broadened the diagnostic arsenal [14]. The conventional diagnostic model for clinical microbiology has been labor-intensive and frequently required days to weeks before test results were available. Moreover, due to the complexity and length of such testing, this service was usually directed at the hospitalized patient population. The physical structure of laboratories, staffing patterns, workflow, and turnaround time all have been influenced profoundly by these technical advances [15]. Such changes will undoubtedly continue and lead the field of diagnostic microbiology inevitably to a truly modern discipline.

CONCLUSION

Molecular diagnostic techniques have a significant role to play in clinical microbiology, although their adoption will never replace conventional methodologies, which continue to be the cornerstone of modern bacteriological methods. Indeed, such molecular diagnostic assays may only be implemented in specialized laboratories to enhance laboratory diagnostic efficiency. It can be inferred that integration of molecular approaches in clinical microbiology will be enhanced through the production of a greater range of diagnostic kits, as well as the existence of more accredited laboratories.

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