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Real-time PCR in Clinical Diagnostic Settings

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Introduction

Real-time PCR is a commonly used technique for the detection of pathogens both in research setting, as well as in diagnostic settings [1]. The goal of this editorial is to introduce or familiarize the reader with the advantages of using the available methodologies in a targeted manner. Real-time PCR combines PCR chemistry with either a fluorescent probe or DNA detection dyes such as syber green, thus allowing visualization of product in "real-time" [2]. The available real-time PCR methodologies provide good sensitivity and specificity. The ease and speed of the design and setup process of qPCR makes it an attractive assay to use. PCR amplification is performed in a closed setting (one tube vs two tube) thus reducing the chances of contamination.

Choice of assay is very important as is the choice of clinical sampling. Clinical samples may consist of body fluids or tissue. The pathogen that is being investigated may be a bacteria, virus or parasite. The virus may be an RNA or DNA virus. To begin with, nucleotide isolation for the clinical specimen will include RNA, DNA or both. There are many available methods for nucleotide isolation. Studies have demonstrated varying levels efficiency of nucleotide isolation with various techniques [3-5]. Our studies and other have demonstrated in a stool sample, isolation of DNA of Bacteriodetes Species is more efficient using a silica-gel column-based method as compared to using phenol chloroform [5]. However the breadth of species representation is improved using phenol-chloroform extraction method. RNA isolation from plasma is more efficient using the silica-gel column method while identification of low-copy numbers viral RNA from human blood is more efficient following ultra-centrifugation.

Sample type and availability is also a factor in the choice of assay (Figure 1). Viral RNA/DNA and bacterial DNA are likely to be present in specific tissues as well as in blood. Identification of microbes starts with the isolation of the appropriate nucleic acid from the sample. For example, in the case of HIV, viral RNA and proviral DNA can be isolated from blood as well as from tissue sources [6]. Analysis of viral RNA will provide information regarding active viral replication and the plasma viral load which is a direct measure of clinical state during the course of HIV infection as well as a measure of effective therapy during HAART. Identification of the level of proviral DNA is a measure of the viral reservoirs during the course of HIV infection and is an important indicator during therapy. In this case, depending on which parameter is being investigated, the nucleic acid isolation methods and the choice of tissue sample vary. Identification of causative agents in disease states is also simplified by the use of real-time PCR assays. These assays provide a fast and specific method to identify bacterial pathogens in sepsis [7-9] and pneumonia [10-14], viral agents in hepatitis [15-18] and respiratory diseases [19].

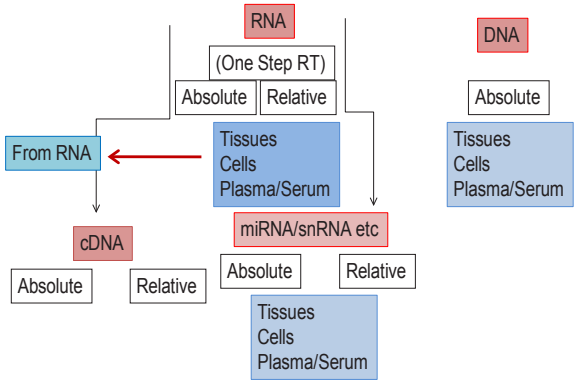

Figure1: Schematic of Possibilities.

A majority of the workflow utilized in this technology is automated with many hundreds of core facilities available around the world. The main factor that distinguishes between a successful assay and an unsuccessful one is the level of understanding of the outcomes available from the assay. Thus, the pre analytical steps that are followed prior to utilization of this methodology are critical to the success of the assay. This includes planning of the collection and storage of samples as well as a detailed plan of processing and analysis. The planning stage should include optimization, analysis of positive and negative controls and a practice run. Once all steps are in place, the real-time PCR platform provides a fast and convenient way to detect microbes of all origins in clinical samples.

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