

Applications of Real-Time PCR in Clinical Microbiology

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Abstract

The introduction of real-time PCR assays to the clinical microbiology laboratory has led to significant improvements in the diagnosis of infectious disease. There has been an explosion of interest in this technique since its introduction and several hundred reports have been published describing applications in clinical bacteriology, parasitology and virology. There are few areas of clinical microbiology which remain unaffected by this new method. It has been particularly useful to detect slow growing or difficult to grow infectious agents. However, its greatest impact is probably its use for the quantitation of target organisms in samples. The ability to monitor the PCR reaction in real-time allows accurate quantitation of target sequence over at least six orders of magnitude. The closed-tube format which removes the need for post-amplification manipulation of the PCR products also reduces the likelihood of amplicon carryover to subsequent reactions reducing the risk of false-positives. As more laboratories begin to utilise these methods standardisation of assay protocols for use in diagnostic clinical

microbiology is needed, plus participation in external quality control schemes is required to ensure quality of testing.

Introduction

The first PCR methods to be described for clinical microbiology utilised gel electrophoresis for the detection of PCR amplification products. Although these assays proved useful, their specificity and sensitivity was compromised by this rather cumbersome end-point detection method. Specificity of detection could be improved by incorporating a solid phase hybridisation such as Southern blotting; however, this was labour intensive and time consuming requiring further manipulation of the PCR product. Detection of PCR products by solid phase hybridisation also limited the numbers of samples that could be processed, and the methods used were difficult to standardise between laboratories. The overall time taken to produce a result from a PCR assay could be two or three days and the test required a significant level of technical skill limiting the use of PCR to specialised laboratories only. The introduction of enzyme-linked hybridisation probe formats (PCR-ELISA) for the detection of amplification products did improve the detection process however they still required manipulation of the amplification products following PCR. Manipulation of the amplified product increases the likelihood of contaminating subsequent PCR reactions leading to false-positives a phenomenon known as amplicon carryover. PCR-ELISA facilitated the introduction of quantitative PCR (QPCR) assays however the range and accuracy of quantitation was limited. The more recent introduction of real-time platforms for PCR has revolutionised molecular diagnostic detection methods in clinical microbiology. These closed-tube systems virtually eliminate the risk of amplicon carryover because the samples are not opened following thermal cycling. Many of these new platforms process samples more rapidly than conventional block-based thermal cyclers making pathogen testing much more rapid. In addition, the ability to monitor the reaction in real-time provides results immediately after cycling and facilitates quantitation of the original target sequence over many orders of magnitude. Real-time platforms can differentiate between several closely related sequences within the same reaction therefore

