
Preface

PCR methods for the detection of microbial pathogens have made relatively little impact in diagnostic microbiology laboratories. In contrast, diagnostic virology has been revolutionized by the wholesale adoption of PCR and, in particular, real-time PCR methods. Those microbiology laboratories that have introduced PCR methods have often opted to use expensive commercially produced tests, rather than the far cheaper alternative of developing their own tests or introducing tests developed by other workers.

This book makes the case for using “home brew” PCR methods to detect microbial pathogens. There is no *a priori* reason why such tests should not be as good as or better than a commercially produced equivalent. The relative cheapness of thermal cyclers and automated DNA extraction platforms makes this approach increasingly attractive, especially for high-volume tests to detect pathogens described in the chapters on MRSA and *Clostridium difficile*. Alternatively components of a commercial test can be judiciously incorporated into “in-house” methods to develop a test as shown here in the chapter on the identification and resistance detection in mycobacteria.

The weakness of many books of PCR protocols is that they are just that—a collection of unrelated methods which may or may not appeal to a reader, depending on whether they have an interest in the particular pathogen being described. Little attention is paid to the general problems of developing and introducing a PCR test into the lab, save the oft-stated advice that separate rooms should be used for separate aspects of the PCR. Although the majority of the chapters in this book are devoted to the detection of specific pathogens, the first chapters in this book should appeal to anyone working in this field regardless of their particular interests.

The relative ease of PCR and especially real-time PCR has meant that virtually any test devised will give a result of some sort. Numerous tests have been developed and introduced into practice without any knowledge of factors such as the efficiency of the reaction, its detection limits, and the specificity of the assay. In some cases this has severely hampered the acceptance of PCR for the detection of a disease, for example, invasive aspergillosis. These aspects and several others are discussed in the first chapter of this book with particular attention to the detection of *Aspergillus fumigatus*.

Another major problem with the use of PCR is the presence of PCR inhibitors in clinical specimens. The next chapter in the book gives a very comprehensive guide to PCR inhibition in different sample types and the methods used to overcome it. Because PCR of blood poses its own inhibition problems, these are discussed separately in the following chapter.

The last of the general chapters in the book looks in detail at the whole area of quality control of PCR. This has been one of the most important factors holding up the introduction of PCR into the diagnostic lab where robust QC has become an increasingly important

issue and laboratories have often not felt completely confident in their results compared to those obtained by more established methods.

The main part of the book is devoted to describing methods for the detection of a wide range of pathogens and from widely different specimens and situations. The list of pathogens detected is not intended to be comprehensive but have been chosen as exemplars to illustrate the wide range of solutions that have been devised to cope with particular problems. The majority of chapters, but not all, use real-time PCR technology rather than gel-based approaches, but these may still have their place where, for example, low sample numbers are processed at a time and expensive probes may degrade before much use has been made of them. One chapter uses an approach which is not strictly a PCR at all. Loop-mediated isothermal amplification (LAMP) is a relatively new technique which has several advantages over PCR, which are well described and are bound to find increasing application in the diagnostic lab. Most chapters concentrate on the detection of a single pathogen but some use multiplex PCR to detect closely related or widely different pathogens.

Whether the reader finds his or her exact pathogen or clinical specimen that he or she is interested in, the sheer variety of approaches used together with the general principles described in the first chapters should enable the reader to design and introduce diagnostic tests in the routine laboratory with confidence.

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