Molecular-based diagnostics, including future trends

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Abstract

Microbiological diagnosis has traditionally relied on phenotypic methods involving culture and biochemical testing to identify and characterize clinically relevant pathogens. These techniques have several disadvantages including poor sensitivity and long turn-around time. Molecular and mass spectrometry techniques are rapidly changing infection diagnosis and management of patients. Compared with conventional culture-based techniques, these modern approaches provide substantially more rapid and specific information on organism identification and on the presence of resistance mechanisms. These methods are expected to contribute substantially to enhancing antimicrobial stewardship and to decreasing 'time to appropriate antibiotics', one of the most important factors in improving the prognosis of patients with life-threatening infections. The UK government review on antimicrobial resistance has clearly laid out the real implications of spreading drug resistance, and provides an overview of how rapid diagnostics can play an important role in reducing unnecessary antimicrobial use and/or allowing early antibiotic de-escalation.[1] This article gives an overview of some of the practical applications of these newer technologies.

Keywords Communicable diseases; mass; matrix-assisted laser desorption-ionization molecular diagnostic techniques; MRCP; nucleic acid amplification test; polymerase chain reaction; spectrometry

Key points

- Traditional microbiological techniques involving culture and biochemical identification are slow, labour-intensive and often poorly sensitive
- New models of care and reorganization of pathology services have created opportunities to introduce new diagnostic techniques, including at the point of care
- The global threat of emerging antimicrobial resistance has highlighted the need for improved diagnostics to enhance clinical decision-making. Better diagnostics can significantly improve good antimicrobial stewardship by reducing their unnecessary use and guiding the selection of narrower spectrum agents
- An abundance of new molecular tests are now available that can be used in a variety of settings; they could also allow better infection prevention and control interventions to be rapidly implemented

Introduction

The molecular methods of nucleic acid amplification tests (NAATs) and mass spectrometry are two of the approaches most commonly adopted by clinical laboratories for routine detection and identification of organisms. These methods also allow rapid identification of microbial sequences that confer drug resistance, and can potentially overcome some of the shortcomings of traditional culture-based methods. There are several limitations to traditional culture-based techniques, as follows:

Turn-around time – traditional techniques rely upon growth of pure bacteria from primary samples (e.g. sputum, blood, urine) on incubated agar plates. Despite supplementation of growth media with various enrichment factors, most samples require 12–18 hours of incubation. It can be 48–72 hours after specimen collection before biochemical identification and antimicrobial susceptibility tests are complete. Patients are given empirical antibiotics based on 'best guess' principles in the intervening period. These are usually broad-spectrum agents and, because patients often present non-specifically, this can lead to inappropriate or unnecessary treatment, thus driving antimicrobial resistance.

Non-culturable organisms – a range of 'fastidious organisms' are unlikely or difficult to grow using traditional culture. These include *Bartonella*, *Borrelia*, *Brucella*, *Campylobacter*, *Helicobacter*, *Coxiella*, *Legionella*, *Leptospira*, *Mycobacterium*, *Mycoplasma*, *Nocardia* and *Rickettsia*. Detection of these organisms is dependent upon serology, antigen detection or NAAT testing.

Inhibition or suppression by antibiotics – patients are often given antibiotics that suppress the growth of pathogens before obtaining samples for microbiological analysis (e.g. administration of a third-generation cephalosporin for suspected bacterial meningitis before obtaining a cerebrospinal fluid (CSF) sample). Manufacturers of blood culture system supplement their media with proprietary formulations of antibiotic-binding resin beads to minimize this problem.

Variability of results – most traditional microbiology is based on pattern recognition and interpretation of the visual appearance and morphology of organisms (e.g. Gram staining). Despite the use of standard operating procedures, this is operator-dependent and reliant upon subjective judgement. Automation of molecular techniques can help to standardize this and reduce errors resulting from variations in reporting.

Poor sensitivity – bacterial culture depends on a critical mass of viable organisms surviving transportation. When the number of live bacteria in a primary specimen is very low, or a significant proportion have not survived the transportation process, low sensitivity can result. Molecular techniques enhance sensitivity by amplifying stretches of bacterial nucleic acid, so that lower

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numbers of the target bacteria are needed. Optimal transportation conditions are also less critical as nucleic acid is detectable even in dead cells.

Nucleic acid amplification tests

NAATs, such as polymerase chain reaction (PCR) and related technologies, rely on amplification (replication) of a specific sequence that is later detected (usually by fluorescent probes). The name of PCR is derived from the fact that the DNA polymerase enzyme (e.g. Taq) is responsible for DNA synthesis, while 'chain reaction' means exponential growth (at the rate of 2^n where n denotes the number of generations) (Figure 1).

The following are examples of practical uses of molecular techniques in the modern microbiology laboratory.

Detection of slow-growing organisms, e.g. *Mycobacterium tuberculosis*

M. tuberculosis is slow growing, often taking 6–8 weeks. Microscopic examination of specimens after Ziehl–Neelsen or auramine staining is simple to perform and rapid. However, it is

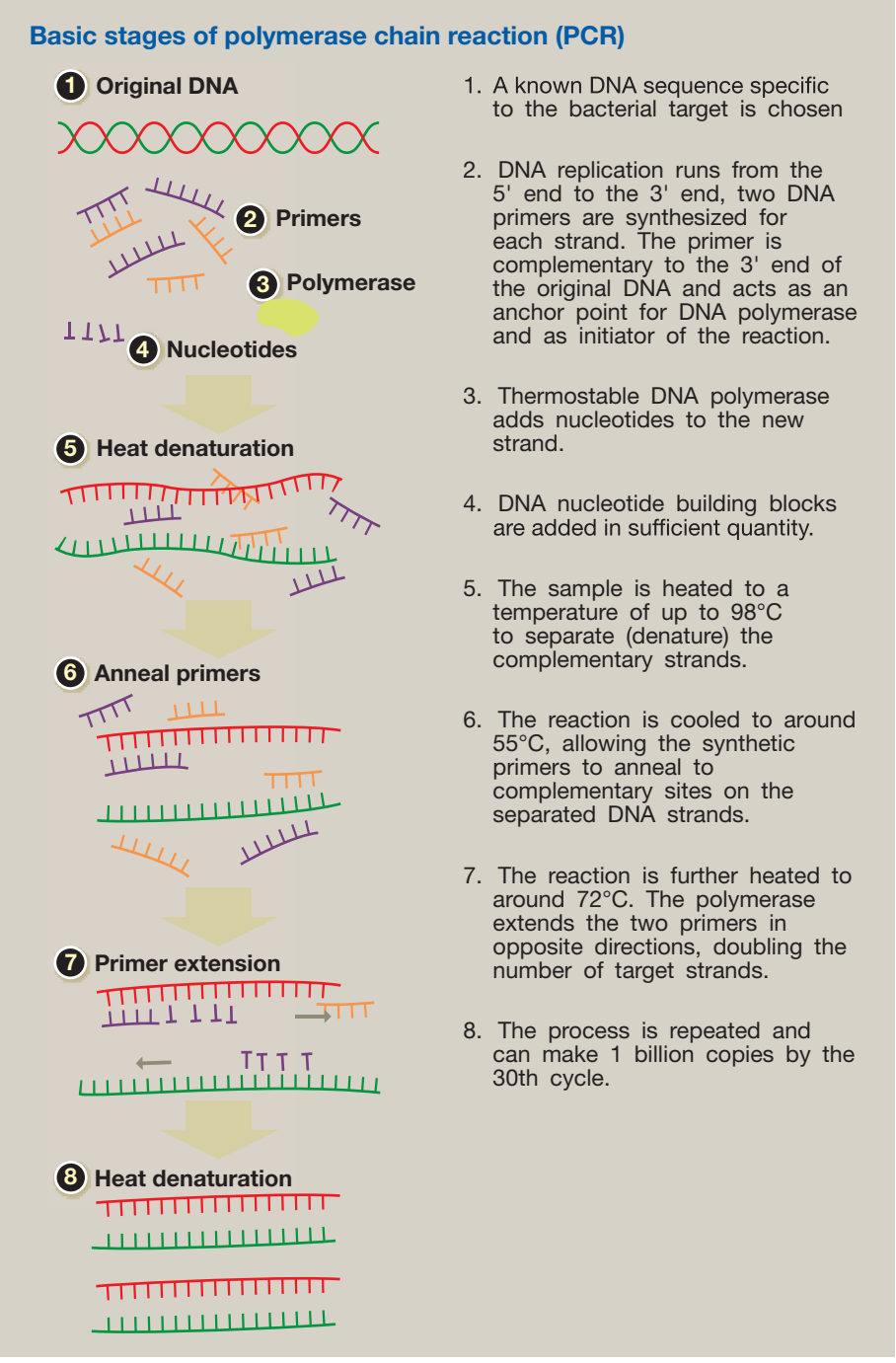

Figure 1