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Molecular-based diagnostics, including future trends

Simon Goldenberg

Abstract

Microbiology laboratories have traditionally relied upon phenotypic methods involving culture and biochemical testing to identify and characterize clinically important pathogens. These techniques have several disadvantages including poor sensitivity and long turn-around time. Molecular and mass spectroscopy techniques are rapidly changing infection diagnosis and management. 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 antibiotic stewardship and to improving 'time to appropriate antibiotics', one of the most important factors in improving the prognosis of patients with life-threatening infections. This article gives an overview of some of the practical applications of these newer technologies.

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

Molecular methods such as nucleic acid amplification tests (NAATs) and mass spectrometry are two of the most common approaches that are being adopted into 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 or urine) on incubated agar plates. Despite supplementation of growth media with various enrichment factors, most samples require 12–18 hours of incubation. It may be 48–72 hours after the specimen was collected once biochemical identification and antimicrobial susceptibility tests are complete. Patients receive 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, so driving antimicrobial resistance.

Non-culturable organisms – there is a range of 'fastidious organisms' that are unlikely to grow using traditional culture.

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These include *Bartonella*, *Borrelia*, *Brucella*, *Campylobacter*, *Helicobacter*, *Coxiella*, *Legionella*, *Leptospira*, *Mycobacterium*, *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). Blood culture system manufacturers 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 organism surviving transportation. When the amount of live bacteria in a primary specimen is very low, or because a significant proportion have not survived the transportation process, low sensitivity may be experienced. Molecular techniques enhance sensitivity by amplifying stretches of bacterial nucleic acid, so that lower numbers of the target bacteria are needed. Optimal transportation conditions are also less critical, since nucleic acid is detectable 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 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.

Mycobacterium tuberculosis

Mycobacterium 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, but is poorly sensitive (only 40–50% of patient with pulmonary tuberculosis (TB) are smear-positive)¹ and cannot differentiate *M. tuberculosis* from non-tuberculous mycobacteria such as *M. avium* complex, etc. PCR tests are available for use on smear-positive and -negative samples that can detect TB in under 2 hours. The Cepheid GeneXpert[®] system has the advantage of detecting *rpoB*, the gene conferring rifampicin resistance, which is a marker for multi-drug resistance.²

Meticillin-resistant *Staphylococcus aureus* (MRSA)

In England there is a programme mandating that all patients admitted to hospital be screened for MRSA. If found to be positive they are isolated and given topical suppression therapy, and may be

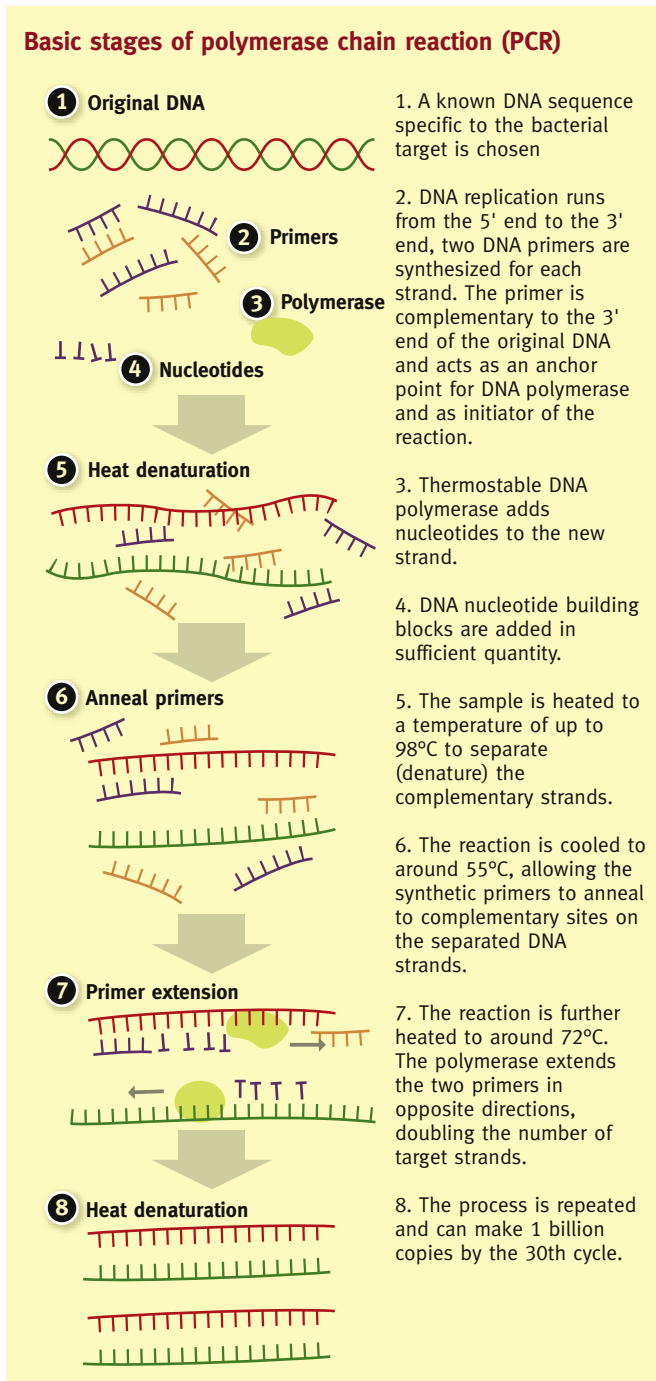


Figure 1

given antibiotic prophylaxis if they require certain types of surgery. Traditional culture takes 48–72 hours during which time there may be uncertainty about how to manage the patient, particularly if they presented as an unplanned or emergency admission. Use of commercially available NAATs reduces turn-around time, inappropriate pre-emptive isolation and associated costs.^{3–5}

Clostridium difficile

Gold standard methods for detection of this anaerobic organism are slow and labour intensive, requiring access to cell culture facilities to demonstrate cytotoxigenicity. Laboratories quickly

adopted enzyme immunoassays for the direct detection of toxins A and B from stool samples when they became available, because they simplified the procedure and allowed results to be obtained within around 6 hours. Unfortunately, the sensitivity of many of the commercially available tests is suboptimal and the Health Protection Agency now recommends using a two-stage testing algorithm, which may incorporate a molecular test. Several of these are available, helping to improve the accuracy of diagnosis. As molecular tests detect the presence of a gene rather than gene expression, it is important to correlate test results with clinical findings to help differentiate colonization (which may be detected in around 20% of hospitalized patients) from infection).^{6–8}

Sexually transmitted infections

Chlamydia culture is technically demanding and poorly sensitive, whereas gonorrhoea culture is simpler but still lacks sensitivity. Molecular testing has allowed the use of less invasive samples (e.g. urine rather than urethral swabs), which patients may prefer. Co-infections are common and multiplex molecular testing allows detection of two or more pathogens from the same sample. If introduced as point-of-care tests, these have the potential to reduce unnecessary pre-emptive treatment as well as the requirement for patients to re-attend the clinic and can be cost saving. Performing the test in the clinic can avoid the delay of sample transportation and the bottleneck of batching in centralized laboratories.^{9–11}

Sepsis and blood stream infections

Sepsis has a high excess mortality that increases by approximately 10% for every hour that antibiotics are delayed,¹² but symptoms of sepsis are often non-specific and blood cultures frequently fail to identify a causative organism. There are several commercial tests for detection of blood stream infections (e.g. SepsiT_{est}[®]; Molzym, Bremen, Germany, VYOO[®]; SIRS-Lab, Jena, Germany and Light-Cycler[®] SeptiFast; Roche, Mannheim, Germany),^{13,14} which could help to guide antibiotic therapy more accurately. However, currently available tests lack sufficient sensitivity and specificity, require specimen batching and a minimum of 6 hours processing time, and are technically complex.

Syndromic surveillance using multiplex panels

Where several pathogens may be responsible for the same symptoms it may be beneficial to apply a molecular test that has a panel of multiple targets rather than performing individual tests sequentially. Several molecular technologies are particularly suited to multiplexing (e.g. Luminex xTAG[®], Seegene Seeplex[®] and BioFire FilmArray[®]). These commercial products allow detection of 15–20 targets in one test, allowing differentiation of pathogens causing similar symptoms (e.g. gastroenteritis, respiratory tract infection and meningitis).^{15–17} This approach simplifies test requesting and minimizes the chance that an infection will be missed because of failure to request the correct pathogen. Some of these tests are significantly faster than traditional methods (culture for *Campylobacter*, *Salmonella* and *Shigella* can take up to 3 days), and so allow more efficient use of side rooms.

Universal broad range 16S rRNA/18S PCR

It is often unclear which pathogens are responsible for disease, samples may be difficult to obtain and there may be limited amounts available for testing (e.g. CSF or tissue samples obtained during surgery). Most molecular techniques require the requester to know what they are looking for so that probes and/or primers specific to a particular organism can be designed. 16S and 18S PCR allow detection of a broad range of bacteria and fungi respectively, using gene sequences containing hypervariable regions that can provide species-specific signature sequences.¹⁸

Sequencing technologies

Molecular methods have allowed sequencing of bacterial genomes, including whole genome sequencing. Next-generation techniques allow whole genome sequencing of an organism in under 8 hours at a cost of around £500, and prices continue to fall in this rapidly developing area. The rich resulting data allow detection of virulence determinants and antimicrobial resistance, as well as in-depth study of the epidemiology of disease. This has been particularly beneficial when investigating outbreaks of infection, and in characterizing newly emergent pathogens, such as the novel coronavirus (nCoV) causing severe respiratory infection.^{19–22}

MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry)

This technique uses a laser to ionize and accelerate bacterial and fungal molecules, which separate according to their mass-to-charge ratio, reaching a detector at the other end of the instrument at different times. In this way each molecule yields a distinct signal that, when compared with a database of known organisms, provides identification to species level. It is highly accurate and inexpensive (after initial outlay cost), and results are available from pure colonies in minutes. MALDI-TOF can also be used for the direct identification of bacteria and yeasts isolated in blood culture broths, and to detect phenotypic antimicrobial resistance by analysis of bacterial metabolites.^{23–25} The Abbott Plex-ID[®] system couples up-front PCR with electron spray mass spectrometry of resulting amplicons (pieces of DNA/RNA generated during PCR) to provide identification without the need for prior culture of organisms, yielding results in approximately 8 hours.

Future trends

The move towards reorganization and consolidation of pathology laboratories to improve efficiency and quality may be a driving force in the adoption of these newer techniques and could potentially open up new models of diagnostic testing (for example, point-of-care or near-patient diagnostics).

As these technologies develop and diagnostics companies offer increasing choice, cost and multiplexing capability is likely to improve. The speed and cost of whole genome sequencing, including deep sequencing to analyse whole populations of organisms, are rapidly improving and this technique is likely to be adopted into routine practice in the near future. The computing power and bioinformatics available to analyse

the resulting data are likely to be the limiting factors in its widespread adoption.

Considerations

Although these techniques offer several clear advantages, this must be balanced with the additional cost and expertise that some of these technologies require. Additionally, these techniques do not allow easy archiving of samples for future study, as the bacteria are not normally being isolated in pure culture; this could limit epidemiological surveillance – for example, many laboratories send a variety of isolates to Public Health England for typing. Most of these techniques by their nature are genomic and give no information about whether particular genes are being expressed, and this must be borne in mind when interpreting results. ◆

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