# Modern microbiology – a quiet revolution with many benefits

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## EDITORIAL

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## Abstract

In the clinical microbiology laboratory, classical culture and identification methods are rapidly giving way to molecular techniques with many benefits for clinicians and patients. Building on the discovery of the structure of DNA and the genetic code, four main scientific advances have been made which underpin these techniques (hybridisation probes, polymerase chain reaction, the observation that the microbial species signature can be read in the ribosomal genes and also in the proteins). Early discoveries have paved the way for new diagnostic methods, which are rapid, highly sensitive and specific. Automation has provided high throughput for large numbers of clinical specimens combined with reasonable cost. The benefits for the clinician and patient include confirmation of clinical diagnoses and information about antimicrobial susceptibility within hours compared to days for conventional methods. In resource-poor settings, molecular techniques and automated systems may seem unaffordable but new public-private partnerships, initiatives by the World Health Organization and new, innovative laboratory methods offer the promise of benefit for all.

**Key Words**
Microbiology, molecular methods, resource-poor

## Introduction

For some time, a quiet revolution has been taking place inside the hospital microbiology laboratory. Molecular techniques, based on the detection of microbial DNA, RNA or proteins, have moved out of the research laboratory and into the domain of routine diagnostic testing. These techniques offer the promise of faster specimen turn around time, more accurate pathogen identification and greater sensitivity, facilitating confident clinical decision-making. Combined with automation, modern microbiology offers high throughput options for large numbers of specimens at reasonable cost. However, while the cost of processing each specimen may be attractive, the price of the required equipment may not. In this article, the development of modern microbiology is traced from the discovery of the structure of DNA to applications for the clinical laboratory. The advantages of these methods are discussed and the question of affordability for resource-poor countries is raised. The aim of this article is not to review all the new technologies available but to provide the non-specialist with an insight into the inner workings of the modern clinical microbiology laboratory and the benefits provided for both patient and clinician.

### History

Four main scientific advances form the basis of modern microbiology. These are: (i) invention of the hybridisation probe; (ii) discovery of the polymerase chain reaction; (iii) the observation that the microbial species signature can be read in the ribosomal genes; and (iv) that it can also be read in the proteins. First off the block were radioactively labelled hybridisation probes developed by Joseph Gall and Mary Lou Pardue in the 1960s. This technology arose from Watson and Crick's discovery of the structure of DNA and the genetic code. Hybridisation probes are DNA or RNA fragments which can bind to complementary sequences in the microbial chromosome. More user-friendly fluorophores replaced the radioactive labels leading to the development of fluorescence in situ hybridisation (FISH). Currently, FISH technology is most commonly used to identify bacteria and fungi in blood cultures by targeting specific microbial DNA sequences and is particularly valuable for candidaemia.[1]

In the 1970s, the painstaking and often controversial work of Carl Woese in studying ribosomal genes led to the first scientifically based tree of life, a map of the large-scale organisation of life showing the early course of evolution.[2] His work paved the way for a new method of identifying microbes based on the nucleotide sequence of the genes encoding the small 16S ribosomal RNA subunit for bacteria and the 18S rRNA subunit for eukaryotic organisms such as fungi.[3] As well as conserved regions, these subunits contain hypervariable regions that can provide genus and species specific signatures. The establishment of sequence databases in GenBank and the Ribosomal Database Project allows comparison of the test result with known bacterial and fungal species.

**Figure 1: Carl Woese – author of the Phylogenetic Tree of Life (picture by permission Don Hamerman from: http://commons.wikimedia.org/wiki/File:Carl_Woese.jpg)**

Also in the 1970s, Anhalt and Fenselau observed differences in mass spectra of bacterial extracts.[4] This idea was further developed by allowing a laser to ionise biomolecules (e.g. bacterial/fungal constituents) in the presence of a matrix. The ionised molecules are then accelerated into an electrical field and enter a flight tube where they are allowed to drift towards a detector, the time of flight mass spectrometer. Matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) can be used to identify bacteria and fungi cultured from clinical specimens by analysing the abundant proteins present and comparing the test sample with an extensive database of protein signatures.[5] Bacteria or fungi present in blood cultures can be directly identified by this method providing there is an incubation step to allow microbial growth.

Developed in 1983 by Kary Mullis, polymerase chain reaction (PCR) enables a target stretch of DNA to be copied thousands or millions of times. The product of the PCR reaction can then be visualised by staining with a fluorescent dye following gel electrophoresis. The method can be adapted for RNA (e.g. RNA viruses) using the enzyme reverse transcriptase, which creates a complementary DNA transcript (cDNA). By targeting specific virulence genes or the 16S rDNA, pathogens can now be detected and identified directly in clinical samples without the need for culture. Initially, the PCR process was quite cumbersome and most unsuited to the clinical laboratory. In addition, clinical specimens such as stool and blood contained PCR inhibitors. Most of the barriers to using PCR as a diagnostic laboratory tool have been overcome and the invention of gel-free quantitative PCR, which monitors amplification in real time, was a significant breakthrough. In qPCR systems both PCR and amplified product detection are generally completed within one hour and because the reaction is carried out in a closed vessel, the risk of amplified products being released into the environment and causing cross-contamination is low.[6] Melting curve analysis is performed to confirm that the correct portion of the target gene has been amplified; melting curves differ according to the PCR product, based on the guanine and cytosine content of the amplified nucleic acid. There are a number of variations on the PCR theme including the ligase chain reaction (LCR), which has greater specificity.

Other molecular methods that are currently making the journey from the research to the clinical laboratory include microarrays and whole genome sequencing; no doubt there will be many others.

**Do we need it?**

Developments in the fields of surgery, medical technology and pharmacology have facilitated treatment of a wide range of malignant and non-malignant disease that were previously untreatable. These advances have led to an increased life expectancy and quality of life. Nevertheless, there is a downside, consisting of a surge in the number of patients who are particularly vulnerable to infection because of surgical and other invasive procedures, treatment with immunosuppressive drugs or simply aging of the immune system. These patients may become infected with a wide range of microorganisms, including those that are not normally pathogenic. There is also an increasing recognition of unusual clinical presentations of infection and pathogens taking up residence in areas of the body that they are not usually associated with. There are several such examples in recent issues of the Australasian Medical Journal.[7–10] With ability to detect non-culturable or difficult-to-culture microbes, molecular techniques offer the promise of rapid detection of the new and unusual. Not all molecular systems are designed for all pathogens, but it is likely that the range of detectable microbes will increase in the future. Modern microbiology also offers hope for the reduction of infections in developing countries, where the

burden of disease is highest. For example, Cepheid's Xpert®MTB/RIF has the potential to revolutionise tuberculosis diagnosis by simultaneously detecting *Mycobacterium tuberculosis* and the rifampicin resistance gene (a marker for multi-drug resistance) delivering results in two hours. Current testing for multi-drug resistant *M. tuberculosis* can take more than four weeks, leading to further spread of resistant strains.[11]

Laboratory tests are an important tool for the clinician in dealing with patients with invasive infection. The incidence of sepsis has increased in some parts of the world and there is a pressing need for rapid identification of the causative microbe.[12] Roche LightCycler® SeptiFast system is designed to identify the main bacterial and fungal causes of bloodstream infections directly in whole blood samples within hours and has the option for identifying the methicillin resistance gene. Multiple studies have established the overall greater sensitivity and specificity of modern molecular methods compared with conventional culture and identification techniques. The detection times are also impressive, 0.2–6 hours for rapid molecular methods compared with 24–48 hours for conventional methods.[13] For some of the molecular methods there is still a need to culture the offending microbe but incubation times can often be shortened because of the greater sensitivity of the test. In addition, there are molecular methods for the detection of antibiotic resistance genes, enabling optimisation of antimicrobial therapy to take place at an earlier stage thus assisting hospital antibiotic stewardship programs.[13]

**Who can afford it?**

Fluorescence microscopes, thermocyclers, qPCR machines, hybridisation ovens, automated expert systems, specialised reagents - these are the more expensive requirements of the modern microbiology laboratory. In some regions of the world uptake of the new technologies has been slow. For resource-poor areas, the obstacles can seem insurmountable because significant funding must be allocated for upgrading laboratory infrastructure and training of staff as well as major equipment purchases. At the same time, procuring the required equipment, reagent supplies and after-sales service can be difficult.[11] An article by Petti et al written in 2006, points out that of the 12 million people who die in sub-Saharan Africa each year, most will probably succumb to an infectious disease.[14] However, at that time, relatively little funding was allocated for laboratories to confirm clinical diagnoses, conduct infectious disease surveillance and direct public health care policy. Limited access to good laboratory testing leads to reliance on clinical algorithms, but without laboratory confirmation misdiagnosis can be common leading to inadequate treatment, increased mortality and lack of knowledge about the true prevalence of infectious diseases. For example, a Nigerian study showed the accuracy of clinical diagnosis of typhoid fever was only about 50% when compared with laboratory culture confirmation.[14] More recently, the coordinated efforts of public, private, national and international partners have resulted in successful laboratory capacity building initiatives in resource-poor areas, particularly where HIV-tuberculosis co-infection is a problem.[11] In addition, new molecular techniques have recently been developed which do not require specialised equipment, such as loop mediated isothermal amplification (LAMP). DNA amplification takes place at a constant temperature (60–65$^{o}$C) and the presence of product inferred from the turbidity in the tube or increased fluorescence caused by by-products in the amplification mix. This method shows great promise for the detection of *Mycobacterium tuberculosis* in clinical specimens.[15]

It is to be hoped that initiatives by the World Health Organization and other stakeholders, combined with new innovations at the laboratory bench, will continue to increase laboratory standards and capacity in resource-poor settings so that the quiet revolution can be adopted more widely, benefiting all.

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## CONFLICTS OF INTEREST

The author declares no competing interests.