Diagnostic microbiology in the 21st century – a quiet revolution

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It is a very exciting time to be a microbiologist. In the early 21st century we are seeing a maturation of molecular biology methods and their translation into the clinical microbiology laboratory, in combination with improvements in existing methods and substantial increases in the power of information technology.

From the early days of microbiology, the diagnosis of bacterial, fungal or viral infections has shared a common platform – culture. This has been the mainstay of diagnostic microbiology followed by immunoassays and molecular tests. The latter group of tests, based largely on the PCR and genomic sequence analysis, have increased sensitivity by several orders of magnitude, lowered test turnaround times, and improved reproducibility and specificity. This makes diagnostic microbiology tests more clinically relevant in a shorter time frame, which is very important in modern health care with its emphasis on shorter lengths of stay, ambulatory style care and also in public health.

In this complete electronic issue with the theme on Diagnostic Microbiology, the articles range from the use of improved specimen collection swabs to new generation sequencers and mass spectrometry. Optimal specimen collection is obviously critical for successful diagnostic tests. The attendant demands of successful microbiological culture have strongly influenced specimen sampling and collection. Improved swabs that are suitable for both culture and molecular tests have enabled the collection of higher amounts of specimens, minimising the need for more invasive collection techniques that have been used traditionally *viz.* nasopharyngeal aspirates.

Rapid detection of the organism or virus in specimens presents a diagnostic challenge, in particular with low target concentrations, unless culture or PCR is used to 'amplify' the agent or nucleic acid sequence. The use of chromogenic selective agar media for target organisms, in particular from specimens with high or mixed commensal microflora has been reported to offer higher culture rates. Subsequent identification of the selected colonies may circumvent PCR inhibition or interference with large amounts of contaminating host and commensal DNA from specimens. PCR inhibitors *viz.* heme molecule, lipopolysaccharides and others are known to affect test sensitivity, in particular those with low target copy numbers.

The requirements for subjective readings, incubation periods of several days to weeks and the need for viable agents have limited the use of culture. In particular, virus isolation has a more limited range of cell types that can be used, than with the much wider range of bacterial or fungal culture media. This has led to a significant increase in the use of molecular tests which – in most diagnostic laboratories – have supplanted virus isolation or immunoassays for antigen detection. The subtyping, as well as detection, of known agents, for example, the large range of enteroviruses, antibiotic resistance genes/plasmids in *Enterobacteriaceae*, identification of new or emerging agents and other confirmatory tests will require the increased use of molecular sequence analysis. The next generation of faster and higher capacity sequencers with smaller footprints will be of greater attraction to microbiologists.

The complex nature of persistence and recrudescence/reactivation with herpesvirus infections present additional diagnostic challenges. These are compounded by different clinical presentations with immunocompetent versus immunocompromised patients. A comprehensive range of virological 'tools' are needed in differential diagnosis for specific antibody response, viral nucleic acid detection, quantification and culture.

Molecular microbiology has brought greater demands on test accuracy, precision and quality control/assurance. This is particularly evident in viral load tests. The high sensitivity and short linear amplification range of PCR are two parameters,

amongst others, that are expected to affect intra and inter assays. These variations may be minimised by increased staff training and the use of high-quality automated precision equipment.

The modern microbiologist has an impressive toolkit with molecular and non-molecular tests to choose from. Sometimes the choice of test for a given pathogen varies between different clinical scenarios, as exemplified by the herpes viruses. The fast turnaround time in many of the new methods makes diagnostic microbiology much more clinically relevant for routine testing, whereas in the past, the long delay in culturing organisms in order to issue antibiotic sensitivity results made this testing less clinically relevant. The fast turnaround time of the new tests also results in rapid recognition of pathogens of public health significance such as multidrug-resistant bacteria, pandemic organisms and bioterrorism agents. More rapid turnaround time is also beneficial for infections which are rapidly progressive and dangerous, such as bloodstream infections.

Digital technology is based on the binary system and this has obviously produced quantum improvements in many facets of our daily lives. Nature, however, has provided us with a more sophisticated quaternary system – the four nucleotides in our genetic code. Microbiologists, as well as other life scientists, have exploited this system to develop and extend molecular tests for rapid detection, identification and confirmation of infection to the molecular level.

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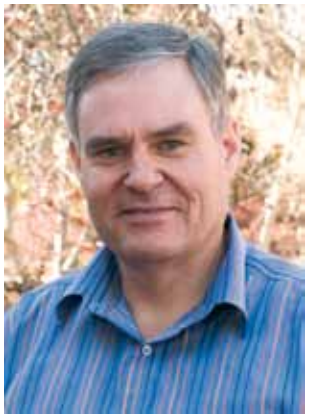
Biographies

Tuckweng Kok is a virologist with 30 years' comprehensive experience in translational research, viral diagnostics and public health virology. He has been seconded to the WHO, Beijing as Virology Consultant for SARS, Human and Avian Influenza assessments. His research work focuses on neutralising antibodies to HIV and Influenza viruses and rapid viral and mycoplasma diagnostics. He has a passion for teaching and is one of the initiators of the postgraduate virology master class course at the University of Adelaide.

Iain Gosbell is a microbiologist and infectious diseases physician, and was previously the Director of the Department of Microbiology and Infectious Diseases, Sydney South West Pathology Service – Liverpool, and took up the position of Professor of Microbiology and Infectious Diseases in the School of Medicine, University of Western Sydney in late 2009. He has published on antibiotic-resistant bacteria, particularly community methicillin-resistant *Staphylococcus aureus* (MRSA). Recently in conjunction with Slade Jensen and Björn Espedido he helped co-found the Antibiotic Resistance and Mobile Elements Group (ARMEG) at the School of Medicine, which will examine resistance elements in multiresistant organisms (MROs), especially MRSA.

Rapid sequencing and analysis for pathogen confirmation



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Genetic amplification methods and especially those based on the polymerase chain reaction (PCR) are revolutionising diagnostic microbiology. Genetic amplification is rapidly becoming the method of choice to detect viruses, 'single pathogen' infections such as Chlamydia and Gonorrhoea, slow growing or difficult-to-grow bacteria such as mycobacteria and legionella, or toxigenic bacteria such as Shigatoxigenic *Escherichia coli* and *Clostridium difficile*. This trend has been accelerated with the use of fluorescent, probe-based, real-time PCR, high-throughput thermocyclers and the use of robotics. Traditional biochemistry-based identification is adequate for many bacterial isolates, as is the specificity afforded by real-time probes for direct PCR detection. However, the sequencing of amplified products (amplicons) is also often performed where increased resolution is required, even though longer amplicons than those generated by real-time PCR are required. Two benefits are clear. Firstly, sequence is constructed solely from the four nucleotides so can be likened to a digital signal. In comparison, biochemical identifications are more like an analogue signal, where weak or equivocal reactions can create ambiguity. Secondly, the resolution afforded by biochemical reactions (less than 100 character states i.e. 48 reactions that can be positive or negative) is easily