Diagnostics into the future



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This edition of *Microbiology Australia* is dedicated to the issue of emerging technology in diagnostics and its suitability to the response to emerging animal and human disease. It is fittingly entitled 'Diagnostics into the future'. There is valid argument that the current pattern of infectious diseases is not historically unprecedented[1] and has major driving forces of human population translocation, transhumance, exposure of naïve populations, increasing contact with wildlife and intensive agriculture[1-4]. However, what is clear is that emergence and re-emergence of diseases globally requires rapid responses built on sophisticated epidemiological understandings of the disease in question. Increasingly, understanding of the complex webs from which disease emerges is built on the continual emergence of new diagnostic methodologies[1]. There are numerous examples of emerging and re-emerging infectious diseases with complex epidemiological bases. It is estimated that 58% of human pathogens are zoonoses and that 73% of emerging diseases of people are zoonoses or have a distinct link to animal disease[2]. Rapid identification of causative agents and subsequent exhaustive epidemiological investigation are our best weapons in responding to these emerging diseases[1]. They allow a rational and targeted approach to disease control and prevention in the face of hitherto unknown disease. Emerging technology and innovative application of existing technology streamline the processes and allow researchers unprecedented rapidity and accuracy of investigation.

An interesting example of this is the emergence of cryptococcosis in Canada over the past 15 years or so[5,6]. Not a new disease, cryptococcosis caused by *Cryptococcus gattii* displayed a dramatic shift in geographical occurrence (from a tropical or subtropical distribution to a temperate North American distribution) and epidemiological features (from a sporadic disease to an outbreak), with disease in animals sentinel to an emerging human caseload[5]. Detailed expression microarray analyses of outbreak isolates have revealed genotypes of high virulence[7] and phylogenetic and recombination analyses of global *C. gattii* isolates have suggested emergence from a recombinant event in the Amazonian rainforest of South America[8]. This illustrates how existing and emerging technologies may be adapted and applied to emerging problems.

Another higher profile global example has been the emergence of the severe respiratory tract diseases Sudden Acute Respiratory Syndrome (SARS)[9] and Middle East Respiratory Syndrome (MERS)[10], both caused by coronaviruses. These diseases, along with a number of others, appear to have emerged from a bat reservoir, often involving an intermediate host, thus allowing for more effective human adaptation[9]. Discovery of these complex webs have intrigued researchers for a number of years, and have required sophisticated technological and multidisciplinary approaches in order to affect appropriate understanding of these diseases.

In this context, 'Diagnostics into the future' is a mix of articles on newly emerging technologies and their applications, and existing technologies adapted to newly emerging problems, of course all in the context of the inextricably linked nature of human and animal disease. Of equal importance in our endeavours as diagnostic microbiologists are emerging technologies and how these may be used to increase our understanding of disease. The topics addressed in this issue include a review of nanotechnologies, high throughput molecular diagnostics, digital PCR as a quantification tool, massively parallel sequencing and the evolution of microarray technology and instrumentation in diagnostic molecular microbiology. In addition, the application of these techniques are discussed in articles describing the epidemiological investigation of Bairnsdale ulcer and Q fever in humans, including the use of next generation sequencing in the epidemiological investigation of protozoal disease.

The article on the use of nanoparticles in diagnostics by Ranzoni *et al*. describes how nanoparticle technology may be integrated with existing technologies to dramatically increase the sensitivity of current diagnostic applications but also to increase the range of specimen suitability, thus reducing preparation time and increasing flexibility. This contribution makes the conceptual bases of these technologies accessible to all and demonstrates how microbiological research can be brought to diagnostics.

An important emerging technology is matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS). The article by Whiley *et al*. describes the use of this technology in the molecular characterisation of microorganisms in a cost-effective manner, using large-scale multiplex SNP detection and analysis. Its subsequent application for genotyping and antimicrobial resistance detection is discussed. This technology is being increasingly applied in infectious diseases, molecular genetics and cancer research.

PCR technology is now commonly used for many diagnostic and research applications. However, the ability to quantify microbial loads using conventional PCR still has significant limitations. Kerry Emslie, from the National Measurement Institute, introduces another method of quantification using digital PCR, which is gaining increasing utility through advances in microfluidic technology. Digital PCR is now providing accuracy of quantification and high reproducibility, and is likely to replace traditional PCR in many settings in the near future.

Deciphering DNA sequences is essential for virtually all branches of microbial research. A significant step in our ability to do so efficiently and cost-effectively was the development of capillary electrophoresis (CE)-based Sanger sequencing. This technology has become widely adopted in laboratories around the world, yet has been hampered by inherent limitations in throughput, scalability, speed and resolution. The late 1990s saw the development of the next-generation sequencing technologies that parallelise the sequencing process, producing thousands or millions of sequences concurrently, at a lower cost than standard dye-terminator methods. The advantages of massively parallel sequencing in the initial investigations of emerging disease, in complex disease, and in microbial community profiling are discussed by David Warrilow and Richard Allcock.

The future of microbial diagnostics is increasingly shaped by new technology and instrumentation. Sepehr Tabrizi examines instrumentation that is rapidly becoming more easily available to microbiologists in all settings, and which will even extend into quite small microbiology laboratories in the near future. Included in this new technology revolution is the use of microarrays, or DNA chips, which have been hailed as the ultimate experimental tool for research, drug discovery and diagnostics. Microarrays have the potential to perform a multitude of molecular tests simultaneously and to produce a wealth of information from a single clinical sample. Joanne Mercer presents the advantages of array technology, and introduces recent advances that appear to effectively manage the potential for cross contamination.

Many of the new molecular technologies are applied to increase our understanding of newly emerging diseases including zoonoses. In particular, Q fever, a disease caused by the bacterium *Coxiella burnetti* in humans, is commonly associated with the livestock and meat industries. However, Norris *et al*. present recent evidence of an alternative epidemiology for this disease, detailing human exposure to cat and dog parturition as a potential significant risk factor.

A fascinating account of the emerging links between human and animal disease in Buruli/Bairnsdale ulcer, a devastating mycobacterial disease found in sub-Saharan Africa and Australia is presented by Janet Fyfe and Carolyn O'Brien. The combination of a feline specialist veterinarian and a laboratory scientist has resulted in a fresh and comprehensive investigation of this disease, illustrating the advantages of a comparative approach to disease investigation (One Health). The globally unique evidence of this disease in animals and in particular the potential role of ringtail possums in the ecology of the pathogen and epidemiology of the disease, may present a breakthrough in understanding of this disease globally and suggest the role of different animals in the pathogen's ecology.

Finally, Jan Šlapeta presents the example of a devastating disease in echidnas caused by systemic coccidiosis. The disease itself has been recognised for some time, but current understanding is limited to whether extraintestinal disease and intestinal disease are caused

by the same or different coccidian protozoa. 454 pyrosequencing technology was applied to answer this question and is a good example of wildlife disease investigations in parasitology.

This issue of *Microbiology Australia* highlights the microbiologist as the technologist and key intellect in the adaptation and implementation of research tools into diagnostic processes. Also, it emphasises the importance of microbiology as a discipline that spans species (a component of comparative pathology) as the basis of One Health. We trust that the contributions presented in this issue stimulate your imagination and promote future collaboration using hitherto unfamiliar technologies in the investigation of human and animal disease.

References

1. Gummow, B. (2010) Challenges posed by new and re-emerging infectious diseases in livestock production, wildlife and humans. *Livest. Sci.* **130**, 41–46. doi:10.1016/j.livsci.2010.02.009
2. Jones, B.A. *et al*. (2013) Zoonosis emergence linked to agricultural intensification and environmental change. *Proc. Natl. Acad. Sci. USA* **110**, 8399–8404. doi:10.1073/pnas.1208059110
3. Ka-Wai Hui, E. (2006) Reasons for the increase in emerging and re-emerging viral infectious diseases. *Microbes Infect.* **8**, 905–916. doi:10.1016/j.micinf.2005.06.032
4. Quilliam, R.S. *et al*. (2013) Subclinical infection and asymptomatic carriage of gastrointestinal zoonoses: occupational exposure, environmental pathways, and the anonymous spread of disease. *Epidemiol. Infect.* **141**, 2011–2021. doi:10.1017/S0950268813001131
5. Krockenberger, M.B. and Lester, S.J. (2011) Cryptococcosis–clinical advice on an emerging global concern. *J. Feline Med. Surg.* **13**, 158–160. doi:10.1016/j.jfms.2011.01.015
6. D'Souza, C.A. *et al*. (2011) Genome variation in *Cryptococcus gattii*, an emerging pathogen of immunocompetent hosts. *mBio* **2**, e00342-10.
7. Ngamskulrungroj, P. *et al*. (2011) *Cryptococcus gattii* virulence composite: candidate genes revealed by microarray analysis of high and less virulent Vancouver Island outbreak strains. *PLoS ONE* **6**, e16076. doi:10.1371/journal.pone.0016076
8. Hagen, F. *et al*. (2013) Ancient dispersal of the human fungal pathogen *Cryptococcus gattii* from the Amazon rainforest. *PLoS ONE* **8**, doi:10.1371/journal.pone.0071148
9. Smith, I. and Wang, L.-F. (2013) Bats and their virome: an important source of emerging viruses capable of infecting humans. *Curr. Op. Virol.* **3**, 84–91. doi:10.1016/j.coviro.2012.11.006
10. CDC. (2013) Update: severe respiratory illness associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV) — worldwide, 2012–2013. *Morb. Mortal. Wkly Rep.* **62**, 480–483.

Biographies

Associate Professor Theo Sloots is the Unit Director of Research at the Queensland Paediatric Infectious Diseases (QPID) laboratory at the Queensland Children's Medical Research Institute, Brisbane. His laboratory conducts basic and applied research in infectious diseases, particularly respiratory and gastrointestinal infections, applies molecular technology to the diagnosis and characterisation of microorganisms, and investigates undiagnosed infectious disease in children.

Cheryl Bletchly completed her BSc(Hons) at the University of Queensland. She spent 10 years at the Sir Albert Sakzewski Virus Research Centre as a research assistant while also completing a PhD on dengue virus. After 5 years in a public health virology laboratory she took up her current position as the Supervising Scientist of the Molecular Diagnostic Unit within Pathology Queensland.

Mark Krockenberger is Associate Professor of Veterinary Pathology in the Faculty of Veterinary Science at the University of Sydney. Mark completed a PhD on cryptococcosis in koalas in 2003 and continues to have a research focus around infectious diseases of animals. He is a Fellow of the Australian and New Zealand College of Veterinary Scientists (Anatomical Pathology) and is currently Director of the Veterinary Pathology Diagnostic Services in the Faculty of Veterinary Science.