New Molecular Platforms for the Diagnosis of Invasive Fungal Infections

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The rapid escalation and high infection-related mortality of invasive fungal infections (IFIs) has prompted the need for rapid, more sensitive and accurate diagnostic tools. Much focus has been on the development of new molecular platforms that enable, broad range detection and identification of fungi from a variety of clinical specimens. However, the wider application of these methods is limited by the lack of standardisation, in part due to the variety of molecular methods. Although there is no universally accepted DNA detection system, three commercial in vitro diagnostic (IVD) assays have recently become available. These are generally faster than in-house assays, less laborious and rigorously maintained by high quality control standards, but are very expensive and still need to be run in parallel with ‘gold standard’ methods as clinical evaluations are ongoing.

**Commercial systems**

Several groups have evaluated the LightCycler® SeptIFast assay (Roche Diagnostics) for the detection of six fungal and 14 bacterial pathogens from whole blood specimens (covering 95% of the most frequently isolated organisms from blood culture) [1-7]. The system utilises dual internal transcribed spacer (ITS)-directed FRET probes and melting curve analysis to provide species identification within 6 h. The detection limit is 30 cfu/ml (100 cfu/ml for *Candida glabrata*) and is potentially more sensitive than blood culture [3]. Studies, however, have shown that the assay is more useful for the detection of bacterial, rather than fungal pathogens.

Further validations are warranted to confirm its clinical and diagnostic utility.

The FXG™: RESP (Asp +) (Mycostica Ltd, Manchester, UK) system was released in 2008 and employs molecular beacons to detect *Aspergillus* and *Pneumocystis jiroveci* in respiratory samples. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) have been reported to be 74%, 93%, 76% and 92%, respectively, for *Aspergillus* detection; and 97.4%, 92.9%, 90.4% and 98.1%, respectively, for *P. jirovecii* detection [8, 9]. Microbial cross-reactions were not apparent (except against *Penicillium* sp.). Prospective and supportive clinical trials are ongoing to evaluate the clinical utility of this kit.

The third available IVD assay is repetitive-sequence-based PCR (rep-PCR; DiversiLab System, Spectral Genomics, Houston, TX). This platform uses primers that target non-coding repetitive sequence regions which are separated on a microfluidic chip (Agilent Bioanalyser 2100) that measures fluorescence intensity and migration time. Resulting fingerprints are compared with known standards, generating a percentage similarity (>85%) and cluster profile for identification. The current rep-PCR library holds patterns for species of *Aspergillus*, *Fusarium* and *Candida*, dermatophytes, *Blastomyces dermatidis*, *Coccidioides* and *Histoplasma capsulatum*. Numerous studies have demonstrated its potential [10-13], but the kit is restrictive as it can only be used on pure cultures and is dependent on an up-to-date rep-PCR library.

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Brian O’Toole is an epidemiologist who has followed a peripatetic research path. He completed his PhD in psychology/neuroscience (Sydney, 1980) and a later Masters in Public Health by research (Sydney, 1990) into risk factors for mortality in 20-40 year old men. With major interests in research design and analysis, psychiatric epidemiology and the psychological effects of trauma, he has pursued largely a research career in community medicine, epidemiology, psychiatry and public health. Milestones have included headship of the only academic survey research centre in Australia; a major set of clinical epidemiologic studies in General Practice Integration research; a national evaluation study of early psychosis intervention programs; and a longitudinal study of the health of Australian Vietnam veterans and their families. He has developed and taught postgraduate courses in epidemiology since the mid-1980s and is a consultant to academic research groups in study design and statistical analysis strategies. He has over 100 published papers and government reports. His current purview at CIDM-PH is to assist staff and students with design and analysis of clinical epidemiological studies.
References


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