Flocked nylon swabs provide a sensitive collection method for culture, rapid, near patient testing and molecular detection of a variety of bacteria and viruses because of their ability to absorb cells then release them effectively to increase the sensitivity of detection of infecting microbes. Flocked swabs are more expensive than cotton or Dacron™-tipped swabs but their dual application for culture and molecular testing can reduce handling and storage costs and test turnaround times.

The move to molecular detection of microbes requires use of optimal collection techniques to maximise their accuracy and sensitivity.

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Biography

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Recent advances in molecular and non-PCR-based platforms for the rapid diagnosis of invasive candidasis in the ICU

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Invasive candidasis (IC) accounts for 15–30% of all nosocomial intensive care unit (ICU) infections [1]. Two-thirds of infections are due to candidemia, a rate 5–10 times higher compared with that in the general hospital population [2]. Crude mortality rates and associated health costs remain high, despite advances in antifungal therapy[3]. *Candida albicans* remains the most frequently isolated species (45%) although there has been a slow but definite shift towards non-*C. albicans* species (especially *Candida glabrata*) infections, which has been attributed to the increased use of fluconazole prophylaxis in some centres. Early antifungal therapy significantly reduces IC-related mortality but is often delayed because 'gold standard' diagnosis by culture and/or histology is insensitive (for example, 50% candidemia cases not detected by blood culture) and slow (at least 48–96 hours required for species detection and identification). This article highlights a number of recent advances in molecular and non-PCR-based technologies that have enabled more rapid diagnosis of IC in the ICU (a recent review [4] provides a comprehensive summary of molecular and serological methods in diagnostic mycology).

Rapid diagnosis from positive blood cultures

Numerous real-time polymerase chain reaction (PCR) assays to rapidly identify yeasts from positive blood cultures have been described with mostly high sensitivities and specificities [5-7]. These are generally simple and easily adapted into the routine workflow with results typically available within three hours of blood

cultures that flag 'positive'. Even though real-time PCR offers many advantages, the majority have been developed in-house and the subsequent lack of standardisation makes it difficult to universally compare the performance characteristics between assays.

Nonetheless, multiplex-tandem PCR (MT-PCR; www.ausdiagnostics.com) platform has recently demonstrated advantages over other published assays by enabling the simultaneous identification of 11 major fungal bloodstream pathogens (including three moulds) direct from positive blood culture specimens within two hours [5]. While identification was dependent on the availability of species primers, the platform's flexibility allowed for customisation of the target panel for particular clinical settings and variability in the number of targets/samples that could be tested in a single run (up to 72). Using automated DNA extraction, the cost per specimen was ~$20.

A novel alternative to DNA amplification is peptide nucleic acid fluorescent *in-situ* hybridisation (PNA FISH; AdvanDx Inc.; Woburn, MA) which utilises *Candida* probes to identify yeasts from positive blood cultures within 2.5 hours via flow cytometry or fluorescent microscopy. Its clinical utility and impact in reducing treatment costs (for example, switching patients from echinocandin to fluconazole therapy when *C. glabrata* has not been detected) have been well-documented with the *C. albicans* and the *C. albicans*/*C. glabrata* probes [8,9]. However, it is the new generation tricoloured PNA FISH probe (Yeast Traffic Light™) that may have the greatest impact because of its ability to rapidly discriminate between fluconazole susceptible and potentially resistant species (*C. albicans*/*C. parapsilosis, C. glabrata*/*C. krusei* and *C. tropicalis*). Further evaluation is required to establish its clinical utility and the system is most likely suited for larger high-throughput laboratories. As yet, the procedure is not automated and the cost/test ranges from US$30 to US$80. False positive results have also been reported.

Although germ tube testing is traditionally performed on cultures from solid media, recent studies have proven it useful for the rapid, presumptive identification of *C. albicans* directly from cell pellets collected from yeast-positive blood cultures (87–92% sensitivity and 100% specificity [10,11]). Such a method offers a practical, simple and cheap alternative for the rapid screening for *C. albicans,* allowing early initiation of treatment whilst awaiting confirmatory results. Nonetheless, false negatives can occur (5%) and false positives with *C. dubliniensis* and *C. tropicalis* have been reported.

Rapid diagnosis from blood specimens

Because the diagnosis of candidemia by blood culture is insensitive and slow [12], efforts to facilitate earlier diagnosis by the direct testing of whole blood, serum and plasma specimens have been made. In a recent retrospective evaluation, the MT-PCR assay (above) was applied to a population of candidemic patients. It was found that pathogen detection and speciation could be achieved up to four days earlier than blood culture in 54/74 (70%) patients when whole blood samples were tested [13].

A higher proportion of fungal isolates (86% versus 43%) was also found in a clinical assessment of the LightCycler™ SeptiFast (Roche Diagnostics) assay when compared with blood culture [14]. This platform provided simultaneous detection of six fungal and 14 bacterial pathogens from whole blood; however, like all closed testing systems, false negatives can occur. Nevertheless, commercial systems have enabled some uniformity to be established but are recommended for use in conjunction with blood culture until extensive, prospective clinical validations are performed.

The low fungal load in blood (<10, and <1 cfu/ml in 25% of cases [15]) and its variation depending on the time of sampling during the disease course have presented some diagnostic challenges [16]. There is also no consensus regarding the optimal blood fraction from which to isolate fungal DNA, with some groups reporting better recovery of fungal DNA from serum and plasma specimens [13,16,17].

Surrogate biomarkers for IC and risk predictive models

Tests that detect surrogate biomarkers of IC have performed well when applied to immunosuppressed patients, but there is currently a lack of data relating specifically to the ICU setting. Nevertheless, detection of the panfungal 1,3-β-D-glucan marker has been an excellent negative predictor and has proven useful for monitoring the effect of antifungal treatment. It is, however, expensive and false positive results can occur. Mannan and anti-mannan antibody tests have also shown increased sensitivity and specificity (>80%) when used in combination but require more clinical evaluation [18].

Specific to the ICU, several risk predictive models which combine *Candida* colonisation and assessment of host-related risk factors have been shown to reduce the incidence of IC by identifying high-risk patients in whom to administer pre-emptive antifungal therapy [19,20]. Prospective validations, however, are still warranted as are measures to improve the absolute risk of IC given the commonality of risk factors (for example, central venous catheters, immunosuppression) among the ICU population. It is also important that risk models be specifically designed for the target population as published algorithms perform differently when adapted to different geographical settings [21].

In conclusion, rapid diagnosis of IC in the ICU remains a challenge. Several technological advances have made it possible to expedite the time to diagnosis. Screening tests which provide presumptive pathogen identification or select for high-risk patients are useful for guiding therapy until confirmatory results become available. Rigorous validation of the above platforms is still required. Quality control and standardisation of molecular assays should become easier given the availability of commercial platforms, automated systems and the recent publication of the MIQE guidelines [22]. Costs may be balanced with potential savings

in treatment and shorter hospital stays. Methods should be selected according to the goals, function and resources of each individual laboratory.

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Biography

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New approaches to enterovirus identification

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The human enteroviruses (HEVs) are members of the Picornaviridae family and cause a diverse range of diseases from respiratory illness to paralysis. Historically, HEVs were isolated in cell culture and subtype identification was by neutralisation using specific antisera. Currently, diagnostic virology laboratories use nucleic-acid-based tests to detect and identify HEVs in clinical specimens.

Human enteroviruses

The human enteroviruses (HEVs) are single-stranded, positive-sense RNA viruses in the *Picornaviridae* family. The International Committee for the Taxonomy of Viruses (ICTV) has classified the HEVs into four species A–D (Figure 1). HEVs are highly infectious, typically transmitted via the faecal-oral route, and cause a wide range of clinical symptoms that can be fatal, including respiratory illness, fever, diarrhoea, meningitis, acute haemorrhagic conjunctivitis, hand-foot-and-mouth disease, poliomyelitis, rash and paralysis. HEVs are also associated with chronic disease and there is mounting evidence for a causal role in the onset of type 1 diabetes [1]. As the symptoms and signs of enteroviral infections are not pathognomonic, prompt laboratory