Fungal Diagnostics

Subjects: Pathology

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For invasive fungal infections late diagnosis results in a poor prognosis. This review summarises the current state of the art in diagnostics used in medical mycology and anticipates the emergence of new generations of boiosensors that will provide new opportunities for rapid, sensitive detection of fungal infections.

Keywords: diagnostic ; medical mycology ; biosensor ; fungi

1. Introduction

Although there have been several significant recent developments, many of the key diagnostic techniques used in front-line diagnosis of invasive fungal infection have not changed substantially in many years. A strong reliance is still made on microscopy and fungal culture in vitro, histopathology, radiographic and CT imaging, serology and antigen detection tests including the use of lateral flow devices[1]. Some of these diagnostic methods have the advantage that they can be developed into point-of-care tests that can be applied under circumstances where advanced bespoke mycological expertise, such as that available in national mycological reference centres, is not available. Increasingly, these foundation methodologies are being complemented by high-technology molecular-based alternative technologies ranging from the use of polymerase chain reaction (PCR) and DNA-sequencing-based approaches, to protein fingerprinting by matrix-assisted laser desorptionionization time of flight (MALDI-TOF) mass spectrometry[2]. The future of diagnostic technologies includes the development of multiplex diagnostics that do not require fungal culture and ideally could include the simultaneous analysis of other important parameters such as providing information about the drug resistance profile of the fungal pathogen. Recent experimental advances have been made in which ultrasensitive laser-based technologies can rapidly scan for specific biomarkers of fungal infection.

2. Conventional Diagnostic Tools

Traditional laboratory-based diagnostic approaches in mycology include microscopy, histology, culture and serology[3]. Direct microscopy and culture from normally sterile or non-sterile sites on the body are routine and represent gold standard diagnostic tools in the detection of fungal infection. Various types of clinical samples, including saliva, urine, blood, cerebrospinal fluid (CSF), bronchoalveolar lavage fluid (BAL), sputum, other body fluids, swabs and tissue, can be used to detect fungal infection by direct microscopy and growth in fungal selective or indicator growth media such as Sabouraud agar, CHROMagar or blood agar[4][5][6][7]. Culture of fungi has its advantages since it may yield the infecting isolate for either phenotypic, proteomic or molecular identification and specific antifungal susceptibility tests[8]. Sub-culture onto CHROMagar™ *Candida*, which is a chromogenic substrate yielding different colony colours for some pathogenic yeast species, or its use as an initial isolation plate, can help to suggest the identification and more importantly can help to ensure that further tests are conducted on pure cultures, rather than mixed yeast species, a common reason for the failure of commercial identification methods. Recently, there has been a new development in this area with the introduction of CHROMagar™ *Candida* Plus, an agar which clearly differentiates the emerging pathogen *Candida auris*, a yeast responsible for multiple nosocomial outbreaks world-wide, from other pathogenic and commensal yeast species. In tests, this was able to differentiate four different clades of *C. auris* from 52 other pathogenic yeast species from 15 genera. It also has potential as a primary isolation medium to test patients for skin colonisation with this pathogen, a common precursor to blood stream infection, and thus facilitate the cohorting of colonised patients[9].

However, it is often not possible to obtain suitable clinical samples, and fungal growth frequently takes from 24–72 h and occasionally many weeks for adequate growth to occur. In particular, blood culture tests are much faster to reveal bacteraemia than candidaemia, which often takes 2–6 days, and fungaemia is often not present in mould infections with the notable exception of infections with *Fusarium, Scedosporium, Aspergillus terreus* and a few other rare mould infections that are spread by haematogenous dissemination. Therefore, faster biomarker and molecular detection

methods have been developed that can be applied directly to serum or other clinical samples, often negating the need for attempted culture. Moreover, the sensitivity of the culture method can also depend on sample collection, transportation and preservation and can be confounded by contamination[7][10][11].

Direct microscopy has been an important diagnostic compliment to obtaining a fungal culture and can often be used to make a preliminary diagnosis within hours of taking a sample. It can be applied to many sample types including tissue biopsies, CSF, BAL, other body fluids, sputum and swabs. Microscopy, can reveal distinctive features of fungi morphology that can be used for provisional identification, thereby providing immediate results. Evidence of fungal cell structures visualised in tissue biopsy specimens by either direct microscopy, cytology or histopathology is considered proof of invasive infection[12]. For example, septate moulds such as *Aspergillus fumigatus* can be distinguished from the pauci-septate Mucorales. The identification of polymorphic species such as *Candida albicans* growing as yeast or hyphae and the encapsulated yeast *Cryptococcus* are also easily revealed. Additionally, staining solutions such as Calcoflour White, Blankophor, Gram staining, India ink and a library of antibody-based immunostains can aid in visualisation of morphological properties of fungi thus enabling accurate diagnosis[13][14][15][16][17]. Calcoflour White is commonly applied to tissue samples to detect fungal elements such as hyphae, pseudohyphae and yeast cells, whereas India ink and Gram staining is recommended for the specific detection of Cryptococcal infections because the dye particles are excluded by the gelatinous capsule creating a visible halo around yeast cells that are suspended in a dark colloid of dye[18][19][20]. Automated microscopic examinations have been devised that have recently been applied to faecal samples to detect fungi that may be associated with gut diseases, although, classically, faecal samples have not been considered as good samples for mycological diagnosis due to the frequent carriage of commensal yeast. A two-step system based on artificial neural networks (ANNs) processes, identifies and quantifies different types of fungal cells in microscopy images based on set parameters. While the system corrects for some common observation errors and significantly enhances throughput, it is limited to the parameters set by the operator and potentially can miss observations that experienced observers would identify[21].

Another development involves combining molecular diagnostics and microscopy such as in PNA-FISH (peptide nucleic acid fluorescent in-situ hybridisation)[22][23]. PNA-probes consist of a fluorescent molecule attached to oligonucleotide bases that bind to species-specific ribosomal RNA[24]. Positive cultures generate fluorescent cells that can be detected by microscopy. The FDA (Food and Drug Administration) has approved a Yeast Traffic Light PNA-FISH kit that accurately identifies *Candida* species in 96% of tested blood cultures[25][26]. Devices that deploy magnetic bead traps have been used to capture low numbers of microbial pathogens in blood samples[26] and may help to increase the sensitivity of direct microscopical techniques when the number of free circulating fungal cells is low.

Under the umbrella of microscopy-based diagnostics, histological examination is a broadly and widely used technique used to detect fungal cells in tissues, and the finding of fungal elements constitutes proven fungal infection[12][27]. Tissue histology can also provide indication of the host immune response to invading fungi by observation of infiltrating white cells. Routine staining techniques include hematoxylin and eosin (H&E), Grocott (methanmine) silver (GMS) stain, Fontana–Masson stain, Ziehl–Neelsen stain and Periodic acid-Schiff (PAS)[28][29][30]. For a detailed review on the application of histology in the diagnosis of fungal infections, see Guarner et al. (2011). Furthermore, guidelines on the detection of fungi in histological samples have also been published[31][32]. Histology provides relatively rapid diagnosis and is particularly useful where live cultures cannot be obtained. However, biopsy for histology from sterile body sites often involves highly invasive procedures and may not be possible in thrombocytopenic or otherwise severely ill patients. Furthermore, similarities in fungal morphology and the diversity of clinical manifestations of different fungi, may require supporting confirmation using more specific diagnostic tools. For example, *Aspergillus* species are often difficult to differentiate from other hyaline, septate causes of hyalohyphomycosis and even some dematiaceous fungi using H&E staining alone. Immunohistochemistry (IHC) can provide greater specificity as it utilises antibodies that bind to species-specific fungal antigens[33][34]. However, wide-spread application of IHC is hindered by limitation in the number of commercially available species-specific antibodies and a high degree of cross-reactivity of some antibodies between some species so they are not widely employed in the clinical setting[35]. More commonly, specific identification of fungal elements is undertaken by the PCR amplification of fungal DNA with *Aspergillus*-specific, *Candida*-specific, mucoraceous mould-specific or panfungal primers, which can be undertaken on formalin-fixed or fresh tissue but has greater sensitivity on the latter. In the case of panfungal primers, the resulting product can then be directly sequenced for specific identification as discussed later in the section on molecular detection methods.

Imaging/Radiology using X-rays, high-resolution computed tomography (HRCT) and magnetic resonance imaging (MRI) can provide essential diagnostic evidence suggestive of invasive fungal infection. Although radiology does not allow for accurate identification of the causative agent or even allow for definitive diagnosis of a fungal aetiology, it does provide indications of the type and extent of infection to inform appropriate lines of treatment and can be useful for guiding biopsy

sampling. Moreover there are some features that can be more suggestive of a fungal aetiology, for example, the presence of large nodules (>1 cm) or perinodular halos in chest radiographs can be indicative of angioinvasive fungal infections, and a reverse halo accompanied by rapid tissue invasion or multiple (≥10) nodules and pleural effusion can be suggestive of mucoraceous mould infection[36][37][38]. Diagnosis of *Pneumocystis* pneumonia can also be suggested by specific "ground-glass densities" in a chest radiograph, where the lungs appear white with vascular markings[39][40]. MRI scanning is particularly helpful in detecting ring-enhancing lesions and other features associated with the diagnosis of fungal infections of the CNS, bulls-eye lesions in liver and spleen tissue and fungal balls in kidney tissue[41].

Serology is a long-established and widely used method of detection of fungal infections. Diagnosis is achieved by identification of proteins usually antibodies in blood or saliva[42][43] using assays such as immunodiffusion (ID), counter-immunoelectrophoresis (CIE), Enzyme-Linked ImmunoSorbent Assays (ELISA), complement fixation (CF), lateral flow assays, radio-immunosorbent assays (RIA) and agglutination assays[44][45]. These techniques allow for the detection of circulating antibodies or fungal antigens depending on the test design. It should be acknowledged that antibody testing will only be helpful in patients able to mount an antibody response such as those with chronic infections including aspergilloma and endocarditis and also those otherwise healthy individuals with acute or chronic infections due to endemic dimorphic pathogens such as coccidioidomycosis and histoplasmosis. Antigen testing will be more helpful in patients with neutropenia or other conditions affecting humoral or cellular immunity[46]. For example, testing for the fungal antigens galactomannan, mannan and β-D-glucan in serum samples and testing for galactomannan in BAL samples are among those routinely performed serological tests for the detection of infections due to *Aspergillus, Candida* and some other mould infections in immunocompromised patients[11][47][48][49][50]. Common diagnostic tests for antibody detection such as, CF and ID are useful markers of infection with histoplasmosis and other endemic dimorphic pathogens, chronic aspergillosis and candidosis. These tests can be qualitative or semi-quantitative in which a titre is determined, providing valuable information on fungal burden that can inform antifungal therapeutic strategies and act as prognostic indicators. Some serological tests offer high sensitivity but there may be caveats that need to be considered. Cross reactivity has also been reported that compromises test specificity[51]. Antibody responses to infection may take 4–8 weeks to become detectable in peripheral blood making early diagnosis difficult. Hence, appropriate titre cut-off values are important to avoid false negative results particularly in the context of early stage infection[52][53]. Regardless of the drawbacks, serological tests are minimally invasive, inexpensive and provide rapid results that usefully inform clinical decisions.

3. Galactomannan Detection

Galactomannan (GM) is one of the most common biomarkers for the detection of *Aspergillus* infection and was one of the first to be commercially developed. GM is a 20 kDa polysaccharide located in the outer cell wall layer of *Aspergillus*, *Penicillium* and certain other fungal species and is shed from fungal hyphae during growth. Platelia™ *Aspergillus* Enzyme Immunoassay (EIA) (Bio-Rad, Marnes-la-Coquette, France) is a commercially available kit based on a one-stage immunoenzymatic sandwich micro-plate assay that detects galactofuranosyl-containing molecules using a rat monoclonal antibody directed at *Aspergillus*, although there are cross-reactions with some other fungal groups including *Fusarium, Geotrichum, Histoplasma*, *Paecilomyces*, *Penicillium,* and *Rhodotorula.* Moreover, there may also be cross reactions with galactomannan resorbed from the intestinal tract in patients with severe mucocitis, and certain batches of antibiotics have been shown to contain galactomannan leading to serum positivity in the absence of aspergillosis. In invasive aspergillosis, the highest concentrations of GM are released in the terminal phases of invasive disease and certainly after angioinvasion had occurred; thus, the test may lack sensitivity for early diagnosis[54][55]. Serum GM can be useful in predicting the outcome and assessing the response to antifungal therapy, but providing a higher cut-off index value is used it has been demonstrated that the galactomannan test conducted on BAL fluid is more sensitive and specific as an early diagnostic test for pulmonary aspergillosis[56]. It is generally considered that the galactomannan test displays optimal sensitivity and specificity when combined with other tests

4. Mannan

Mannan is a major component of the yeast cell wall, and tests developed specifically to detect *Candida* infection include commercially available tests to detect mannan or mannoproteins, such as the Platelia™ *Candida* Ag Plus EIA (Bio-Rad, Marnes-la-Coquette, Paris, France) and the CandTec latex agglutination test (Ramco Laboratories, Stafford, TX, USA). There are two confounding factors: one is that *Candida* spp. are common human commensals and heavy colonisation can cause positive results in serum, and the second is that *Candid*a mannan is rapidly cleared from the circulation, so frequent testing is important. Repeated high *Candida* mannan tests can help to confirm chronic candidosis, usually seen in

immunocompromised patients with consistent radiology revealing bulls-eye lesions in the liver and/or spleen. The best results generally seem to be achieved with a combination of mannan antigen and antimannan antibody detection (Platelia™ *Candida* Ab Plus; Bio-Rad)[47][57].

5. β-(1,3)-D-Glucan

The main structural polysaccharide components of the cell wall of fungal pathogens are glucan, chitin, and mannan. Of these, β-(1,3)-D-glucan is the most important and abundant polysaccharide component of many cell walls and is a common component of the cell walls of most pathogenic fungi[58][59]. Fungitell® is one of the commercially available assay kits capable of detecting and quantifying the presence of β-(1-3)-D-glucan in serum and cerebrospinal fluid using a colorimetric method. Although it is generally considered a sensitive, non-specific pan-fungal test there are certain fungal groups which produce less (1-3)-β-D-glucan such as *Cryptococcus* spp. *Blastomyces* spp. and the mucoraceous moulds so will not be detected[60]. Detection of high beta-glucan levels is often encountered during *Pneumocyctis jirovecii* infection thus lack of detection is helpful in excluding this diagnosis[61]. Further, the conformational behaviour of linear oligo-β-(1-3)-D-glucosides was studied using NMR experiments and molecular modelling[62]. This theoretical study revealed that conformational properties of disaccharide fragments depended on neither their position in the chain nor the length of the chain. Interestingly, monoclonal antibodies were developed specifically recognizing β-(1-3)-D-glucan using hybridoma technology antigen. The developed antibodies interacted with species from *Aspergillus*, *Candida*, *Penicillium* genera and *Saccharomyces cerevisiae*, but not bacteria[63].

6. Cryptococcal Capsular Polysaccharide

One of the earliest fungal biomarkers to be commercially developed was the latex agglutination kit (IMMY Immuno-Mycologics, Norman, OK, USA) for the detection of the cryptococcal capsular antigen glucuronoxylmannan. For several decades, this test has proved invaluable in the early diagnosis of cryptococcal meningitis or other systemic infection and can be conducted on serum or CSF with sensitivity and specificity >90% often reported[64]. Tests have to be conducted on neat and 1:10 dilutions to allow for a prozone effect at high concentrations of the antigen. More recently, the same company has developed a lateral flow device (LFD) for the detection of the same capsular antigen and this has shown great promise as a point-of-care test with a reported sensitivity and specificity of 93% and 100%, respectively[65]. It is especially useful in less developed countries due to its low cost and long shelf-life even at ambient temperature and although it is usually conducted on serum samples, thus requiring centrifugation, or on CSF, recent studies have demonstrated its utility when used with direct finger-stick blood samples producing 100% concordance between whole blood, serum and plasma[66]. This is the first truly point-of-care test (POCT) for detecting a fungal infection.

7. Point-of-Care Tests (POCT) in Fungal Diagnosis

A point-of-care test (POCT) is defined by the College of American Pathologists as ‘testing that is performed near or at the site of a patient with the result leading to a possible change in the care of the patient.’ POCTs are usually performed with minimal equipment by individuals that have not had laboratory training such as physicians, nurses, nursing assistants and sometimes even the patients themselves; these currently include such tests as home pregnancy tests and blood glucose monitoring. POCT diagnostics have great potential for front line interventions in the treatment of disease and can be split into those that require little equipment and are simple to perform which makes them ideal for use in areas of the world lacking sophisticated laboratory equipment, and those that although they make use of sophisticated techniques are rapid to perform and can be miniaturised and run directly on untreated blood, urine, or other body fluids directly at the patients bed-side, these are often referred to as ‘lab on a chip tests’ (LOC)[57]. Although such devices would have high acquisition costs, they could also be utilised in resource-limited environments. The World Health Organization comments on the desirability for them to be affordable, sensitive, specific, robust and user friendly[67][68]; portability would also be an essential attribute.

Currently in the field of medical mycology it is the lateral flow devices with techniques based on immunochromatography that show the greatest promise and indeed on occasion are already employed as POCTs. As previously described, there is a commercial lateral flow device for the detection of cryptococcal capsular antigen (IMMY Immuno-Mycologics, Norman, OK, USA) which can be used on fingerstick bloods and has revolutionised the diagnosis of cryptococcal meningitis in less developed countries where cryptococcal disease is a major cause of mortality within the HIV-infected populations[66]. There has also been commercial development of a lateral flow device for the detection of *Aspergillus* antigen (OLM diagnostics, Newcastle-upon-tyne, UK, USA). This was initiated by Thornton and colleagues (2008), who developed a specific monoclonal antibody (mAb JF5) that targets an early germ tube specific glycoprotein (JF5) of *Aspergillus* species[69]. Detection of this antigen in human serum or BAL samples is indicative of active invasive infection and can be

detected at an early stage of infection. An added advantage of this test is that it will not detect fungal spores, as it requires the cells to germinate and start to invade tissues before the target glycoprotein is synthesised; thus it is much less susceptible to false positive results than other *Aspergillus*-specific diagnostic tests due to the confounding presence of fungal spore contamination. The device can be used with serum but can also be applied to BAL samples for the diagnosis of pulmonary aspergillosis, which means it could be employed in bronchoscopy suites as near-patient testing. Studies comparing results with the lateral flow device, galactomannan and beta-glucan detection as well as *Aspergillus*-specific PCR tests conclude that it is suitable as a POCT and is a useful adjunct to other biomarker tests in the diagnosis of chronic pulmonary aspergillosis and invasive aspergillosis[70][71]. Galactomannan-like antigens were also targeted using IgM mAb476 to detect *Aspergillus* infection[72]. Several companies have introduced β-(1,3)-D-glucan detection kits, including Fungitec G-Test MK (Seikagaku) and Fungitell (Associates of Cape Cod), the kinetic turbidimetric β-Glucan Test Wako (Wako Pure Chemical Industries) and the endopoint chromogenic B-G Star kit (Maruha). These differ somewhat in their dynamic range and positive cut offs[73]. As mentioned above, the CHROMagar™ *Candida* Plus agar could also be utilised as a near-patient surveillance test with patient skin swabs being sub-cultured directly onto the agar to detect olonization with the pathogenic yeast *Candida auris*, although this agar would then require incubation for 48 h before yielding the results.

8. Nucleic Acids Based Diagnostics

Various in-house polymerase chain reaction (PCR) tests have been developed and employed in fungal diagnostics in a wide range of settings. Molecular tests include conventional PCR, nested PCR, real-time PCR (RT-PCR), PCR based on ITS regions and rDNA, PCR-ELISA, multiplex PCR, and direct DNA sequencing. This range of methodologies brings obvious benefits in terms of the specificity of diagnosis, as primers can be designed to detect specific pathogens; however, there are challenges in terms of sensitivity and reproducibility, in particular in the generation of false negative results. Although conventional PCRs are rapid and can offer advantages in sensitivity, there are no standard FDA-approved protocols and hence results can be subject to lab-to-lab variability[1][74][75][76]. This reality is well recognised even in experienced molecular labs where PCR methodologies are used routinely and there have been efforts to standardise various aspects of testing with recommendations from the European *Aspergillus* PCR Initiative (EAPCRI)/ISHAM (International Society for Human and Animal Mycology) working group[77]. A recent publication by the American Thoracic Society recommended PCR to confirm *Aspergillus* infection in immunocompromised patients using blood or serum samples[78]. Modified “nested PCR” protocols have been established for improved specificity and sensitivity. This is achieved by subjecting samples to two consecutive PCR reactions using two sets of primers, thus allowing for the detection of fungal DNA as low as 1 fg with 100% specificity; however, this is dependent on sample type and concentration and is extremely vulnerable to contamination[79]. Endoscopic sinus surgery specimens were found to be particularly unsuitable for this technique perhaps due to contamination with high levels of environmental fungi in the nasal cavity[80].

One of the drawbacks of organism-targeted PCR is the requirement of a hypothesis to be made of the nature of the suspected aetiological agent. An alternative strategy is to undertake a preliminary diagnosis to confirm that the sample is positive in a pan-fungal DNA amplification which is then further tested using target-specific primers designed to confirm the presence of specific fungal organisms. A draw-back of this approach is that panfungal primers are by their nature designed to detect conserved sequences present throughout the kingdom and are thus less sensitive than specific PCR primers. Several commercial PCR tests exist for *Aspergillus* and *Candida* and there are also commercial tests for *Pneumocystis* and mucoraceous moulds. A recent evaluation found all the commercial *Aspergillus* PCR tests to have comparable sensitivity and specificity on serum samples but noted that sensitivity was significantly lower on serum than on respiratory samples. Two tests— the MycAssay *Aspergillus*® (Myconostica, Cambridge, UK, USA) and the AsperGenius® (PathoNostics B.V., Maastricht, The Netherlands) assays—were recommended for routine PCR-detection of *Aspergillus* spp. DNA in respiratory samples[81]. Two of the kits, AsperGenius® and MycoGenie® (Ademtech, Pessac, France), have the useful additional attribute of detecting the resistance markers TR_{34}/L98H and TR_{46}/Y121F/T289A associated with the environmental azole resistance now being reported more widely in *A. fumigatus* isolates from patients[81][82]. All can be used directly on patient samples but could also be applied to panfungal products. Alternatively, the product of a pan-fungal PCR can be sequenced and matched to databases to reveal the identification with the proviso that care should be taken when interrogating public databases due to the presence of erroneous entries. Pan-fungal PCR amplifications have targeted ribosomal DNA for 18S, 5.8S and 28S ribosomal RNA subunit genes. These ribosomal genes exist in close proximity in the genome and are separated by internal transcribed regions (ITS1 and ITS2) which differ between fungal species and can be exploited to achieve an identification at the species level. These regions have therefore proved very fruitful in developing tests to identify fungi isolated from clinical samples[83]. These PCR tests are highly sensitive, so care must be taken when being used for direct diagnostic purposes to consider possible confounding

positives originating from olonizing or contaminating organisms in patient samples[84]. Real-time PCR (RT-PCR) allows for the quantification of fungal burden, thereby helping to differentiate between the presence of actively growing fungi and contaminating fungal spores. Additionally, multiplex PCR has been developed, which allows for the rapid and simultaneous amplification of several targets by using multiple primer sets in the same sample[85]. In a recent study, a multiplex PCR assay showed 100% sensitivity and 94.1% specificity in the detection of *Candida* spp. in samples acquired from 58 patients with candidaemia[86]. PCR-ELISA is a less commonly employed molecular method, but it does have some unique qualities. These tests quantitatively detect digoxigenin-labelled PCR products that hybridize to target-specific probes immobilised on ELISA plates[87][88]. However, sensitive and quantitative, PCR-ELISA methods have been increasingly replaced by RT-PCR due to its inherent higher accuracy.

PCR-based diagnostics are also subject to some common limitations. The sensitivity of PCR is dependent on the type, complexity and processing of samples. For example, the sensitivity of PCR is significantly reduced in formalin-fixed paraffin-embedded samples as fixation damages nucleic acids, thus inhibiting the PCR reaction. However, the technique often does prove helpful on such samples, especially if fresh tissue samples are not available[89]. Secondly, the design and choice of PCR primers affects the outcome of a PCR reaction. Primers that have reduced target specificity may increase the likelihood of false-positive results. Alternately, if primers bind weakly to the target, this may lead to false-negative results. Furthermore, it is estimated that up to 20% of the unedited sequences in the GenBank have incorrect lineage designations which will affect identification of fungal species that depend on DNA sequencing of DNA and primer design and hence the efficiency of PCR amplification[90].

A new, FDA-approved, commercial platform, the T2 Magnetic Resonance (T2MR) (T2 Biosystems), is being evaluated in several centres for its utility in detecting the five most common pathogenic *Candida* species in blood samples, alongside a large array of other blood stream pathogens. This automated qualitative molecular diagnostics system utilizes T2 magnetic resonance (T2MR) in which particles coated with target-specific agents form clusters when they encounter the target pathogen in clinical samples. Complexes formed in this way alter the micro-environment of water molecules around the target which is then quantified in the presence of a magnetic field. Although acquisition costs are high and individual sample costs are significant, the system can detect low numbers of *Candida* cells within 5 h without prior sample purification steps[91].

A recent advance in fungal diagnostics is the application of MALDI-TOF MS (Matrix-assisted laser desorption ionization-time of flight mass spectrometry). This technique identifies species-specific fungal peptides instead of nucleic acids. Of the commercially available MALDI-TOF MS platforms, Bruker Biotyper (Germany) and Vitek MS (France) are approved by the FDA. Various studies have reported sensitivity of this diagnostic technique in identification of various fungal species including yeast and mould isolates[92][93][94]. In a recent survey, MALDI-TOF MS accurately identified 92.7% of tested fungal species[95]. The technique offers a rapid and accurate identification of closely related species and even uncommon pathogenic fungi such as *Paecilomyces* species[96][97]. However, while intact cells have been subjected to MALDI-TOF MS analysis, cell lysates are preferred and sample preparation can be particularly challenging for moulds and spores of fungi due to their robust cell walls. Additionally, the quality of reference database and analysis methods (e.g., spectrum scoring) can influence the efficiency of fungal identification. These databases and methods can vary between labs as well as between MALDI-TOF MS platforms. While reference databases are constantly updated and can provide up to 99% accuracy in the identification of fungal species, a number of these are still based on in-house libraries which are not always available publicly[98][99]. To date, in the field of medical mycology, this technique has been used predominantly as an identification tool for yeast species and in centres that have invested in the technology that has replaced phenotypic carbohydrate assimilation and other phenotypic tests, thus reducing the identification of common yeast isolates from 48 h to as little as 30 min[100]. Recent developments also suggest some utility in the detection of antifungal drug resistance[101]. Latterly, many mould species isolated from clinical specimens have also been added to databases, thus increasing the clinical utility of this approach[102]. However, there have also been direct applications to clinical samples, usually yeast detection in blood samples, in an attempt to reduce the time required for culture, thereby allowing for earlier diagnosis and identification of the infecting organism facilitating the prompt administration of appropriate antifungal therapy[103][104][105].

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