Chapter 2

Fungal Diagnostics: Review of Commercially Available Methods

Javier Yugueros Marcos and David H. Pincus

Abstract

Fungi and yeasts are critical causes of acute infection. As such, the detection and identification of these organisms are crucial in the diagnosis of affected patient populations. There is a vast array of commercial tests currently available for diagnostic purposes. These vary from traditional culture and biochemical methods to advanced multiparameter molecular tests. Recent technological advances have driven the development of rapid tests which are complementing and in some cases replacing the more traditional methods of detection. Irrespective of the method used the ultimate goal is timely detection of the infectious agent allowing appropriate treatment and improved outcome for the patient.

Key words: Commercial test, Fungal diagnostics, Direct examination, Chromogenic media, Yeast identification, Antigen-based test, Antibody-based test, Nucleic acid probes, Molecular biology

1. Introduction

Fungi have progressively emerged as an important cause of life-threatening infections. Patient populations at greatest risk are those critically ill and/or immunocompromised, including surgical intensive care unit (ICU), advanced human immunodeficiency virus (HIV) infected, inherited immunodeficiency-prolonged neutropenia, and solid organ and hematopoietic stem cell transplant (HSCT) recipients (1, 2).

Significant progress has been made over the last 10 years in the field of fungal detection and identification. Technological advances and introduction of new technologies have led to availability of a wide variety of commercial tests. Their use in the clinical setting has been successful, some moving into clinical

Louise O'Connor and Barry Glynn (eds.), *Fungal Diagnostics: Methods and Protocols*, Methods in Molecular Biology, vol. 968, DOI 10.1007/978-1-62703-257-5_2,

Table 1
Common histological stains used for diagnosis of fungal infections

Stain[a]	Fungal elements	Background
GMS	Brown to black	Green to blue
PAS	Pink to red	Pale pink to pale purple
GF	Pink to red to purple	Yellow
H&E	Pink to red to purple	Pink

[a]*GMS* Gomori methenamine silver, *PAS* periodic acid-Schiff, *GF* Gridley fungus, *H&E* hematoxylin and eosin

guidelines for fungal infection management. This chapter reviews commercially available methods for detection and identification of the fungi most commonly associated with infection. The aim is to provide the reader with an overview of the extensive range of methods available focusing on tests, kits, assays, or systems for use in Europe, USA, or Japan.

2. Direct Examination of Clinical Samples

Direct examination of clinical samples for fungi is made possible by several stains enabling a more obvious appearance of fungal elements. Classical stains used in histopathology include Gomori methenamine silver, periodic acid-Schiff, Gridley fungus, and hematoxylin and eosin stains (3) (Table 1). Alternatively, calcofluor white (CW) can be used with a fluorescent microscope to observe fungal elements in clinical samples. CW binds β-glycosidic linkages of polysaccharides in the fungal cell wall but also binds nonspecifically to keratin and human connective tissue elements (4).

3. Culture-Based Methods for Fungal Detection

3.1. Nonspecific Detection of Fungi

Culture media used to cultivate fungi include various non-selective agars such as Sabouraud dextrose agar (SDA), potato dextrose agar, and brain heart infusion (BHI) agar. In order to inhibit growth of bacteria or saprophytic fungi, selective agents such as chloramphenicol, gentamicin, and cycloheximide are added. Broths (e.g., Sabouraud dextrose broth, BHI broth, etc.) are used occasionally to grow or enrich for fungi. Most fungi are non-fastidious and grow on most media (including trypticase soy blood agar) that

supply sources of nitrogen (e.g., peptone) and carbon (e.g., glucose). The disadvantage of these media is that many fungi look alike and additional methods and more incubation are needed for identification. That delay in diagnosis was the impetus for development of more specific methods such as the chromogenic media which will be described later in this review.

Automated blood culture systems are also available and include the BACTEC™ (BD™ Diagnostic Systems, Sparks, MD, USA), BacT/Alert® 3D (bioMérieux®, Marcy l'Etoile, France), and VersaTREK® (TREK Diagnostic Systems, Cleveland, OH, USA). Aside from using different broth components and/or volumes, these systems employ different detection methods to monitor growth and metabolism, i.e., CO_2 generation and subsequent pH decreases detected by fluorimetric BACTEC™ (5) or colorimetric BacT/Alert® 3D (6) sensors or headspace gas changes detected by VersaTREK®'s pressure sensors (7).

3.2. Specific Detection of Fungi

The germ tube test described in 1960 (8) was the main method for rapid identification of *C. albicans* until the advent of enzymatic tests and chromogenic media. Some labs still use the germ tube test for rapid inexpensive detection of *C. albicans* but as it relies on microscopic observation and technical expertise, it has been largely displaced by enzymatic tests. Table 2 shows commercial tests that use a combination of substrates to detect presence of *N*-acetyl-β-D-galactosaminidase and L-proline arylamidase. While

Table 2
Rapid (30–60 min) identification of *Candida albicans* using NGL/PRO[a]

Test	Vendor	No. isolates tested	% SN	% SP	Reference
BactiCard® Candida	Remel, Lenexa, KS, USA	583	99.3	99.6	(9)
		133	100.0	100.0	(10)
		536	97.0[b]	97.1[b]	(11)
		196	97.8	92.5	(12)
Candida albicans Test Kit (MUREX *C. albicans* 50)	Remel	583	98.7	99.6	(9)
		502	94.6	97.8	(13)
		133	100.0	100.0	(10)
O.B.I.S. albicans	Oxoid, Basingstoke, UK	219	100.0	100.0	(14)
AlbiQuick™ Test Kit	Hardy Diagnostics, Santa Maria, CA, USA	NR	NR	NR	NR

[a] *NGL* β-galactosaminidase, *PRO* proline arylamidase, *SN* sensitivity, *SP* specificity, *NR* not reported, *TP* true positive
[b] Recalculated to include *Candida dubliniensis* as TP

both enzymes are present in *C. albicans*, they are also found in some less common species, e.g., *C. dubliniensis*, *C. rugosa*, and *Trichosporon* spp.

Staib (15) first described birdseed (*Guizotia abyssinica*) agar as a useful screening tool for detection and rapid identification of *C. neoformans*. Most strains of *C. neoformans* are detected within 72 h incubation on this medium where phenoloxidase activity results in dark brown colored colonies. A comparison of birdseed agar to conventional media (SDA and Mycosel™ agar; BD™ Diagnostic Systems) with 35 clinical samples from AIDS patients showed 100% sensitivity and specificity with plates incubated at 30°C. Authors suggested that this or a higher temperature (37°C) could be more selective and avoid rare false-positives reported for non-*C. neoformans* cryptococci. Although no problems have been reported with bacterial overgrowth, antibiotic supplementation has been suggested to aid screening samples with heavy background bacterial flora (16). Another commercially available medium used for identification of *C. neoformans* and *C. albicans*, within 24 h from previously isolated colonies, is TOC (tween 80-oxgall-caffeic acid) agar (17). However, its use as a primary isolation medium requires extended incubation of 3–5 days. Other investigators (18, 19) recommended urease for rapid recognition of *C. neoformans*, but this lacks specificity and needs to be followed by a more reliable method. Rapid urease can be useful for screening respiratory isolates when the target species are limited to *C. neoformans* and *C. gattii*.

The rapid trehalose assimilation test and its modifications can be used for rapid identification of *C. glabrata* (Table 3). Rapid identification of *C. glabrata* is critical due to its resistance or reduced susceptibility to triazole antifungals, e.g., fluconazole (28).

3.3. Chromogenic Media

Chromogenic media allow direct identification of the most common clinical *Candida* species. They also allow for recognition of mixed yeast cultures that may be difficult or impossible to see on conventional mycological media, e.g., SDA. They incorporate substrates, e.g., halogenated indoxylic derivatives, used to detect specific enzymes. Substrate cleavage results in formation of brightly colored indigo dyes that are retained within yeast cells resulting in specific color development in colonies of target species. These media also contain one or more antibiotics used to prevent growth of contaminating bacterial flora. *Candida albicans* is identified by β-hexosaminidase activity, which is also found in the less common and closely related species *C. dubliniensis*. Some of these media, e.g., CHROMagar™ Candida (CHROMagar™, Paris, France), allow for presumptive identification of other yeasts, e.g., *C. tropicalis*, through detection of additional enzymes, e.g., phosphatase (29). In this case, *C. tropicalis* forms blue colonies by hydrolysis of both chromogenic substrates. Presumptive identification may be

Table 3
Rapid identification of *Candida glabrata*[a]

Test	Vendor	No. isolates tested	% SN	% SP	Reference
Glabrata RTT	Fumouze Diagnostics, Levallois-Perret Cedex France	330	≥94.0	≥97.3	(20)[b]
		1,174	95.8	98.9	(21)
		168	98.4	100.0	(22)
		332	≥91.1	≥83.9	(23)[c]
Rapid Trehalose Assimilation Broth	Remel	320	91.5	96.3	(24)
Rapid trehalose + sucrose assimilation with Rosco disks (Diatabs™)	Rosco Diagnostica A/S, Taastrup, Denmark	440	100.0	100.0	(25)
Rapid trehalase (solution from Diatabs™ w/Clinistix®)	Rosco Diagnostica A/S; Bayer, Tarrytown, NY, USA	482	≥98.2	≥95.3	(26)
Rapid trehalase/maltase	Rosco/Bayer	255	96.9	98.4	(26)
RAT (Mayo Clinic method)	Scientific Device Laboratory, Des Plaines, IL, USA	91	≥81.3	100.0	(27)[d]
GlabrataQuick™ Kit	Hardy Diagnostics	NR	NR	NR	NR

[a]*SN* sensitivity, *SP* specificity, *NR* not reported
[b]Four isolation media tested; % SN range was 94.0–98.0 and % SP range was 97.3–98.6
[c]Five isolation media tested; % SN range was 91.1–96.7 and % SP range was 83.9–99.6
[d]Two isolation media tested; % SN range was 81.3–100.0 and % SP was 100.0

possible for other species, e.g., *C. krusei* or *C. glabrata*, but performance is related to expertise in recognition of morphologic or discrete color differences from other similar species (30).

Chromogenic media available for the detection of Candida species include Brilliance™ Candida (Oxoid, Basingstoke, UK) (31), Candi*Select*™ 4 (Bio-Rad, Marnes-la-Coquette, France) (32), CHROMagar™ Candida (33, 34), and chromID™ Candida (bioMérieux®, Marcy l'Etoile, France) (35).

4. Post-culture Identification Methods

All of the non-molecular commercial identification systems described in Subheadings 4.1–4.3 require inoculation with a pure culture isolate while the molecular methods in Subheading 4.4 allow processing from positive blood culture bottles. The different methods relying on pure culture isolates have varying culture requirements, i.e., isolation medium, culture age, and inoculum suspension density.

4.1. Manual Identification Systems

The early manual identification systems on the market were limited in the scope of species that could be identified, which was a function of limited number of substrates and immature databases. As commercial methods evolved, so did the numbers/types of substrates and their databases. Table 4 shows a summary of the available manual yeast identification methods. For the purpose of consistency, some species and/or biotype claims listed in the various product package inserts were abbreviated in order to keep in line with current taxonomy, i.e., *C. albicans*/*C. stellatoidea*, *C. albicans* 1/*C. albicans* 2, *C. paratropicalis*/*C. tropicalis*, etc. that are conspecific were counted as single claims.

4.2. Automated Identification Systems

With the introduction of automation, workflow became easier, and reaction and final result interpretations became objective and more reproducible. These advances allowed accurate differentiation of a much wider range of clinically relevant taxa. Table 5 summarizes the systems available commercially.

4.3. Matrix-Assisted Laser Desorption Ionization: Time of Flight Mass Spectrometry

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is one of the newest methods used for fungal identification. This is a very accurate and rapid technique that requires minimal expertise or hands-on time. The method is based primarily on ribosomal proteins. Whole cell preparations or extracts are embedded in a chemical matrix and ionized by a laser. The resultant molecules migrate through a charged field in a vacuum tube toward a detector. The differences in time of flight for these ions translate to different mass peaks in the range of 2–20 kDa. These spectra are then compared to a database of known species to arrive at an identification within minutes of the ionization step. Results have shown that this method is less dependent on culture conditions than many other commercial phenotypic methods and therefore allows for fairly broad ranges of growth conditions, i.e., isolation media, incubation time, and temperature.

There are currently two commercial systems available the BioTyper MALDI-TOF (microflex™ LT instrument; Bruker Daltonics, Bremen, Germany) and Vitek® MS™ (bioMérieux®).

BioTyper MALDI-TOF: As of the end of 2011, this system had a European Conformity (CE; Conformité Européene) mark and could be used for in vitro diagnostic (IVD) reporting in European Union countries. In other countries, e.g., USA, the format was research use only (RUO) prior to other regulatory, e.g., Food and Drug Administration (FDA), approvals.

Vitek® MS™: This MALDI-TOF MS system uses similar principles to the Bruker system and became available in early 2011 with the CE-IVD mark. Prior to development of commercial software and other regulatory approvals, this system also used an RUO

Table 4
Manual comprehensive yeast identification methods

Product (vendor)	No. strains	% Correct	% NoID	% MisID	Author
api® Candida (bioMérieux®)	609	97.4	0.7	2.0	Fricker-Hidalgo et al. (36)
	198	91.4	5.1	3.5	Bernal et al. (37)
	156	82.7	4.5	12.8	Buchaille et al. (38)
	159	91.8	0.6	7.5	Campbell et al. (39)
	202	97.0	2.5	0.5	Paugam et al. (40)
api® 20C AUX (bioMérieux®)	505	96.2	0.0	3.8	Buesching et al. (41)
	1,093	97.3	2.7	0.0	Land et al. (42)
	126	77.0	19.8	3.2	Bergan et al. (43)
	178	89.3	5.6	5.1	Schuffenecker et al. (44)
	206	94.7	0.0	5.3	Willemsen et al. (45)
	243	93.0	6.2	0.8	Ramani et al. (46)
	171	96.5	1.2	2.3	Sand and Rennie (47)
	201	100.0	0.0	0.0	Wadlin et al. (48)
	116	99.1	0.0	0.9	Gündes et al. (49)
Auxacolor™ (Bio-Rad)	178	88.8	5.6	5.6	Schuffenecker et al. (44)
	97	79.4	15.5	5.2	Milan et al. (50)
	206	62.1	NR	NR	Willemsen et al. (45)
	156	85.9	10.3	3.8	Buchaille et al. (38)
	105	91.4	4.8	3.8	Sheppard et al. (51)
	159	91.2	8.2	0.6	Campbell et al. (39)
	202	94.1	4.9	1.0	Paugam et al. (40)
	100	94.0	6.0	0.0	Romney et al. (52)
Candifast® (ELITech Group)	178	51.1	25.3	23.6	Schuffenecker et al. (44)
	116	88.8	5.2	6.0	Gündes et al. (49)
Fungichrom® (ELITech Group)	156	84.6	10.9	4.5	Buchaille et al. (38)
	202	92.1	5.4	2.5	Paugam et al. (40)
	116	95.7	2.6	1.7	Gündes et al. (49)
Fungifast® (ELITech Group)	156	77.6	19.9	2.6	Buchaille et al. (38)
	202	88.1	10.9	1.0	Paugam et al. (40)
ID 32 C (bioMérieux®)	69	76.8	0.0	23.2	Latouche et al. (53)
	156	98.1	1.9	0.0	Buchaille et al. (38)
	243	88.9	10.3	0.8	Ramani et al. (46)
	250	93.6	0.8	5.6	Marklein et al. (54)
RapID™ Yeast Plus (Remel)	300	99.3	0.0	0.7	Kitch et al. (55)
	156	78.2	5.8	16.0	Buchaille et al. (38)
	447	90.6	2.5	6.9	Espinel-Ingroff et al. (56)
	133	94.0	0.0	6.0	Heelan et al. (57)
	201	99.0	0.0	1.0	Wadlin et al. (48)
	750	95.5	2.4	2.1	Sanguinetti et al. (58)
Uni-Yeast-Tek® (Remel)	623	99.8	0.0	0.2	Bowman and Ahearn (59)
	229	99.1	0.0	0.9	Bowman and Ahearn (60)
	206	94.2	NR	NR	Cooper et al. (61)
	126	88.7	0.0	11.3	Bergan et al. (43)
	489	37.2	49.7	13.1	Salkin et al. (62)

Table 5
Automated comprehensive yeast identification methods

Product (vendor)	No. strains	% Correct	% NoID	% MisID	Reference
Biolog YT Microplate™ (Biolog)	129	48.8	12.4	38.8	(63)
	171	48.5	8.8	42.7	(47)
MicroScan® Rapid YS (Siemens)	437	85.4	NR	8.2	(64)
	357	96.6	0.6	2.8	(65)
	150	85.3	NR	NR	(66)
Sherlock® MIS (MIDI, Inc.)	550	68.0	16.2	15.8	(67)
	477	70.2	6.1	23.7	(68, 69)
Vitek® YBC (bioMérieux®)	253	95.7	0.0	4.3	(70)
	352	84.9	0.0	15.1	(80)
	1,106	93.4	NR	NR	(71)
	221	83.3	2.7	14.0	(81)
	398	97.2	1.3	1.5	(72)
	222	80.6	14.4	5.0	(74)
	150	95.0	NR	NR	(66)
	409	89.7	3.2	7.1	(73)
	55	87.3	0.0	12.7	(53)
	171	96.5	0.6	2.9	(47)
	201	91.0	4.0	5.0	(48)
Vitek® 2 YST (bioMérieux®)	97	99.0	0.0	1.0	(75)
	172	93.6	3.5	2.9	(76)
	623	98.6	0.5	0.9	(77)
	750	98.4	0.5	1.1	(58)
	136	94.1	1.2	4.7	(78)
	68	97.1	0.0	2.9	(79)

format. Pre-2011 publications address studies of this RUO format (SARAMIS™ software) and although most evaluated bacterial identification, fungi were also tested.

4.4. Tests Utilizing Nucleic Acids for Fungal Detection

FISH has routinely been employed as a method of detection of fungi for some time. One commercial system which utilizes the method is the Yeast Traffic Light® PNA-FISH® (AdvanDx, Woburn, MA, USA). This is a peptide nucleic acid fluorescent in situ hybridization (PNA FISH) method employing fluorescein-labeled probes that hybridize with 26S ribosomal RNA (rRNA) of target species, e.g., *C. albicans*, that can be identified directly using smears of positive blood cultures. Other nucleic acid based methods using cultured materials as sample include Prove-it™ Fungi and BlackLight® Fungal ID kit.

Prove-it™ Fungi (Mobidiag, Helsinki, Finland) is a rapid DNA-based test intended for identification of 13 yeast species:

C. albicans, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. lusitaniae*, and *C. guilliermondii* as well as six more species (*C. dubliniensis*, *C. pelliculosa*, *C. kefyr*, *C. norvegensis*, *C. haemulonii*, and *Saccharomyces cerevisiae*) enclosed in the so-called pan-yeast panel. The method, based on extraction of DNA from either positive blood culture bottles or pure culture samples, uses polymerase chain reaction (PCR) and a small microarray placed at the bottom of the tube, to detect fluorescence signals emitted when specific yeast sequences hybridize. The total turnaround time is approximately 3 h once DNA is extracted and purified.

The BlackLight® Fungal ID kit (2B BlackBio S.L., Madrid, Spain) applies the principle of pyrosequencing, which relies on detection of pyrophosphate release at each nucleotide incorporation during sequencing reaction. The test starts from a 15 μl dry blood spot (1 mm diameter), which is used directly for PCR without pretreatment. The subsequent sequencing of a small region of the 18S rRNA gene allows the test to identify the most relevant *Candida* species as well as *Aspergillus fumigatus* and *A. niger*.

The assay is available in Europe for IVD purposes and at the moment this review was written, no clinical evaluation was yet published. The proof-of-principle description and data on analytical performance of the assay were discussed in a recent paper, where they showed 100% agreement with standard microbiological methods, when testing a collection of clinical blood culture isolates (82).

5. Antigen and Antibody-Based Methods for Fungal Detection

Detection of antigenic compounds from the fungal cell wall (e.g., chitin, β-glucans, and mannoproteins) or the respective antibodies generated by the host immune system is commonly used, in conjunction with other diagnostic procedures (e.g., microbiological cultures, histological examination of biopsy samples, and radiographic evidence), as an aid in the diagnosis of fungal infections.

5.1. Antigen- and Antibody-Based Tests for Candida

Mannan, a polysaccharide non-covalently bound to the yeast cell wall, can act as an antigen, generating an immune response that leads to production of host antibodies (83). Detection of mannan or respective antibodies led to commercialization of one of the most widely used tests for the laboratory detection of *Candida*: Platelia™ Candida Ag and Platelia™ Candida Ab (Bio-Rad). These are sandwich enzyme-linked immunosorbent assay (ELISA) tests for quantitative detection of *Candida* mannan or anti-mannan

antibodies, in serum of subjects at-risk or suspected of invasive candidiasis. Both assays have been available in Europe for about 10 years, but neither has received FDA approval for USA IVD use.

Overall sensitivity of mannan detection seems to be in the range of 60% and specificity around 90%, according to analysis of 12 published studies on adult populations (84). Similar to better figures were also found in pre-term infants in a neonatal ICU, suggesting that regular serology surveillance of circulating mannan would be a good complement to blood cultures for early detection of invasive candidiasis in this particular population (85).

Due to rapid elimination of mannan during infection, a negative result from the Platelia™ Candida Ag test cannot rule out diagnosis of invasive candidiasis. The manufacturer recommends concomitant screening of anti-mannan antibodies, by means of the Platelia™ Candida Ab assay, a two-step indirect immunoenzymatic microplate assay that allows quantitative detection of antibodies in human serum (86). This guidance is supported by several studies and literature analysis, reporting sensitivity improvements up to 85–90% (84, 87–89), and was recently endorsed by the experts report from the 3rd European Conference on Infections in Leukemia (90).

Other tests and kits based on the use of mannan and other specific antigens for *Candida* detection have been developed and commercialized by different companies. Serion Immunodiagnostica GmbH (Würzburg, Germany) commercializes the Serion ELISA antigen Candida and the Serion ELISA classic *Candida albicans* IgG/IgM/IgA, two immunoassays for either quantitative or qualitative use. The first is based on a mixture of cytoplasmic antigen structures such as enolase, and a certain amount of other cell-wall components, allowing detection of *Candida* infection in serum or plasma. The second is aimed to detect human antibodies directed against *C. albicans*. Very few studies describing and comparing performance of these assays have been published to date (91–93). Wulf and coworkers showed that the antibody-based test could pinpoint evidence for invasive candidiasis significantly earlier (approximately 22 days) (93).

Indirect tests for *Candida* detection based on the principle of latex agglutination (LA) are also commercially available. The Pastorex™ Candida (Bio-Rad) uses latex particles coated with specific antibodies that react with the polysaccharide antigen from *Candida* through agglutination, making it visible to the naked eye. Even though clinical specificity is pretty high, the clinical sensitivity remains quite low, around 25% (94, 95).

The Cand-Tec™ (Ramco Laboratories Inc. Stafford, TX, USA) is another agglutination assay for *Candida* detection in human samples. One retrospective study compared its sensitivity and specificity with the Pastorex™ Candida assay, in a very limited cohort of patients. While sensitivity values for Cand-Tec™ were

much better (68.8% vs. 16.7%), specificity was significantly lower (57.1% vs. 100%) (95). The use of this assay in combination with indirect hemagglutination seems to improve clinical performance in ICU patients (sensitivity 100% and specificity 83.3%) (96).

Based on the principle of indirect immunofluorescence and targeting a mannoprotein located on the germ tube cell wall surface, Vircell Microbiologists (Granada, Spain) commercializes an assay for detection of IgG antibodies against *C. albicans* germ tube antigens (CAGTA). This assay showed sensitivity of 83–85% and specificity of 86–95% through different studies (97, 98). Titers can be lower in case of invasive infections caused by *Candida* species other than *C. albicans*, and also in immunocompromised patients, even though overall performance does not seem affected (98, 99). Interestingly, a recent study suggested its potential value as a prognostic marker, and although further investigations are needed, their findings did show significantly lower mortality rates in patients with increased CAGTA titers, concomitantly treated with antifungal agents (100).

5.2. Antigen- and Antibody-Based Tests for Cryptococcus

Cryptococcal antigen detection has shown very high sensitivity and specificity, making it very helpful and reliable for diagnosis of cryptococcal pulmonary infection and meningitis.

Several commercial methods are available. The specificity of most cryptococcal antigen tests can be obscured by presence of rheumatoid interference factors in specimens, as they can lead to false-positive reactions. This is especially true for serum samples but much less for cerebrospinal fluid (CSF) specimens, which rarely contain antiglobulins responsible for this cross-reaction. For this reason, most manufacturers (e.g., IMMY, Meridian Biosciences Inc., and Bio-Rad) propose and recommend use of pronase. Pretreatment of biological specimens with pronase was shown to significantly reduce nonspecific interference of noisy substances and consequently, reduce false-positive results (101, 102). Additional factors that could lead to false-positive results are infection by *Trichosporon* species, use of starch, some disinfectants, and soap (103), and use of BBL™ Port-A-Cul™ anaerobic vials for specimen transport (104). Furthermore, it is recommended to process CSF specimens prior to culture as immersion of a platinum wire/loop containing trace amounts of surface condensation from agar has been described as a source of interference (105). Sample storage is also important, as increased pH and high temperature induced degradation of cryptococcal antigen and therefore loss of reactivity for serological tests (106). Commercially available tests for *Cryptococcus* species are summarized in Table 6.

5.3. Antigen- and Antibody-Based Tests for Aspergillus

Detection of circulating galactomannan (GM) is generally utilized for diagnosis and management of invasive aspergillosis. GM is a heteropolysaccharide component of the cell walls of *Aspergillus*

Table 6
Commercially available antigen or antibody-based tests for *Cryptococcus* detection

Format	Assay name	Manufacturer	Reference
Latex agglutination	Cryptococcal Antigen Latex Agglutination System (CALAS®)	Meridian Bioscience, Inc. Cincinnati, OH, USA	(105–107)
	Latex-Cryptococcus Antigen Test	IMMY	Not reported
	Pastorex™ Crypto Plus	Bio-Rad	(99, 108)
	Cryptococcus Antigen Test	Remel	(106, 107)
	Crypto-LA®	Wampole Laboratories Inc., Cranbury, NJ, USA	(107)
	Eiken Latex test	Eiken, Tokyo, Japan	(108, 109)
Enzymatic Immuno-Assays	Premier™ Cryptococcal Antigen	Meridian Bioscience Inc.	(105, 107)

and *Penicillium* species and was the first circulating antigen found in animal models with invasive aspergillosis. The Platelia™ Aspergillus EIA (enzyme immunoassay; Bio-Rad) is a one-stage immunoenzymatic sandwich microplate assay which uses rat monoclonal antibody EBA-2, for detection of *Aspergillus* GM antigen in serum. CE-IVD marked by the end of the 1990s, it was also FDA approved in 2003. Three recent publications review its use, performance variability, and assay limitations, providing guidance for better results interpretation (110–112). A wide variety of factors affect performance of Platelia™ Aspergillus EIA including host conditions, concomitant treatments, presence of other fungal species, handling and processing of biological specimens, etc. Even though other authors provided more exhaustive lists and further description of false-positive and false-negative results (111–113), Table 7 highlights some of the most common sources of interference that can confer misleading results. Measurement of GM is currently recommended by the international guidelines for management of patients with invasive aspergillosis, where it is clearly highlighted that clinical judgment must prevail when interpreting test results (2).

Even though the manufacturer does still not claim use of the Platelia™ Aspergillus EIA for samples other than serum, several different reports since 2004 pointed out the interest of GM measurement in other specimens, e.g., urine, BAL, or CSF, because of higher sensitivity and potential early detection over the course of infection in immunocompromised patients (114–118). However, its use in BAL samples from immunocompetent patients seems to have no added value (119). The utility of the Platelia™ Aspergillus EIA in pediatric patients is still unclear and contradictory results

Table 7
Most common variables associated with false-positive and false-negative results for the Platelia™ Aspergillus EIA test (Bio-Rad)

Factor and/or situation that can lead to false-positive results	
Host related	Renal failure Mucositis Food intake of galactofuranose[a] Gut colonization and potential translocation of *Bifidobacterium* Gastrointestinal microflora of neonates
Iatrogenic	Blood derivatives Intravenous solutions containing gluconate Treatment with antibiotics derived from the fermentation of *Penicillium* species (e.g., piperacilin-tazobactam, amoxicilin-clavulanic acid) Use of cyclophosphamide in cancer patients
Sample collection and/or processing	Use of materials such as cotton swabs and cardboard Inappropriate cut-off value (too low)
Environmental	Presence of other non-*Aspergillus* fungi such as *Penicillium*, *Alternaria*, *Paecilomyces*, *Geotrichum*, *Histoplasma*, and even *C. neoformans*[b]
Factor and/or situation that can lead to false-negative results	
Host conditions	Chronic granulomatose disease
Iatrogenic	Treatment with antifungals
Sample collection and/or processing	Long-term storage of samples Inappropriate cut-off value (too high)

[a]Stabilizing agent commonly used in some cereal-derived products and some cream desserts
[b]Epitope contained within its galactoxylomannan has been shown to cross-react (113)

have been reported among different studies. Although still not recommended by the manufacturer further validation studies might help to clarify its positioning within this critical population (120). In all cases, the significance of positive results in patients without confirmed aspergillosis always requires further evaluation.

Another assay format to detect GM in serum is proposed with Pastorex™ Aspergillus (Bio-Rad), a qualitative test, which uses a simple agglutination technique. The lowest concentration of GM detectable is 15 ng/ml, around ten times more than the 1 ng/ml claimed by the Platelia™ Aspergillus EIA. Definitely less cumbersome to perform, it was shown to be less sensitive and more specific than its EIA companion (121, 122).

Detection of human antibodies against *Aspergillus* is also commercially available through different vendors. The Platelia™ Aspergillus IgG (Bio-Rad) is a two-phase indirect microplate immunoenzymatic technique used to detect IgG anti-*Aspergillus* antibod-

ies in human serum or plasma. The VIRION/SERION ELISA classic *Aspergillus fumigatus* IgG/IgM/IgA (Serion Immundiagnostica & Institut Virion\Serion GmbH) is another quantitative alternative to detect antibodies against *A. fumigatus* in serum or plasma. In this case, separate detection of individual immunoglobulin classes offers a more detailed view for mycological monitoring of at-risk patients. Since an immune response is needed for antibodies to be present, this kind of assay is mostly used within the context of allergic or chronic *Aspergillus*-related pathologies (123, 124).

Two alternatives for *Aspergillus* detection, provided by IMMY, are available in Europe and USA. The first is based on the principle of Complement Fixation. As with other assays previously described, although a positive result strongly suggests *Aspergillus* infection or allergy, the organism must be repeatedly cultured or observed by microscopy for a definitive diagnosis. The second is an Immunodiffusion Test for aspergillosis, which is mostly restricted to diagnosis of allergic bronchopulmonary aspergillosis and aspergilloma.

5.4. Beta-D-Glucan Assays

Glucans are (1→3)-β-D-linked polymers of glucose that are part of the outer fungal cell wall. They can also be found in some bacteria, e.g., *Alcaligenes*, *Pseudomonas*, and *Streptococcus*, which produce glucan or glucan-like polymers, as well as in plant cellulose (125, 126). These polymers can be released or actively secreted from the cell wall as exopolymers and are well known to activate the immune system of mammals (127). Detection of β-D-glucan is commercially available through different manufacturers of assays based on the ability of β-D-glucan to react with *Limulus* Amebocyte Lysate (LAL). This compound, from horseshoe crab blood cells, was recently discovered to react with β-D-glucan (as well as endotoxin), by activation of factor G of the coagulation cascade (128).

The Fungitell™ assay (Associates of Cape Cod, Inc., East Falmouth, MA, USA) is CE-marked and FDA approved since 2004. In Japan, three companies propose assays for β-D-glucan detection. The Fungitec® G Test MK (Seikagaku Corporation, Tokyo, Japan) and B-G-Star B-D-glucan test (Maruha Nichiro Foods, Inc., Tokyo, Japan) are in vitro chromogenic endpoint quantitative assays, whereas the Wako test (Wako Pure Chemical Industries) is a kinetic turbidimetric test.

In summary, detection of circulating β-D-glucan seems to be a good indication to exclude invasive fungal infections, due to its high negative predictive value (112, 129, 130). General recommendation is to combine use of β-D-glucan with other surrogate markers such as mannan, GM, or fungal DNA, as in the majority of cases this combination not only provides species identification of the etiologic agent but also tends to increase β-D-glucan diagnostic accuracy (97). Assessment of the optimal sampling strategy for at-risk patients, the criteria to define a positive test result, the optimal cut-off value, and how to interpret the influence of concurrent

bacteremia on diagnostic performance might be very helpful to determine optimal utilization of β-D-glucan testing for diagnosis of IFIs. Even though several commercially available solutions exist, the assay is still technically demanding and highly susceptible to external contamination during processing. Efforts need to be undertaken to simplify procedures and reduce the risk of false-positive results, although some of them are inherent to the almost omnipresence of glucans in the environment.

6. Molecular Biology-Based Methods

Recent progress in molecular biology, especially the arrival of real-time amplification and fluorescence-based detection tools, has allowed development and commercialization of different tests for detection and identification of fungi. Their development has often been linked to continuous efforts towards detection of pathogens associated with sepsis, a life-threatening condition associated with an altered inflammatory response to infection. Therefore, the vast majority of molecular biology-based tests commercially available for fungal detection are often coupled to that of bacteria and some antibiotic resistance markers. A summary of tests described in this section and their main characteristics is shown in Table 8. It is important to highlight that all these tests and kits are only available in the European market either for RUO or IVD purposes. The following sections will describe the tests available for sepsis as well as those tests available for specific detection of fungal species.

6.1. Sepsis-Based Assays

The LightCycler® SeptiFast Test M^{GRADE} (Roche Diagnostics GmbH, Mannheim, Germany) was the first test of this series of platforms dedicated to detection and identification of both bacteria and fungi directly from whole blood, as an aid in management of septic patients. This test is based on real-time PCR targeting species-specific ITS regions between the 18S and 5.8S rRNA genes. The assay can detect and identify five *Candida* species and *Aspergillus fumigatus*. As a constant for this type of assay, three main steps are applied from sample collection to final results, sample preparation, amplification, and real-time detection.

The initial sample for SeptiFast Test M^{GRADE} is whole ethylenediaminetetraacetic acid (EDTA) anticoagulated human blood, which is mechanically lysed using the SeptiFast Lyse Kit M^{GRADE} (Roche Diagnostics GmbH) and the MagNA Lyser® (Roche Diagnostics GmbH). While the method originally started from 3 ml of whole blood, the current version recommends starting with 1.5 ml. Only one-third of the total genomic DNA, purified by means of the SeptiFast Prep Kit M^{GRADE} (Roche Diagnostics

Table 8
Main features of the different molecular biology-based tests, commercially available, for fungal detection

Assay (manufacturer)	Species claimed	Sample type (volume)	Main features for		
			Sample preparation	PCR	Final identification
LightCycler® SeptiFast Test M^{GRADE} (Roche Diagnostics GmbH)	*Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *Aspergillus fumigatus*	Whole blood (1.5 ml)	Isolation of total DNA Mostly manual[a]	Broad-range ITS PCR	Fluorescence real-time PCR signals and melting curves
Vyoo® Assay (SIRS-Lab GmbH)	Idem as above	Whole blood (up to 5 ml)	Selective enrichment of microbial DNA Automated process	Broad-range rDNA PCR	Fluorescence signals read on an automated array[b]
Magicplex™ Sepsis Real-Time Test (Seegene Inc.)	Idem as above	Whole blood	Selective human cell lysis and degradation of human DNA Manual or automated	Broad-range rDNA PCR	Fluorescence real-time PCR signals
SepsiTest™ Molzym GmbH & Co.KG)	Pan-yeast assay	Whole blood, heart valve tissue, and synovial fluid		Broad-range rDNA PCR	Sequencing of amplicons
Affigene® Aspergillus tracer (Cepheid AB)	*Aspergillus* spp.	Whole blood and plasma samples (0.5 ml)	Not specified[c]	Broad-range rDNA PCR	Fluorescence real-time PCR signal
MycAssay™ Aspergillus (Myconostica Ltd.)	*Aspergillus* spp.	Respiratory and serum samples	MycXtra DNA extraction for respiratory specimens Roche High Pure Template DNA kit for serum Manual processing	Broad-range 18S rDNA PCR	Fluorescence real-time PCR signal
MycAssay™ Pneumocystis (Myconostica Ltd.)	*Pneumocystis jirovecii*	Respiratory samples	MycXtra DNA extraction Manual processing	Mitochondrial large subunit PCR	Fluorescence real-time PCR signal

[a]Some published reports combine it with automated methods such as MagNA Pure® (Roche Diagnostics GmbH) or EasyMAG® (bioMérieux®)
[b]Former format used electrophoretic separation of amplicons
[c]Published report used Roche HighPure PCR Template Preparation Kit (Roche Diagnostics GmbH) (131)

GmbH), is dedicated to specific fungal detection. Amplification of targets occurs in the LightCycler® 2.0 instrument (Roche Diagnostics GmbH) and final interpretation of results is done by dedicated software (SIS SeptiFast Identification Software—Roche Diagnostics GmbH), which calculates melting point values and links the peak heights to a particular fungal species. The whole procedure is validated by reading positive signals coming from the internal control provided with the kit. Turnaround time ranges from 6 to 8 h.

The utility and performance of the LightCycler® SeptiFast Test M^{GRADE} in the clinical setting have been reported in more than 15 publications. A summary of results observed for detection of fungal pathogens is provided in Table 9. While blood culture is generally used as the reference for comparison, some authors prefer to evaluate performance through analysis of the overall sepsis or febrile episode and only a few studies have focused the analysis on how the test performed for detection of IFIs, based on published consensus definitions. To date, no clear evidence has been shown whether the LightCycler® SeptiFast Test M^{GRADE} can have an impact on management of patients with IFIs. None of the studies has tested serial specimens collected during the period at-risk, before blood cultures are drawn, and therefore it is still unclear whether fungal DNA can be considered or not as an early marker in development of invasive candidiasis (84). Nevertheless, results published thus far suggest that its use in combination with other methods (e.g., blood culture or GM detection) could be valuable. Whether LightCycler® SeptiFast Test M^{GRADE} has the potential to facilitate early evidence-based treatment decisions, antimicrobial selection, and adequacy of antimicrobial treatment, effectively designed observational or even interventional studies are needed to prove its clinical value in improving appropriate treatment and patient outcomes (142).

Another commercially available multiplex PCR sepsis-based test is the Vyoo® assay (SIRS-Lab GmbH, Jena, Germany). The Vyoo® assay can accommodate up to 5 ml EDTA-whole blood, hence increasing probability to detect low fungal burden. Furthermore, it uses a specific DNA-binding protein able to recognize unmethylated DNA motifs, much less frequent in human DNA than in bacteria, yeast, or molds (143). After a first step of mechanical lysis, microbial DNA is processed and enriched by affinity chromatography, which can significantly improve the signal-to-noise ratio between human and fungal DNA. The final detection of amplicons can be done either by electrophoretic separation or by hybridization methodologies (144). Available in Europe for diagnostic purposes since 2007, a new updated version was recently released including changes related to automated DNA isolation and enrichment as well as final readout of results which is now based on software-supported microarrays analysis.

Table 9
Summary of some published reports evaluating fungal detection performance of the LightCycler® SeptiFast Test M^{GRADE} (SF)

Population (# of patients)	Reference method (RM)	# of positive cases for the RM	# of positive cases for SF	Concordance (SF out of total by RM)	Comments	Reference
HU–FNP ($n=34$)	Blood culture	None	*A. fumigatus* ($n=2$)	*A. fumigatus* results linked to invasive infection confirmed by clinical data and mold isolation from BAL, 1 week later	Levels of galactomannan for both patients were continuously borderline	(134)
HU–FNP ($n=70$)	FNE	Candidemia ($n=0$) *A. fumigatus* ($n=12$)[a]	*Candida* ($n=3$) *A. fumigatus* ($n=5$)	*Candida* ($n=1$ of 3)[b] *A. fumigatus* ($n=5$ of 12)	*Aspergillus* infection link made on the basis of repetitive high levels of circulating GM	(133)
HU–FNP ($n=86$)	IFI definition[c] (3)	Probable aspergillosis ($n=2$) Possible aspergillosis ($n=1$) Possible candidiasis ($n=3$) Possible IFI ($n=1$)	*Candida* ($n=7$) *A. fumigatus* ($n=2$)	Probable aspergillosis (none of 2) Possible aspergillosis (1 of 1) Possible candidasis (3 of 3) Possible IFI (1 of 1)	Blood culture remained negative SeptiFast diagnosis preceded standard diagnosis in three cases	(135)
ICU & IC ($n=77$)	Blood culture	Candida ($n=4$)	*Candida* ($n=5$) *A. fumigatus* ($n=1$)	*Candida* (3 of 4)	Antifungal treatment against *Candida* could have been either adjusted earlier or implemented in one and three cases, respectively	(136)
ICU, ER, SW ($n=436$)	Sepsis episode	*Candida* ($n=9$)	*Candida* ($n=10$) *A. fumigatus* ($n=7$)	6//9 *Candida* (6 of 9) All 7 *A. fumigatus* PCR findings judged as workflow contamination	Study not designed for method comparison, not providing any conclusion on clinical utility of therapy adjustments	(132)
ICU, ER, GM ($n=200$)	Blood culture	*Candida* ($n=4$)	*Candida* ($n=4$) *A. fumigatus* ($n=0$)	*Candida* (2 of 4)		(137)

Not reported ($n=359$)	Blood culture	*Candida* ($n=10$)	*Candida* ($n=20$) *A. fumigatus* ($n=12$)	*Candida* (5 of 10) Two out of five patients linked to *A. fumigatus* infection	*A. fumigatus* PCR positive results in five patients For three of them, no test was performed to confirm *Aspergillus*	(138)
ICU & HU ($n=110$)	IFI definition (3)[c]	Probable aspergillosis ($n=3$) Possible aspergillosis ($n=4$) Candidemia ($n=0$) Candida colonization ($n=2$)	*Candida* (n=0) *A. fumigatus* (n=2)	Probable aspergillosis (2 of 3) Possible aspergillosis (none of 4) *Candida* colonization (none of 2)	No false-positive results for *Candida* spp. occurred, even in patients heavily colonized	(139)
HU, ICU ($n=86$)[d]	Blood culture	*Candida* ($n=1$)	*Candida* ($n=1$) *A. fumigatus* ($n=1$)	*Candida* (1 of 1) *Aspergillus* considered false-positive result[e]	Fungemia defined by microbial growth in one or more blood culture sets	(140)
ICU, SW, ER, OU, HU, NU, PU ($n=811$)	Blood culture	*Candida* ($n=16$)	*Candida* ($n=27$)	*Candida* ($n=13$ of 16)	No *Aspergillus* found Higher rate of positive results for patients undergoing empirical antimicrobial therapy	(141)

ICU intensive care unit, *IC* immunocompromised, *SW* surgical ward, *GM* general medicine, *HU* hematological unit, *OU* oncology unit, *PU* pediatrics unit, *NU* neonates unit, *FNP* febrile neutropenic patients, *IFI* invasive fungal infection, *FNE* febrile neutropenic episode

[a]Positive levels of galactomannan (>0.7 optical density index)

[b]Succesive positive PCR results

[c]Linked to high levels of procalcitonin

[d]33 febrile neutropenic patients

[e]Analytical galactomannan negative results on serum and no clinical evidence of aspergillosis

Furthermore, a positive control was included to guarantee complete validity of results. The total turnaround time is around 7 h.

The SepsiTest™ (Molzym GmbH & Co. KG, Bremen, Germany) is another bundled CE-IVD marked kit for the detection of bacteria, yeasts, and molds in whole blood and other body fluid samples. It uses a proprietary technology, which gets rid of human DNA through degradation of released DNA after selective lysis of human cells. Microorganisms are therefore enriched and subsequently lysed for final elution of their DNA. This manual processing can today be combined with selectNA™, an automated platform able to handle up to 12 samples, reducing hands-on time needed for sample preparation. The eluted DNA is then combined with broad-range rDNA PCR, which provides molecular detection of pathogens. SepsiTest™ supplies two separate assays with broad-range primers targeting eubacterial and fungal targets. The final identification needs sequencing of the amplification product, which is then compared to the highly variable region sequences contained in the online identification tool, provided by the manufacturer as well. The total turnaround time is around 8 h and the species currently identified include *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *Cladosporium cladosporioides*, and *Malassezia* spp.

One of the latest CE-IVD marked sepsis-based kits is the Magicplex™ Sepsis Real-time Test (Seegene, Seoul, Korea). It claims detection of bacterial and fungal targets from EDTA-whole blood samples and is commercially available since November 2010. The same six fungal species as the LightCycler® SeptiFast Test M^{GRADE} are detected, by means of a broad-range PCR, compatible with different PCR platforms, including their own Seecycler™ (Seegene, Seoul, Korea). The company recently announced its partnership with Molzym GmbH & Co. KG to apply their microbial DNA enrichment technology and the selectNA™ automated platform to the assay process. At the moment this review was written, no study evaluating this test had been published.

6.2. Specific Assays

The MycAssay™ Aspergillus (Myconostica Ltd., Manchester, UK) is a CE-IVD marked, real-time PCR assay for the specific detection of *Aspergillus* DNA in serum and lower respiratory tract specimens. Myconostica Ltd., a diagnostic company spin-off from the University of Manchester, launched in 2010 this assay together with a product for extracting fungal DNA from human respiratory samples (MycXtra® DNA extraction).

The MycAssay™ Aspergillus PCR assay was validated for use with different platforms such as SmartCycler® (Cepheid, Sunnyvale, CA, USA) AB7500 (Life Technologies™, Carlsbad, CA, USA), LightCycler® 2.0, and Stratagene® M×3000™ (Agilent Technologies, Santa Clara, CA, USA). The gene targeted is the 18S rDNA and the analytical limit of detection (LoD) achieved is below 50 target copies. One interesting feature is that it provides a

clinical cut-off for better interpretation of results in the clinical setting. The assay includes an internal amplification control in every reaction to highlight false-negative results. Due to its pan-*Aspergillus* capacity, the assay is presented in closed tube reactions and single-use reagents to reduce the risk of contamination.

Another PCR-based specific test for *Aspergillus* detection is available through Cepheid AB (Bromma, Sweden). The Affigene® Aspergillus tracer is a CE-IVD marked qualitative assay for detection of *Aspergillus* DNA directly from human whole blood and plasma samples. The kit provides reagents in a ready-to-use, single-use format, compatible with several real-time PCR platforms such as Mx3000P/3005P (Stratagene, La Jolla, USA), the iCycler iQ/iQ5 (BioRad Laboratories Inc, Hercules, CA, USA), the Rotor-Gene 3000 (Corbette Research, Sydney, Australia), and the ABI 7300 (Applied Biosystems, Foster City, USA). The analytical sensitivity in both plasma and whole blood is below 0.5 genome equivalents per microliter. The assay was evaluated in a population of neutropenic patients where two patients diagnosed as having proven or probable aspergillosis according to the EORTC/MSG criteria were the only ones showing a positive PCR result (145).

Myconostica Ltd., recently acquired by another English company (Lab 21 Ltd., Cambridge, UK), also commercializes the MycAssay™ Pneumocystis assay: the first commercially validated (CE-IVD marked) real-time PCR assay, for diagnosis of *Pneumocystis* infection in human clinical respiratory specimens, targeting the mitochondrial large subunit of *P. jirovecii*. It is recommended to be combined with MycXtra® DNA extraction and was validated for use on the same platforms as the MycAssay™ Aspergillus. Its LoD is below 35 target copies and an internal amplification control is used in every reaction. A recent multicenter study on 110 patients compared results to clinical diagnosis of *Pneumocystis* respiratory infection determined by non-molecular methods in accordance with local diagnostic procedures. Sensitivity and specificity were 93% and 90%, respectively, compared to values from Merifluor-*Pneumocystis* direct fluorescent antigen (146).

7. Unmet Needs and Trends for the Future

Diagnosis of fungal infections is still challenging and there is need for improvement. Efforts must continue towards development of less invasive, more standardized and reliable IVD tools, contributing to earlier and more accurate diagnosis of fungal infections, which will lead to more appropriate therapeutic decisions and improved outcomes of at-risk patients. Standardization and combination are two key concepts to keep in mind for successful

development of future commercial tests, in addition to implementation of new technological advances in the field of diagnostics.

The standardization of techniques, especially those based on the detection of fungal nucleic acids is a must, and some international initiatives are already pursuing consensus guidelines for use of such techniques (147–149). This standardization is not only needed for the techniques but also for the design of evaluation studies. The heterogeneity of the different attained or at-risk populations hampers interpretation of results and the clinical utility of the evaluated techniques. Efforts must be made towards standardization of methodologies, which will indeed help enable formal validation of new tools, such as PCR-based ones (150).

Combination of information provided by different markers, or combination of distinct technologies, seems to be improving diagnostic accuracy. Examples have been provided regarding utility of merging information provided by detection of mannan and anti-mannan antibodies in the case of *Candida* infections (90), but this example has also been successfully implemented when combining information provided by detection of fungal antigens and nucleic acids by means of PCR-based assays (87, 139). Clinical guidelines also emphasize importance of combining information from different IVD tools (2).

Combining classical methodologies with the most recent technological advances is also how some manufacturers propose to move forward within the unmet diagnostic need. Probably the most relevant examples are those addressing positive blood cultures to rapidly and accurately identify the presence of fungal species—Yeast Traffic Light® PNA-FISH, Prove-it™ Fungi, and the BlackLight® Fungal ID kit.

Implementation of the newest and most recent technological advances will also warrant development of improved products. Appearance of technologies such as Digital PCR and better accessibility to next-generation sequencing technologies are examples of technologies that could arrive in the near future to the diagnostic field. Such technological jumps which occurred previously with serology were seen again with PCR and have recently occurred with the successful translation of mass spectrometry in the IVD field. MALDI-TOF mass spectrometry provides today an impressive way to rapidly identify fungal species from isolated colonies. Additional work is needed to better identify clinically relevant molds and since their ability to utilize carbohydrates and enzymatic substrates is quite diverse, the classical microbiological methods appear less promising than the potential of MALDI-TOF mass spectrometry. Future technological advances will make possible its use directly from positive blood culture bottles and maybe directly from the biological specimen.

Another example of technological combinations, even though not yet available for diagnostic purposes is the one provided by the Plex-ID (Abbot Wiesbaden, Germany). This system, based on the use of eubacterial and panfungal PCR, is able to detect nearly all known fungal targets by coupling genome-specific amplification and electrospray ionization mass spectrometry. It allows determination of the base composition from small quantities of nucleic acids in a complex mixture, which is then compared to the genomic information contained in a curated database, a key element of the system. The Ibis Bioscience database needs to be constantly updated with the latest information on newly identified microorganisms. The time to result is around 8 h and some papers have been published describing analytical performance and proof-of-principle on blind panels of bacterial isolates (151).

In addition to all facts previously mentioned, the future must also bring more automation. This would make tests easier and more standardized and in some cases bring them closer to the bedside, allowing reduced time to results, often crucial in some clinical situations (e.g., febrile neutropenia).

Acknowledgments

We would like to thank Karine Kaiser, Carole Vachon, and Ira Salkin for their helpful comments and critical review of this manuscript.

References

1. Fridkin SK (2005) The changing face of fungal infections in health care settings. Clin Infect Dis 41:1455–60
2. Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Segal BH, Steinbach WJ, Stevens DA, van Burik JA, Wingard JR, Patterson TF (2008) Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 46:327–360
3. Chandler FW, Kaplan W, Ajello L (1980) Color atlas and text of the histopathology of mycotic diseases. Year Book Medical Publishers, Chicago
4. Monheit JE, Cowan DF, Moore DG (1984) Rapid detection of fungi in tissue using calcofluor white and fluorescence microscopy. Arch Pathol Lab Med 108:616–618
5. Nolte FS, Williams JM, Jerris RC, Morello JA, Leitch CD, Matushek S, Schwabe LD, Dorigan F, Kocka FE (1993) Multicenter clinical evaluation of a continuous monitoring blood culture system using fluorescent-sensor technology (BACTEC 9240). J Clin Microbiol 31:552–557
6. Thorpe TC, Wilson ML, Turner JE, DiGuiseppi JL, Willert M, Mirrett S, Reller LB (1990) BacT/Alert: an automated colorimetric microbial detection system. J Clin Microbiol 28:1608–1612
7. Mirrett S, Hanson KE, Reller LB (2007) Controlled clinical comparison of VersaTREK and BacT/ALERT blood culture systems. J Clin Microbiol 45:299–302
8. Taschdjian CL, Burchall JJ, Kozinn PJ (1960) Rapid identification of *Candida albicans* by filamentation on serum and serum substitutes. Am J Dis Child 99:212–215
9. Crist AE Jr, Dietz TJ, Kampschroer K (1996) Comparison of the MUREX C. albicans, Albicans-Sure, and BactiCard Candida test kits with the germ tube test for presumptive

identification of *Candida albicans*. J Clin Microbiol 34:2616–2618

10. Heelan JS, Siliezar D, Coon K (1996) Comparison of rapid testing methods for enzyme production with the germ tube method for presumptive identification of *Candida albicans*. J Clin Microbiol 34:2847–2849
11. Carrillo-Muñoz AJ, Quindós G, Cárdenes CD, Alonso-Vargas R, Brió S, Arévalo P, Pemán J, Estivill D, Pontón J (2003) Performance of Bacticard Candida compared with the germ tube test for the presumptive identification of *Candida albicans*. Mycoses 46:467–470
12. Cárdenes CD, Carrillo-Muñoz AJ, Arias A, Rodríguez-Alvarez C, Torres-Lana A, Sierra A, Arévalo MP (2004) Comparative evaluation of four commercial tests for presumptive identification of *Candida albicans*. J Microbiol Methods 59:293–297
13. Fenn JP, Segal H, Blevins L, Fawson S, Newcomb-Gayman P, Carroll KC (1996) Comparison of the Murex *Candida albicans* CA50 test with germ tube production for identification of C. albicans. Diagn Microbiol Infect Dis 24:31–35
14. Baalham T, Brown A, Dunsmuir R, Shankland GS, Blackman M (2005) Comparative evaluation of a new biochemical test kit for the presumptive identification of *Candida albicans*. 15th European Congress of Clinical Microbiology and Infectious Diseases Copenhagen / Denmark. Abstract number: 1134_01_304 http://www.blackwellpublishing.com/eccmid15/abstract.asp?id=36567 Accessed 19 July 2011
15. Staib F (1962) *Cryptococcus neoformans* und *Guizotia abyssinica* (syn. G. oleifera D.C.). Farbreaktion für *Cr. neoformans*. Z Hyg Infektionskr Med Mikrobiol Immunol Virol 148:466–475
16. Denning DW, Stevens DA, Hamilton JR (1990) Comparison of *Guizotia abyssinica* seed extract (birdseed) agar with conventional media for selective identification of *Cryptococcus neoformans* in patients with acquired immunodeficiency syndrome. J Clin Microbiol 28:2565–2567
17. Fleming WH, Hopkins JM, Land GA (1977) New culture medium for the presumptive identificaion of *Candida albicans* and *Cryptococcus neoformans*. J Clin Microbiol 5: 236–243
18. Roberts GD, Horstmeier CD, Land GA, Foxworth JH (1978) Rapid urea broth test for yeasts. J Clin Microbiol 7:584–588
19. Zimmer BL, Roberts GD (1979) Rapid selective urease test for presumptive identification of *Cryptococcus neoformans*. J Clin Microbiol 10:380–381
20. Freydière AM, Robert R, Ploton C, Marot-Leblond A, Monerau F, Vandenesch F (2003) Rapid identification of *Candida glabrata* with a new commercial test, GLABRATA RTT. J Clin Microbiol 41:3861–3963
21. Freydière AM, Perry JD, Faure O, Willinger B, Tortorano AM, Nicholson A, Peman J, Verweij PE (2004) Routine use of a commercial test, GLABRATA RTT, for rapid identification of *Candida glabrata* in six laboratories. J Clin Microbiol 42:4870–4872
22. Pemán J, Aparisi N, García-Esteban C, Gobernado M (2004) Rapid identification of *Candida glabrata* using a new commercial kit. Rev Iberoam Micol 21:82–84
23. Willinger B, Wein S, Hirschl AM, Rotter ML, Manafi M (2005) Comparison of a new commercial test, GLABRATA RTT, with a dipstick test for rapid identification of *Candida glabrata*. J Clin Microbiol 43:499–501
24. Fenn JP, Billetdeaux E, Segal H, Skodack-Jones L, Padilla PE, Bale M, Carroll K (1999) Comparison of four methodologies for rapid and cost-effective identification of *Candida glabrata*. J Clin Microbiol 37: 3387–3389
25. Lopez J, Dalle F, Mantelin P, Moiroux P, Nierlich AC, Pacot A, Cuisenier B, Vagner O, Bonnin A (2001) Rapid identification of *Candida glabrata* based on trehalose and sucrose assimilation using Rosco diagnostic tablets. J Clin Microbiol 39:1172–1174
26. Freydière A-M, Parant F, Noel-Baron F, Crepy M, Treny A, Raberin H, Davidson A, Odds FC (2002) Identification of *Candida glabrata* by a 30-second trehalase test. J Clin Microbiol 40:3602–3605
27. Murray MP, Zinchuk R, Larone DH (2005) CHROMagar Candida as the sole primary medium for isolation of yeasts and as a source medium for the rapid-assimilation-of-trehalose test. J Clin Microbiol 43: 1210–1212
28. Odds FC, Hanson MF, Davidson AD, Jacobsen MD, Wright P, Whyte JA, Gow NA, Jones BL (2007) One year prospective survey of *Candida* bloodstream infections in Scotland. J Med Microbiol 56:1066–1075
29. Orenga S, James AL, Manafi M, Perry JD, Pincus DH (2009) Enzymatic substrates in microbiology. J Microbiol Methods 79: 139–155

30. Perry JD, Freydière AM (2007) The application of chromogenic media in clinical microbiology. J Appl Microbiol 103:2046–2055
31. Ghelardi E, Pichierri G, Castagna B, Barnini S, Tavanti A, Campa M (2008) Efficacy of Chromogenic Candida Agar for isolation and presumptive identification of pathogenic yeast species. Clin Microbiol Infect 14: 141–147
32. Sendid B, François N, Standaert A, Dehecq E, Zerimech F, Camus D, Poulain D (2007) Prospective evaluation of the new chromogenic medium CandiSelect 4 for differentiation and presumptive identification of the major pathogenic *Candida* species. J Med Microbiol 56:495–499
33. Odds FC, Bernaerts R (1994) CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J Clin Microbiol 32:1923–1929
34. Powell HL, Sand CA, Rennie RP (1998) Evaluation of CHROMagar Candida for presumptive identification of clinically important *Candida* species. Diagn Microbiol Infect Dis 32:201–204
35. Baumgartner C, Freydière AM, Gille Y (1996) Direct identification and recognition of yeast species from clinical material by using albicans ID and CHROMagar Candida plates. J Clin Microbiol 34:454–456
36. Fricker-Hidalgo H, Vandapel O, Duchesne MA, Mazoyer MA, Monget D, Lardy B, Lebeau B, Freney J, Ambroise-Thomas P, Grillot R (1996) Comparison of the new API Candida system to the ID 32C system for identification of clinically important yeast species. J Clin Microbiol 34:1846–1848
37. Bernal S, Martín Mazuelos E, Chávez M, Coronilla J, Valverde A (1998) Evaluation of the new API Candida system for identification of the most clinically important yeast species. Diagn Microbiol Infect Dis 32:217–221
38. Buchaille L, Freydière AM, Guinet R, Gille Y (1998) Evaluation of six commercial systems for identification of medically important yeasts. Eur J Clin Microbiol Infect Dis 17:479–488
39. Campbell CK, Davey KG, Holmes AD, Szekely A, Warnock DW (1999) Comparison of the API Candida system with the AUXACOLOR system for identification of common yeast pathogens. J Clin Microbiol 37:821–823
40. Paugam A, Benchetrit M, Fiacre A, Tourte-Schaefer C, Dupouy-Camet J (1999) Comparison of four commercialized biochemical systems for clinical yeast identification by colour-producing reactions. Med Mycol 37:11–17
41. Buesching WJ, Kurek K, Roberts GD (1979) Evaluation of the modified API 20C system for identification of clinically important yeasts. J Clin Microbiol 9:565–569
42. Land GA, Harrison BA, Hulme KL, Cooper BH, Byrd JC (1979) Evaluation of the new API 20C strip for yeast identification against a conventional method. J Clin Microbiol 10:357–364
43. Bergan T, Hollum AB, Vangdal M (1982) Evaluation of four commercial biochemical test systems for identification of yeasts. Eur J Clin Microbiol 1:217–222
44. Schuffenecker I, Freydière A, de Montclos H, Gille Y (1993) Evaluation of four commercial systems for identification of medically important yeasts. Eur J Clin Microbiol Infect Dis 12:255–260
45. Willemsen M, Breynaert J, Lauwers S (1997) Comparison of Auxacolor with API 20 C Aux in yeast identification. Clin Microbiol Infect 3:369–375
46. Ramani R, Gromadzki S, Pincus DH, Salkin IF, Chaturvedi V (1998) Efficacy of API 20C and ID 32C systems for identification of common and rare clinical yeast isolates. J Clin Microbiol 36:3396–3398
47. Sand C, Rennie RP (1999) Comparison of three commercial systems for the identification of germ-tube negative yeast species isolated from clinical specimens. Diagn Microbiol Infect Dis 33:223–229
48. Wadlin JK, Hanko G, Stewart R, Pape J, Nachamkin I (1999) Comparison of three commercial systems for identification of yeasts commonly isolated in the clinical microbiology laboratory. J Clin Mcrobiol 37:1967–1970
49. Gündes SG, Gulenc S, Bingol R (2001) Comparative performance of Fungichrom I, Candifast and API 20C Aux systems in the identification of clinically significant yeasts. J Med Microbiol 50:1105–1110
50. Milan EP, Malheiros ES, Fischman O, Colombo AL (1997) Evaluation of the AUXACOLOR system for the identification of clinical yeast isolates. Mycopathologia 137:153–157
51. Sheppard DC, deSouza E, Hashmi Z, Robson HG, René P (1998) Evaluation of the Auxacolor system for biochemical identification of medically important yeasts. J Clin Microbiol 36:3726–3727
52. Romney MG, Bryce EA, Rennie RP, Sand CA (2000) Rapid identification of clinical yeast isolates using the colorimetric

AUXACOLOR system. Diagn Microbiol Infect Dis 36:137–138

53. Latouche GN, Daniel HM, Lee OC, Mitchell TG, Sorrell TC, Meyer W (1997) Comparison of use of phenotypic and genotypic characteristics for identification of species of the anamorph genus *Candida* and related teleomorph yeast species. J Clin Microbiol 35:3171–3180
54. Marklein G, Josten M, Klanke U, Müller E, Horré R, Maier T, Wenzel T, Kostrzewa M, Bierbaum G, Hoerauf A, Sahl HG (2009) Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J Clin Microbiol 47:2912–2917
55. Kitch TT, Jacobs MR, McGinnis MR, Appelbaum PC (1996) Ability of RapID Yeast Plus System to identify 304 clinically significant yeasts within 5 hours. J Clin Microbiol 34:1069–1071
56. Espinel-Ingroff A, Stockman L, Roberts G, Pincus D, Pollack J, Marler J (1998) Comparison of RapID yeast plus system with API 20C system for identification of common, new, and emerging yeast pathogens. J Clin Microbiol 36:883–886
57. Heelan JS, Sotomayor E, Coon K, D'Arezzo JB (1998) Comparison of the rapid yeast plus panel with the API20C yeast system for identification of clinically significant isolates of Candida species. J Clin Microbiol 36: 1443–1445
58. Sanguinetti M, Porta R, Sali M, La Sorda M, Pecorini G, Fadda G, Posteraro B (2007) Evaluation of VITEK 2 and RapID Yeast Plus systems for yeast species identification: experience at a large clinical microbiology laboratory. J Clin Microbiol 45:1343–1346
59. Bowman PI, Ahearn DG (1975) Evaluation of the Uni-Yeast-Tek kit for the identification of medically important yeasts. J Clin Microbiol 2:354–358
60. Bowman PI, Ahearn DG (1976) Evaluation of commercial systems for the identification of clinical yeast isolates. J Clin Microbiol 4:49–53
61. Cooper BH, Johnson JB, Thaxton ES (1978) Clinical evaluation of the Uni-Yeast-Tek system for rapid presumptive identification of medically important yeasts. J Clin Microbiol 7:349–355
62. Salkin IF, Land GA, Hurd NJ, Goldson PR, McGinnis MR (1987) Evaluation of YeastIdent and Uni-Yeast-Tek yeast identification systems. J Clin Microbiol 25: 624–627
63. McGinnis MR, Molina TC, Pierson DL, Mishra SK (1996) Evaluation of the Biolog MicroStation system for yeast identification. J Med Vet Mycol 34:349–352
64. Land GA, Salkin IF, El-Zaatari M, McGinnis MR, Hashem G (1991) Evaluation of the Baxter-MicroScan 4-hour enzyme-based yeast identification system. J Clin Microbiol 29: 718–722
65. St Germain G, Beauchesne D (1991) Evaluation of the MicroScan rapid yeast identification panel. J Clin Microbiol 29:2296–2299
66. Riddle DL, Giger O, Miller L, Hall GS, Woods GL (1994) Clinical comparison of the Baxter MicroScan yeast identification panel and the Vitek Yeast Biochemical Card. Am J Clin Pathol 101:438–442
67. Crist AE Jr, Johnson LM, Burke PJ (1996) Evaluation of the Microbial Identification System for identification of clinically isolated yeasts. J Clin Microbiol 34:2408–2410
68. Kellogg JA, Bankert DA, Chaturvedi V (1998) Limitations of the current Microbial Identification System for identification of clinical yeast isolates. J Clin Microbiol 36: 1197–1200
69. Kellogg JA, Bankert DA, Chaturvedi V (1999) Variation in Microbial Identification System accuracy for yeast identification depending on commercial source of Sabouraud dextrose agar. J Clin Microbiol 37: 2080–2083
70. Oblack DL, Rhodes JC, Martin WJ (1981) Clinical evaluation of the AutoMicrobic system Yeast Biochemical Card for rapid identification of medically important yeasts. J Clin Microbiol 13:351–355
71. Land G, Stotler R, Land K, Staneck J (1984) Update and evaluation of the AutoMicrobic yeast identification system. J Clin Microbiol 20:649–652
72. el-Zaatari M, Pasarell L, McGinnis MR, Buckner J, Land GA, Salkin IF (1990) Evaluation of the updated Vitek yeast identification data base. J Clin Microbiol 28: 1938–1941
73. Fenn JP, Segal H, Barland B, Denton D, Whisenant J, Chun H, Christofferson K, Hamilton L, Carroll K (1994) Comparison of updated Vitek Yeast Biochemical Card and API 20C yeast identification systems. J Clin Microbiol 32:1184–1187
74. Dooley DP, Beckius ML, Jeffrey BS (1994) Misidentification of clinical yeast isolates by using the updated Vitek Yeast Biochemical Card. J Clin Microbiol 32:2889–2892
75. Aubertine CL, Rivera M, Rohan SM, Larone DH (2006) Comparative study of the new colorimetric VITEK 2 yeast identification card fluorometric card and of CHROMagar

Candida as a source medium with the new card. J Clin Microbiol 44:227–228

76. Loïez C, Wallet F, Sendid B, Courcol RJ (2006) Evaluation of VITEK 2 colorimetric cards versus fluorimetric cards for identification of yeasts. Diagn Microbiol Infect Dis 56: 455–457
77. Hata DJ, Hall L, Fothergill AW, Larone DH, Wengenack NL (2007) Multicenter evaluation of the new VITEK 2 advanced colorimetric yeast identification versus the older card. J Clin Microbiol 45:1087–1092
78. Valenza G, Strasen J, Schafer F, Frosch M, Kurzai O, Abele-Horn M (2008) Evaluation of new colorimetric Vitek 2 yeast identification card by use of different source media. J Clin Microbiol 46:3784–3787
79. Vijgen S, Nys S, Naesens R, Magerman K, Boel A, Cartuyvels R (2011) Comparison of Vitek identification and antifungal susceptibility testing methods to DNA sequencing and Sensititre YeastOne antifungal testing. Med Mycol 49:107–110
80. Hasyn JJ, Buckley HR (1982) Evaluation of the AutoMicrobic system for identification of yeasts. J Clin Microbiol 16:901–904
81. Pfaller MA, Preston T, Bale M, Koontz FP, Body BA (1988) Comparison of the Quantum II, API Yeast Ident, and AutoMicrobic systems for identification of clinical yeast isolates. J Clin Microbiol 26:2054–2058
82. Quiles-Melero I, García-Rodriguez J, Romero-Gómez MP, Gómez-Sánchez P, Mingorance J (2011) Rapid identification of yeasts from positive blood culture bottles by pyrosequencing. Eur J Clin Microbiol Infect Dis 30:21–24
83. Fukazawa Y (1989) Antigenic structure of *Candida albicans*. Immunochemical basis of the serologic specificity of the mannans in yeasts. Immunol Ser 47:37–62
84. Bille J (2010) New nonculture-based methods for the diagnosis of invasive candidiasis. Curr Opin Crit Care 16:460–464
85. Oliveri S, Trovato L, Betta P, Romeo MG, Nicoletti G (2008) Experience with the Platelia Candida ELISA for the diagnosis of invasive candidosis in neonatal patients. Clin Microbiol Infect 14:391–393
86. Platelia™ Candida AB/AC/AK (2009) Bio-Rad instructions for use. <http://www3.bio-rad.com/cmc_upload/Literature/inserts/62799_881046_GB.pdf> Accessed 01 May 2011
87. Alam FF, Mustafa AS, Khan ZU (2007) Comparative evaluation of (1, 3)-β-D-glucan, mannan and anti-mannan antibodies, and Candida species-specific snPCR in patients with candidemia. BMC Infect Dis 7:103–111
88. Arendrup MC, Bergmann OJ, Larsson L, Nielsen HV, Jarløv JO, Christensson B (2010) Detection of candidaemia in patients with and without underlying hematological disease. Clin Microbiol Infect 16:855–862
89. Sendid B, Caillot D, Baccouch-Humbert B, Klingspor L, Grandjean M, Bonnin A, Poulain D (2003) Contribution of the Platelia Candida-specific antibody and antigen tests to early diagnosis of systemic *Candida tropicalis* infection in neutropenic adults. J Clin Microbiol 41:4551–4558
90. Mikulska M, Calandra T, Sanguinetti M, Poulain D, Viscoli C, Third European Conference on Infections in Leukemia Group (2010) The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. Crit Care 14:R222
91. Downey LC, Smith PB, Benjamin DK, Cohen-Wolkowiez M (2010) Recent advances in the detection of neonatal Candidiasis. Curr Fungal Infect Rep 4:17–22
92. Lunel FM, Mennink-Kersten MA, Ruegebrink D, van der Lee HA, Donnelly JP, Blijlevens NM, Verweij PE (2009) Value of Candida serum markers in patients with invasive candidiasis after myeloablative chemotherapy. Diagn Microbiol Infect Dis 64:408–15
93. Wulf GG, Conradi I, Rüchel R (2004) Diagnostic value of antibody detection against *Candida* germ tube antigens in a patient with hepatosplenic candidosis. Mycoses 47(Suppl 1):11–14
94. Mitsutake K, Miyazaki T, Tashiro T, Yamamoto Y, Kakeya H, Otsubo T, Kawamura S, Hossain MA, Noda T, Hirakata Y, Kohno S (1996) Enolase antigen, mannan antigen, Cand-Tec antigen, and β-glucan in patients with candidemia. J Clin Microbiol 34: 1918–1921
95. Mori T, Matsumura M (1999) Clinical evaluation of diagnostic methods using plasma and/or serum for three mycoses: aspergillosis, candidosis, and pneumocystosis. Nippon Ishinkin Gakkai Zasshi 40:223–230
96. Bär W, Hecker H (2002) Diagnosis of systemic *Candida* infections in patients of the intensive care unit. Significance of serum antigens and antibodies. Mycoses 45:22–28
97. Pazos C, Moragues MD, Quindós G, Pontón J, Palacio A (2006) Diagnostic potential of (1→3)- β-D-glucan and anti-*Candida albicans* germ tube antibodies for the diagnosis and therapeutic monitoring of invasive candidiasis in neutropenic adult patients. Rev Iberoam Micol 23:209–215

98. Moragues MD, Ortiz N, Iruretagoyena JR, García-Ruiz JC, Amutio E, Rojas A, Mendoza J, Quindós G, Pontón-San Emeterio J (2004) Evaluation of a new commercial test (*Candida albicans* IFA IgG) for the serodiagnosis of invasive candidiasis. Enferm Infecc Microbiol Clin 22:83–88
99. Pemán J, Zaragoza R, Quindós G, Alkorta M, Cuétara MS, Camarena JJ, Ramírez P, Giménez MJ, Martín-Mazuelos E, Linares-Sicilia MJ, Pontón J, The study group Candida albicans Germ Tube Antibody Detection in Critically Ill Patients (CAGTAUCI) (2011) Clinical factors associated with a *Candida albicans* germ tube antibody positive test in intensive care unit patients. BMC Infect Dis 11:60–65
100. Zaragoza R, Pemán J, Quindós G, Iruretagoyena JR, Cuétara MS, Ramírez P, Gómez MD, Camarena JJ, Viudes A, Pontón J, On behalf of the study group Candida albicans Germ Tube Antibody Detection in Critically Ill Patients (CAGTAUCI) (2009) Clinical significance of the detection of *Candida albicans* germ tube-specific antibodies in critically ill patients. Clin Microbiol Infect 15:592–595
101. Temstet A, Roux P, Poirot JL, Ronin O, Dromer F (1992) Evaluation of a monoclonal antibody-based latex agglutination test for diagnosis of cryptococcosis: comparison with two tests using polyclonal antibodies. J Clin Microbiol 30:2544–2550
102. Tanner DC, Weinstein MP, Fedorciw B, Joho KL, Thorpe JJ, Reller L (1994) Comparison of commercial kits for detection of cryptococcal antigen. J Clin Microbiol 32:1680–1684
103. Blevins LB, Fenn J, Segal H, Newcomb-Gayman P, Carroll KC (1995) False-positive cryptococcal antigen latex agglutination caused by disinfectants and soaps. J Clin Microbiol 33:1674–1675
104. Wilson DA, Sholtis M, Parshall S, Hall GS, Procop GW (2011) False-positive cryptococcal antigen test associated with use of BBL Port-A-Cul transport vials. J Clin Microbiol 49:702–703
105. Heelan JS, Corpus L, Kessimian N (1991) False-positive reactions in the latex agglutination test for *Cryptococcus neoformans* antigen. J Clin Microbiol 29:1260–1261
106. McFadden DC, Zaragoza O, Casadevall A (2004) Immunoreactivity of cryptococcal antigen is not stable under prolonged incubations in human serum. J Clin Microbiol 42:2786–2788
107. Saha DC, Xess I, Biswas A, Bhowmik DM, Padma MV (2009) Detection of *Cryptococcus* by conventional, serological and molecular methods. J Med Microbiol 58:1098–1105
108. Jaye DL, Waites KB, Parker B, Bragg SL, Moser SA (1998) Comparison of two rapid latex agglutination tests for detection of cryptococcal capsular polysaccharide. Am J Clin Pathol 109:634–641
109. Babady NE, Bestrom JE, Jespersen DJ, Jones MF, Beito EM, Binnicker MJ, Wengenack NL (2009) Evaluation of three commercial latex agglutination kits and a commercial enzyme immunoassay for the detection of cryptococcal antigen. Med Mycol 47:336–338
110. Mennink-Kersten MA, Donnelly JP, Verweij PE (2004) Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. Lancet Infect Dis 4:349–57
111. Verdaguer V, Walsh TJ, Hope W, Cortez KJ (2007) Galactomannan antigen detection in the diagnosis of invasive aspergillosis. Expert Rev Mol Diagn 7:21–32
112. Thornton CR (2010) Detection of invasive aspergillosis. Adv Appl Microbiol 70:187–216
113. Chen SC, Kontoyiannis DP (2010) New molecular and surrogate biomarker-based tests in the diagnosis of bacterial and fungal infection in febrile neutropenic patients. Curr Opin Infect Dis 23:567–77
114. Klont RR, Mennink-Kersten MA, Verweij PE (2004) Utility of *Aspergillus* antigen detection in specimens other than serum specimens. Clin Infect Dis 39:1467–1474
115. Husain S, Clancy CJ, Nguyen MH, Swartzentruber S, Leather H, LeMonte AM, Durkin MM, Knox KS, Hage CA, Bentsen C, Singh N, Wingard JR, Wheat LJ (2008) Performance characteristics of the Platelia *Aspergillus* enzyme immunoassay for detection of Aspergillus galactomannan antigen in bronchoalveolar lavage fluid. Clin Vaccine Immunol 15:1760–1763
116. Hage CA, Knox KS, Davis TE, Wheat LJ (2011) Antigen detection in bronchoalveolar lavage fluid for diagnosis of fungal pneumonia. Curr Opin Pulm Med 17:167–171
117. Maertens J, Maertens V, Theunissen K, Meersseman W, Meersseman P, Meers S, Verbeken E, Verhoef G, Van Eldere J, Lagrou K (2009) Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. Clin Infect Dis 49: 1688–1693
118. Hsu LY, Ding Y, Phua J, Koh LP, Chan DS, Khoo KL, Tambyah PA (2010) Galactomannan testing of bronchoalveolar lavage fluid is use-

ful for diagnosis of invasive pulmonary aspergillosis in hematology patients. BMC Infect Dis 10:44–49

119. Nguyen MH, Jaber R, Leather HL, Wingard JR, Staley B, Wheat LJ, Cline CL, Baz M, Rand KH, Clancy CJ (2007) Use of bronchoalveolar lavage to detect galactomannan for diagnosis of pulmonary aspergillosis among nonimmunocompromised hosts. J Clin Microbiol 45:2787–2792
120. Steinbach WJ (2010) Invasive aspergillosis in pediatric patients. Curr Med Res Opin 26: 1779–1787
121. Verweij PE, Stynen D, Rijs AJ, de Pauw BE, Hoogkamp-Korstanje JA, Meis JF (1995) Sandwich enzyme-linked immunosorbent assay compared with Pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromised patients. J Clin Microbiol 33:1912–1914
122. Machetti M, Feasi M, Mordini N, Van Lint MT, Bacigalupo A, Latgé JP, Sarfati J, Viscoli C (1998) Comparison of an enzyme immunoassay and a latex agglutination system for the diagnosis of invasive aspergillosis in bone marrow transplant recipients. Bone Marrow Transplant 21:917–921
123. Fricker-Hidalgo H, Coltey B, Llerena C, Renversez JC, Grillot R, Pin I, Pelloux H, Pinel C (2010) Recombinant allergens combined with biological markers in the diagnosis of allergic bronchopulmonary aspergillosis in cystic fibrosis patients. Clin Vaccine Immunol 17:1330–1336
124. Tsagarakis N, Kentrou N, Margariti V, Malgarinou A, Maurea S, Tsagarakis I, Anastasakou E (2009) Anti-aspergillus antibodies and galactomannan antigen detection for serodiagnosis of aspergillosis. ECCMID Meeting. Abstract number: P905
125. Stone BA, Clarke AE (1992) Chemistry and biology of (1→3)-β-glucans. La Trobe University Press, Melbourne, Australia
126. Mennink-Kersten MA, Ruegebrink D, Verweij PE (2008) *Pseudomonas aeruginosa* as a cause of 1,3-β-D-glucan assay reactivity. Clin Infect Dis 46:1930–1931
127. Suzuki T, Ohno N, Saito K, Yadomae T (1992) Activation of the complement system by (1→3)-beta-D-glucans having different degrees of branching and different ultrastructures. J Pharmacobiodyn 15:277–285
128. Ohki M, Nakamura T, Morita T, Iwanaga S (1980) A new endotoxin sensitive factor associated with hemolymph coagulation system of horseshoe crab (Limulidae). FEBS Lett 120:217–20
129. Marty FM, Koo S (2009) Role of (1→3)-β-D-glucan in the diagnosis of invasive aspergillosis. Med Mycol 47(Suppl 1):S233–240
130. Maertens J, Meersseman W, Van Bleyenbergh P (2009) New therapies for fungal pneumonia. Curr Opin Infect Dis 22:183–190
131. Torelli R, Posteraro B, De Carolis E, De Pascale G, Caira M, Bello G, Antonelli M, Pagano L, Fadda G, Sanguinetti M (2011) Comparison of MycAssay Aspergillus test with galactomannan detection in bronchoalveolar lavage (BAL) fluid samples of hematological and ICU patients. Poster 1941. 21st European society of clinical microbiology and infectious diseases meeting, Milan, Italy
132. Lehmann LE, Alvarez J, Hunfeld KP, Goglio A, Kost GJ, Louie RF, Raglio A, Regueiro BJ, Wissing H, Stüber F (2009) Potential clinical utility of polymerase chain reaction in microbiological testing for sepsis. Crit Care Med 37:3085–3090
133. von Lilienfeld-Toal M, Lehmann LE, Raadts AD, Hahn-Ast C, Orlopp KS, Marklein G, Purr I, Cook G, Hoeft A, Glasmacher A, Stüber F (2009) Utility of a commercially available multiplex real-time PCR assay to detect bacterial and fungal pathogens in febrile neutropenia. J Clin Microbiol 47:2405–2410
134. Mancini N, Clerici D, Diotti R, Perotti M, Ghidoli N, De Marco D, Pizzorno B, Emrich T, Burioni R, Ciceri F, Clementi M (2008) Molecular diagnosis of sepsis in neutropenic patients with hematological malignancies. J Med Microbiol 57:601–604
135. Lamoth F, Jaton K, Prod'hom G, Senn L, Bille J, Calandra T, Marchetti O (2010) Multiplex blood PCR in combination with blood cultures for improvement of microbiological documentation of infection in febrile neutropenia. J Clin Microbiol 48:3510–3516
136. Dierkes C, Ehrenstein B, Siebig S, Linde HJ, Reischl U, Salzberger B (2009) Clinical impact of a commercially available multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis. BMC Infect Dis 9:126–132
137. Louie RF, Tang Z, Albertson TE, Cohen S, Tran NK, Kost GJ (2008) Multiplex polymerase chain reaction detection enhancement of bacteremia and fungemia. Crit Care Med 36:1487–92
138. Westh H, Lisby G, Breysse F, Böddinghaus B, Chomarat M, Gant V, Goglio A, Raglio A, Schuster H, Stuber F, Wissing H, Hoeft A (2009) Multiplex real-time PCR and blood culture for identification of bloodstream

pathogens in patients with suspected sepsis. Clin Microbiol Infect 15:544–51

139. Maubon D, Hamidfar-Roy R, Courby S, Vesin A, Maurin M, Pavese P, Ravanel N, Bulabois CE, Brion JP, Pelloux H, Timsit JF (2010) Therapeutic impact and diagnostic performance of multiplex PCR in patients with malignancies and suspected sepsis. J Infect 61:335–42
140. Yanagihara K, Kitagawa Y, Tomonaga M, Tsukasaki K, Kohno S, Seki M, Sugimoto H, Shimazu T, Tasaki O, Matsushima A, Ikeda Y, Okamoto S, Aikawa N, Hori S, Obara H, Ishizaka A, Hasegawa N, Takeda J, Kamihira S, Sugahara K, Asari S, Murata M, Kobayashi Y, Ginba H, Sumiyama Y, Kitajima M (2010) Evaluation of pathogen detection from clinical samples by real-time polymerase chain reaction using a sepsis pathogen DNA detection kit. Crit Care 14:R159
141. Sachse S, Straube E, Lehmann M, Bauer M, Rußwurm S, Schmidt K-H (2009) Truncated hCGBP improves NAT-based detection of bacterial species in human samples. J Clin Microbiol 47:1050–1057
142. Kortgen A, Bloos F, Sachse S, Lehmann M, Straube E, Reinhart K, Bauer M (2009) Clinical relevance of fungal DNAemia detected by multiplex PCR in septic patients. 4th international congress of the german sepsis society, Weimar, Germany.
143. An assessment of the potential clinical utility of a new multiplex-PCR assay (Vyoo®) in the management of ICU patients with sepsis. http://apps.who.int/trialsearch/Trial.aspx?TrialID=DRKS00000612 Accessed 19 July 2011
144. Wellinghausen N, Kochem AJ, Disqué C, Mühl H, Gebert S, Winter J, Matten J, Sakka SG (2009) Diagnosis of bacteremia in whole-blood samples by use of a commercial universal 16S rRNA gene-based PCR and sequence analysis. J Clin Microbiol 47:2759–65
145. Westh H, Lisby G, Breysse F, Böddinghaus B, Chomarat M, Gant V, Goglio A, Raglio A, Schuster H, Stuber F, Wissing H, Hoeft A (2009) Multiplex real-time PCR and blood culture for identification of bloodstream pathogens in patients with suspected sepsis. Clin Microbiol Infect 15:544–51
146. Maubon D, Hamidfar-Roy R, Courby S, Vesin A, Maurin M, Pavese P, Ravanel N, Bulabois CE, Brion JP, Pelloux H, Timsit JF (2010) Therapeutic impact and diagnostic performance of multiplex PCR in patients with malignancies and suspected sepsis. J Infect 61:335–42
147. Hauser PM, Bille J, Lass-Flörl C, Geltner C, Feldmesser M, Levi M, Patel H, Muggia V, Alexander B, Hughes M, Follett SA, Cui X, Leung F, Morgan G, Moody A, Perlin DS, Denning DW (2011) Multicenter, prospective clinical evaluation of respiratory samples from subjects at risk for Pneumocystis jirovecii infection by use of a commercial real-time PCR assay. J Clin Microbiol 49:1872–8
148. White PL, Perry MD, Loeffler J, Melchers W, Klingspor L, Bretagne S, McCulloch E, Cuenca-Estrella M, Finnstrom N, Donnelly JP, Barnes RA, European Aspergillus PCR Initiative (2010) Critical stages of extracting DNA from Aspergillus fumigatus in whole-blood specimens. J Clin Microbiol. 48:3753–3755
149. Klingspor L, Loeffler J (2009) Aspergillus PCR formidable challenges and progress. Med Mycol 47(Suppl 1):S241–7
150. Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP (2009) Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. Lancet Infect Dis 9:89–96
151. Baldwin CD, Howe GB, Sampath R, Blyn LB, Matthews H, Harpin V, Hall TA, Drader JJ, Hofstadler SA, Eshoo MW, Rudnick K, Studarus K, Moore D, Abbott S, Janda JM, Whitehouse CA (2009) Usefulness of multilocus polymerase chain reaction followed by electrospray ionization mass spectrometry to identify a diverse panel of bacterial isolates. Diagn Microbiol Infect Dis 63:403–408