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Review

Immunological methods for the detection of structural components and metabolites of bacteria and fungi in blood

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Summary — This review compares the clinical usefulness of immunological methods for the detection of structural components and metabolites of bacteria and fungi. Bacterial antigens (especially those of *Mycobacterium*, *Neisseria*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Escherichia coli*, *Salmonella*, *Chlamydia*, and *Brucella*) are best detected by enzyme-linked immunosorbent assay. Methods involving antibodies are more expensive and are effective only when performed in series. The detection of antibodies that recognize *S aureus* teichoic acid merely confirms the presence of a metastatic complication. Tissue invasion by *Candida albicans* is not yet reliably detectable by the presence of a specific antigen. Simple, but not completely reliable methods are available such as the latex test for mannans detection and/or agglutination with liposomes for detecting 48-kDa cytoplasmic protein antigen and an assay for detecting enolase antigen. A latex agglutination test has also been developed for the mannans antigen of *Aspergillus* and for *Cryptococcus neoformans* capsular polysaccharide ; the latter test is more cost effective. The sensitivity of both tests is improved by serial assays. A negative finding with hemmagglutination-based antibody tests rules out *C albicans* infection, and titers of 1/640 or higher have been associated with disseminated infection by *Aspergillus*. Concentrations of *C albicans* blastopore antigen antibodies higher than 400 IU/ml can be seen in disseminated candidiasis. High concentrations of endotoxin are indicative of imminent septic shock. Some biological indicators (C reactive protein, angiotensin converting enzyme, fibronectin, elastase–α_1-antitrypsin complex, tumor necrosis factor and interleukin-6) have been used to rule out a bacterial cause of fever. Such methods, when used in combination, appear to be useful only in excluding a microbial cause of the disease.

antibody / antigen / diagnosis / septicemia

Résumé — Méthodes immunologiques pour la détection des composants structuraux et des métabolites des bactéries et des champignons dans le sang. *Cette revue compare l'utilité clinique des méthodes immunologiques de détection des composants structuraux et des métabolites des bactéries et des champignons. Les antigènes bactériens (en particulier ceux de* Mycobacterium, Neisseria, Staphylocccus aureus, Yersinia enterocolitica, Escherichia coli, Salmonella, Chlamydia *et* Brucella*) sont très bien détectés par la méthode Elisa. Les méthodes utilisant des anticorps sont plus coûteuses, et ne sont efficaces que lorsqu'elles sont réalisées en série. La détection des anticorps qui reconnaissent l'acide teichoïque de* S aureus *confirme simplement la présence d'une complication métastatique. L'invasion tissulaire par* Candida albicans *n'est pas encore facilement détectable par la présence d'un antigène spécifique. Des méthodes simples mais pas complètement fiables telles que le test au latex pour la détection du mannane, l'agglutination avec des liposomes sont disponibles pour détecter un antigène protéique cytoplasmique de 48 kDa ainsi qu'un dosage pour détecter l'antigène enolase. Un test d'agglutination du latex a également été développé pour l'antigène mannane de* Aspergillus *et pour les polysaccharides capsulaires de* Cryptococcus neoformans *; ce dernier test a la meilleure efficacité par rapport à son coût. La sensibilité des deux tests est améliorée par des dosages en série. Les méthodes utilisant des anticorps sont plus efficaces lorsqu'elles sont réalisées en série. Un résultat négatif avec des tests basés sur l'hémagglutination élimine l'infection par* C Albicans *et des titres de 1/640 ou plus ont été retrouvés, associés à une infection disséminante par* Aspergillus. *Les concentrations d'anticorps dirigés contre des antigènes des blastopores de* C albicans *plus élevés que 400 IU/ml ont été trouvés dans des candidoses disséminées. Des concentrations élevées d'endotoxines indiquent un choc septique imminent. Certains indicateurs biologiques (C réactive protéine, enzyme de conversion de l'angiotensine, fibronectine, complexe élastase-α_1-antitrypsine, facteur de nécrose tumorale et interleukine-6) ont été utilisés pour éliminer la cause bactérienne d'une fièvre. Ces méthodes, lorsqu'elles sont combinées, ne semblent être utiles que pour exclure une cause microbienne de maladie.*

anticorps / antigène / diagnostic / septicémie

Introduction

Many culture-dependent and culture-independent methods have been developed to improve the speed and sensitivity of septicemia detection. Culture-dependent methods are hampered by the disadvantages of slow bacterial growth. Culture-independent methods are faster, but may lack specificity or sensitivity [1].

The presence of antigens from microorganisms, circulating antibodies against these molecules, biological indicators and microbial metabolites can be detected in biological fluids. Such studies are particularly useful when the culture is negative, or in bacteremia caused by slow-growing microorganisms. The detection of a given antigen provides an earlier and more precise diagnosis than the detection of antibodies, since the presence of the former is independent of the host's immune response. Monitoring the antigen levels has the additional advantage of providing feedback on the response to treatment and more accurately reflects the patient's improvement. Because antigens may form immunocomplexes,

they may need to be dissociated with physical or chemical methods before detection is possible. Antigens may also go undetected because they appear only transiently in circulation, or because of the inadequate sensitivity of the detection method used.

Many studies have compared the usefulness of modern and conventional systems. Because the conclusions reached have often been contradictory, the present review will attempt to clarify the relative merits of older and newer approaches to investigate bacterial and fungal components and metabolites in peripheral blood.

Bacteria

Detection of circulating antigens

Latex agglutination and co-agglutination

The main problem with latex agglutination and co-agglutination is their low sensitivity leading some authors to question their use in routine hospital practice despite their high specificity. Our results have varied depending on the microorganism, supplier and sample (serum, concentrated or dilute urine). Because carriers may test positive, a positive result does not confirm the diagnosis nor does a negative result rule out a given microorganism.

Although latex agglutination is not effective in detecting antigens of *Streptococcus pneumoniae* [2, 3] or *Streptococcus agalactiae* [4, 5], it is useful for D group streptococci, *Escherichia coli* K1, *Neisseria meningitidis, Staphylococcus aureus* and *Haemophilus influenzae* type B [2, 3, 6, 7].

Counterimmunoelectrophoresis

In comparison with latex methods, counterimmunoelectrophoresis is less sensitive in detecting *H influenzae, N meningitidis, S agalactiae and S pneumoniae*. Samples should be free of hemoglobin, which can interfere with the results. If the antigen is present at a high concentration, it may cross-react with other antibodies or give rise to the prozone phenomenon. The productivity of the system can be enhanced by concentrating the antigen with polyacrylamide gel absorption, however, this method is more painstaking than the latex test, and no purified antigens for quality control are available.

Immunoassay

Immunological methods can be used to detect antigens of *Mycobacterium tuberculosis, Mycobacterium avium-intracellulare* and *Mycobacterium gordonae* (radio-immunoassay, RIA; enzyme-linked immunosorbent assay, Elisa) [8, 9], *Neisseria gonorhoeae* (Elisa), *S aureus* (Elisa), *Yersinia enterocolitica* (Elisa), *Chlamydia* spp (Elisa), *Salmonella* spp (Elisa) [10], *Brucella* spp (Elisa) and *E coli* (RIA, Elisa). Studies with *S pneumoniae* have thus far failed to demonstrate the effectiveness of immunological techniques for the detection of this microorganism [2, 11].

Antibody detection

The detection of specific antibodies in the diagnosis of sepsis is beset by three major problems: 1) diagnosis takes considerable time, a requirement that ceases to be a disadvantage in the case of slow-growing microorganisms; 2) false positives can occur because of ubiquitous microorganisms; and 3) false negatives can occur because the amount of antibody produced is sometimes insufficient for diagnosis.

S aureus

Anti-teichoic acid antibody methods are based on the detection of anti-IgG/IgM antibodies by double diffusion, counterimmunoelectrophoresis (the least sensitive methods), Elisa and RIA [12]. Antibodies are present at 10–15 days in complicated sepsis; titers decreasing during the following 2–4 weeks and becoming negative after 2–5 months. Titers greater than 1/4 (precipitation reactions) indicate complications (endocarditis, multiple metastatic abscesses, hematogenic osteomyelitis) [13]. These methods have been shown useful in follow-up and prognosis during prolonged treatment. The main problems are cross-reactions with coagulase-negative staphylococci [14] and low antibody titers in the general population. A negative result alone, without clinical confirmation, does not rule out severe infection [13, 15, 16]. The results obtained by different authors are contradictory [17]. In summary, tests based on anti-teichoic acid antibody detection are of use to confirm the suspected diagnosis but lack the necessary weight in themselves to determine the definitive diagnosis.

Other antibodies have thus far proven to be of limited value. Antibodies against protein A are of low sensitivity and specificity in detecting infectious endocarditis [18]. With antibodies against α-hemolysin, values above 10 IU/ml are associated with complications, but specificity is low [19]. The main disadvantage of antibodies against peptidoglycan is that they cross-react with other microorganisms [18].

Fungi

Antigen detection

Candida albicans

A number of *C albicans* antigens can reach the bloodstream in subjects with invasive candidiasis. The molecules investigated to date include a thermostable 48-kDa protein antigen, a thermostable mannan antigen, a thermolabile antigen believed to be a glycoprotein, and a series of metabolites.

Candida antigens are difficult to study for several reasons. They often form immunocomplexes, and may appear in circulation only briefly when the lesion is localized [20]. Sera must be previously treated with physical (heat) or chemical methods (acid or alkaline hydrolysis) to dissociate the antigen from the immunocomplex. Mannans seem to be more useful than cytoplasmic antigens, since dissociation from immunocomplexes is easier with a stable polysaccharide. False negatives can result from adsorption of the antigen to the walls of the tube; moreover, this antigen may not accurately reflect invasiveness.

Because the concentration of antigens in serum is very low (in the nanogram per milliliter range), they are not readily detected by many conventional immunological methods [21]. Most problems with availble tests are related with their lack of sensitivity rather than their lack of specificity [20], many tests requiring days to yield a positive result. It is unclear whether the serotype of *C albicans* influences the sensitivity of the test [22]. The presence of rheumatoid factor may lead to false positives, a problem which needs to be solved by the suppliers of the kit [21].

Mannan antigen. The mannan antigen can reach concentrations of 1–10 ng/ml in the bloodstream. Cross-reactions have been shown between *Saccharomyces cerevisae, C albicans, C tropicalis, C guillermondii,* and *C pseudotropicalis* mannan [23].

Mannans have been detected in 47–100% of patients with invasive candidiasis, depending on the series and on the techniques used [24]. This antigen is not detectable in healthy subjects or in patients colonized or having superficial infection, patients with bacterial infection or other mycoses, although one study reported false positive results in 9% of the patients with superficial mycosis [23].

RIA and Elisa are the most sensitive and reliable methods for detecting mannans, although they are also the most painstaking and complex methods for the rapid analysis of individual samples in hospital laboratory practice [21, 23, 25]. Agglutination tests are straightforward, and some are commercially available as kits. However, the transient nature and low levels of antigenemia make frequent, sequential use necessary [26] to achieve a clinically useful degree of sensitivity when disseminated or catheter-related candidiasis is suspected. The evolution of mannan titers can reflect the patient's clinical status, and indicate the most appropriate management and treatment. High concentrations of antigen suggest invasive rather than transitory, catheter-related fungemia [27]. Two commercial tests based on latex particle agglutination are currently available: i) Pastorex (Pasteur Laboratories) uses latex particles coated with antimannan IgM, which cross-reacts with several species of *Candida,* The particles react with the polysaccharide antigen of candida through an agglutination which is visible with the naked eye. This test provides quantitative data for positive results. Although a positive test is highly specific, a negative result does not rule out the disease due to the moderate sensitivity of the test. The limit of detection is 2.5 ng/ml. This method requires previous heat treatment to dissociate the antigen from immunocomplexes ; and ii) LA-Candida (Immuno-Mycologics Laboratories) uses a system of proteolytic enzymes to dissociate immunocomplexes, destroy antibodies, and rheumatoid factor. The limits of detection are between 5 ng/ml and 5 µg/ml, and the reaction is stopped by the addition of an enzyme inhibitor. This method does not appear to be very useful in the detection of candidemia [28].

The sensitivity of the methods described thus far is, in decreasing order: Elisa (54–100%), RIA (51%), hemagglutination (HAP) (44%), latex agglutination (41%). False positives are most frequent with HAP (3,5%) and Elisa (3%) [21] .

Cytoplasmic antigen or enolase antigen. A commercial system that detects cytoplasmic antigens is available. Directigen (Becton-Dickinson Laboratories) is a colorimetric immunoassay that uses liposome-bound reagents to detect 48-kDa cytoplasmic candida antigen on the membrane. Monoclonal antibody specific for this antigen is immobilized on a membrane incorporated into a test device. Antigen in sera is bound to the immobilized antibody as the specimen passes through the membrane. Polyclonal rabbit antibodies directed against the antigen are bound to the trapped antigen to enhance the number of reactive sites. Liposome detector particles containing a red dye and coated with goat anti-rabbit antibodies are then bound, resulting in the formation of a colored image on the membrane for a positive. Sensitivity of the assay in detecting antigen in serum seeded with known quantities of purified antigen is 0.5 to 1 ng/ml. The test is straightforward, effective (67% sensitivity) and specific, yielding negative results in subjects colonized by *C albicans* [29]. In a prospective study, the expression of antigen in the serum of patients with cancer who were at high risk for deeply invasive candidiasis was evaluated. Use of these markers appears valid; antigen positivity was not associated with either colonization or superficial infection in tissue from immunocompromised or immunocompetent patients. The antigen marker becomes more sensitive as multiple samples are tested. It also may be the earliest marker to identify invasive candidiasis and probably is present before true fungemia [31]. Of the experimental methods that have been tested thus far, Elisa has given consistently positive results in invasive candidiasis. A dot-immunoassay is also being developed [30].

Glycoprotein antigen. Cand-Tec (Ramco Laboratories) is a latex agglutination test that detects circulating thermolabile glycoproteins of *C albicans*. The results obtained to date are variable and inconclusive, hence the method shoud not be considered completely reliable. The titer recommended by the manufacturer as the criterion for dissemination (1/4) is of little diagnostic use, however, a threshold of 1/8 is more informative, and may be more useful in distinguishing between colonization and systemic infection [25, 28].

Aspergillus spp

The galactomannan antigen of *Aspergillus* spp is detectable in serum. Because it may form immunocomplexes with antibodies, previous dissociation with heat or dialysis and freeze-drying is recommended. Cross-reactions have been observed with galactomannans of *Aspergillus fumigatus, A flavus, A versicolor, A niger and A terreus.*

In addition, *C albicans* and *A fumigatus* may share cell wall antigens.

Several methods have been proposed, including counterimmunoelectrophoreris, Elisa, RIA and latex agglutination [21, 32]. Despite their high specificity, sensitivity is limited in part by low circulating levels of antigen (ng/ml range).

The Pastorex system uses thermal treatment to dissociate immunocomplexes, followed by latex agglutination with IgM antibodies to detect galactomannan, and provides titers of use in orienting the diagnosis. The limit of sensitivity is 15 ng/ml ; a negative result does not necessarily rule out fungal infection, due to the transitory nature of antigenemia. False negatives can occur due to the adsorption of galactomannan to the plastic walls of the reaction vessels when non-fresh serum is used, and false positives can be caused by contaminated samples. One study claimed to achieve a high rate of sensitivity, however, these figures are questionable [33].

The clinical effectiveness of these systems, in detecting *Candida* and *Aspergillus* antigens for the diagnosis of invasive diseases has yet to be determined

Cryptococcus neoformans

The capsular antigen of *C neoformans* is present in the blood of patients with lower respiratory tract infection, meningitis (50–70% of all patients) and fungemia. The sensitivity of latex agglutination methods in detecting this antigen ranges from 10 to 157 ng/ml, depending on the reagents used [34]. Several commercial systems provide titers of positive results. A positive result gives an indication of the effectiveness of antifungal treatment, and of the prognosis. False positives occur due to cross-reaction with DF-2, *Trichosporum beigelii*, rheumatoid factor and other unknown factors. Titers may remain weakly positive after antifungal treatment when the fungus is no longer viable. False negatives may occur in samples from chronic patients whose disease is of low virulence, and from immunodepressed patients in whom the production of capsular polysaccharide is decreased; the prozone phenomenon can also cause false negative results. Previous treatment of the sera with pronase (Latex-Cryto Antigen, IMMY Laboratories) eliminates false positives resulting from the subjective interpretation of agglutination, and antigen detection is enhanced by eliminating the prozone phenomenon and other sources of false-negatives [34]. An Elisa method is currently being developed to detect circulating *Cryptococcus* antigen [35].

Antibody detection

The ubiquitousness of fungi, and their predilection for producing systemic infection in immunodepressed patients, have contributed to the frequency of spurious results obtained with serological analyses of antibodies. At present, laboratory methods based on the detection of specific antibodies are used as complementary tests, more for their prognostic than their diagnostic value [36]. They are of little use in anergic patients with fulminating infection [27].

An effective serological test should provide standardized, quantitative data. Single determinations are of little use; serial runs are much more informative. An increase in the antibody titer in the space of a few days may indicate fungal invasion, while stable or decreasing titers may rule out systemic invasion; however, a sudden sharp decrease will probably reflect the formation of antigen-antibody complexes. The persistence of high titers suggests unremitting infection, a sign that may be informative in patients with disseminated candidiasis and in carriers of valvular infection, where such a finding may alert the clinician to possible endocarditis [37].

Detection systems

Passive hemagglutination. A negative result rules out, with reasonable certainty, infection due to *Candida* spp. Titers of 1/640 or higher have been observed in the course of disseminated infection due to *Aspergillus* spp. When lower titers are obtained, it is advisable to repeat the test 15 days later.

Indirect immunofluorescence. This technique detects total antibodies against candida blastopores. Values higher than 400 IU/ml are seen in disseminated candidiasis. The disease can cause, with a rapid increase in antibody titers, a decrease to negative titers (due to uptake of the antigen by circulating antibodies), or persistence of positive titer from the moment of diagnosis. Experimental studies have reported the value of IgG/IgA antibodies against the germinal tube, and preliminary studies have demonstrated the usefulness of these methods in distinguishing between systemic disease and superficial infection without tissular invasion [38].

Counterimmunoelectrophoresis. A positive result with counterimmunoelectrophoresis may support the identification of candida as the etiological agent. However, the technical demands of this method, together with its low sensitivity, limit its usefulness. It can nonetheless be used to detect antibodies against *Aspergillus* spp. In comparison with hemagglutination tests, it is less sensitive but more specific.

Elisa. This method is effective in detecting the presence of antibodies against *Aspergillus* spp [39].

Immunoblotting. This technique has attained a sensitivity of 95% in detecting antibodies against *C albicans* mycelium antigens with molecular masses between 80 and 90 kDa present only in disseminated candidiasis [40].

Detection of microbial metabolites

Chromatography

Chromatographic methods identify microorganisms on the basis of their metabolites, alcohols and fatty acids or their fatty acid components of the cell wall. The presence of butyric, isobutyric and isovaleric acid indicates infection by anaerobic bacteria, but says nothing about the genus or

species. Characteristic profiles have been described for infection by *C albicans* (mannose, arabinitol, adonitol and xylitol), *C neoformans*, *Neisseria* spp, strict anaerobes, mycobacteria, staphylococci, streptococci and non-fermenting Gram-negative rods [38, 41–45]. The complexity of these techniques, together with the rates of false positives and false negatives recorded, have kept them from becoming standard practice in the hospital microbiology laboratory, although they may in time be simplified enough to provide a rapid method of diagnosis.

However, studies with gas chromatography-mass spectrometry in combination with selection monitoring (GC-MS-SIM) have shown the *m/e* 268 ion to be a useful marker of microorganisms commonly found in bacterial meningitis, with 88% sensitivity and 98% specificity [46]. Tuberculostearic acid, identified as a specific marker for mycobacterial infection, is rapidly detectable, with a high rate of sensitivity, by GC-MS-SIM [47].

Endotoxin detection

In patients with Gram-negative bacteremia, the concentration of endotoxin in biological fluids increases rapidly after the administration of antibiotics, and may be associated with the appearance of septic shock. A commercially available quantitative assay (Coatest Endotoxin, Kabi Diagnostica Laboratories) measures plasma levels of endotoxins, detecting concentrations from 0.1 to 1.2 ng/ml. In principle, Gram-negative bacterial endotoxin catalyses the activation of a proenzyme in the Limulus Amoebocyte Lysate. The enzyme formed splits of p-nitroaniline (pNA) from the substrate. After stopping the reaction with acetic acid, the rate at which the pNA has been released is measured photometrically at 405 nm. The findings correlated well with the results of blood cultures, although the latter method was more sensitive in diagnosing bacteremia, requiring as little as 1.5×10^3 cells of Gram-negative bacteria to produce a positive result. The functional status of the liver, which removes endotoxins, influences the percentage of positive reactions. False positives can be caused by localized infections, the intestinal absorption of endotoxin and Gram-positive sepsis, although this last source is controversial. The major application of endotoxin-based methods of detection seems to be in the quantification of endotoxin as a predictive marker of septic shock caused by Gram-negative organisms [44, 48].

Other markers of infection

C reactive protein

Between 50 and 90% of all cases of septicemia course with raised levels of C reactive protein, although a certain number of false positives and false negatives have been reported [49–51].

Angiotensin converting enzyme.

Serum activity of angiotensin converting enzyme is raised in miliary tuberculosis [50, 52].

Fibronectin

A drop in fibronectin concentration, together with an increase in leukocyte bands, predicts serious infection with 71% reliability [53].

Elastase-α_1-antitrypsin complex

When neutrophils are activated, elastase is released into the bloodstream, where it is neutralized by α_1-antitrypsin. The concentration of these complexes is increased in all children with sepsis or meningitis. A negative test is a fully reliable indicator of the absence of bacterial infection, while a positive test predicts infection in only 41% of the cases. This method can provide earlier results than tests for C reactive protein in the diagnosis of sepsis [54–57].

Tumor necrosis factor (TNF)

A RIA developed to detect TNF has proved useful in the diagnosis and prognosis of septicemia [58]. Concentrations of TNF greater than 40 pg/ml indicate sepsis, with 71% sensitivity, 98% specificity and 91% diagnostic probability. An unfavorable prognosis is associated with concentrations greater than 250 pg/ml. Anti-TNF-α agents such as monoclonal antibodies have been proposed for use in treatment, although the results published to date are contradictory [59].

Interleukin-6

At concentrations below 40 UI/ml, the cytokine interleukin-6 is associated with a favorable prognosis. An unfavorable course of sepsis is associated with concentrations greater than 7500 UI/ml [60].

Determination of the concentration of D-lactate.

D-Lactic is a specific metabolite of lower forms of life and its presence in sterile fluids is a sign of active bacterial metabolism [61]. The enzymatic test with D-lactate dehydrogenase is a rapid detection method that provides useful information on whether the disease in question is infectious or non-infectious; the test is also accurate in patients who have received antibiotic therapy [62].

The clinical effectiveness of these markers of infection often depends on their combined use to rule out rather than to confirm sepsis [63, 64].

In conclusion (tables I, II), many immunological methods have been developed to detect bacterial or fungal infection in blood. The most straightforward, rapid and reliable methods for use in the hospital laboratory appear to be Elisa to detect bacterial antigens, and agglutination tests to detect fungi such as *Candida* spp, *Aspergillus* spp and *C neoformans*. In the future, a large number of rapid immunological methods that will become available to microbiologists for the detection of pathogens in clinical samples will not be dependent on culture of the germ in the laboratory.

Table I. Summary of commercially available methods for the detection of bacterial antigens, antibodies against *S aureus*, microbial metabolites and other biological parameters[a] in biological fluids.

Detection	*Test*	*Advantages*	*Disadvantages*	*Use*
Antigen	Agglutination	Rapid, specific	Low sensitivity, false positives and negatives	Infrequent, pending improvement
Antigen	Counterimmuno-electrophoresis	Specific	Low sensitivity	Infrequent, pending improvement
Antigen	Elisa/RIA	High sensitivity and specificity	Radioactivity, time-consuming	Increasing
Antibody	*S aureus*	Variety of test	False negatives and positives	Confirmation of endocarditis
Metabolites	Chromatography	Good sensitivity and specificity	Technically complex	Infrequent
	Endotoxin	Adequate sensitivity	False negatives, painstaking and time consuming	Increasing, pending improvement

[a]Other parameters, including C-reactive protein, angiotensin-converting enzymes, elastase-α_1-antitrypsin, fibronectin, tumor necrosis factor, interleukin-6 and D-lactate.

Table II. Methods for the detection of antigens and antibodies against fungi in serum.

Detection	*Test*	*Advantages*	*Disadvantages*	*Use*
Antigens	*C albicans*	Rapid, specific	Low sensitivity; questionable use in detecting invasion	Only test available; sequential use
Antigens	*Aspergillus* spp	Rapid, specific	Low sensitivity questionable use in detecting invasion	Only test available
Antigens	*C neoformans*	Rapid	Room for improvement in sensitivity and specificty	Adequate
Antibody	*C albicans*	Rapid	False negatives and positives; questionable value in detecting invasion	Only test available; sequential use

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