L.J. Wheat

Review article

Rapid diagnosis of invasive aspergillosis by antigen detection

Key words:
aspergillosis; diagnosis; immunoassay; PCR; antigen detection

Acknowledgements:
Dr Christopher Bentsen, of Bio-Rad Laboratories, provided useful information about the test that has not yet been published. The author thanks Michelle Durkin for assistance with graphics, Ann Lemont and John Witt for proofreading the manuscript, and Blair Wheat for editorial assistance.

Abstract: Aspergillosis is a serious and often fatal infection in the bone marrow or organ transplant patient, for which improved methods of diagnosis are desperately needed. Currently, the diagnosis is most often made based on clinical findings and radiographic findings, which are nonspecific, and toxic therapies are initiated empirically, often without ever establishing the diagnosis. Without a definitive diagnosis, physicians often withhold or reduce the doses of the antifungal agent when toxicity develops or the patient improves, permitting progression of disease in those with invasive aspergillosis. The Platelia *Aspergillus* galactomannan antigenemia assay may assist physicians in making these decisions. With a sensitivity of 81% and a specificity of 89% in the studies leading to its FDA clearance, physicians still must be aware of the potential for false-positive and false-negative results; the test does not replace careful microbiological and clinical evaluation. This report will review the relevant literature and provide guidelines for use of the test in patient management.

Author's affiliation:

L.J. Wheat

MiraVista Diagnostics and MiraBella Technologies
Indianapolis, Indiana, USA

Correspondence to:
L. Joseph Wheat, MD
MiraVista Diagnostics
4444 Decatur Blvd
Suite 300
Indianapolis, IN 46241
USA
Tel: 1 317 856 2681
email:
jwheat@miravistalabs.com

Aspergillosis continues to be a serious and common opportunistic infection in immunocompromised subjects. Invasive aspergillosis (IA) occurs in 5–20% of individuals who undergo allogeneic stem cell transplantation, while a lower proportion occurs in solid organ allograft recipients (1–4). Furthermore, mortality remains high, above 50% in most studies (1). While the effect of the serious underlying disease has a profound impact on outcomes, delayed diagnosis contributes to mortality.

Diagnosis of IA may be difficult (5). Although air-crescent and halo signs seen on radiographs or computed tomography (CT) scans suggest IA (6), they are neither specific nor sensitive, and often are not correctly identified (7, 8). Often, definitive diagnosis by biopsy is not feasible because of coagulation abnormalities. Bronchoscopy to obtain specimens for cytology or culture may be possible in such patients, but the sensitivity for diagnosis is only about 25% (9). More often, antifungal therapy is initiated empirically, and the diagnosis is not proven.

Today, empiric therapies may include voriconazole (8) or caspofungin (10, 11), which, while active against *Aspergillus*, may not be effective

Received 19 August 2003,
accepted for publication 11 September 2003

Transplant Infectious Disease . ISSN 1398-2273

Transpl Infect Dis 2003: **5**: 158–166

against other molds, an emerging problem following bone marrow (12) or solid organ transplantation (13). The availability of rapid, specific, and non-invasive diagnostic tests could be highly useful in such cases, permitting earlier initiation of effective treatment. Furthermore, negative tests for aspergillosis may alert the clinician to modify empiric therapy to include agents that are active against other molds, or to pursue additional diagnostic procedures.

Antigen detection for diagnosis of IA was first reported in the late 1970s, and was made a reality by the production of monoclonal antibodies (14) and creation of a standardized and reproducible assay in the early 1990s (15). Available in Europe for over 5 years, the Platelia® *Aspergillus* antigen immunoassay, produced by Bio-Rad Laboratories (Hercules, CA, USA), was cleared by the Food and Drug Administration (FDA) for diagnostic use in the US in May 2003. This review will focus on the clinical uses of the new *Aspergillus* antigen assay, as well as its limitations.

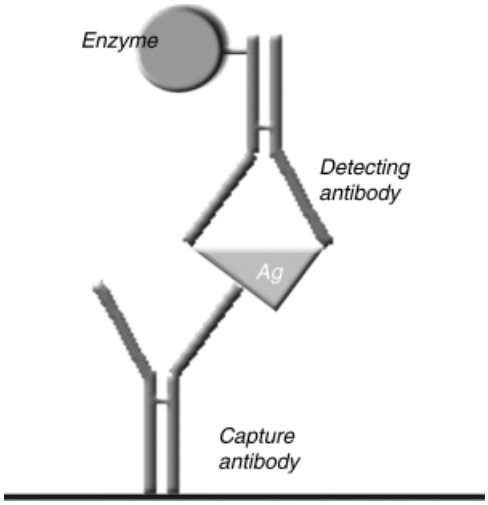

Fig. 1. Schematic describing the steps of the assay. The test uses microplates that are pre-coated with the captured antibody. Next, the detector antibody and test serum are added in that order, and the plates are incubated at 37°C for 90 min. The plates are aspirated and rinsed, and then TMB chromogen substrate is added, and the plate is incubated in the dark for 30 min at 30°C. After the reaction is stopped with H_2SO_4, wells that contain antigen become yellow, and the color is recorded using a microplate reader.

Methodology

The assay is a sandwich enzyme immunoassay using rat monoclonal antibodies to *Aspergillus fumigatus*. This antibody was produced by immunizing rats with a mycelial extract of *A. fumigatus*, and it recognizes a galactomannan epitope that contains β (1 → 5)-linked galactofuranose (14). The antibody reacts with several *Aspergillus* species, including *A. fumigatus, A. flavus, A. niger, A. versicolor,* and *A. terres* (14); and with exoantigens from several other molds: *Penicillium digitatum, Trichophyton rubrum* and *interdigitalis, Botrytis tulipae, Wellemia sebi,* and *Cladosporium cladosporioides* (14); *Cladosporium herbarum, Acremonium spp, Alternaria alternata, Fusarium oxysporum, Wangiella dermatitidis*, and *Rhodotorula rubra* (16); *Paecillomyces variotii* and *Penicillium chrysogenum* (17).

Testing procedure

The test serum is first boiled for 3 min in the presence of 4% ethylenediaminetetraacetic acid (EDTA) to dissociate immune complexes and destroy interfering substances. The resultant coagulum is centrifuged at 10,000 × g for 10 min, and the supernatant is removed and may be stored at 2–8°C for up to 72 h before testing. *Aspergillus* grows well in contaminated serum stored at 2–8°C, highlighting the importance of careful specimen processing and storage.

Testing is performed by adding a peroxidase-linked detector antibody followed by 50 µl of the test specimen into the pre-coated microplate wells, then incubating at 37°C for 90 min (Fig. 1). Next, a tetramethylbenzidine (TMB) chromogen substrate is added, and the plate is incubated in the dark for 30 min at 30°C; if antigen is present, then a blue color appears. The enzyme reaction is stopped by adding H_2SO_4 stopping solution, which changes the color to yellow. The microplate wells are aspirated and washed between steps and read in a microplate reader at both 450 and 620/630 nm wavelengths after the last step.

Calculation of result

A positive control well, negative control well, and two cut-off control wells are included for quality control and calculation of antigen results, reported as an Index. The results are determined by comparison with the cut-off control. The optical density (OD) of the test specimen is divided by the mean OD of the cut-off control, and results with an Index value of 0.5 or higher are considered to be positive. In some reports, several specimens containing known amounts of galactomannan were included to permit more exact quantitation. However, the assay is not linear at ODs above 2.0 and may not accurately reflect galactomannan concentration in such specimens.

Sensitivity

The sensitivity of the Bio-Rad Platelia® *Aspergillus* EIA was 80.7% in the studies used for FDA approval in the US. In other studies, the sensitivity has ranged from less than 50% to over 90%. A few large recent

prospective studies will be reviewed more fully to provide an understanding of the indications and limitations of the assay in patient management (Table 1) (7, 18–21). Other studies, summarized in Table 2, are presented to provide a comprehensive review of the literature (15, 22–35).

Maertens et al. (7) prospectively monitored allogeneic hematopoietic stem cell transplantation (HSCT) patients. Patients underwent aggressive evaluation for IA, including frequent chest radiographs and CT scans, weekly surveillance cultures, and *Aspergillus* antigen testing twice weekly. An assay cut-off of 1.5 was considered to be positive, and consecutive positive results were required to be classified as a true-positive assay result. Based on the autopsy-updated classification, the sensitivity was 94.4%. These findings substantiate their earlier experience, where they reported a sensitivity of 92.6% in autopsy-confirmed cases (28). Diagnosis by detection of antigenemia was more sensitive and specific than other procedures, including radiography, CT scan, and culture (Table 3).

Herbrecht et al. (18) found the test to be less sensitive. They prospectively evaluated neutropenic patients undergoing workup for fever, patients under investigation for suspected IA, and patients undergoing routine monitoring following HSCT. Their cut-off for a positive result was 1.5, and a single positive result was considered to be diagnostic. The sensitivity was 64.5% with definite, 16.4% with probable, and 25.5% with possible IA. If the cut-off was reduced to 0.7 and the analysis was restricted to adult non-allogeneic HSCT patients, the sensitivity increased to 73.1% in definite cases.

Early diagnosis

The detection of antigenemia during twice-weekly monitoring facilitates early diagnosis of IA. Antigenemia preceded CT findings by 1 week and preceded initiation of antifungal therapy in nearly 90% of cases (7), confirming earlier findings (36) (Table 4). Sulahian et al. (32) reported that antigenemia preceded CT evidence of IA by more than a week in 65% of cases. Others reported similar findings in retrospective cohorts (21, 37), while some investigators have not shared this experience (25, 26, 30, 34, 38).

Specificity

False-positive results have been observed in all studies, but the prevalence has varied considerably. Major variables affecting specificity include the selection of the cut-off to define positivity and the requirement for demonstration of persistent positivity for classification as true-positive. Maertens et al. (7) reported a specificity of 98.8% if two or more positive results were required vs. 85.4% if only one was required for classification as true-positive. However, false-positive results also have been reported in up to 20% of cases in studies requiring consecutive positive results (7, 17, 23, 24, 28, 32). Herbrecht et al. reported a specificity of 99.4% using a cut-off of 1.5, 93.9% with a cut-off of 0.7, but only 88.7% with a cut-off of 0.6. McLaughlin et al. (21), however, reported only a small drop in specificity from 99.7% to 97% when the cut-off was reduced from 1.5 to 0.5.

Sensitivity and specificity of Platelia® *Aspergillus* galactomannan immunoassay

Reference	Sensitivity Positive/total (%)	Specificity Negative/total (%)	Comment
Bio-Rad package insert	Prov/prob 25/31 (81)	132/148 (89)	Retrospective, stored specimens from bone marrow transplant and leukemia patients at 3 US centers, adults and children, cut-off 0.5, single positive
Maertens (7)	Prov 17/18 (94) Prob 0/0 Pos 0/6 (0)	72/73 (99)	Prospective monitoring allo-HSCT, 2 × /week, cut-off 1.5, consecutive positive
Herbrecht (18)	Prov 20/31 (64) Prob 11/67 (16) Pos 14/55 (25)	607/640 (95)	Prospective monitoring and diagnostic evaluation of suspected IA, neutropenic and HSCT, cut-off 1.5, single positive
Pinel (19)	Prov 0/3 (0) Prob 14/31 (45)	748/751 (99)	Prospective monitoring during neutropenia and ICU, cut-off 1.0, consecutive positive
Becker (20)	Serum 8/17 (47) BAL 17/17 (100) BAL[1] 19/22 (85)	134/143 (93) 143/143 (100) 176/176 (100)	Prospective diagnostic evaluation during neutropenia, CT-guided BAL, blinded, cut-off 1.0
McLaughlin (21)	Prov 12/13(92)[1] Prob 8/11 (73)	589/607 (97)[2]	Retrospective, HSCT patients, cut-off 0.5, single positive

[1]Second non-blinded study.
[2]Specimens.
Prov, proven; Prob, probable; Pos, possible; HSCT, hematopoietic stem cell transplantation; IA, invasive aspergillosis; CT, computed tomography; BAL, bronchoalveolar lavage; ICU, intensive care unit.

Table 1

Other studies evaluating sensitivity and specificity of galactomannan immunoassay

Reference	Sensitivity Positive/total (%)	Specificity Negative/total (%)	Comment
Stynen (15)	9/9 (100)	81/88 (92)	Retrospective, neutropenia and bone marrow transplant, cut-off 4 SD above mean of negative controls
Verweij (22)	9/10 (90)	43/51 (84)	Retrospective, neutropenia, cut-off 1 ng/mL galactomannan standard, multiple sera/patient
Rohrlich (23)	10/10 (100)	21/27 (78)	Prospective, neutropenia, 2 $\times$/week, cut-off mean negative controls plus 4 SD, consecutively positive
Sulahian (24)	Prov 19/25 (76) Prob 14/15 (93)	138/169 (82)	Retrospective, bone marrow transplant, cut-off mean negative controls plus 5 SD
Bretagna (25)	Prov or Prob 6/6 (100)	31/35 (89)	Prospective, hematology patients, once weekly testing, cut-off >1 ng/mL galactomannan, single positive
Bretagna (26)	Prov 6/6 (100) Prob 8/12 (75)	18/19 (95)	Retrospective, hematology patients, cut-off >1 ng/mL, single positive
Siemann (27)	Prov 5/5 (100) Prob 2/6 (33) Suspected 4/8 (50)	Unlikely IA 25/25 (100) Premature infants 1/6 (17)	Retrospective, hematologic malignancy or ICU, cut-off 1.5, single specimen
Maertens (28)	Prov 25/27(93) Prob 2/6 (33)	42/44 (95.4)	Prospective monitoring, neutopenia or steroids, cut-off 1.0, consecutive positive
Kawamura (29)	4/4 (100)	90/90 (100)	Inadequate description of diagnostic criteria or patient groups, cut-off 1.0
Ulusakarya (30)	Prov 8/10 (80) Prob 3/6 (50)	118/135 (96)	Prospective monitoring, neutropenia, cut-off 1.5, consecutive positive
Salonen (31)	Prov 6/6 (100) Prob 1/1 (100) Suspected 10/15 (67)		Prospective, hematology and bone marrow transplantation, cut-off 1.5, consecutive positive in 15 of 17
Sulahian (32)	Prov or Prob 48/53 (91)	700/744 (94)	Prospective, children with hematologic malignancy or HSCT, cut-off 1.5, consecutive positive
Fortun (33)	Prov or Prob 5/9 (56)	31/33 (94)	Retrospective, liver transplantation, cut-off 1 ng, consecutive positive
Kami (34)	19/33 (58)	86/89 (97%)	Retrospective and prospective, hematologic malignancy, cut-off 1.5
Sanguinetti (35)	BAL 20/20 (100)	BAL 0/24 (100)	Retrospective, cut-off 1.5

SD, standard deviation; Prov, proven; Prob, probable; IA, invasive aspergillosis; ICU, intensive care unit; HSCT, hematopoietic stem cell transplantation; BAL, bronchoalveolar lavage.

Table 2

False-positive results may occur more frequently in children. Herbrecht et al. (18) reported a specificity of 48% of children vs. 98% of adults. Sulahian et al. (32) noted a specificity of 97.5% in adult patients compared with 89.9% in children. Others noted false-positive results in 83% of premature infants (27). Hayden et al. (37), however, reported the specificity to be 98.4% in pediatric cases using a cut-off of 0.5. Some suggest that galactomannan present in milk, rice, or protein-rich nutrients is the cause of false-positive results in children (27, 32, 39, 40), a conclusion that cannot explain the high rate of false-positivity in premature infants who do not receive cereal (27) (Table 5).

False-positive results may be more common in allogeneic HSCT patients (6.6%) than in other patient groups (0.6%) (18). When used for monitoring for IA following allogeneic HSCT, false-positive results have occurred most often during the first 2 weeks after cytoreductive therapy (28). This higher positive rate was ascribed to increased absorption of dietary galactomannan made possible by the breakdown of the intestinal mucosa caused by chemotherapy and irradiation (18).

Cyclophosphamide metabolites were suspected to cause false-positive results (41), and perhaps explained positive results before infection in an experimental model of aspergillosis in which the animals received cyclophosphamide 2 days before infection (42). Others, however, failed to observe false-positive results in patients who had received cyclophosphamide (28).

One concern is that *Aspergillus* colonization could cause antigenemia, resulting in the incorrect diagnosis of aspergillosis. Of note, however, is that Maertens et al. (36) did not observe false-positivity in patients who

Comparison of diagnostic tests in proven invasive aspergillosis[1]

Finding	*Sensitivity* (*N* = 18)	*Specificity* (*N* = 82)
Chest radiograph	94%	60%
CT of lung[2]		
Any abnormality	78%	7%
"Halo sign"	28%	93%
BAL growing *Aspergillus*[3]	50%	92%
Aspergillus galactomannan EIA:		
1 result	94%	85%
⩾ 2 results	94%	99%

[1]Data from Maertens et al. (7).
[2]Computed tomography (CT) done in 15 cases and 15 controls.
[3]Bronchoscopy and bronchoalveoloar lavage (BAL) performed in 16 patients and 26 controls.

Table 3

Temporal onset of galactomannan antigenemia in invasive aspergillosis[1]

Finding	*Antigen first*[2]	*Days prior*[3]
Radiogram chest	12/15 (80%)	8
CT lung	12/15 (80%)	6
Positive culture	16/18 (89%)	9
Definite diagnosis IA	16/18 (89%)	14
Initiation therapy	16/18 (89%)	6
Death	17/18 (94%)	14

[1]Data from Maertens et al. (7).
[2]The data depict the number of cases in which the positive antigen result preceded the finding.
[3]Median number of days by which the positive antigen preceded the finding.
CT, computed tomography; IA, invasive aspergillosis.

Table 4

were colonized. Furthermore, Rohrlich et al. (23) evaluated patients with cystic fibrosis, who exhibited persistent airway colonization with *Aspergillus*, and failed to detect antigenemia. However, occult *Aspergillus* infection may occur in some colonized patients with presumed false-positive results, as shown by Maertens et al. (7): three patients presumed to have false-positive results during life were proven to have IA at autopsy.

False-positive results also may be caused by infection with organisms that share cross-reacting antigens with *Aspergillus*. The monoclonal antibody used in the assay reacts with antigens from members of several fungal genera besides *Aspergillus* (14). Kappe and Schulze-Berger (16) reported a false-positive result caused by contamination of a specimen with *Penicillium chrysogenum*, and cautioned laboratories to inspect carefully for contamination. Bretagne et al. (25) described a false-positive result in a patient with pneumonia and sinusitis caused by *Phialophora americana*, although antigens extracted from this organism did not react in the assay. Positive results using the same monoclonal antibody in a latex agglutination assay were observed in guinea pigs that were infected with *Penicillium marneffei* (43).

False-positive results have also been reported in neutropenic patients with bacteremia caused by staphylococci, enterococci, *Corynebacterium jeikeium, Pseudomonas, Escherichia coli*, and fungemia with *Candida albicans* (17). Interestingly, exoantigens from these organisms were not reactive in the assay, and others failed to observe false-positive results in bacteremic patients (28).

Anti-animal antibodies may cause false-positive results in sandwich assays (44). However, pre-treatment with EDTA and boiling should overcome this problem in the Platelia® assay. Nevertheless, a false-positive result was reported in a patient with graft-versus-host disease (GVHD) who had auto-reactive antibodies (45).

Finally, miscellaneous other causes for false-positive results have been observed or proposed. Glucopyranose present in cellulose was postulated to cause false-positive results when material on a cotton swab was tested in the latex agglutination format of the *Aspergillus* antigen assay (46). Galactomannan was detected in several drugs that originated from fungal organisms, including co-amoxyclav, piperacillin (39), piperacillin/tazobactam (Thomas Walsh, unpublished communication, 2003), and uricase (19). Undoubtedly, other causes for false-positive results will be discovered as the test is used more often in the US.

Other body fluids

Antigen has been detected in body fluids other than serum. Although these specimens were not included in the FDA clearance, they may be superior to serum for testing in certain circumstances. The detection of antigen in bronchoalveolar lavage fluid (BAL) was described in the initial report of the test (47). Becker et al. (20) reported detection of antigen in the BAL of all 18 cases of IA, while antigenemia was present in only 47%. Others have also reported the detection of antigen in BAL (48, 49). More recently, Sanguinetti et al. (35) described the detection of galactomannan in BAL of all 20 hematology patients with IA, but did not compare the results to antigenemia testing. The results were negative in all control specimens in that study. Airway colonization, however, may cause false-positive results in BAL specimens.

The central nervous system (CNS) is often involved in patients with disseminated aspergillosis, and four studies have reported detection of antigen in the cerebrospinal fluid (CSF) (50–53). However, antigen in the CSF was also found in control patients with pulmonary IA who lacked

Causes for false-positive *Aspergillus* galactomannan antigenemia

Condition	Mechanism	Reference
GVHD	Auto-antibodies	Hamaki (45)
Allogeneic HSCT	Intestinal breakdown and dietary galactomannan	Herbrecht (18)
Children	Dietary galactomannan	Herbrecht (18), Sulahian (32)
Premature infants	Unknown	Siemann (27)
Contamination with cotton	Shared glucopyranose	Dalle (46)
Penicillium chrysogenum contamination	Shared galactomannan	Kappe (16)
Penicillium marneffei or *Paecilomyces* infection	Shared galactomannan	Stynen (14)
Cyclophosphamide treatment	Cross-reactive metabolite	Hashiguchi (41)
Fungemia or bacteremia	Cross-reactive antigen	Swanink (17), Herbrecht (18)
Undiagnosed aspergillosis	Occult infection	Maertens (7)
Medications of fungal origin: antibiotics and uricase	Contaminating galactomannan	Pinel (19), Ansorg (39)
Reproducible low-positive results followed by negative results	Concomitant anti-fungal therapy, low fungal burden, variable antigen release	C. Bentsen (Personal communication)
Sporadic positive results that are not reproducible	Poor technique, splashing of adjacent positive wells, poor washing	Platelia® *Aspergillus* package insert

GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation.

Table 5

Indications and guidelines for use of *Aspergillus* galactomannan immunoassay

Indications for use:
Twice-weekly monitoring during severe immunosuppression
Follow-up testing of positive specimens before accepting as a true positive
Diagnosis of suspected IA in patients with compatible clinical findings
Monitoring once or twice weekly to assess response to treatment
Guideline for use:
Results may be falsely positive or falsely negative, and must be correlated with clinical and laboratory findings
Antigenemia testing does not replace other tests for IA
Testing should precede empiric antifungal therapy to maximize sensitivity
Positive results should be confirmed on a new specimen to maximize specificity

IA, invasive aspergillosis.

Table 6

CNS aspergillosis (51). The detection of antigen in the CSF in the absence of antigenemia would provide compelling evidence of CNS aspergillosis, in the appropriate clinical setting.

Antigen may also be detected in urine, but with a lower sensitivity than in serum. Stynen et al. (15) detected antigen in the urine of 5 of 7 cases, all of which exhibited antigenemia. The concentration of antigen, however, was greater in the serum than in the urine. In another study, antigen was detected in the urine of only 2 of 6 antigenemic patients (48). These studies suggest that urine is inferior to serum for antigen testing in aspergillosis.

Effect of therapy

Monitoring for antigen clearance or rebound may provide useful information for assessing the effectiveness of therapy. Bretagne et al. (25) and Maertens et al. (36) described declining levels in patients who responded to therapy and rising concentrations in those with fatal outcomes. Rohrlich et al. (23) reported clearance of antigenemia in patients who responded to therapy and reappearance in those who relapsed. More recently, Boutboul et al. (54) noted stable levels in responding patients and rising levels in failing patients. Becker et al. (20) observed that antigen was no longer detectable in BAL after three days of therapy and others have reported a decline in CSF antigen concentration with therapy (50, 52).

Monitoring antigenemia may assist in patient management. Failure of antigenemia to decline would suggest treatment failure, and support consideration of modifying therapy, including combination therapy (11). Conversely, clinical deterioration in the presence of falling antigen levels would support investigation of other causes for the clinical deterioration, including fungal infection with organisms that are resistant to therapy.

Rebound antigenemia after treatment was stopped suggests relapsing infection and the need for resumption of therapy.

Comparison of *Aspergillus* EIA and PCR

While this is an area of intense investigation that dates back to the early 1990s, a clinically applicable and reliable method has yet to be identified and the findings are inconsistent between studies. Focusing on studies comparing polymerase-chain reaction (PCR) to the Platelia® *Aspergillus* EIA, Kami et al. (34) reported a sensitivity of 79% by PCR compared with 58% by EIA. Specificity was 92% by PCR and 97% by EIA (34). Bretagne et al. (26) noted a lower sensitivity for PCR than antigenemia, 50% vs. 77.8%, respectively. In another report using a rat model of IA, antigenemia testing was superior to PCR (55). Kawamura et al. (29), however, noted lower sensitivity for antigenemia than PCR.

PCR methods for the detection of *Aspergillus* DNA in specimens other than blood have also been described. *Aspergillus* DNA has been detected in BAL specimens, but comparison with antigenemia testing remains incomplete (47, 56–58). Kami et al. (53) reported the detection of *Aspergillus* DNA in the CSF of 5 patients with CNS aspergillosis, 4 of whom also had elevated levels of antigen by EIA. Spiess et al. (59) evaluated a real-time PCR assay on BAL and blood from patients with IA and controls, noting it to be specific but less sensitive than a previously described nested PCR. However, they did not compare PCR with antigen detection (59). Sanguinetti et al. (35) recently compared real-time PCR and nested PCR to galactomannan antigen detection in BAL from patients with hematologic disorders. Using EORTC criteria, the sensitivity of galactomannan detection was 100%, compared with 90% for both real-time and nested PCR (35). The results were negative in control patients.

Research is required to define the most appropriate PCR method before molecular diagnostics can be accurately compared with antigenemia testing.

Proposed uses and limitations

To provide accurate results, the laboratory must be proficient in performance of the assay; to use them appropriately in patient management, the clinician must be aware of the assay's limitations. The indications and guidelines for use of antigenemia testing are summarized in Table 6. The most important point is that a negative test cannot rule out the diagnosis of IA, and a positive test alone is not diagnostic of the infection. Accordingly, the tests must be used in conjunction with and not in place of other diagnostic procedures and expert clinical judgment. Also of note is the importance of confirming positive results before accepting them as diagnostic of IA. The cost of frequent antigenemia testing is a cause for concern. Will the benefits of early diagnosis and appropriate therapy justify these added costs of care (36)? Prospective studies are needed to assess the optimal, cost-effective use of the test.

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