Clinical Review

Use of serum Aspergillus galactomannan antigen as a biomarker of invasive aspergillosis in patients with hematologic malignancy and recipients of allogeneic hematopoietic stem cell transplants for the purpose of treatment development

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Materials Reviewed	Sponsor submission 23 Journal articles for analysis (Appendix 1) 44 Journal articles for reference (Footnotes)

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Executive summary

Invasive Aspergillosis
Aspergillus is a ubiquitous filamentous fungus (mould) that can cause a spectrum of diseases whose manifestations are related to host factors. The hallmark of invasive aspergillosis (IA) is vascular invasion with subsequent tissue infarction. IA is a major cause of mortality in patients with hematologic malignancy and recipients of allogeneic hematopoietic stem cell transplants (HSCT). The most common sites involved are the lungs, sinuses, and central nervous system.

Definite diagnosis of IA is challenging. Clinical signs and symptoms are not sensitive or specific. Blood and respiratory cultures are usually negative, and the patients' underlying morbidities often prohibit obtaining tissue specimen for histologic and culture demonstration of invasive mould, the conventional gold standard for diagnosis. Suggestive lung radiologic findings including nodular infiltrates with "halo" sign, cavitation, and pleural-based wedge density, are not pathognomonic. In 2002, the European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) published criteria for diagnosis of invasive fungal infections which classify IA into proven, probable, or possible based on host factors, clinical or radiologic features, and microbiologic features. The criteria were revised in 2008, refining the definitions. According to the revised EORTC criteria, proven IA requires culturing the organism from normally sterile material, or demonstrating invasive fungal elements in a tissue specimen. The diagnosis of probable IA requires defined clinical and radiologic manifestations in a susceptible host with supportive microbiologic features that include positive cultures from non-sterile material or positive galactomannan (GM) antigen in the serum, bronchoalveolar lavage, or cerebrospinal fluid. Possible IA refers to cases with host, clinical and radiologic features, but without microbiologic support.

The current treatment of choice for IA is voriconazole, a triazole antifungal approved by the FDA in 2002 for the primary treatment of IA. In a key study comparing voriconazole to amphotericin B deoxycholate, eligible immunocompromised patients with definite or probable IA were evaluated. This study predated both the publication of the EORTC/MSG criteria and the availability of the GM assay, but was designed under the aegis of a steering committee that included EORTC. The definitions of IA categories were largely similar to EORTC criteria, except that some patients who would be now be classified by the criteria as probable were classified as proven, and some patients who would be classified by the criteria as possible were classified as probable. Global response (complete or partial resolution of radiologic signs and clinical symptoms at 12 weeks) was 53% and 32% in subjects with proven and probable IA who received voriconazole and amphotericin B respectively. Survival at Day 84 was 71% and 58% respectively.

Review of the literature indicates that the median prevalence of IA in patients at risk for IA because of a hematologic malignancy or HSCT is approximately 12% (range 2.6 –

34). The prevalence of IA in at risk hematology patients who also have clinical or radiologic signs suggestive of invasive fungal infection is expected to be higher. Because obtaining diagnostic procedures in these patients may not be clinically feasible, and because the reference modalities used to diagnose proven IA (culture and microscopy) have poor sensitivity, the prevalence of IA in at risk hematology patients who also have clinical and radiologic manifestations will be underestimated. Our review estimates the prevalence in this population at 36% (95% CI 26.1, 45.9), with higher prevalence estimates obtained from autopsy studies.

Galactomannan antigen

Galactomannan antigen is a cell wall polysaccharide component of *Aspergillus* species. In 2003, the FDA cleared the Platelia™ Bio-Rad assay for detection of serum GM as an aid for the diagnosis of IA "when used in conjunction with other diagnostic procedures such as microbiologic cultures, histologic examination of biopsy samples, and radiologic evidence of infection". The assay is a one-stage immuno-enzymatic sandwich micro-plate assay that detects galactofuranosyl-containing molecules using a rat monoclonal antibody directed at *Aspergillus* galactomannan. The result is expressed as an optical density index. The FDA accepted a serum GM index of 0.5 as the cutoff for positivity.

Review of the published literature indicates that serum GM can be detected a week prior to the appearance of signs or symptoms of IA in approximately 50% of IA patients who have a hematologic malignancy or HSCT. In these patients, GM titer has been correlated with disease burden, and has been reported to progressively increase in patients with uncontrolled infection, and decrease in response to appropriate therapy. Resolution of serum GM titer has been correlated with 12 and 16-week survival.

Analytically, false positive results occur due to cross-reaction with other fungal species, due to administration of Plasmalyte, and in patients receiving piperacillin-tazobactam or amoxicillin-clavulanate. Review of the published literature indicates that the cross reacting fungal species are clinically uncommon in patients with hematologic malignancies, accounting for less than 2% of all fungal infections (compared to *Aspergillus* which accounts for 60-75% of all fungal infections in this population). Patients receiving Plasmalyte, piperacillin-tazobactam, or amoxicillin-clavulanate should be excluded from IA treatment trials that incorporate GM in the diagnostic criteria.

In the hematology population at risk (regardless of the presence of manifestations suggestive of fungal infection), the median sensitivity and specificity of two consecutive serum GM indices ≥ 0.5 are 93% and 91% respectively, and the PPV and NPV are 71% and 98% respectively. The median sensitivity, median specificity, PPV, and NPV of two consecutive specimen indices ≥ 1.0 are 88%, 98%, 85%, and 98% respectively. However, the specificity of GM is underestimated because the diagnostic modalities against which it is evaluated (culture and microscopy) have poor sensitivity. Using the lower end of the 95% CI for prevalence, and the lower end of the 95% CI for sensitivity and specificity of GM in all hematology patients at risk, the PPV of two consecutive samples ≥ 1.0 is approximately 82%. However, a study that evaluated the performance of GM assay at a

cutoff of ≥ 1.0 obtained ante-mortem in patients demonstrated to have IA at autopsy reported a PPV of 93%.

Future treatment trials of IA are likely to be of non-inferiority design with voriconazole (the current drug of choice) as the active control. Assessment of the data generated from the study comparing voriconazole to amphotericin B (which will likely make up the bulk of any future NI margin justification) allow estimation of historical drug effect. Although some patients in that study may be re-classified in a different diagnostic category, the data show similar efficacy difference between the two treatment groups across all IA diagnosis categories. The data suggest that the constancy assumption will be met and that there is adequate historical sensitivity to drug effect using a population similar to that which would be enrolled in a future study using GM as a microbiologic entry criterion.

Proposed Future Role of GM

The Mycoses Study Group (MSG) is seeking FDA's acceptance of serum GM as a sole microbiologic diagnostic criterion in patients with hematologic malignancy and prolonged neutropenia, and recipients of HSCT who have clinical signs or symptoms compatible with invasive fungal infection as defined by EORTC criteria for the purpose of enrollment in clinical trials.

The Mycoses Study Group proposes using two consecutive serum GM index measurements of ≥ 0.5 as a sole microbiologic criterion to classify patients as having probable IA. Our analysis indicates that the PPV of two consecutive measurements of ≥ 1.0 is higher than the PPV of two consecutive measurements of ≥ 0.5, and would be more appropriate to implement in future trials to minimize type I error. However, in clinical practice, a serum GM index cutoff of 0.5 is currently accepted for diagnosis and initiation of therapy for IA. A concern would be that adopting a cutoff for trial enrollment that is higher than the cutoff for diagnosis or initiation of therapy may discourage clinicians from enrolling patients into treatment trials evaluating new modalities of antifungal therapy, thus potentially increasing the difficulty of conducting such trials. However, our review indicates that approximately 80% of patients diagnosed with proven or probable IA (based on histopathology or cultures) have a GM index ≥ 1.0 at presentation. Therefore adopting an index of 1.0 rather than an index of 0.5 as the optimal cutoff is unlikely to discourage physicians from enrolling patients into future IA treatment trials and unlikely to impede patient care.

Recommendations

Based on review of the published literature, the reviewer recommends acceptance of serum *Aspergillus* galactomannan antigen (GM) as detected by the Platelia™ Bio-Rad assay (or an essay with similar performance characteristics) as a biomarker of invasive aspergillosis for treatment trial enrollment with the following restrictions

Serum Galactomannan Platelia Assay for the diagnosis of Invasive Aspergillosis in Patients with hematologic malignancy or recipients of HSCT

- Patients with hematologic malignancy and recipients of allogeneic hematopoietic stem cell transplants (HSCT) who have clinical signs/symptoms or radiologic findings suggestive of invasive fungal infection as defined by EORTC criteria

- Two consecutive serum indices of 1.0 or higher, obtained 48-72 hours apart

- Exclusion of patients concomitantly receiving antibiotics known to result in a false positive GM (piperacillin/tazobactam, amoxicillin/clavulunate), or concomitantly receiving Plasmalyte

Because of insufficient data, the reviewer does not recommend the use of the GM assay as a microbiologic criterion in lieu of positive culture or histology for enrollment of patients with immunodeficiencies other than hematologic malignancy or recipients of allogeneic HSCT in IA treatment trials.

Background

Epidemiology of IA

Aspergillus species are ubiquitous filamentous fungi (moulds). *Aspergillus* spores are usually acquired by inhalation. Aspergillosis encompasses a spectrum of diseases whose manifestations are related to host factors. Invasive aspergillosis (IA) is a major concern in certain immnocompromised hosts. Traditional patient populations at risk include those with hematologic malignancies and prolonged neutropenia, recipients of hematopoietic stem cell transplants (HSCT) or solid organ transplants, patients with advanced AIDS or chronic granulomatous disease (CGD), and patients with pre-existing structural lung disease[1] . New populations at risk include patients receiving immunosuppressive agents that result in prolonged deficiency of B and T cell mediated immunity (for example, monoclonal antibodies as rituximab and almetuzumab)[2].

In patients with hematologic malignancy and recipients of allogeneic HSCT, the mean prevalence of IA is 12 - 18%[3,4,5] , but can be as high as 44% in some centers[5,6]. IA accounts for 60 - 75% of all invasive fungal infections in HSCT[5,6,7], and for 75 - 94% of all filamentous fungal infections in patients with hematologic malignancy[5,7,8].

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[1]Segal BH. Aspergillosis. N Engl J Med 2009;360:1870-1884

[2]Maertens J. et al. Advances in the serological diagnosis of invasive aspergillosis in patients with hematologic disorders. Mycoses 2007;50(S1):2-17

[3]Penack O. et al. Aspergillus galactomannan testing in patients with long-term neutropenia: implications for clinical management. Annals Oncology 2008; 19:1-6

[4] Slobbe L, et al. Outcome and medical costs of patients with invasive aspergillosis and acute myelogenous leukemia-myelodysplastic syndrome treated with invasive chemotherapy: an observational study. Clin Infect Dis 2008; 47:1507-12

[5]Lefflang MM, et al. Galactomannan detection for invasive aspergillosis in immunocompromised patients. The Cochrane Database of Systematic Reviews, 2008, Issue 4. Art. No.: CD007394.DOI:10.1002/14651858.CD007394

[6] Neofytus D. et al. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of multicenter Prospective Antifungal Therapy (PATH) alliance. Clin Infect Dis 2009; 48:265-273

[7] Pagano L. et al. Fungal infections in recipients of hematopoietic stem cell transplant: results of SEIFEM B-2004 study. Clin Infect Dis 2007;45:1161-1170

[8] Pagano L., et al. Infections caused by filamentous fungi in patients with hematologic malignancies. A report of 391 cases by GIMEMA infection program. Hematologica 2001;86:862-870

Clinical Manifestations of IA

The most commonly affected sites are the lungs, sinuses and central nervous system. The hallmark is of IA vascular invasion with subsequent tissue infarction/necrosis. Clinical signs and symptoms of IA, including persistent fever, cough, pleuritic chest pain, and hemoptysis are not sensitive or specific. Lab findings and chest radiography are also not sensitive or specific[1]. Blood and respiratory cultures are usually negative in patients with IA, and the underlying patients' morbidities often prohibit obtaining tissue specimen for histologic and microbiologic (culture) diagnosis. Chest computerized tomography (chest CT) typically shows nodular lesions, at times with a halo sign or cavitation[9]. However, CT findings are not specific for aspergillosis and can be seen in a variety of other mould or bacterial infections[1,2,10,11].

Definitions/Classification of IA

In 2002, The European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the Mycoses Study Group (EORTC/MSG) developed definitions of "proven", "probable" and "possible" invasive fungal infections in patients with hematologic malignancies or recipients of HSCT based on host factors, and clinical and microbiologic features[10] . The EORTC revised these definitions in 2008[11]. The revisions expanded the definition of susceptible hosts, and refined the definitions of clinical and radiologic features to include more specific descriptions of clinical and radiologic findings. According to EORTC criteria, proven invasive aspergillosis requires culturing the organism from sterile material, or demonstration of invasive fungal elements in histopathologic tissue specimen. Diagnosis of probable IA requires combination of clinical/radiologic features, and culture of the organism from a non-sterile site or antigen detection in the serum, CSF, or bronchoalveolar lavage (BAL) in a susceptible host (Table 1). Possible IA includes cases with host and clinical/radiologic criteria, but without microbiologic support.

Table 1: 2008 EORTC/MSG criteria for the diagnosis of invasive aspergillosis

Host factors

- Recent history of neutropenia (< 500 neutrophils/mm^3 for more than 10 days) temporally related to the onset of fungal disease
- Receipt of an allogeneic stem cell transplant

[9]Greene RE., et al. Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. Clin Infect Dis 2007;44:373-379

[10]Ascioglu et al. Defining opportunistic fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplant. An international concensus. Clin Infect Dis 2002; 34:7-14

[11]De Pauw B. et al. Revised definitions of invasive fungal disease from EORTC/IFI cooperative group and the NIAID MSG consensus group. Clin Infect Dis 2008; 46:1813-1821

- Prolonged use of corticosteroids (excluding patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone-equivalent for 13 weeks
- Treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF-α blockers, certain monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days
- Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency)

Clinical criteria

- Lower respiratory tract fungal disease
    - The presence of 1 of the following 3 signs on CT:
        - Dense, well-circumscribed lesions(s) with or without a halo sign
        - Air-crescent sign
        - Cavity
    - Tracheobronchitis
        - Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis
- Sinonasal infection
    - Imaging showing sinusitis plus at least 1 of the following 3 signs:
        - Acute localized pain (including pain radiating to the eye)
        - Nasal ulcer with black eschar
        - Extension from the paranasal sinus across bony barriers, including into the orbit
- CNS infection
    - One of the following 2 signs:
        - Focal lesions on imaging
        - Meningeal enhancement on MRI or CT

Mycological/Microbiologic criteria

- Direct test (cytology, direct microscopy, or culture):
    - Mould in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following:
        - Presence of fungal elements indicating a mould
        - Recovery by culture of a mould

- Indirect tests (detection of antigen or cell-wall constituents)
    - Galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or CSF

Adapted from[11] De Pauw B. et al. Revised definitions of invasive fungal disease from EORTC/IFI cooperative group and the NIAID MSG consensus group. Clin Infect Dis 2008; 46:1813-1821

Treatment of IA

Voriconazole, a triazole antifungal, was approved by the FDA in 2002 for the treatment of IA and is currently considered the drug of choice for IA based on a landmark study that compared voriconazole to amphotericin B deoxycholate[12]. Immunocompromised patients with allogeneic or autologous HSCT, hematologic cancer, aplastic anemia, myelodysplastic syndrome, or other immunocompromising conditions including AIDS, receipt of corticosteroid therapy, and solid-organ transplantation were enrolled. Definite/proven IA was defined as a clinically compatible illness plus one or more of the following:

- isolation of *Aspergillus* species from a normally sterile site
- hyphae consistent with the presence of *Aspergillus* in a biopsy specimen or aspirate, plus culture of *Aspergillus* from the same organ
- radiologic evidence of pulmonary lesions that were not attributable to other factors and a culture of BAL fluid that was positive for *Aspergillus* in a patient who had undergone allogeneic HSCT or who had a neutropenic hematologic condition
- tracheobronchial lesions confirmed by bronchoscopy, with a positive culture for *Aspergillus*

Probable IA was defined as a clinically compatible illness plus one or more of the following:

- hyphae consistent with the presence of *Aspergillus* in a biopsy specimen or aspirate but without culture
- In subjects who had undergone allogeneic HSCT or who had a neutropenic hematologic condition
    - the presence of a halo or an air-crescent sign on a chest
    - radiologic evidence of new pulmonary lesions that were not attributable to other factors with either hyphae consistent with *Aspergillus* in BAL fluid or sputum or a sputum culture that was positive for *Aspergillus*
    - clinical evidence of sinusitis, opacification of a sinus on CT or MRI, and positive histopathological examination or culture of *Aspergillus* from a lesion in the nose or paranasal sinus
- radiologic evidence of new pulmonary lesions that were not attributable to other factors and BAL fluid that was positive for *Aspergillus* on a smear or culture in a patient with another immunocompromising condition (other than lung transplantation)
- tracheobronchial lesions confirmed by bronchoscopy and a positive finding on histopathological or microscopic examination of a biopsy specimen or BAL fluid.

The study predated the publication of EORTC criteria but was designed under the aegis of an international steering committee that included EORTC, and the statistical analysis was conducted by the EORTC Data Center. The above eligibility criteria largely adhere

[12] Herbrecht R., et al. Voriconazole versus amphotericin B for the primary therapy of invasive aspergillosis. NEJM 2002;347:408-415

to the 2002 EORTC guidelines with two exceptions: patients with neutropenic hematologic disease or recipients of allogeneic HSCT with clinically compatible illness and a halo sign or air-crescent sign on chest CT and positive culture of BAL fluid were considered as having proven aspergillosis, and patients with neutropenic hematologic disease or recipients of allogeneic HSCT with clinically compatible illness and a halo sign or air-crescent sign on chest CT but without microbiologic support were considered as having probable aspergillosis. These patients would be classified as having probable and possible IA respectively by the updated 2008 criteria. Of note, GM determination was not widely available and was not obtained in this study.

In patients with proven and probable IA, global response at 12 weeks (complete or partial resolution of all attributable symptoms, signs, radiographic/bronchoscopic abnormalities present at baseline) was seen in 53% of voriconazole treated patients compared to 32% of amphotericin B treated patients. Survival at Day 84 was 71% in patients who received voriconazole compared to 58% in patients who received amphotericin B (Table 2).

Table 2: Overall Efficacy of voriconazole in the Primary Treatment of Proven and Probable Acute Invasive Aspergillosis

	Voriconazole (%)	Amphotericin B (%)	Difference (95% CI)
Global Response	76/144 (52.8%)	42/133 (31.6%)	21.2% (9.8, 32.6)
Survival at Day 84	102/144 (70.8%)	77/133 (57.9%)	12.9% (2.1, 24.2)

Prognosis of IA

The mortality of IA remains high. However, with the introduction of diagnostic criteria, improved diagnostic modalities that allow earlier diagnosis (including high resolution CT scan, and galactomannan antigen detection), and new treatment options, the 12 week mortality rates have decreased from 60-90% before 2003-2004, to 22% - 45%[4,6,13].

Objective of the Review

The objective of this review is to determine the acceptability of using serum GM as a biomarker for invasive aspergillosis in patients at risk because of hematologic malignancies and allogeneic HSCT recipients for the purpose of enrollment in clinical trials for treatment development. The sponsor's submission and the published literature are reviewed.

This review will address the following:

- Analytic sensitivity and specificity of the assay: analytic causes of false positive and false negative values

[13] Upton A. et al. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. Clin Infect Dis 2007;44:531-40

- Clinical utility of the assay in the proposed population: correlation with disease onset, activity, progression, resolution, and outcome
- Clinical sensitivity and specificity of the assay at different cut-off values used to define positive assay in patients with hematologic malignancy and recipients of HSCT
- Impact of accepting the proposed biomarker on clinical trial enrollment in non-inferiority trials evaluating antifungal therapy

Analytic sensitivity and specificity of serum Galactomannan antigen assay

Galactomannan is a cell wall polysaccharide component of *Aspergillus*. It is released by growing hyphae, and not by conidia that colonize the airways. Therefore, its detection in an individual more likely reflects invasive disease[2,14] .

Platelia™ *Aspergillus* EIA is a one-stage immuno-enzymatic sandwich micro-plate assay that detects galactofuranosyl-containing molecules using a rat monoclonal antibody directed at *Aspergillus* galactomannan. The result is expressed as Optical Density Index (ODI) or Galactomannan Index (GMI), which is the OD of the clinical specimen divided by the mean OD of 2 "cutoff" controls supplied in the kit. In 2003, the FDA cleared the Platelia™ assay as an aid for the diagnosis of IA "when used in conjunction with other diagnostic procedures such as microbiologic cultures, histologic examination of biopsy samples, and radiologic evidence of infection". The FDA accepted an index of ≥ 0.5 as a cutoff for positivity[15].

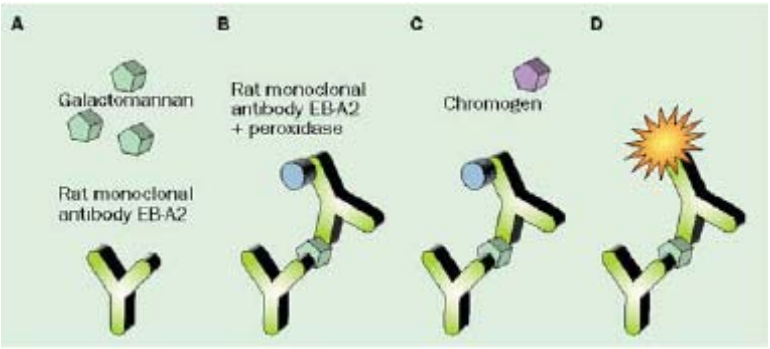

Figure 1 GM Platelia Assay.
Adapted from Mennink-Kersten M. et al. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. Lancet Infect Dis, 2004: 4:349-357.

[14] Klont R. et al. Utility of Aspergillus antigen detection in specimens other than serum specimens. Clin Infect Dis, 2004. 39(10): p. 1467-74.

[15] Platelia Package Insert, Bio-Rad, Marnes-La-Coquette

Causes of false positive and false negative results for the GM assay are listed in Tables 3 and 4.

Table 3: Causes of false positive GM assay

- Cross reaction with other fungi including *Penicillium*, *Geotrichum*, *Trichophyton*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Nigrospora oryzae*, and *Paecillomyces lilacinus* [2,16,17,18,19]
- Cross reaction with *Bifidobacterium*[16]
- Piperacillin-tazobactam and amoxicillin-clavulanate[16,20,21,22]
- Plasmalyte[16]

Table 4: Causes of false negative GM assay

- Receipt of mould active antifungal agent[23]
- Presence of neutralizing antibodies against *Aspergillus*[24]

[16] Mennink-Kersten M. et al., Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. Lancet Infect Dis, 2004;4:349-357.

[17] Wheat LJ, Walsh TJ. Diagnosis of invasive aspergillosis by galactomannan antigenemia detection using an enzyme immunoassay. Eur J Clin Microbiolol Infect Dis 2006; 27:245-251

[18] Cummings JR. et al. Cross reactivity of non Aspergillus fungal species in the Aspergillus galactomannan enzyme immunoassay. Diagnostic Microbiol and Infect dis 2007;59:113-115

[19]Bonini A. et al. Galactomannan detection in Geotrichum capitatum invasive infections: report of 2 new cases and review of diagnostic options. Diagnostic Microbiol Infect Dis 2008;62:450-452

[20] Aubry A. et al. Occurrence and kinetics of false positive Aspergillus galactomannan test results following treatment with beta lactam antibiotics in patients with hematological disorders. J Clin Microbiol 2006; 44:398-394

[21]Orlopp K. et al. False positivity of the Aspergillus galactomannan Platelia ELISA because of piperacillin/tazobactam treatment: does it represent a clinical problem? J antimicrobial chemother 2008;62:1109-1112

[22] Weisser M. et al. Galactomannan does not precede major signs on a pulmonary computerized tomography scan suggestive of invasive aspergillosis in patients with hematological malignancies. Clin Infect Dis 2005;41:1143-1149

[23] Marr, K.A., Antifungal therapy decreases sensitivity of the Aspergillus galactomannan enzyme immunoassay. Clin Infect Dis, 2005;40:1762-1769.

[24] Herbrecht R. et al. Aspergillus galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. J Clin Oncol 2002;20:1898-1906

False positive GM results

False positive serum GM due to infection with fungal species other than *Aspergillus* is not common in patients with hematologic malignancy or recipients of allogeneic HSCT. The PATH (Prospective Antifungal Therapy) alliance registry[6] reported 250 episodes of invasive fungal infections occurring in 234 patients who had received a HSCT. *Aspergillus* accounted for approximately 60% of all fungal infections, followed by *Candida* (approximately 25%), *Zygomyces* (approximately 7 %), and other moulds (approximately 7%). *Fusarium* accounted for about third of infections in the "other moulds" category. Only one patient had histoplasmosis.

In a report of 391 patients with hematologic malignancies who had proven or probable invasive mould infection[8], *Aspergillus, Zygomyces*, *Hyalohyphomycetes*, and other moulds (*Fusarium, Scedosporium, Scopulariopsis, Acremonium*) accounted for approximately 76%, 12%, 10%, and 2% of infections respectively. In another report of 121 patients who had received HSCT and were diagnosed with invasive fungal infections, approximately 25% were due to *Candida*, and 71% were due to *Aspergillus* species[7].

The combined results of these three reports are summarized in Table 5.

Table 5: Invasive fungal infections in patients with hematologic malignancy or recipients of HSCT

	Approximate Prevalence %
Yeast	25%
Candida species	25
Other yeast	< 1
Mould	75%
Aspergillus species	60
Zygomyces	7-8
Fusarium/Scedosporium	3-4
Other	2-5

These reports indicate that the fungi that cross react with *Aspergillus* in the GM assay are clinically uncommon, representing less than 2% of invasive fungal infections, and less than 5% of all mould infections. Therefore, for the purpose of clinical trial enrollment for IA treatment development, the impact of false positive GM indices due to infections with these fungi is expected to be minimal.

Cross reactivity with the bacterium *Bifidobacterium* is theorized to play a role in the observation of false positive titers in neonates and possibly in adults with severe mucositis due to bacterial gut dislocation.

Cross reactivity of certain beta-lactam antibiotics, namely piperacillin-tazobactam and amoxicillin-clavulanate is well described[15,16,19,21,23] . Patients receiving these antibiotics should be excluded from clinical trials of IA therapy. Patients receiving Plasmalyte should also be excluded.

False negative GM results
False negative results can occur in patients receiving antifungal agents with activity against *Aspergillus*[22], and in patients who have neutralizing antibodies against *Aspergillus*[22,23].

Serum GM Assay – Clinical Performance

Correlation of GM titer with disease activity, disease burden, and outcome

In a rabbit model of IA pulmonary infection, the GM antigen serum titer correlated with pulmonary fungal burden[22].

Multiple clinical studies demonstrate that GM antigen, when present, can appear in the serum 6.9 – 8.4 days prior to chest radiographic manifestations in 50-65% of patients, prior to onset of fever in 40% of patients, and prior to other clinical symptoms in 40% of patients[15,25,26,27,28]. Serum GM index progressively increases in patients who demonstrate progression of the disease [26,29], and decreases in patients who respond to appropriate therapy[3,22,23,26,27,28,30].

[25] Maertens J. et al. Autopsy-Controlled Prospective Evaluation of Serial Screening for Circulating Galactomannan by a Sandwich Enzyme-Linked Immunosorbent Assay for Hematological Patients at Risk for Invasive Aspergillosis. J Clin Microbiol 1999;37:3223-3228

[26] Sulahian A. et al. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units using a 4-year prospective study. Cancer 2001;91:311-8

[27] Maertens J. et al. Galactomannan and CT-based pre-emptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. Clin Infect Dis 2005;41:1242-59

[28] Maertens JA. et al. Optimization of the cutoff value for Aspergillus double sandwich enzyme immunoassay. Clin Infect Dis 2007; 44:1329-1336

[29] Boutboul F. et al. Invasive aspergillosis in allogeneic stem cell transplant recipients: increasing antigenemia is associated with progressive disease. Clin Infect Dis 2002;34:939-943

[30]Maertens J. et al. Galactomannan serves as a surrogate endpoint for outcome of pulmonary invasive aspergillosis in neutropenic hematology patients. Cancer 2009; 115:355-62

In addition, recent clinical studies indicate that persistence of GM in the serum correlates with mortality[28,30,31,32,33]. Patients who normalize GMI are significantly more likely to survive than patients who do not ($p < 0.001$)[31]. Resolution of GMI correlates with resolution of clinical signs and symptoms at 12 weeks (KCC 0.8857 – 0.9123) and at 4 months (KCC 0.9712)[30].

Clinical Sensitivity, Specificity, Positive and Negative Predictive values

Sponsor's analysis

The sponsor performed a literature review from 1996 to 2008, and summarized the relevant studies in Table 6. The majority of studies were in patients with hematologic malignancy or recipients of allogeneic HSCT, and defined IA according to 2002 EORTC/MSG criteria. The studies used variable cutoffs (CO) ranging from 0.5 to 1.5 to define a positive test. The frequency of GM testing in most of the studies was once or twice a week. When available, comparison of sensitivity and specificity at different positivity cutoffs were included.

Table 6: Summary of studies evaluating GM assay in the diagnosis of IA- Sponsor's submission

AuthorYear	Study design	Population, Number cases/controls	Reference standard used	ELISA test- index cutoff &frequency	SN, SP, PPV, NPV, ROC
Package insert		Adult HSCT, Heme Malignancy		CO ≥ 0.5	SN: 81% SP: 89% PPV: 55 NPV: 97
Rohrlich 1996	Prospective cohort 18-mo period	37 pediatric patients: 15 HSCT; 22 neutropenic Cases: 10 probable Controls: 27		CO ≥ 1.0 consecutive positive sample 2 x/week	SN: 100% SP: 92.5% PPV: 83.3 NPV: 100
Sulahian 1996	Prospective cohort 1992-1994	211 adult HSCT recipients Cases: 48: 25 proven; 15 probable; (8 suspected were not included in final analysis) Controls: 163	No EORTC/ MSG criteria	CO ≥ 0.8 1 x/week during 1st mo, then monthly	SN: 82.5% SP: 92.6% PPV: 73.3 NPV: 95.5
Bretagne 1997	Prospective cohort	50 at risk hematology patients	No EORTC/ MSG criteria	CO ≥ 0.8 consecutive	SN:100% SP: 76.2%

[31] Woods G. et al. Serum Aspergillus galactomannan antigen values strongly correlate with outcome of invasive aspergillosis. A study of 56 patients with hematologic cancer. Cancer 2007;110:830-4

[32] Maertens J, et al. Screening for galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. Blood 2001;97:1604-1610

	1994	Cases: 3 proven; 3 probable; (9 suspected were not included in final analysis) Controls: 21		positive samples 1 x/week	PPV: 54.5 NPV: 100
Machetti 1998	Prospective cohort 6-mo period	22 allogeneic HSCT recipients Cases: 5: 1 proven; 3 probable; (1 possible was not included in final analysis) Controls: 17	No EORTC/ MSG criteria	CO > 1.5 3 x/week during 1st mo, then 1 x/week	SN: 60% SP: 82.3% PPV: 50 NPV: 87.5
Maertens (1999)	Prospective cohort 1997-1998	186 patients with hematological malignancy; 92% neutropenic Cases: 27 autopsy proven Controls: 44	Autopsy	CO ≥ 1.0 consecutive positive samples 2 x/week	SN: 92.6% SP: 95.4% PPV: 92.6 NPV: 95.4
Sulahian 2001	Prospective cohort 1995-1998	347 children with hematological malignancies Cases:9: 5 proven and 4 probable 450 allogeneic stem cell transplant recipients Cases: 44, 22 proven, 22 probable	Mimicking EORTC/ MSG criteria	CO ≥1.5 consecutive positive samples 2 x/week	SN: 100% SP: 89.9% PPV: 20 NPV: 100 SN: 88.6% SP: 97.5% PPV: 79 NPV: 98
Maertens 2001	Prospective cohort 1997-2000	362 prolonged neutropenic episodes in 191 adult patients Cases: 39: 30 proven; 9 probable; (54 possible were not included in final analysis); Controls: 264	EORTC/ MSG criteria	CO ≥ 1.0 consecutive positive samples 2 x/week	SN: 89.7% SP: 98.1% PPV: 87.5 NPV: 98.4
Ulusakarya 2001	Prospective cohort 1997-1998	135 prolonged neutropenic hematology patients Cases: 18: 10 proven; 6 probable; (2 possible were not included in final analysis) Controls: 117	No EORTC/ MSG criteria	CO > 1.5 CO > 1.0 1 x/week	SN: 68.7% SP: 99.5% PPV: 68.7 NPV: 99.5 SN: 100% SP: 92.3% PPV: 62.5 NPV: 100
Maertens 2002	Prospective Cohort 1997-2001	100 myeloablative adult (< 16y) HSCT Cases: 18 autopsy proven Controls: 73	EORTC/ MSG criteria	CO ≥ 1.0 consecutive positive samples 2 x/week	SN: 94.4% SP: 98.8% PPV: 94.4 NPV: 98.8
Herbrecht 2002	Prospective 1997-2001	4 cohorts of adult and pediatric onco-hematology patients - FUO (n=261) Cases: 1 possible - suspected pulmonary infection (n=297)	EORTC/ MSG criteria	CO ≥ 1.5 Variable frequency	SN: 100% SP: 95% PPV: 7 NPV: 100 SN: 27.5% SP: 98.6%

		Cases: 25 proven; 67 probable; 53 possible - suspected extrapulmonary (n=28) Cases: 5 proven - surveillance in transplant recipients (n=211) Cases: 1 proven; 1 possible **Overall (n=797)**			PPV: 95.2 NPV: 58.3 SN: 40% SP: 100% PPV: 100 NPV: 88 SN: 100% SP: 91.3% PPV: 10 NPV: 100 **Overall** SN: 29.4% SP: 94.8% PPV: 57.7 NPV: 84.9
Becker 2003	Prospective cohort 1999-2000	160 adult patients with hematological malignancies and neutropenic Cases: 17: 2 proven; 11 probable; 4 suspected Controls: 117	Modified EORTC/ MSG criteria[1]	CO ≥ 1.0 consecutive positive samples 2 x/week	SN: 47% SP: 93% PPV: 73 NPV: 82
Pinel 2003	Prospective cohort 1998-2001	807 Hematology patients and intensive care patients Cases: 56: 3 proven; 31 probable; (22 possible were not included in final analysis) Controls: 751	EORTC/ MSG criteria	CO ≥ 1.0 consecutive positive samples 1 or 2 x/week	SN: 50% SP: 99.6% PPV: 85 NPV: 97.7
Rovira 2004	Prospective cohort 1991-2001	74 adult allogeneic HSCT recipients Cases: 8: 1 proven; 5 probable; 2 possible Controls: 66	EORTC/ MSG criteria	CO ≥ 1.5 2 x/week	SN: 75% SP: 100% PPV: 100 NPV: 97
Maertens 2004	Prospective cohort 2001-2002	124 prolonged neutropenic episodes in 104 adult patients Cases: 29: 16 proven; 13 probable; (21 possible were not included in final analysis) Controls: 74	EORTC/ MSG criteria	CO ≥ 0.5 consecutive positive samples 2 x/week	SN: 96.5% SP: 98.6% PPV: 98.6 NPV: 98.4
Kawazu 2004	Prospective cohort 2001-2002	96 adult patients with hematological malignancies: 149 consecutive neutropenic and/or GvHD episodes Cases: 11: 9 proven; 2 probable; (13 possible were not included in final analysis) Controls: 125	EORTC/ MSG criteria	CO ≥ 0.6 CO ≥ 0.5 consecutive positive samples 1 x/week	SN: 100% SP: 93% PPV: 55 NPV: 100 SN: 100% SP: 84% PPV: 35 NPV: 100
Marr 2004	Prospective cohort	67 adult allogeneic stem cell transplant recipients	Modified EORTC/ MSG criteria[2]	CO ≥ 0.5 Variable frequency	SN: 87.5% (no AF) SP: 77.1% (no AF)

		Cases: 24: 13 proven; 11 probable; (8 possible were not included in final analysis) Controls: 35			SN: 81.8% (AF) SP: 77.1% (AF)
Pazos 2005	Prospective cohort 2001-2002	40 adult patients with hematological malignancies Cases: 11: 5 proven; 3 probable; (3 possible were not included in final analysis) Controls: 29	EORTC/ MSG criteria	CO ≥ 1.5 2 x/week	SN: 87.5% SP: 89.6% PPV: 70 NPV: 96.3
Marr 2005	Prospective cohort	315 patients with hematologic disorders: neutropenic and HSCT recipients Cases: 46: 20 proven; 26 probable Controls: 269	EORTC/ MSG criteria	CO ≥ 1.5 CO ≥ 1.0 CO ≥ 0.5	SN: 43.4% SP: 93.3% PPV: 52.6 NPV: 90.6 SN: 47.8% SP: 88.4% PPV: 41.5 NPV: 90.8 SN: 69.5% SP: 69.8% PPV: 28.3 NPV: 93
Yoo 2005	Prospective cohort 2002-2003	128 febrile neutropenic patients unresponsive to broad-spectrum antibiotics Cases: 14: 2proven; 12 probable Controls: 114	EORTC/ MSG criteria	CO ≥ 0.5 consecutive positive samples 2 x/week	SN: 85.7% SP: 78% PPV: 32.4 NPV: 97.8 ROC: .85
Busca 2006	Prospective cohort 2002-2004	74 adult allogeneic HSCT recipients Cases: 9: 2 proven; 7 possible	EORTC/ MSG criteria	CO ≥ 1.0 2 x/week	SN: 100% SP: 93% PPV: 64 NPV: 100
Suankrtay 2006	Prospective cohort 2002-2004	50 episodes in 44 adult patients with hematological disorders at risk: prolonged neutropenia and HSCT recipients Cases:17: 5 proven; 12 probable Controls:	EORTC/ MSG criteria	CO ≥ 1.0 CO ≥ 0.75 CO ≥ 05 consecutive positive samples 1 or 2 x/week	SN: 88.2% SP: 97% PPV: 93.8 NPV: 94.1 ROC: .98 SN: 94.1% SP: 78.8% PPV: 69.6 NPV: 96.3 SN: 94.1% SP: 66.7% PPV: 59.3 NPV: 95.7
Steinbach 2007	Prospective cohort 2003-2004	64 pediatric HSCT recipients with neutropenia and/or GVHD Cases: 1 probable Controls: 63	EORTC/ MSG criteria	CO ≥ 0.5 consecutive positive samples 2 x/week	SN: NR SP: 87.3%
Maertens 2007	Retrospective multi-center study	239 prolonged neutropenic episodes in 203 adult patients Cases: 38: 19 proven; 19 probable; (no possible cases	EORTC/ MSG criteria	CO ≥ 0.5 consecutive positive samples 2 x/week	SN: 92.1% SP: 97.5% PPV: 87.5 NPV: 98.4 ROC: .843

		included) Controls: 201			& .808
Lai 2007	Prospective cohort 2004-2005	189 patients at risk for IA; mixed population but predominantly hematology-oncology Cases: 40: 5 proven; 9 probable; (26 possible were not included in final analysis) Controls: 149	EORTC/ MSG criteria	CO ≥ 1.5 consecutive positive samples Frequency not mentioned	SN: 78.6% SP: 93.9% PPV: 55 NPV: 97.9
Yoo 2007	2004-2005	78 febrile neutropenic patients unresponsive to broad-spectrum antibiotics Cases: 22: 7 probable; (15 possible were not included in final analysis) Controls: 56	EORTC/ MSG criteria	CO ≥ 0.5 consecutive positive samples 2 x/week	SN: 71.4% SP: 98.2% PPV: 83.3 NPV: 96.4
Penack 2008	Prospective cohort	200 adult patients with hematological malignancies and prolonged neutropenia or HSCT recipients Cases: 17 proven and probable	EORTC/ MSG criteria	CO ≥ 0.5 2 x/week	SN: 100% SP: 93.8% PPV: 60.7 NPV: 100
Fortun 2001	Retrospective case-control study	Liver transplant recipients		CO ≥ 1 (+ retest)	SN: 55.6% SP: 93.9% PPV: 71.4 NPV: 88.6
Kwak 2004	Prospective cohort 2001-2002	154 liver transplant recipients Cases: 1 probable Controls: 153	EORTC/ MSG criteria	CO ≥ 0.5 (+ retest) 2 x/week	SN: NR SP: 87% PPV: NR NPV: NR
Husain 2004	Prospective cohort 2001-2002	70 lung transplant recipients Cases: 12: 9 proven; 3 probable Controls: 58	EORTC/ MSG criteria	CO ≥ 0.5 (+ retest) 2 x/week	SN: 25% SP: 75.8% PPV: 17.6 NPV: 83

Sensitivity, specificity, and positive predictive values varied considerably depending on the cutoff used, patient characteristics, and IA prevalence at the centers conducting the study. The sensitivity of two consecutive GM indices ≥ 0.5 ranged from 27 to 100%, specificity ranged from 67 to 100%, PPV from 28 to 100%, and NPV from 58 to 100%.

Published literature

The reviewer performed a PubMed search using "galactomannan" as the search term on March 27, 2009. A total of 711 articles in the English language were retrieved. Twenty-seven (27) articles were selected after excluding

- general review articles
- articles not about testing
- duplicates
- case reports
- studies that did not use Platelia assay

- studies that did not report diagnostic accuracy
- studies that addressed cross-reactivity only
- studies that did not specify the nature of immunosuppression
- studies that used a cutoff GM index other than 0.5, 1.0 or 1.5
- studies that evaluated GM in samples other than serum
- studies that did not use EORTC criteria, and
- studies evaluating PCR only

Two of the 27 articles were meta-analyses, 23 reported studies of patients with hematologic malignancies or recipients of HSCT and two reported studies of patients with solid organ transplants. The 23 articles included in the analysis are listed in Appendix 1.

Pfieffer et al.[33] performed a meta-analysis on studies published between 1966 and February 2005. They excluded review articles, case reports, assays not based on Platelia™, and duplicate articles. Their analysis included 27 articles evaluating GM for IA diagnosis in a variety of hosts (hematologic malignancy, recipients of HSCT, recipients of solid organ transplant, and pediatric and adult patients). Diagnostic criteria conformed to EORTC guidelines in 19 studies. Some studies demonstrated an incorporation bias, since GM is part of the microbiologic reference standard for diagnosis of probable IA in the EORTC criteria. There was considerable heterogeneity of the population tested, and in the cutoff used to define positivity and the number of samples required for positivity. Five studies used 0.5 as the cutoff for positivity (1 study required a single positive GM, 4 studies required consecutive positive GM), 13 studies used 1.0 as the cut-off (6 required a single positive GM, 7 required consecutive positive GM), and 11 studies used 1.5 as the cut-off (4 required a single positive GM, 7 required consecutive positive GM).

Selected findings are summarized in Tables 7, 8 and 9

Table 7: GM assay performance, published meta-analyses 27 studies, patients with proven and probable IA

Cutoff	Sensitivity % (95% CI)	Specificity% (95% CI)
0.5	79 (69-87)	86 (83-89)
1.0	65 (57-72)	94 (92-95)
1.5	48 (41-56)	95 (93-96)

Adapted from Pfeiffer et al.

Table 8: GM assay performance, published meta-analyses 27 studies, patients with proven IA

Cutoff	Sensitivity % (95% CI)	Specificity% (95% CI)
0.5	27 (6-61)	79 (74-83)

[33] Pfeiffer CD. et al. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis 2006;42:1417-1427

1.0	79 (71-87)	87 (85-88)
1.5	68 (58-76)	92 (91-93)

Adapted from Pfeiffer et al.

Table 9: GM performance, published meta-analyses 27 studies, all cutoffs, patients with proven IA

Population – all cutoffs	Sensitivity % (95% CI)	Specificity % (95% CI)
Hematologic malignancy	70 (62-77)	92 (90-93)
HSCT recipient	82 (70-90)	86 (83-88)
Solid organ transplant recipients	22 (3-60)	84 (78-88)

Adapted from Pfeiffer et al.

The Cochrane collaboration[5] reviewed articles published between August 2005 and April 2007. After excluding all articles that were narrative reviews, were not original research, did not describe diagnostic accuracy, used an obsolete ELISA assay, or were duplicate publications, 30 articles were included in the meta-analysis review. All 30 studies were in hematology patients. The median prevalence of proven and probable IA was 12% (range 0.8% to 44%).

Of the 30 studies, five reported data on more than one cutoff value and four reported results when subsequent positive results were available versus single sample results. Seven studies (901 patients) reported results for an Optical Density Index (ODI) of 0.5 as cut-off value. The results are summarized in Tables 10 and 11.

Table 10: Summary of test performance for different cutoffs (adapted from Cochrane review) – sensitivity and specificity

Subgroup	Variable 95% CI	N of participants (studies)	Prevalence range
Cutoff 0.5	Sensitivity 79 (61-93) Specificity 82 (71-92)	901 (7)	Median 9.9% (0.8 to 34%)
Cutoff 1.0	Sensitivity 71 (61-81) Specificity 90 (87-94)	1744 (12)	Median 12.4% (0.8 to 44%)
Cutoff 1.5	Sensitivity 62 (45-79) Specificity 95 (92-98)	1600 (17)	Median 7.4% (0.8 to 34%)

Adapted from Lefflang et al. 3 studies included all 3 cutoffs and 2 studies included cutoffs of 1.0 and 1.5

Specificity increases and sensitivity decreases when two consecutive samples are required to indicate a positive GM result.

Table 11: Effect of number of samples - Cochrane review

Cutoff		Studies	Sensitivity (95% CI)	Specificity (95% CI)
0.5	Any sample	7	78 (61-89)	81 (72-88)
	Single sample	1	94 (82-100)	61 (3-91)

	Subsequent sample	6	74 (60-88)	83 (77-90)
1.0	Any sample	12	75 (59-86)	91 (84-95)
	Single sample	6	83 (70-95)	84 (74-84)
	Subsequent sample	6	61 (51-81)	95 (91-98)
1.5	Any sample	17	64 (50-77)	95 (91-97)
	Single sample	10	62 (44-81)	92 (87-98)
	Subsequent sample	7	68 (49-87)	97 (94-99)

Adapted from Lefflang et al.

Reviewer's analysis – Hematologic malignancy and HSCT recipients

The reviewer retrieved 23 studies evaluating the performance of serum GMI assay in patients with hematologic malignancies or recipients of HSCT. The prevalence of IA (proven and probable) ranged from 2.6% to 34.0%, with a median of 11.5% and mean of 14.0 +/- 8.5%. Reported sensitivity from individual studies ranged from 12% to 100%, specificity from 34% to 100%, negative predictive value from 73% to 100%, and positive predictive value from 12% to 100%.

Statistical analyses were performed by Dr. Cheryl Dixon, Division of Biometrics IV. A summary of the results is shown in Tables 12, 13, and 14.. When available, assay performance in the same population but at different cutoffs was compared. In addition, to eliminate incorporation bias, cases designated as probable solely based on a positive GM test were not included in the analyses.

Table 12: Serum GMI assay performance in patients with hematologic malignancy or recipients of HSCT

	Single > 0.5	**Single > 1.0**	**Single > 1.5**	**Consec > 0.5**	**Consec > 1.0**	**Consec >1.5**
Rohrlich, 1996					SN 10/10 (100) SP 25/27 (92.5) PPV 10/12 (83.3) NPV 25/25 (100)	
Machetti, 1998						SN 3/4 (75) SP 14/17 (82.3) PPV 3/6 (50) NPV 14/15 (93
Ulusakarya, 2000		SN 16/16 (100) SP 108/117 (92.3) PPV 16/25 (64.0)	SN 11/16 (68.7) SP 112/117 (95.7) PPV 11/16 (68.7)			

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	Single > 0.5	**Single > 1.0**	**Single > 1.5**	**Consec > 0.5**	**Consec > 1.0**	**Consec >1.5**
		NPV 108/108 (100)	NPV 112/117 (95.7)			
Maertens, 2001		SN 36/39 (92.3) SP 250/264 (94.6) PPV 36/50 (72.0) NPV 250/253 (98.8)			SN 35/39 (89.7) SP 259/264 (98.1) PPV 35/40 (87.5) NPV 259/263 (98.4)	
Sulahian, 2001 Pediatric population Adult population						SN 9/9 (100) SP 304/338 (89.9) PPV 9/43 (20.9) NPV 304/304 (100) SN 39/44 (88.6) SP 396/406 (97.5) PPV 39/49 (79.6) NPV 396/401 (98.8)
Herbrecht, 2002			SN 31/98 (31.6) SP 607/640 (94.8) PPV 31/64 (48.4) NPV 607/674 (90.1)			
Maertens, 2002		SN 17/18 (94.4) SP 70/82 (85.4) PPV 17/29 (58.6) NPV 70/71 (98.6)			SN 17/18 (94.4) SP 81/82 (98.8) PPV 17/18 (94.4) NPV 81/82 (98.8)	
Becker, 2003		SN 10/17 (59) SP 33/44 (75) PPV 10/21 (48) NPV 33/40 (83)	SN 3/17 (18) SP 37/44 (84) PPV 3/10 (30) NPV 37/51 (73)		SN 8/17 (47) SP 41/44 (93) PPV 8/11 (73) NPV 41/50 (82)	SN 2/17 (12) SP 42/44 (95) PPV 2/4 (50) NPV 42/57 (74)
Pinel, 2003					SN 17/34	

	Single > 0.5	**Single > 1.0**	**Single > 1.5**	**Consec > 0.5**	**Consec > 1.0**	**Consec >1.5**
					(50.0) SP 748/751 (99.6) PPV 17/20 (85) NPV 748/765 (97.7)	
Buchheidt, 2004						SN 1/7 (14.3) SP 92/93 (98.9) PPV 1/2 (50.0) NPV 92/98 (93.9)
Maertens, 2004	SN 28/29 (96.5) SP 63/74 (85.1) PPV 28/39 (71.8) NPV 63/64 (98.4)	SN 27/29 (93.1) SP 74/74 (100.0) PPV 27/27 (100) NPV 74/76 (97.4)	SN 24/29 (82.7) SP 74/74 (100) PPV 24/24 (100) NPV 74/79 (93.7)	SN 28/29 (96.5) SP 73/74 (98.6) PPV 28/29 (96.5) NPV 74/71 (98.6)	SN 23/29 (79.3) SP 74/74 (100) PPV 29/29 (100) NPV 74/80 (92.5)	SN 18/29 (62.0) SP 74/74 (100) PPV 18/18 (100) NPV 74/85 (87.1)
Kawazu, 2004	SN 11/11 (100.0) SP 42/125 (34) PPV 11/94 (12) NPV 42/42 (100.0)	SN 11/11 (100) SP 107/125 (86) PPV 11/29 (38) NPV 107/107 (100)	SN 9/11 (82) SP 112/125 (90) PPV 9/22 (41) NPV 112/114 (98)	SN 11/11 (100) SP 105/125 (84) PPV 11/31 (35) NPV 105/105 (100)	SN 7/11 (64) SP 122/125 (98) PPV 7/10 (70) NPV 122/126 (97)	SN 5/11 (45) SP 122/125 (98) PPV 5/8 (63) NPV 122/128 (95)
Rovira, 2004			SN 4/6 (66.7) SP 66/66 (100) PPV 4/4 (100) NPV 66/68 (97.1)			
Marr, 2005	SN 32/46 (69.5) SP 188/269 (69.8) PPV 32/113 (28.3) NPV 188/202	SN 22/46 (47.8) SP 238/269 (88.4) PPV 22/53 (41.5) NPV 238/262 (90.8)	SN 20/46 (43.4) SP 251/269 (93.3) PPV 20/38 (52.6) NPV 251/277 (90.6)			

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	Single > 0.5	**Single > 1.0**	**Single > 1.5**	**Consec > 0.5**	**Consec > 1.0**	**Consec >1.5**
	(93.1)					
Pazos, 2005						SN 7/8 (87.5) SP 26/29 (89.6) PPV 7/10 (70) NPV 26/27 (96.3)
Yoo, 2005				SN 12/14 (85.7) SP 89/114 (78) PPV 12/37 (32.4) NPV 89/91 (97.8)		
Busca, 2006					SN 2/2 (100) SP 60/65 (92.3) PPV 2/7 (28.6) NPV 60/60 (100)	
Suankrtay, 2006				SN 16/17 (94.1) SP 22/33 (66.7) PPV 16/27 (59.3) NPV 22/23 (95.7)	SN 15/17 (88.2) SP 32/33 (97) PPV 15/16 (93.8) NPV 32/34 (94.1)	SN 13/17 (76.5) SP 33/33 (100) PPV 13/13 (100) NPV 33/37 (89.2)
Foy 2007	SN 6/12 (50.0) SP 102/109 (93.6) PPV 6/13 (46.0) NPV 102/108 (94.4)					
Lai, 2007			SN 11/14 (78.6) SP 140/149 (93.9) PPV 11/20 (55) NPV 140/143			

Serum Galactomannan Platelia Assay for the diagnosis of Invasive Aspergillosis in Patients with hematologic malignancy or recipients of HSCT

	Single > 0.5	**Single > 1.0**	**Single > 1.5**	**Consec > 0.5**	**Consec > 1.0**	**Consec >1.5**
			(97.9)			
Maertens, 2007	SN 37/38 (97.4) SP 182/201 (90.5) PPV 37/56 (66.1) NPV 182/183 (99.5)	SN 31/38 (81.6) SP 194/201 (96.5) PPV 31/38 (81.6) NPV 194/201(96.5)	SN 29/38 (76.3) SP 196/201 (97.5) PPV 29/34 (85.3) NPV 196/205 (95.6)	SN 35/38 (92.1) SP 196/201 (97.5) PPV 35/40 (87.5) NPV 196/199 (98.5)		
Yoo, 2007				SN 5/7 (71.4) SP 55/56 (98.2) PPV 5/6 (83.3) NPV 55/57 (96.5)		
Penack, 2008	SN 17/17 (100) SP 172/183 (93.8) PPV 17/28 (60.7) NPV 172/172 (100)					
Overall	**SN 131/153 (85.6) SP 749/961 (77.9) PPV 131/343 (38.2) NPV 749/771 (97.1)**	**SN 170/214 (79.4) SP 1074/1176 (91.3) PPV 170/272 (62.5) NPV 1074/1118 (96.1)**	**SN 142/275 (51.6) SP 1595/1685 (94.7) PPV 142/232 (61.2) NPV 1595/1728 (92.3)**	**SN 107/116 (92.2) SP 540/603 (89.6) PPV 107/170 (62.9) NPV 540/559 (96.6)**	**SN 134/177 (75.8) SP 1442/1465 (98.4) PPV 134/157 (85.4) NPV 13442/1485 (97.1)**	**SN 97/146 (66.4) SP 1103/1159 (95.2) PPV 97/153 (63.4) NPV 11103/1152 (95.7)**

Table 13: Summary - Effect of cutoff and number of samples – mean and 95% CI

Cutoff	# Samples	# Studies	Sensitivity	Specificity	PPV	NPV
0.5	Single	6	86 (64, 100)	78 (53, 100)	47 (23, 72)	98 (94, 100)
	Consecutive	6	90 (81, 100)	87 (73, 100)	66 (37, 95)	98 (96, 99)

1.0	Single	8	84 (67, 100)	90 (83, 96)	63 (45, 81)	96 (91, 100)
	Consecutive	9	79 (63, 95)	97 (94, 99)	80 (63, 96)	96 (91, 100)
1.5	Single	9	61 (43, 79)	94 (90, 98)	65 (45, 84)	92 (86, 99)
	Consecutive	9	62 (38, 87)	95 (90, 99)	65 (44, 85)	92 (86, 98)

Table 14: Summary - Effect of cutoff and number of samples – median and interquartile range

Cutoff	# Samples	# Studies	Sensitivity	Specificity	PPV	NPV
0.5	Single	6	97 (65-100)	88 (61-94)	53 (24-68)	99 (94-100)
	Consecutive	6	93 (83-97)	91 (75-98)	71 (34-90)	98 (96-99)
1.0	Single	8	93 (65-99)	90 (86-96)	61 (43-79)	98 (92-100)
	Consecutive	9	88 (57-97)	98 (93-99)	85 (72-94)	98 (93-99)
1.5	Single	9	69 (38-80)	95 (92-99)	55 (45-93)	96 (90-98)
	Consecutive	9	75 (30-88)	98 (90-99)	63 (50-90)	94 (88-98)

The overall results of this analysis are in agreement with the Cochrane Collaboration analysis. As the cutoff for positivity increases, the specificity of the assay increases but the sensitivity decreases. The NPV is high at any cut-off. The PPV is highest for two consecutive samples at a cutoff of 1.0.

Reviewer's analysis – Hematologic malignancy and HSCT recipients with clinical/radiologic features suggestive of fungal infection

The studies cited above reported the performance of serum GM detection in all patients at risk for IA because of hematologic malignancy or receipt of HSCT regardless of the presence or absence of other clinical/radiologic manifestations. The prevalence of IA in the subset of patients who also have clinical or radiologic manifestations suggestive of invasive fungal infection (the population expected to enroll in clinical trials) is expected to be higher. The predictive values of serum GM are also accordingly expected to be higher.

In two studies of immunocompromised patients who presented with a "halo sign" on chest CT and underwent needle biopsy, the prevalence of IA in neutropenic patients was approximately 45%[34,35]. Other studies in patients with hematologic malignancy or recipients of HSCT who had radiologic signs on chest CT and had the diagnosis confirmed at autopsy or pre-mortem by biopsy culture reported prevalence of 65%[36],

[34] Primack S., et al. Pulmonary nodules and the CT halo sign. Radiology 1994; 190:513-515

[35] Jin Wong H., et al. Invasive pulmonary aspergillosis: prediction at thin section CT in patients with neutropenia– a prospective study. Radiology 1998; 208:777-782

[36] Becker MJ et al. Galactomannan detection in computerized tomography-based broncho-alveolar lavage fluid and serum in haematological patients at risk for invasive pulmonary aspergillosis. Br J Haematol 2003; 121: 448-457.

45%[37], 31% [24], 35% [3], 30%[38], 14.9%[39], and 31.3%[40]. The pooled estimate and 95% CI using a random effects model by De Sermonian and Laird is 36.0% (26.1, 45.9). However, this estimate is quite conservative, because diagnostic procedures are not always feasible to obtain in these patients and even when obtained, the sensitivity of cultures is poor. In one study[41], only 36% of patients proven to have IA at autopsy were diagnosed ante-mortem by culture or histology. In another study[37], the sensitivity of either culture or microscopy in patients with proven IA was approximately 50%.

In support of the above, an analysis of patients with hematologic malignancy or recipients of HSCT who had IA at autopsy, the sensitivity, specificity, PPV and NPV of two consecutive positive GM at a cutoff of $\geq$ 1.0 performed ante-mortem were approximately 93%, 95%, 93%, and 95% respectively[25]. In addition, the performance of the serum GM assay in at risk hematology patients who had radiologic manifestations was evaluated in two studies (Table 15). Herbrecht[24]evaluated 294 at risk hematology patients who had a suspected pulmonary infection. At a cutoff of 1.5 on a single sample, GM assay sensitivity was 27.5%, specificity 99%, PPV 95.2%, and NPV 58.3%. In 27 patients who had a suspected extra-pulmonary infection, sensitivity was 40%, specificity 100%, PPV 100%, and NPV 88%. In the same study, when GM assay was done for surveillance in transplant recipients (211 patients), or patients with fever of unknown origin (261 patients), the PPV was only 10% and 7% respectively. Penack[3] (2008) evaluated 48 neutropenic patients with evidence of pulmonary infiltrates on high resolution CT scan. At a cutoff of 0.5 on a single sample, sensitivity was 78.3%, specificity and PPV were 100%, and NPV was 83%. In 55 patients who had persistent fever after 6 days of antibiotic therapy (a clinical indicator suggestive of fungal infection), sensitivity was 91.3% whereas specificity and PPV of the assay were 100% and NPV was 94.1%.

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[38] Verweij PE, et al. Comparison of antigen detection and PCR assay using bronchoalveolar lavage fluid for diagnosing invasive pulmonary aspergillosis in patients receiving treatment for hematological malignancies. J Clin Microbiol 1995; 33: 3150-3153.

[39] Bergeron, A et al. Contribution of Galactomannan antigen detection in BAL to the diagnosis of pulmonary aspergillosis in patients with hematologic malignancies. Chest 2010;137:410-415

[40] Maertens J et al. Galactomannan and computerized tomography based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. Clin Infect Dis 2005;41:1242-50

[41] Subira M et al. Clinical applicability of the new EORTC/MSG classification for invasive pulmonary aspergillosis in patients with hematological malignancies and autopsy-confirmed invasive aspergillosis. Ann Hematol 2003; 82:80-82

Table 15: Effect of presence of culture confirmation, clinical symptoms or radiologic manifestations

	Sensitivity	Specificity	PPV	NPV
Pulmonary infiltrates (cutoff 1.5)	27.5%	99.0%	95.2%	58.3%
Pulmonary infiltrates (cutoff 0.5)	78.3%	100%	100%	83%
Suspected extra-pulmonary infection (cutoff 1.5)	40%	100%	100%	88%
Fever > 6 days not responsive to antibiotics (cutoff 0.5)	91.3%	100%	100%	94.1%

As expected, the PPV of serum GM assay increases significantly where the prevalence of IA is higher. Therefore the PPV is higher when performed in a subset of patients with clinical or radiologic indicators of fungal infection than when assay is performed in all patients at risk.

The specificity of GM assay is likely to be underestimated. The poor sensitivity of other diagnostic methods for IA will lower the estimated specificity of serum GM because subjects deemed positive by GM will not be considered positive by the reference diagnostic modalities.

Table 16 summarizes the estimated PPV and NPV in patients at risk due to hematologic malignancy or recipients of HSCT,with clinical or radiologic manifestations. These values were determined from the median sensitivity and specificity, and the upper and lower bound of the prevalence CIs.

Table 16: Estimated PPV and NPV in hematology patients with clinical or radiologic manifestations of an invasive fungal infection for the range of estimated prevalences

Prevalence			30%		40%		50%	
	SN %	SP %	PPV %	NPV %	PPV %	NPV %	PPV %	NPV %
Cons 0.5	93	91	81.6	96.8	87.3	95.5	91.2	92.8
Cons 1.0	88	98	95.0	95.0	96.7	92.5	97.8	89.1

(SN and SP as calculated in Table 13)

As indicated in Table 16, the PPV is above 90% when two consecutive samples of ≥ 1.0 define positivity at all prevalence within the 95% CI range, whereas the PPV of two consecutive samples of ≥ 0.5 is above 90% at a prevalence of 50%.

If the lower bound of the 95% CI for sensitivity and specificity are used for calculation, the PPV of two consecutive samples of ≥ 1.0 is 81.8% at a prevalence of 30%, and 94% at a prevalence of 60%. Of note, the study that evaluated the performance GM assay at a cutoff of ≥ 1.0 obtained ante-mortem in patients demonstrated to have IA at autopsy reported a PPV of 93%[25]. For the purpose of enrollment in non-inferiority treatment trials, it would be more appropriate to accept serum GM cutoff of 1.0 rather 0.5.

Three trials[42,43,44] evaluated the performance of serum GMI in the same patients at different cutoffs. Serum GM index was ≥ 1.0 at the time of IA diagnosis in 45/57 (79.9%) of patients, and ≥ 0.5 in 55/57 (96.5%) of patients.

Assay performance in immunocompromised non-hematology patients

Two studies that met the reviewer's criteria (please refer to the Clinical Performance section, Published Literature) were retrieved, one in liver transplant recipients, and one in lung transplant recipients. The study by Fortun (2001) used a single sample at a cutoff of 1.0 to define positivity. The study by Hussain (2004) used a single sample at a cutoff of 0.5 to define positivity. Prevalence of IA was 5.8% and 17.0% respectively.

Table 17: Assay performance in patients with solid organ transplants.

	Population	Assay performance (%)
Fortun 2001 Single sample Cutoff 1.0	Liver transplant	SN 5/9 (55.6%) SP 31/33(93.9%) PPV 5/7(71.4%) NPV 31/35 (88.6)
Hussain 2004 Single sample Cutoff 0.5	Lung transplant	SN 3/12 (25.0%) SP 44/58 (75.8%) PPV 3/17 (17.6%) NPV 44/53 (83.0%)

This data is insufficient for analysis.

Impact of accepting Galactomannan antigen assay as an invasive aspergillosis biomarker in clinical trials of drug development

[42] Maertens J. et al. Prospective clinical evaluation of lower cut-offs for galactomannan detection in adult neutropenic cancer patients and haematological stem cell transplant recipients. Br J Haematol 2004; 126:852-860

[43] Kawazu M, et al. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1-3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. J Clin Microbiol 2004; 42:2733-41

[44] Suankrtay C. et al. Galactomannan antigenemia for the diagnosis of invasive aspergillosis in neutropenic patients with hematological disorders. J Medical Association of Thailand 2006; 89:1851-1858.

Future treatment trials for IA are likely to be non-inferiority (NI) design, with voriconazole (the current drug of choice) as the comparator. Non-inferiority trials require assay sensitivity in order to be interpretable. Assay sensitivity is the ability of the trial to detect a specified difference between treatments, if such a difference was present. It is based on three considerations (1) historical evidence of sensitivity of drug effect, (2) similarity of the new NI trial to the historical trials (constancy assumption) and (3) quality of the new trial (ruling out defects that would tend to minimize differences between treatments). Regarding this last point, a test with a high positive predictive value (high pre-test probability) and high specificity would be needed to ensure that patients who actually have the disease are enrolled. However, this may not be a concern if the future NI trial enrolls a similar population of subjects as the historical data on which assay sensitivity and the estimate of the historical evidence of drug effect is based (i.e., if points 1 and 2 above regarding assay sensitivity are met).

To make the assumption of constancy and to estimate the historical sensitivity of drug effect, it is important to conduct a full NI margin justification. This would require knowledge of the design of the future NI study either from a protocol or a guidance document. However, the landmark study that compared voriconazole to amphotericin B[12] would most likely make up the bulk of any future NI margin justification. The data from that study suggest that there is adequate historical sensitivity to drug effect using a population similar to that which would be enrolled in a future study using the galactomannan as one possible entry criterion. Although the voriconazole data included patients who would be classified in a different category using the 2008 EORTC/MSG criteria, the data shows similar drug effect across all categories of subjects as shown in Table 18.

Table 18: Voriconazole drug effect according to IA diagnosis classification

IA category	Voriconazole (V) Global Response Rate	Active Control (C) Global Response Rate	Difference (V-C)	95% CI of V-C Difference
Proven	30/67 (44.8%)	8/41 (19.5%)	25.3%	(8.3, 42.3)
Probable	46/77 (59.7%)	34/92 (37.0%)	22.7	(8.0, 37.4)
Proven and probable*	76/144 (52.8%)	42/133 (31.6%)	21.2%	(9.8, 32.6)
Possible	22/53 (41.5%)	12/61 (23.1%)	21.8%	(5.2, 38.4)
Proven, probable, and possible**	98/197 (49.7%)	54/194(27.8%)	21.9%	(12.5, 31.3)

* Modified Intention-to-treat population from Herbrecht, 2002[12]

**Intention-to-treat population from Herbrecht, 2002

If the above is not acceptable since a future protocol or guidance document is not available in order to conduct a full NI margin justification, it may not be possible to determine that there is adequate support for the lack of inflation of type I error. Assuming that all subjects would be enrolled using the galactomannan as the mycological factor, in order to reduce the potential type I error rate inflation to a tolerable level of less

than 1/3 of a one-sided 2.5% test, a PPV in the range of 95% or higher would be needed. The prevalence of invasive aspergillosis in the subjects tested with the galactomannan would need to be at least 70%. As already noted in the Clinical Performance section of this document, the conservatively estimated prevalence of IA in hematologic patients with clinical and radiographic symptoms is 36.0% (95% CI 26.1, 45.9), and there is little evidence to lead one to believe that it would be as high as 70%. However, the three studies cited in Reviewer's analysis – Hematologic malignancy and HSCT recipients with clinical/radiologic features suggestive of fungal infection section demonstrate that the PPV noted at autopsy or in patients with pulmonary infiltrates is 93-95%.

Note that for superiority add-on trials, none of these issues exist and a positive superiority trial with the use of the galactomannan would be entirely interpretable without further assumptions other than lack of bias or poor study conduct which is assessed for all studies.

Of note, a serum GM cutoff of 0.5 is adopted clinically to define positivity, and prompts initiation of clinical workup and/or initiation of anti-mould therapy. Because earlier therapy improves survival, clinicians treating a patient with clinical signs and symptoms may not be willing to withhold therapy until the index is 1.0. This raises the concern that adopting a cut-off of two consecutive indices at ≥ 1.0 may make it difficult for investigators and sponsors to enroll patients in clinical trials, and make these trials unfeasible. However, our analysis indicates that serum GM is ≥ 1.0 in approximately 80% of patients at the time of diagnosis. Therefore, adopting a cutoff of 1.0 for the purpose of inclusion in non-inferiority clinical trials is not likely to hinder trial enrollment or to impede clinical practice.

Recommendations

Based on review of the published literature, the reviewer recommends acceptance of serum *Aspergillus* galactomannan antigen (GM) as detected by the Platelia™ Bio-Rad assay (or an essay with similar performance characteristics) as a biomarker of invasive aspergillosis for treatment trial enrollment with the following restrictions

- Patients with hematologic malignancy and recipients of allogeneic hematopoietic stem cell transplants (HSCT) who have clinical signs/symptoms or radiologic findings suggestive of invasive fungal infection as defined by EORTC criteria

- Two consecutive serum indices of 1.0 or higher, obtained 48-72 hours apart

- Exclusion of patients concomitantly receiving antibiotics known to result in a false positive GM (piperacillin/tazobactam, amoxicillin/clavulunate), or concomitantly receiving Plasmalyte

Because of insufficient data, the reviewer does not recommend the use of the GM assay as a microbiologic criterion in lieu of positive culture or histology for enrollment of

patients with immunodeficiencies other than hematologic malignancy and prolonged neutropenia or recipients of allogeneic HSCT in IA treatment trials.

Appendix 1

1- Maertens J. et al. Prospective clinical evaluation of lower cut-offs for galactomannan detection in adult neutropenic cancer patients and haematological stem cell transplant recipients. Br J Haematol 2004; 126:852-860

2- Suankrtay C. et al. Galactomannan antigenemia for the diagnosis of invasive aspergillosis in neutropenic patients with hematological disorders. J Medical Association of Thailand 2006; 89:1851-1858.

3- Penack O. et al. Aspergillus galactomannan testing in patients with long-term neutropenia: implications for clinical management. Ann Oncol 2008; 19:1-6

4- Maertens JA. et al. Optimization of the cutoff value for Aspergillus double sandwich enzyme immunoassay. Clin Infect Dis 2007; 44:1329-1336

5- Marr, K.A., Antifungal therapy decreases sensitivity of the Aspergillus galactomannan enzyme immunoassay. Clin Infect Dis 2005:40:1762-1769

6- Kawazu M, et al. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1-3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. J Clin Microbiol 2004; 42:2733-41

7- Yoo J-H, et al. Application of nucleic acid sequence-based amplification for diagnosis of and monitoring the clinical course of invasive aspergillosis in patients with hematologic diseases. Clin Infect Dis 2005; 40: 392-398.

8- Yoo J-H, et al. Comparison of the real-time nucleic acid sequence-based amplification (RTi-NASBA) with conventional NASBA, and galactomannan assay for the diagnosis of invasive aspergillosis. J Korean Med Sci 2007; 22: 672-676

9- Foy PC, et al. Galactomannan antigen enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2007;13:440-443

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11- Rohrlich P, et al. Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. Ped Infect Dis J 1996; 15:232-237

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14- Becker MJ, et al. Galactomannan detection in computerized tomography-based broncho-alveolar lavage fluid and serum in hematological patients at risk for invasive pulmonary aspergillosis. Br J Haematol 121:448-57, 2003

15- Pinel C, et al. Detection of circulating Aspergillus fumigatus galactomannan: value and limits of the Platelia test for diagnosing invasive aspergillosis. J Clin Microbiol 2003; 41: 2184-2186

16- Sulahian A, et al. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. Cancer 2001; 91:311-318

17- Machetti M, et al. Comparison of an enzyme immunoassay and a latex agglutination system for the diagnosis of invasive aspergillosis in bone marrow transplant recipients. Bone Marrow Transplant 1998; 21: 917-921

18- Herbrecht R, et al. Aspergillus galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. J Clin Oncol 2002; 20:1898-1906

19- Pazos C, et al. Contribution of (1→3)-β-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. J Clin Microbiol 2005; 43: 299-305

20- Lai C-C, et al. Assessment of Platelia Aspergillus enzyme immunoassay for the diagnosis of invasive aspergillosis. J Microbiol Immunology and Infection 2007; 40: 148-153

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Serum Galactomannan Platelia Assay for the diagnosis of Invasive Aspergillosis in Patients with hematologic malignancy or recipients of HSCT
22- Buchheidt, D et al. Prospective clinical evaluation of a LightCyclerTM-mediated polymerase chain reaction assay, a nested-PCR assay and a galactomannan enzyme-linked immunosorbent assay for detection of invasive aspergillosis in neutropenic cancer patients and haematological stem cell transplant recipients. Br J Haematol 2004; 125: 196–202

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