Temporary Compliance Waiver Notice

At the time of initial posting on October 28, 2014 the attached PDF document may not be fully accessible to readers using assistive technology. A fully accessible version of the document is in preparation and will be posted as soon as it is ready. We regret any inconvenience that this may cause our readers.

In the event you are unable to read this document or portions thereof, please contact Division of Drug Information in Office of Communications at 301-796-3634 or email druginfo@fda.hhs.gov.

Summary Review

Biomarker Qualification for Detection of Galactomannan in Serum and Bronchoalveolar Lavage Fluid by the Platelia *Aspergillus* Enzyme Immunoassay (manufactured by Bio-Rad Laboratories and Sanofi Diagnostics)

Applicant: On behalf of the Mycoses Study Group:

Peter G. Pappas, M.D.
Division of Infectious Diseases
University of Alabama, Birmingham
Birmingham, AL 35294

Paul E. Verweij, M.D.
Department of Medical Microbiology
University Hospital Nijmegen
The Netherlands

The overall conclusions of this Summary Review supersede the recommendations of the previous reviews[1] related to galactomannan testing in serum and bronchoalveolar lavage fluid as a biomarker for invasive aspergillosis in patients with hematologic malignancies and recipients of allogeneic hematopoietic stem cell transplants.

[1] Previous reviews are summarized in the memorandum from Shukal Bala, Ph.D. archived under PIND 112,146 on July 11, 2011.

Summary Review

Galactomannan Antigen, detected by Platelia® *Aspergillus* enzyme immunoassay in Serum or Broncho-alveolar Lavage Fluid, as a Biomarker of Invasive Aspergillosis in Patients with Hematologic Malignancies and Recipients of Allogeneic Hematopoietic Stem Cell Transplants for Use in Clinical Trials for Drug Development

1. Executive Summary

The purpose of the biomarker qualification review is to determine whether the detection of galactomannan (GM) by Platelia® *Aspergillus* enzyme immunoassay (EIA), in serum and broncho-alveolar lavage (BAL) fluids, in conjunction with clinical and host factors, is appropriate for the proposed context of use that is "to serve as a sole microbiological criterion to diagnose patients as having probable invasive aspergillosis (IA) in clinical trials conducted to evaluate the efficacy and safety of antifungal drugs for the treatment of invasive aspergillosis."

The Platelia® *Aspergillus* enzyme immunoassay (EIA) assay is cleared by the FDA Center for Devices and Radiological Health (CDRH) for testing of serum and BAL samples. The package insert for this device instructs that "Platelia® *Aspergillus* EIA is a test which, when used in conjunction with other diagnostic procedures such as microbiological cultures, histological examination of biopsy samples and radiological evidence can be used as an aid in the diagnosis of invasive aspergillosis." For the use of the device/assay by physicians for the treatment of patients in clinical practice, high sensitivity is important to guide the prompt initiation of therapy in patients with IA. Whereas, from a clinical trial perspective, high specificity and positive predictive value (PPV) of the GM assay are important considerations for the enrollment of patients with IA.

The FDA has not previously accepted, as a basis for approval, the detection of GM in serum or BAL fluids as the sole microbiologic criterion in clinical trials evaluating therapies for IA. In these trials, the primary analysis population includes patients with proven and probable IA documented by positive fungal culture and/or cytology. Accepting GM positive findings as the sole microbiologic criterion, in conjunction with host [hematologic cancer patients or recipients of hematopoietic stem cell transplants (HSCT)] and clinical factors, would change the patient classification from possible IA to probable IA, and the patient would be included in the primary analysis population.

GM is a polysaccharide component of the fungal cell wall, and is present in *Aspergillus* and other fungi. The presence of these other fungal species, as well as the presence of antimicrobials (such as piperacillin/tazobactam, amoxicillin/clavulanate, amoxicillin and ampicillin) and the electrolyte solution, PlasmaLyte, may yield false positive results.

We have concluded that GM results by Platelia® *Aspergillus* EIA are appropriate for the proposed context of use and can be used to classify a subject enrolled into an aspergillosis treatment trial as having probable invasive aspergillosis under the following conditions:

Specimen Source and Results:

Serum: A positive result should be based on a cut-off GM index ≥ 0.5 based on testing of two separate serum samples or a single sample with a value of ≥ 1.0.
Broncho-alveolar lavage fluid: A positive result should be based on a cut-off GM index ≥1.0 based on testing of two aliquots of a single BAL fluid sample.

Patient Population:

- The positive GM test results should be used in patients with hematologic malignancies or hematopoietic stem cell transplants (HSCT) who also have clinical and radiologic features consistent with fungal infection as defined by the 2008 European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria.

The context of use including assay results and patient clinical characteristics for aspergillosis clinical trials are expected to result in a high PPV for IA in clinical trials and will minimize the enrollment of patients with false positive results. A number of other criteria and additional details concerning this context of use are detailed in Section 4 (Recommendations) of this review.

2. Background Information

Galactomannan (GM) is a cell wall polysaccharide component of *Aspergillus*, a ubiquitous filamentous fungus (mold) that may cause invasive disease in hosts with neutrophil or macrophage dysfunction. In 2008, the Mycoses Study Group proposed using this antigen as an indicator of invasive aspergillosis in lieu of culture in patients with hematologic malignancies and recipients of allogeneic hematopoietic stem cell transplants (HSCT) and who also have radiologic evidence suggestive of invasive fungal infection.

- ***Invasive Aspergillosis***

The clinical manifestations of *Aspergillus* infections are dependent on host factors. Invasive aspergillosis (IA) is characterized by vascular invasion with subsequent tissue infarction/necrosis. Patients with defects in neutrophil and macrophage function are particularly at risk, including those with hematologic malignancies and prolonged neutropenia, recipients of HSCT or solid organ transplants, patients with chronic granulomatous disease and recipients of immunosuppressive agents that result in prolonged deficiency of B and T cell mediated immunity. The most common sites of infection are the sinuses, lungs, and central nervous system.[2]

IA may be difficult to diagnose ante-mortem. The clinical manifestations (such as fever, cough, pleuritic chest pain and hemoptysis) can be non-specific. Blood and respiratory cultures are usually negative. Chest computerized tomography (Chest CT) typically shows nodular lesions, at times with a halo sign or cavitation, however, these findings can be seen in a variety of other invasive fungal or bacterial infections. In patients with hematologic malignancies, invasive procedures (such as needle biopsies) may be too dangerous to perform due to thrombocytopenia. In addition, the yield of invasive procedures for cultures is not optimal. In one study, only 36% of patients proven at autopsy to have IA were diagnosed ante-mortem by culture or histology.[3] In another study, the sensitivity of either culture or microscopy in patients with proven IA was approximately 50%.[4]

Prior to 2002, IA was frequently fatal, with 12 week mortality between 60-90%.[5,6] In 2002, the European Organization for Research and Treatment of Cancer/Invasive Fungal

---

[2] Segal BH. Aspergillosis. *N Engl J Med* (2009) **360**:1870-1884.

[3] Subira M, Martino R, Rovira M, VasquezL, Serrano D, and De la Camara R. 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.

[4] Maertens J, Maertens V, Theunissen K, Meersseman W, Meersseman P, Meers S, Verbeken E, Verhoef G, Van Eldere J, and Lagrou K. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. *Clin Infect Dis* (2009) **49**: 1688-1693.

[5] Neofytus D, Horn D, Anaissie E, Steinbach W, Olyaei A, Fishman J, Pfaller M, Chang C, Webster K, and marr K,. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant

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.[7] These criteria were revised in 2008.[8] According to the EORTC/MSG criteria, proven IA requires culturing the organism from normally sterile specimens, or demonstration of invasive fungal elements in a histopathologic tissue specimen. Diagnosis of probable IA requires a combination of clinical/radiologic features, and culture of the organism from a non-sterile site or GM antigen detection in the serum, cerebrospinal fluid, or broncho-alveolar lavage (BAL) in a susceptible host. Possible IA includes cases with host and clinical/radiologic criteria, but without microbiologic support. These criteria were developed to enhance the early diagnosis of IA, as early diagnosis and treatment improve survival. According to treatment guidelines[9], voriconazole, an antifungal approved by the FDA in 2002 for the treatment of IA, is recommended for the treatment of invasive IA in most patients. Subsequent to the introduction of new diagnostic modalities (high resolution CT scan, GM detection) and new treatment options, the 12 week mortality has decreased to 22-45%. The FDA has not previously accepted detection of GM as a sole microbiologic criterion in clinical trials evaluating therapies for IA. Patients considered to have probable IA on the basis of positive GM by EORTC/MSG criteria would have been classified for the purpose of trial analysis as having possible IA. In clinical trials for treatment of IA, the primary analysis population includes patients with proven and probable IA.

Voriconazole was approved based on a study comparing the drug to amphotericin B deoxycholate.[10] Eligible immunocompromised patients with definite or probable IA were

---

recipients: analysis of multicenter Prospective Antifungal Therapy (PATH) alliance. *Clin Infect Dis* (2009) **48**: 265-273.

[6] Upton A, Kirby KA, Carpenter P, Boeckh M, and Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. *Clin Infect Dis* (2007) **44**: 531-540.

[7] Ascioglu S, Rex JH, De Pauw B, Bennett JE, Bille J, Crokaert F, Denning DW, Donnelly JP, Edwards JE, Erjavec Z, Fiere D, Lortholary O, Maertens J,Meis JF, Patterson TF, Ritter J, Selleslag D, Shah PM, Stevens DA, and Walsh TJ. Defining Opportunistic Invasive Fungal Infections in Immunocompromised Patients with Cancer and Hematopoietic Stem Cell Transplants: An International Consensus. *Clin Infect Dis* (2002) 34: 7-14.

[8] De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Munoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, and Bennett JE. Revised definitions of invasive fungal disease from EORTC/IFI cooperative group and the NIAID MSG consensus group. *Clin Infect Dis* (2008) 46: 1813-1821.

[9] Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Segal BH, Steinbach WJ, Stevens DA, van Burik JA, Wingard JR, Patterson TF. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis*. (2008) 46(3):327-60.

[10] Herbrecht, R, Denning, DW, Patterson, TF, Bennett, JE, Greene, RE, Oestmann, JW, Kern, WV, Marr, KA, Ribaud, P, Lortholary, O, Sylvester, R, Rubin, RH, Wingard, JR, Stark, P, Durand, C, Caillot, D,

evaluated. This study predated both the publication of the 2002 EORTC/MSG criteria and the availability of the Platelia® *Aspergillus* enzyme immunoassay (EIA), but was designed under the aegis of a steering committee that included EORTC/MSG. The definitions of IA categories were largely similar to the 2002 and 2008 EORTC/MSG criteria except for GM based classification. Global response (complete or partial resolution of radiologic signs and clinical symptoms at 12 weeks) was 53% and 32% in subjects with IA (proven and probable) who received voriconazole and amphotericin B, respectively; survival at Day 84 was 71% and 58%, respectively.

IA trials are difficult to perform. The above voriconazole trial enrolled 391 patients from 95 centers in 19 countries over 4 years. The availability of a serum and BAL GM biomarker, as the sole microbiological criteria, is likely to greatly enhance the pace of recruitment and increase the number patients included in the primary analysis population by changing the classification from possible IA to probable IA.

- ***Galactomannan Antigen***

GM is released by growing hyphae, and not by conidia that colonize the airways. Its detection in an individual is therefore thought to more likely indicate invasive disease rather than simple colonization. The 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* GM. In 2003, the Center for Devices and Radiological Health (CDRH), FDA, cleared detection of serum GM by this 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 test was cleared for testing of BAL fluids in 2011. The CDRH cleared the test with an index of ≥ 0.5 as a cut-off for both serum and BAL for the diagnosis of patients with aspergillosis; the results of Platelia® *Aspergillus* EIA are to be used in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples, and radiological evidence of disease.

- **Analytic Performance of Platelia® *Aspergillus* EIA**

Infection with fungi other than *Aspergillus* may also result in positive GM. These fungi include *Penicillium*, *Geotrichum*, *Trichophyton*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Nigrospora oryzae*, and *Paecilomyces lilacinus.* However, infections with these fungi account for less than 2% of all fungal infections in patients with hematologic malignancies or recipients of HSCT, whereas *Aspergillus* accounts for approximately 60%.[5] These cross-reactions are therefore not likely to be clinically relevant or to significantly impact the clinical specificity and positive predictive value (PPV) of the assay.

PlasmaLyte may cause a positive result, and its use would preclude relying upon a GM result. This is addressed in the Recommendations section of this document. . Receipt of piperacillin/tazobactam and amoxicillin/clavulanate has also been reported to result in positive reactions. The latter antibacterial drug is unlikely to be used in the patient

---

Thiel, E, Handrasekar, PHC, Hodges, MR, Schlamm, HT, Troke, PF, and de Pauw, B. Voriconazole versus amphotericin B for the primary therapy of invasive aspergillosis. *NEJM* (2002) **347**:408-415.

population at risk for IA. The original reviews for this qualification request recommended that patients receiving piperacillin/tazobactam be excluded from clinical trials. However, as the cross-reactivity is due to manufacturing issues and as this antibacterial drug is commonly used to empirically treat patients with febrile neutropenia, the MSG has embarked on a study to show that newer preparations do not cause cross-reactivity. The use of piperacillin/tazobactam (including the name of the manufacturer and time of administration relative to GM measurement) should be recorded on case report forms and data sets so that a sensitivity analysis can be performed on these patients. If the MSG study shows cross-reactivity due to administration of the drug, then patients administered piperacillin/tazobactam should not be considered evaluable in the primary efficacy analyses if the sole microbiologic criterion for classifying the patient as probable IA was a positive GM finding.

3. Clinical Performance of Platelia® *Aspergillus* EIA

- **Serum**

Future trials are likely to be non-inferiority (NI) trials and a biomarker with a high PPV is required to assure enrollment of subjects who have the disease. PPV is dependent on the sensitivity and specificity of the assay, and on the prevalence of the disease in the target population.

To estimate the PPV of serum GM in patients with hematologic malignancies and recipients of HSCT, a PubMed literature search using the term "galactomannan" was performed 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 GM testing
- duplicates
- case reports
- studies that did not use the Platelia® *Aspergillus* EIA
- 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 cut-off 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/MSG criteria, and
- studies evaluating polymerase chain reaction (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 in solid organ transplant recipients (see Appendix A for the list of 23 studies). Some of these publications were included in the applicant's submission.

The results from the 23 studies in the hematologic population were pooled. 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%. When available, assay performance at various cut-offs

and number of samples required for positivity was compared in the same population using the "gold standard" of positive culture, histopathology, or autopsy. To eliminate incorporation bias, cases designated as probable solely based on a positive GM test were not included in the analyses. The results are summarized in Table 1.

Table 1: Summary - Effect of Cut-off and Number of Samples – Median and Interquartile Range

Cut-off	# 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 highest PPV is obtained for two consecutive samples at a cut-off of 1.0 (Table 1; text highlighted in red). In addition, the performance at a cut-off of 1.0 obtained from testing one sample was similar to that of a cut-off of 0.5 obtained from testing 2 consecutive samples (Table 1; text highlighted in blue).

The performance characteristics of the GM assay is likely to be underestimated due to the poor sensitivity of other diagnostic methods (culture/microscopy) for IA. In addition, the above performance characteristics were estimated using studies that enrolled subjects with hematologic malignancies or recipients of HSCT regardless of the presence or absence of symptoms or radiologic findings suggestive of fungal infection, whereas the proposed population for enrollment in clinical trials will also have radiologic features suggestive of fungal infection. The prevalence of IA is expected to be considerably higher in this population subset compared to the general population at risk. To estimate the prevalence in this population subset, studies in neutropenic patients with a halo sign who underwent a needle biopsy and 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 were identified (see Appendix B for the list of studies). The pooled estimate and 95% CI using a random effects model by De Sermonian and Laird is 36.0% (26.1, 45.9). This estimate is conservative, because diagnostic procedures are not always feasible in these patients and even when performed, the sensitivity of cultures is poor.

Using a conservative estimate of 30% prevalence for the proposed sub-population and assuming the median sensitivity and specificity observed for consecutive serum GM measurements of at least 1.0, the estimated PPV is 95%. Assuming the median sensitivity and specificity observed for a single GM measurement of at least 1.0 or two consecutive measurements of 0.5, the estimated PPV is 82%. As discussed above, because the specificity of GM and the prevalence of IA in patients with radiologic manifestations are likely to be underestimated, we have concluded that the PPV of a single GM measurement of at least 1.0 or two consecutive measurements of 0.5 is likely greater than estimated. A study evaluating 48 neutropenic patients with evidence of pulmonary infiltrates on high resolution CT scan reported a sensitivity of 78% and

specificity and PPV of 100% for a single GM value of ≥0.5 supporting our conclusion.[11] In the same study, 55 patients who had persistent fever after 6 days of antibacterial drug therapy (a clinical indicator suggestive of fungal infection), sensitivity of a single GM value of ≥ 0.5 was 91.3% whereas specificity and PPV of the assay were 100%.

Impact of Accepting Serum GM as a Biomarker for IA in Clinical Trials

The interpretability of a future NI trial 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. A test with a high PPV (high pre-test probability) and high specificity would be needed to ensure that patients who actually have the disease are included in the primary analysis population. As long as a future NI trial using GM 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) concerns regarding bias towards NI will be of less concern. As voriconazole is recommended in clinical care guidelines as the treatment for most patients with IA, a future trial will likely use this drug as the active comparator and the study comparing voriconazole to amphotericin (the study that led to voriconazole approval) is likely to serve as the foundation for future NI margin justification. Although that study predated the availability of GM and the EORTC/MSG criteria, the data from the trial shows similar drug effect across all categories of subjects ranging from including only those with proven disease to including all those with proven, probable, or possible disease (Table 2).

Table 2: Voriconazole Drug Effect According to IA Diagnosis Classification

IA category	Voriconazole Global Response Rate*	Active Control Global Response Rate*	Difference	95% CI of 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 (19.7%)	21.8%	(5.2, 38.4)
Proven, probable, and possible***	98/197 (49.7%)	54/194 (27.8%)	21.9%	(12.5, 31.3)

* Global response rate represents complete or partial resolution of radiologic signs and clinical symptoms at 12 weeks

** Modified Intention –to-treat population from Herbrecht *et al*., 2002

***Intention-to treat population from Herbrecht *et al*., 2002

In summary, the performance characteristics of the Platelia® *Aspergillus* EIA that led to our previous conclusion that two consecutive serum measurements of at least 1.0 were required to classify a patient as having probable IA were obtained from studies evaluating patients with hematologic malignancy or recipients of HSCT regardless of clinical or radiologic features suggestive of fungal infection, where the median prevalence of IA is 10-12%. However, the population to be enrolled in IA trials will also have clinical and radiologic features consistent with fungal infection. The estimated point prevalence of IA in this population is 36%. Because the reference diagnostic methods for IA (culture and

[11] Penack O, Rempf P, Graf B, Blau IW, Thiel E. Aspergillus galactomannan testing in patients with long-term neutropenia: implications for clinical management. *Annals Oncology* (2008) **19**: 984-989.

microscopy) are not very sensitive, the performance characteristics of Platelia® *Aspergillus* EIA are most likely underestimated. Based on these assumptions, we have concluded that the PPV of two measurements of ≥0.5 or one measurement of ≥1.0 are sufficiently high and are acceptable to now conclude that a patient with predisposing hematologic condition who also has features suggestive of fungal infection should be classified in the primary analysis population as probable IA in the absence of other confirmatory microbiologic criteria. In addition, even if some patients are misclassified in a different diagnostic category in future trials, the treatment effect of voriconazole, the expected comparator in future studies, is similar across IA categories.

Our previous review of galactomannan had recommended that the two consecutive serum GM tests specimens be obtained 48-72 hours apart. The basis for that recommendation was that GM was determined twice weekly in the studies. However, these studies evaluated screened patients at risk of IA rather than patients with radiological evidence consistent with IA. At this time, considering practical considerations and study feasibility, the time interval between the two serum GM determinations of at least 0.5 will not be limited. The samples should be obtained separately.

- **Broncho-alveolar lavage fluid**

A majority of the studies measured performance of the Platelia® *Aspergillus* EIA, in BAL fluids, at a GM index of ≥1.0 (Table 3). The negative predictive value was consistent across studies (range 96% -100%), suggesting that a GM negative result correlated with absence of disease. The specificity of the test varied (range 79% - 100%; median 92%). The positive predictive value of the GM assay in BAL fluids ranged from 54% to 100% with a mean and median of 81% and 76%, respectively. For more details see the joint clinical, microbiology and statistics review for Detection of Galactomannan in Broncho-alveolar Lavage Fluids by Platelia® *Aspergillus* EIA[12].

[12] Archived under PIND 112,146 on July 11, 2011.

Table 3: Summary of Performance Characteristics of the Galactomannan Assay in Patients with Hematologic Malignancy or HSCT

Reference, Country [a]	Host factor	Total	Proven IPA	Probable IPA	Possible IPA	Without IPA	Estimated Prevalence	BAL GMI ≥ 0.5				BAL GMI ≥ 1.0			
								Sn[c]	Sp	PPV	NPV	Sn[c]	Sp	PPV	NPV
Becker *et al*., 2003[13] Netherlands	Hematological malignancies (retrospective)	29	1	6	2	18	28%					100%	100%	100%	100%
	Hematological malignancies (prospective)	53	3	9	12	23	34%					92%	100%	100%	96%
Musher *et al*., 2004[13] United States	HSCT	99	49			50		76%	94%			61%	98%		
Verweij *et al*., 1995[13] Netherlands	Hematological malignancies	19		7	2	10						71%	90%		
Desai *et al*., 2009[13] United States	Hematological malignancies	85	9		37	39	19%	78%	84%			78%	92%	54%[d]	97%
Penack *et al.*, 2008[13] Belgium	Hematological malignancies	45	17			28	12%*					100%	79%	74%	100%
Maertens *et al.*, 2009[13] Belgium	Hematological malignancies	128	31	27	29	41	45%	97%	80%	68%	98%	91%	88%	76%	96%
Overall Mean (Median)							**23% (23%)**	**83% (78%)**	**86% (84%)**			**85% (91%)**	**92% (92%)**	**81% (76%)**	**98% (97%)**

Note: GMI = galactomannan index; HSCT = hematopoietic stem cell transplant recipients; Sn = sensitivity; Sp = specificity; PPV= positive predictive value; NPV = negative predictive value;
Shaded regions = No information available
[a] The EORTC/MSG (2002) criteria were used for all studies except the study by Verweij PE *et al*., 1995 and Maertens *et al*., 2009
[b] Estimated Prevalence = (proven + probable IPA) / (proven+probable+without IPA). IPA = invasive pulmonary disease.
[c] Sensitivity = proportion with proven + probable IPA based on the Platelia® Aspergillus EIA.
[d] Includes possible IPA patients and patients without IPA

Same as Table of 1A of BAL galactomannan clinical, microbiology and statistics review

[13] See Appendix C for list of references

4. Recommendations

We have concluded that galactomannan results by Platelia® *Aspergillus* EIA can be used for the proposed context of use to classify a patient enrolled into an aspergillosis treatment trial as having probable invasive aspergillosis under the following conditions:

Specimen Source and Results:

Serum: A positive result should be based on a cut-off GM index ≥ 0.5 based on testing of two separate serum samples or a single value of ≥ 1.0.

Broncho-alveolar lavage fluid: A positive result should be based on a cut-off GM index ≥1.0 based on testing of two aliquots of a single BAL fluid sample.

- The quantitative value of the GM index should be documented in the case report form and included in the database.

Patient Populations:

- The qualification is limited to patients with hematologic malignancies or hematopoietic stem cell transplants (HSCT) who also have clinical and radiologic features suggestive of fungal infection as defined by the 2008 European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria.

Interaction of GM with Certain Drug Products:

- This qualification does not apply to patients concomitantly receiving PlasmaLyte.
- The use of piperacillin/tazobactam (including the name of the manufacturer and time of administration relative to GM measurement) should be recorded on case report forms and data sets so that a sensitivity analysis can be performed on these patients.
- This qualification does not apply to patients concomitantly receiving amoxicillin/clavulanate.

Continued efforts to obtain specimens for culture:

- As is standard practice, continued efforts should be made to obtain specimens for fungal smear, culture or histopathologic confirmation according to EORTC/MSG criteria; all positive and negative results should be documented on case report forms and in the datasets. Presence of bacterial infections should be reported. This qualification does not apply to patients with identified pathogens which cross-react with the Platelia® *Aspergillus* EIA, such as *Penicillium, Paecilomyces, Geotrichum* and *Histoplasma* (for details see Platelia® *Aspergillus* EIA test brochure).

Analysis of Study Results:

- The primary efficacy analysis in clinical trials for treatment of IA should be performed on the modified intent to treat (MITT) population which is defined as all proven and probable IA patients. Proven and probable IA is defined as per the 2008 EORTC/MSG criteria. However, the use of a positive GM (as described above) to classify a patient as probable will be restricted to only patients with hematological malignancies or HSCT.

- A subset analysis should be done for patients that are diagnosed based on a positive culture and/or histopathology excluding those whose microbiological diagnosis is based only on a GM positive result.

- Patients with hematologic malignancy or recipients of HSCT who have a positive serum GM assay without clinical or radiologic findings defined by the EORTC/MSG criteria should not be enrolled, or if enrolled should be excluded from analysis of efficacy.

Appendix A

Studies used to estimate the clinical performance of Platelia® *Aspergillus* enzyme immunoassay in serum

1. Maertens J, Theunissen K, Verbeken E, Lagrou K, Verhaegen J, Boogaerts M, and Van Eldere J. 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. Suankratay C, Kanitcharaskul P, and Arunyingmongkol K. 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, Rempf P, Graf B, Blau IW, and Thiel E. Aspergillus galactomannan testing in patients with long-term neutropenia: implications for clinical management. *Ann Oncol* (2008) **19**: 984-989.

4. Maertens JA. Klont R, Masson C, Theunissen K, Meersseman W, Lagrou K, Heinen C, Cre´pin B, Van Eldere J, Tabouret M, Donnelly JP, and Verweij PE. Optimization of the cutoff value for Aspergillus double sandwich enzyme immunoassay. *Clin Infect Dis* (2007) **44**:1329-1336.

5. Marr KA, Laverdiere M, Gugel A, and Leisenring W. Antifungal therapy decreases sensitivity of the Aspergillus galactomannan enzyme immunoassay. *Clin Infect Dis* (2005) **40**:1762-1769.

6. Kawazu M, Kanda K, Nannya Y, Aoki K, Kurokawa M, Chiba S, Motokura T, Hirai H, and Ogawa S. 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**:2733-2741.

7. Yoo J-H, Choi J-H, Choi SM, Lee D-G, Shin W-S, Min W-S, and Kim C-C. 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, Choi SM, Lee-D-G, Park S-H, Choi J-H, Kwon E-Y, and Shin W-S. 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, Van Burik JH, and Weisdorf DJ. Galactomannan antigen enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* (2007) **13**:440-443.

10. Maertens J, Verhaegen J, Lagrou K, Van Eldere J, and Boogaerts M. 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.

11. Rohrlich P, Sarfati J, Mariani P, Duval, M, Agnes C, Saint-Martin C, Bingen EL, Latge J-P, and Vilmer E. 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.

12. Ulusakarya A, Chachaty E, Vantelon J-M, Youssef A, Tancrede C, Pico J-L, Bourhis J-H, Fenaux P, and Munck J-N. Surveillance of Aspergillus galactomannan antigenemia for invasive aspergillosis by enzyme-linked immunosorbent assay in neutropenic patients treated for hematological malignancies. *The Hematology Journal* (2000) **1**: 111-116.

13. Maertens J, Van Eldere J, Verhaegen J, Verbeken E, Verschakelen J, and Boogaert M. Use of circulating galactomannan screening for early diagnosis of invasive aspergillosis in allogeneic stem cell transplant recipients. *J Infect Dis* (2002) **186**:1297-1306.

14. Becker MJ, Martin J, Lugtenburg EJ, Cornelissen JJ, van der Schee C, Hoogsteden HC, and Marie SD. Galactomannan detection in computerized tomography-based broncho-alveolar lavage fluid and serum in hematological patients at risk for invasive pulmonary aspergillosis. *Br J Haematol* (2003) **121**:448-457.

15. Pinel C, Fricker-Hidalgo H, Lebeau B, Garban F, Hamidfar R, Ambroise-Thomas P, and Grillot R. 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, Boutboul F, Ribaud P, Leblanc T, Lacroix C, and Derouin F. 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, Feasil M, Mordini N, Van Lint MT, Bacigalupo A, Latge´ JP, Sarfati J, and Viscoli C. 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, Letscher-Bru V, Oprea C, Lioure B, Waller J, Campos F, Villard O, Liu K-L, Natarajan-Ame´ S, Lutz P, Dufour P, Bergerat J-P, and Candolfi E. Aspergillus galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* (2002) **20**:1898-1906.

19. Pazos C, Ponton J, and del Palacio A. 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, Hsu HL, Lee LN, and Hsueh P-R. Assessment of Platelia Aspergillus enzyme immunoassay for the diagnosis of invasive aspergillosis. *J Microbiol Immunology and Infection* (2007) **40**: 148-153.

21. Busca, A, Locatelli F, Barbui A, Limerutti G, Serra R, Libertucci D, and Falda M. Usefulness of Sequential *Aspergillus galactomannan* Antigen Detection Combined With Early Radiologic Evaluation for Diagnosis of Invasive Pulmonary Aspergillosis in Patients Undergoing Allogeneic Stem Cell Transplantation. *Transplantation Proceedings* (2006) **38**: 1610–1613.

22. Buchheidt D, Hummel M, Schleiermacher D, Spiess B, Schwerdtfeger R, Cornely OA, Wilhelm S, Reuter S, Kern W, Sudhoff T, Morz H, and Ru¨diger. 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.

23. Rovira M, Jimenéz M, De La Bellacasa JP, Mensa J, Rafel M, Ortega M, Almela M, Martínez C, Fernández-Avilés F, Martínez JA, Urbano-Ispizua A, Carreras E, and Montserrat E. Detection of Aspergillus galactomannan by enzyme immunoabsorbent assay in recipients of allogeneic hematopoietic stem cell transplantation: A Prospective study. *Transplantation* (2004) **77**: 1260–1264.

Appendix B

Studies used to estimate the prevalence of IA in patients with hematologic malignancy or recipients of HSCT

1. Primack S, Hartman TE, Lee KS and Muller NL. Pulmonary nodules and the CT halo sign. *Radiology* (1994) **190**: 513-515.

2. Won JH, Lee KS, Cheon JE, Hwang JH, Kim TS, Lee HG, and Han J. Invasive pulmonary aspergillosis: prediction at thin section CT in patients with neutropenia– a prospective study. *Radiology* (1998) **208**: 777-782.

3. Becker MJ, Martin J, Lugtenburg EJ, Cornelissen JJ, van der Schee C, Hoogsteden HC, and Marie SD. 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.

4. Maertens J, Maertens V, Theunissen K, Meersseman W, Meersseman P, Meers S, Verbeken E, Verhoef G, and Van Eldere J. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. *Clin Infect Dis* (2009) **49**: 1688-1693.

5. Verweij PE, Latge JP, Rijs AJ, Melchers WJ, De Pauw BE, Hoogkamp-Korstanje JA, and Meis JF. 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.

6. Bergeron A, Belle A, Sulahian A, Lacrois C, Chevret S, Raffoux E, Amulf B, Socie G, Ribaud P, and Tazi A. Contribution of Galactomannan antigen detection in BAL to the diagnosis of pulmonary aspergillosis in patients with hematologic malignancies. *Chest* (2010) **137**: 410-415.

7. Maertens J, Theunissen K, Verhoef G, Verschakelen J, Lagrou K, Verbeken E, Wilmer A, Verhaegen J, Boogaerts M, and Van Eldere J. 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**: 1242-1250.

8. Herbrecht R. Letscher-Bru V, Oprea C, Lioure B, Waller J, Campos F, Villard O, Liu K-L, Natarajan-Ame´ S, Lutz P, Dufour P, Bergerat J-P, and Candolfi E. Aspergillus galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* (2002) **20**: 1898-1906.

9. Penack O, Rempf P, Graf B, Blau IW, and Thiel E. et al. Aspergillus galactomannan testing in patients with long-term neutropenia: implications for clinical management. *Annals Oncology* (2008) **19**: 984-989.

Appendix C

Studies used to estimate the clinical performance of Platelia® *Aspergillus* enzyme immunoassay in bronchoalveolar lavage fluid

1. Becker MJ, Lugtenburg EJ, Cornelissen JJ, Van Der Schee C, Hoogsteden HC, and De Marie S. 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.

2. Musher B, Fredricks D, Leisenring W, Balajee SA, Smith C, and Marr KA. *Aspergillus* galactomannan enzyme immunoassay and quantitative PCR for diagnosis of invasive aspergillosis with bronchoalveolar lavage fluid. *J Clin Microbiol* (2004) **42**: 5517-5522.

3. Verweij PE, Latge JP, Rijs AJ, Melchers WJ, De Pauw BE, Hoogkamp-Korstanje JA, and Meis JF. 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.

4. Desai R, Ross LA, and Hoffman JA. The role of bronchoalveolar lavage galactomannan in the diagnosis of pediatric invasive aspergillosis. *Pediatr Infect Dis J* (2009) **28**: 283-286.

5. Penack O, Rempf P, Graf B, Blau IW, and Thiel E. *Aspergillus* galactomannan testing in patients with long-term neutropenia: implications for clinical management. *Ann Oncol* (2008) **19**: 984-989.

6. Maertens J, Maertens V, Theunissen K, Meersseman W, Meersseman P, Meers S, Verbeken E, Verhoef G, Van Eldere J, and Lagrou K. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. *Clin Infect Dis* (2009) **49**: 1688-1693.