MAJOR ARTICLE

Optimization of the Cutoff Value for the *Aspergillus* Double-Sandwich Enzyme Immunoassay

Johan A. Maertens,[1] Rocus Klont,[4] Christine Masson,[6] Koen Theunissen,[1] Wouter Meersseman,[2] Katrien Lagrou,[3] Christine Heinen,[6] Brigitte Crépin,[6] Johan Van Eldere,[3] Marc Tabouret,[7] J. Peter Donnelly,[5] and Paul E. Verweij[5]

Departments of [1]Hematology, [2]Intensive Care Medicine, and [3]Medical Microbiology, Universitaire Ziekenhuizen, Catholic University Leuven, Leuven, Belgium; Departments of [4]Hematology and [5]Medical Microbiology, Radboud University Nijmegen Medical Center and Nijmegen University Center for Infectious Diseases, Nijmegen, The Netherlands; and [6]Bio-Rad Laboratories, Marnes-La-Coquette, and [7]Bio-Rad Laboratories, Steenvoorde, France

Background. Many health care centers worldwide use the Platelia *Aspergillus* enzyme immunoassay (PA-EIA; Bio-Rad Laboratories) for diagnosis of invasive aspergillosis (IA). A cutoff optical density (OD) index of 1.5 was originally recommended by the manufacturer, but in practice, most institutions use lower cutoff values. Moreover, a cutoff OD index of 0.5 was recently approved in the United States. In the present study, we set out to optimize the cutoff level by performing a retrospective analysis of PA-EIA values for samples that had been obtained prospectively from adult patients at risk for IA at 2 European health care centers.

Methods. In total, 239 treatment episodes were included of which there were 19 episodes of proven IA and 19 episodes of probable IA. Per-episode and per-test analyses and receiver operating characteristic curves were used to determine the optimal cutoff value.

Results. In the per-episode analysis, lowering the cutoff OD index for positivity from 1.5 to 0.5 increased the overall sensitivity by 21% (from 76.3% to 97.4%) but decreased the overall specificity by 7% (from 97.5% to 90.5%). Requiring 2 consecutive samples with an OD index $\geq$0.5 resulted in the highest test accuracy, with an improved positive predictive value. At a cutoff OD index of 0.5, the antigen test result was positive during the week before conventional diagnosis in 65% of cases and during the week of diagnosis in 79.5% of cases.

Conclusions. A cutoff OD index of 0.5—identical to the approved cutoff in the United States—improves the overall performance of the PA-EIA for adult hematology patients.

Infections due to *Aspergillus* species are a leading cause of morbidity and mortality among immunocompromised patients, especially among patients who undergo intensive chemotherapy for hematological malignancies and those who undergo hematopoietic stem cell transplantation [1–4]. A high crude mortality rate has been observed; this stems, in part, from the inability to make a timely and reliable diagnosis [5]. For this reason, novel techniques based on the detection of specific fungal antigens, such as galactomannan and β-D-glucan, and on the amplification of fungal DNA by PCR techniques have become the focus of clinical study [6].

During the past decade, considerable experience has been gained with the Platelia *Aspergillus* galactomannan EIA (PA-EIA; Bio-Rad Laboratories), which uses the galactofuranose-specific rat monoclonal antibody EB-A2 to both capture and detect galactomannan [7–17]. The test has been commercially available in Europe since the mid-1990s and was approved for diagnostic use by the US Food and Drug Administration in 2003 [18, 19]. However, there has been a major difference between the cutoff OD indices. In Europe, the manufacturer originally recommended interpreting an optical density (OD) index $\geq$1.5 as a positive result and an OD index <1.0 as a negative result, with indices of 1.0–1.5 being indeterminate. By contrast, in the United States, an OD index $\geq$0.5 is interpreted as a positive result [19]. Lowering the threshold for negative samples from 1.0 to 0.8 and for positive samples from 1.5 to 1.0 was already suggested in 1998 by Verweij et al. [20].

Received 3 January 2007; accepted 1 February 2007; electronically published 4 April 2007.

Presented in part: 16th European Congress of Clinical Microbiology and Infectious Diseases, Nice, France, 3 April 2006 (S 277).

Reprints or correspondence: Dr. Johan A. Maertens, University Hospital Gasthuisberg, Dept. of Hematology, Acute Leukemia and Hematopoietic Stem Cell Transplantation Unit, Herestraat 49, B-3000 Leuven, Belgium (johan.maertens@uz.kuleuven.ac.be).

Clinical Infectious Diseases 2007; 44:1329–36

1058-4838/2007/4410-0011$15.00
DOI: 10.1086/514349

Also, in practice, many European centers already use a lower cutoff OD index of 0.5 or 0.7 to classify positive results [17, 21]. Not surprisingly, these differences may account for much of the variation in the performance of the test, especially because lower cutoff values are likely to increase test sensitivity at the expense of test accuracy [22, 23]. The frequency of sampling may also play a role [24].

In the present study, we assessed the performance of the PA-EIA using different cutoff OD indices. We used plasma and serum samples that had been obtained prospectively. Contrary to previous studies, most of which involved single health care centers [8–16, 21], this study was able to compare the performance (receiver operating characteristic [ROC] analysis) of the PA-EIA at 2 health care centers that have lengthy experience in *Aspergillus* antigen screening for patients who are at high risk for the development of invasive aspergillosis (IA). Interestingly, the frequency of sampling and the frequency of IA differed significantly in both centers.

SUBJECTS, MATERIALS, AND METHODS

Patients and serum samples. The patients included in this retrospective study were adults (age, ⩾16 years) at high risk of developing IA because of prolonged neutropenia that had resulted from intensive chemotherapy for underlying hematologic disorders (mainly acute leukemia/myelodysplastic syndrome) or from myeloablative conditioning therapy to prepare for a hematopoietic stem cell transplantation. Samples had been collected from patients admitted to the University Hospital Gasthuisberg (Leuven, Belgium) during the period from November 2001 through July 2004 and the Radboud University Nijmegen Medical Center (Nijmegen, The Netherlands) during the period from January 2002 through March 2005. Both health care centers had implemented a screening program for patients at high risk of IA. In Leuven, blood samples were tested at least twice weekly (during November 2001–December 2002) or daily (during January 2003–July 2004) from the start of chemotherapy or conditioning therapy until discharge from the hospital or death. In Nijmegen, blood samples were tested twice weekly (on Monday and Thursday) from hospital admission until the end of hospitalization or death. In Nijmegen, patients received no mold-active antifungal prophylaxis, but those with oropharyngeal candidiasis or gastrointestinal colonization with *Candida albicans* received fluconazole (200 mg per day). In Leuven, patients treated for acute leukemia/myelodysplastic syndrome received prophylaxis with fluconazole (400 mg per day), and hematopoetic stem cell transplant recipients were given itraconazole oral solution (2.5 mg/kg twice per day). Piperacillin-tazobactam and amoxicillin-clavulanate were not given routinely. A diagnosis of pulmonary IA was established using high-resolution CT and bronchoscopy with bronchoalveolar lavage. Therapy for proven or probable IA included both licensed and investigational antifungal agents. The study was approved by the Leuven and Nijmegen Institutional Review Boards.

Patients with IA and control subjects were identified by examining microbiologic and histopathologic records, followed by a chart review to determine the presence or absence of proven or probable invasive fungal disease, as defined by the European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [25]. In brief, patients were considered to have proven IA if hyphae compatible with *Aspergillus* species were seen in a tissue biopsy specimen or if *Aspergillus* species was recovered from it. Patients were considered to have probable IA if there were clinicoradiological signs and symptoms of infection and if *Aspergillus* species had been seen in or recovered by culture of specimens obtained from the lower respiratory tract (i.e., bronchoalveolar lavage, sputum, or bronchial aspirate). To avoid incorporation bias, detection of circulating galactomannan was not included among the diagnostic criteria [26]. Possible cases of IA were excluded from the study altogether. Demographic data, including age, sex, underlying disease, type of transplantation, use of antifungal prophylaxis, date of diagnosis of IA, and treatment data were collected by chart review.

Performance of the galactomannan EIA. The amount of galactomannan in each sample was measured using the Platelia *Aspergillus* kit (Bio-Rad Laboratories) by technicians blinded to the source of the sample and the clinical data. Plasma and serum samples were analyzed in accordance with the manufacturer's instructions [27]. In brief, 300 μL of test serum was mixed with 100 μL of 4% EDTA treatment solution and heated at 100°C for 3 min, to dissociate immune complexes and to precipitate any serum proteins that might interfere with the test. Fifty μL of the supernatant was added to 50 μL of the reaction mixture, and the 100-μL solution was added to microtitration plates precoated with the antibody EB-A2. After 90 min of incubation at 37°C, the plates were washed extensively before adding 100 μL of a substrate-chromogen solution containing tetramethylbenzidine. The plates were then incubated for another 30 min in the dark at room temperature, after which 100 μL of 1.5 N sulfuric acid was added to stop the reaction. The OD was read at 450/620 nm. All reagents were purchased from Bio-Rad Laboratories. Positive and negative controls were included in each run, as were threshold control samples that were provided by the manufacturer. Results were recorded as an OD index relative to the mean OD of the threshold controls.

Statistical analyses. Sensitivity and specificity were calculated on both a per-episode and a per-test outcome basis. For the per-episode calculation, each episode was classified as positive if there had ever been a positive test result, which was

determined for a range of static cutoff OD indices (range, 0.5–1.5) and a dynamic cutoff criterion defined by OD indices ⩾0.5 in 2 consecutive samples. Sensitivity was calculated as the proportion of episodes with a diagnosis of proven and probable IA that yielded positive test results in accordance with different criteria. Specificity was calculated as the proportion of control episodes that yielded negative results. Positive predictive values and negative predictive values were also calculated. Ninety-five percent CIs were calculated using standard methods for binominal distributions.

For the per-test analysis, each test result was interpreted as the unit of observation [28]. As such, sensitivity was calculated as the proportion of all test results for an episode of proven or probable IA that yielded positive results, as determined for a range of cutoff OD indices (range, 0.2–1.5). Conversely, specificity was the proportion of all test results for control episodes that yielded negative results. Center-specific ROC curves were calculated to illustrate the trade-off in rates of true-positive results (sensitivity) versus false-positive results (1-specificity) as the cutoff for the test was shifted from high (1.5) to low (0.2) OD indices. A test that yields no predictive information will generate a straight diagonal line, whereas tests that discriminate well between 2 groups will have high rates of true-positive results and low rates of false-positive results, yielding an elliptical curve [29].

To determine the impact of shifting OD index cutoffs on the interval between presumptive and formal diagnosis, the number of days between the first positive test result (using 3 different cutoffs, including 1.5, 0.5, and 2 × ⩾0.5) and the day of formal diagnosis (day 0, according to conventional methods) was calculated for the cohort of patients with proven and probable aspergillosis.

RESULTS

Characteristics of treatment episodes. Because a patient could be included in the study with each subsequent cycle of therapy, a per-episode rather than a per-patient analysis was performed. A total of 239 treatment episodes from 203 patients were identified for evaluation. The data set was restricted to include only data from adult patients with proven or probable IA (with plasma or serum samples obtained before diagnosis) and from patients without IA (the control group). The episodes were grouped as follows: 19 episodes of proven IA, 19 episodes of probable IA, and 201 control episodes; the rate of true-positive results was 15.9% (16.3% for Leuven and 15.5% for Nijmegen). Demographic and treatment characteristics are shown in table 1.

A total of 4884 serum samples were available; 3952 serum samples were from Leuven (mean, 32 samples per episode; range, 4–96 samples per episode), and 932 serum samples were from Nijmegen (mean, 8 samples per episode; range, 1–47 samples per episode). Of these, 700 serum samples were analyzed from 19 episodes of proven IA, 480 from 19 episodes of probable IA, and 3704 from 201 control episodes. The distribution of serum samples per episode and per center is shown in table 1.

Performance of the PA-EIA. In the per-episode calculations, each episode was categorized according to whether any positive test result was available. Sensitivity and specificity calculations using different OD index cutoff values are shown in table 2. The overall sensitivity to detect proven plus probable IA increased by 21.1% (from 76.3% to 97.4%) when the OD index value to define positivity (based on a single assay) was decreased from ⩾1.5 to ⩾0.5. This shift of the OD index value decreased the per-episode specificity by 7% (from 97.5% to 90.5%). The differences were more pronounced for the Nijmegen center (data not shown). A lowering of the OD cutoff for positivity from 1.5 to 0.5 resulted in a marked reduction of 19.2% in the positive predictive value, from 85.3% (95% CI, 69.9%–95.1%) to 66.1% (95% CI, 52.2%–78.2%), whereas the negative predictive value remained virtually unchanged, from 95.6% (95% CI, 91.8%–98.0%) to 99.4% (95% CI, 97.0%–99.9%). By contrast, requiring 2 consecutive samples with an OD index ⩾0.5 to define positivity resulted in an improved specificity and positive predictive value (97.5% [95% CI, 94.3%–99.2%] and 87.5% [95% CI, 73.2%–95.8%], respectively) while maintaining an overall sensitivity of 92.1% and a negative predictive value of 98.5% (95% CI, 95.7%–99.7%).

For the per-test analysis, each test result was interpreted as the unit of observation. This analysis can be considered to be a "worst case scenario," because all of the screening tests are included in the calculation. Per-test calculations yielded sensitivities ranging from 17.8% to 31.7% and from 34.7% to 60.1% using OD index cutoff values of 1.5 and 0.5, respectively (figure 1). Thus, when compared with the manufacturer's original threshold for positivity of 1.5, an OD index cutoff of 0.5 resulted in a doubling of the per-test sensitivity in both centers without affecting the specificity of the assay.

To determine whether better performance of the assay could be achieved by lowering the OD index cutoff to define positivity, center-specific ROC curves were calculated; the diagnostic accuracy, as given by the area under the ROC curve (AUC), was 0.843 for Leuven (95% CI, 0.825–0.861) and 0.808 for Nijmegen (95% CI, 0.775–0.842) (figure 2*A* and 2*B*). In view of the significantly higher mean number of serum samples analyzed in the cohort from Leuven, this data set was reanalyzed assuming a twice-weekly sampling frequency (Monday and Thursday), as was used at the Nijmegen health care center. This approach reduced the total number of serum samples for the Leuven center to 1150, but it did not alter the AUC (0.849; 95% CI, 0.817–0.880) of the ROC curve (data not shown).

In addition, an analysis was performed to determine the effect of temporal proximity of sample collection to the day of

Table 1. Demographic characteristics of patients and characteristics of episodes of invasive aspergillosis (IA).

Characteristics	Proven IA group	Probable IA group	Control group	All subjects or episodes
No. of patients	16	19	168	203
No. of episodes	19	19	201	239
Sex				
Male	11	15	94	120
Female	5	4	74	83
Age range, years	18–68	18–69	16–76	16–76
Underlying disorder or risk factor				
ALL	5	1	28	34
AML	3	3	81	87
MDS	0	7	2	9
Other	1	1	33	35
Allogeneic HSCT	10	7	57	74
Leuven, Belgium, health care center				
No. of samples	570	309	3073	3952
No. of samples per episode				
Mean	44	45	30	32
Median (range)	40 (4–87)	36 (17–95)	27 (7–96)	28 (4–96)
Nijmegen, The Netherlands, health care center				
No. of samples	130	171	631	932
No. of samples per episode				
Mean	22	15	7	8
Median (range)	19 (10–44)	14 (1–45)	3 (1–47)	4 (1–47)

NOTE. Data are number of patients, unless otherwise indicated. ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; HSCT, hematopoietic stem cell transplantation; MDS myelodysplastic syndrome.

conventional diagnosis (day 0); all serum specimens obtained from day 7 before diagnosis (day −7) to day 7 after diagnosis (day +7) were included in the analysis. The per-sample analysis (table 3) shows that the sensitivity is better for tests performed during the week of diagnosis, especially when using the index cutoff value of 0.5. In addition, the AUC of the ROC curve increased to 0.916 (95% CI, 0.879–0.953) and 0.984 (95% CI, 0.970–0.997) for samples obtained during week −1 and week 0, respectively (data from the Leuven center only).

A total of 3704 serum samples were analyzed from 201 control episodes (mean, 18.4 serum samples per episode; range, 1–96 serum samples per episode). Six serum samples (0.16%) from the control group yielded false-positive results when using a cutoff OD index of 1.5. After lowering the threshold for positivity to 0.5, a total of 3682 serum samples (99.4%) tested negative, whereas 22 serum specimens (0.6%) tested positive; this finding was confirmed by a subsequent sample in only 6 (3%) of the control episodes.

We also estimated the time between the day of diagnosis of IA (in accordance with European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria) and the day of the first positive test result, using different OD indices to define a positive result (data not shown). Overall, a lowering of the OD index cutoff from 1.5 to 0.5 increased the interval between presumptive and conventional diagnosis from 2 to 10 days and from 2 to 7 days in Leuven and Nijmegen, respectively.

DISCUSSION

The present analysis is based on data derived from 2 European centers that have used the PA-EIA routinely as a screening assay in high-risk neutropenic patients for many years. As evidenced by the AUC of the ROC curves, the diagnostic accuracy (or performance) of the assay was high and was identical in both centers. Our analysis presents supportive evidence that an OD index cutoff of 0.5—identical to the approved cutoff in the United States—can be used reliably to define a case of probable IA in this particular patient population. The increased accuracy of the assay at a lower than previously recommended cutoff (OD index, ⩾1.5) can be explained by the elimination of major sources of bias and variation in the design and interpretation in our study and by better knowledge about and avoidance of causes of false-positive and false-negative assay results [24].

A high overall per-episode sensitivity of 97.4%, an acceptable specificity of 90.5%, and a high negative predictive value of 99.4% were achieved; the per-episode positive predictive value

Table 2. Sensitivity and specificity of the Platelia *Aspergillus* EIA (Bio-Rad Laboratories), according to selected cutoff optical density (OD) indices and European Organization for Research and Treatment of Cancer/Mycoses Study Group classification of episodes of invasive aspergillosis (IA).

OD index cutoff value, episode classification	No. of episodes with positive results/no. of episodes tested	Sensitivity, % (95% CI)	No. of episodes with negative results/no. of episodes tested	Specificity, % (95% CI)
OD index ⩾1.5				
Proven IA	19/19	100 (85.4–100)	…	…
Probable IA	10/19	52.6 (28.9–75.5)	…	…
Overall	29/38	76.3 (59.8–88.6)	…	…
Control group	…	…	196/201	97.5 (94.3–99.2)
OD index ⩾1.0				
Proven IA	19/19	100 (85.4–100)	…	…
Probable IA	12/19	63.2 (38.4–83.7)	…	…
Overall	31/38	81.6 (65.7–92.3)	…	…
Control group	…	…	194/201	96.5 (93.0–98.6)
OD index ⩾0.5				
Proven IA	19/19	100 (85.4–100)	…	…
Probable IA	18/19	94.7 (74.0–99.9)	…	…
Overall	37/38	97.4 (86.2–99.9)	…	…
Control group	…	…	182/201	90.5 (85.6–94.2)
OD index ⩾2 × 0.5				
Proven IA	19/19	100 (85.4–100)	…	…
Probable IA	16/19	84.2 (60.4–96.6)	…	…
Overall	35/38	92.1 (78.6–98.3)	…	…
Control group	…	…	196/201	97.5 (94.3–99.2)

was only 66.1%, but it increased significantly (without affecting the other statistical parameters) to 87.5% when 2 consecutive samples with an OD index ⩾0.5 were required to define positivity. This latter observation is in line with previous findings and once more underscores the importance of the demonstration of a gradual increase in the level of antigenemia, rather than targeting a specific threshold [21]. A further lowering of the index cutoff for positivity should be discouraged, because

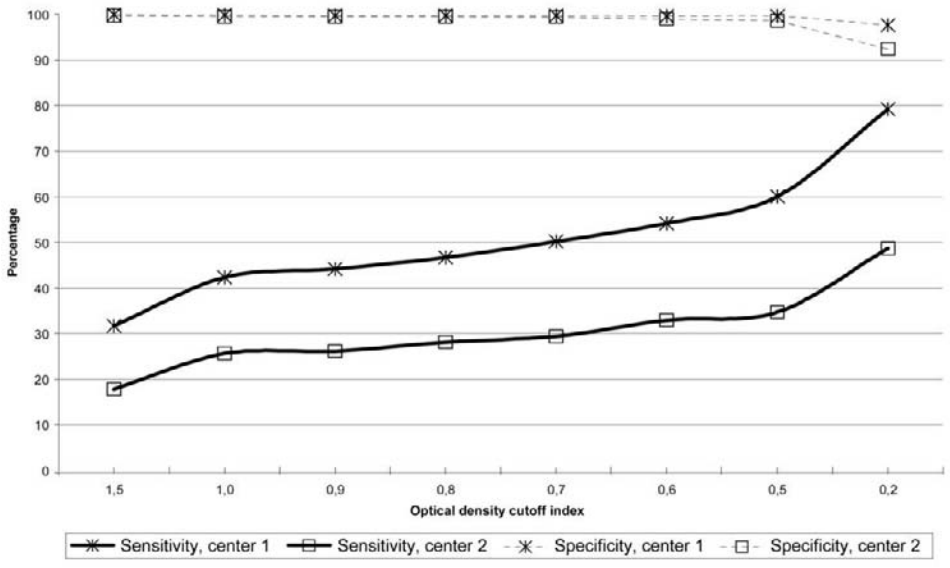

Figure 1. Health care center–specific sensitivity and specificity, from a per-test analysis, for proven and probable invasive aspergillosis at variable static optical density index cutoffs for positivity (x-axis). Center 1, Leuven, Belgium; center 2, Nijmegen, The Netherlands.

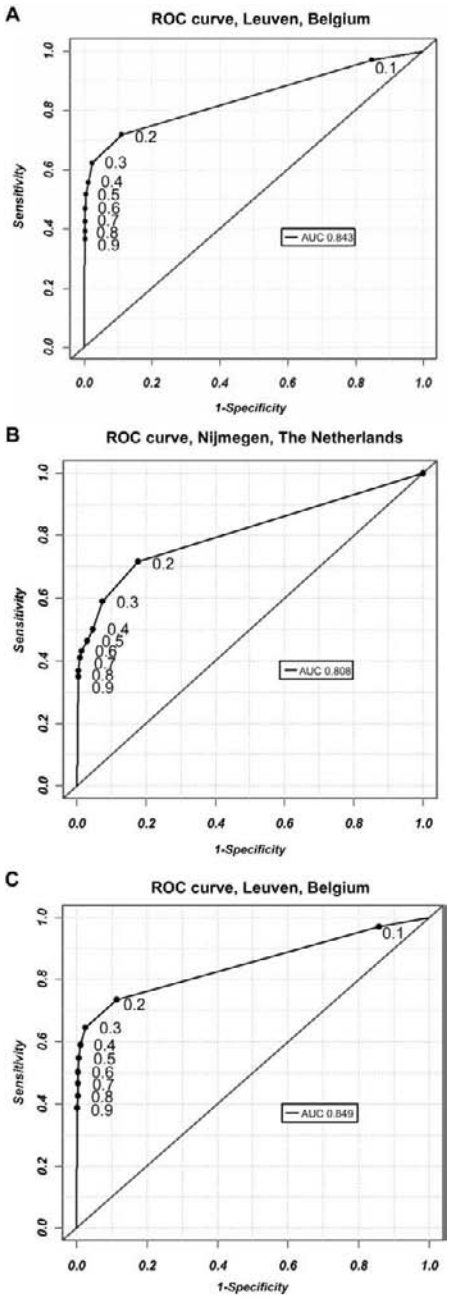

Figure 2. Receiver operating characteristic (ROC) curves graphing sensitivity (true-positive results) versus 1-specificity (false-positive results) using multiple optical density index cutoff values to define positivity. The optical density index cutoff value decreases from high to low values as the curves move from left to right. The performances of tests obtained in Leuven, Belgium, and in Nijmegen, The Netherlands, are shown in panels *A* and *B. C,* Performance of the tests obtained in Leuven while mimicking a twice-weekly sampling frequency (identical to Nijmegen). *Diagonal lines,* empirical ROC. AUC, area under the ROC curve.

it would lead to an unacceptably high frequency of false-positive test results. In addition, compared with higher cutoffs and with conventional diagnostic methods, this lower cutoff increased the interval between presumptive and formal diagnosis; this finding has recently also been reported by others [18].

It has been suggested that a high sampling frequency can bias diagnostic classification when using per-episode or per-patient analyses [30]. Although we cannot provide a definitive answer to the question of how often the test should be performed from our analysis, figure 2*A* and 2*C* show that the accuracy of the assay was not increased by a higher sampling frequency than twice weekly.

In line with previous reports [20–22], we observed that the specificity of the test—in per-episode as well as per-test analysis—remained high over a wide range of thresholds for test positivity. In this high-risk neutropenic population, few assays (0.6%) with false-positive results were seen when a cutoff of 0.5 was used, and overall, only 3% of the control episodes tested repeatedly positive. This low rate of false-positive results may simply have resulted from exclusion of children [31] and cases of possible IA from the study, as well as from the avoidance of antibiotics that can be associated with false-positive PA-EIA test results, including piperacillin-tazobactam [32] and amoxicillin-clavulanate [33]

As recently suggested by Marr et al. [28], we looked at the

Table 3. Changes in sensitivity of the Platelia *Aspergillus* EIA (PA-EIA; Bio-Rad Laboratories) over time for proven and probable invasive aspergillosis (IA), calculated according to test result at different optical density (OD) index cutoff values.

	Time to diagnosis	
Patient population, OD	Week −1	Week 0
Leuven, Belgium		
No. of samples	105	131
OD index cutoff value		
⩾1.5	24.8 (16.9–34.1)	42.0 (33.4–50.9)
⩾1.0	44.8 (35.1–54.8)	58.0 (49.1–66.6)
⩾0.5	66.7 (56.8–75.6)	84.0 (76.6–89.8)
Nijmegen, The Netherlands[a]		
No. of samples	35	40
OD index cutoff value		
⩾1.5	34.4 (16.8–49.3)	47.5 (31.5–63.9)
⩾1.0	42.9 (26.3–60.6)	55.0 (38.5–70.7)
⩾0.5	60.0 (42.1–76.1)	65.0 (48.3–79.4)
Overall		
No. of samples	140	171
OD index cutoff value		
⩾1.5	26.4 (19.3–34.5)	43.3 (35.7–51.0)
⩾1.0	44.3 (35.9–52.9)	57.3 (49.5–64.8)
⩾0.5	65.0 (56.5–72.9)	79.5 (72.7–85.3)

NOTE. Data are percentage (95% CI) of PA-EIA–positive samples, unless otherwise indicated. Week −1 corresponds to day 7 through day 1 before diagnosis; week 0 corresponds to day of diagnosis through day 7 after diagnosis.

[a] Includes data from 15 episodes (5 proven cases and 10 probable cases of IA).

performance of the assay according to a per-test analysis and confirmed the higher sensitivity of the PA-EIA at lower thresholds. In addition, sensitivity was markedly increased when the sample was obtained closer to the date of diagnosis, especially when a low OD index value cutoff was used to define positivity.

In summary, the results of the present study support the use of an OD index of 0.5 as a cutoff for positivity for the PA-EIA when used to screen neutropenic adult patients and recipients of allogeneic hematopoetic stem cell transplants, and this cutoff may allow for earlier diagnosis of IA. Because it is identical to the OD index cutoff for positivity approved by the US Food and Drug Administration in the United States, the adoption of a single uniform cutoff worldwide not only will contribute to standardization of the PA-EIA, but it will also facilitate clinical trials in which assay results are used as a criterion for diagnosis.

Acknowledgments

Financial support. Bio-Rad Laboratories.

Potential conflicts of interest. J.A.M. and P.E.V. have received research support and speaker's honoraria from Bio-Rad. C.M., C.H., B.C., and M.T. are employees of Bio-Rad, France. All other authors: no conflicts.

References

1. Kuderer NM, Dale DC, Crawford J, et al. Mortality, morbidity, and cost associated with febrile neutropenia in adult cancer patients. Cancer **2006**; 106:2258–66.
2. Pagano L, Caira M, Candoni A, et al. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. Haematologica **2006**; 91:1068–75.
3. Fukuda T, Boeckh M, Carter RA, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood **2003**; 102: 827–33.
4. Subira M, Martino R, Franquet T, et al. Invasive pulmonary aspergillosis in patients with hematologic malignancies: survival and prognostic factors. Haematologica **2002**; 87:528–34.
5. Hope WW, Walsh TJ, Denning DW. Laboratory diagnosis of invasive aspergillosis. Lancet Infect Dis **2005**; 5:609–22.
6. McLintock LA, Jones BL. Advances in the molecular and serological diagnosis of invasive fungal infection in haemato-oncology patients. Br J Haematol **2004**; 126:289–97.
7. Stynen D, Goris A, Sarfati J, et al. A new sensitive sandwich ELISA to detect galactofuran in patients with invasive aspergillosis. J Clin Microbiol **1995**; 33:497–500.
8. Verweij PE, Stynen D, Rijs AJ, et al. Sandwich enzyme-linked immunosorbent assay compared with Pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromised patients. J Clin Microbiol **1995**; 33:1912–4.
9. Rohrlich P, Sarfati J, Mariani P, et al. Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. Pediatr Infect Dis J **1996**; 15:232–7.
10. Sulahian A, Tabouret M, Ridbaud P, et al. Comparison of enzyme immunoassay and latex agglutination test detection of galactomannan in the diagnosis of invasive aspergillosis. Eur J Clin Microbiol Infect Dis **1996**; 15:139–45.
11. Bretagne S, Marmorat-Khuong A, Kuentz M, et al. Serum *Aspergillus* galactomannan antigen testing by sandwich ELISA: practical use in neutropenic patients. J Infect **1997**; 35:7–15.
12. Maertens J, Verhaegen J, Demuynck H, 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: 3223–8.
13. Williamson ECM, Oliver DA, Johnson EM, et al. *Aspergillus* antigen testing in bone marrow transplant recipients. J Clin Pathol **2000**; 53: 362–6.
14. Ulusakarya A, Chachaty E, Vantelon JM, et al. Surveillance of *Aspergillus* galactomannan antigenemia for invasive aspergillosis by enzyme-linked immunosorbent assay in neutropenic patients treated for hematological malignancies. Hematol J **2000**; 1:111–6.
15. Sulahian A, Boutboul F, Ridbaud P, et al. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric haematology units during a 4-year prospective study. Cancer **2001**; 91:311–8.
16. Maertens J, Verhaegen J, Lagrou K, et al. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. Blood **2001**; 97:1604–10.
17. Herbrecht R, Letscher-Bru V, Oprea C, et al. *Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. J Clin Oncol **2002**; 20:1898–906.
18. Marr KA, Balajee SA, McLaughlin, et al. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. J Infect Dis **2004**; 190: 641–9.
19. Wheat LJ. Rapid diagnosis of invasive aspergillosis by antigen detection. Transpl Infect Dis **2003**; 5:158–66.
20 Verweij PE, Erjavec Z, Sluiters W, et al. Detection of antigen in sera of patients with invasive aspergillosis: intra- and interlaboratory reproducibility. The Dutch Interuniversity Working Party for Invasive Mycoses. J Clin Microbiol **1998**; 36:1612–6.
21. Maertens J, Theunissen K, Verbeken E, 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**; 126:852–60.
22. Mennink-Kersten MA, Donnelly JP, Verweij PE. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. Lancet Infect Dis **2004**; 4:349–57.
23. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis **2006**; 42: 1417–27.
24. Maertens J, Theunissen K, Deeren D, Meersseman W, Van Eldere J. Defining a case of invasive aspergillosis by serum galactomannan. Med Mycol **2006**; 44(Suppl):173–8.
25. Ascioglu S, Rex JH, De Pauw B, et al.; Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer and Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. 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.
26. Whiting P, Rutjes AWS, Reitsma JB, et al. Sources of variation and bias in studies of diagnostic accuracy: a systematic review. Ann Intern Med **2004**; 140:189–202.
27. Platelia *Aspergillus* [package insert]. Marnes-La-Coquette, France: Bio-Rad.
28. Marr KA, Laverdiere M, Gugel A, Leisenring W. Antifungal therapy decreases sensitivity of the *Aspergillus* galactomannan enzyme immunoassay. Clin Infect Dis **2005**; 40:1762–9.
29. Walter SD. Properties of the summary receiver operating characteristics curve for diagnostic test data. Stat Med **2002**; 21:1237–56.
30. Marr KA, Leisenring W. Design issues in studies evaluating diagnostic test for aspergillosis. Clin Infect Dis **2005**; 41(Suppl 6):S381–6.

31. Mennink-Kersten M, Ruegebrink D, Klont R, et al. Bifidobacterial lipoglycan as a new cause for false-positive Platelia *Aspergillus* enzyme-linked immunosorbent assay reactivity. J Clin Microbiol **2005**; 43: 3925–31.
32. Aubry A, Porcher R, Bottero J, et al. Occurrence and kinetics of false-positive *Aspergillus* galactomannan test results following treatment with β-lactam antibiotics in patients with hematological disorders. J Clin Microbiol **2006**; 44:389.
33. Maertens J, Theunissen K, Verhoef G, Van Eldere J. False-positive *Aspergillus* galactomannan antigen test results. Clin Infect Dis **2004**; 39: 289–90.