AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Prospective Evaluation of a New *Aspergillus* IgG Enzyme Immunoassay Kit for Diagnosis of Chronic and Allergic Pulmonary Aspergillosis

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Anti-*Aspergillus* IgG antibodies are important biomarkers for the diagnosis of chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA). We compared the performance of a new commercial enzyme immunoassay (EIA) (Bordier Affinity Products) with that of the Bio-Rad and Virion\Serion EIAs. This assay is novel in its association of two recombinant antigens with somatic and metabolic antigens of *Aspergillus fumigatus*. In a prospective multicenter study, 436 serum samples from 147 patients diagnosed with CPA (136 samples/104 patients) or ABPA (94 samples/43 patients) and from 205 controls (206 samples) were tested. We obtained sensitivities of 97%, 91.7%, and 86.1%, and specificities of 90.3%, 91.3%, and 81.5% for the Bordier, Bio-Rad, and Virion\Serion tests, respectively. The Bordier kit was more sensitive than the Bio-Rad kit ($P < 0.01$), which was itself more sensitive than the Virion\Serion kit ($P = 0.04$). The Bordier and Bio-Rad kits had similar specificity ($P = 0.8$), both higher than that of the Virion\Serion kit ($P = 0.02$). The area under the receiver operating characteristic (ROC) curves confirmed the superiority of the Bordier kit over the Bio-Rad and the Virion\Serion kits (0.977, 0.951, and 0.897, respectively; $P < 0.01$ for each comparison). In a subset analysis of 279 serum samples tested with the Bordier and Bio-Rad kits and an in-house immunoprecipitin assay (IPD), the Bordier kit had the highest sensitivity (97.7%), but the IPD tended to be more specific (71.2 and 84.7%, respectively; $P = 0.10$). The use of recombinant, somatic, and metabolic antigens in a single EIA improved the balance of sensitivity and specificity, resulting in an assay highly suitable for use in the diagnosis of chronic and allergic aspergillosis.

Aspergillus fumigatus is the species most frequently implicated in pulmonary aspergillosis (1, 2). Chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA) occur in immunocompetent hosts (3–9). CPA usually affects patients with underlying lung disease, such as mycobacterial infections, chronic obstructive pulmonary disease (COPD), ABPA, and emphysema (7, 9, 10). It has been estimated that >4.8 million asthmatic patients worldwide suffer from ABPA, and that about 240,000 people in Europe have CPA (11). Patients suffering from asthma or cystic fibrosis (CF) may become sensitized to *Aspergillus* antigens, resulting in ABPA, *Aspergillus*-related bronchitis, or severe asthma (1, 12).

The diagnosis of CPA and ABPA remains challenging and is based on a combination of clinical, radiological, biological, and mycological criteria (8, 13, 14). The detection of anti-*Aspergillus* antibodies is considered to be an important criterion (1, 2, 6, 7, 12, 14–16). Several serological methods are available, and those based on immunoprecipitin detection (IPD) are used for confirmation purposes, due to their high specificity (16) However, they have not been standardized and are not easy to perform. Thus, screening tests to detect anti-*Aspergillus* IgG by indirect hemagglutination, indirect immunofluorescence, or enzyme immunoassay (EIA, also called enzyme-linked immunosorbent assay [ELISA]) are often preferred (16–18). The use of EIAs also facilitates quantitative evaluation of the antibody response and automation, leading to rapid and easy routine selection (16–18).

We conducted a prospective multicenter study to evaluate the performance of a new commercial anti-*Aspergillus* IgG kit, the *A. fumigatus* IgG ELISA kit (Bordier Affinity Products). This assay is novel in terms of its antigen composition, because it combines two recombinant antigens with somatic and metabolic antigens from *A. fumigatus*. We compared this assay with two other commercial EIAs, the Platelia *Aspergillus* IgG (Bio-Rad) and the ELISA classic *Aspergillus* IgG (Virion\Serion), and an in-house IPD method (19).

Received 15 December 2015 **Returned for modification** 7 January 2016
Accepted 10 February 2016

Accepted manuscript posted online 17 February 2016

Citation Dumollard C, Bailly S, Perriot S, Brenier-Pinchart MP, Saint-Raymond C, Camara B, Gangneux JP, Persat F, Valot S, Grenouillet F, Pelloux H, Pinel C, Cornet M, Grenoble *Aspergillus* Committee. 2016. Prospective evaluation of a new *Aspergillus* IgG enzyme immunoassay kit for diagnosis of chronic and allergic pulmonary aspergillosis. J Clin Microbiol 54:1236–1242. doi:10.1128/JCM.03261-15.

Editor: D. W. Warnock

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MATERIALS AND METHODS

Study design, patients, and serum samples. This prospective study was conducted in the five French university hospitals of Grenoble, Rennes, Lyon, Dijon, and Besançon. Immunocompetent patients with suspected noninvasive aspergillosis were included between January 2013 and April 2015. Patients were assigned to one of six groups on the basis of clinical, radiological, and biological criteria, in accordance with the classifications established by the international committees of experts available at the time of the study (Table 1) (6–8, 13, 20). Cystic fibrosis (CF) patients were excluded from the group of colonized patients (group 2), because their *Aspergillus* IgG levels have been reported to be high even in the absence of ABPA, and persistent colonization may itself induce IgG responses in these patients (12, 21–23). CPA patients were assigned to group 3, 4, or 5, and group 6 included patients with ABPA. Immunocompromised patients at risk of invasive aspergillosis were excluded.

Laboratory methods. All serum samples were stored at −20°C until processing. Each was tested at each center with the three EIAs: Platelia *Aspergillus* IgG (Bio-Rad, Marnes-la-Coquette, France), ELISA classic *A. fumigatus* IgG (Virion\Serion, Würzburg, Germany), and ELISA *A. fumigatus* IgG (Bordier Affinity Products, Crissier, Switzerland), according to the manufacturers' recommendations. If the result of at least one test was positive or equivocal, the serum sample was subjected to testing with an in-house IPD method (19). Testing by this last method was centralized at Grenoble University Hospital to ensure standardization.

Platelia *Aspergillus* IgG. The Platelia *Aspergillus* IgG (Bio-Rad) assay relies on one recombinant antigen that is coated on the ELISA microplate. Values of ≥10 AU/ml were considered positive, values of 5 to 10 AU/ml were classified as equivocal, and values of <5 AU/ml were considered negative. Samples yielding >80 AU/ml were diluted and retested.

ELISA classic *A. fumigatus* IgG. The antigenic composition of the ELISA classic *A. fumigatus* IgG assay (Virion\Serion) is not available from the manufacturer. The measured optical density (OD) was converted into concentration in arbitrary units per milliliter by reference with the standard curve equation provided in each batch. Values of ≥70 AU/ml were considered positive, values of 50 to 70 AU/ml were classified as equivocal, and values of <50 AU/ml were considered negative.

ELISA *A. fumigatus* IgG. The wells in the ELISA *A. fumigatus* IgG (Bordier Affinity Products) were coated with two recombinant antigens, dipeptidyl peptidase type V (chymotrypsin) and RNase (mitogillin), and the somatic and metabolic antigens (24). An OD index was calculated by OD of the sample/OD of a cutoff provided in the kit. OD index values of ≥1 were considered positive, values of 0.8 to 1 were considered equivocal, and values of <0.8 were considered negative.

Immunoprecipitin detection. We used a double-diffusion gel electrophoresis technique with in-house metabolic and somatic antigens different from those used in the Bordier EIA (19). Briefly, we dispensed 10 μl of antigen solution into the agarose wells (1%) (Agarose NA; Amersham Biosciences). After migration, we added 200 μl of serum to the troughs. After incubation, catalase activity was detected by adding 20% hydrogen peroxide. The precipitin bands were stained with Amidoschwarz (Merck, USA). The result of the test was considered positive if ≥2 precipitin bands were detected and equivocal if only one precipitin band was detected (19). If catalase activity was detected, the result was considered positive, regardless of the number of precipitin bands (25).

Statistical analysis. The baseline characteristics of the groups of patients were compared in Fisher's exact tests and Wilcoxon tests for qualitative and quantitative variables, respectively. We used the Cochran Q test, followed by McNemar *post hoc* tests with Holm correction for multiple comparisons to compare the sensitivities and specificities of the assays. We calculated that a sample size of 223 serum samples from patients and 192 serum samples from controls would be sufficient to detect a significant difference with a power of 0.9. We calculated the Youden's index (sensitivity + specificity − 1) and the diagnostic odds ratio (DOR), as previously described (26). We carried out two secondary analyses, one after the exclusion of patients diagnosed solely on the basis of the presence of anti-*Aspergillus*-specific IgG and/or precipitins, to prevent overestimation, and the other after the exclusion of the sera showing equivocal results. The EIAs were compared by calculating the area under the receiver operating characteristic curve (ROC-AUC). The interassay reproducibility (coefficient of variation [CV] and standard deviation [SD]) of the Bordier test was evaluated on the basis of 39 measurements on the same sample, as an internal quality control. A P value of <0.05 was considered significant. Statistical analyses were performed with SAS 9.3 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Patients and sera. We included 352 patients in total. They had a median age of 58.9 years (quartile 1, 47.0 years; quartile 3, 71.5 years), 5% were minors (age, <18 years), and 55% were male. Serum samples were collected at the university hospitals of Grenoble, Rennes, Lyon, Dijon, and Besançon (n = 239, 74, 70, 31, and 22 samples, respectively). The commonest underlying lung conditions in the controls and patients are shown in Table 2. The distributions of the 352 patients and the 436 serum samples are detailed in Tables 1 and 3.

Test performances. The performances of the three EIAs when equivocal results were treated as positive are shown in Table 3. Overall, the sensitivity of the Bordier assay (97%) was significantly higher than that of the Bio-Rad assay (91.7%), which was itself higher than that of the Virion\Serion assay (86.1%) (McNemar's $P < 0.05$). The specificities of the Bordier and Bio-Rad tests were similar (90.3% and 91.3%, respectively; McNemar's $P = 0.8$) but significantly higher than that of the Virion\Serion assay (81.5%; McNemar's $P = 0.02$ for both comparisons). According to Youden's index, the Bordier assay provided the best balance of sensitivity and specificity. The Bordier assay showed the best DOR, and the Bio-Rad assay had a DOR that was greater than that of the Serion assay. The 95% confidence intervals (CIs) confirm that the Bio-Rad and Bordier assays are more discriminatory than the Serion assay. These results were confirmed by the ROC-AUC analysis, which showed that the performances of the Bordier and Bio-Rad assays were excellent, with AUCs of >0.9 (Fig. 1). The AUC of the Bordier assay (0.977; 95% CI, 0.962 to 0.991) was even greater than that of the Bio-Rad and Virion\Serion assays (0.951; 95% CI, 0.928 to 0.974; and 0.897; 95% CI, 0.863 to 0.931, respectively) ($P < 0.01$) (Fig. 2).

Based on these results, we decided to compare the performances of the best two EIAs (Bordier and Bio-Rad) with that of the IPD assay for the 279 serum samples for which one of the three EIAs gave a positive or equivocal result and for which a sufficiently large volume of sample remained for additional testing. The Bordier assay was again found to be the most sensitive (McNemar's $P < 0.05$) (Table 4). The Bio-Rad assay was more sensitive than the IPD assay only if equivocal results were considered to be negative (McNemar's $P = 0.049$; data not shown). The IPD tended to be more specific than the Bordier assay (84.7% and 71.2%, respectively; $P = 0.10$). Youden's index favored the IPD assay, but Bordier had the better DOR compared to the Bio-Rad and IPD assays.

In a secondary analysis performed after exclusion of the 20 patients diagnosed solely on the basis of the presence of anti-*Aspergillus* antibodies, the comparisons of the three EIAs and of the AUCs were unchanged, and the same differences were observed ($P < 0.01$ for the Cochran Q test and $P < 0.05$ for the McNemar tests in all comparisons). In the comparison of the IPD assay with the best two EIAs, we observed a difference in the results obtained from the pattern described above. The sensitivity of the

TABLE 1 Classification and distribution of the patients (adapted from references 7, 8, 13, and 20)

Patient group (*n* [%])[a]	Inclusion criteria[b]			
	General	Radiological	Clinical manifestation	*Aspergillus* species evidence
Control groups (205)				
Group 1 (191 [54.1])	Patients with respiratory symptoms in whom an *Aspergillus*-related disease had been ruled out			
Group 2, colonized patients (14 [4])	Patients with colonization defined as the recovery of *Aspergillus* spp. from respiratory specimens at least twice within a period from 10 days to 1 yr and who did not match criteria for CPA or ABPA diagnosis and had no major respiratory symptoms			
Group 3, simple aspergilloma (17 [4.8])		One "fungus ball" (mass within a lung cavity surrounded by the "air crescent" on CT scan or X ray with no progression over ≥3 mo	No alteration of general condition and no hemoptysis	*Aspergillus* species isolated from a respiratory sample AND/OR histological evidence of *Aspergillus* species hyphae AND/OR positive anti-*Aspergillus* serum precipitins
CPA (104)				
Group 4, CCPA or CFPA (62 [17.6])		At least one cavitary lesion (CCPA) in the lung, with or without a fungus ball, with progression over ≥3 mo; CFPA = fibrotic destruction after CCPA	Alteration of general condition (fever, wt loss, asthenia), chronic cough, and/or hemoptysis progressing for ≥3 mo	*Aspergillus* species isolated from a respiratory sample AND/OR histological evidence of *Aspergillus* species hyphae AND/OR positive anti-*Aspergillus* serum precipitins
Group 5, CNPA (25 [7.1])		Expanding cavities, nodules, consolidations with progression over ≥1 mo	Mild immunodeficiency (diabetes, alcoholism, immunosuppressive drugs); alteration of general condition (fever, wt loss, asthenia), chronic cough, and/or hemoptysis progression ≥1 mo	*Aspergillus* species isolated from a respiratory sample AND/OR histological evidence on a biopsy or surgical resection showing *Aspergillus* species hyphae AND/OR positive anti-*Aspergillus* serum precipitins and/or *Aspergillus* antigen detected in serum
ABPA (43)				
Group 6 (43 [12.2])	Asthma or CF and the triad of alteration of respiratory function, presence of anti-*Aspergillus*-specific IgE (>0.35 kAU/liter), and high total serum IgE (>1,000 IU/ml) (children: total IgE more than twice normal value for age); OR, two of the following criteria: high eosinophil count (>500 cells/μl), recent pulmonary lesions/worsening of existing lesions, serum precipitins, or anti-*Aspergillus* IgG antibodies			

[a] CPA, chronic pulmonary aspergillosis; CCPA, chronic cavitary pulmonary aspergillosis; CFPA, chronic fibrosing pulmonary aspergillosis; CNPA, chronic necrotizing pulmonary aspergillosis; ABPA, allergic bronchopulmonary aspergillosis.
[b] CT, computed tomography; CF, cystic fibrosis.

TABLE 2 Underlying lung conditions

Previous pulmonary history[a]	No. (%) for: Controls ($n = 167$)	Diseased patients ($n = 85$)
Allergic bronchopulmonary aspergillosis	0 (0)	25 (29.4)[b]
Asthma	36 (21.6)	9 (10.6)[c]
No previous respiratory history	30 (18)	1 (1.2)
Cystic fibrosis	11 (6.6)	30 (35.3)
Bronchiectasis	4 (2.4)	7 (8.2)
Aspergillus sinusitis	1 (0.6)	0 (0)
Previous pulmonary aspergillosis	1 (0.6)	15 (17.6)
Previous *Aspergillus* colonization	0 (0)	5 (5.9)
Previous aspergilloma	0 (0)	9 (10.6)
Emphysema	23 (13.8)	8 (9.4)
Chronic obstructive pulmonary disease	45 (26.9)	19 (22.4)
Respiratory deficiency	18 (10.8)	7 (8.2)
Previous mycobacterial infection	12 (7.2)	9 (10.6)
Assessment of respiratory symptoms[d]	20 (12)	2 (2.4)
Previous cancer history	1 (0.6)	8 (9.4)
Assessment before immunosuppression[e]	17 (10.2)	0 (0)
Other[f]	16 (9.6)	6 (7.1)

[a] Previous pulmonary history was missing for $n = 100$ (38 controls and 62 diseased patients). The patients and controls may have more than one underlying condition.
[b] Twenty patients also had cystic fibrosis.
[c] No patient had CF.
[d] Respiratory symptoms, such as shortness of breath, cough, and wheezing rhonchi.
[e] Including before treatment with biotherapy or before a transplantation (lung, heart, liver, or kidney).
[f] Pneumothorax, asbestosis or solvent exposure, sarcoidosis, pulmonary hypertension, sleep apnea, pulmonary embolism, and previous hemoptysis.

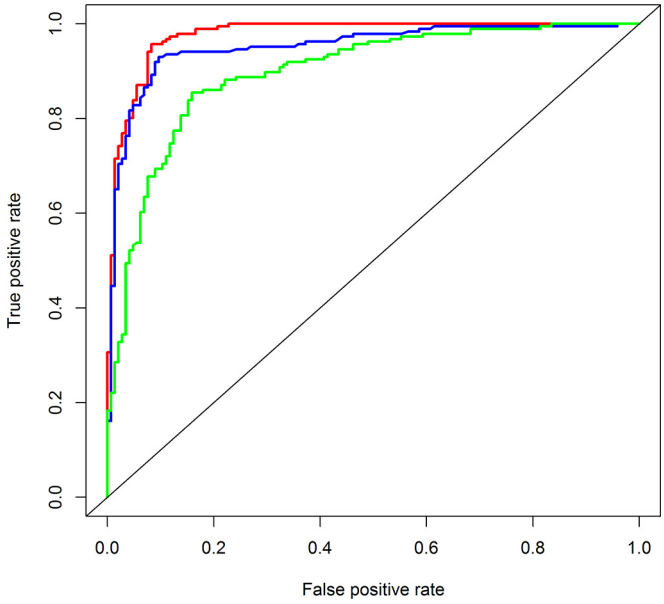

FIG 1 Receiver operating characteristic (ROC) curves of Platelia *Aspergillus* IgG (Bio-Rad) (blue curve), ELISA classic *A. fumigatus* IgG (Virion\Serion) (green curve), and ELISA *A. fumigatus* IgG (Bordier Affinity Products) (red curve) assays.

Bordier assay tended to be higher than that of the Bio-Rad assay; however, the difference did not reach statistical significance (98% and 94.5%, respectively; McNemar's $P = 0.06$), but both were more sensitive than the IPD assay (88%; McNemar's $P < 0.01$ and 0.01, respectively).

An analysis after exclusion of the equivocal results showed the same trend in the differences between the three EIAs. The sensitivity of the Bordier assay (96.1%) tended to be higher than that of the Bio-Rad assay (91.7%) (McNemar's $P = 0.07$) and was significantly superior to that of the Virion\Serion assay (85.6%) (McNemar's $P < 0.01$). The specificities of the Bordier and the Bio-Rad assays were comparable (94.4% and 95.5%, respectively; McNemar's $P = 0.68$) and tended to be higher than the specificity of Virion\Serion (88.2%; McNemar's $P = 0.06$).

An analysis of test performances by patient category, and after correction for multiple comparisons, indicated that the new Bordier EIA was more sensitive than the Bio-Rad, Virion\Serion, and IPD tests for group 5 of the CNPA, with a sensitivity at 100% (Tables 3 and 4 and Fig. 2). In the other groups of patients, the

TABLE 3 Performances of three anti-*Aspergillus* IgG enzyme immunoassays for the diagnosis of chronic pulmonary aspergillosis and allergic bronchopulmonary aspergillosis[a]

Performance measure[b]	Group (n)	Bordier	Bio-Rad	Virion\Serion	*P* value: Cochran Q test	McNemar *post hoc* tests: Bordier/Bio-Rad	Bordier/Virion\Serion	Bio-Rad/Virion\Serion
Se (% [95% CI])	All sera (230)	97.0 (94.7, 99.2)	91.7 (88.2, 95.3)	86.1 (81.6, 90.1)	<0.01	<0.01	<0.01	0.04
	Group 3 (23)	95.6 (78.0, 99.9)	95.6 (78.0, 99.9)	78.3 (56.3, 92.5)	0.02	NA[c]	0.12	0.12
	Group 4 (78)	97.4 (91.0, 99.7)	92.3 (84.0, 97.1)	82.0 (71.7, 89.8)	<0.01	0.12	<0.01	0.06
	Group 5 (35)	100 (90.0, 100)	91.4 (76.9, 98.2)	82.9 (66.3, 93.4)	<0.01	NA	NA	0.51
	Group 6 (94)	95.7 (89.4, 98.8)	90.4 (82.6, 95.5)	92.6 (85.2, 96.9)	0.20	0.20	0.45	0.73
Sp (% [95% CI])	All sera (206)	90.3 (86.2, 94.3)	91.3 (87.4, 95.1)	81.5 (76.3, 86.9)	<0.01	0.8	0.02	0.02
	Group 1 (192)	91.7 (86.8, 95.2)	92.7 (88.1, 96.0)	81.8 (75.6, 87.0)	<0.01	0.79	<0.01	<0.01
	Group 2 (14)	71.4 (41.9, 91.6)	71.4 (41.9, 91.6)	78.6 (49.2, 95.3)	0.87	NA	0.65	0.71
Youden's index	All sera (436)	0.873	0.830	0.676				
DOR (95% CI)	All sera (436)	296 (122, 715)	116 (59, 228)	27 (16, 45)				

[a] Equivocal results were considered positive.
[b] Se, sensitivity; 95% CI, 95% confidence interval; Sp, specificity. Youden's index was calculated by sensitivity + specificity − 1.
[c] NA, not applicable.

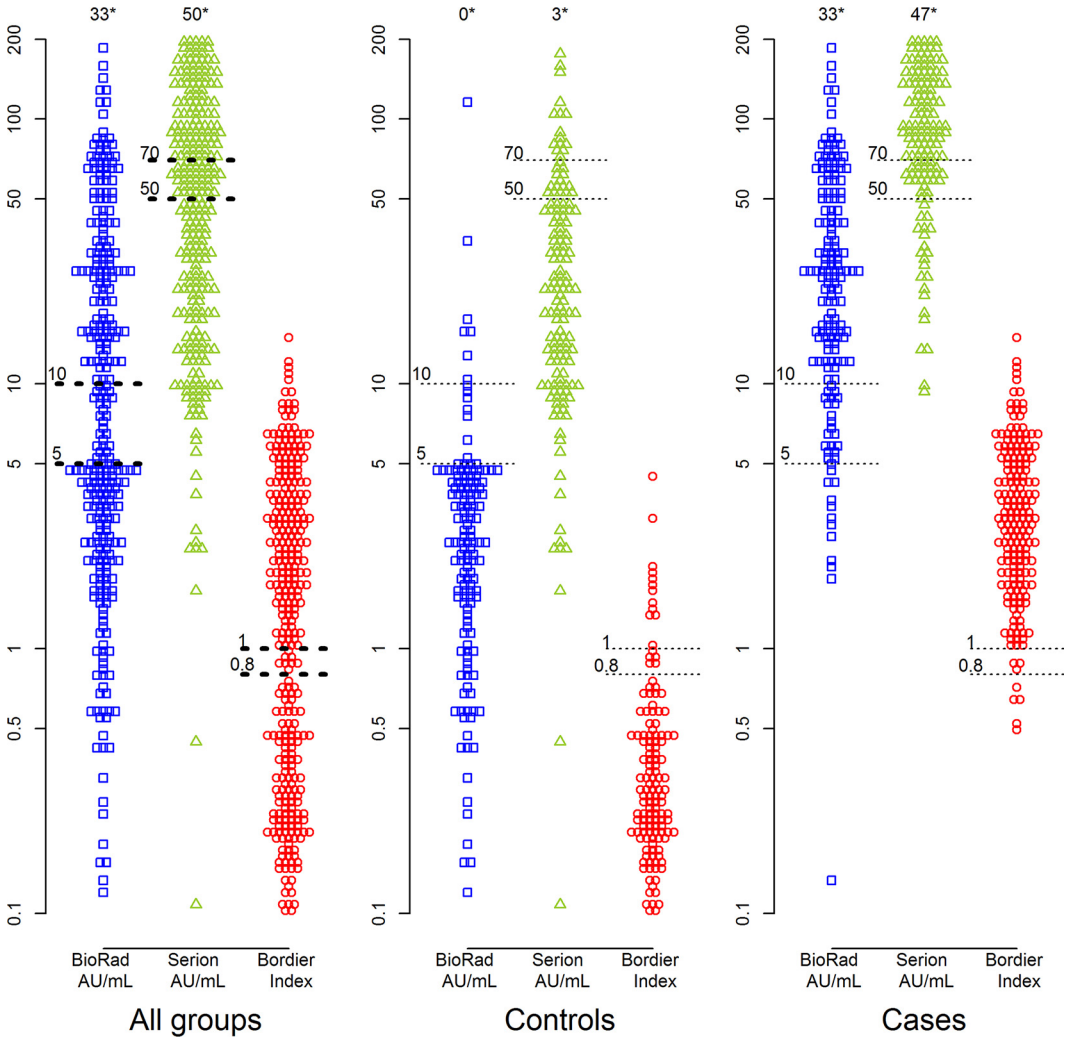

FIG 2 Quantitative results and distribution of antibodies obtained with three anti-*Aspergillus* IgG enzyme immunoassays for the diagnosis of chronic pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. *, number of values >200 AU/ml for the Bio-Rad and Virion\Serion assays; dotted line, positive and negative cutoffs; blue squares, Platelia *Aspergillus* IgG assay (Bio-Rad); green triangles, ELISA classic *A. fumigatus* IgG assay (Virion\Serion); red circles. ELISA *A. fumigatus* IgG assay (Bordier Affinity Products); controls, groups 1 and 2; cases, patients with chronic or allergic aspergillosis (groups 3 to 6). The *y* axis is logarithmic. The R package beeswarm was used to create the graph.

only differences between sensitivities remaining significant after correction for multiple comparisons were those for group 4, with the Bordier assay being more sensitive than the Virion\Serion and IPD assays (McNemar's $P < 0.01$ and 0.01, respectively). The per-group analysis of group 1 revealed specificity results similar to those for the total control group. The sample size for group 2 was insufficiently large for a separate analysis.

The interassay CV of the Bordier EIA was 20% (SD = 0.366). Quantitative results of the assays are detailed in Fig. 2. The Bordier EIA provided significantly fewer equivocal results (2.8%) than the Bio-Rad (6.7%) and the Virion\Serion assay (10.1%) ($P < 0.01$ for each comparison with the Bordier assay).

DISCUSSION

We show here that the new commercially available Bordier EIA for the detection of anti-*Aspergillus* IgG antibodies is suitable for the diagnosis of CPA and ABPA in immunocompetent patients. In this large prospective multicenter cohort of 352 patients providing 436 serum samples, this assay had a high sensitivity (97%) and specificity (90.3%), and its AUC value of 0.977 was excellent. This new assay was more sensitive than the other two EIAs used in routine clinical practice and the IPD assay used for confirmation. The sensitivity of the Bordier assay was remarkably high, at 100%, in the group of patients with chronic necrotizing pulmonary aspergillosis. Its specificity was similar to that of the Bio-Rad assay and higher than that of the Virion\Serion assay but tended to be lower than that of the IPD assay. Overall, the best Youden's index (indicating the trade-off between sensitivity and specificity) and the best DOR (indicating the discriminatory power) were obtained with the Bordier assay.

The Bio-Rad and Virion\Serion EIAs have already been compared in a large retrospective study (17). The sensitivity and specificity obtained were 93.8% and 87.3%, respectively, for the Bio-Rad assay and 90.6% and 75.7%, respectively, for the Virion\Serion assay. Our findings confirm the superiority of the Bio-Rad assay over the Virion\Serion assay. Another recent

TABLE 4 Performances of two anti-*Aspergillus* IgG enzyme immunoassays and the immunoprecipitin method[a]

Performance measure[b]	Group (*n*)	Bordier	Bio-Rad	IPD	*P* value			
						McNemar *post hoc* tests		
					Cochran Q test	Bordier/Bio-Rad	Bordier/IPD	Bio-Rad/IPD
Se (% [95% CI])	All sera (220)	97.7 (95.8, 99.7)	93.2 (89.8, 96.5)	89.1 (85.0, 93.2)	<0.01	0.03	<0.01	0.14
	Group 3 (23)	95.6 (78.0, 99.9)	95.6 (78.0, 99.9)	91.3 (72.0, 98.9)	0.16	NA[c]	0.56	0.56
	Group 4 (72)	98.6 (92.5, 99.9)	95.8 (88.3, 99.1)	86.1 (75.9, 93.1)	<0.01	0.5	0.01	0.08
	Group 5 (34)	100 (90.0, 100)	91.2 (76.3, 98.1)	85.2 (68.9, 95.1)	<0.01	NA	NA	0.69
	Group 6 (91)	96.7 (90.7, 99.3)	91.2 (83.4, 96.1)	92.3 (84.8, 96.9)	0.25	0.18	0.34	0.76
Sp (% [95% CI])	All sera (59)	71.2 (59.6, 82.7)	76.3 (65.4, 87.1)	84.7 (75.6, 93.9)	0.14	0.80	0.10	0.36
	Group 1 (52)	75.0 (61.0, 86.0)	78.9 (65.3, 88.9)	86.5 (74.2, 94.4)	0.28	0.79	0.21	0.45
	Group 2 (7)	42.9 (9.9, 81.6)	57.1 (18.4, 90.1)	71.4 (29.0, 96.3)	0.37	NA	0.50	0.56
Youden's index	All sera (279)	0.689	0.695	0.738				
DOR (95% CI)	All sera (279)	106 (37, 303)	44 (20, 98)	45 (20, 103)				

[a] Equivocal results were considered positive.
[b] Se, sensitivity; 95% CI, 95% confidence interval; Sp, specificity. Youden's index was calculated by sensitivity + specificity − 1.
[c] NA, not applicable.

study compared two EIAs, including the Bio-Rad EIA, and an IPD method based on commercial antigens in a prospective cohort (18). Again, the Bio-Rad assay had high sensitivity (93%), but its reproducibility was low, with an interassay CV of 33%, which, according to the authors, precludes its use for the monitoring of patients. Another drawback of this test is the need to dilute and retest all samples yielding values of >80 AU/ml. Furthermore, one recent study evaluated a commercial Western blot kit for the detection of anti-*Aspergillus* IgG (27). The authors reported a sensitivity of 90.0% to 93.8% in CPA and ABPA patients, with the specificity (94%) being estimated solely on the basis of the results for healthy blood donors as controls. Further studies are required to compare the EIAs and Western blot assay for the detection of anti-*Aspergillus* IgG.

We found that the new Bordier EIA outperformed the Bio-Rad and Virion\Serion EIAs. This better performance may be explained by the association of two selected recombinant proteins with metabolic and somatic custom-produced antigens. The chosen recombinants yielded the best performances in tests carried out with eight proteins and the purified galactomannan antigen (24). Preliminary results obtained through separate analyses of the two sets of antigens, recombinant versus the metabolic/somatic custom produced, suggested that the combination of these antigens was beneficial in terms of both sensitivity and specificity (data not shown). The gain in sensitivity and the parallel loss of specificity are counterbalanced by the use of the metabolic and somatic antigens of *A. fumigatus* included in this kit. This might also explain the greater discrimination of the Bordier test, which provided less equivocal results than those of the other tests (Fig. 2).

Our study confirmed the high specificity (84.7%) of the IPD, based on in-house antigens that we have been using for >20 years, which was even greater (96.6%) when equivocal results were treated as negative (19). However, it was not significantly higher than that of the Bordier assay or the Bio-Rad assay, probably due to the loss of statistical power in the smaller sample size. Our selection of serum samples giving positive or equivocal results with one of the three EIAs for the analysis of the IPD assay may have resulted in a slight overestimation of the sensitivity.

Our results suggest that the Bordier EIA is suitable for use as a screening test in a two-step strategy for the detection of anti-*Aspergillus* antibodies. Our findings confirm that the IPD assay is an appropriate specific method for precipitin detection and confirmation of the EIA results. In this case, equivocal results in the IPD assay should be considered negative to increase specificity. If a one-step strategy is preferred, our results suggest that the Bordier EIA would be the best choice, as it gave the best compromise between sensitivity and specificity (Youden's index, 0.873; Table 3).

The classification of colonized patients remains challenging. We decided to consider them controls, as a diagnosis of infection had been ruled out in these patients. Conversely, *Aspergillus* species colonization may be considered a prerequisite or initial stage of infection, accounting for the grouping together of colonized and infected patients in other studies (27). We also performed an analysis in which colonized individuals were grouped with the patients. The only modification to the results concerned the relative specificities of the Bordier, Bio-Rad, and IPD assays, which became comparable when equivocal results were considered negative (data not shown).

In conclusion, given its high Youden's index and diagnostic odds ratio, indicating a good balance between sensitivity and specificity, this new Bordier EIA is suitable for the detection of anti-*Aspergillus* IgG for the diagnosis of chronic and allergic pulmonary aspergillosis. Further studies are required to confirm its use for monitoring clinical status in patients. Finally, our results confirmed that immunoprecipitin detection was an appropriate method for confirming EIA results.

ACKNOWLEDGMENTS

We thank Renée Grillot for her support and advice, which were very helpful in the development of the in-house antigens and ELISA leading to the creation of the Bordier EIA. We thank M. Monod for producing the recombinant antigens of *A. fumigatus*. We also thank all the technicians of the Parasitology-Mycology Laboratory of Grenoble Hospital for technical assistance.

M. Cornet has received travel grants from Gilead, Pfizer, and Merck and received remuneration for talks on behalf of Pfizer. The Parasitology

and Mycology Laboratory of Grenoble University Hospital, where M. Cornet, M. P. Brenier-Pinchart, and H. Pelloux currently work, produces and sells *A. fumigatus* somatic and metabolic antigens to Bordier Affinity Products. Bordier Affinity Products provided the kits for the three EIAs analyzed here but did not participate in any phase of the study, from its design to the analysis of the results, the conclusions reported here, and the writing of the manuscript. H. Pelloux and M. Cornet report having received research grants for the Parasitology and Mycology Laboratory of Grenoble University Hospital from Virion\Serion Immundiagnostica GmbH and Bordier Affinity Products.

C. Dumollard and S. Perriot contributed to the design of the study, performed the assays, collected the data, analyzed the results, and contributed to the writing of the manuscript. S. Bailly performed the statistical analysis and contributed to the writing of the manuscript. M. P. Brenier-Pinchart and H. Pelloux contributed to the classification and diagnosis of patients, coordinated the Grenoble *Aspergillus* Committee, and contributed to the writing of the manuscript. C. Saint-Raymond, B. Camara, J. P. Gangneux, F. Persat, S. Valot, and F. Grenouillet contributed to the recruitment of the patients and their clinical management, patient classification and diagnosis, and the writing of the manuscript. C. Pinel and M. Cornet contributed to the development of the antigens used in both the Bordier EIA and the in-house IPD method, the study design, and the writing of the manuscript. Collaborators of the Grenoble *Aspergillus* Committee contributed to the recruitment, management, diagnosis, and classification of the patients.

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