Prospective evaluation of a new Aspergillus IgG EIA kit for the diagnosis of chronic and allergic pulmonary aspergillosis

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Full-length paper

Prospective evaluation of a new *Aspergillus* IgG EIA kit for the diagnosis of chronic and allergic pulmonary aspergillosis

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Abstract

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 EIA (Bordier Affinity Products) with those of the Bio-Rad and Virion\Serion EIAs. This assay is novel in the association of two recombinant antigens with somatic and metabolic antigens of *A. fumigatus*. In a prospective multicentre study, 436 serum samples from 147 patients diagnosed with CPA (136 sera/104 patients) or ABPA (94 sera/43 patients) and from 205 controls (206 sera) 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 specificities ($p=0.8$), both higher than that of the Virion\Serion kit ($p=0.02$). The areas under the 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 sera 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 between sensitivity and specificity, resulting in an assay highly suitable for use in the diagnosis of chronic and allergic aspergillosis.

Introduction

A. 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 over 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 or 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 multicentre study to evaluate the performance of a new commercial anti-*Aspergillus* IgG kit, the *Aspergillus fumigatus* IgG ELISA kit (Bordier Affinity Products). This assay is novel in terms of its antigen composition, because it

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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).

Materials and Methods

Study design, patients and sera

This prospective study was conducted in the five French university hospitals of Grenoble, Rennes, Lyon, Dijon, and Besançon. Immunocompetent patients with suspected non-invasive 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 colonised patients (group 2), because their *Aspergillus* IgG levels have been reported to be high even in the absence of ABPA, and persistent colonisation may itself induce IgG responses in these patients (12, 21–23). CPA patients were assigned to groups 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 centre with the three EIAs: Platelia *Aspergillus* IgG (Bio-Rad, Marnes-la-Coquette, France), ELISA Classic *Aspergillus fumigatus* IgG (Virion\Serion, Würzburg, Germany) and ELISA *Aspergillus fumigatus* IgG (Bordier Affinity Products, Crissier, Switzerland), according to the manufacturers' recommendations. If the result of at least one test was positive or equivocal,

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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 standardisation.

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

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

ELISA *Aspergillus fumigatus* IgG (Bordier Affinity Products).The wells were coated with the two recombinant antigens, dipeptidyl peptidase type V (chymotrypsin) and ribonuclease (mitogillin), and the somatic and metabolic antigens (24). An OD index was calculated by OD of the sample / OD of a cut off provided in the kit. OD index values ≥1 were considered positive, values of 0.8-1 were considered equivocal, and values <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 10µl of antigen solution into the agarose wells (1%) (Agarose NA, Amersham Biosciences). After migration, we added 200µl 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 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 ROC curve (ROC AUC). The inter-assay reproducibility (coefficient of variation (CV) and standard deviation (SD)) of the Bordier test was evaluated on the basis of 39 measurement on the same sample, as an internal quality control. A p value <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 [47.0-71.5], 5% were minors (age<18), 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, 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 sera 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 3A. 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 $p<0.05$). The specificities of the Bordier and Bio-Rad tests were similar (90.3% and 91.3%, respectively; McNemar $p=0.8$) but significantly higher than that of the Virion\Serion assay (81.5%; McNemar $p=0.02$ for both comparison). According to Youden's index, the Bordier assay provided the best balance between sensitivity and specificity. Bordier showed the best DOR and Bio-Rad had a greater DOR than Serion. The 95% confidence intervals (CIs) confirm that Bio-Rad and Bordier are more discriminatory than Serion. These results were confirmed by the area under the ROC curve (AUC) analysis, which showed that the performances of the Bordier and Bio-Rad assays were excellent, with AUCs exceeding 0.9. The AUC of the Bordier assay (0.977; 95%CI [0.962; 0.991]) was even greater than those of the Bio-Rad and Virion\Serion assays (0.951 [0.928; 0.974] and 0.897 [0.863; 0.931]) respectively) ($p<0.01$) (Figure 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 sera for which one of the three EIAs gave a positive or equivocal result and for which a sufficiently large volume of the

sample remained for additional testing. The Bordier assay was again found to be the most sensitive (McNemar $p<0.05$) (Table 3B). The Bio-Rad assay was more sensitive than the IPD assay only if equivocal results were considered to be negative (McNemar $p=0.049$; 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 favoured the IPD assay but Bordier had the better DOR compared to Bio-Rad and IPD.

In a secondary analysis performed after the 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 the 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 Bordier assay tended to be higher than that of the Bio-Rad one, however the difference did not reached statistical significance (98% and 94.5%, respectively; McNemar $p=0.06$) but both were more sensitive than the IPD assay (88%; McNemar $p<0.01$ and $p=0.01$, respectively).

An analysis after exclusion of the equivocal results showed the same trend in the differences between the three EIAs. The sensitivity of Bordier (96.1%) tended to be higher than the one of Bio-Rad (91.7%) (McNemar p=0.07) and was significantly superior to the one of Virion\Serion (85.6%) (McNemar p<0.01). The specificity of the Bordier and the Bio-Rad assays were comparable (94.4% and 95.5 %, respectively; McNemar p=0.68) and tended to be higher than that of Virion\Serion (88.2 %; McNemar 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% (Table 3A, 3B and Figure2). In the other groups of patients, the 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 $p<0.01$ and $p=0.01$, respectively). The per-group analysis of group 1 revealed similar specificity results as for the total control group. The sample size for group 2 was insufficiently large for a separate analysis.

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

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 multicentre cohort of 352 patients providing 436 sera, 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 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 inter-assay 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 greater than 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%-93.8% in CPA and ABPA patients, 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 set 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 could also explain the greater discrimination of the Bordier test, which provided less equivocal results than the other tests (Figure 2).

Our study confirmed the high specificity (84.7%) of the IPD based on in-house antigens that we have been using for more than 20 years which was even greater (96.6%)

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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 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 3A).

The classification of colonised patients remains challenging. We decided to consider them as controls, as a diagnosis of infection had been ruled out in these patients. Conversely, *Aspergillus* spp. colonisation may be considered a prerequisite or initial stage of infection, accounting for the grouping together of colonised and infected patients in other studies(27).We also performed an analysis in which colonised 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

274 patients. Finally, our results confirmed that immunoprecipitin detection was an appropriate
275 method for confirming EIA results.
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Transparency declaration

Muriel 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 analysed here but did not participate in any phase of the study, from its design to the analysis of the results and conclusions reported here and the writing of the manuscript.

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Author contribution

C. Dumollard, S. Perriot contributed to the design of the study, performed the assays, collected the data, analysed the results and contributed to the writing of the manuscript. Dr S. Bailly performed the statistical analysis and contributed to the writing of the manuscript. Dr M.P Brenier-Pinchart and H. Pelloux contributed to the classification and diagnosis of patients, co-ordinated 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.

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Grenouillet contributed to the recruitment of the patients and their clinical management and topatient classification and diagnosis and the writing of the manuscript. Dr C. Pinel and M. Cornet contributed to the development of the antigens used in both the Bordier EIA and the in-house IPD method, and to 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.

Collaborators of the Grenoble *Aspergillus* Committee are Thiebaut-Bertrand A., Maubon D., Bosseray A., Hamidfar R., Epaulard O., Cognet O., Dard C., Garnaud C., El-Zeenni N., Murat J.B., Bartoli M., Saviuc P., Wintenberger C., Tonini J., Honnorat E.

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393 **TABLE 1. Classification and distribution of the patients (adapted from** (7, 8, 13, 20)**).**
394

		Inclusion criteria		
Control groups (*n*=205)	**Group 1** (*n*=191; 54.1%)	Patients with respiratory symptoms in whom an *Aspergillus*-related disease had been ruled out		
	Group 2 Colonised patients (*n*=14; 4%)	Patients with colonisation defined as the recovery of *Aspergillus* spp. from respiratory specimens at least twice within a period from 10 days to one year and who did not match the criteria for CPA or ABPA diagnosis and had no major respiratory symptoms		
		Radiological criteria	**Clinical manifestation**	***Aspergillus* sp. evidence**
CPA (*n*= 104)	**Group 3** simple aspergilloma (*n*=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 months	No alteration of the general condition and no haemoptysis	*Aspergillus* sp isolated from a respiratory sample AND/OR Histological evidence of *Aspergillus* sp hyphae AND/OR Positive anti-*Aspergillus* serum precipitins
	Group 4 CCPA or CFPA (*n*=62; 17.6%)	At least one cavitary lesion (CCPA) in the lung, with or without a "fungus ball", with progression over ≥ 3 months. CFPA = fibrotic destruction after CCPA	Alteration of the general condition (fever, weight loss, asthenia), chronic cough and/or haemoptysis progressing for ≥ 3 months	*Aspergillus* sp isolated from a respiratory sample AND/OR Histological evidence of *Aspergillus* sp hyphae AND/OR Positive anti-*Aspergillus* serum precipitins

	Group 5 CNPA (*n*=25; 7.1%)	Expanding cavities, nodules, consolidations with progression over ≥ 1 month.	Mild immunodeficiency (diabetes, alcoholism, immunosuppressive drugs). Alteration of the general condition (fever, weight loss, asthenia), chronic cough and/or haemoptysis progression ≥ 1 month.	*Aspergillus* sp isolated from a respiratory sample AND/OR Histological evidence on a biopsy or surgical resection showing *Aspergillus* sp hyphae AND/OR Positive anti-*Aspergillus* serum precipitins and/or *Aspergillus* antigen detected in serum
			Inclusion criteria	
ABPA (*n*=43)	**Group 6** (*n*=43; 12.2%)	Asthma or CF and the triad of alteration of respiratory function, presence of anti-*Aspergillus* specific IgE (>0.35 kAU/l) and high total serum IgE (> 1000 IU/ml) (children: total IgE > twice the 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		

CPA. Chronic pulmonary aspergillosis
CCPA. Chronic cavitary pulmonary aspergillosis
CFPA. Chronic fibrosing pulmonary aspergillosis
CNPA. Chronic necrotizing pulmonary aspergillosis
ABPA. Allergic bronchopulmonary aspergillosis

TABLE 2. Underlying lung conditions

Previous pulmonary history	Controls (*n*=167)	Diseased patients (*n*=85)
Allergic bronchopulmonary aspergillosis	0 (0)	25 (29.4)*
Asthma	36 (21.6)	9 (10.6)ϕ
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* colonisation	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†	20 (12)	2 (2.4)
Previous cancer history	1 (0.6)	8 (9.4)
Assessment before immunosuppresion£	17 (10.2)	0 (0)
Others¥	16 (9.6)	6 (7.1)

Missing previous pulmonary history: *n*=100 (38 controls and 62 diseased patients)

Patients and controls may have more than one underlying condition.

* 20 patients also had cystic fibrosis.

ϕ No patient had CF

† Respiratory symptoms, such as shortness of breath, cough, wheezing rhonchi

£ Including before treatment with biotherapy or before a transplantation (lung, heart, liver or kidney)

¥Others: pneumothorax, asbestosis or solvent exposure, sarcoidosis, pulmonary hypertension, sleep apnoea, pulmonary embolism, previous haemoptysis.

TABLE 3:

(A) Performances of three anti-Aspergillus IgG enzyme immunoassays for the diagnosis of chronic pulmonary aspergillosis and allergic bronchopulmonary aspergillosis and

(B) Performances of two anti-Aspergillus IgG enzyme immunoassays and the immunoprecipitation method

[1] Univocal results were considered positive

A

		Bordier		Bio-Rad		Virion-Serion		*p*-value*	McNemar		
									Bordier/ Bio-Rad	Bordier/ Virion-Serion	Bio-Rad/ Virion-Serion
Se % [95% CI]	All sera *n*=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 *n*=23	95.6	[78.0 ; 99.9]	95.6	[78.0 ; 99.9]	78.3	[56;3 ; 92.5]	0.02	NA	0.12	0.12
	Group 4 *n*=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 *n*=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 *n*=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 *n*=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 *n*=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 *n*=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[1]	All sera *n*= 436	0.873		0.830		0.676					
DOR		296	[122 ; 715]	116	[59 ; 228]	27	[16 ; 45]				

B								*p*-value*	McNemar		
		Bordier		Bio-Rad		IPD			Bordier/ Bio-Rad	Bordier/ IPD	Bio-Rad/ IPD
Se % [95% CI]	All sera *n*=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 *n*=23	95.6	[78.0 ; 99.9]	95.6	[78.0 ; 99.9]	91.3	[72.0 ; 98.9]	0.16	NA	0.56	0.56
	Group 4 *n*=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 *n*=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 *n*=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 *n*=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 *n*=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 *n*=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 *n*= 279	0.689		0.695		0.738					
DOR		106	[37 ; 303]	44	[20 ; 98]	45	[20 ; 103]				

Se: Sensitivity Sp: specificity

*__Cochran Q test__

†Equivocal results were considered positive

¥McNemar post-hoc tests showed no significant difference

‖Youden's index = sensitivity + specificity – 1

NA = not applicable

Figure Legends

Figure 1.Receiver operating characteristic (ROC) curves ofPlatelia *Aspergillus* IgG (Bio-Rad), ELISA Classic *Aspergillus fumigatus* IgG (Virion\Serion) and ELISA *Aspergillus fumigatus* IgG (Bordier Affinity Products) assays.

Blue curve Platelia *Aspergillus* IgG (Bio-Rad)

Green curve. ELISA Classic *Aspergillus fumigatus* IgG (Virion\Serion)

Red curve ELISA *Aspergillus fumigatus* IgG (Bordier Affinity Products)

Figure 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.

* Indicates the number of values higher than 200 AU/ml for the Bio-Rad and Virion\Serion assays

The Y scale is logarithmic

dotted line: positive and negative cut-offs

R package beeswarm was used to perform the graph.

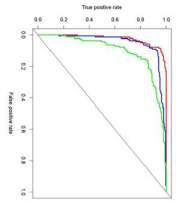
Blue square. Platelia *Aspergillus* IgG (Bio-Rad)

Green triangle. ELISA Classic *Aspergillus fumigatus* IgG (Virion\Serion)

Red circles. ELISA *Aspergillus fumigatus* IgG (Bordier Affinity Products)

Controls : groups 1 and 2

Cases : Patients with chronic or allergic aspergillosis (groups 3 to 6)


True positive rate
0.0
0.2
0.4
0.6
0.8
1.0
False positive rate