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

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1 Full-length paper

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

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27

28 **Abstract**

29 Anti-*Aspergillus* IgG antibodies are important biomarkers for the diagnosis of chronic  
30 pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA). We  
31 compared the performance of a new commercial EIA (Bordier Affinity Products) with those  
32 of the Bio-Rad and Virion\Serion EIAs. This assay is novel in the association of two  
33 recombinant antigens with somatic and metabolic antigens of *A. fumigatus*. In a prospective  
34 multicentre study, 436 serum samples from 147 patients diagnosed with CPA (136 sera/104  
35 patients) or ABPA (94 sera/43 patients) and from 205 controls (206 sera) were tested. We  
36 obtained sensitivities of 97%, 91.7%, and 86.1%, and specificities of 90.3%, 91.3%, and  
37 81.5% for the Bordier, Bio-Rad and Virion\Serion tests, respectively. The Bordier kit was  
38 more sensitive than the Bio-Rad kit ( $p<0.01$ ), which was itself more sensitive than the  
39 Virion\Serion kit ( $p=0.04$ ). The Bordier and Bio-Rad kits had similar specificities ( $p=0.8$ ),  
40 both higher than that of the Virion\Serion kit ( $p=0.02$ ). The areas under the ROC curves  
41 confirmed the superiority of the Bordier kit over the Bio-Rad and the Virion\Serion kits  
42 (0.977, 0.951 and 0.897, respectively;  $p<0.01$  for each comparison). In a subset analysis of  
43 279 sera tested with the Bordier and Bio-Rad kits and an in-house immunoprecipitin assay  
44 (IPD), the Bordier kit had the highest sensitivity (97.7%), but the IPD tended to be more  
45 specific (71.2 and 84.7%, respectively;  $p=0.10$ ).

46 The use of recombinant, somatic and metabolic antigens in a single EIA improved the balance  
47 between sensitivity and specificity, resulting in an assay highly suitable for use in the  
48 diagnosis of chronic and allergic aspergillosis.

49

50 **Introduction**

51 *A. fumigatus* is the species most frequently implicated in pulmonary aspergillosis (1, 2).  
52 Chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA)  
53 occur in immunocompetent hosts (3–9). CPA usually affects patients with underlying lung  
54 disease, such as mycobacterial infections, chronic obstructive pulmonary disease (COPD),  
55 ABPA and emphysema (7, 9, 10). It has been estimated that over 4.8 million asthmatic  
56 patients worldwide suffer from ABPA and that about 240,000 people in Europe have CPA  
57 (11). Patients suffering from asthma or cystic fibrosis (CF) may become sensitized to  
58 *Aspergillus* antigens, resulting in ABPA, *Aspergillus*-related bronchitis or severe asthma (1,  
59 12).

60

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

71

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

75 combines two recombinant antigens with somatic and metabolic antigens from *A. fumigatus*.  
76 We compared this assay with two other commercial EIAs — the Platelia *Aspergillus* IgG  
77 (Bio-Rad), and the ELISA Classic *Aspergillus* IgG (Virion\Serion) — and an in-house IPD  
78 method (19).

79

## 80 **Materials and Methods**

### 81 **Study design, patients and sera**

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

93

### 94 **Laboratory methods**

95 All serum samples were stored at -20°C until processing. Each was tested at each centre with  
96 the three EIAs: Platelia *Aspergillus* IgG (Bio-Rad, Marnes-la-Coquette, France), ELISA  
97 Classic *Aspergillus fumigatus* IgG (Virion\Serion, Würzburg, Germany) and ELISA  
98 *Aspergillus fumigatus* IgG (Bordier Affinity Products, Crissier, Switzerland), according to the  
99 manufacturers' recommendations. If the result of at least one test was positive or equivocal,

100 the serum sample was subjected to testing with an in-house IPD method (19). Testing by this  
101 last method was centralized at Grenoble University Hospital, to ensure standardisation.

102

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

107

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

113

114 ELISA *Aspergillus fumigatus* IgG (Bordier Affinity Products). The wells were coated with the  
115 two recombinant antigens, dipeptidyl peptidase type V (chymotrypsin) and ribonuclease  
116 (mitogillin), and the somatic and metabolic antigens (24). An OD index was calculated by  
117 OD of the sample / OD of a cut off provided in the kit. OD index values  $\geq 1$  were considered  
118 positive, values of 0.8-1 were considered equivocal, and values  $< 0.8$  were considered  
119 negative.

120

121 Immunoprecipitin detection. We used a double-diffusion gel-electrophoresis technique with  
122 in-house metabolic and somatic antigens different from those used in the Bordier EIA (19).  
123 Briefly, we dispensed 10 $\mu$ l of antigen solution into the agarose wells (1%) (Agarose NA,  
124 Amersham Biosciences). After migration, we added 200 $\mu$ l of serum to the troughs. After

125 incubation, catalase activity was detected by adding 20% hydrogen peroxide. The precipitin  
126 bands were stained with Amidoschwarz (Merck, USA). The result of the test was considered  
127 positive if  $\geq 2$  precipitin bands were detected and equivocal if only one precipitin band was  
128 detected (19). If catalase activity was detected, the result was considered positive regardless  
129 of the number of precipitin bands (25).

130

### 131 **Statistical analysis**

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

147

## 148 **Results**

### 149 **Patients and sera**



150 We included 352 patients in total. They had a median age of 58.9 years [47.0-71.5], 5% were  
151 minors (age<18), and 55% were male. Serum samples were collected at the university  
152 hospitals of Grenoble, Rennes, Lyon, Dijon, and Besançon ( $n= 239, 74, 70, 31$  and  $22,$   
153 respectively). The commonest underlying lung conditions in the controls and patients are  
154 shown in Table 2. The distributions of the 352 patients and the 436 sera are detailed in Tables  
155 1 and 3.

156

### 157 **Test performances**

158 The performances of the three EIAs when equivocal results were treated as positive are shown  
159 in Table 3A. Overall, the sensitivity of the Bordier assay (97%) was significantly higher than  
160 that of the Bio-Rad assay (91.7%), which was itself higher than that of the Virion\Serion assay  
161 (86.1%) (McNemar  $p<0.05$ ). The specificities of the Bordier and Bio-Rad tests were similar  
162 (90.3% and 91.3%, respectively; McNemar  $p=0.8$ ) but significantly higher than that of the  
163 Virion\Serion assay (81.5%; McNemar  $p=0.02$  for both comparison). According to Youden's  
164 index, the Bordier assay provided the best balance between sensitivity and specificity. Bordier  
165 showed the best DOR and Bio-Rad had a greater DOR than Serion. The 95% confidence  
166 intervals (CIs) confirm that Bio-Rad and Bordier are more discriminatory than Serion. These  
167 results were confirmed by the area under the ROC curve (AUC) analysis, which showed that  
168 the performances of the Bordier and Bio-Rad assays were excellent, with AUCs exceeding  
169 0.9. The AUC of the Bordier assay (0.977; 95%CI [0.962; 0.991]) was even greater than those  
170 of the Bio-Rad and Virion\Serion assays (0.951 [0.928; 0.974] and 0.897 [0.863; 0.931])  
171 respectively ( $p<0.01$ ) (Figure 2).

172 Based on these results, we decided to compare the performances of the best two EIAs  
173 (Bordier and Bio-Rad) with that of the IPD assay, for the 279 sera for which one of the three  
174 EIAs gave a positive or equivocal result and for which a sufficiently large volume of the

175 sample remained for additional testing. The Bordier assay was again found to be the most  
176 sensitive (McNemar  $p < 0.05$ ) (Table 3B). The Bio-Rad assay was more sensitive than the IPD  
177 assay only if equivocal results were considered to be negative (McNemar  $p = 0.049$ ; data not  
178 shown). The IPD tended to be more specific than the Bordier assay (84.7% and 71.2 %  
179 respectively;  $p = 0.10$ ). Youden's index favoured the IPD assay but Bordier had the better  
180 DOR compared to Bio-Rad and IPD.

181 In a secondary analysis performed after the exclusion of the 20 patients diagnosed solely on  
182 the basis of the presence of anti-*Aspergillus* antibodies, the comparisons of the three EIAs and  
183 of the AUCs were unchanged and the same differences were observed ( $p < 0.01$  for the  
184 Cochran Q test and  $p < 0.05$  for the McNemar tests in all the comparisons). In the comparison  
185 of the IPD assay with the best two EIAs, we observed a difference in the results obtained from  
186 the pattern described above. The sensitivity of the Bordier assay tended to be higher than that  
187 of the Bio-Rad one, however the difference did not reach statistical significance (98% and  
188 94.5%, respectively; McNemar  $p = 0.06$ ) but both were more sensitive than the IPD assay  
189 (88%; McNemar  $p < 0.01$  and  $p = 0.01$ , respectively).

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

196 An analysis of test performances by patient category, and after correction for multiple  
197 comparisons, indicated that the new Bordier EIA was more sensitive than the Bio-Rad,  
198 Virion\Serion and IPD tests for group 5 of the CNPA with a sensitivity at 100% (Table 3A,  
199 3B and Figure 2). In the other groups of patients, the only differences between sensitivities

200 remaining significant after correction for multiple comparisons were those for group 4, with  
201 the Bordier assay being more sensitive than the Virion\Serion and IPD assays (McNemar  
202  $p<0.01$  and  $p=0.01$ , respectively). The per-group analysis of group 1 revealed similar  
203 specificity results as for the total control group. The sample size for group 2 was insufficiently  
204 large for a separate analysis.

205 The inter-assay CV of the Bordier EIA was 20% (SD=0.366). Quantitative results of the  
206 assays are detailed in Figure 2. Bordier EIA provided significantly fewer equivocal results  
207 (2.8%) than the Bio-Rad (6.7%), and the Virion\Serion (10.1%) ( $p<0.01$  for each comparison  
208 with Bordier).

209

## 210 Discussion

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

223 The Bio-Rad and Virion\Serion EIAs have already been compared in a large retrospective  
224 study (17). The sensitivity and specificity obtained were 93.8% and 87.3% respectively, for

225 the Bio-Rad assay and 90.6% and 75.7%, respectively, for the Virion\Serion assay. Our  
226 findings confirm the superiority of the Bio-Rad assay over the Virion\Serion assay. Another  
227 recent study compared two EIAs, including the Bio-Rad EIA, and an IPD method based on  
228 commercial antigens in a prospective cohort (18). Again, the Bio-Rad assay had high  
229 sensitivity (93%), but its reproducibility was low, with an inter-assay CV of 33% which,  
230 according to the authors, precludes its use for the monitoring of patients. Another drawback of  
231 this test is the need to dilute and retest all samples yielding values greater than 80 AU/ml.  
232 Furthermore, one recent study evaluated a commercial western blot kit for the detection of  
233 anti-*Aspergillus* IgG (27). The authors reported a sensitivity of 90.0%-93.8% in CPA and  
234 ABPA patients, the specificity (94%) being estimated solely on the basis of the results for  
235 healthy blood donors as controls. Further studies are required to compare the EIAs and  
236 western-blot assay for the detection of anti-*Aspergillus* IgG.

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

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

250 when equivocal results were treated as negative (19). However it was not significantly higher  
251 than that of the Bordier assay or the Bio-Rad assay, probably due to the loss of statistical  
252 power in the smaller sample size. Our selection of serum samples giving positive or equivocal  
253 results with one of the three EIAs for the analysis of IPD assay may have resulted in a slight  
254 overestimation of the sensitivity.

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

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

270 In conclusion, given its high Youden's index and diagnostic odds ratio, indicating a  
271 good balance between sensitivity and specificity, this new Bordier EIA is suitable for the  
272 detection of anti-*Aspergillus* IgG for the diagnosis of chronic and allergic pulmonary  
273 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.  
276

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283 **Transparency declaration**

284 Muriel Cornet has received travel grants from Gilead, Pfizer and Merck and received  
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286 The Parasitology and Mycology Laboratory of Grenoble University Hospital, where M.  
287 Cornet, M.P. Brenier-Pinchart, and H. Pelloux currently work, produces and sells *A.*  
288 *fumigatus* somatic and metabolic antigens to Bordier Affinity Products.

289 Bordier Affinity Products provided the kits for the three EIAs analysed here but did not  
290 participate in any phase of the study, from its design to the analysis of the results and  
291 conclusions reported here and the writing of the manuscript.

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294 GmbH and from Bordier Affinity Products.

295 **Author contribution**

296 C. Dumollard, S. Perriot contributed to the design of the study, performed the assays,  
297 collected the data, analysed the results and contributed to the writing of the manuscript. Dr S.  
298 Bailly performed the statistical analysis and contributed to the writing of the manuscript. Dr  
299 M.P Brenier-Pinchart and H. Pelloux contributed to the classification and diagnosis of  
300 patients, co-ordinated the Grenoble *Aspergillus* Committee, and contributed to the writing of  
301 the manuscript. C. Saint-Raymond, B. Camara J.P. Gangneux, F. Persat, S. Valot, and F.

302 Grenouillet contributed to the recruitment of the patients and their clinical management and  
303 topatient classification and diagnosis and the writing of the manuscript. Dr C. Pinel and M.  
304 Cornet contributed to the development of the antigens used in both the Bordier EIA and the  
305 in-house IPD method, and to the study design and the writing of the manuscript. Collaborators  
306 of the Grenoble *Aspergillus* Committee contributed to the recruitment, management, diagnosis  
307 and classification of the patients.

308 **Collaborators of the Grenoble *Aspergillus* Committee** are Thiebaut-Bertrand A., Maubon  
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393 **TABLE 1. Classification and distribution of the patients (adapted from (7, 8, 13, 20)).**  
 394

Inclusion criteria				
<b>Control groups</b> (n=205)	<b>Group 1</b> (n=191; 54.1%)	Patients with respiratory symptoms in whom an <i>Aspergillus</i> -related disease had been ruled out		
	<b>Group 2</b> Colonised patients (n=14; 4%)	Patients with colonisation defined as the recovery of <i>Aspergillus</i> 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	<i>Aspergillus</i> sp. evidence
<b>CPA</b> (n= 104)	<b>Group 3</b> 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 $\geq 3$ months	No alteration of the general condition and no haemoptysis	<i>Aspergillus</i> sp isolated from a respiratory sample AND/OR Histological evidence of <i>Aspergillus</i> sp hyphae AND/OR Positive anti- <i>Aspergillus</i> serum precipitins
	<b>Group 4</b> CCPA or CFPA (n=62; 17.6%)	At least one cavitory lesion (CCPA) in the lung, with or without a "fungus ball", with progression over $\geq 3$ months. CFPA = fibrotic destruction after CCPA	Alteration of the general condition (fever, weight loss, asthenia), chronic cough and/or haemoptysis progressing for $\geq 3$ months	<i>Aspergillus</i> sp isolated from a respiratory sample AND/OR Histological evidence of <i>Aspergillus</i> sp hyphae AND/OR Positive anti- <i>Aspergillus</i> serum precipitins

<b>Group 5</b> CNPA (n=25; 7.1%)	Expanding cavities, nodules, consolidations with progression over $\geq$ 1 month.	Mild immunodeficiency (diabetes, alcoholism, immunosuppressive drugs). Alteration of the general condition (fever, weight loss, asthenia), chronic cough and/or haemoptysis progression $\geq$ 1 month.	<i>Aspergillus</i> sp isolated from a respiratory sample AND/OR Histological evidence on a biopsy or surgical resection showing <i>Aspergillus</i> sp hyphae AND/OR Positive anti- <i>Aspergillus</i> serum precipitins and/or <i>Aspergillus</i> antigen detected in serum
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**Inclusion criteria**

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<b>ABPA</b> (n=43)	<b>Group 6</b> (n=43; 12.2%)	Asthma or CF and the triad of alteration of respiratory function, presence of anti- <i>Aspergillus</i> 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/ $\mu$ l, recent pulmonary lesions/worsening of existing lesions, serum precipitins or anti- <i>Aspergillus</i> IgG antibodies
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395

- 396 CPA. Chronic pulmonary aspergillosis  
 397 CCPA. Chronic cavitary pulmonary aspergillosis  
 398 CFPA. Chronic fibrosing pulmonary aspergillosis  
 399 CNPA. Chronic necrotizing pulmonary aspergillosis  
 400 ABPA. Allergic bronchopulmonary aspergillosis

401 **TABLE 2. Underlying lung conditions**  
402

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) <sup>‡</sup>
No previous respiratory history	30 (18)	1 (1.2)
Cystic fibrosis	11 (6.6)	30 (35.3)
Bronchiectasis	4 (2.4)	7 (8.2)
<i>Aspergillus</i> sinusitis	1 (0.6)	0 (0)
Previous pulmonary aspergillosis	1 (0.6)	15 (17.6)
Previous <i>Aspergillus</i> 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 <sup>†</sup>	20 (12)	2 (2.4)
Previous cancer history	1 (0.6)	8 (9.4)
Assessment before immunosuppression <sup>‡</sup>	17 (10.2)	0 (0)
Others <sup>‡</sup>	16 (9.6)	6 (7.1)

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

404 Patients and controls may have more than one underlying condition.

405

406 \* 20 patients also had cystic fibrosis.

407 <sup>‡</sup> No patient had CF

408 <sup>†</sup> Respiratory symptoms, such as shortness of breath, cough, wheezing rhonchi

409 <sup>‡</sup> Including before treatment with biotherapy or before a transplantation (lung, heart, liver or kidney)

410 <sup>‡</sup>Others: pneumothorax, asbestosis or solvent exposure, sarcoidosis, pulmonary hypertension, sleep  
411 apnoea, pulmonary embolism, previous haemoptysis.

412

**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**

Equivocal results were considered positive

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A								<i>p</i> -value*	McNemar		
		Bordier		Bio-Rad		Virion-Serion			Bordier/ Bio-Rad	Bordier/ Virion- Serion	Bio-Rad/ Virion- Serion
Se % [95% CI]	All sera <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <sup>†</sup>	All sera <i>n</i> = 436	0.873		0.830		0.676					
		DOR	296	[122 ; 715]	116	[59 ; 228]	27	[16 ; 45]			



B		<i>p</i> -value*							McNemar		
		Bordier		Bio-Rad		IPD		Bordier/ Bio-Rad	Bordier/ IPD	Bio-Rad/ IPD	
Se % [95% CI]	All sera <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <i>n</i> =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 <sup>†</sup>	All sera <i>n</i> = 279	0.689		0.695		0.738					
DOR		106	[37 ; 303]	44	[20 ; 98]	45	[20 ; 103]				

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421 **Se: Sensitivity Sp: specificity**422 <sup>\*</sup>Cochran Q test423 <sup>†</sup>Equivocal results were considered positive424 <sup>‡</sup>McNemar post-hoc tests showed no significant difference425 <sup>†</sup>Youden's index = sensitivity + specificity – 1

426 NA = not applicable

427

428

429 **Figure Legends**

430 **Figure 1. Receiver operating characteristic (ROC) curves of Platelia *Aspergillus* IgG (Bio-Rad),**

431 **ELISA Classic *Aspergillus fumigatus* IgG (Virion\Serion) and ELISA *Aspergillus fumigatus* IgG**

432 **(Bordier Affinity Products) assays.**

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

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

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

436

437

438 **Figure 2. Quantitative results and distribution of antibodies obtained with three anti-**

439 ***Aspergillus* IgG enzyme immunoassays for the diagnosis of chronic pulmonary**

440 **aspergillosis and allergic bronchopulmonary aspergillosis.**

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

442 The Y scale is logarithmic

443 dotted line: positive and negative cut-offs

444 R package beeswarm was used to perform the graph.

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

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

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

448 **Controls : groups 1 and 2**

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

450



