Performance of two *Aspergillus* IgG EIA assays compared with the precipitin test in chronic and allergic aspergillosis

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Abstract

Detection of *Aspergillus* IgG antibodies is important in the diagnosis of chronic pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. Immunoprecipitation techniques to detect these antibodies appear to lack sensitivity and accurate quantitation compared with enzyme immunoassays (EIA). This study assessed the performance of two commercial EIAs compared with counterimmunoelectrophoresis (CIE). This was a prospective cohort study of 175 adult patients with chronic or allergic pulmonary aspergillosis. *Aspergillus* IgG antibodies were detected using CIE, Phadia ImmunoCap *Aspergillus* IgG and Bio-Rad Platelia *Aspergillus* IgG. Inter-assay reproducibility was determined for each method and 25 patients had two serum samples analysed within a 6-month interval. When compared with CIE, both ImmunoCap and Platelia *Aspergillus* IgG had good sensitivity (97 and 93%, respectively) for detection of *Aspergillus* IgG antibodies. The level of agreement between the two EIAs for positive results was good, but the concentration of antibodies was not correlated between the tests or with CIE titre. ImmunoCap IgG inter-assay coefficient of variation was 5%, whereas Platelia IgG was 33%. Median ImmunoCap IgG values for CPA and allergic aspergillosis were 95 and 32 mg/L, respectively, whereas Platelia IgG values were >80 and 6 AU/mL. The direction of CIE titre change over 6 months was mirrored by ImmunoCap IgG levels in 92% of patients, and by Platelia IgG in 72% of patients. Both ImmunoCap and Platelia *Aspergillus* IgG EIAs are sensitive measures of *Aspergillus* IgG antibodies compared with CIE. However, ImmunoCap appears to have better reproducibility and may be more suitable for monitoring patient disease.

Keywords: Allergic bronchopulmonary aspergillosis, *Aspergillus*, chronic pulmonary aspergillosis, enzyme immunoassay, IgG antibody
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Introduction

Aspergillus is a ubiquitous fungus that causes a large number of pulmonary and non-pulmonary diseases [1]. Chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA) are two of the commonest pulmonary manifestations in non-immunocompromised patients [2]. Diagnosis of these conditions relies on a number of clinical, radiological and microbiological parameters. Important to the diagnosis of CPA and ABPA is the detection of *Aspergillus* IgG antibodies [3, 4]. Traditionally, IgG antibodies were measured using immunoprecipitation techniques (*Aspergillus* precipitins) [5, 6]. Counterimmunoelectrophoresis (CIE) largely replaced immunodiffusion (agar gel double diffusion), as its performance is comparable, but the time taken to perform this method is significantly less [7]. However, CIE has definite limitations, including poor sensitivity, subjective qualitative results and labour intensity [8]. There has therefore been a drive to produce commercial enzyme-linked immunosorbant assays (ELISA) with the potential for full automation and quantitative results. IgG antibody specificity in the diagnosis of aspergillosis has not been addressed with any rigour.

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The National Aspergillosis Centre (NAC) in Manchester provides long-term management for patients with CPA and ABPA. The monitoring of *Aspergillus* precipitins has been used for many years to aid both diagnosis and response to therapy. However, with emerging resistance to azole therapies it is becoming more important to have technically consistent and fully quantitative *Aspergillus* IgG antibody results to guide early investigation and management [9]. Therefore, this study aimed to compare two commercial *Aspergillus* IgG antibody tests, Phadia ImmunoCap™ fluorenzymeimmunoassay (FEIA) (Phadia, Uppsala, Sweden) and Bio-Rad Platelia™ *Aspergillus* IgG EIA (Marnes-la-Coquette, France), with CIE to detect and monitor *Aspergillus* IgG antibodies in patients with chronic and allergic aspergillosis.

Materials and Methods

Patients and sera collection

This was a prospective cohort study. Sera were collected from 175 adult patients attending routine outpatient appointments at the NAC in Manchester, UK, between February 2010 and September 2010. Patients were grouped, following serum collection, according to previously diagnosed disease. One hundred and sixteen patients had CPA as defined by the criteria of Denning and colleagues [3], forty-one patients had ABPA as defined by Greenberger's criteria [4], five had severe asthma with fungal sensitization (SAFS) [10], and 13 patients did not meet the criteria for any of these conditions (three with fungal rhinosinusitis, two with extrinsic allergic alveolitis and eight with no evidence of fungal disease or sensitization but chronic lung disease). For data analysis, ABPA and SAFS patients were grouped together. None of the patients had cystic fibrosis and any immunosuppression was confined to inhaled and low-dose oral corticosteroids. None of the patients had received a transplant or had malignant haematological disease.

IgG levels over 6 months

Serum was collected at two separate appointments within a 6-month period from 25 patients receiving antifungal therapy, to observe changing IgG levels over time. The first 25 patients to have a routine repeat blood test within the study period were selected. No details were collected regarding clinical features or serum drug levels.

Serolgical analysis

Sera were tested for specific *Aspergillus* IgG antibodies by three separate techniques.

CIE. Aspergillus fumigatus somatic and culture filtrate antigens were obtained from Microgen Bioproducts (Camberley, Surrey, UK) at dilutions of 20 and 2 mg/mL. The test was performed by CIE in an agarose gel and electrophoresed in a boric acid/EDTA buffer, pH 8.2, for 90 min. Results were expressed as a titre, based on the highest serum dilution where a precipitation line was visible. A titre of ≥ 1 (neat serum) was considered positive. For purposes of inter-assay reproducibility, 40 serum samples were tested twice by different technicians. Technicians processing these samples were blinded to the results of other tests or patient details.

ImmunoCap Aspergillus IgG. Specific *A. fumigatus* IgG concentrations were determined using Gm3 ImmunoCaps on an ImmunoCap 250 machine. Gm3 ImmunoCaps are derived from whole extract of *Aspergillus* conidia and mycelium. Serum samples were instrument-diluted to 1 in 100 and the measurement range was 2–200 mg/L. For samples with an antibody level >200 mg/L, serum was diluted to 1 in 200 and processed again, results being determined by multiplying the concentration obtained by 2. A level of >40 mg/L was considered positive. This is the cut-off level used by UK laboratories following agreement at a consensus meeting between six UK laboratories and Phadia, which analysed unpublished data from eight UK centres. Fifty samples were processed twice to determine inter-assay reproducibility. Technicians processing these samples were blinded to the results of other tests and patient details.

Platelia Aspergillus IgG. The Platelia EIA detects *Aspergillus* IgG antibodies against a purified, unspecified recombinant antigen. Serum samples were diluted to 1 in 400 and processed manually according to the manufacturer's instructions. The optical density was measured at 450 nm and the IgG concentration was calculated by drawing a calibration curve from the five calibrators provided with the assay. The manufacturer recommends a concentration <5 AU/mL as negative, 5–10 AU/mL as intermediate and ≥ 10 AU/mL as positive. For samples with a titre of >80 AU/mL the manufacturer recommends that the sample be repeated with an additional 1 in 5 dilution if optical density (OD) <3.0 or 1 in 60 dilution if OD ≥ 3.0. Eleven sera were randomly selected for each dilution, while the remaining results were expressed as >80 AU/mL without further dilution due to financial constraints. Seventy-eight serum samples were processed twice by different operators to determine inter-assay reproducibility.

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Correlation of results between tests was calculated by global agreements and Gwet's AC_1, which gives more accurate percentages of agreement than Cohen's Kappa

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coefficient when one agreed category has a small percentage [11]. Concordance for Gwet's AC_1 is measured on the same scale as Landis and Koch's kappa criteria: >0.8 excellent, 0.61–0.8 substantial, 0.41–0.6 moderate, 0.21–0.4 fair, <0.2 poor [12]. Reproducibility of each test was calculated by coefficients of variation (CVs), Spearman's correlation coefficient and Bland Altman plots of agreement between repeated measures. Concentrations of IgG antibody between disease groups were compared using Kruskal–Wallis tests and Mann–Whitney *U*-tests.

Results

Sera were collected from 175 patients attending the NAC. The mean age was 62 years (range 20–84) and 54% of patients were male.

Test performance

Using the predetermined cut-off levels for positive and negative results, ImmunoCap had a 97% sensitivity and Platelia 93% sensitivity compared with CIE. However, the Platelia and ImmunoCap tests produced more positive results in all disease categories than CIE (Table 1). Comparing Platelia and Immunocap, 82% of results were in complete agreement and Gwet's AC_1 was 0.65, indicating substantial inter-test concordance.

There were 31 discordant results between the ImmunoCap and Platelia tests. Sixteen Platelia tests were positive when ImmunoCap was negative, and 15 Platelia tests were negative when ImmunoCap was positive (Table S1, Supporting Information). The CIE result was in agreement with ImmunoCap in 71% and with Platelia in 29% of the discordant group.

Inter-assay reproducibility

Fifty serum samples were run through the ImmunoCap assay twice. Five patient samples produced a result of >200 mg/L in both assay runs. The CV was 5% and Spearman's correlation coefficient was 0.996. A Bland Altman plot of agreement showed that the mean difference between the two results was 0.18 mg/L (SD 3.6 mg/L) (Supporting Information Fig. S1). Seventy-eight serum samples were run through the Platelia assay twice. Of the 156 results, 53 (34%) were reported to have a value >80 AU/mL. Fifteen patient samples had a result >80 AU/mL in both assay runs. This made calculations of reproducibility more difficult as results had to be rounded to 80 AU/mL, leading to a likely under-estimation of CV and SD. The CV was therefore calculated at ≥33% and Spearman's correlation coeffcient was 0.634. A Bland Altman plot of agreement between measures showed the mean difference between the two results was −7.0 AU/mL (SD ≥ 26 AU/mL). Lastly, 40 serum samples were run through the CIE assay twice. The CV was 2% and Spearman's correlation coefficient was 0.81. The mean difference between the two tests was 0.15 (SD 1.1).

IgG antibody concentrations between tests

The correlation between CIE titre and both the ImmunoCap and Platelia tests is shown in Fig. 1. There was poor correlation between both tests and CIE titre (Spearman's correlation coefficient ImmunoCap $r = 0.54$, Platelia $r = 0.52$). Sixty-three (36%) of the Platelia samples gave a result >80 AU/mL necessitating dilution, whereas 15 (9%) of the ImmunoCap samples required dilution. Twenty-two Platelia samples were diluted (11 for each of the 1 in 5 and the 1 in 60 dilutions) and 15 ImmunoCap samples. Following dilution, the mean value for the Platelia results was 586 AU/mL (range 114–1806) and the mean value for the ImmunoCap results

TABLE 1. ***Aspergillus fumigatus*** **IgG antibody detection rates compared with CIE, according to disease category, for Platelia and ImmunoCap assays**

	CIE positive	CIE negative		CIE positive	CIE negative
CPA (n = 116)					
Platelia positive	62	37	ImmunoCap positive	63	37
Platelia negative	3	14	ImmunoCap negative	2	14
ABPA (n = 46)					
Platelia positive	6	15	ImmunoCap positive	6	13
Platelia negative	1	24	ImmunoCap negative	1	26
Other (n = 13)					
Platelia positive	0	2	ImmunoCap positive	1	4
Platelia negative	1	10	ImmunoCap negative	0	8

	Platelia		ImmunoCap		CIE	
Disease	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
CPA (n = 116)	99 (85)	17 (15)	100 (86)	16 (14)	65 (56)	51 (44)
ABPA/SAFS (n = 46)	21 (46)	25 (54)	19 (41)	27 (59)	7 (15)	39 (85)
Other (n = 13)	2 (13)	11 (87)	5 (38)	8 (62)	1 (8)	12 (92)

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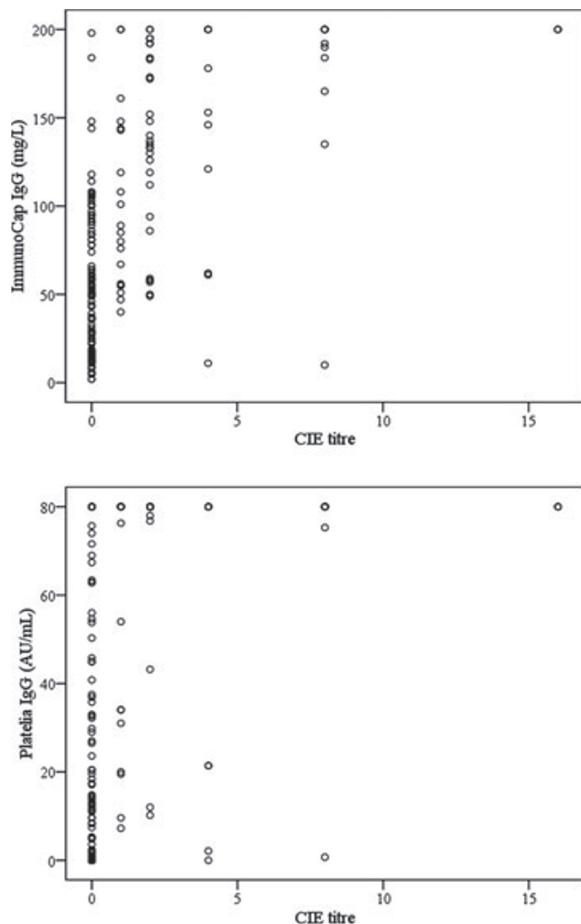

FIG. 1. Correlation between ImmunoCap and Platelia *Aspergillus* IgG levels and precipitin (CIE) titre. There was one precipitin titre of 32, which has not been included in the figures due to scale: the ImmunoCap IgG level was 76 mg/L while the Platelia IgG was >80 AU/mL.

was 623 mg/L (range 200–1641). A comparison between pre-dilution results for the ImmunoCap and Platelia results is shown in Fig. 2. Spearman's correlation coefficient was $r = 0.68$, coefficient of determination 46%, but it is clear from Fig. 2 that there was a very wide range of ImmunoCap results when Platelia was >80 AU/mL. When negative results and those beyond the upper limit of the assays were removed the correlation coefficient was 0.04.

IgG antibody concentrations between disease categories

There was a wide range of values for all tests against each disease category (Fig. 3). A Kruskal–Wallis test revealed statistically significant differences in Platelia IgG concentrations between the disease categories (χ^2 (d.f. 2, $n = 175$) = 56, p <0.001). Median values for each group were: CPA > 80 AU/mL, ABPA 6 AU/mL, 'Other' 0.3 AU/mL. Post-hoc Mann–Whitney *U*-tests with Bonferroni correction of calculated

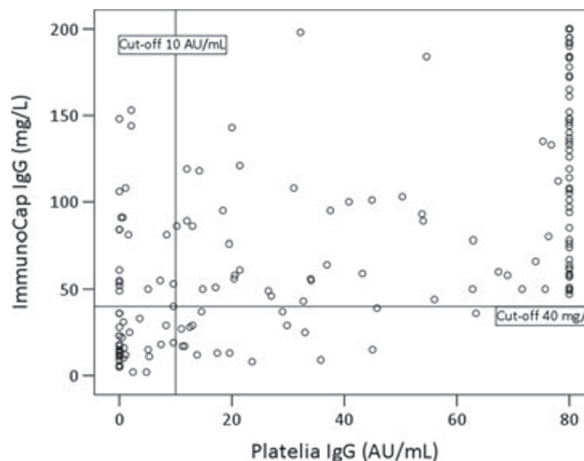

FIG. 2. Correlation between ImmunoCap and Platelia *Aspergillus* IgG concentrations. All 15 ImmunoCap results with a titre of >200 mg/L had a Platelia IgG concentration >80 AU/mL. For Platelia results >80 AU/mL there was a wide variation of ImmunoCap results (45–200 mg/L).

p-values showed that patients with CPA had statistically higher IgG concentrations than those with ABPA/SAFS (p <0.001) or those with other diseases (p <0.001). There was no significant difference between ABPA/SAFS patients and patients with 'Other' diseases (p 0.08). For ImmunoCap IgG concentrations the Kruskal–Wallis test again revealed statistically significant differences in IgG concentrations between the disease categories (χ^2 (d.f. 2, $n = 175$) = 38, p <0.001). Median values for each group were: CPA 95 mg/L, ABPA/SAFS 32 mg/L, 'Other' 36 mg/L. Post-hoc tests showed similar results to the Platelia test, with CPA patients having statistically higher IgG concentrations than ABPA/SAFS patients (p <0.001) and 'Other' diseases (p 0.003) but no significant difference between ABPA/SAFS and 'Other' diseases (p 0.70). CIE titres were in agreement with these findings.

IgG levels over 6 months

Twenty-five patients had two blood samples taken within a 6-month interval. Each sample was tested for *Aspergillus* IgG antibodies by the three tests under investigation (Table 2). Significant changes were defined as a change of >2 SD of the mean as per earlier measures of reproducibility. Seven patients had Platelia IgG levels >80 on both occasions. One of these had ImmunoCap concentrations >200 mg/L, four had a fall in ImmunoCap IgG and two had a rise in ImmunoCap IgG. These changes were mirrored by the same directional change in CIE titres. Ten patients demonstrated the same directional change in results between all three tests. The remaining eight patients had discordant results for the three tests. Overall, excluding those with results above the assay limit of detection,

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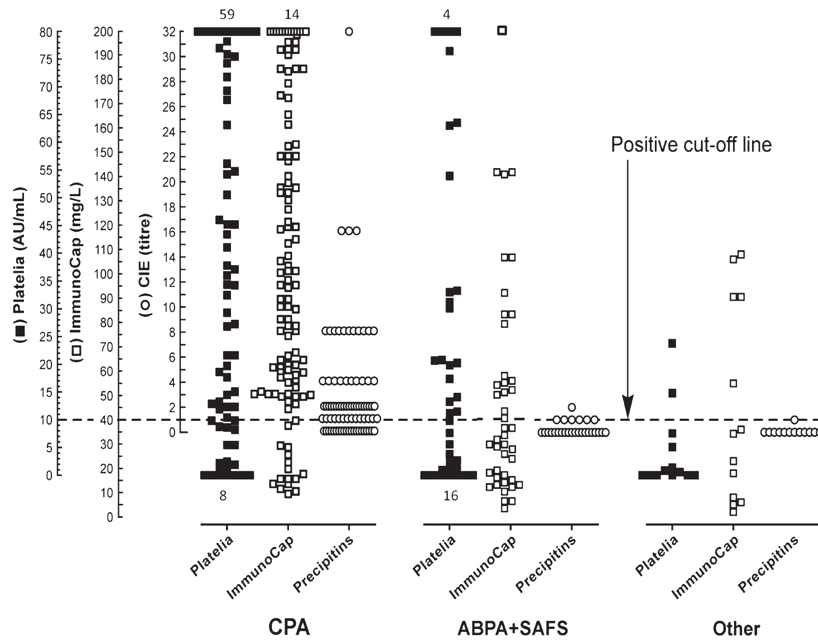

FIG. 3. Distribution of *Aspergillus* IgG concentrations in each patient group. Where the number of patients with a particular IgG concentration is indistinct, these have been labelled.

TABLE 2. ***Aspergillus* IgG concentrations from two blood samples taken within 6 months**

Patient	Platelia IgG (AU/mL)		ImmunoCap IgG (mg/L)		CIE (titre)	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	>80	>80	>200	>200	1	2
2	>80	>80	>200	133	8	2
3	>80	>80	190	173	8	2
4	>80	>80	107	62	8	0
5	>80	>80	>200	148	8	1
6	>80	>80	183	195	2	4
7	>80	>80	97	114	0	1
8	17	>80	13	49	0	2
9	75	>80	135	146	4	8
10	34	51	55	56	1	1
11	41	38	100	95	1	1
12	>80	69	78	58	1	0
13	10	5	19	15	0	0
14	0	0	15	13	0	0
15	0	0	12	9	0	0
16	0	1	11	10	0	0
17	4	0	33	28	0	0
18	13	16	29	27	0	0
19	56	27	44	46	0	0
20	27	55	49	89	0	1
21	10	21	86	121	2	4
22	38	>80	85	85	1	1
23	8	67	81	60	1	0
24	45	63	101	78	0	0
25	1	1	108	91	0	0

Significant changes in results are defined as a change in value >2 SD of the mean from reproducibility tests: Platelia 52 AU/mL and ImmunoCap 8 mg/L.

ImmunoCap mirrored CIE change in 22 (92%) of 24 patients, and Platelia in 14 (78%) of 18 patients.

Discussion

This study has evaluated the performance of two commercial EIAs used to detect and monitor *Aspergillus* IgG precipitating antibodies and compared it with CIE in patients with chronic and allergic aspergillosis. Both assays had good sensitivity when compared with CIE. In fact, sensitivity appeared to be significantly better than that of CIE, with ImmunoCap and Platelia tests detecting *Aspergillus* IgG antibodies in 29–30% more CPA patients and in 26–31% more ABPA/SAFS patients. The actual sensitivity of these assays to detect disease cannot be determined from this data as many patients were already on

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antifungal medication at the time of serum collection and there was no control group. However, it is noteworthy that the eight patients with no evidence of fungal disease were negative on both assays. The ImmunoCap and Platelia IgG tests showed good levels of agreement (82%) and inter-rater reliability (Gwet's AC_1 0.65). For discordant results there was an equal mix of positive Platelia and positive ImmunoCap results, but CIE results agreed with ImmunoCap in 72% of cases but only 29% of Platelia IgG results. However, the Platelia IgG test detected 14 positive results in the CPA/ABPA groups and two in the 'Other' group, whereas ImmunoCap detected 11 positive results in the CPA/ABPA groups and four in the 'Other' group. Interpreting this in the context of sensitivity and specificity for disease identification is not possible due to the lack of a control group and concurrent antifungal treatment for those with CPA/ABPA.

There was a significant difference between the performance of the ImmunoCap and Platelia assays during tests of inter-assay reproducibility. The Platelia IgG assay was more difficult to analyse due to a large number of results with a value >80 AU/mL. The Platelia IgG assay was run on 78 samples twice by different operators and the CV was $\geq$33% with mean differences of −7 AU/mL and a wide standard deviation of $\geq$26 AU/mL. With a cut-off value of 5 AU/mL this could seriously affect disease monitoring. The inter-assay CV reported by the manufacturer is up to 16.2% (product insert), which is likely to still be significant in the context of disease monitoring. A limitation of our study is that sera were not tested on the same day, although sera were stored at −80°C between testing, and furthermore, tests were carried out manually, introducing potential operator error. We found that the ImmunoCap assay had a much better inter-assay CV of 5% and mean differences of <1 mg/L, which would be very unlikely to affect disease monitoring. We did not analyse intra-assay reproducibility for either test but Guitard *et al.* [13] recently reported the intra-assay precision of the Platelia *Aspergillus* IgG assay to be CV 5%, using four sera repeated over 15 runs on an automated machine. This is similar to the manufacturer data of up to 3.6% (product insert).

A further drawback of the Platelia *Aspergillus* IgG test is that, in this group of patients, 36% (63) of results were above the upper limit of detection (>80 AU/mL). While this may not be important for disease detection, it is important for disease monitoring. To obtain an accurate level with which to monitor treatment, the assay has to be re-run after additional dilution, causing considerable expense and laboratory time. This study was unable to analyse all samples >80 AU/mL due to cost implications. There was a wide distribution of ImmunoCap results for these samples (45 to >200 mg/L). Only 15 ImmunoCap IgG results had concentrations >200 mg/L, all of which had Platelia IgG levels >80 AU/mL. The Platelia IgG test takes over 3 h to perform and is very labour intensive if not using automated equipment. Automated ELISA equipment is currently not standard in UK hospital laboratories. ImmunoCap IgG is fully automated and run on the same machine as other common tests of allergy, to which most UK hospitals have access.

The correlation of concentrations of IgG antibodies between the two tests was poor, which may in part reflect the poor reproducibility of the Platelia IgG test or may be explained in part by the use of different *Aspergillus* antigens. The ImmunoCap assay is directed against whole extracts of *A. fumigatus* conidia and mycelium containing a wide variety of allergens, whereas the Platelia assay uses an unspecified purified recombinant *Aspergillus* antigen (product insert), and detects species other than *A. fumigatus*. This is of great importance when trying to compare sensitivities and IgG concentrations.

Monitoring of *Aspergillus* IgG antibody levels in 25 patients over 6 months was performed well by the ImmunoCap assay compared with CIE titre. Twenty-two (92%) of the 24 results showed the same directional change or stability as the CIE titre (one excluded as results above assay limit of detection). The Platelia IgG test was more difficult to analyse as the poor reproducibility has an impact on analysis, but if the seven patients with results >80 AU/mL are excluded, 14 (78%) of 18 patients showed the same directional change as CIE titre.

All three tests showed statistically higher IgG concentrations in those with CPA than ABPA. Up to 59% of ABPA/SAFS patients were negative for *Aspergillus* IgG antibodies. However, many patients were on antifungal treatment and IgG concentrations may fall quicker in ABPA compared with CPA, where the aim of therapy is not cure but control of disease and prevention of progression. There is currently no defined cut-off level for either assay in specific disease groups. One study suggested an ImmunoCap cut-off of 90 mg/L for diagnosis of ABPA in cystic fibrosis with a sensitivity of 91% and specificity of 88% [14]. A further study reported an ImmunoCap cut-off of 70 mg/L as giving a sensitivity of 70% in patients with invasive aspergillosis/ABPA and 98% specificity in healthy controls [15]. The Platelia *Aspergillus* IgG test has been evaluated in the diagnosis of non-invasive aspergillosis in one retrospective study, which found good sensitivity (90–94%) for patients with CPA and ABPA and a specificity of 99% using control sera from healthy pregnant women [13].

A limitation of this study is the lack of documented clinical features and details of concurrent antifungal treatment, which may have provided more precise interpretation of results, particularly for patients in whom more than one serum sample was tested over time. An area not properly explored by this or any study of *Aspergillus* IgG serology is that of specificity. Blood

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donors or other healthy controls provide a baseline figure, but are not useful in the context of a respiratory service where consideration of some form of aspergillosis is present in the vast majority of patients. In a sense then, only matched patients can be used to determine specificity, but in this case invasive, chronic and allergic aspergillosis, as well as *Aspergillus* bronchitis [16], need to be excluded. In a separate study, in cystic fibrosis patients, we have addressed this by prospective recruitment, testing all patients with the same protocol, latent class analysis, to define discrete patient groups, and then performing ROC analysis on the relevant tests within each patient grouping [17]. To truly establish the specificity of *Aspergillus* IgG for CPA and ABPA, this should be done with matching controls, which is easier for ABPA (asthma) than for CPA (multiple underlying conditions). Exclusion of *Aspergillus* disease to establish a true negative population is now easier with *Aspergillus* PCR on sputum, which is far more sensitive than culture [18,19]. Therefore, this study cannot definitively determine the performance of any of the assays for CPA and ABPA, but points the way towards assay selection for such a study, and addresses dilution considerations and issues of reproducibility.

In summary, both the ImmunoCap and Platelia *Aspergillus* IgG EIAs are sensitive assays for detecting *Aspergillus* IgG antibodies. However, the ImmunoCap appears to have better inter-assay reproducibility, making it more suitable for monitoring patient disease in a routine service setting. Sensitive assays are required for the diagnosis of CPA as radiology is often not specific and cultures are insensitive.

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Author Contributions

Dr C. Baxter contributed to the study conception and design and acquisition, interpretation and analysis of data, drafted and revised the manuscript critically for important intellectual content and provided final approval of the version to be published. Professor D. Denning contributed to the study conception and design and interpretation of data, revised the manuscript critically for important intellectual content and provided final approval of the version to be published. Dr A. Jones contributed to the study conception and design, revised the manuscript critically for important intellectual content and provided final approval of the version to be published. Dr A. Todd contributed to the acquisition of data, revised the manuscript critically for important intellectual content and provided final approval of the version to be published. Dr C. Moore contributed to the study conception and design and interpretation of data, revised the manuscript critically for important intellectual content and provided final approval of the version to be published. Professor M. Richardson contributed to the study conception and design and acquisition and interpretation of data, revised the manuscript critically for important intellectual content and provided final approval of the version to be published.

Transparency Declaration

Caroline Baxter has received travel grants from Schering Plough and Pfizer and has been paid for talks on behalf of Astellas. Malcolm Richardson acts as an advisor/consultant to Astellas Pharma, Merck and Gilead Sciences, and has received grant support and sponsorship from these companies. Caroline Moore has received a travel grant from Astellas, been paid for talks on behalf of Pfizer and has received grant support from Pfizer. David Denning holds founder shares in F2G Ltd, a University of Manchester spin-out company, and has received grant support from F2G as well as the Fungal Research Trust, the Wellcome Trust, the Moulton Trust, The Medical Research Council, The Chronic Granulomatous Disease Research Trust, the National Institute of Allergy and Infectious Diseases, National Institute of Health Research, the European Union and AstraZeneca. He acts as an advisor/consultant to F2G and Myconostica (now part of the Lab21 group) as well as other companies over the last 5 years, including Pfizer, Schering Plough (now Merck), Nektar, Astellas and Gilead. He has been paid for talks on behalf of Merck, Astellas, Novartis, Merck, Dainippon and Pfizer. Andrew Jones and Anthony Todd report no potential conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Bland Altman plots of agreement between repeated measures.

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Table S1. Discordant results between Platelia and ImmunoCap *Aspergillus* IgG antibody tests.

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