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Between-laboratory quality control of automated analysis of IgG antibodies against *Aspergillus fumigatus*☆

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ABSTRACT

Measurement of IgG antibodies against *Aspergillus fumigatus* is an important criterion for the diagnosis of aspergilloma, allergic bronchopulmonary aspergillosis, and extrinsic allergic alveolitis. In the present study, we compared IgG antibody analysis against *A. fumigatus* using 2 widely used automated immunochemistry systems. In a between-laboratory quality control program, good agreement was found between the results from laboratories that used the ImmunoCAP system; however, a laboratory that used the Immulite 2000 system found approx. 2-fold higher antibody levels in the quality control samples than did the ImmunoCAP system. Measurements of IgG against *A. fumigatus* in patient sera were significantly correlated ($r_s = 0.77$, $P < 0.0001$). These results demonstrate that analysis of IgG antibodies against *A. fumigatus* with these 2 systems has reached a level of standardization that allows for direct comparison of quantitative results from different laboratories. For longitudinal analysis of IgG against *A. fumigatus*, reagents from the same manufacturer should be used.

1. Introduction

Measurement of IgG antibodies against the mold *Aspergillus fumigatus* is an important criterion for the diagnosis of bronchial aspergilloma, allergic bronchopulmonary aspergillosis (ABPA), and mold-induced extrinsic allergic alveolitis (Stevens et al., 2000). In nonneutropenic patients with invasive aspergillosis, the detection of IgG antibodies is thought to be the best noninvasive means of establishing the diagnosis of subacute invasive aspergillosis (Hope et al., 2005). Furthermore, IgG antibody testing in patients with hematologic malignancies prior to their becoming immunocompromised has a predictive value for development of invasive aspergillosis (Sarfati et al., 2006).

Initially, antibodies against *A. fumigatus* were determined using the double immunodiffusion (DID) technique according to Ouchterlony or with the immunoelectrophoresis technique (Barton, 2010; Longbottom and Pepys, 1964). However, these methods are time consuming, labor intensive, require relatively large amounts of *A. fumigatus* extract and patient serum, and give at best only semiquantitative results. At present, the DID technique has been largely replaced by the enzyme-linked immunosorbent assay (ELISA), which is a considerably more rapid and sensitive technique that produces quantitative results with significantly less *A. fumigatus* extract and patient serum per test, and is easily automated (Barton, 2010). These benefits stimulated many laboratories to develop their own in-house ELISA for *A. fumigatus*–specific IgG antibodies (Barton, 2010; Kauffman et al., 1983; Sepulveda et al., 1979), later followed by a number of commercial suppliers. Because each of these assays expressed its results in different, arbitrarily chosen quantitative units, and because the *A. fumigatus* preparations used were not standardized, comparison of results between laboratories was difficult.

The ImmunoCAP system (Phadia, Nieuwegein, Netherlands) is a widely used ELISA technique for automated analysis of specific IgE antibodies. This system uses standardized allergen extracts covalently coupled to a solid phase. Meier and Müller (1998) described the use of this ImmunoCAP system for the assay of venom-specific IgG antibodies during venom immunotherapy. Kränke et al. (2001) used ImmunoCAP allergens produced for analysis of specific IgE antibodies for measurement of IgG antibodies against *A. fumigatus* and several other molds. Subsequently, the manufacturer (Phadia) introduced ImmunoCAP allergens certified for analysis of specific IgG antibodies against these antigens. Since then, the results of this ELISA for IgG antibodies have been compared with clinical data and with results of the Ouchterlony test against these antigens in a number of studies (Barton et al., 2008; Makkonen et al., 2001; Van Hoeyveld et al., 2006).

Another widely used system for automated analysis of IgE antibodies is the Immulite 2000 system (Siemens, Breda, Netherlands) (Ollert et al., 2005). This analyzer also facilitates the measurement of specific IgG antibodies. In the present study, we compared the analysis of IgG antibody against *A. fumigatus* on these 2 automated immunochemistry analyzers and describe their performance in a between-laboratory quality control program.

☆ Conflict of interest: None.

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2. Methods and sera

2.1. Methods

Routine analysis of specific IgG against *A. fumigatus* was performed every week with the ImmunoCAP 100 system or its enlarged version, the ImmunoCAP 250 system (from 2007 onwards), according to the manufacturer's instructions (Phadia). Results are expressed in milligrams of antigen-specific IgG per liter (mg_A/L). The calibration for antigen-specific IgG analysis (Yman, 2001) follows the same principle as the heterologous calibration that is used for allergen-specific IgE analysis (Plebani, 2003; Yman, 2001). Analysis of specific IgG against *A. fumigatus* with the Immulite 2000 system was performed according to the manufacturer's instructions (Siemens). The Immulite 2000 also uses the heterologous standardization principle for quantitation of IgG antibodies against *A. fumigatus*.

2.2. Sera

After routine analysis of specific IgG against *A. fumigatus* with the ImmunoCAP system, sera were frozen at −20 °C. For use as between-run quality control samples, selected sera were pooled and refrozen after aliquoting. Moreover, every year new serum pools that contained various levels of IgG antibodies against *A. fumigatus* were prepared for use in an external quality control scheme. These serum pools were composed of approx. 25 individual sera. NaN_3 was added as a preservative at a final concentration of 0.01%. Samples ($n = 3$) were distributed together each year to a number of laboratories in Belgium and the Netherlands by SKML, section for humoral immunology, type III allergy (http://www.skml.nl). Furthermore, 70 sera with *A. fumigatus*–specific IgG levels evenly distributed over the range 0–200 mg_A/L by analysis with the ImmunoCAP system were selected for analysis on the Immulite 2000.

The Institutional Review Board of Erasmus University Medical Center approves the use of leftover samples for quality control purposes.

2.3. Statistical analysis

GraphPad Prism for Windows version 5.01 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis of results. Outliers were assessed with the Grubbs' test (http://www.graphpad.com/quickcalcs).

3. Results

3.1. Reference values

After analysis of 30 sera collected from healthy blood donors in 2003, a mean value of 13.16 mg_A/L was found, with a standard deviation of 14.27 mg_A/L. The median value was 8.75 mg_A/L. Statistical analysis showed that the results were not normally distributed. Therefore the upper 97.5% level was estimated by nonparametric statistical analysis at 35 mg_A/L. At a level of 62 mg_A/L of anti–*A. fumigatus* IgG antibodies, the between-run coefficient of variation (CV) was 8.1% for routine assay runs conducted during 2006 using the ImmunoCAP 100. At a level of 92 mg_A/L of anti–*A. fumigatus* IgG antibodies, the between-run CV was 12.9% for routine assay runs conducted during 2008 using the ImmunoCAP 250.

In 2005, 152 sera from healthy blood donors were tested for IgG antibodies against *A. fumigatus* using the Immulite 2000 system. A mean value of 13.71 mg_A/L was found with a standard deviation of 2.68 mg_A/L. The median value was 13.20 mg_A/L. Analysis with the EP Evaluator software (Siemens) gave an upper reference value of 19.3 mg_A/L. At a level of 9.7 mg_A/L, the between-run CV was 10.0%.

3.2. Between-laboratory results

Fig. 1 shows the results of analyses conducted with ImmunoCAP and Immulite 2000 on 12 different serum pools received during 2008, 2009, 2010, and 2011 by laboratories in Belgium and the Netherlands. All participants that used the ELISA technique correctly identified the sera with the lowest and highest level of IgG antibodies against *A. fumigatus*. In 2008, 2009, 2010, and 2011, 20, 22, 19, and 20 laboratories, respectively, used the ImmunoCAP system for analysis of anti–*A. fumigatus* IgG. After exclusion of outliers, the between-laboratory CVs for participants that used the ImmunoCAP system were 13.7%, 10.8%, and 9.8% for QC samples 2008A, 2008B, and 2008C, respectively; 16.6%, 12.9%, and 13.3% for QC samples 2009A, 2009B, and 2009C, respectively; 16.3%, 11.7%, and 18.1% for QC samples 2010A, 2010B, and 2010C, respectively; and 12.8%, 7.3%, and 11.4% for QC samples 2011A, 2011B, and 2011C, respectively.

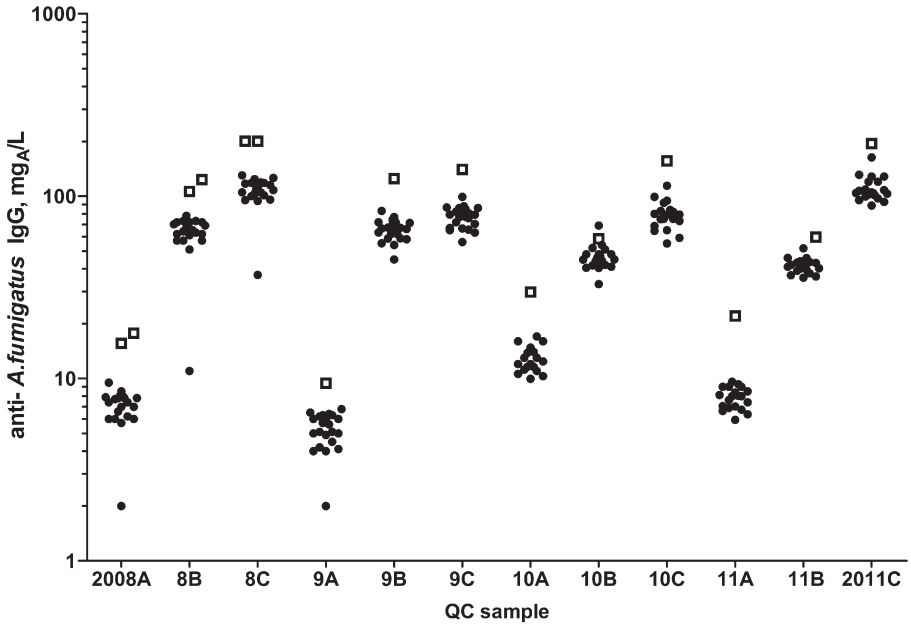

Fig. 1. Between-laboratory results for analysis of anti–*A. fumigatus* IgG in quality control samples. Closed circles: ImmunoCAP system. Open squares: Immulite 2000 system.