Letter to the Editor

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Between-laboratory analysis of IgG antibodies against *Aspergillus fumigatus* in paired quality control samples

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To the Editor,

The ImmunoCAP system (Thermo Fisher, Uppsala, Sweden) is a widely used ELISA technique for automated analysis of specific IgE antibodies. This system has also been found useful for automated analysis of IgG antibodies against a number of mold and bird antigens as part of the diagnosis of aspergillosis and extrinsic allergic alveolitis [1–3]. This method has now largely replaced the time-consuming and labor-intensive Ouchterlony double immunodiffusion technique, also known as the precipitin test [1, 2]. In Belgium and the Netherlands, an external quality control (QC) scheme, 'Type III Allergy', is operational within the section 'Humoral Immunology' of the SKML (www.skml.nl). In 2012, the results of four annual QC rounds were reported [4]. Here, we further analyze these results on the basis of paired QC samples. The outcomes suggest that such a paired analysis may uncover analytical bias in individual laboratories.

Routine analysis of specific IgG antibodies was performed every week with the ImmunoCAP 250 system according to the manufacturer's instructions (ImmunoCAP 250 User Manual, version 1.4, November 2012, Thermo Fisher), as described previously [4]. After routine analysis of specific IgG against *Aspergillus fumigatus* with the ImmunoCAP 250 system, sera were frozen at –20 °C. Every year, three new serum pools that contained a low, intermediate and high level of IgG antibodies against *A. fumigatus* were prepared for use in the external QC scheme [4]. These serum pools were composed of approximately 25 individual sera. NaN_3 was added as a preservative at a final concentration of 0.01%. After aliquoting by SKML, the three QC samples were distributed together each year to a number of laboratories in Belgium and the Netherlands.

The Institutional Review Board of Erasmus University Medical Center approves the use of leftover samples for QC purposes. GraphPad Prism for Windows, version 5.01 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis of the results.

In the external QC scheme, good agreement between the results from laboratories that used the ImmunoCAP system was previously reported [4]. In a further analysis of these QC data, the results for the intermediate and high IgG antibody QC samples in the individual laboratories were plotted against each other (Figure 1). A significant correlation was observed in all four annual QC rounds (2008: Spearman rank $r_s = 0.82$, $p < 0.0001$; 2009: $r_s = 0.84$, $p < 0001$; 2010: $r_s = 0.82$, $p < 0001$; 2011: $r_s = 0.83$, $p < 0.0001$). This result was unexpected: an independent distribution of the results for the intermediate- and high-IgG antibody samples was anticipated. Comparison of the results of the low IgG antibody QC samples with those for the intermediate IgG antibody QC samples showed a similar, although less significant, pattern (2008: $r_s = 0.41$, $p = 0.07$; 2009: $r_s = 0.46$, $p = 0.04$; 2010: $r_s = 0.80$, $p < 0.0001$; 2011: $r_s = 0.70$, $p = 0.0008$).

The pattern, depicted in Figure 1, points to a constant ratio between the results of the paired QC samples analyzed in the different laboratories. This finding suggests that the paired results from the participating laboratories were read from standard curves with a similar shape, which nonetheless produced different quantitative results in the various laboratories.

A possible explanation could be calibration curve drift. Phadia (now Thermo Fisher) has introduced a procedure for the ImmunoCAP system, in which the same

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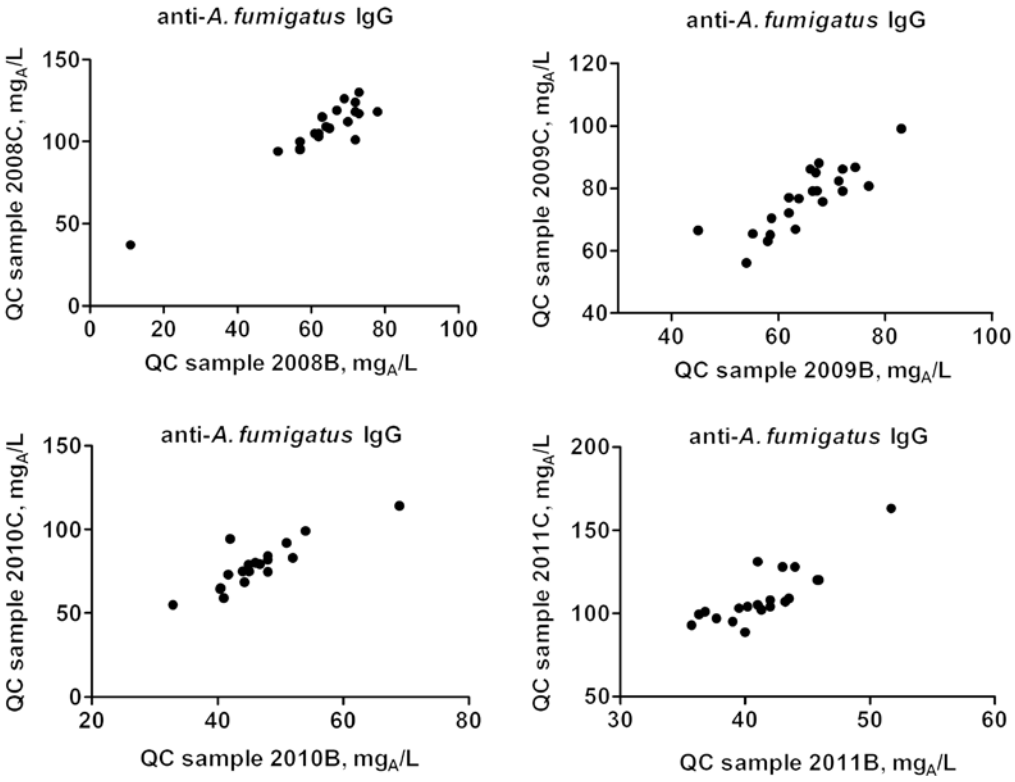

Figure 1: Between-laboratory results for analysis of intermediate B and high C levels of anti-*A. fumigatus* IgG in paired QC samples. mg_A/L, milligrams of antigen-specific IgG per liter.

calibration curve is used in several sequential assay runs, which are approved only when the results of two 'curve control' samples are in a defined range, with acceptance limits of 0.023–0.057 mg/L, and 0.200–0.410 mg/L, respectively. The same calibration curve can be used for up to 28 days (ImmunoCAP 250 User Manual, version 1.4, November 2012). In our department, during a 1-year period, three of every four assay runs for specific IgG antibody analysis against mold and bird antigens on the ImmunoCAP 250 instrument were conducted with a stored calibration curve. Many manufacturers of immunochemistry systems provide a master calibration curve for their immunoassays. The user calibration consists of running two or three adjuster calibrators with known concentrations, and an algorithm to adjust the master calibration curve [5]. In contrast, no adjustment of the stored calibration curve is implemented in the ImmunoCAP 250 system.

Another possible explanation could be the different handling of the calibrators and the patient samples. Calibrators and curve controls are processed undiluted, whereas patient samples are diluted 100-fold (in two sequential 10-fold steps) by the ImmunoCAP 250 instrument before analysis of IgG against bird and mold antigens. The effective dilution may have drifted away from the 100-fold dilution in some participating laboratories.

Curve controls of total and specific IgE, tryptase, and a number of autoimmunity tests are also applied at analysis on the ImmunoCAP 250 instrument (Thermo Fisher Product Catalogue 2016). In these autoimmunity tests, patient sera are also prediluted by the ImmunoCAP 250 instrument. Analysis of between-laboratory QC data for these assays could reveal whether a pattern, depicted in Figure 1, can also be found for these tests.

Today, bias is quantitatively the most important component of uncertainty for measurement results in clinical chemistry between laboratories [6]. Analytical bias may lead to inaccurate reference values and misclassification of individual patients.

In summary, the results suggest that between-laboratory analysis of paired QC samples may uncover analytical bias in individual laboratories.

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