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Performance evaluation of ImmunoCAP® ISAC 112: a multi-site study

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Abstract

Background: After the re-introduction of ImmunoCAP® ISAC sIgE 112 on the market, we undertook a study to evaluate the performance of this multiplex-based immunoassay for IgE measurements to allergen components.
Methods: The study was carried out at 22 European and one South African site. Microarrays from different batches, eight specific IgE (sIgE) positive, three sIgE negative serum samples and a calibration sample were sent to participating laboratories where assays were performed according to the manufacturer's instructions.
Results: For both the negative and positive samples results were consistent between sites, with a very low frequency of false positive results (0.014%). A similar pattern of results for each of the samples was observed across the 23 sites. Homogeneity analysis of all measurements for each sample were well clustered, indicating good reproducibility; unsupervised hierarchical clustering and classification via random forests, showed clustering of identical samples independent of the assay site. Analysis of raw continuous data confirmed the good accuracy across the study sites; averaged standardized, site-specific ISU-E values fell close to the center of the distribution of measurements from all sites. After outlier filtering, variability across the whole study was estimated at 25.5%, with values of 22%, 27.1% and 22.4% for the 'Low', 'Moderate to High' and 'Very High' concentration categories, respectively.
Conclusions: The study shows a robust performance of the ImmunoCAP® ISAC 112 immunoassay at different sites. Essentially the same results were obtained irrespective of assay site, laboratory-specific conditions and instruments, operator, or the use of microarrays from different batches.

Keywords: allergen component; component resolved diagnostics; ImmunoCAP® ISAC 112; microarray; molecular allergology; specific IgE.

Introduction

The use of allergen extracts to determine sensitizations underlying allergic symptoms is often confounded by cross-reacting allergens and poly-sensitizations [1–3]. Measuring specific immunoglobulin E (sIgE) antibodies against individual components (allergen molecules) can separate genuine (primary) and cross-reactive (secondary) sensitizations, thus providing valuable information on the individual IgE-repertoire and improving diagnosis of allergic diseases [2–6].

The ImmunoCAP® ISAC (Immuno Solid-phase Allergen Chip) sIgE 112 (ISAC 112) is a microarray-based immunoassay that enables simultaneous measurement of specific antibodies against an array of 112 recombinant or purified native allergen components derived from over 50 allergen sources [7–9]. Fluorescently labeled anti-IgE antibodies detect specific IgE from a serum or plasma test sample that binds to the immobilized allergen components. Fluorescence intensity is measured by a microarray scanner, converted to arbitrary concentration units, and is

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then displayed both in arbitrary units ISAC Standardized Units for IgE (ISU-E), being internally cross-calibrated to ImmunoCAP singleplex kU_A/l, and as semi-quantitative values (classes). The technical and clinical performances of the ISAC 112 have been validated in several studies that – depending on the allergen component – report comparable results to quantitative singleplex ImmunoCAP allergen component tests [3, 10–19].

After customers having experienced problems with increasing background signals, the product was retracted by the manufacturer, and re-introduced after improvements in the production process had been made. The relaunched ImmunoCAP ISAC 112, is slightly modified with respect to allergen lay-out, and therefore requires an adapted software tool. The array is unchanged with respect to allergen composition – the only change is that the purity of the native Timothy allergen Phl p 4 has been improved.

The ISAC 112 platform provides a minimally invasive, rapid and efficient means of testing IgE sensitizations to a broad panel of allergen components [3], and has been used as an aid in the diagnosis and risk assessment of allergic patients [20–25], to predict allergic symptoms as well as for prescription and monitoring of immunotherapy [26–30], sensitization profiling [31–34], and for the study of allergic diseases [16, 35–37].

The objective of this study was to evaluate the performance of the ISAC 112 microarray in a real life setting using identical assays performed by different operators in multiple sites worldwide.

Materials and methods

Study design

The study was designed to cover as many relevant parameters as possible, including number of laboratories, geographic areas and batches of the array which would guarantee the largest possible between assay variation. The number of positive and negative sera and the assay design were governed by creating enough positive and negative results for every component on the chip in order to be able to perform useful statistical analysis.

Twenty-three sites, 22 in Europe and one in South Africa, participated in the study (see Supplemental Data, Table 1). Microarrays, sourced randomly from ten different batches, and aliquots of eight sIgE positive serum samples (Pos 1–8), three sera with sIgE < 0.1 kU_A/l (Neg 1–3) as well as the calibration sample were sent out to participating sites within the same week and the assays were performed according to the manufacturer's instructions during the following 4 weeks.

The eight positive samples contained sIgE to 110 of the 112 allergen components present on the microarray (Table 1) at different levels covering the whole measuring range of the assay (0.3–100 ISU-E).

ISAC 112 method

ImmunoCAP ISAC 112 (Phadia AB, Uppsala, Sweden) was run according to the instructions of the manufacturer. Briefly, 30 µL of serum samples was added to each microarray and incubated at room temperature for 120 min. After washing, 30 µL fluorescence-labeled anti-human IgE antibodies were added. Following incubation for 30 min, unbound labeled antibodies were removed by washing and fluorescence was measured in an appropriate microarray scanner available in the respective laboratory. Fluorescence measurements from anti-IgE antibodies were compared with a calibration curve and expressed as arbitrary ISU-E. In addition to reporting continuous ISU-E values, test results were analyzed with Phadia Microarray Image Analysis (MIA) Software and ISU-E measurements were assigned to one of four categories on a semi-quantitative ordinal scale ranging from 'Undetectable or Very Low' (0–0.3 ISU-E), to 'Low' (0.3–3 ISU-E), 'Moderate to High' (3–15 ISU-E), and 'Very High' (>15 ISU-E).

The assay was calibrated using a sample containing known amounts of humanized antibodies with several different allergen specificities. The calibration sample used contains 14 chimeric IgE antibodies specific for 14 different allergen components, and is mixed in ratios to obtain concentrations covering the entire measuring range. The concentration of each individual chimeric antibody is determined by singleplex ImmunoCAP assays for the corresponding allergen component (ImmunoCAP®, Phadia AB, Uppsala, Sweden). The calibration sample is thus cross-calibrated against singleplex specific IgE, which in turn is linked by heterologous interpolation to the World Health Organization (WHO) International Reference Reagent 75/502 for total serum IgE.

Data analysis

Homogeneity analysis was carried out on discrete semi-quantitative data from the eight positive serum samples as described by De Leeuw and Mair [38]. To evaluate if measurements from identical samples assayed at different sites clustered into identical groups, we assessed clustering of the entire dataset into sets of samples. Hierarchical

Table 1: Number of IgE-reactive components for each of the positive serum samples and numbers of sample-component interactions per semi-quantitative concentration category.

Serum sample	Number of IgE-reactive components, n	Number of IgE responses per semi-quantitative category		
		'Low' (0.3–3 ISU-E)	'Moderate to High' (3–15 ISU-E)	'Very High' (>15 ISU-E)
Pos 1	13	5	5	3
Pos 2	76	24	38	14
Pos 3	59	26	24	9
Pos 4	46	18	18	10
Pos 5	88	29	42	17
Pos 6	95	23	20	52
Pos 7	78	20	24	34
Pos 8	79	29	34	16

clustering and clustering using random forests was performed using positive and negative serum samples as described [39].

For quantitative analyses, raw continuous ISU-E measurements were used after outlier filtering to obtain an aggregate score of accuracy across sites. Outliers were defined using a local outlier factor algorithm with a fixed number of neighbors (n = 8); the outlier factor was set so that < 5% of the total data was discarded, thus reflecting outlying results in the quality control practice [40].

Standardized ISU-E values were calculated from sample-component assay result combinations with values ≥ 0.3 ISU-E from at least 22 of the 23 sites. In total, 444 of 896 possible combinations met these criteria. For each of the 444 sample-component combinations, standardized ISU-E values were calculated by dividing the site-specific values by the mean value from all sites. Site-specific aggregate ISU-E means were calculated as the mean of all 444 standardized ISU-E values from that site.

Site-specific coefficients of variation (CV) were calculated using the quantitative ISU-E values obtained from the 444 sample-component combinations. The CV across all sites (CV_{Total}) was estimated as the median of all combination-based estimates of variation from the study sites.

User questionnaire

A questionnaire including three questions on ease of handling was sent to each site; questions and answer options were:

1. In your opinion, was performing of the updated ISAC 112 assay; (Easy, Neither easy nor difficult, Difficult)
2. Did you receive an approved calibration curve the first time you ran the quality control assay? (Yes or no)
3. How was the usability of the updated MIA software, version 1.2.4? (Easy, Neither easy nor difficult, Difficult).

Results

Semi-quantitative analysis

Discrete, categorized, IgE measurements from unfiltered data are shown by study site and by sample in Figure 1. We observed a similar pattern of results for each of the 11 samples across the 23 sites (Figure 1A). Categorized values for the three negative samples and one positive sample are presented in Figure 1B, showing that ISU categories for negative and positive samples were consistent between the different sites. Of 7728 measurements of the three negative samples (3 samples and 112 allergen components at 23 sites), only one yielded a signal slightly above 0.3 ISU, corresponding to a low frequency (0.014%) of false positive results.

A homogeneity analysis of semi-quantitative measurements was performed to visualize the degree of dispersion between the different sites and within the assay runs. Figure 2 shows the projection for the eight positive samples at each of the 23 sites, where the number of dimensions have been reduced from 110, based on the number of IgE-reacting allergens components for each sample, down to two, allowing for easy visualization. This projection illustrates the degree of similarity/disparity between the samples, since samples with similar properties, i.e. sensitization pattern, cluster in each other's vicinity. Samples having clusters with low levels of dispersion demonstrate a less reactive sensitization pattern, e.g. positive sample 1 with 13 IgE-reacting allergen components displayed the lowest degree of within-sample dispersion, while other samples with 46–95 IgE-reacting allergen components demonstrated a higher degree of dispersion. Although the degree of dispersion differed slightly between the samples, the 23 measurements for each sample were well clustered, indicating good reproducibility of the assay.

Using unsupervised hierarchical clustering as well as classification via random forests, we observed clustering of samples with the same identity regardless of the assay site. The visual impression of correct classification was confirmed by the classification error of zero (Supplemental Data, Figures 1 and 2).

Quantitative analysis

Of 896 possible combinations of samples and allergen components (8 positive samples and 112 allergen components) around half (n = 444) gave measured values ≥ 0.3 ISU-E at > 95% of the sites. Figure 3 shows the distribution of standardized ISU-E values of the 444 measured values from all 23 sites (n = 10,212 in total), and aggregate means of standardized ISU-E values for each site. Precision of the assay at each site is indicated by the proximity of the aggregate mean value to the center of the distribution. Aggregate mean values fell close to the center of the distribution with a similar number on either side; only one site had a value outside the range 0.5–1.5.

Total variability

Median between-site variation in ISU-E values for the 444 sample-component combinations with values ≥ 0.3 ISU at > 95% of the sites was calculated to estimate total precision. After filtering outliers, which correspond to < 5% of total measurements, CV_{total} was estimated at 25.5%. Including the outlying values led to an estimate of approximately 29.9%. Estimates of variability for each of the positive ISU

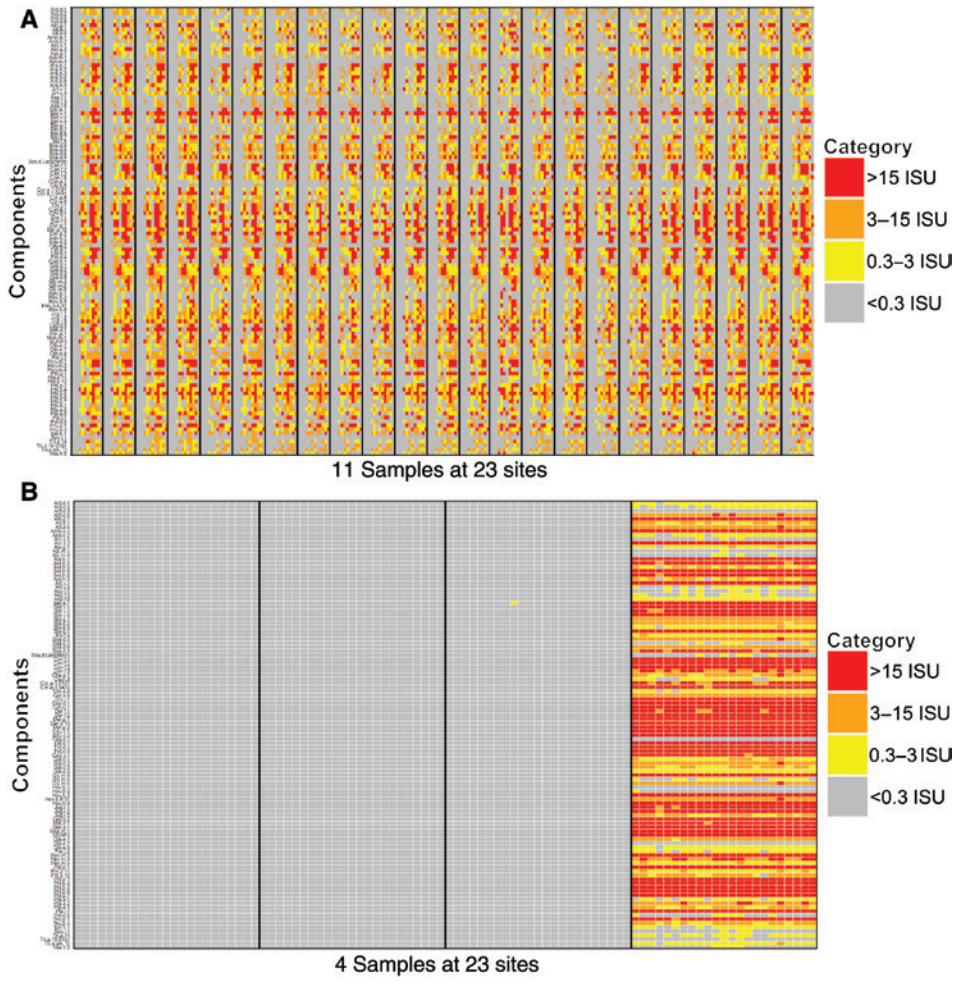

Figure 1: Heat map representation of semi-quantitative data by assay site and sample.
(A) Sites are separated by the solid vertical lines. At each site, samples from left to right are: 1–3 negative, 4–12 positive, and the reference sample; the 112 components are distributed on the vertical axis. (B) Samples are separated by the solid vertical line. From left to right these are three negative and one positive sample. The assay sites are distributed on the horizontal axis between the vertical lines for each sample. The 112 components are distributed on the vertical axis.

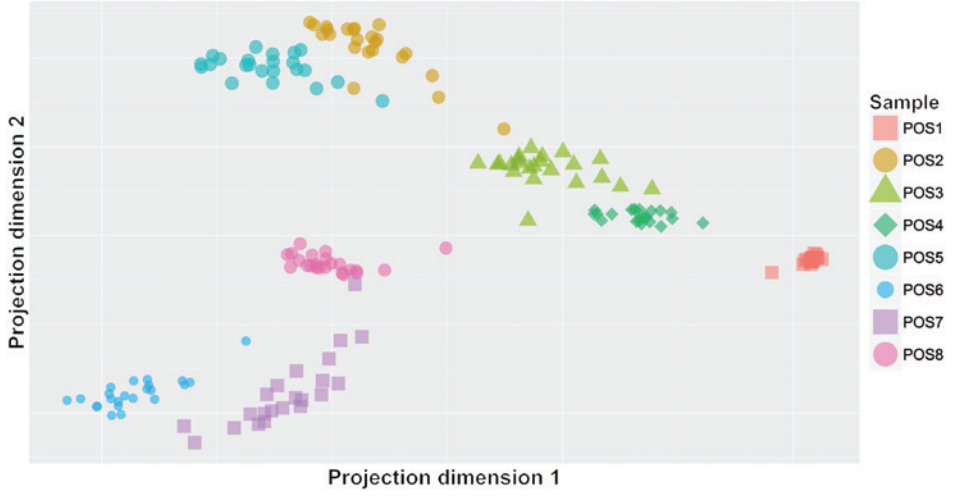

Figure 2: Projection of measurements based on all 112 components onto a 2-dimensional plane for eight positive samples at each of the 23 sites. Dispersion within each cluster is a function of the sample reactivity.

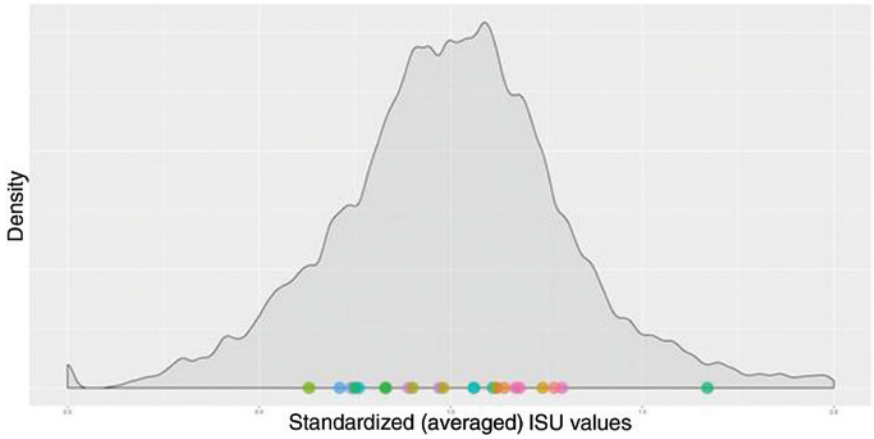

Figure 3: Distribution of standardized ISU values and site means.
Aggregate means of standardized ISU values for the 23 individual sites are indicated by the colored dots.

categories for filtered data were: 'Low' 22%, 'Moderate to High' 27.1%, and 'Very High' 22.4%.

User questionnaire

Fifteen of the users found the assay easy to perform and 14 found the evaluation process using the software MIA easy. Five users answered that the performance of the assay and the evaluation process was neither easy nor difficult, while one user found the software difficult to use. Twenty users were able to run the assay, including an approved calibration, without any complications at their first attempt, while two users reported having difficulties at the first try.

Discussion

This is the most extensive real-life evaluation of ISAC 112 to date, incorporating a range of varying factors. The performance of the ISAC 112 microarray immunoassay was tested at multiple sites worldwide under different laboratory conditions with different operators using microarrays from several batches with the aim of maximizing the possible sources of variability. The obtained results show good consistency in analytical performance across sites, and the low frequency of false positive results confirms the high specificity of the assay, with a low and stable background that was consistent between sites.

The semi-quantitative nature of ISAC means that at IgE levels close to the cut-off points, the output is likely to fluctuate between concentration categories in different assays. However, visualization of discrete data showed that assigned categories for a given component were consistent between samples and sites, and unsupervised clustering showed perfect alignment of samples into expected groups, regardless of assay location. Despite the semi-quantitative nature of ISAC we have also chosen to treat the ISAC results as if they were continuous allowing comparison with other reported evaluations of ISAC. Site-specific means from continuous data (standardized ISU-E) were close to the center of the distribution of standardized ISU-E values, further indicating that the performance at different sites was stable and precise. One European site had a mean standardized ISU-E value that was slightly higher than the others. When assays were re-run at the site, results in better agreement with that of other sites were obtained; however, no information was available on the cause of the outlying values in the original assays.

A previous version of the ISAC microarray (ISAC 103) was reported to show high inter-assay variability for certain components [12] that resulted in poor performance in detecting sensitizations to certain allergen sources [41]. Inter-assay CV of ISAC 103 was >100%, 33% and 13.2% for Low, Medium and High ISU-E categories, respectively [17]; however, definitions of the Low and Medium categories for ISAC 103 differ from the current definitions for ISAC 112. These previous estimates of total variation were based on within- and between-assay estimates of variability obtained using a reproducibility study where all assays were run at one site [17]. In the present study the category-specific estimates of variation ranged from 22 to 27% and the overall estimate, CV_{Total}, was 25.5%. Given the design of the current study, which encompassed a wide array of variable factors, the obtained results demonstrate consistent performance of the ISAC 112 assay over the entire measurement range. Furthermore, results from this multi-site

study show good coherence with the estimated total CV of 17–26.4% from assays performed in one location by a single operator using identical instruments and microarrays from the same batch (according to the directions for use, see Supplemental Data, Table 2).

The results of the present study are also consistent with those of other groups. A recent study of the repeatability and reproducibility of ISAC 112, carried out in two different laboratories, reported that the reproducibility was 'very good' for 73 allergens on the microarray and 'good' for 22 allergens according to the classification of Fleiss [42] (intra-class correlation coefficients of >0.90, and 0.71–0.90, respectively) [11]. A study of the MeDALL custom microarray using a slightly different methodology, did not report the overall CV; however, mean CV (all concentrations) for the Bet v 1 and Phl p 2 components were ≤ 14.1% and ≤ 16.3%, respectively [16].

In conclusion, the outcomes of this study show a very robust performance of ISAC 112 across the 23 participating sites. Essentially the same results were obtained regardless of assay site, laboratory-specific conditions, operator, and different batches of the microarray.

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