

The importance of EN ISO 15189 accreditation of allergen-specific IgE determination for reliable *in vitro* allergy diagnosis

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Abstract

Background: Allergen-specific serum immunoglobulin E detection and quantification have become an important step in allergy diagnosis and follow-up. In line with the current trend of laboratory test accreditation to international standards, we set out to design and assess an accreditation procedure for allergen-specific serum IgE.

Methods: Method validation according to the accreditation procedure under the EN ISO 15189 standard was carried out for allergen-specific immunoglobulin E determination using the fluoroimmunoassay method ImmunoCAP[®] (ThermoFisher). Data were produced by 25 hospital laboratories in France. A total of 29 allergen specificities including mixes, extracts, and molecular allergens were assayed. Allergen-specific serum immunoglobulin E concentrations ranged from 0.1 to 100 kU_A/l.

Results: Repeatability, reproducibility, and accuracy results fulfilled method validation criteria for automated laboratory tests and proved similar irrespective of the allergen specificity, allergen-specific serum immunoglobulin E concentration, or individual laboratory.

Conclusion: Allergen-specific serum immunoglobulin E determination with the fluoroimmunoassay method ImmunoCAP[®] is a highly repeatable, reproducible, and accurate method which may be considered as a single analyte assay in view of the EN ISO 15189 accreditation procedure.

Allergen-specific immunoglobulin E (IgE) detection by skin prick or blood testing proves allergic sensitization and is therefore required for allergy diagnosis (1–3). Allergen-specific serum IgE (sIgE) may distinguish allergy from sensitization in a number of well-defined conditions and help with the follow-up of allergic patients (4–6). Over the last 20 years, technical performances of sIgE measurement methods have gained paramount importance in clinical laboratory practice (7–10). Accurate quantitative determination of sIgE remains challenging, due to the very low amount of circulating IgE, to the polyclonal and interindividually variable sIgE responses, and to lack of standardized allergenic material (11–14). Methods have become highly automated, allergenic extracts or components have been significantly improved, certification standards have been set for both manufacturers and clinical laboratories. Accreditation is the process leading to the certification to ISO 15189 standard for medical laboratories (<http://iso.org>, 14–16). In France, it was recently decided by law that clinical laboratories were due to validate the accreditation process by November 2020 (17, 18). This requirement applies to thousands of laboratory tests, but allergy work-up stands out. Indeed, sIgE determination is unique among blood tests in that virtually unlimited numbers of allergen specificities are assessed with a single method. Thus, sIgE may be considered as a unique analyte, irrespective of allergen specificity; alternatively, each allergenic reagent may be viewed as a separate unique test (19). The former view was shared by Belgian and Swiss laboratories and local accreditation organisms, but was a matter of debate in France. With respect to the Fluoroenzymatic immunoassay (FEIA) method, our group recently reviewed manufacturer's quality control testing and recommendations, external and internal quality control (IQC) schemes, and literature data (20). We thereafter assumed that a global accreditation procedure in line with the ISO 15189 standard could rely on extensive assessment of a limited number of allergen specificities. In this study, we report experimental evidence supporting the choice of this accreditation procedure for sIgE determination with the FEIA method. Data were produced by 25 French laboratories from the AllergoBioNet network (comprising French, Belgian, and Swiss hospitals).

Methods

Accreditation procedure and parameters

The accreditation procedure under the EN ISO 15189 standard involves several steps, including method validation. The latter addresses a number of technical parameters which must

perform according to the standard. For sIgE FEIA method, parameters such as repeatability, reproducibility, and accuracy need to be characterized in the clinical laboratory. Conversely, linearity, sensitivity (low limit of detection and low limit of quantification), specificity, and the absence of intersample contamination are checked and guaranteed by the manufacturer as part of the CE marking for *in vitro* diagnosis medical devices and procedures (CE DM IVD) label. The manufacturer is bound to supply adequate documentation to end users.

Repeatability evaluates the dispersion of a measure by repeated assays of a given sample during a single experimental procedure using the same batch of allergen.

Reproducibility evaluates the dispersion of a measure by repeated assays of a given sample during multiple experimental procedures over time (e.g., multiple operators, normal sample storage, different runs of the same machine).

Accuracy (or bias) compares results of a given laboratory with target values of the analyte in an external quality control (EQC) sample. As a rule, the target value is defined as the mean value of results collected from a panel of peer laboratories.

Method validation scheme for sIgE determination in the accreditation procedure

sIgE determination

sIgE were measured with the ImmunoCAP[®] system, according to the manufacturer's instructions (ThermoFisher Scientific, Uppsala, Sweden). sIgE concentrations were expressed in allergen-specific units (kU_A/l). A typical standard curve for sIgE determination is built with 6 points: 0, 0.35, 0.70, 3.5, 17.5, and 100 kU_A/l and displays an S-shape (sigmoid curve). A common standard curve is shared for all sIgE measures, irrespective of allergen specificity. This curve is plotted against the international WHO standard for total IgE determination (WHO 75/502). Linearity of sIgE determination was guaranteed by the manufacturer for a working range of 0.10–100 kU_A/l .

Test samples and ethical aspects

The study was based on a prospective, noninterventional laboratory work-up. Samples for the study were selected in each laboratory among routine leftover batches based on appropriate specificity and concentration of sIgE to be tested. Selected samples were anonymized prior to test procedures, data collection, and analysis. Assays were conducted on undiluted sera. Prior to sIgE determination for the study, samples had been stored at 4°C for less than a week or at –20°C for longer periods. Clinical data (precedence, final diagnosis)

Abbreviations

CCD, cross-reactive carbohydrate determinants; CE IVD DM, CE marking for *in vitro* diagnosis medical devices and procedures; COFRAC, Comité Français d'Accréditation; CV, coefficient of variability; EC, European community; EN ISO, European Norm–International Organization for Standardization; EQC, external quality control; FEIA, Fluoroenzymatic immunoassay; IgE, Immunoglobulin E; IQC, internal quality control; IU/L, International unit per unit volume; SD, standard deviation; sIgE, allergen-specific serum IgE; U_A/l , allergen-specific unit per unit volume; UK NEQAS, United Kingdom National External Quality Assessment Service.

were not collected for this analytical study. Total IgE levels were measured in 10 random samples and found <1000 kIU/l (not shown), which is the manufacturer's alert level for possible interference with quantitation of very low levels of allergen-specific IgE (Thermo Fisher guidelines for accreditation).

Assays of method validation parameters

Repeatability was measured for three frequent sIgE specificities: house dust mite *Dermatophagoides* (*D.*) *pteronysinus*, environmental fungus *Aspergillus* (*A.*) *fumigatus*, and food allergen peanut *Arachis* (*A.*) *hypogaea*. For each sIgE specificity, two sIgE concentrations were assayed: 0.8 and 10 kU_A/l. Each participant center performed 20 serial determinations on two *D. pteronyssinus* sIgE samples and 10 serial determinations on two *A. fumigatus* and two *A. hypogaea* sIgE samples.

Repeatability was further assayed for a panel of 24 other sIgE specificities including mixes, airborne allergenic extracts, food extracts, venoms, drugs, and molecular allergens with sIgE concentrations ranging from 0.14 to 70 kU_A/l. These allergens were tested at one per center by means of 10 serial sIgE measurements.

Reproducibility was evaluated through collection and analysis of IQC results of each center. IQC samples were either commercially available samples (ThermoFisher) or pooled patient samples.

Accuracy was evaluated through retrospective analysis of data from EQC programs collected as part of the routine external quality assessment.

Data collection, data processing, and statistical analysis

Data were collected by CL and AS from 25 hospital laboratories in France (location: Amiens; Angers; Bordeaux; Caen; Clermont-Ferrand; Le Mans; Lille; Limoges; Lyon; Marseille; Nantes; Nice; Paris, Ambroise-Paré; Paris, Bichat; Paris,

Necker; Paris, Trousseau; Poitiers; Reims; Rennes; Rouen; Saint-Denis; Saint-Etienne; Strasbourg; Toulouse; and Tours). For each serum determination, concentrations of sIgE were expressed as kU_A/l. For each laboratory, mean and standard deviation (SD) were calculated for each data set. The coefficient of variability (CV) defined as (SD × 100)/mean was expressed as a percentage. Statistical comparison of sIgE results was performed with analysis of variance tests and Student's *t*-test. *P* values of 0.05 or less were considered as statistically significant.

Results

Repeatability

sIgE concentration was serially measured in the same machine run in six samples per laboratory. Each laboratory performed 20 serial measurements for sIgE to *D. pteronyssinus* and 10 serial measurements for sIgE to *A. hypogaea* and *A. fumigatus*. For each laboratory, samples were selected among local ones that had been previously shown to titrate to 0.8 or 10 kU_A/l. Intralaboratory coefficients of variation (CV) of sIgE measurements ranged from 1.00% to 9.90% (one outlier at 11.2%) (Table 1). Interlaboratory mean CV ranged from 4.09% to 5.61%. The analysis of variance showed CV for the three allergens and both levels was distributed uniformly among laboratories, allergen specificities, and sIgE concentration. Moreover, CV was not altered between the 20 serial measurements scheme (*D. pteronyssinus*) and the 10 serial measurements scheme (*A. hypogaea* and *A. fumigatus*).

Repeatability assays were extended to 24 further allergens for a total of 34 samples and a broader concentration range from 0.1 to 70 KU_A/l. Each laboratory assayed 1 or 2 samples with 10 serial measures (Table 2). Two samples were assayed for the allergenic mixes Phadiatop® (common

Table 1 Interlaboratory repeatability of sIgE measures performed with the Fluoroenzymatic immunoassay (FEIA) method for three common allergens

Allergens assayed for sIgE repeatability	Reagent code	Target value (kU _A /l)	Minimum and maximum intralaboratory coefficient of variation (%)	Interlaboratory mean coefficient of variation (%)
<i>Dermatophagoides pteronyssinus</i>	d1	10	1.70–7.90	4.09
<i>Aspergillus fumigatus</i>	m3		2.40–9.60	5.16
Peanut (<i>Arachis hypogaea</i>)	f13		2.40–11.20	4.36
<i>D. pteronyssinus</i>	d1	0.8	2.80–7.90	4.73
<i>A. fumigatus</i>	m3		2.20–9.90	5.61
Peanut (<i>A. hypogaea</i>)	f13		1.00–9.60	4.17
				Interlaboratory mean CV for any allergen = 4.85%

Each laboratory selected six local samples titrating to 10 or to 0.8 kU_A/l for sIgE to one of three commonly used allergenic extracts: *Dermatophagoides pteronyssinus* (house dust mite), *A. hypogaea* (peanut), and *A. fumigatus* (airborne mold). Twenty (*D. pteronyssinus*) or 10 (peanut, *A. fumigatus*) serial assays were performed on the corresponding samples. Coefficient of variation (CV) was calculated for each series of measures in each laboratory and then as an interlaboratory mean. Values of individual (intralaboratory) and mean (interlaboratory) CV are shown.

airborne allergen mix) and fx26 (cow's milk, egg white, peanut, mustard), and the ratio between the FEIA signal and the background noise was calculated. In the other 32 samples, sIgE concentrations were considered as low (0.1–2 kU_A/l, $n = 7$), medium (2–10 kU_A/l, $n = 19$), and high (12 kU_A/l or higher, $n = 6$). Mean intralaboratory CV was 3.81% (range 1.10–8.40%, one outlier at 10.28). Analysis of variance showed no difference in the CV when considering food vs airborne allergens, extracts vs molecular allergens, or high, medium, and low levels of sIgE.

Reproducibility

Reproducibility of sIgE determination was evaluated using sequential measurements of sIgE in IQC samples over a period of up to 6 months. For each sample ($n = 47$), each allergen ($n = 7$), and each laboratory, sIgE concentrations were

measured at least 20 times. For a given allergen, intralaboratory CV ranged from 3.58 to 12% (Table 3). Mean intralaboratory CV was 7.45% (5.34% to 9.18%, not shown). Mean interlaboratory CV was 7.17% (range 5.19% to 9.50%), not including sIgE against *Alternaria alternata*, which served as IQC in only one laboratory. Analysis of variance showed no difference in the CV of sequential IQC measurements with respect to allergen, sIgE concentration, or laboratory.

Accuracy

Interlaboratory data comparison for a given sample is available monthly through the Quality Club® program (ThermoFisher). The sIgE report of January 2014 of laboratories using the ImmunoCAP® system displayed a CV of 9.28% for sIgE to *D. pteronyssinus* (31.5 ± 2.93 kU_A/l, $n = 497$, two outliers); 8.59% for sIgE to timothy grass (*Phleum pratense*)

Table 2 Intralaboratory repeatability of sIgE measures performed with the Fluoroenzymatic immunoassay (FEIA) method for 24 allergens

Allergens assayed for sIgE repeatability		Reagent code	Mean value (kU _A /l)	Range (kU _A /l)	Mean coefficient of variation (%)
Mixes	Common airborne allergen mix	Phadiatop®	3.10	3.00–3.30	3.30
	Cow's milk, egg white, peanut, mustard	fx26	0.62	0.49–0.70	10.28
Airborne allergen extracts	<i>ermatophagoides farinae</i>	d2	6.70	6.50–6.90	1.60
	<i>D. farinae</i>	d2	10.30	9.60–11.30	5.10
	<i>D. farinae</i>	d2	61.60	57.70–68.10	4.60
	Cockroach (<i>Blatella germanica</i>)	i6	14.8	13.20–16.60	5.70
	Cockroach (<i>B. germanica</i>)	i6	1.1	0.91–1.20	7.90
	Cat dander	e1	3.10	2.98–3.20	2.00
	Horse dander	e3	6.10	5.92–6.50	2.9
	Hamster epithelium	e84	2.70	2.36–2.80	4.50
	Birch pollen	t3	6.10	5.90–6.40	2.00
	Olive tree pollen	t9	4.50	4.21–4.66	3.00
	Olive tree pollen	t9	0.17	0.16–0.19	5.90
	Orchard grass (<i>Dactylis glomerata</i>) pollen	g3	4.70	4.60–5.00	2.60
Food allergen extracts	Egg white	f1	69.50	65.20–74.80	4.20
	Egg white	f1	12.30	12.20–12.80	2.00
	Egg white	f1	1.20	1.14–1.20	2.30
	Cow's milk	f2	4.80	4.40–5.00	4.50
	Cow's milk	f2	0.24	0.22–0.26	5.70
	Barley (<i>Hordeum vulgare</i>) grains	f6	4.98	4.34–5.30	6.10
Other allergen extracts	Common wasp (<i>Vespula</i> spp) venom	i3	5.30	5.16–5.60	2.50
	Common wasp (<i>Vespula</i> spp) venom	i3	3.90	3.40–4.50	8.40
	Pholcodine	c261	4.90	4.55–5.40	6.10
	Quaternary ammonium	c260	5.50	4.95–6.30	8.20
Allergen components	rPhl p 1 + rPhl p 5	g213	6.00	5.70–6.20	2.30
	rBet v 1	t215	6.20	6.14–6.30	1.10
	rBet v 1	t215	0.14	0.13–0.15	4.50
	rPru p 1	f419	65.16	61.40–68.10	2.80
	rCor a 1	f428	13.40	12.80–13.80	2.20
	rCor a 8	f425	6.70	6.50–7.00	2.60
	rAra h 2	f423	3.90	3.70–4.10	3.10
	rHev b 5	k218	4.70	4.43–5.00	3.70
	rHev b 5	k218	0.18	0.17–0.19	3.20
	MUXF3 (plant carbohydrate determinant)	o214	0.87	0.82–0.97	5.30
	Interlaboratory mean CV for any allergen = 4.24%				

Each laboratory selected one or two local samples on the basis of sIgE concentration to a given allergen. Ten serial measurements were performed on each sample. Mean value, range of sIgE results, and the coefficient of variation (CV) are shown for each series of measures.

Table 3 Interlaboratory reproducibility of sIgE measures performed with the fluoroenzymatic immunoassay (FEIA) method

Allergenic extracts assayed for sIgE reproducibility	Reagent code	Minimum and maximum intralaboratory coefficient of variation (%)	Mean coefficient of variation (%)
<i>Dermatophagoides pteronyssinus</i>	d1	4.67–12.00	7.66
Cat dander	e1	4.54–9.12	6.51
Birch pollen	t3	4.52–9.05	6.46
Ragweed pollen	w1	3.58–6.80	5.19
<i>Aspergillus fumigatus</i>	m3	9.27–9.80	9.50
<i>Alternaria alternata</i>	m6	ND	7.36
Peanut	f13	4.58–9.90	7.52

Sequential results for sIgE determinations on internal quality control samples were collected and analyzed. In each laboratory, each sample had been assayed for at least 20 times over a period of up to 6 months. A total of 47 samples were assayed. Coefficient of variation (CV) was calculated for each series of measures in each laboratory and then as an interlaboratory mean. Values of individual (intralaboratory) and mean (interlaboratory) CV are shown. ND, not determined.

pollen (3.11 ± 0.27 kU_A/l, $n = 432$, two outliers); and 8.24% for sIgE to silver birch (*Betula verrucosa*) pollen (9.75 ± 0.8 kU_A/l, $n = 443$, two outliers).

Interlaboratory comparison is also available through the UK NEQAS program for immunology, immunochemistry, and allergy, an EQC scheme of six annual samples and 15 allergen specificities (<http://www.immqas.org.uk>). The sIgE report of March 2014 showed CV ranging from 8.8% (cat dander, 3.97 ± 0.35 kU_A/l, $n = 202$ and timothy grass, 2.58 ± 0.23 kU_A/l, $n = 164$) to 13.8% (hazelnut, 6.88 ± 0.95 kU_A/l, $n = 171$) for ImmunoCAP® 250 reports. Reported values of sIgE determination were similar for ImmunoCAP® 100 and 1000 (<30 participants each). By contrast, alternative methods yielded different results, for example, from 1.94 to 10.21 kU_A/l for cat dander.

Discussion

Using the widespread FEIA method, we sought to characterize sIgE determination as a single analyte assay according to EN ISO 15189 accreditation criteria (20). Repeatability, reproducibility, and accuracy of sIgE determination were addressed by 25 hospital laboratories for a total of 29 allergens and 231 serum samples. Our results can be summarized as follows:

- 1 The coefficient of variation for sIgE determination was independent of allergen specificity and sIgE levels. Mixes, airborne allergenic extracts, food extracts, venoms, drugs, and molecular allergens displayed similar results for intralaboratory and interlaboratory repeatability, reproducibility, and accuracy. This was also true when comparing high, medium, and low sIgE concentrations including those <0.35 kU_A/l.
- 2 The mean coefficient of variation for all allergens, all sIgE concentrations, and all laboratories was <10%, which is the upper limit an automated blood test should not exceed (19).

In light of these findings, sIgE determination with the FEIA method fulfills the requirements for EN ISO 15189 certification with respect to repeatability, reproducibility, and accuracy. Technical performances are similar irrespective of

the allergenic extract or component, the concentration of sIgE, and the participant center. Our results confirm and extend those obtained through the UK NEQAS program for immunology, immunochemistry, and allergy, which compares allergen extracts only and does not exceed 15 distinct specificities. Thus, sIgE determination with the FEIA method may be considered as a single analyte test.

Further support for this view is found in the current environment of the FEIA method: high degree of automation, sample, and reagent monitoring with respect to common standard curve, working range, limit of detection, linearity, temperature, humidity, and expiry date. As previously indicated, all these parameters are guaranteed by the manufacturer as part of its CE IVD report.

Altogether, our study demonstrates that method validation for FEIA sIgE determination does not need to be replicated for each allergen in each clinical laboratory. Instead, it may rely on 20 serial sIgE measurements for one allergen in a single sample (repeatability), 20 sequential sIgE measurements for one allergen in another single sample (reproducibility), and checking accuracy by means of scheduled EQC programs. As long as these criteria are met, interlaboratory variations are negligible. Under the current economic circumstances, we provide here a solid argument for a cost-efficient accreditation procedure of sIgE testing. Its strength resides in data collection and processing for an extended number and type of allergens (exceeding the panel available from current EQC programs), range of sIgE concentrations, and number of participating laboratories.

Current commercially available sIgE assay formats share the principle of multiple allergen reagents as the only variable of sIgE assessment (19). Thus, our study may be considered as a proof of concept for methods other than FEIA. Nevertheless, it would be crucial to conduct similar experimental studies with other commercially available methods.

Our group was unable to perform such experiments, as no laboratory of the AllergoBioNet network is currently employing an alternative commercial method for sIgE determination.

With respect to quantitation of very low levels of sIgE, between 0.10 and 0.35 kU_A/l, we report here the good per-

formance of the FEIA method. In our study, we included four allergen specificities which were assayed for repeatability at both medium (5–6 kU_A/l) and very low (0.14–0.24 kU_A/l) levels: olive pollen, cow's milk, Bet v 1, and Hev b 5 (Table 2). Coefficients of variation did not exceed 6% for any of the very low levels, which was indeed an unexpectedly good result. No negative result showed up while repeating the sIgE assays on these very low levels.

In our study, the effect of potential confounders such as very high total IgE levels and allergen-specific antibodies of different isotypes has not been assessed, mainly because these parameters are not consensual as part of basic *in vitro* allergy assessment (21–24). According to Thermo Fisher guidelines for sIgE accreditation, IgE levels up to 1000 kIU/l do not interfere with the measurement of sIgE levels above 0.35 kU_A/l. Allergen-specific sIgA and sIgG (including sIgG1 and sIgG4) responses are highly variable with respect to levels and epitope diversity depending on the allergen which is assessed (food, airborne) and the existence of immune therapeutic interventions (e.g., specific immunotherapy to *Hymenoptera* venoms or food oral tolerance induction) (25, 26). Interference of allergen-specific isotypes has been reported for ELISA and immunoblotting methods (27), but experimental evidence of interference with currently available commercial methods is lacking.

Our study was dedicated to analytical aspects of sIgE determination. It is important to remind that the detection of allergen sIgE, be it at high levels in some patients, needs to be confronted to clinical history to get the accurate allergy diagnosis.

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In brief, we confirm here that quantitative sIgE determination with the FEIA method complies with EN ISO 15189 requirements with allergenic reagents including mixes, extracts, and proteins behaving similarly and being therefore well suited for use as a technically reliable diagnostic and prognostic factor in allergy management.

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Author contributions

CL and AS designed the research, analyzed data, performed statistical analysis, and wrote the manuscript. JV wrote and critically revised the manuscript for important intellectual content. All authors performed experiments, collected data, and read and approved the final version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest with respect to the present work.

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