



Third-party verification of total and specific immunoglobulin E on analyzer Immulite® 2000XPi



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ABSTRACT

Background: Although Immulite®2000XPi (Siemens) allergy testing is routinely used in laboratories worldwide; a well-designed comprehensive third-party verification of its analytical performance has not yet been published. Our aim was to verify stability, precision and reference intervals for total and some frequent specific IgEs, test trueness for several most common allergens and assess the comparability of fluorescent- and chemiluminescent-enzyme immunoassay for total IgE.

Methods: Verification was based on EP15-A3, EP9-A3 and EP28-A3c CLSI guidelines. Stability was tested on serum pools stored at 2–8 °C, during 7 days. Manufacturer's reference intervals were verified for children and adults. Acceptance criteria were based on manufacturer specifications and national External Quality Assessment provider data.

Results: Samples for total IgE analysis were stable at 2–8 °C for 7 days. Precision was within manufacturer's specifications for most of tested allergens, except for total IgE (92.5 kIU/L, CVs 5.6% and 7.6%) and w1 (9.3 kIU/L, CV = 5.9%). Bland-Altman plot revealed statistically significant constant (–32.4 kIU/L) and proportional bias (–20.2%) for total IgE between two methods. Passing Bablok showed statistically but not clinically significant biases between two Immulite analyzers for betula verrucosa (constant and proportional), house dust mite and egg white allergens (proportional). For adults and children, total IgE results were within reference intervals declared by Siemens.

Conclusions: Immulite®2000XPi assay has acceptable precision and trueness. Samples for total IgE are stable even longer than declared by the manufacturer. Declared reference intervals can safely be adopted for both adult and children population in our laboratory. Fluorescent- and chemiluminescent-enzyme immunoassay results of total IgE are not comparable.

1. Introduction

Stability as well as transferability of reference intervals (for children and adults) for total immunoglobulin E (tIgE) CLEIA assay (Immunité®2000XPi) have not been demonstrated by a third-party. Moreover, the existing literature reports about the interchangeability of FEIA (ImmunoCAP 100, Thermo Fisher Scientific Inc., Waltham, MA, USA) and CLEIA (Immunité®2000XPi, Siemens Healthcare Diagnostics, Erlangen, Germany) tIgE are contradictory. Thus, a comprehensive third-party verification of the analytical performance of Immunité®2000XPi is obviously missing.

Total and specific IgE in vitro determinations, along with anamnesis

(e.g. physical examination) and skin prick tests (SPT), are an essential part in diagnosis, monitoring and treatment of allergy diseases. Detection of sIgE points to the allergic sensitization in an individual and predicts risk of allergies in later life, while tIgE quantification provides useful information on allergen exposure and treatment monitoring. Early detection of the clinically relevant allergen is crucial for necessary allergen avoidance or treatment indication. Sometimes, serological determinations of sIgE and tIgE are the only option for diagnosis when SPTs cannot be performed due to eczema, dermatitis, or possible risk of anaphylactic reaction [1].

First-generation of sIgE assays were mainly done using the modified radioallergosorbent test (mRAST), second-generation methods were

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based on fluorescent enzyme immunoassays (FEIA) whereas third-generation assays were mostly enzyme amplified chemiluminescent immunoassays (CLEIA). CLEIA brought many advantages, such as reduced analysis time, liquid reagents, stable calibration curve and higher sensitivity over conventional methods [2]. Although none of these methods is a true “gold standard”, fluorescent enzyme immunoassay has been generally accepted and widely used due to its good comparability with skin prick test results [3]. Literature findings about the comparability and interchangeability of FEIA and CLEIA are still contradictory [4,5]. Considering methodological differences, verification of the comparability of two or more assays is extremely important for proper interpretation of allergy test results. In our laboratory, analysis of inhalant and food allergens i.e. determination of tIgE and sIgE concentrations, was performed by FEIA on ImmunoCAP 100 analyzer until September 2018. Since then, new method (CLEIA) on Immulite® 2000XPI has been implemented. Therefore, the aim of our study was to i) verify measurand stability, precision and reference intervals for children and adult population, for tIgE and some most frequent sIgE, ii) assess comparability of CLEIA sIgEs measurements on two Immulite® 2000XPI analyzers and iii) assess comparability of FEIA and CLEIA assays for tIgE.

The following sIgE were included (aims i and ii): house dust mite (d1), cat dander (e1), egg white (f1), dactylis glomerata (g3), betula verrucosa (t3) and ambrosia elatior (w1).

2. Materials and methods

Study was conducted from May to July of 2018. For verification of stability, precision and reference intervals, concentrations of tIgE and sIgE were measured using CLEIA assays, on Immulite® 2000XPI automated analyzer with system reagents, calibrators and controls. Total IgE assay (IMMULITE® 2000 Total IgE) was calibrated according to manufacturer's instructions with high and low total immunoglobulin E (TIE) Adjustors. System accuracy and precision were monitored daily using commercial control material (Total IgE Control Module). Specific IgE assays (IMMULITE® 2000 3gAllergy™ Specific IgE Universal Kit) were calibrated with high and low specific immunoglobulin E (SPE) Adjustors and checked with 3gAllergy™ Specific IgE Universal Kit Controls. The same reagent kits were used for stability and precision evaluations, there were no changes nor calibrations during this period. Linearity ranges for tIgE and sIgE assays are 1.00 up to 2000 kIU/L and 0.1 up to 100 kIU/L, respectively [6,9]. Manufacturer also declared limits of detection for both assays (1.0 kIU/L for tIgE; 0.1 kIU/L for sIgE), but limit of quantification was declared only for sIgE (0.2 kIU/L).

2.1. Total IgE stability verification

According to the manufacturer, serum sample for determination of tIgE can be stored at 2–8 °C for 3 days, respectively [6]. However, we have assessed the stability of total IgE in serum, at 2–8 °C during 7 days. Stability of tIgE in serum was examined on serum pools which were generated from leftover patient samples collected from 6 mL serum clot activator tubes (Vacutest Kima s.r.l., Piove di Sacco, Italy). According to tIgE concentration, four different pools were collected: (a) concentration within reference interval (N1), (b) concentration near the upper limit of reference interval (N2), (c) high concentration (P), and (d) very high concentration (PP). Hemolyzed, lipemic or icteric samples were excluded from this study. Pools N1 and N2 were collected from patients in which the likelihood for increased tIgE concentrations was low (i.e. Clinic for Eye Disorders, Department of Urology, Clinic for Neurology, Department of Endocrinology, Department of Hematology patients, and from hospital outpatients). Samples for pathological pools (P and PP) were collected randomly from samples referred for allergy testing to the Immunology laboratory of the Department of Medical Laboratory Diagnostics, at the University Hospital “Sveti Duh”.

Pools were tested in triplicate for 7 days. According to CLSI, median

of triplicate measurements was used for statistical analysis [7]. Upon initial testing, pools were stored at 2–8 °C in the refrigerator (Smeg S.p.A, Guastalla, Italy) with integrated automatic temperature control. Absolute (kIU/L) and relative (%) biases were calculated according to the formula:

Absolute bias

$$= \text{median at specified time point} - \text{median at initial value.}$$

$$\text{Relative bias} = (\text{absolute bias} / \text{median at initial value}) \times 100$$

For tIgE stability assessment, acceptance criteria provided by National EQA provider (CROQALM, Croatian Centre for Quality Assessment in Laboratory Medicine) were 15% (if the initial concentration of tIgE was higher than 40 kIU/L) and ± 6 kIU/L (if the initial concentration of tIgE was lower than 40 kIU/L). Moreover, calculated biases were also compared to criteria declared by manufacturer (Siemens) as follows: 5.1%, 6.7% and 6.4% for tIgE concentrations 50, 107 and 600 kIU/L [6].

2.2. Verification of precision

Verification of precision for tIgE and frequent sIgEs (d1, e1, g3, w1) was performed according to CLSI E15-A3 guidelines [8]. Precision evaluation included repeatability (within-run precision) and total imprecision (day-to-day precision).

For tIgE, precision was verified on two serum pools, one with concentration near the upper limit of reference interval declared by manufacturer (tIgE N, 87 kIU/L) and the other one with high concentration (tIgE P). Pools were collected from leftover patient samples using the protocol described above (under verification of stability). For sIgEs, two different serum pools were used. In each pool, two different sIgEs were tested, as follows: d1 and w1 in the first pool and g3 and e1 in the second pool. Pools were made from leftover samples from hospital patients in whom allergic testing was requested and stored in the refrigerator with integrated constant temperature monitoring at 2–8 °C.

Precision was assessed on each pool in five replicate measurements in one run and repeated for five consecutive days (five runs). Within-run precision was expressed as coefficient of variation (CV) for each day. Day-to-day precision was expressed as the overall CV of all five days. Obtained coefficients of variations for within-run and day-to-day precision were compared to CVs declared by the manufacturer as shown in Table 1 [6,9].

2.3. Verification of reference intervals

Reference intervals for tIgE, provided by the manufacturer, were verified for population served by our laboratory. Although manufacturer declared four different reference intervals for children < 1, 1 to 2, 2 to 3 and 3 to 9 years of age, we decided to combine those together and verify only one reference interval for all children (< 9 years of age). Reference intervals (suggested by the manufacturer) for tIgE are as follows: < 87 kIU/L for adults and < 52 kIU/L for children (< 9

Table 1
Manufacturer and EQA criteria for within-run and day-to-day precision.

Analyte	Concentration	Siemens	
		Within-run precision (CV, %)	Day-to-day precision (CV, %)
tIgE	107.0 kIU/L	4.5	6.7
	223.0 kIU/L	4.4	6.7
sIgE d1	0.5 kIU/L	10.0	12.0
sIgE g3	1.5 kIU/L	5.3	6.0
sIgE w1	9.5 kIU/L	5.6	6.8
sIgE e1	1.1 kIU/L	7.3	7.3

CV – coefficient of variation.

years of age) [6]. Verification of reference intervals was done on 20 reference adult individuals and 20 children (< 9 years of age). The samples from adult participants in this verification were recruited from patients' samples submitted for regular healthcare check-up in University hospital, Sveti Duh". For children reference intervals, leftover samples were used from children at the University hospital, Sveti Duh" and Children's Hospital Zagreb who had their laboratory testing done as a part of the periodic healthcare checkup or for preoperative diagnostics. All participants did not have a personal history of allergy in their medical record. Verification of reference intervals was performed according to CLSI EP28-A3c guideline [10].

2.4. Method trueness (comparability of CLEIA measurements on two Immulite analyzers)

Comparison of specific IgEs on two Immulite® 2000XPi analyzers (CLEIA) was performed according to CLSI EP9-A3 guidelines [11]. Method comparison for the most common sIgEs (d1, f1, g3, t3, w1) was performed between two Immulite analyzers, one in University hospital, Sveti Duh" and the other one in Children's Hospital Srebrnjak. Leftover inpatient and outpatient samples (N = 200; 40 for each sIgE) collected in Children's Hospital Srebrnjak, had been stored to ensure wide range sIgE concentrations and classes. Samples were stored at -20 °C for two weeks and then transported to University hospital "Sveti Duh" for analysis. Analysis was done in different batches and different days. According to CROQALM, acceptance criterion for bias was 15%.

2.5. Method trueness (comparability of FEIA and CLEIA assays)

Comparison of FEIA (ImmunoCAP 100) and CLEIA (Immulite® 2000XPi) methods for total IgEs was performed according to CLSI EP9-A3 guidelines [11]. Leftover samples (N = 46) from inpatients referred for tIgE testing to the Immunology laboratory of the Department of Medical Laboratory Diagnostics, at the University Hospital "Sveti Duh" have been used. Samples were centrifuged, aliquoted and stored in a freezer at -20 °C until analysis (up to 1 week). All samples were analysed for tIgE in one batch by both methods and within 4 h of thawing. Acceptance criterion for bias was 15% according to CROQALM.

2.6. Ethical approvals

This study has been granted ethical approval by the institutional ethical boards of the University hospital "Sveti Duh" (for the entire study protocol), Children's Hospital Srebrnjak (for method comparison of sIgE) and Children's Hospital Zagreb (for verification of reference intervals for children population).

2.7. Statistical analysis

For stability assessment, bias from initial concentration was calculated and expressed as absolute (kIU/L) or relative (%) bias for each day. Bland Altman analysis and Passing Bablok regression were used for method comparison. Additionally, level of agreement between sIgE classes was determined with kappa statistics. Acceptable kappa was 0.80.

Stability and precision data were calculated in Microsoft Excel 2007 (Microsoft, Redmond, Washington, USA). Data from method comparison was carried out using the MedCalc 12.5.0.0 (Ostend, Belgium) statistical software.

3. Results

3.1. Total IgE stability verification

Absolute (kIU/L) and relative (%) biases from tIgE initial concentrations in four tIgE pools (N1, N2, P and PP) are shown in Table 2.

Mean relative biases for each tIgE pool were acceptable according to Siemens and CROQALM criteria, except for the pool with the lowest concentration of tIgE (N1, 27.6 kIU/L) which exceeded manufacturer criteria (5.1%) with mean relative bias of -7.7%.

3.2. Verification of precision

Within-run and day-to-day precision results for two concentrations of tIgE and one concentration of each sIgE included in our study are presented in Table 3. Most of coefficients of variation (CVs) for tIgE satisfied manufacturer's (Siemens) criteria. The only exception in tIgE was N pool (concentration 92.5 kIU/L) where CV for day-to-day precision (7.8%) exceeded criteria (6.7%) declared by Siemens. Moreover, day-to-day precision was acceptable for all four allergens, according to Siemens. Within-run-precision was satisfactory for all, apart from tIgE (N pool) and w1 sIgE of which CVs (5.6% and 5.9%) were slightly higher than declared (4.5% and 5.6%) by Siemens, respectively.

3.3. Verification of reference intervals

For each group of participants (N = 20), adults and children < 9 years of age, tIgE results were within reference intervals declared by the manufacturer. For included participants, medians of age and 95% confidence intervals (CIs) were 47 (39–56) and 3 (1–7) years. Corresponding medians of tIgE and their 95% CIs were 17.00 (8.22–28.08) kIU/L for adults and 11.80 (3.90–20.28) kIU/L for children, respectively. Therefore, reference intervals declared by manufacturer can safely be adopted for both adult and children population in our laboratory.

3.4. Method trueness (comparability of CLEIA measurements on two Immulite analyzers)

Method comparison for five most common sIgEs (d1, f1, g3, t3, w1), which was performed between two Immulite analyzers, is presented in Fig. 1. Passing Bablok regression showed statistically significant constant and proportional bias for t3 sIgE. Moreover, statistically significant proportional biases were observed for f1 and d1 allergens, while constant biases were absent. However, observed deviations were within CROQALM criteria and were thus considered as minor. For other included sIgEs (g3 and w1) no constant and proportional biases were found. Moreover, there were no significant deviations from linearity ($P > 0.10$). Levels of agreement among classes, expressed as kappa values, were almost perfect for each sIgE (0.94 for d1, 0.92 for f1, 0.93 for g3, 0.96 for t3 and 0.95 for w1), respectively.

3.5. Method trueness (comparability of FEIA and CLEIA assays)

The comparison of two common methods for tIgE determination, FEIA (ImmunoCAP 100) and CLEIA (Immulite® 2000XPi), is presented in Figs. 2 and 3. Bland-Altman statistics revealed statistically significant constant (-32.4 kIU/L) and proportional bias (-20.2%) between these two methods. Moreover, this deviation did not meet CROQALM acceptance criteria (15%). Passing Bablok regression plot (described by the equation $y = -3.79 (-5.42 \text{ to } -0.80) + 0.94 (0.90\text{--}0.98)x$) also showed statistically significant constant and proportional biases. Furthermore, no significant deviation from linearity was found ($P > 0.100$).

4. Discussion

In this study we have verified analytical performance of Immulite® 2000XPi. Our findings show that serum samples for tIgE analysis can be stored at 2–8 °C up to 7 days. Although the precision was acceptable for Immulite analyzer, method comparison between FEIA and CLEIA methods for tIgE revealed statistically significant constant and

Table 2

Absolute (kIU/L) and relative (%) deviations (biases) from the initial value of tIgE in pools (N1, N2, P, PP) stored at 2–8 °C and measured during 7 days.

Pool	tIgE (N1)		tIgE (N2)		tIgE (P)		tIgE (PP)	
Initial concentration of tIgE (kIU/L)	27.6		80.2		128.0		959.0	
	Bias (kIU/L)	Bias %	Bias (kIU/L)	Bias %	Bias (kIU/L)	Bias %	Bias (kIU/L)	Bias %
1 day	−1.7	−6.2	−4.1	−5.1	−4.0	−3.1	105.0	10.9
2 day	−2.3	−8.3	−6.9	−8.6	−5.0	−3.9	107.0	11.2
3 day	−2.6	−9.4	−3.8	−4.7	−4.0	−3.1	−26.0	−2.7
4 day	−1.3	−4.7	−1.4	−1.7	−7.0	−5.5	94.0	9.8
5 day	−2.6	−9.4	−6.5	−8.1	−13.0	−10.2	1.0	0.1
6 day	−2.2	−8.0	−5.2	−6.5	0.0	0.0	−18.0	−1.9
7 day	−2.2	−8.0	−5.5	−6.9	2.0	1.6	−2.0	−0.2
Mean	−2.1	−7.7*	−4.8	−5.9	−4.4	−3.5	37.3	3.9

* Result that did not meet Siemens acceptance criteria.

Table 3

Within-run and day-to-day precision results (CV, %) for two concentrations of tIgE and one concentration of each sIgE (d1, g3, e1, w1).

	tIgE (kIU/L) and sIgE (kU/L) concentration	Within-run precision (CV, %)	Day-to-day precision (CV, %)
tIgE N	92.47	5.57*	7.89*
tIgE P	339.40	4.16	6.59
sIgE d1	0.47	2.71	2.90
sIgE g3	1.41	3.44	3.99
sIgE e1	1.24	2.40	2.40
sIgE w1	9.34	5.85*	5.85

– CV – coefficient of variation.

*Result that did not meet Siemens acceptance criteria.

proportional biases. Moreover, slight statistically significant biases between two Immulite analyzers were observed for t3 (constant and proportional), d1 and f1 sIgEs (proportional). Reference intervals declared by Siemens are acceptable for population served by our

laboratory.

Sample stability verification is important for every analyte which could be analysed after prolonged transportation or storage. Some previous studies stated that stabilities of total and specific IgEs in serum are no longer than 3 and 7 days stored at 2–8 °C, respectively [12]. Manufacturer specifications in term of sample stability for tIgE and sIgE analysis were also based on these studies [6,9]. Stability of tIgE in our study was longer than declared by the manufacturer, as minimal biases were observed during 7 days of storage (2–8 °C). Even though these biases were within acceptance criteria, we have observed some unusual and unexpected variations during the stability study. During the study period, system performance (accuracy and precision) was closely monitored on a daily basis, by using commercial control material. Moreover, all analysis were done in triplicate. We therefore consider our results trustworthy but cannot provide any logical explanation for these variations other than some random effects.

Development of third-generation immunoassay brought extended measurement range for sIgE (down to 0.1 kU/L) and increased precision

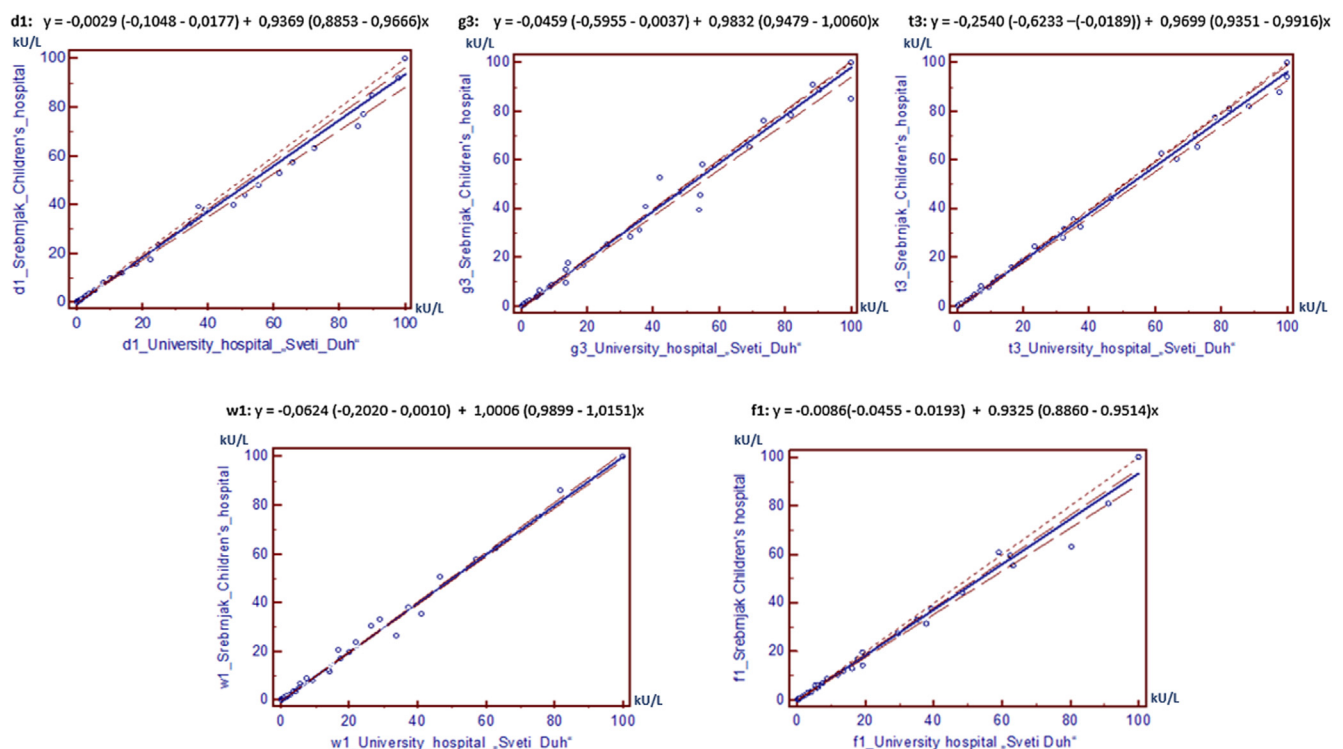


Fig. 1. Passing Bablok regression plots and following equations for five most common sIgEs showing the comparison of two Immulite® 2000XPi analyzers (one in University hospital, Sveti Duh” and the other one in Children’s Hospital Srebrnjak). – Solid line represents regression line, dotted lines represent 95% confidence interval.

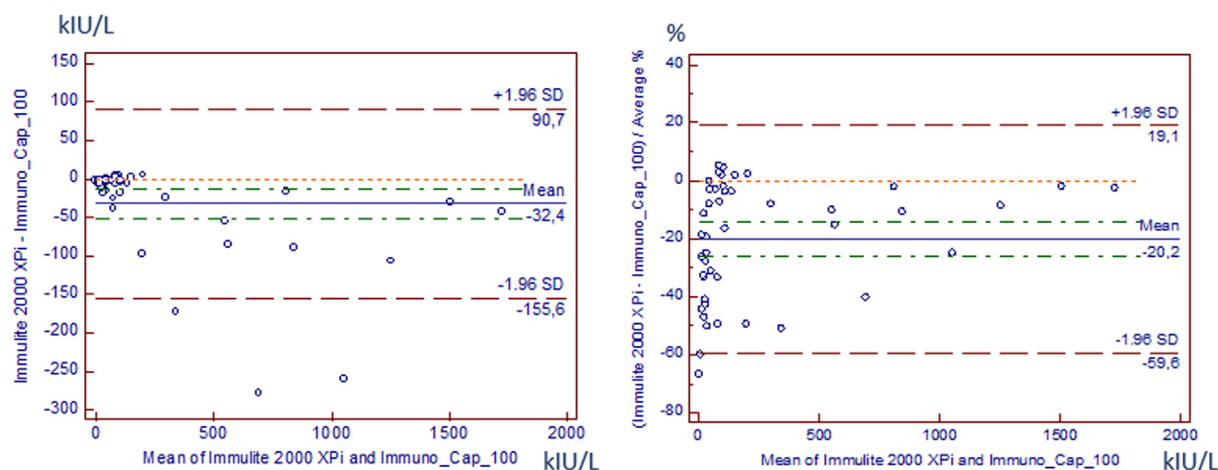


Fig. 2. Bland Altman plots showing the differences in tIgE measured by FEIA (ImmunoCAP 100, Thermo Fisher Scientific Inc., Waltham, MA, USA) and CLEIA (Immulite® 2000XPI, Siemens Healthcare Diagnostics, Erlangen, Germany). – Solid line (mean) represents mean difference, dotted lines represent 95% confidence interval of mean difference and dashed lines ± 1.96 standard deviation of mean difference.

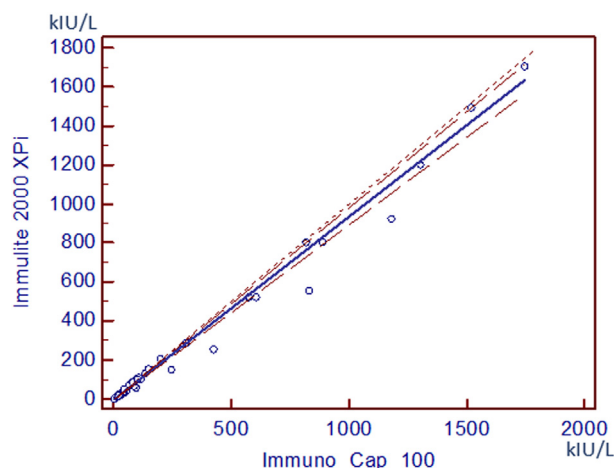


Fig. 3. Passing Bablok regression plot showing the comparison of ImmunoCAP (Thermo Fisher Scientific Inc., Waltham, MA, USA) and Immulite® 2000XPI (Siemens Healthcare Diagnostics, Erlangen, Germany) for tIgE measurements. – Solid line represents regression line, dotted lines represent 95% confidence interval.

and analytical sensitivity [13]. Li TM et al. have studied analytical performance of Immulite® 2000XPI and they reported good precision results – total imprecision CVs were $\leq 15\%$ near the cut-off concentration of 0.35 kU/L [2,14]. In our study, precision was even better as all CVs were below 10% ($\leq 3\%$ near the cut-off concentration). This may be considered as an important improvement of previous immunoassay generations as it has been reported that precision of FEIA near the cut-off concentration (0.35 kU/L) was poor [14]. Obviously, this advantage provides useful additional information for risk establishment and possible prevention of allergy diseases in later life.

Consistent with the study conducted by Li TM et al., intra-assay comparison of two Immulite® 2000XPI analyzers for five common sIgEs showed very good agreement in our study [14]. Depending on specific allergen, for only a few sIgEs statistically significant constant and proportional biases were observed. Moreover, high kappa values (almost perfect) among classes for those sIgEs confirmed that this lack of agreement was negligible and clinically irrelevant. Previous studies evaluating sIgE inter-assay comparison observed inconsistent results on whether CLEIA (Immulite) and FEIA (ImmunoCAP) should be used interchangeably. Bulat Lokas et al. recently reported fair to perfect agreement for some allergens measured by these two methods. They

have also observed strong correlations between two methods for tIgE and majority of studied sIgEs [5]. This was confirmed by some other recent studies [15–17]. Although these studies included a large number of samples, they were mostly carried out on an Asian population (Korea, Japan). In contrast to earlier findings, Graham et al. compared egg white (f1) results measured on Immulite® 2000XPI and ImmunoCAP [18]. Although they also reported high correlation between two systems, f1 allergen results were 3-fold higher when measured on Immulite® 2000XPI compared to results analysed on ImmunoCAP. Poor agreement between these methods was again emphasized in study of Hamilton et al. [19]. Inconsistency among these studies were mostly due to tested allergens. Discordance between paired sIgEs, measured by two methods, may be due by different constitution and quality of allergenic extracts and different antibody used in method. Therefore, for some allergens, reagents were more similar than for others. Obviously, some effort for standardization, e.g. the same allergenic components in reagents, is highly desired. Moreover, Szecsi et al. studied the differences between allergen extracts and recombinant allergens by comparing them on Immulite and ImmunoCAP [4]. They have found substantially higher results of allergenic extracts assayed on Immulite, however, results of recombinant allergens were twice as high on Immulite as on ImmunoCAP. Considering that most of previous studies were somehow limited by small number of samples or tested allergens, further comparative studies involving bigger patient groups are highly warranted. Lack of standardization and potential heterogeneity of the extracts, along with technical differences (e.g. allergen binding, signal detection and time analysis) between these two methods cause major discrepancies in the results. Such differences may cause patient misclassification leading to inappropriate clinical decisions and therapy prescriptions. Although many literature data exist about sIgE comparisons, only few studies reported comparison of tIgE between two different methods – FEIA and CLEIA [5,13]. Consistent with the literature, statistically significant constant and proportional biases were observed in our study. The results of total IgE obtained on Immulite were about 30 kIU/L higher than results obtained on ImmunoCap. It has also been noted that difference between the two methods is greater with higher concentrations of tIgE.

It has been known that immune system of elderly is weakened, however previous studies reported that concentrations of tIgE vary between different age groups [20]. De Amici et al. stated that concentration of tIgE generally increases, while concentrations of sIgEs significantly decrease over the age, following different trends [21]. These observations are in line with upper limits of reference intervals provided by Siemens, which are higher for adult population (87 kIU/L)

than for children (52 kIU/L). Even though previously mentioned studies were based on populations of Italy and USA, we have also successfully verified declared reference intervals.

Our study has some limitations. Unfortunately, we investigated stability for total IgE only, because we were not able to collect samples and prepare pools with higher concentrations of specific allergens. Method comparison (FEIA and CLEIA) of tIgE was performed in one batch, thus it was logistically challenging to provide equally distributed concentrations and more samples in high measurement range. Furthermore, due to some organizational issues we were not able to collect data on provocation and skin prick tests for the patients involved in this study.

As for strengths of this study, it should be noted that precision evaluation was conducted in accordance with the most recent CLSI protocol E15-A3, whilst most of the previous studies were carried out following an outdated guideline version (20 consecutive days measurements), which does not allow the estimation of the within-run precision. Furthermore, such a comprehensive report on tIgE stability, precision and trueness has not yet been published in the literature.

In conclusion, we report very good analytical performance of Immulite® 2000XPI analyzer implemented in our laboratory. We were able to confirm manufacturer's declarations for stability of serum tIgE (7 days at 2–8 °C), method precision and reference intervals for adults and children. Moreover, close agreement between two Immulite analyzers was observed for most frequent sIgEs. As expected, our study has provided evidence of poor comparability of tIgE results measured by FEIA and CLEIA. Thus, longitudinal monitoring of tIgE and sIgE in allergic patients, should always be done using the same assay. When replacing one of these methods with another, clinicians have to be properly informed about it and how will this affect interpretation of test results and patient management.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cca.2019.09.015>.

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