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Performance validation of a third-generation allergen-specific IgE assay in the clinical laboratory: Interlaboratory and intermethod comparison

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Abstract

Background: Allergen-specific IgE (sIgE) measurements are used to help identify causative allergenic agents and to determine the degree of sensitization to facilitate treatment decisions. We examined the performance of a new third-generation chemiluminescent enzyme immunoassay for allergen-specific IgE (sIgE) on the continuous random access Immulite® 2000 system.

Methods: Detection limit and dilutional linearity were determined. Within-run and total precision were assessed according to the NCCLS EP5 guideline. Interlaboratory comparison of the new Immulite 2000 third-generation allergen-specific IgE assay was performed, as well as intermethod comparison against the Pharmacia FEIA, a second-generation assay.

Results: The detection limit was <0.1 kU/l. Dilutional linearity held from 100 down to 0.2 kU/l. Regression analysis of the interlaboratory comparison results yielded: Immulite 2000(Laboratory 1)=1.07 Immulite 2000(Laboratory 2)+0.18 kU/l; $r=0.98$, $n=3588$ results. Intermethod comparison showed the following: Immulite 2000=0.83 (Pharmacia FEIA)+0.42 kU/l; $r=0.79$, $n=512$ results. Bland–Altman analysis of the interlaboratory and intermethod comparisons indicated no systematic bias.

Conclusions: We confirmed the reported performance characteristics of the third-generation sIgE assay and found reasonably good interlaboratory and intermethod agreement. The extended range capability of the third-generation assay provides a new tool for investigating cutoffs and trends in childhood allergy disease progression at concentrations <0.35 kU/l.

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Keywords: Immunoglobulin-E; Radioallergosorbent test; Evaluation studies; Reproducibility of results

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1. Introduction

Clinical sIgE measurements are performed using the traditional first-generation sIgE assay or modified radioallergosorbent test (mRAST) or else by the second-generation fluorescent enzyme immunoassays (FEIA) [1–4]. The traditional first-generation sIgE assay is limited by cyanogen bromide (CNBR) immobilization that gives high and variable nonspecific binding with different allergens [1,2].

Traditional first-generation mRAST allergy blood testing utilizes a single 25 kU/l IgE calibrator with 2 overnight incubations. Allergens are immobilized onto paper discs. Samples are classified as positive or negative, based on a cutoff of 500 counts/min. Results are reported in mRAST class scores that can be used as grounds for instituting immunotherapy.

Second-generation allergen-specific IgE assays are quantitative enzyme immunoassays on a batch analyzer. Measuring range is from 0.35 to 100 kU/l. The 2 incubations require 3 h in total. The Pharmacia FEIA [3,4] is an example of a second-generation sIgE test. It uses cellulose sponge as the solid support and gives quantitative results, but continues to use CNBR immobilization. The detection limit is 0.35 kU/l, by default.

With the recent recognition that early sensitization to allergens can lead to allergic diseases in later life, early detection of very low and rising sIgE concentrations can provide useful information on the likelihood of getting asthma [5–9]. Early detection also facilitates treatment options. Sasai et al. [5] demonstrated that early detection of sIgE predicts the risk of allergic disease at later life. House dust mite-specific IgE values between 0.23 and 0.35 kU/l at 6 months of age proved to be one of the significant predictors of a positive reaction to house dust mite sIgE at 5 y. Likewise, Boyano Martinez et al. [6] determined that food challenge is not necessary for children <2 y with sIgE concentrations for egg white of over 0.43 kU/l. For egg yolk, ovalbumin and ovomucoid, the optimal decision point was 0.35 kU/l, which is the default detection limit of the second-generation FEIA. Due to the technological limitation of the 0.35 kU/l detection limit in FEIA, it is difficult to determine whether there is a better decision point <0.35 kU/l with this methodology. Likewise, probability curves derived from logistic regression analysis of FEIA results start at 0.35 kU/l according to the analysis by Soder-

strom et al. [7] and Malandain [8]. Sampson [9] reported that “low concentrations of food-specific IgE antibodies are associated with a certain risk of clinical reactivity”. Boyano Martinez et al. [6] also emphasized that it was not possible to establish a better decision point than 0.35 kU/l for egg yolk, ovalbumin and ovomucoid, because specific IgE concentrations cannot be quantified below this point with the CAP system FEIA technique.

An sIgE assay able to give precise results at lower concentrations and with a detection limit as low as 0.1 kU/l would provide investigators with a better tool for exploring decision points and looking for trends <0.35 kU/l. Such a third-generation sIgE assay (with a measuring range extending to 0.1 kU/l and a functional sensitivity of 0.2 kU/l) has recently been developed [10,11]. We report the validation, performed in a clinical laboratory setting, of this new quantitative third-generation chemiluminescent enzyme immunoassay for sIgE on the continuous random access Immulite 2000 system.

2. Methods and materials

This study was conducted within the Department of Pathology and Laboratory Medicine at the Harbor UCLA Medical Center, Torrance, CA 90509.

2.1. Patient specimens

Sixty-nine serum samples from atopic individuals with increased total IgE concentrations were obtained from commercial sources with informed consent. All serum samples were submitted to the laboratory as blind clinical samples.

2.2. Allergens

Tests for sIgE antibodies were requested on each submitted sample for the following 52 allergens commonly assessed during diagnosis of allergic problems: *Dermatophagoides pteronyssinus* (D1), *Dermatophagoides farinae* (D2), cat dander epithelium (E1), dog epithelium (E2), pork (F26), orange (F33), potato (F35), wheat (F4), strawberry (F44), apple (F49), oat (F7), corn (F8), rice (F9), Johnson grass (G10), Bahia grass (G17), Bermuda grass (G2), orchard grass (G3),

meadow fescue (G4), rye grass (G5), June grass (Kentucky Blue) (G8), house dust-Greer (H1), house dust-Hollister-Stier (H2), cockroach (I6), latex (K82), *Stemphylium botryosum* (M10), *Phoma betae* (M13), *Candida albicans* (M5), *Alternaria tenuis* (M6), *Fusarium moniliforme* (M9), maple (T1), walnut (T10), cottonwood (T14), white ash (T15), eucalyptus (T18), mesquite (T20), pecan (T22), beech (T5), mountain cedar (T6), oak (T7), white mulberry (T70), common ragweed (W1), lambs quarters (W10), Russian thistle (W11), golden rod (W12), cocklebur (W13), rough marsh elder (W16), firebush (W17), western ragweed (W2), giant ragweed (W3), false ragweed (W4), mugwort (W6), and English plantain (W9).

2.3. Assays

2.3.1. Enzyme-enhanced chemiluminescent assay

The sIgE assay on the Immulite 2000 system is a solid phase (bead), 2-site sequential chemiluminescent (1,2-dioxetane phosphate) enzyme (alkaline phosphatase) immunoassay. It has a very low detection limit due to efficient washing and high specific signal. The reaction tube is spun rapidly on its vertical axis, expelling the liquid phase into a waste sump. The signal is generated by hydrolysis of dioxetane-phosphate ester. The destabilized dioxetane decomposes, yielding chemiluminescence. Reportable results for the Immulite 2000 third-generation sIgE assay cover the range of 0.1 to 100 kU/l.

2.3.2. Fluorescence enzyme immunoassay (FEIA)

The Pharmacia UniCAP® 100 system FEIA (Pharmacia Diagnostics, Uppsala, Sweden) used in the intermethod comparison, employs solid phase allergens covalently bound to a cellulose sponge encased in a capsule. sIgE is detected with the use of anti-IgE antibody labeled with beta-galactosidase, generating fluorescence. Reportable results for the Pharmacia UniCAP 100 system sIgE assay cover the range of 0.35 to 100 kU/l.

2.4. Detection limit

Twenty determinations were performed in a single run on an sIgE-free (0 kU/l concentration) calibrator provided by the manufacturer. The mean and standard deviation (SD) for the counts of the 0 calibrator

replicates were calculated. The counts 2 SD above the mean were then translated into concentration via the calibration curve information provided by the manufacturer, yielding an estimate of the assay's detection limit in kilounits per liter (kU/l).

2.5. Precision

In accordance with the NCCLS EP5 guideline, within-run and "total" coefficients of variation (CVs) were determined by analysis of variance (ANOVA) using multiple concentrations, ranging from 0.25 to 75 kU/l, of sIgE sera with multiple allergen specificities (D1, E2, F4, G2, H2, I6, K82, M1, T8 and W14). These were analyzed in duplicate, twice per day, in runs separated by a minimum of 2 h. The NCCLS study yields total CVs that provide a measure of reproducibility across time (20 days) within a single laboratory.

2.6. Dilutional linearity

Multiple dilutions of samples, targeted for the range from 0.2 to 100 kU/l, were prepared by serial 2-fold dilution, mixing serum samples and the 100 kU/l calibrator with a negative control serum which came from a nonatopic donor. The calibrator was provided by the manufacturer. The observed results (y) and dilution factor values (x) were plotted in log–log coordinates for visual inspection and analyzed using regression analysis.

2.7. Interlaboratory comparison

The 69 serum samples were each divided into 1.5 to 2 ml aliquots, given an identification number, then stored at -20°C . The sera were then simultaneously analyzed blindly on the Immulite 2000 systems in a neighboring and the UCLA laboratories.

The manufacturer's package insert assay procedure was followed to obtain Immulite 2000 quantitative and extended scoring system [11] results for the samples, which were analyzed blindly in the two laboratories. Individual laboratory reports for each of the serum samples were collected and statistical analysis was performed by linear regression. Bland–Altman analysis [12,13] was also used to compare the interlaboratory results for concentrations covering the range of approximately 0.1 to 100 kU/l.

2.8. Intermethod comparison

Those patient samples with Immulite 2000 sIgE results ranging from 0.35 to 100 kU/l were also analyzed by the Pharmacia UniCAP assay. The manufacturer's package insert assay procedure was followed to obtain quantitative UniCAP results. Linear regression was used to analyze the paired results obtained with the Immulite 2000 and UniCAP systems for those samples for which the UniCAP result was also in the 0.35 to 100 kU/l range. Bland–Altman analysis [12,13] was also used.

3. Results

3.1. Detection limit

Fig. 1 indicates that the detection limit estimated for the Immulite 2000 sIgE assay was 0.035 kU/l in this study. This is well below the manufacturer's claim of 0.1 kU/l. Both are substantially superior to the detection limit of 0.35 kU/l for second-generation methods [3,4].

3.2. Reproducibility

Total CVs for the Immulite 2000 sIgE assay obtained from the NCCLS EP5 studies conducted in

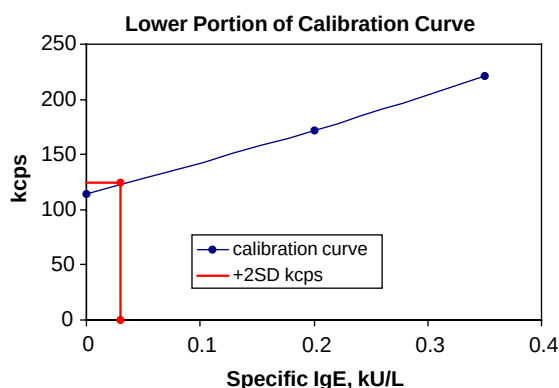


Fig. 1. Low end of a representative calibration (“dose–response”) curve for the Immulite 2000 sIgE assay, illustrating the results of a detection limit experiment, with the counts 2 SD above the average for 20 replicates of the assay's 0 calibrator (vertical axis) translated through the calibration curve to the corresponding concentration value (horizontal axis).

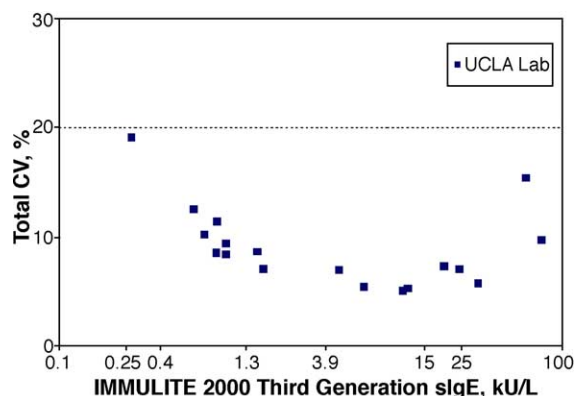


Fig. 2. Total (interassay) CVs from the NCCLS EP5 precision studies of the Immulite 2000 sIgE assay conducted in the UCLA laboratory. The allergens included D1, E2, F4, G2, H2, I6, K82, M1, T8 and W14.

the UCLA laboratory are displayed in Fig. 2. Total CVs for the different allergen sera pools were concentration dependent and similar to results previously reported [11]. Total CVs from the two studies are comparable—the 2 studies showed essentially the same patterns of concentration-dependent total CVs across the Immulite 2000's working range of 0.1 to 100 kU/l.

Note that close to the 0.35 kU/l cutoff, most second-generation assays exhibit high imprecision [14,15]. As illustrated in Fig. 2, the availability of a third-generation allergy blood test such as the Immulite 2000 sIgE assay allows physicians to obtain explicit, reproducible results near and below the standard 0.35 kU/l cutoff concentration.

3.3. Dilutional linearity

Fig. 3 shows a dilutional linearity plot for the Immulite 2000 sIgE method for concentrations ranging from 0.2 to 100 kU/l. Visual inspection and regression analysis demonstrate that the Immulite 2000 sIgE method is linear over the range examined.

3.4. Distribution of sIgE concentrations among the atopic samples

Table 1 gives the percentage distribution of the 3588 sIgE results for the 69 atopic patients and 52 allergens studied according to the extended classification system which was developed to approximate the mRAST classification [11]. Note that 49% of the results are

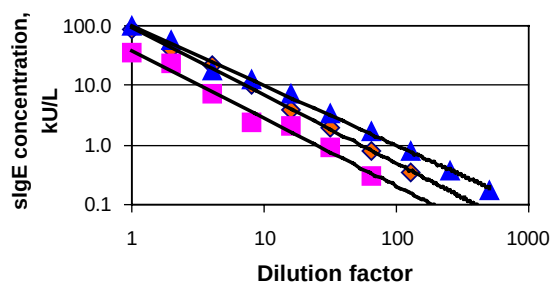


Fig. 3. Dilutional linearity for the Immulite 2000, a third-generation sIgE assay. From top to bottom: calibrator (100 kU/l) and samples positive for F1 and G2.

<0.1 kU/l and 9.8% of the results fall between 0.11 and 0.39 kU/l. Out of a total of 3588 determinations, the Immulite 2000 assay yielded 1479 results >0.35 kU/l, the traditional cutoff (which is also the lowest concentration measurable by second-generation sIgE assays), but 1827 results (over 23% more) above 0.1 kU/l (the Immulite 2000 assay's detection limit).

3.5. Interlaboratory comparison

Comparison of Immulite 2000 sIgE results between the 2 laboratories is displayed as a Bland–Altman plot in Fig. 4. The regression equation for the interlaboratory comparison was $\text{Immulite 2000(Laboratory 1)} = 1.07 \text{ Immulite 2000(Laboratory 2)} + 0.18 \text{ kU/l}$; $r=0.98$, $n=3588$ results. Since the slope and correlation coefficient are close to 1.0 and the intercept is not significantly different from 0, the Immulite 2000 systems used in the two laboratories demonstrated highly comparable sIgE results. The Bland–Altman plot in Fig. 4 also revealed close agreement

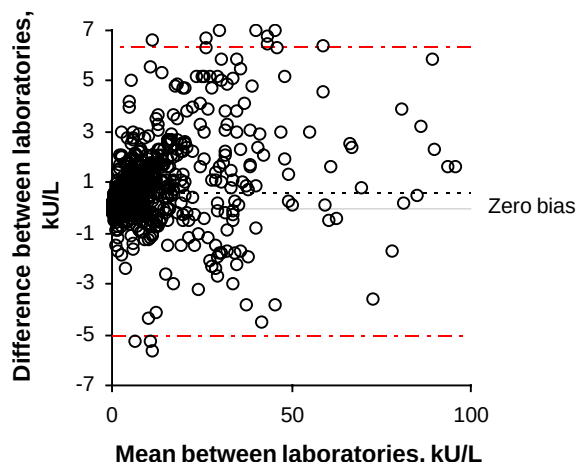


Fig. 4. Bland–Altman difference plot: interlaboratory comparison of the Immulite 2000 results obtained from 2 laboratories. The difference between laboratories, in kilounits per liter (kU/l), is plotted as a function of the mean between laboratories, also in kilounits per liter. The line of identity (difference=0) is shown. The upper and lower horizontals enclose the central 95% of the observed differences.

(within +7 and −5 kU/l over the range of 0 to 100 kU/l) between results obtained in the 2 laboratories.

3.6. Intermethod comparison

For the intermethod comparison, the regression equation was as follows: $\text{Immulite 2000} = 0.83 (\text{Pharmacia FEIA}) + 0.42 \text{ kU/l}$; $r=0.79$, $n=512$ results.

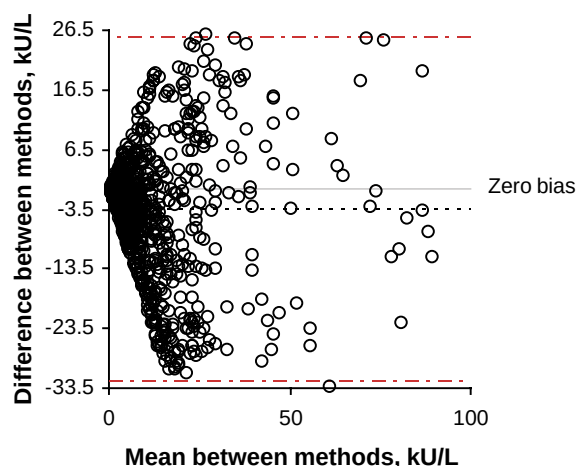


Fig. 5. Bland–Altman difference plot: intermethod comparison of the Immulite 2000 to the Pharmacia FEIA, a second-generation sIgE assay.

Table 1
Percentages of detectable sIgE results: 69 atopic samples and 52 allergens

Concentration, kU/l	Extended classification system	Percentages of results in each class
0 to 0.1	Class 0	49.1
0.11 to 0.24	Class 0/1	6.1
0.25 to 0.39	Class I	3.7
0.4 to 1.29	Class II	10.7
1.3 to 3.89	Class III	10.1
3.9 to 14.99	Class IV	8.4
15 to 24.99	Class V	2.3
≥25	Class VI	9.8

Since the 2 methods do not necessarily use the same extract materials, the regression statistics are not expected to be perfect [3]. The Bland–Altman plot in Fig. 5 revealed acceptably close intermethod agreement.

4. Discussion

This study confirmed the performance characteristics of a third-generation sIgE assay that was reported previously [11]. This third-generation sIgE assay is characterized by several features, including use of a true 0 calibrator during generation of the stored master curve, a detection limit of 0.1 kU/l and a functional sensitivity of 0.2 kU/l [10]. In addition, use of liquid allergens and enzyme-enhanced chemiluminescence enable the new method to reduce time-to-first-result to about 1 h, compared to the overnight or 3-h time-to-first-result characteristic of first- and second-generation assays, respectively.

The new Immulite 2000 third-generation sIgE method evaluated here in an independent study showed a detection limit of >0.1 kU/l, a functional sensitivity of 0.2 kU/l. Method comparison with the FEIA methodology was acceptable. The current study demonstrates interlaboratory reproducibility for the Immulite 2000 sIgE assay over the 0.1 to 100 kU/l working range.

The availability of the third-generation sIgE assay alleviates the loss of sIgE information <0.35 kU/l during investigations of the childhood allergy march or allergic disease progression [16]. Whereas the cutoff for total IgE is based on the upper reference limit for a normal infant population, a serum sIgE concentration >0.35 kU/l, or RAST class 1, for sIgE is traditionally used as evidence that sensitization has occurred [16]. Second-generation sIgE assays with a 0.35 kU/l detection limit do not allow investigators to ascertain whether there is a better cutoff to define sensitization. Information <0.35 kU/l can also give a better definition of probability curves. Probability curves based on logistic regression analysis of quantitative, second-generation sIgE results start at 0.35 kU/l [7]. Thus probability curve and optimal decision point studies reported to date [5–9] should be revisited now that a more precise and sensitive sIgE assay is available. Reference ranges for sIgE in normal

infant populations can now be determined with the new Immulite 2000 third-generation sIgE method and appropriate cutoffs can now be applied to indicate allergen sensitization [16]. As a start, studies should be done utilizing specific IgE assays to relevant allergen[s] in infants such as egg white and house dust mite.

The current study also indicates that the third-generation assay has good precision ($<15\%$ total CV) at the 0.35 kU/l boundary between class 0 and class 1 in the standard scoring system [11]. This is to be contrasted with first- and second-generation assays where reproducibility of the class boundary between negative and positive is poor, presumably because of poor precision close to the 0.35 kU/l concentration [13].

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