

# Accuracy of IgE antibody laboratory results

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**Background:** Studies have demonstrated that the magnitude of sensitization as evidenced by specific IgE (sIgE) levels provides significant information as to whether a sensitized individual is likely to be truly reactive. However, it is not clear that quantitative sIgE results provided by different laboratories using different technologies are comparable.

**Objective:** To investigate whether similar results were obtained from Clinical Laboratory Improvement Act–certified laboratories that used 3 common systems for sIgE antibody determination with serum samples and mouse-human IgE chimeric antibodies with known specificity and quantity.

**Methods:** Sixty samples for peanut and 20 for soy were submitted for sIgE determination on 3 different systems: ImmunoCAP, Immulite, and Turbo radioallergosorbent test (RAST). Mouse-human chimeric IgE antibodies specific for the major birch allergen Bet v 1 and for the dust mite allergen Der p 2 were also included.

**Results:** A qualitative evaluation using a cutoff of 0.35 kUA/L showed some differences in the ability to detect sIgE sensitization, with the Turbo RAST being most variable. However, considerable differences were found with quantitative evaluation, with Immulite overestimating and Turbo RAST underestimating sIgE compared with ImmunoCAP. Similar discrepancies were seen with the mouse-human chimeric IgE antibody samples.

**Conclusion:** These findings have potentially serious clinical implications, since each of these systems is widely used. It is therefore important that all laboratories clarify which system they are using. Just because 2 systems present their results in the same units does not mean that the results are necessarily correct or interchangeable.

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## INTRODUCTION

When patients seek medical help for suspected allergic disease, possible offending allergens and the degree of sensitization must be identified. Such evaluation is usually done with either skin testing or serologic tests for specific IgE (sIgE).<sup>1,2</sup> Most studies to date have investigated the relationship between allergic disease and sensitization from a dichotomous (positive or negative) perspective based on arbitrarily chosen analytical cutoff points.<sup>1–4</sup> However, recent studies, particularly in food allergy, have demonstrated that the magnitude of sensitization as evidenced by the quantitative level of food-specific antibodies provides significant guidance as to whether a sensitized individual is likely to be truly reactive to a specific food.<sup>5–14</sup> Similar studies on asthma and inhalant allergy have also demonstrated the relevance of quantitative sIgE levels in the context of clinical disease.<sup>15–19</sup>

During the past 3 decades a variety of laboratory methods have been developed for the measurement of sIgE antibodies. Unfortunately, some studies have noted large discrepancies among results obtained by different methods.<sup>20–24</sup> One of the

challenges in caring for patients is that physicians frequently are not able to choose their preferred method of testing because different insurance systems and health care professionals contract with different laboratories. Further confusion in this area has arisen because different manufacturers report their sIgE results in seemingly identical classes or units, which can camouflage possible differences in their performance. This problem has not been seriously addressed in detail or placed in evidence in a formalized manner, although undoubtedly this field would benefit from improved standardization.<sup>25,26</sup>

In this study we sought to investigate this issue by performing a blinded comparison of the results from representative laboratory systems for 2 important allergens: peanut and soy. In addition, we sought to provide insight into the reasons for discrepant laboratory values by analyzing the results from repetitive analysis of dilutions of mouse-human chimeric IgE antibodies directed against birch pollen (Bet v 1) and house dust mite (Der p 2), with defined antibody specificity and protein concentration. In such experiments, if measured correctly, the sIgE in all samples should be equivalent to the total IgE (tIgE).

## MATERIALS AND METHODS

### *Serum Samples*

Sixty routine serum samples previously tested for sIgE to peanut and 20 samples previously tested for sIgE to soy were chosen from random clinical samples sent for routine analysis in the serum bank of a commercial reference laboratory to cover the range of sIgE levels from lower than 0.1 kU<sub>A</sub>/L up to 100 kU<sub>A</sub>/L. All patient identifications were removed. The

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samples were thawed, aliquoted in sample tubes, and shipped according to the clinical routine for shipment of clinical samples for laboratory analysis.

#### Chimeric Antibodies

Mouse-human chimeric IgE antibodies specific for the major birch pollen allergen Bet v 1 and the house dust mite allergen Der p 2 were included in the study. The chimeric anti-Bet v 1 antibody was provided by Phadia AB (Uppsala, Sweden) and the chimeric anti-Der p 2 antibody originally produced by Dr Martin Chapman was purchased from Indoor Biotechnologies Inc (Charlottesville, Virginia). The scientific generation of these chimeric IgE monoclonal antibodies has been described in detail elsewhere.<sup>27–29</sup> In both cases the constant part of the immunoglobulin heavy chain of the original mouse monoclonal antibody has been exchanged with the constant part of the human IgE heavy chain by use of molecular biology engineering. The protein concentration of the anti-Bet v 1 construct was, according to Phadia AB, determined by amino acid analysis to be 608.25 µg/mL. The anti-Der p 2 IgE chimeric protein concentration of 10.5 µg/mL was given by the manufacturer. The translation factor among tIgE, sIgE, and protein concentration has previously been established as 1 kU IgE = 1 kU<sub>A</sub> = 2.42 µg using ImmunoCAP.<sup>30</sup> Both sets of chimeric antibodies were aliquoted in human plasma that contained less than 0.2 kU/L of tIgE. Concentrations of 96.8 µg/L, equal to 40 kU<sub>A</sub>/L, and thereafter 2-, 10-, and 50-fold dilutions were prepared. If measured correctly the obtained values would be similar for tIgE and sIgE in all these samples.

#### IgE Analysis

The serum samples with peanut and soy sIgE antibodies were coded and sent to 2 Clinical Laboratory Improvement Act-licensed national reference laboratories for determination of allergen sIgE by the following methods: (1) ImmunoCAP 1000 (Phadia AB), (2) Immulite 2000 Standard (Diagnostic Products Corporation, Los Angeles, California) both run in the same commercial laboratory, and (3) Turbo radioallergosorbent test (RAST) (Hycor Biomedical Inc, Garden Grove, California) run in another commercial laboratory. The samples were tested in triplicate. The samples that contained the chimeric antibodies were randomized, coded, and sent on 3 separate occasions to the same laboratories for blinded analysis of tIgE, birch, and mite sIgE. On the ImmunoCAP and Immulite systems, duplicate samples were also analyzed for further precision analysis. The laboratory that used the Turbo RAST for sIgE used ADVIA Centaur (Bayer Diagnostics, Tarrytown, New York) for tIgE determinations. The reagents for these tests were proven by the manufacturers to be IgE specific.

#### Statistical Analysis

The obtained results from Immulite, Turbo RAST, and ImmunoCAP were compared. Based on its wide use and standardization both from a technical and clinical perspective as verified in more than 3,000 peer-reviewed publications, Im-

munoCAP was used as the reference system. Weighted linear regressions, using weights proportional to the scales, were used for comparing the 3 systems for the peanut and soy samples. For the regression slope, 95% confidence intervals (CIs) were estimated. Bland-Altman logarithmic differences between Immulite and Turbo RAST vs ImmunoCAP were also calculated for the peanut and soy samples. Means and SDs were estimated from the logarithmic differences and compared with the expected means and SDs under the null hypothesis that no differences exist among the methods examined. The SD for the logarithmic differences would, in this case, be approximately 10% and yield control limits for the average logarithmic difference of approximately ±0.03 for the peanut samples and approximately ±0.05 for the soy samples.

For the chimeric samples, the average concentration for each dilution was calculated from the duplicate run performed 3 different times. For ImmunoCAP and Immulite, the within- and between-assay run coefficient of variance (CV) was estimated using restricted maximum likelihood. For Turbo RAST, the ordinary sample variance was estimated. All CVs are expressed as percentages.

## RESULTS

### *Dichotomous Comparison of Assay Performances With Peanut and Soy Antibodies in Serum*

As indicated in Table 1 using a dichotomous analysis and the well-established cutoff level of 0.35 kU<sub>A</sub>/L of sIgE, 50 of the peanut samples were classified as positive and 10 were classified as negative by ImmunoCAP. Of these 50 positive samples, Immulite revealed 47 as positive, whereas Turbo RAST detected 39 as positive. Immulite classified all of the 10 negative peanut samples as negative, whereas Turbo RAST classified 1 of these samples as positive. Using 0.10 kU<sub>A</sub>/L as a cutoff level, ImmunoCAP classified 55 samples as positive and 5 as negative. Immulite classified 1 of the 55 positive samples as negative and 1 of the 5 negative samples as positive. Using this cutoff, Turbo RAST classified 48 of the positive samples as positive and all 5 negative samples as negative (Table 1).

Table 1. Dichotomous Comparison of Assay Performances With Peanut and Soy Antibodies in Serum

| Assays              | Peanut antibodies |          | Soy antibodies |          |
|---------------------|-------------------|----------|----------------|----------|
|                     | Positive          | Negative | Positive       | Negative |
| Cutoff of 0.35 kU/L |                   |          |                |          |
| ImmunoCAP           | 50                | 10       | 17             | 3        |
| Immulite            | 47                | 10       | 16             | 3        |
| Turbo RAST          | 39                | 9        | 12             | 3        |
| Cutoff of 0.10 kU/L |                   |          |                |          |
| ImmunoCAP           | 55                | 5        | 17             | 3        |
| Immulite            | 54                | 4        | 17             | 3        |
| Turbo RAST          | 48                | 5        | 17             | 3        |

Abbreviation: RAST, radioallergosorbent test.

For the 20 soy samples and a cutoff of 0.35 kU<sub>A</sub>/L, ImmunoCAP classified 17 samples as positive. Of the 17 positive samples, Immulite classified 16 and Turbo RAST detected 12 samples as positive. Using a cutoff of 0.10 kU<sub>A</sub>/L, all of the systems classified 17 samples as positive and all negative samples were equally classified (Table 1).

#### Comparisons of Quantitative Ability of the Different Assays

All 3 assays present their quantitative results in kilounits of allergen sIgE per liter (kU<sub>A</sub>/L). To evaluate the quantitative agreement among the methods, the results for sIgE to peanut obtained with Immulite and Turbo RAST were plotted against the values obtained with ImmunoCAP in both a linear plot and a logarithmic Bland-Altman plot (Fig 1a and b). The linear plot showed that for samples with more than 10 kU<sub>A</sub>/L of sIgE on ImmunoCAP, the results obtained with Immulite always tended to be higher. For Turbo RAST the variability of the results made no such conclusion possible. In addition, for values below 10 kU<sub>A</sub>/L, the logarithmic Bland-Altman plot showed considerable variability for both Immulite and Turbo RAST.

To further quantify the graphic results, weighted linear regression lines were fitted to the data in Figure 1a, and the average deviation and the variation of the deviations were estimated for the data in Figure 1b. The results of the weighted linear regression indicated significantly higher values for Immulite than for ImmunoCAP (slope, 1.594; 95% CI, 1.343–1.846;  $P < .001$ ). This finding was also demonstrated by the significant average logarithmic difference of 0.24 ( $P = .02$ ; CV, 78%). For Turbo RAST the weighted linear regression did not exclude a linear relationship with ImmunoCAP (slope, 1.124; 95% CI, 0.897–1.351). However, despite the high variation between the logarithmic differences (CV, 123%), Turbo RAST showed a significant negative

logarithmic average deviation of 0.34 compared with the ImmunoCAP results ( $P = .04$ ).

For the sIgE to soy, Immulite showed a large variation among individual samples compared with ImmunoCAP (CV, 84%), which gave a nonsignificant logarithmic average difference (−0.20) and a possible linear relationship (slope, 1.149; 95% CI, 0.67–1.629) (Fig 2a and b). For Turbo RAST, the results were significantly much lower than for ImmunoCAP in both the weighted linear regression (slope, 0.339; 95% CI, 0.243–0.436) and the average logarithmic difference (−1.08) ( $P < .001$ ). In addition, for Turbo RAST results, the variation for the deviation was high compared with ImmunoCAP (CV, 87%).

#### Comparison of Results Measuring Chimeric Antibodies

All 3 of these assay systems use calibration curves stated to be linked to the World Health Organization tIgE standard and report their results in kU<sub>A</sub>/L. Since their results are widely disparate, this raises the question of which, if any, are reporting the true value of sIgE. One method to determine this would be to use samples in which all the IgE protein in a sample is directed toward a single specificity and in which the amount of immunoglobulin is known. Mouse-human chimeric antibodies fulfill this requirement and thus provide the opportunity to determine if a given assay for sIgE is performing correctly, assuming that the measurement reagents are specific for the IgE structure.

The average results of 3 different determinations of dilutions of the chimeric antibodies for all 3 systems demonstrated a fairly close agreement for the tIgE results, indicating their respective abilities to correctly quantify tIgE (Fig 3a). However, when each assay was used to measure the sIgE in the same samples, widely divergent results were obtained (Fig 3b and c). The ImmunoCAP system gave similar results

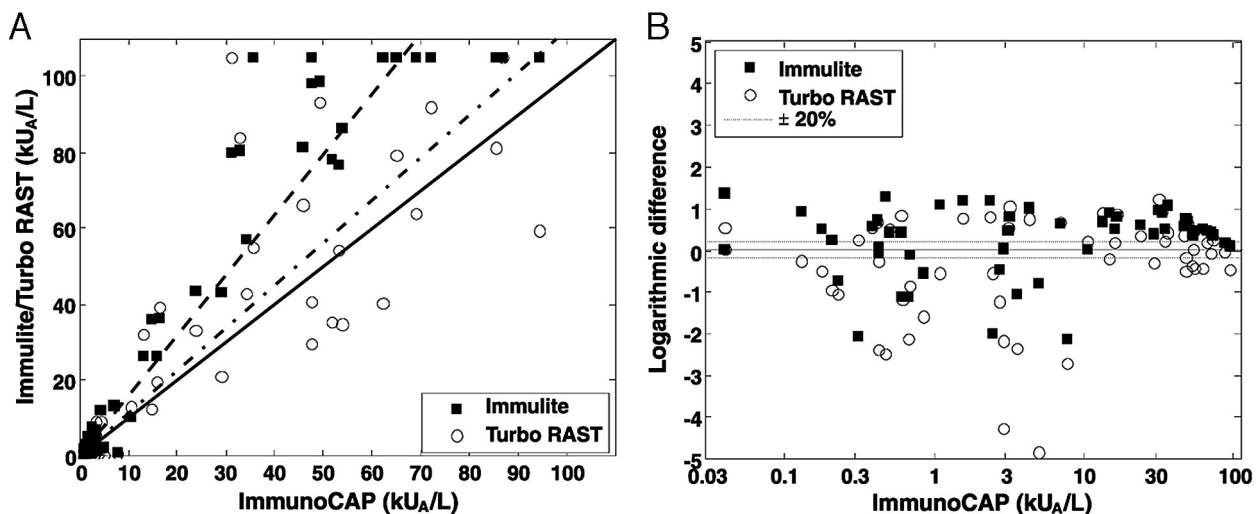


Figure 1. Comparison of the analysis of peanut IgE antibodies of Immulite and Turbo radioallergosorbent test (RAST) compared with ImmunoCAP as reference. A, Linear correlation plot; B, logarithmic Bland-Altman plot. Dotted lines indicate  $\pm 20\%$ , which is accepted as the deviation for results that are similar.

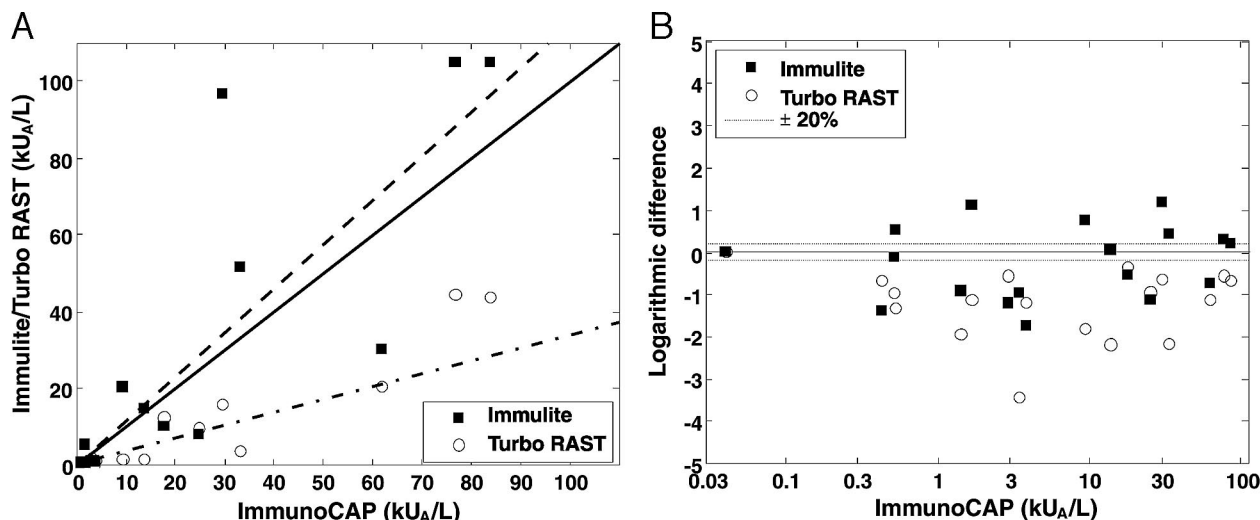


Figure 2. Comparison of the analysis of soy IgE antibodies of Immulite and Turbo radioallergosorbent test (RAST) compared with ImmunoCAP as reference. A, Linear correlation plot; B, logarithmic Bland-Altman plot. Dotted lines indicate  $\pm 20\%$ , which is accepted as the deviation for results that are similar.

for the tIgE and sIgE results in all samples. In contrast, the Immulite reported sIgE values considerably higher (nearly 4-fold) than their own tIgE results for both chimeric antibody preparations. In contrast, the Turbo RAST system consistently underestimated the sIgE antibody levels for both chimeric antibodies by approximately 10-fold compared with the tIgE levels determined by the same laboratory. Since these results mimic those seen herein with the analysis of sIgE to peanut and soy on these different systems, they substantiate that the ImmunoCAP system results are correct and that the other systems deviate from true values.

To dispel the theory that the observed differences occurred just by chance, the precision of the determinations with the 3 systems was analyzed. For the allergen sIgE chimeric antibodies, similar good precision was found for the ImmunoCAP and Immulite systems, whereas for Turbo RAST the precision expressed as CV varied for the different dilutions from 11% to 172%, being lower for the birch chimeric antibody (mean, 48%) than for the mite chimeric antibody (mean, 118%) (Table 2). For tIgE, good precision was obtained for both the ImmunoCAP and Immulite systems. For Turbo RAST, the precision was good for the 2 highest concentrations, whereas for the 2 most diluted samples the levels were reported as too low for determination (Table 2). Thus, although the tIgE results were relatively comparable in all 3 systems, only ImmunoCAP reported close agreement between the sIgE and tIgE levels in all samples tested. In contrast, the sIgE results reported with Immulite were much higher than the defined amounts of IgE protein present in the samples, whereas the Turbo RAST system reported considerably lower values.

## DISCUSSION

This study demonstrates generally poor agreement among these 3 assay systems for the measurement of sIgE to peanut

and soy, a finding that is consistent with some prior reports to other allergens<sup>20–22</sup> but in contrast to other reports.<sup>23,24</sup> This finding further confirms that it is not possible to equate allergen sIgE antibody levels determined in any of these systems with results from the others. Since the argument of selection bias is sometimes raised, it is important to reiterate that these samples were picked randomly and not selected by any other criteria than their apparent content of sIgE.

The discrepancies among the systems investigated were further demonstrated using the chimeric antibodies. Thus, with these chimeric antibodies, it was clearly demonstrated that the laboratory that used Immulite considerably overestimated sIgE levels and the laboratory that used Turbo RAST underestimated sIgE levels. Furthermore, the correct levels of sIgE in these samples compared with total IgE protein were precisely determined using ImmunoCAP. Furthermore, the specificity of the reagents in the different assays was validated, since all the results agreed closely for the measurements of tIgE. All 3 sIgE and tIgE assays use calibrators stated to be linked to the World Health Organization standard 75/502.

Although a variety of methods for the quantification of sIgE have been developed during the past 20 to 30 years, currently in the United States most in vitro allergy testing is performed with the 3 assays that were included in this study: the ImmunoCAP, Immulite, and Turbo RAST. In the present study, we chose to use the ImmunoCAP as the reference system, since its performance has been well documented. This system has been demonstrated to measure  $1 \text{ kUA/L} = 1 \text{ KU/L} = 2.42 \text{ } \mu\text{g/L}$  with a very high precision,<sup>30</sup> a finding that is confirmed in the present study. It has also undergone vast documentation with more than 3,000 peer-reviewed publications and with much research focused on its clinical utility.<sup>3–19</sup> Similar information is not available for the other systems used in this investigation.

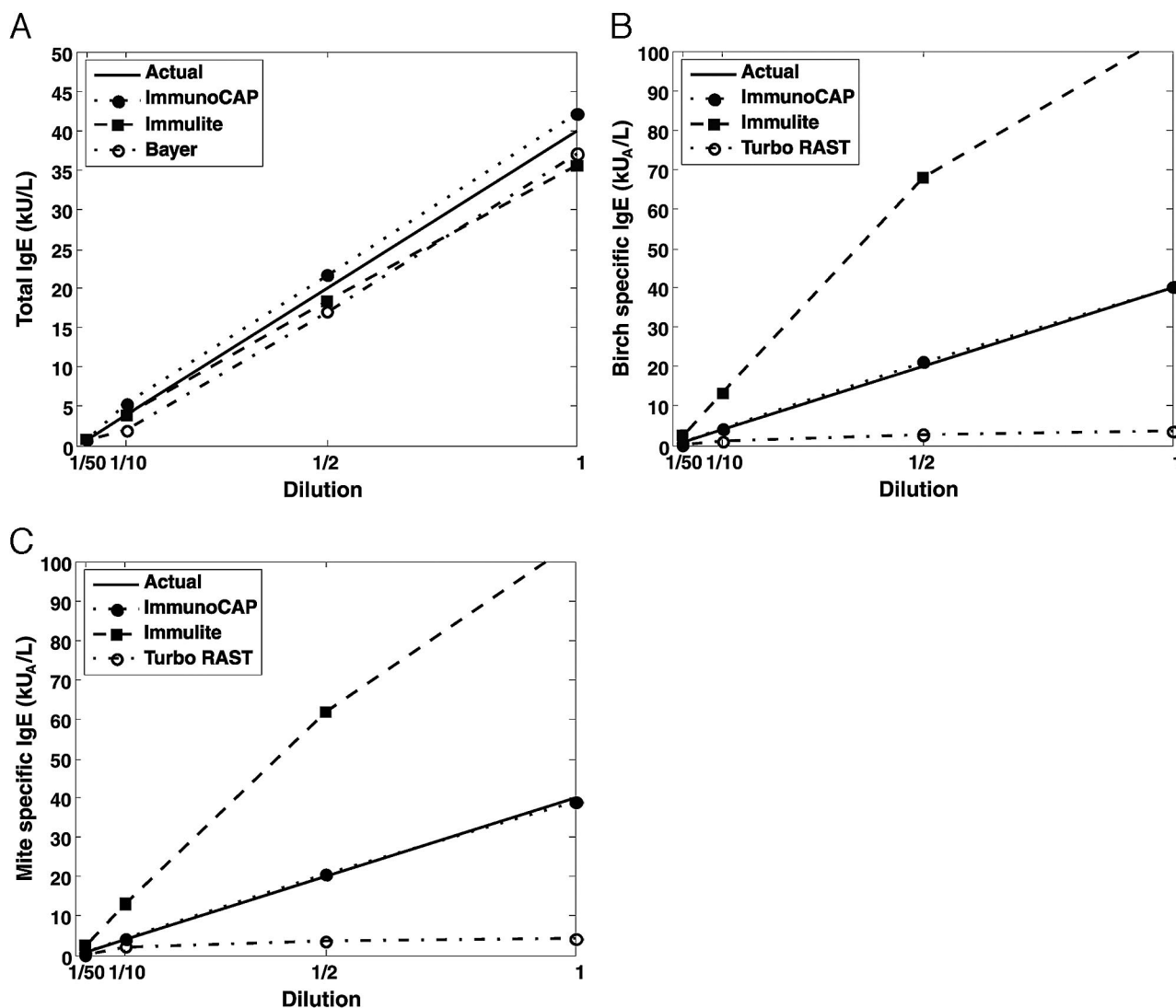


Figure 3. Chimeric IgE results of the 3 systems (the laboratory using Turbo radioallergosorbent test [RAST] analyzed total IgE [tIgE] with Centaur) compared with the actual values of undiluted and diluted samples. A, tIgE; B, Bet v 1; and C, Der p 2. Each point represents the average of triplicate determinations as given in Table 2. Results of more than 100 were given the value of 100 and results of less than 2 were given the value of 0.

Several studies in the past have compared different assay performances on a simple dichotomous positive or negative basis. In the present study, which used cutoffs in the low range, we found that the ImmunoCAP and Immulite gave similar results, whereas the Turbo RAST results were less consistent. Such findings have been reported previously for sIgE measurements to inhalant allergens but not for food allergens.<sup>20–22</sup>

As has been recommended for the management of patients with suspected food allergy, clinical decisions can be based on the sIgE level.<sup>5–14</sup> With the advent of more quantitative methods for sIgE measurement, it is clear that the comparison of assays on a dichotomous basis is no longer adequate. For optimal patient care, the quantitative abilities of these assays

should be fully elucidated. As in this study, some previous studies have indicated that Immulite overestimates and Turbo RAST underestimates sIgE levels compared with ImmunoCAP to several allergens,<sup>20–22</sup> although some studies have disputed this.<sup>23,24</sup> The College of American Pathologists offers periodic voluntary survey samples to laboratories to compare their performances in determining sIgE. These proficiency surveys are considered proprietary, and they used to report the results as classes rather than quantitative units. This approach would actually hide some of the discrepancies demonstrated in this report. However, for a variety of different allergens even using classes can reveal similar discrepancies among these assays as in the present study. Different allergens (peanut and soy) were tested in this study, and in fact

Table 2. Precision of the Determination of Chimeric Antibodies by the 3 Systems<sup>a</sup>

| Dilution factor                          | ImmunoCAP         |                  |       | Immulite          |                  |       | Turbo RAST        |                  |        |
|------------------------------------------|-------------------|------------------|-------|-------------------|------------------|-------|-------------------|------------------|--------|
|                                          | Between-assay run | Within-assay run | Total | Between-assay run | Within-assay run | Total | Between-assay run | Within-assay run | Total  |
| Specific IgE antibodies to birch Bet v 1 |                   |                  |       |                   |                  |       |                   |                  |        |
| 1                                        | 1.36              | 5.83             | 5.99  |                   |                  |       |                   |                  | 90.08  |
| 2                                        | 3.42              | 4.85             | 5.93  | 2.60              | 2.88             | 3.88  | ... <sup>b</sup>  | ...              | 25.92  |
| 10                                       | 2.15              | 0.90             | 2.33  | 1.73              | 4.36             | 4.69  | ...               | ...              | 11.47  |
| 50                                       | 0.00              | 3.03             | 3.03  | 0.00              | 5.56             | 5.56  | ...               | ...              | 14.19  |
| Pooled                                   | 2.13              | 4.11             | 4.63  | 1.80              | 4.41             | 4.76  | ...               | ...              | 47.75  |
| Specific IgE antibodies to mite Der p 2  |                   |                  |       |                   |                  |       |                   |                  |        |
| 1                                        | 4.29              | 2.82             | 5.13  |                   |                  |       |                   |                  | 172.39 |
| 2                                        | 3.36              | 2.21             | 4.02  | 2.89              | 4.41             | 5.27  | ...               | ...              | 140.64 |
| 10                                       | 4.71              | 1.40             | 4.92  | 0.00              | 2.67             | 2.67  | ...               | ...              | 12.23  |
| 50                                       | 2.76              | 1.74             | 3.26  | 3.88              | 5.71             | 6.90  | ...               | ...              | 80.51  |
| Pooled                                   | 3.86              | 2.11             | 4.40  | 2.79              | 4.44             | 5.24  | ...               | ...              | 118.46 |

Abbreviation: RAST, radioallergosorbent test.

<sup>a</sup> Data are the coefficients of variation presented in percentages.

<sup>b</sup> Ellipses indicate no replicates were available.

they behaved differently in this respect, with an even worse precision for the soy antibodies compared with the peanut antibodies. This may be in part because fewer samples are available for the soy determination, but poor precision in the soy assays likely adds to this picture.

Precision is an important component of any assay's performance, and poor precision may confuse concordance between tests. In this study as in others, the high precision of the ImmunoCAP was again demonstrated,<sup>20,22</sup> whereas in particular the poor reproducibility of Turbo RAST is of concern. In previous studies that considered precision, similar results have been reported for the Turbo RAST system.<sup>20,22</sup>

The use of chimeric IgE antibodies directed against birch and mite allergens clearly and objectively verified the discrepancies of the 3 systems studied. These tools verified that the ImmunoCAP system gave accurate results and that again the Immulite system overestimated and the Turbo RAST system underestimated their measurements of sIgE. In view of this, the interpretation and the lack of differences among the systems reported in some studies<sup>23,24</sup> may need to be reevaluated.

Our findings are of additional interest in lieu of the fact that all 3 systems determined the levels of tIgE similarly and that all 3 use tIgE as a calibrator to interpolate levels of sIgE. Thus, the observed discrepancies must arise from these assays' mechanics.<sup>31-33</sup> The reason given for an assay underestimating levels of sIgE usually entails inadequate relevant allergen binding sites on their solid phase. In addition, one must suspect the mechanics of the assay if wide variance is seen between repeated assays. With respect to this study, both problems seem to be inherent in the Turbo RAST system. Reasons that an assay may overestimate measurement of sIgE are less clear. One may speculate that the signal developed by increased binding is amplified in a nonlinear manner or that

improper extrapolation methods have been used. Regardless of the reason(s) it is clear that the Immulite system considerably overestimates sIgE not only compared with the ImmunoCAP system but also compared with its own measurement of tIgE, whereas the Turbo RAST system contrarily considerably underestimates the sIgE results.

Importantly, this study does not demonstrate any means by which the results of these systems can be reliably correlated with one other. This finding further implies that one cannot apply data developed with one system to another system and that only results from a system that has undergone sufficient clinical study using valid end points, such as blinded oral food challenges, can be interpreted clinically. For example, the levels of sIgE antibodies that have been published for the interpretation of food allergy using ImmunoCAP<sup>5-14</sup> can clearly not be used for the other 2 methods used in this study. However, similar studies for these other methods would certainly be welcomed.

In summary, there is little doubt about the potential value of diagnostic tests that provide accurate, precise, and quantitative measurement of sIgE to all different allergens. The findings presented herein, although restricted to only 4 specificities, have serious clinical implications, particularly since each of these systems has been and is currently in wide clinical use. Because of these implications, all laboratories must clarify which system is being used and users must understand these differences. In particular, just because different systems present their results in the same apparent units (kU<sub>A</sub>/L) or classes, it by no means signifies that the results are correct or interchangeable. Although the chimeric antibody studies unequivocally demonstrate the quantitative ability of the ImmunoCAP system, the present study does not demonstrate any means by which these different systems can be reliably correlated.

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1. d
2. b
3. d
4. true, true, false, false, false
5. a
6. c