

LETTER TO THE EDITOR

Two commercial Gal d1 specific IgE assays show major differences in performance

To the Editor

Egg allergy is one of the most frequent food allergies in children, and the diagnosis of egg allergy is based on the history, IgE tests, and standardized food challenges.¹ Measurement of egg-specific IgE can also be useful in predicting allergic reactions in oral food challenge (OFC) tests and in monitoring oral immunotherapy. Probability curves providing the likelihood of a positive outcome of an OFC/positive symptoms with the help of specific IgE measurements have been established for both egg and ovomucoid.²⁻⁴

It is known that allergic reactions to egg may occur either to any form of egg or, in some patients, mainly to raw or lightly boiled/baked eggs.^{5,6} In this context, it has been demonstrated that ovomucoid (Gal d 1)-specific IgE antibody (Ab) measurements can clinically be used to predict tolerance to hard boiled or baked eggs. Positive and negative decision points for levels of Gal d 1-specific IgE have been reported. In one recent study by Palosuo et al,⁷ all subjects with levels over 14 kU_A/L (ImmunoCAP) failed an egg OFC while the cutoff for passing the egg OFC was 0.9 kU_A/L. Similar results were reported by Ando et al,⁸ defining decision point levels of 10.8 kU_A/L and 1.6 kU_A/L, respectively. Thus, high test precision not only in the high concentration region but also in the low concentration region is clinically important when measuring Gal d 1-specific IgE antibodies. Besides Gal d 1, there are other important egg white components such as ovalbumin (Gal d 2) and lysozyme (Gal d 4).

To take advantage of these findings, it is of the utmost importance that the sIgE measurements are truly specific. There are three commercially available automated specific IgE immunoassay platforms in Japan, of which IMMULITE and ImmunoCAP are the predominant. Both allow the analysis of a variety of allergens, including Gal d 1. However, commercially available Gal d 1 has been reported to be contaminated with lysozyme (Gal d 4)⁹ to an extent that could significantly affect the outcome of IgE measurements. The aim of this study was to compare the IMMULITE and ImmunoCAP Gal d 1-specific IgE assays with respect to their contents of Gal d 1 and Gal d 4.

For this purpose, two allergen-specific mouse-human chimeric monoclonal antibodies were used, each comprising of the antigen-specific variable domain from a mouse monoclonal antibody Ab and the constant part of the heavy chain of human IgE. The Ab were obtained from Thermo Fisher Scientific (TFS), Uppsala, Sweden, and reacted specifically with Gal d 1 and Gal d 4, respectively, and could be measured with the specific and total IgE assays of both assay platforms. The chimeric Ab were obtained at a high concentration (total

IgE 70 kU/L), and a 5-step dilution series was prepared, up to a dilution factor of 16. Hence, samples with six different concentrations of each Ab were available for the assay comparisons. The assays were performed in the clinical laboratory of Kotobiken (IMMULITE), and at the scientific laboratory of TFS IDD (ImmunoCAP). Each of the two Ab came from the same batch, respectively, and furthermore, all assays were run on a single detection base. Also, all assays were performed in accordance with the manufacturer's instructions and the principles of good laboratory practice.

Both the ImmunoCAP and IMMULITE Gal d 1 assays displayed good dilution linearity for the anti-Gal d 1 chimeric Ab (Figure 1A). However, a clear difference between these assays was observed when anti-Gal d 4 chimeric IgE Ab was applied to the two Gal d 1 assays (Figure 1B). While negative results (<0.1 kU_A/L) were obtained with all six dilutions of the Gal d 4 IgE Ab in the ImmunoCAP assay, positive results ranging between 5.6 and 61.9 kU_A/L were obtained with IMMULITE Gal d 1 assay, demonstrating that this assay detects not only Gal d 1-specific IgE but also Gal d 4-specific IgE.

In their report on impurities in commercially available egg allergens, Everberg et al⁹ described a method for removal of contaminating Gal d 4 from Gal d 1 and demonstrated the importance of such purification for preventing the occurrence of false-positive results in an assay for Gal d 1-specific IgE. With the ImmunoCAP system, we have found in our clinic a prevalence of sensitization to Gal d 4 of 54% among egg-sensitized patients (data not shown). Taken together with the findings presented here, we believe that the IMMULITE Gal d 1 sIgE test may frequently generate falsely elevated positive results.

In a sample where all IgE is represented by a single allergen specificity, measurements of total and specific IgE should yield comparable concentration values. With the ImmunoCAP total IgE and Gal d 1-specific IgE assays, approximately equal results were obtained for all six dilutions of the chimeric Gal d 1 IgE Ab, with an average sIgE/tIgE ratio of 1.17 (range 1.10-1.26) (Figure 2). In contrast, IMMULITE gave specific IgE levels that were substantially higher than the corresponding total IgE values in all six dilutions of the chimeric Gal d 1 IgE Ab, with an average sIgE/tIgE ratio of 4.07 and a range of 3.46-4.75 (Figure 2). A similar finding was previously reported by Szecsi & Stender.¹⁰ The discrepant results obtained with IMMULITE can only be attributed to the specific IgE assay since a close agreement of total IgE concentrations measured with IMMULITE and ImmunoCAP has been demonstrated,¹¹ indicating the ability of both assays to correctly quantify total IgE.

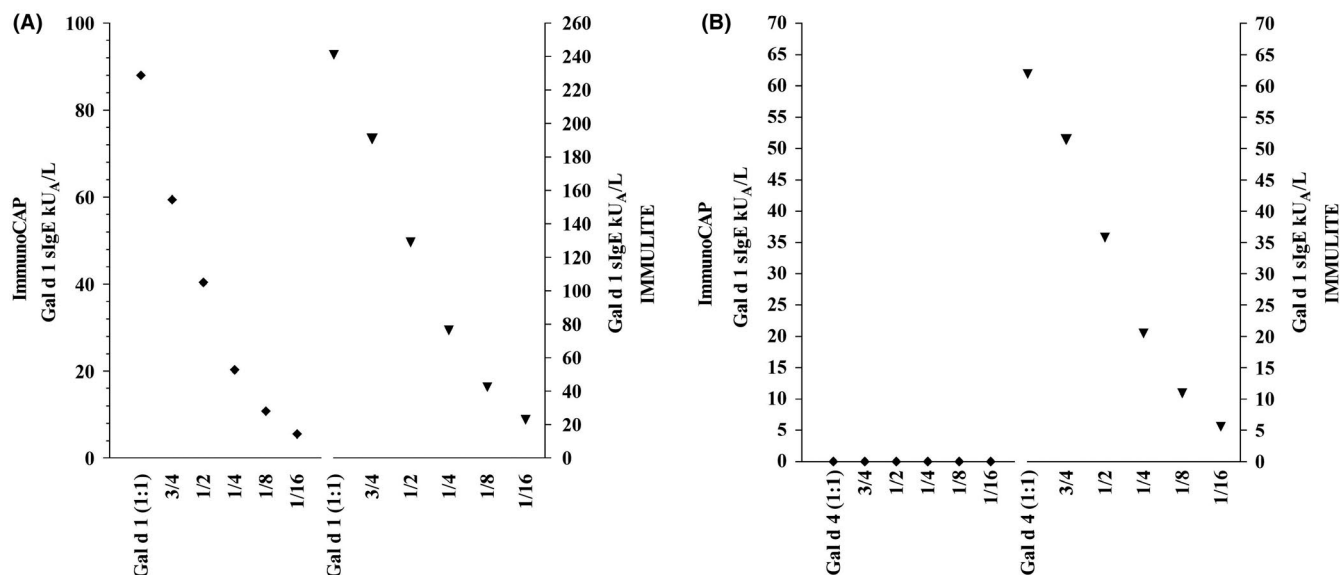


FIGURE 1 Measurement of anti-Gal d 1 (A) and anti-Gal d 4 (B) chimeric IgE antibodies at six different dilutions with ImmunoCAP (left y-axis) and IMMULITE (right y-axis) Gal d 1-specific IgE tests. Gal d 4-specific IgE antibody should not be detected by a test for Gal d 1-specific IgE

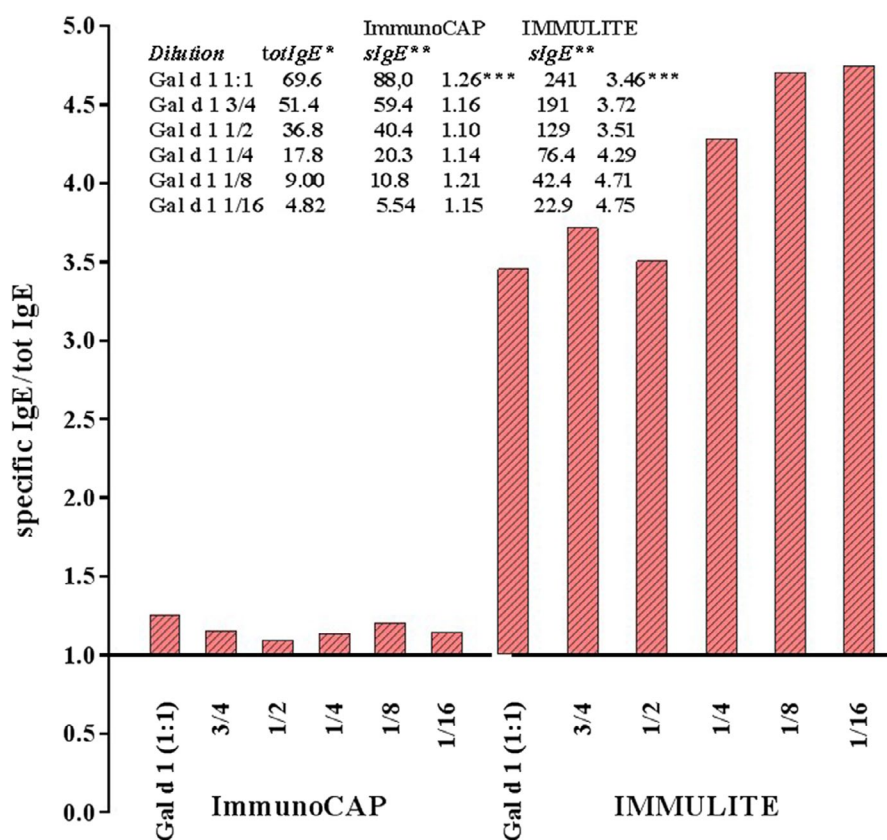


FIGURE 2 Accuracy of measurement of Gal d 1-specific IgE assessed by means of comparison to total IgE measurement of the same samples of anti-Gal d 1 chimeric IgE antibody at 6 different dilutions. Ratios of specific over total IgE measurements are shown for ImmunoCAP and IMMULITE. The closer to the value 1 on the y-axis, the more accurate is the test. Inset: concentration values obtained with total IgE and Gal d 1-specific IgE assays. *kU/L; **kU_A/L; ***ratio specific IgE/total IgE

Both assays are calibrated against the current WHO IgE reference preparation. In this study, we could therefore safely use the total IgE values measured with ImmunoCAP as reference in the sIgE/tIgE comparisons.

Limitations in the study were, despite covering the entire measuring range of the samples, the relatively small number of samples, where only single-run analysis was performed which in turn limited

the statistical evaluations. Furthermore, the anti-Gal d 4 allergen-specific mouse-human chimeric monoclonal Ab donated by TFS bind only to one epitope on Gal d 4 and do not prove the presence of the complete protein.

Despite these limitations, this study has shown that both ImmunoCAP and IMMULITE Gal d 1 sIgE assays display favorable dilution linearity properties but while that the Gal d 1 ImmunoCAP

was not contaminated with Gal d 4, the IMMULITE assay may generate false-positive results for samples containing IgE antibodies to Gal d 4, presumably as a result of impurity of the Gal d 1 preparation used. One solution to the impurities in the IMMULITE Gal d 1 sIgE test can be using recombinant allergens instead of native ones. However, recombinant egg white allergen components are not, to our knowledge, yet available. Additionally, while the ImmunoCAP Gal d 1 sIgE assay was found to accurately quantify Gal d 1-specific IgE, the IMMULITE assay appeared to overestimate the Gal d 1-specific IgE concentration 4-fold on average.

This study was approved by the ethics committee at Dokkyo University on May 31, 2019, with approval number 29026. The authors are grateful to TFS IDD Japan for their donation of the chimeric IgE antibodies.

CONFLICT OF INTEREST

The authors have no conflict of interest in relation to this work.

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REFERENCES

1. Benhamou AH, Caubet JC, Eigenmann PA, et al. State of the art and new horizons in the diagnosis and management of egg allergy. *Allergy*. 2010;65(3):283-289.
2. Komata T, Soderstrom L, Borres MP, Tachimoto H, Ebisawa M. The predictive relationship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. *J Allergy Clin Immunol*. 2007;119(5):1272-1274.
3. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol*. 1997;100(4):444-451.
4. Sato S, Ogura K, Takahashi K, Sato Y, Yanagida N, Ebisawa M. Usefulness of antigen-specific IgE probability curves derived from the 3gAllergy assay in diagnosing egg, cow's milk, and wheat allergies. *Allergol Int*. 2017;66(2):296-301.
5. Eigenmann PA. Anaphylactic reactions to raw eggs after negative challenges with cooked eggs. *J Allergy Clin Immunol*. 2000;105(3):587-588.
6. Leonard SA, Sampson HA, Sicherer SH, et al. Dietary baked egg accelerates resolution of egg allergy in children. *J Allergy Clin Immunol*. 2012;130(2):473-480.e1.
7. Palosuo K, Kukkonen AK, Pelkonen AS, Makela MJ. Gal d 1-specific IgE predicts allergy to heated egg in Finnish children. *Pediatr Allergy Immunol*. 2018;29(6):637-643.
8. Ando H, Moverare R, Kondo Y, et al. Utility of ovomucoid-specific IgE concentrations in predicting symptomatic egg allergy. *J Allergy Clin Immunol*. 2008;122(3):583-588.
9. Everberg H, Brostedt P, Oman H, Bohman S, Moverare R. Affinity purification of egg-white allergens for improved component-resolved diagnostics. *Int Arch Allergy Immunol*. 2011;154(1):33-41.
10. Szecsi PB, Stender S. Comparison of immunoglobulin E measurements on IMMULITE and ImmunoCAP in samples consisting of allergen-specific mouse-human chimeric monoclonal antibodies towards allergen extracts and four recombinant allergens. *Int Arch Allergy Immunol*. 2013;162(2):131-134.
11. Wood RA, Segall N, Ahlstedt S, Williams PB. Accuracy of IgE antibody laboratory results. *Ann Allergy Asthma Immunol*. 2007;99(1):34-41.