

Evaluation of Allergenetics Fluorometric Assays for IgE and IgE Antibody

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Dear Sir,

In the development of diagnostic routine laboratory tests use of the microtiter plate configuration in the test set-up has been suggested as an improvement. Recently, a new product has appeared where microtiter systems are used for measurement of IgE and IgE antibody (3), (Allergenetics, Mountain View, Ca, US). The purpose of this study was to evaluate the performance of these tests by comparing the results with those obtained with the reference Phadebas IgE PRIST® and Phadebas® methods. In such comparisons special attention should be given to:

Standard curve; detection limit, measuring range.

Correlation of positive sera to reference method.

Non-specific binding; healthy individuals, atopic controls.

Reproducibility; within-between assay, reagent batch variation.

The Allergenetics total IgE FAST™ test measuring range was investigated by testing a serum of 13,400 kU IgE/l (Phadebas IgE PRIST). We found the serum to be scored as 83 kU/l indicating a severe hook effect in the Allergenetics total IgE FAST system, thus limiting accurate measurement to concentrations below 80 kU/l.

The IgE values obtained for serum samples from 21 patients with documented allergy were only half of those obtained with Phadebas IgE PRIST.

As the lowest calibrator of the kit is 10 kU/l,

the measuring range of Allergenetics total IgE FAST test is 10-80 kU/l, which in reality is 5-40 kU/l, since all values were only 50 % of expected levels. A value registered as above 80 kU/l in the Allergenetics system can reflect any concentration of IgE up to infinity due to the hook effect. One possible explanation for this phenomenon is the use of monoclonal tracer antibody, which in our experience is more sensitive to hooking. Also the tandem assay principle used in this test is more often disturbed by a significant hook effect than sandwich assays.

The performance of Allergenetics IgE FAST™ for the assay of specific IgE was also investigated. We first prepared serial dilutions of the highest calibrator using the diluent supplied in the kit and measured in the Allergenetics system. The dilution curve was not parallel with the supplied standard curve (Fig. 1). Assuming that the lowest calibrator informs about the lowest value acceptable by the reagents, the measuring range of the Allergenetics IgE FAST test is 2-20 "IU/ml". These results were confirmed by measurement of IgE antibody concentrations in the calibrators by Phadebas RAST.

Allergenetics IgE FAST test results are scored as IU/ml. We are not aware of the existence of International Units for IgE antibody, however, our data indicate that Allergenetics "IU/ml" are similar to Phadebas RAST Units/ml (PRU/ml), at least in the upper part of the measuring range.

The results from sera of patients with documented allergy were studied by measuring eight to 11 sera with high scores in Phadebas RAST

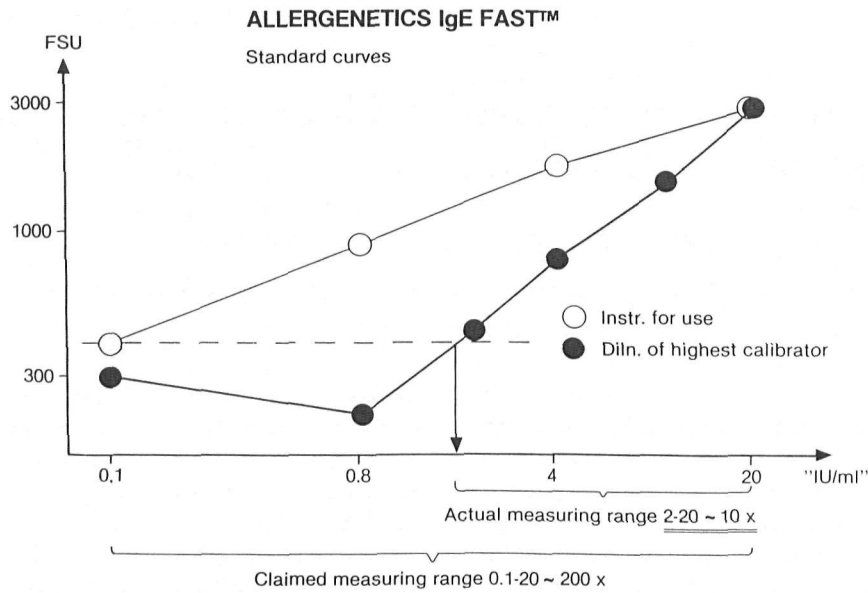


Fig. 1. Standard curve of Allergenetics IgE FAST™ test as run according to instructions from the manufacturer (○). Closed circles (●) show the results obtained when the highest calibrator was serially diluted with the diluent supplied in the kit and then measured in the Allergenetics system.

against each of seven different allergens (g6 *Phleum pratense*, t1 *Acer negundo*, w1 *Ambrosia elatior*, w9 *Plantago lanceolata*, d2 *Dermatophagoides farinae*, e1 cat and f4 wheat) in the Allergenetics IgE FAST test. The correlation was poor ($r = 0.49$), (Fig. 2). A similar random correlation between the results of Allergenetics IgE FAST and skin prick testing has been reported (1). In contrast, the clinical correlation for RAST (1) and Phadebas/Phadezym RAST (2) is 75-100 %.

The non-specific binding of negative and 1,000 kU IgE/l spiked serum was below the lowest calibrator in both Allergenetics IgE FAST test and Phadebas RAST for all allergens investigated.

We were able to compare two different batches of Allergenetics short ragweed allergen-coated wells. There was a more than 10-fold difference in uptake, indicating either problems with production of the wells by the manufacturer or poor stability of the final product.

In conclusion: Allergenetics total IgE FAST test has a practical measuring range of 5-40 kU IgE/l with the values assigned by the test being only half of those established by Phadebas IgE

PRIST. The Allergenetics IgE FAST test for specific IgE assay has a poor sensitivity (1-2

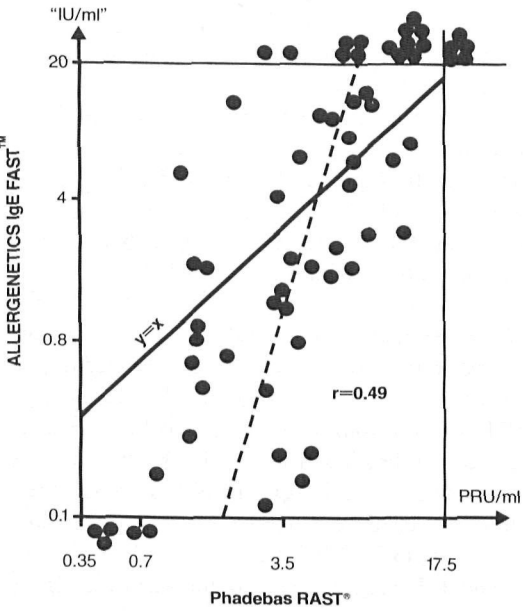


Fig. 2. Correlation between Phadebas RAST® and Allergenetics IgE FAST™ test. Sera from eight to 11 patients with documented allergy were tested against each of seven different allergens.

PRU/ml) and the scores show a bad correlation to those obtained with the reference Phadebas/Phadezym RAST method and with skin prick testing. There are also indications of poor reproducibility between batches of Allergenetics allergen-coated wells.

This investigation clearly shows the need for documentation of new commercially available tests for the diagnosis of diseases in humans.

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