

Short Communication

Capture EIA for determination of IgE to diphtheria and tetanus toxins in comparison to RIA

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A capture IgE assay in EIA with diphtheria toxin and tetanus toxoid as model systems was developed and evaluated in relation to RIA in a DT booster study. The EIA and the RIA gave very similar results, although the EIA determined IgE over a wider range of concentrations, without interference by other subclasses. The comparison to the capture EIA indicated that the RIA gave more reliable results when using a toxin as antigen. Weak correlations between local side effects and IgE responses and differences between vaccine groups were noted by RIA but not by EIA, which correlated better with the clinical findings. © 1998 Elsevier Science Ltd. All rights reserved

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Immunoglobulin E (IgE) response has been reported to a variety of bacterial^{3,8,11–13} and viral antigens^{3,12}, and after vaccination with tetanus^{7,9} and diphtheria⁷ and pertussis vaccines, both after primary immunization^{2,4,5} and booster injections^{2,5,10}. The clinical significance of these antibodies is not clear, although there appears to be a relationship between local reactions and IgE responses¹. Additional antigens in present and future combined vaccines necessitate further investigations of IgE responses to new antigens.

IgE responses are most commonly measured by radioimmunoassay (RIA), but enzyme-immunoassay (EIA) has the advantage of stable reagents and cheap automatic reading apparatus. Also, the RIAs used are indirect assays, where loss of sensitivity can be assumed in the presence of high levels of IgG antibodies. We have previously used RIA in studies of IgE responses to pertussis, tetanus and diphtheria toxins^{2,5,7,10} and have shown in a first study that interference by IgG was not a significant problem in our system⁵. However, in order to allow for more extensive testing of IgE to any type of antigen, we developed a capture IgE assay in EIA for determination of antibodies to diphtheria

and tetanus toxins as model systems and evaluated the EIA in relation to RIA in a booster study of DT vaccines^{6,7}.

MATERIALS AND METHODS

Antigen-alkaline phosphatase conjugate

In order to obtain stable antigen-alkaline phosphatase conjugates, glutaraldehyde concentrations in relation to enzyme concentrations and conditions for conjugation, e.g. temperature and time, were investigated. The optimal conditions were found to be mixing 1 mg of diphtheria toxin (2600 Lf ml⁻¹, 6760 µg protein ml⁻¹) or tetanus toxoid (500 Lf ml⁻¹, 1300 µg protein ml⁻¹, SBL Vaccin AB, Stockholm, Sweden) with 5000 units of alkaline phosphatase from bovine intestinal mucosa, Sigma Type VII-T (Sigma Co., St Louis, Mo) and adding PBS pH 7.0 to a final volume of 1.5 ml. While the solution was gently stirred, 0.02 ml of 2.5% glutaraldehyde in PBS pH 7.0 were added by drops. The reaction was allowed to proceed for 7 days at +37°C. The suspension was dialyzed overnight against PBS pH 7.0 and then for 6 h against 0.05 M Tris-HCl pH 8.0, containing 2 mM MgCl₂. The conjugate was centrifuged at 3000 rpm for 10 min and finally diluted to 3.0 ml with 0.05 M Tris-HCl pH 8.0 containing 2 mM MgCl₂. It was stored at 4°C with 0.02% NaN₃ as preservative.

Capture anti-IgE antibody and the capture enzymeimmunoassay (EIA)

Out of several commercial sources of anti-IgE, the most consistent results were obtained with goat anti-

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human IgE from Euro-Diagnostica (Malmö, Sweden) at a coating concentration of $10\text{ }\mu\text{g ml}^{-1}$. Coating of 96-well microplates with the anti-IgE was done overnight at room temperature (RT) in $100\text{ }\mu\text{l}$ volumes, while sera and the antigen-alkaline phosphatase conjugate was added in $50\text{ }\mu\text{l}$ volumes. The alkaline-phosphatase conjugated antigen(s) was added at a concentration of $3.3\text{ }\mu\text{g ml}^{-1}$ and incubation was done on a shaker at RT for 2 h. Each serum sample was tested in five twofold dilutions from 1:8 to 1:128 and incubation was done for 1 h at RT.

IgE concentration by EIA was obtained by plotting log optical density (OD) values at $A_{405\text{ nm}}$ against log serum dilutions, as shown in Figure 1. The cut-off level for the titrations was arbitrarily set at $\log -0.5$, i.e. an OD value of 0.3. The titres were expressed as arbitrary EIA units (EU ml^{-1}), obtained at the intersect of the serum titration curve with the cut-off level. The sensitivity of the EIA was arbitrarily set at $<0.003\text{ EU ml}^{-1}$ ($\log -2.5$), but, as seen from sample 1 in Figure 1, the titration curve of a negative serum does not intersect the horizontal line at this value, which is why a theoretical higher sensitivity could be achieved. The upper limit of detection was 199.5 EU ml^{-1} in the study, representing the highest value among the tested samples, but on theoretical grounds the assay has no upper limit of sensitivity. For some comparisons of results by EIA and RIA, the difference in the range of determinations was adjusted by setting the same limits for EIA as observed for RIA, i.e. at 0.1 and 17.5.

Radioimmunoassay (RIA)

The modified Phadebas RASTTM (Pharmacia, Uppsala, Sweden) was used for measurement of IgE to diphtheria toxin and tetanus toxoid by radioimmunoassay (RIA), as described in detail previously^{2,3}. Briefly, each toxin/toxoid was coupled to CnBr-activated discs by the Special Allergen Service, Pharmacia, Uppsala. Sera were diluted 1:2 and tested in duplicate, extending the incubation time to 24 h under continuous shaking. The detection range was between 0.1 Phadebas RAST Units (PRU) per ml and 17.5 PRU ml^{-1} .

Serum material

The serum material consisted of pre- and post-booster samples of 104 school-children at 10 years of age, who received a regular, aluminium-adsorbed diphtheria-tetanus (DT) vaccine or an experimental, non-adsorbed (fluid) DT vaccine⁶. The sera had previously been analyzed by RIA and the IgE levels correlated to local side effects⁷.

Statistical analysis

Comparisons of titres obtained in the two assays and in the two vaccine groups was made by Mann-Whitney U test.

RESULTS

Figure 2 shows the comparison of the IgE concentrations in the 208 samples to diphtheria toxin and to

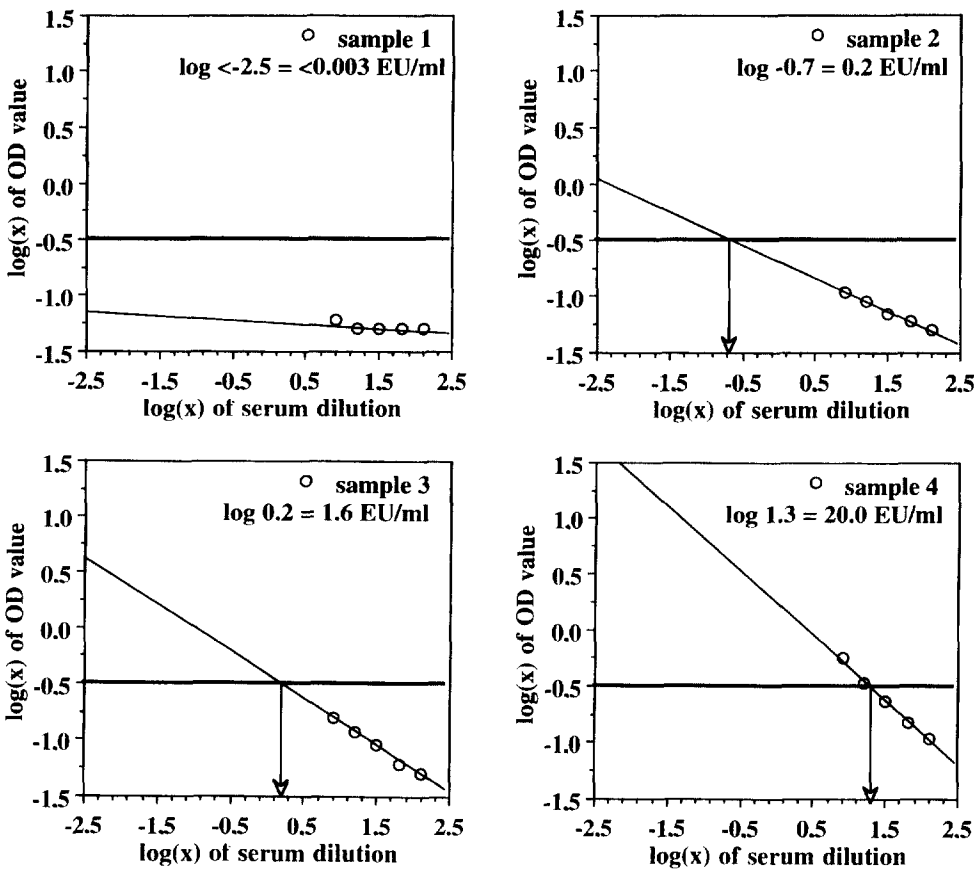


Figure 1 Example of IgE determinations to diphtheria toxin or tetanus toxoid in clinical samples. Sample 1 has no detectable IgE, samples 2 and 3 have moderately elevated concentrations and sample 4 has a high IgE concentration. The arbitrary cut-off level, shown as a horizontal line, was set at the optical density (A_{405}) of $\log -0.5$ (= OD of 0.3)

tetanus toxoid. The correlation coefficient for IgE to diphtheria toxin by both methods was $r = 0.87$, $P = 0.001$ and for tetanus toxoid $r = 0.78$, $P = 0.001$. For adjusted EIA values (lower part of Figure 2), the corresponding data were $r = 0.88$, $P = 0.001$ and $r = 0.80$, $P = 0.001$, respectively. Concordance between the determinations by EIA and RIA is shown in Table 1. Of the 59/416 (14%) discordant determinations, 41 represented positive values by EIA but negative by RIA.

The median IgE concentration in the 104 post-booster sera to diphtheria toxin by EIA was

4.0 EU ml⁻¹ (range <0.003–199.5) and by RIA was 1.8 PRU ml⁻¹ (<0.1–≥17.5). The corresponding data for IgE to tetanus toxoid were 4.0 EU ml⁻¹ (<0.003–177.8) and 0.4 PRU ml⁻¹ (<0.1–≥17.5). A comparison of EIA and RIA with the same cut-off levels showed no significant difference between the results obtained by the two methods for diphtheria ($P = 0.63$), but a significant ($P = 0.04$) difference was detected for tetanus.

When analyzing the responses by type of vaccine, the median post-booster IgE to diphtheria toxin in the adsorbed vaccine group ($n = 51$) was by EIA

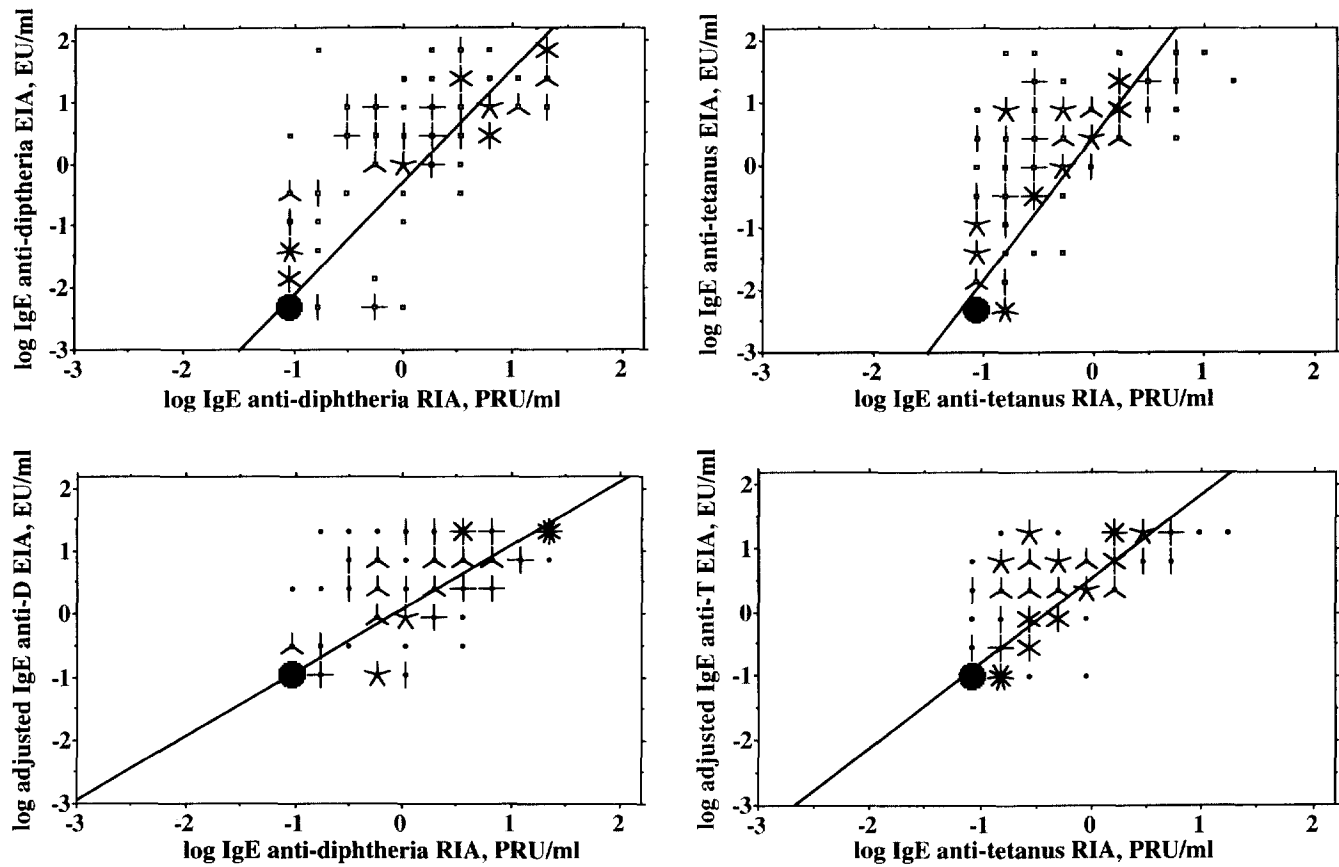


Figure 2 Correlation between IgE concentrations determined by EIA and RIA to diphtheria toxin (left side) and tetanus toxoid (right side) in 208 serum samples. The upper parts of the figure compare the full range of IgE values by EIA to RIA, while the lower parts compare adjusted EIA values, i.e. the lower and upper limits of detection in EIA were set at the same values as for RIA. The size and number of symbols correspond to the number of overlapping observations. A single sample corresponds to a dot, two or more overlapping samples are represented by the corresponding number of lines, while the large number of overlapping, negative values is shown as a filled circle

Table 1 Concordance between RIA and EIA for IgE to diphtheria toxin and tetanus toxoid in 208 sera

	Positive IgE to diphtheria toxin by RIA (≥0.1 PRU ml ⁻¹)	Negative IgE to diphtheria toxin by RIA (<0.1 PRU ml ⁻¹)
Positive IgE to diphtheria toxin by EIA (≥0.003 EU ml ⁻¹)	94	20
Negative IgE to diphtheria toxin by EIA (<0.003 EU ml ⁻¹)	6	88
	Positive IgE to tetanus toxin by RIA (≥0.1 PRU ml ⁻¹)	Negative IgE to tetanus toxin by RIA (<0.1 PRU ml ⁻¹)
Positive IgE to tetanus toxin by EIA (≥0.003 EU ml ⁻¹)	100	21
Negative IgE to tetanus toxin by EIA (<0.003 EU ml ⁻¹)	12	75

2.2 EU ml⁻¹ (<0.003–79.4) and by RIA 1.9 PRU ml⁻¹ (<0.1–≥17.5). In the non-adsorbed vaccine group ($n = 53$), the median post-booster IgE to diphtheria toxin was 5.0 EU ml⁻¹ (<0.003–199.5) and 1.6 PRU ml⁻¹ (<0.1–≥17.5), respectively. The corresponding data for IgE to tetanus toxoid were in the adsorbed vaccine group 5.0 EU ml⁻¹ (range <0.003–177.8) and 0.3 PRU ml⁻¹ (<0.1–8.2), and in the non-adsorbed vaccine group 3.5 EU ml⁻¹ (<0.003–31.6) and 0.6 PRU ml⁻¹ (<0.1–≥17.5), respectively. For diphtheria, the difference between the groups was not significant by either EIA or RIA. For tetanus, however, the difference between the groups was significant by RIA ($P = 0.01$), but not by EIA (0.34).

Weak but significant correlations between IgE in postbooster samples to diphtheria toxoid and immediate pain at the site of injection ($r = 0.2$, $P = 0.019$) and between IgE in postbooster samples to tetanus toxoid and duration of swelling/redness ($r = 0.20$, $P = 0.019$) were noted by RIA, but not observed when the comparison was made by EIA (P values of 0.71 and 0.39, respectively).

DISCUSSION

The capture EIA and the RIA gave very similar results, indicating that IgG antibodies did not significantly interfere with IgE determination by RIA. The few discordant determinations indicate, however, that competition between antibody classes in RIA can occasionally lead to negative results. The determinations of IgE to diphtheria toxin were similar by the two assays. For tetanus toxoid, a significant difference between the assays was found, RIA underestimating IgE concentrations as compared to EIA. A likely explanation for these differences is a less efficient coupling of a toxoid (tetanus) as compared to a toxin (diphtheria) to the disks.

The lack of a difference in IgE responses between the two vaccine groups by EIA corresponds well with the lack of differences in local side effects to the two vaccines⁶. Likewise, the weak correlations noted between some local side effects and post-booster IgE antibody responses by RIA⁷ were not found by EIA. The EIA allowed for the determination of IgE responses by only a few serum dilutions over a wider

range of concentrations than RIA, showing that the capture EIA represents a useful alternative to RIA for measurement of IgE responses to vaccines.

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