

Latex allergosorbent test (LAST): A new immunoassay for specific IgE with latex particles

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We describe a two-step latex (Lx) agglutination assay for the titration of specific anti-Dermatophagoides pteronyssinus IgE. The samples are first incubated with allergen-coated Lx of 2.3 μm diameter. Bound IgE is digested by pepsin and then titrated by its agglutinating activity on 0.8 μm Lx particles coated with antihuman Fc, rabbit F(ab')₂. This latex allergosorbent test detects 100 pg of specific IgE per milliliter and does not depend on the concentration of total IgE. Owing to a tenfold increase in the allergosorbent surface, no competition with the binding of specific anti-D. pteronyssinus IgG is observed. Pepsin digestion eliminates potential interferences caused by autoantibodies against IgE. A good correlation ($r = 0.92$) is found with Phadebas RAST on a series of 91 samples. The latex allergosorbent test does not make use of radioisotopes and can be performed in <6 hours. (J ALLERGY CLIN IMMUNOL 1988;82:35-9.)

Titration of serum-specific IgE has been extensively performed since 1972 with great improvement in the diagnostic accuracy of immediate hypersensitivity. The Phadebas RAST (Pharmacia, Uppsala, Sweden), first described by Wide et al.¹ is still the method of choice, even if this assay suffers from early recognized interferences.²

In recent years, however, the need for a more sensitive assay has arisen, both in clinical practice and experimentation. Consequently, modified RAST³ and, more recently, fluoroallergosorbent tests⁴ have been proposed. We describe here an Lx agglutination assay with improved specificity and sensitivity for specific-IgE measurement compared to the available assays.

MATERIAL AND METHODS

Reagents and buffers

The following reagents were purchased: Lx-0.8 μm , 10% wt/vol, with carboxyl groups (Estapor K1.080) and Lx-2.3 μm (L200), from Rhône-Poulenc, Courbevoie, France; 1-ethyl-3(3-dimethyl-amino-propyl) carbodiimide HCl from Sigma Chemical Co., St. Louis, Mo.; Tween 20 from Tech-

Abbreviations used

BSA:	Bovine serum albumin
BBS:	Borate-buffered saline, 20 mmol/L, pH 8.2
DPT:	<i>Dermatophagoides pteronyssinus</i>
GBS:	Glycine-buffered saline, 50 mmol/L, pH 9.2
GBS-BSA:	GBS containing 1% BSA
GBS-Tween:	GBS containing 0.1% Tween 20
LAST:	Latex allergosorbent test
Lx:	Latex particles
PBS:	Phosphate-buffered saline, 8 mmol/L, pH 7.4
Lx-2.3:	Latex particles of 2.3 μm in diameter
Lx-0.8:	Latex particles of 0.8 μm in diameter
CV:	Coefficient of variation

nicon, Tarrytown, N. Y.; normal rabbit serum from Marloie, Belgium; and BSA and IgE standard serum from Calbiochem, La Jolla, Calif.

Buffers were BBS with 9 gm/L of NaCl and 20 mmol/L of borate, pH 8.2; GBS with 9 gm/L of NaCl, 50 mmol/L of glycine, pH 9.2; GBS-Tween; GBS-BSA; GBS containing 10 or 20 gr/L of dextran T-500 (Pharmacia), and PBS with 9 gm/L of NaCl and 8 mmol/L of phosphate, pH 7.4.

Allergen preparation and coupling

Glycerinated-allergen extract of DPT from Bencard Ltd. (Epsom, Surrey, England) was extensively dialyzed at 4° C

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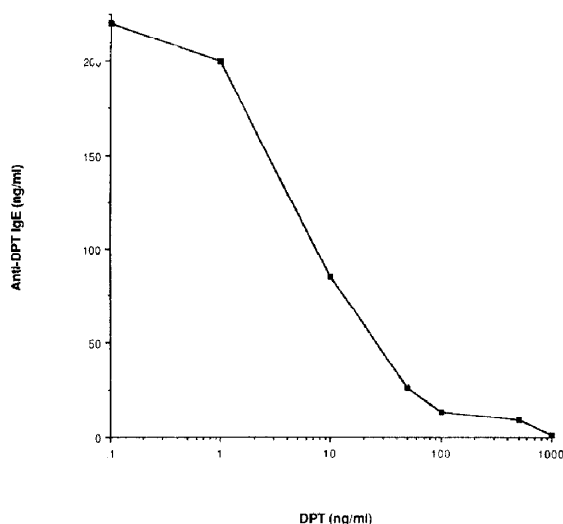


FIG. 1. DPT diluted from 1000 ng/ml to 1 ng/ml was added (vol/vol) to a serum sample containing 220 ng specific anti-DPT IgE per milliliter and the mixture was incubated for 4 hours with DPT-coated Lx-2.3. After washing and centrifugation, IgE bound to Lx-particles was digested with pepsin and titrated by its agglutinating activity on Lx-0.8 coated with rabbit antihuman F_c , $F(ab')_2$.

against PBS containing 1000 units of aprotinin per milliliter and concentrated on an YM-10 ultrafiltration membrane (Amicon Corp., Danvers, Mass.). The solutions were kept at -80°C until just before use. One hundred micrograms of DPT was labeled with ^{125}I by the chloramine-T method.⁵

DPT was insolubilized on cyanogen bromide-activated paper disks (0.6 cm diameter, 541 filter paper, Whatman Ltd., Maidstone, Kent, England).⁶ A mean of 7 μg of allergen was bound to each disk.

Preparation of Lx particles

Lx-2.3 was used as an allergosorbent. One hundred microliters of a 10% (wt/vol) suspension was washed in BBS and resuspended in 200 μl of the same buffer; 100 μl was retrieved and mixed with 100 μl of a 10 mg/ml of carbodiimide solution in water. After a 5-minute incubation at 21°C , 10 μl of a 10 mg/ml of allergen solution in BBS was added. The final volume was adjusted to 1 ml with water, and the suspension was further incubated at 21°C for 4 hours while it was vortexed. After centrifugation at 10,000 g for 5 minutes, Lx-2.3 was resuspended by a short sonication (model B12 sonifier, Branson, Danbury, Conn.), followed by centrifugation successively in GBS-BSA, GBS-Tween 20, and twice in GBS-BSA. The pellet was finally resuspended in 1 ml of GBS-BSA, sonicated, and kept at 4°C . The amount of allergen coupled on Lx-2.3 was estimated by adding a trace amount of radio-labeled DPT during the coupling procedure. After washing and centrifugation, the radioactivity was counted in the pellet. A mean of 108 μg of allergen was coupled per 50 μl of Lx 10%, which corresponded to 5.4 μg of DPT per assay.

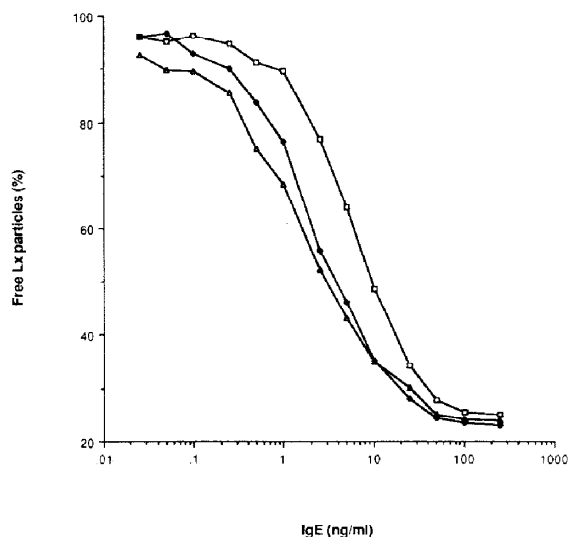


FIG. 2. A serum sample was incubated at concentrations of specific anti-DPT IgE ranging from 0.025 ng/ml to 250 ng/ml with DPT-coated Lx-2.3 for 4 hours at 37°C . After washing and centrifugation, IgE bound to Lx particles was digested with pepsin and titrated by its agglutinating activity on Lx-0.8 coated with rabbit antihuman F_c , $F(ab')_2$. Residual nonagglutinated particles were counted optically. Dextran (1% or 2%) was added to increase the Lx agglutination; agglutination of Lx without dextran (□); with 1% dextran (◆); with 2% dextran (Δ).

Lx-0.8 was coated with $F(ab')_2$ fragments obtained by pepsin digestion of antihuman F_c , rabbit IgG.⁷

RAST

Fifty microliters of serum sample was added to allergen-coated disks that were then incubated for 3 hours at 21°C . The disks were washed with 9 gr/L of NaCl, containing 1% Tween 20, and further incubated overnight at 21°C in the presence of 50 μl (50,000 cpm) of ^{125}I -labeled rabbit antihuman F_c (Pharmacia). After three washings with the same buffer, the radioactivity associated with the disk was counted.

In some experiments, we used the commercially available DPT-coated disks (Phadebas RAST) and expressed the results in classes by reference to an antibirch-pollen standard serum, according to the manufacturer's instructions.

Reference serum

Serum of a DPT-sensitive patient was fully absorbed onto insolubilized DPT, and specific anti-DPT IgG and IgE concentrations were calculated from the amount of IgG and IgE recovered by elution.⁸ Specificity of the eluted antibodies was first evaluated by direct binding to DPT-coated microtitration plates; 83% of eluted IgG and 87% of eluted IgE, respectively, were capable of binding to DPT. The eluate, which contained $>85\%$ IgG, was used as a source of specific anti-DPT IgG. In some assays, it was added at concentrations varying from 0.4 to 50 $\mu\text{g}/\text{ml}$ to the reference serum.

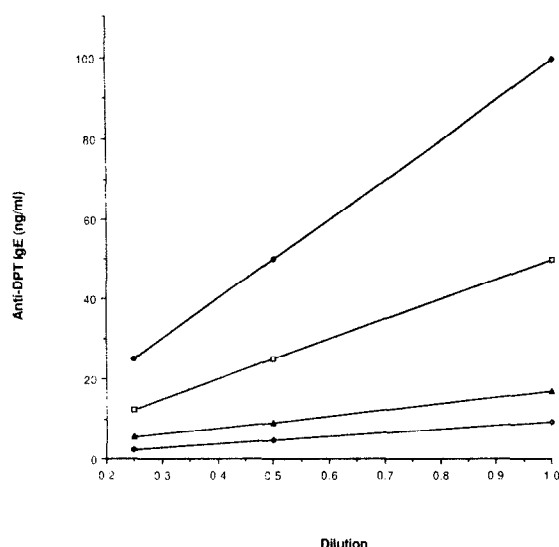


FIG. 3. Four sera with specific anti-DPT IgE ranging from 100 ng/ml to 9.5 ng/ml were incubated at three different dilutions with DPT-coated Lx. Titration of IgE was made after pepsin digestion as in Fig. 2.

LAST

One hundred microliters of the sample, of the reference serum, or of normal rabbit serum as negative control was incubated in a microtest tube 3811 (Eppendorf, Hamburg, Federal Republic of Germany) with 50 μ l of allergen-coated Lx-2.3 (0.5% suspension in GBS) for 4 hours at 37° C, with continuous shaking. The suspension was then centrifuged for 2 minutes at 10,000 revolutions per minute in a Biofuge A (Heraeus Christ, F.R.G.); the pellet was washed with 1 ml of 9 gm/L of NaCl and resuspended in 50 μ l of the same solution. Bound IgE was then digested for 30 minutes at 21° C by addition of 1 mg/ml of pepsin in 80 μ l of 0.15 N HCl; 20 μ l of 2 mol/L of Tris-NaOH was then added to increase the pH.

Thirty microliters of the suspension was mixed with 30 μ l of antihuman Fc_ε Lx-0.8 (0.04% suspension in GBS), and incubated for 30 minutes at 37° C, with continuous shaking. Agglutination was measured by instrumental counting of the residual nonagglutinated particles in the IMPACT system (Acade Diagnostic Systems s.a., Brussels, Belgium), which has been fully described.⁹ Fifty microliters of twofold dilutions of a standard IgE solution in GBS containing 5% BSA was digested with pepsin, as described above, and used to construct a reference curve. Addition of allergen-coated Lx-2.3 did not interfere with this curve. To compare the results of the LAST with the commercially available RAST, we arbitrarily defined five classes in the Lx assay, that is, 0, <0.5 ng/ml; 1+, 0.5 to 2 ng/ml; 2+, 2 to 7.5 ng/ml; 3+, 7.5 to 25 ng/ml, and 4+, >25 ng/ml. Allergen inhibition was performed by mixing 50 μ l of the reference serum and 50 μ l of DPT diluted from 1000 ng/ml to 1 ng/ml in normal rabbit serum just before addition of 50 μ l of Lx-2.3.

RESULTS

Assay specificity

Pepsin digestion of IgE generates Fc_ε fragments, including Fc_ε'' or D_ε1 determinants, which can be titrated by their agglutinating activity on Lx particles coated with rabbit antihuman Fc_ε F(ab')₂. This assay has already been fully evaluated in our laboratory.¹⁰

The allergen specificity was estimated in two ways. First, the addition of soluble DPT during incubation of the samples with Lx-2.3 reduced the amount of detectable IgE (Fig. 1). One microgram (total protein content) of soluble DPT was sufficient to inhibit completely the binding of 220 ng of specific anti-DPT IgE. Second, the addition of 250 ng of polyclonal nonspecific IgE to a serum containing 16 ng/ml of anti-DPT IgE did not affect the amount of anti-DPT IgE detected (data not presented).

Since specific anti-DPT IgG could competitively inhibit the binding of IgE to Lx-2.3, we have added increasing amounts of purified anti-DPT IgG to the reference serum. We observed no competition even with 120 times more IgG than IgE (data not presented).

Assay sensitivity

Twofold dilutions of the reference serum were mixed with Lx-2.3. Representative curves illustrated in Fig. 2 demonstrate the detection of specific anti-DPT IgE from 25 pg/ml to 250 ng/ml, with a maximum sensitivity between 1 and 100 ng/ml. The addition of 1% or 2% dextran to increase Lx agglutination lowers the detection level to 100 pg/ml, corresponding to 2 SDs of the mean agglutination obtained with normal rabbit serum.

Assay linearity and reproducibility

The dilution of a sample had no effect on the titration of IgE (Fig. 3). The intra-assay CVs were calculated by making, on a single day, 30 measurements in each of three samples containing low (1.5 ng/ml), mid-range (57 ng/ml), and high (80 ng/ml) specific IgE concentrations. The CVs were 14.7%, 2.9%, and 1.4%, respectively.

The interassay variation was estimated by titrating specific IgE in three dilutions of a serum (3, 7, and 30 ng/ml of specific IgE) on 15 consecutive days. The CVs were, respectively, 16.7%, 16.8%, and 4.8%.

LAST-RAST correlation

We estimated the correlation coefficient between LAST and RAST on 91 serum samples. For this purpose, we defined arbitrarily different classes in our Lx assay. The correlation coefficient was $r = 0.92$.

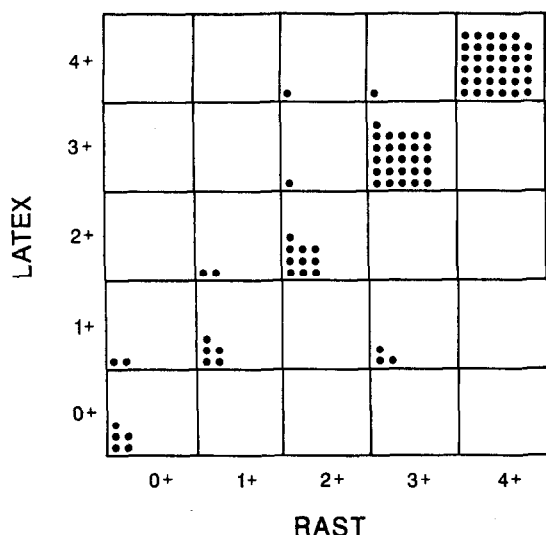


FIG. 4. Specific anti-DPT IgE was titrated in 91 serum samples by LAST and RAST. Different classes were arbitrarily defined for the Lx particle assay and were compared to RAST classes. Both assays were performed as described in Material and Methods. The correlation coefficient was 0.92 with a linear regression line defined as $y = 0.91(x) + 0.29$.

($p < 0.001$), and the linear regression was defined by $Y = 0.29 + 0.91(X)$ (Fig. 4). Eighty-nine percent of the samples was class identical, and 6.6% differed by only one class.

As the antigenic content of our preparation could differ from the DPT used in the Phadebas RAST, we coupled our DPT solution on cyanogen bromide-activated paper disks. The relationship between the IgE titrated by Lx agglutination and the radioactivity bound to the disks is illustrated in Fig. 5. All values <1000 cpm (corresponding to 750 pg/ml) were less than the background level of RAST assay and therefore excluded. The correlation coefficient calculated on 71 samples was $r = 0.83$ ($p < 0.001$) with a linear regression defined by $Y = 1.86 + 1.1 \times 10^{-4}(X)$.

DISCUSSION

Specific IgE titration has been widely used in the assessment of allergic diseases, since the introduction of RAST.¹ Its usefulness as a diagnostic tool has been recently reviewed.¹¹ However, the low antibody-binding capacity of RAST and the relatively high degree of protein nonspecific adsorption have hampered its use. We describe here a quantitative immunoassay for specific anti-DPT IgE. This assay can be compared favorably to RAST in its sensitivity and ease of performance. It has already been successfully applied to other allergens, such as *D. farinae* and grass pollens.

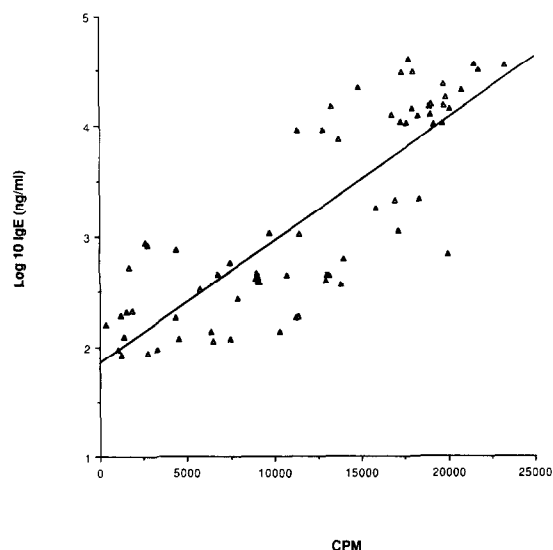


FIG. 5. Specific anti-DPT IgE was titrated in 71 serum samples with LAST and with an allergosorbent test prepared with the same DPT solution. Both assays were performed as in Material and Methods. The correlation coefficient was 0.83 with a linear regression line defined by $y = 1.1 \times 10^{-4}(x) + 1.86$.

A low degree of nonspecific adsorption of proteins on Lx particles, as exemplified by the absence of interference in the assay when the concentration of non-specific IgE was increased, allows good specificity and sensitivity. The addition of soluble allergen completely inhibits the binding of IgE to Lx particles, irrespective of total IgE concentration. Titers as low as 100 pg/ml specific IgE (0.04 IU/ml) can be determined repeatedly with acceptable variation coefficients. This sensitivity increases with the incubation time, without significant modification of the nonspecific protein adsorption; for instance, a 5-hour incubation lowers the sensitivity to <50 pg/ml (data not presented). Such a sensitivity can also be obtained by addition of dextran.¹² This should permit the detection of specific IgE in fluids other than serum, such as bronchoalveolar lavages, and provide a useful research tool, allowing, for instance, titration of specific IgE in cell supernatants. For routine specific IgE titration, however, the assay can be performed in a short time, usually 4 hours, and can be automated. Modifications of RAST³ were intended to improve the sensitivity; however, a prolonged incubation time often leads to an increase in nonspecific IgE binding. The IgE fluoroallergosorbent test is considered to be more sensitive than RAST,⁴ but this assay needs further assessment, since the fine specificity of the monoclonal anti-IgE antibody might critically influence the IgE titration.¹³

The allergen-coated Lx particle offers an exterior surface 10 times larger than the cellulose disk used in the RAST, but the porous nature of the latter is likely to increase the surface accessible for coupling. Indeed, the amount of insolubilized allergen is of the same order of magnitude on both surfaces. The amount of specific antibodies bound to the Lx particle is nevertheless four to five times greater than on the RAST (data not presented). This may be due to an easier access of the antibody to the insolubilized allergen by reduction in steric hindrance or may result from the coupling procedure. Indeed, instead of cyanogen bromide activation as in RAST, we used carbodiimide-activated particles, which is preferable for preservation of the IgE-binding epitopes of the allergen.⁸ We demonstrate here that specific antiallergen IgG does not interfere with LAST, even when it is added in large excess over specific IgE. Other authors have demonstrated that this is not the case with RAST.¹⁴

Specific IgE is expressed quantitatively in our assay. Its increased sensitivity allows for a sharper distinction between atopic and nonatopic individuals. This is especially useful when the clinical symptoms suggest an IgE-mediated reaction but specific IgE is too low to be detected by RAST. Pepsin digestion also eliminates the risk of interferences that could arise from autoantibodies directed against IgE.¹⁵ Moreover, as a standard IgE solution is used for every specific IgE assay, patients with polyspecific IgE immune responses could be more accurately evaluated. The polystyrene tube radioimmunoassay^{14, 16} is quantitative and not influenced by specific immunoglobulins of other isotypes. However, its sensitivity is not better than that of RAST, and its handling is long and somewhat complex.

In both LAST and RAST, the same parameter was measured as could be observed from the 81% linearity between the assays. With paper disks prepared with the same allergen as on the Lx particles, the relationship between Lx and RAST, using a continuous scale, demonstrated a 69% linearity, once again suggesting that the coupling procedure might affect in a different way the allergen accessibility. Centrifugation of allergen-coated Lx-2.3 after its incubation with serum generated sometimes a brittle pellet, requiring some skill to retrieve the supernatant. This difficulty, never encountered after pepsin digestion, was easily overcome with training.

The Lx assay for the titration of specific IgE is a versatile tool for both clinical and research purposes because it elicits a fast, sensitive, and quantitative titration of IgE.

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