

Aeroallergen analyses and their clinical relevance

I. Immunochemical quantification of allergens by RAST-inhibition, Mab-ELISA, basophil histamine release, and counter current immuno electrophoresis

C.R. JOHNSEN¹, L. ABRAHAMSEN¹, P. STAHL SKOV^{1,2}, N. JOHANSEN³
& L.K. POULSEN¹

National University Hospital, ¹Laboratory of Medical Allergology TTA 7542, and ²Reference Laboratory, Copenhagen, Denmark, ³ALK Laboratories, Product Development Department, Hørsholm, Denmark

The aim was to compare IgE and IgG₄ RAST-inhibition assay (RI), monoclonal antibody ELISA (Mab-ELISA), counter current immuno electrophoresis (CCIE) and histamine release from basophil leukocytes (HR) for allergen quantification with special reference to aeroallergen detection. As components of indoor aeroallergens, cat, dog, and *Derm. pter.* allergen extracts were selected for the experiments. To evaluate unspecific interference, these allergens were compared mutually and with *Cladosporium herbarum*. Allergen extracts in varying dilutions were mixed with crushed glass fibre filter materials, eluted, recovered by centrifugation, and allergen concentration quantified by the assays. Equal sensitivity was found for both IgE- and IgG₄-RI assaying cat allergen (in the range 5 - 50 SQ-U/ml) and dog allergen (in the range 10² - 10³ SQ-U/ml). The IgG₄-RI assaying *Derm. pter.* was more sensitive (50 SQ-U/ml) than IgE-RI (2*10³ SQ-U/ml). The ranges of allergen detection limits for the Mab-ELISA were equal for cat and *Derm. pter.* (10 - 10² SQ-U/ml). The range of allergen detection limits for CCIE, assaying dog were 10⁴ - 10⁵ SQ-U/ml. The ranges of allergen detection limits for HR were equal for cat and *Derm. pter.* (10 - 10² SQ-U/ml), and 10² - 10³ SQ-U/ML for dog. Because of cross-reactivity, a minor degree of interference was observed in the IgE-RI and the HR test for the highest concentration of cat and dog allergens.

Key words: aeroallergens; allergen analysis; immunochemical methods; cat; dog; house dust mite.

Accepted for publication 6 January 1991

Most environmental studies detect allergens in dust samples. The techniques used are morphological detection of pollen grains, moulds, and house dust mites or immunochemical quantification of all kind of allergens. Several immunological methods for allergen detection are available today, and many investigations have been made to identify and quantify allergens in the indoor and outdoor environment. The methods used are mostly RAST-inhibition (RI) assays, different immunoelectrophoretic techniques, and enzyme linked immuno sor-

bent assays (ELISA). The aim of this study was to compare 1) IgE- and 2) IgG₄-RI assay with sera from allergic patients, 3) monoclonal antibody ELISA (Mab-ELISA), 4) counter current immuno electrophoresis (CCIE), and 5) histamine release from basophil leukocytes (HR) for allergen analysis with special reference to aeroallergen quantification. These data represent part of a study describing different methods of allergen quantification. The indoor aeroallergens, cat, dog, and the house dust mite *Dermatophagoides pteronyssinus* (*Derm. pter.*)

were selected for the experiments. To evaluate unspecific interference, these three allergens were compared mutually and with *Cladosporium herbarum* (*Clad. her.*) (4). The methods of analysis were evaluated for quantification of allergens sampled on glass fibre filter sheets with special attention to the application of different air sampler devices collecting allergens on filter sheets.

MATERIAL AND METHODS

Chemicals

Buffer for allergen coating: (PBS) = PBS buffer pH 7.4 (sodium phosphate 10 mmol/l, sodium chloride 150 mmol/l, sodium azide 100 mg/l). Washing buffer: (PBST) = PBS added with Tween 20 0.01% v/v. Allergen diluent: (PBSE); PBS added with ϵ -amino-n-caproic acid 0.1 mol/l. Glassfibre filter sheet elution buffer: (STE) = Sørensen phosphate buffer 1/15 pH 7.0, sodium azide 200 mg/l, Tween 20 0.5% v/v, ϵ -amino-n-caproic acid 0.1 mol/l. Mab-ELISA substrate: (HPO/PD) = Substrate HPO/PD (0.1 ml of 3 mmol/l hydrogenperoxide and 5 mmol/l ortophenylene diamine in 0.1 mol/l sodium-citrate buffer, pH 5.0) Histamine release buffer: (PIPES) = Pipes-AMC pH 7.4 (piperazine-N,N (bis-ethane sulphonic acid) 10 mmol/l, sodium acetate 140 μ mol/l, potassium acetate 5 mmol/l, CaCl_2 0.6 mmol/l, MgCl_2 1.1 mmol/l). Histamine fluorometric reagent: (OPT) = o-phthalaldehyde. NaOH/OPT (2 mg of OPT dissolved in 1 ml of methanol and 9 ml of NaOH 0.05 mol/l). All chemicals were of analytical grade.

Allergens

Lyophilised allergen extracts of cat (Aquagen SQ-U 555) and dog (Aquagen SQ-U 553) epithelium and *Dermatophagoides pteronyssinus* (Aquagen SQ-U 503) obtained from ALK, Hørsholm, Denmark were reconstituted in a PBSE buffer to a stock solution of 1 mio SQ-U/ml. Lyophilised allergen extract of *Clad.*

dorsporium herbarum (*Clad. her.*) (Pharmalgen m2) obtained from Pharmacia AB, Uppsala, Sweden, was reconstituted in PBSE buffer to a stock solution of 100,000 BU/ml.

Model for elution from air sampler filter materials

We selected a model for elution of allergens from glass fibre filter sheets with the purpose of quantifying aeroallergens which could be used with different air sampler devices.

Preliminary studies have demonstrated a 10-fold increased recovery, when:

- a) allergen dilutions were mixed with crushed glass fibre filters followed by overnight elution; instead of;
- b) allergen dilutions were sprayed onto filter sheets with subsequent drying of allergens on the filters, crushing of the filter material and finally overnight elution of allergens from the glass fibres.

However, since the above-mentioned method a) is a more convenient and less variable method, it was selected for these experiments to evaluate the different techniques for detection of allergens. The advantage of this methodology was that the difference in allergen recovery from filters was eliminated so that a comparison of allergen analysing methods became possible. The actual recovery as determined by RAST-inhibition and ELISA was more than 95% when analysing the allergen/filter mixtures in comparison with dilutions of allergen stock extracts (data not shown). The presence of Tween in the solution mixture of allergens and powdered filter materials may explain the increased recovery compared with the method of eluting allergens from intact filters.

Two types of glass fibre filter sheet were used in the experiment:

- 1) Whatman EPM 2,000. Size 20 $\times$ 25 cm was punched to a diameter of 80 mm with an average weight of: 443 ± 6 mg;
- 2) Gelman Sciences A/E with a diameter of 37 mm with an average weight of: 72.6 ± 0.5 mg.

The Whatman filter sheets were folded twice, and the Gelman Sciences filter sheets once, enclosed in vellum envelopes and crushed in a hydraulic press (Compac 10, Juelsminde, Denmark) by 500 kg/cm². The powdered filter material was transferred to 5 ml test tubes, and 2 ml dilutions of allergen extracts in STE buffer were added to tubes containing pulverized 37 mm filter sheets, and 2.5 ml dilutions were added to test tubes containing 80 mm filter sheets. Under vertical rotation, this mixture was incubated at room temperature overnight. After this single incubation the soluble allergens were recovered by centrifugation (3,000 g for 10 min).

Varying allergen dilutions (10-fold) and pulverized filter material (37 and 80 mm) were prepared using the allergens cat, dog, house dust mite and *Clad. her.* All preparations were made in duplicate in STE buffer. For each of the allergens to be tested, either cat, dog, or *Derm. pter.*, two dilutions of the irrelevant allergens plus *Clad. her.* were prepared in order to evaluate unspecific reactions for each of the analyses, IgE-RI Mab-ELISA, HR, and CCIE. The design of the study is shown in Table 1.

IgG subclass Maxisorp-RI

Sera from patients allergic to either cat, dog or house dust mite and undergoing maintenance

immunotherapy of at least 6 month's duration with the allergens in question were selected. High titres of specific IgG₄-antibodies are characterizing sera from patients undergoing immunotherapy (1, 18). Several sera were evaluated for content of IgE₁₋₄ antibodies. A serum pool of 2 sera for cat (DJ/KA) and *Derm. pter.* (BM/HF), and 1 serum for dog (BA) containing high titres of IgG₄ were selected for the optimization of the allergen coating and serum concentrations in the RI assay (data not presented). A pool of RAST negative sera from blood donors was used as normal human sera controls (NHS). RAST inhibition was performed at 4°C by overnight incubation of the IgG₄ reference serum/sera pools with allergen dilutions in PBS. Reference sera were diluted 1:500 (cat) or 1:100 (dog and *Derm. pter.*) and allergen/antibody complexes were centrifuged at 3000 g for 20 min. Subsequently, the inhibition mixtures were transferred to test tubes and analysed in the IgG₄ RAST according to Djurup et al. (1).

Briefly, Maxisorp test tubes (NUNC, Roskilde, Denmark) were coated by addition of 500 µl of allergen diluted in PBS (cat 83 SQ-U/ml = 0.25 µg/ml, dog 1350 SQ-U/ml = 2.7 µg/ml) and incubated at 37°C for 2 h. In the *Derm. pter.* system rabbit anti-house dust mite (1:200), was used as catching antibody. Rabbit anti-house dust mite antibody was prepared by immunization with a mixture

Table 1.

Design of comparison between immunochemical methods for allergen quantification. Allergen extracts in varying dilutions were mixed with crushed glass fibre filter materials and recovered by centrifugation. All experiments were made in duplicate for both 37 and 80 mm filters

Allergen		Cat	Dog	<i>Derm. pter.</i>
Analysis	IgE-RI	X	X	X
	Mab-ELISA	X	ND	X
	HR	X	X	X
	CCIE	ND	X	ND
Primary allergen concentration, SQ-U/ml		10 ² -10 ⁵	10 ² -10 ⁵	10 ² -10 ⁴
	10-fold dilutions	+ Buffer	+ Buffer	+ Buffer
Irrelevant allergen concentration	Cat ^a	NA	10 ² + 10 ⁴	10 ² + 10 ⁴
	Dog ^a	10 ² + 10 ⁴	NA	10 ² + 10 ⁴
	<i>Derm. pter.</i> ^a	10 ² + 10 ⁴	10 ² + 10 ⁴	NA
	<i>Clad. her.</i> ^b	10 ² + 10 ⁴	10 ² + 10 ⁴	10 ² + 10 ⁴

NA = not applicable, ND = not done, ^a Concentration in SQ-U/ml, ^b Concentration in BU/ml.

of *Derm. pter.* and *Dermatophagoides farinae* allergen extracts according to Harboe & Ingild (2). Both allergens were obtained from ALK. *Derm. pter.* allergen (666 SQ-U/ml = 0.66 µg/ml) was added as a second layer. Between each step, test tubes were washed 3 times in PBST.

500 µl of the inhibition supernatant were transferred to allergen-coated test tubes and incubated at 37°C for 2 h. Subsequently, 500 µl of monoclonal anti-human IgG₄ (Oxoid, No. M11013, clone RJ4, 1:32000) were incubated at 37°C for 2 h. Finally, 500 µl of radiolabelled rabbit anti-mouse IgG (1:1000, DAKO No. Z109 purified and radiolabelled as described (13)) were added, and the following day the tubes were washed and counted for 1 min in a gamma scintillation counter. All samples were tested in duplicate, and mean cpm values were transformed to:

$$\% \text{ inhibition} = 100 - 100 \times \left(\frac{\text{sample}}{\text{uninhibited}} \right).$$

IgE-Maxisorp-RI

Sera from patients allergic to cat, dog, or house dust mite and not reacting to *Clad. her.* were selected. The patients all had a positive medical history of reaction to the allergen, a positive skin prick test (SPT), and a RAST class 4 for the allergen in question. Furthermore, they did not have a RAST class exceeding 2 for the concomitant allergens. None of the patients had been undergoing immunotherapy. Sera were controlled and found not to have high titres of blocking IgG₁- or IgG₄-antibodies (data not shown). Sera were characterized by immunoblotting according to Jarolim et al. (3) (data not presented), and a serum pool of 8 sera for cat (Cat-0589) and *Derm. pter.* (Dpt-0589) and 9 for dog (Dog-0589) was selected. For each of the serum pools experiments were made to establish the optimal dilutions of allergens for coating of Maxisorp test tubes and dilution of serum pools for inhibition. NHS was used as serum controls.

RAST-inhibition was performed at 4°C by overnight incubation of 400 µl of the IgE-reference sera pools with 400 µl of allergen dilutions in PBS. Reference sera pools (Cat 0589, Dog 0589, and Dpt 0589) were all diluted 1:50 and allergen/antibody complexes were centrifuged at 3000 g for 20 min. Subsequently, the inhibition mixtures were transferred to test tubes and analysed in the IgE-RAST according to Poulsen et al. (12).

Briefly, Maxisorp test tubes were coated by addition of 300 µl of allergen diluted in PBS (Cat and *Derm. pter.* 1:100, and dog 1:50) and incubated at 4°C overnight. In the *Derm. pter.* system, rabbit anti-house dust mite (1:200), was used as catching antibody, as previously described.

From the inhibition mixture, 2 * 300 µl of the supernatant were transferred to each of 2 allergen coated Maxisorp test tubes and incubated overnight on a vibrator at 20°C. After incubation, 300 µl of anti-IgE¹²⁵I (Pharmacia AB, Uppsala, Sweden), diluted 1:12 (about 10,000 cpm) were added and incubated overnight at 20°C and counted for 5 min in a gamma-scintillation counter. Between each step, test tubes were washed 3 times in PBST. The results were expressed as % inhibition according to the previously given formula.

Mab-ELISA

Samples to be tested were assayed in Mab-ELISA for house dust mite major allergen (Der p1)(5), and for cat major allergen (Fel d1)(6), respectively. Briefly, 100 µl of test sample and dilutions (1:10, 1:100, and 1:1000) were transferred to wells in polystyrene microtitre plates (96-well NUNC-Immuno-Plate Maxisorp F96, Nunc A/S, Roskilde, Denmark) previously coated overnight with 10 µg/ml (0.1 ml/well in 0.1 M sodiumcarbonate buffer) monospecific monoclonal mouse antibodies against house dust mite major allergen (Der p1) or Cat major allergen (Fel d1). After 3 h of incubation and repeated washings with PBST, horseradish peroxidase-conjugated affinity purified rabbit polyclonal antibodies to Der p1 or Fel d1, were added.

After 1 h of incubation and repeated washing with PBST, substrate HPO/PD was added to each well, and reaction was completed in darkness for 15 min. The absorption at 492 nm was read 5 min after addition of 1.0 M/l of sulphuric acid. Standard response curves were constructed (absorbance against concentration in a semilogarithmic plot) for each allergen from reference major allergen extract solutions and unknown samples were read from the curve. Results were given in ng/ml for both Fel d1 and Der p1. It was not possible to perform Mab-ELISA for dog allergens; instead we performed CCIE as mentioned below.

CCIE

Briefly, 5 μ l of polyspecific rabbit anti-dog hair/dander antibodies (ALK Laboratories A/S, Hørsholm, Denmark) rendered specific by immunoabsorption on CNBr-activated sepharose 4B column coupled with human serum protein, were applied in wells of 1:1 mixtures of HSA- and LSA-agarose gel on 5 $\times$ 5 cm glass plates. Five μ l in 2-fold dilutions of the dog allergen samples to be tested were placed in opposite wells on the immunoelectrophoresis plate, and electrophoresis was run overnight at 2V/cm. The plates were pressed, washed, dried, and stained with coomassie brilliant blue, and the concentration of dog allergen was expressed as a titre defined as the reciprocal value of the highest dilution of the dog extract giving a visible precipitate (7).

Histamine release from basophil leukocytes

Three patients were selected for histamine release experiments. The patients were selected based on a positive medical history of allergic reactions to the allergen and a positive skin prick test, RAST, and histamine release to either dog or cat epithelium, or house dust mite. Non-allergic persons were selected as controls. A blood sample of 10 ml was drawn from the patients in heparinized tubes and stored at room temperature for up to 24 h.

Dilutions (PIPES 1:10) of allergens or test samples from filter sheet elution were added in the above concentrations in a volume of 25 μ l to glass microfibre-prepared microtitre plates (16). Thereafter 25 μ l of whole blood samples were added to the plates, which were then incubated at 37°C for 1 h (10). The net release of histamine was calculated from a histamine standard, where blood samples were incubated with 0 and 50 ng of histamine, 2HCl/ml. During incubation, released histamine was bound to the microfibre, and interfering substances were removed by washing the plates twice with deionized water in a SLT-microtitre plate washer. Thereafter, histamine was released from the fibres and coupled to OPT by addition of 75 μ l of NaOH/OPT mixture. After 10 min at room temperature, the coupling reaction was terminated by adding 75 μ l of 0.59% HClO₄. The histamine content was determined in a Gilson spectrofluorometer equipped with a flow through cell and microtitre plate sampler (17). The result, expressed as the amount of released histamine in ng/ml, was plotted against concentration of allergens and showed a bell-shaped curve (e.g. Fig 3C) since both a low and a high allergen concentration can give identical histamine release results.

Clad. her. from stock solutions of 10⁵ BU/ml was diluted 10-fold to account for unspecific reactions resulting in a maximum of 30 ng/ml of release histamine (e.g. Fig. 4C - dog-allergic patient).

Quantification of allergen in an unknown sample cannot be read from the bell-shaped curve. Therefore we made a secondary set-up in which allergen extracts in varying dilutions (PBSE) were mixed with crushed glass fibre filter material, incubated overnight, and recovered by centrifugation. Each sample was then diluted 10-fold with PIPES from 10⁻¹ to 10⁻⁶, and the allergen content examined by HR. The same three donors were used in all experiments. The results are presented in Fig. 1A (cat), Fig. 1B (dog), and Fig. 1C (*Derm. pter.*). Each curve represents the 10-fold dilution of the test sample further diluted with PIPES, as indicated on the x-axis. We define

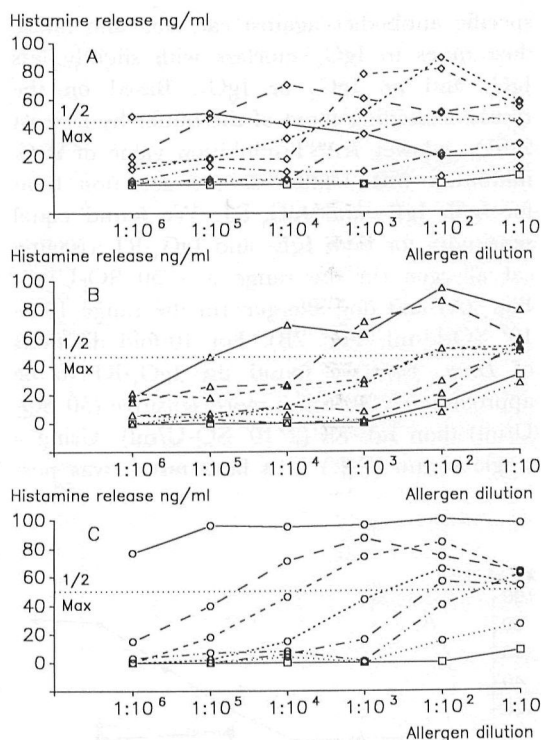


Fig. 1. Relation between 10-fold dilutions of predetermined allergen concentrations in filter mixture eluates or control extract and histamine release in whole blood from an allergic patient. The predetermined allergen concentrations tested in dilutions are:

A. Cat epithelium: stock solution 10⁶ SQ-U/ml ($\diamond$ — $\diamond$); filter mixture eluates in SQ-U/ml: 10⁵ ($\diamond$ — $\diamond$), 10⁴ ($\diamond$ — $\diamond$), 10³ ($\diamond$ — $\diamond$), 10² ($\diamond$ — $\diamond$), 10 ($\diamond$ — $\diamond$), and 1 ($\diamond$ — $\diamond$), and *Clad. her.* filter mixture eluates 10⁴ BU/ml ($\square$ — $\square$).

B. Dog epithelium: stock solution 10⁶ SQ-U/ml ($\triangle$ — $\triangle$); filter mixture eluates in SQ-U/ml: 10⁵ ($\triangle$ — $\triangle$), 10⁴ ($\triangle$ — $\triangle$), 10³ ($\triangle$ — $\triangle$), 10² ($\triangle$ — $\triangle$), 10 ($\triangle$ — $\triangle$), and 1 ($\triangle$ — $\triangle$), and *Clad. her.* filter mixture eluates 10⁴ BU/ml ($\square$ — $\square$).

C. *Derm. pter.* stock solution 10⁶ SQ-U/ml ($\circ$ — $\circ$); filter mixture eluates in SQ-U/ml: 10⁵ ($\circ$ — $\circ$), 10⁴ ($\circ$ — $\circ$), 10³ ($\circ$ — $\circ$), 10² ($\circ$ — $\circ$), 10 ($\circ$ — $\circ$), and 1 ($\circ$ — $\circ$), and *Clad. her.* filter mixture eluates 10⁴ BU/ml ($\square$ — $\square$).

allergen concentration in the test sample with the unknown allergen concentration by interpolation from 10-fold filtration curves of known allergen concentrations, inducing half maximum histamine release. The allergen content in an unknown sample can be quantified by

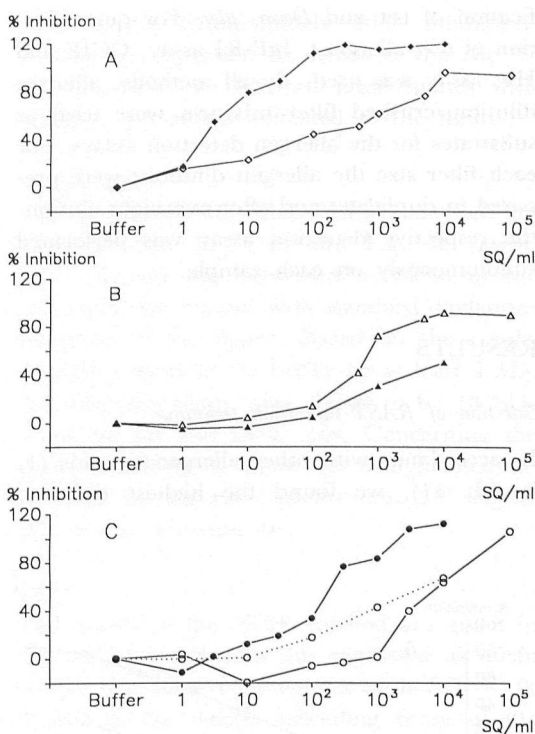


Fig. 2. Comparison between IgE and IgG₄ Maxisorp RAST-inhibition standard curves for:

A. Cat epithelium; IgE-0589 ($\diamond$ — $\diamond$) and IgG₄ ($\blacklozenge$ — $\blacklozenge$) sera pools; B. Dog epithelium; IgE-0589 ($\triangle$ — $\triangle$) and IgG₄ ($\blacktriangle$ — $\blacktriangle$) sera pools; C. *Derm. pter.*; IgE-0589 ($\circ$ — $\circ$), IgE-SJ ($\circ$ — $\circ$) and IgG₄ ($\bullet$ — $\bullet$) sera pools.

diluting it 10-fold from 10⁻¹ to 10⁻⁸ in PIPES, correcting for the dilution factor, and comparing the results of the HR with the curves in Fig. 1A - 1C. Only results representing the lowest allergen concentrations (before the plateau on the curves) should be compared.

Comparisons

To select one assay for RI, a preliminary comparison was made between IgE-RI and IgG-subclass-RI assays using 10-fold dilutions of lyophilised extracts of cat, dog, and *Derm. pter.* diluted in PBSE. Based on this study we continued with a comparison between IgE-RI assay, Mab-ELISA assay, and HR for quanti-

fication of cat and *Derm. pter.* For quantification of dog allergens, IgE-RI assay, CCIE and HR assay was used. By all methods, allergen dilutions/crushed filter mixtures were used as substrates for the allergen detection assays. For each filter size the allergen dilutions were prepared in duplicate, and after overnight elution, the respective detection assay was performed simultaneously on each sample.

RESULTS

Selection of RAST-inhibition technique

In accordance with other allergen systems (1, 9, 13, 14), we found the highest titres of

specific antibodies against cat, dog and house dust mites in IgG₄ subclass with slightly less IgG₁ and no IgG₂ or IgG₃. Based on the results exceeding those of the buffer by at least 2 SD, a lower RAST-inhibition value of 20% inhibition was defined as the detection limit for both IgE- and IgG₄-RI. We found equal sensitivity for both IgE- and IgG₄-RI assaying cat allergen (in the range 5 - 50 SQ-U/ml, Fig. 2A) and dog allergen (in the range 10² - 10³ SQ-U/ml, Fig. 2B). For 10-fold dilutions of *Derm. pter.* we found the IgG₄-RI to be approximately 40 times more sensitive (50 SQ-U/ml) than IgE-RI (2*10³ SQ-U/ml). Using a single serum (S.J.) in a later test it was pos-

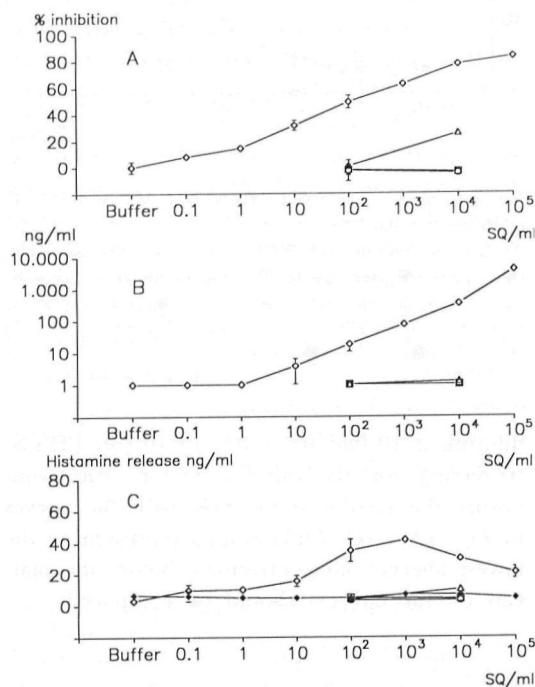


Fig. 3. Comparison between IgE Maxisorp RAST-inhibition (A), Mab-ELISA (B) and HR (C) assaying cat allergen in filter mixture eluates. Predetermined allergen concentrations in SQ-U/ml of cat epithelium (◇—◇), dog epithelium (△—△) and *Derm. pter.* (○—○), and in BU/ml of *Clad. her.* (□—□) were detected. (◆—◆) in C represents HR testing in whole blood from a non-allergic person. Missing error bars indicate SD smaller than line symbol size.

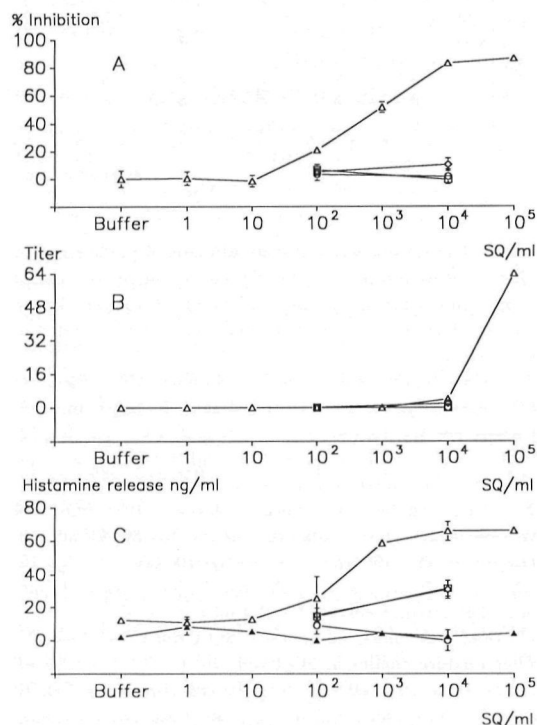


Fig. 4. Comparison between IgE Maxisorp RAST-inhibition (A), CCIE (B), and HR (C) assaying dog allergen in filter mixture eluates. Predetermined allergen concentrations in SQ-U/ml of dog epithelium (△—△), cat epithelium (◇—◇) and *Derm. pter.* (○—○), and in BU/ml of *Clad. her.* (□—□) were detected. (▲—▲) in C represents HR testing in whole blood from a non-allergic person. Missing error bars indicate SD smaller than line symbol size.

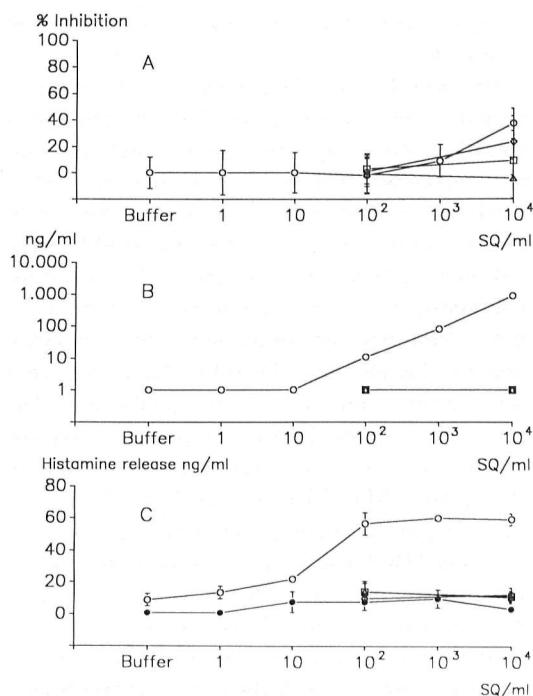


Fig. 5. Comparison between IgE Maxisorp RAST-inhibition (A), Mab-ELISA (B) and HR (C) assaying *Derm. pter.* allergen in filter mixture eluates. Predetermined allergen concentrations in SQ-U/ml of *Derm. pter.* (○—○), cat epithelium (◇—◇) and dog epithelium (△—△), and in BU/ml of *Clad. her.* (□—□) were detected. (●—●) in C represents HR testing in whole blood from a non-allergic person. Missing error bars indicate SD < line symbol size.

sible to improve the sensitivity for the IgE-RI (10² SQ-U/ml) as shown in Fig. 2C.

With both assays it was possible to achieve acceptable standard curves for allergen quantification. We chose to proceed with IgE-RI.

IgE-RT

As in the former set-up the results of IgE-RI showed equal sensitivity, for the three allergens, when IgE-RI, Mab-ELISA, and HR were compared simultaneously. Fig. 3a (cat), Fig. 4a (dog), and Fig. 5a (*Derm. pter.*). Concerning the irrelevant allergens we found a certain degree of unspecificity for the highest concentrations (10⁴ SQ-U/ml) of dog assaying cat allergens and for cat assaying mite aller-

gens, giving approximately 20% inhibition. Results are expressed as means of the two 37 mm and the two 80 mm filter eluates with standard deviations indicated in the figure.

Mab-ELISA

The results of the Mab-ELISA method are given in Fig. 3b (Cat) and Fig. 5b (*Derm. pter.*). Results are expressed as mean of the two filter type eluates with standard deviations indicated in the figure. Based on the results exceeding those of the buffer by at least 2 SD, the detection limits were found to be 10 SQ-U/ml for cat and *Derm. pter.* Concerning the irrelevant allergens, there was a slight unspecificity for the highest concentration (10⁴ SQ-U/ml) of dog assaying cat.

CCIE

The results of the CCIE method are given in Fig. 4b (dog). Results are expressed as mean of the two filter type eluates with SD = 0. Based on the results exceeding those of the buffer by at least 2 SD, the detection limits were found to be 10⁴ SQ-U/ml (titre 4). Concerning the irrelevant allergens, a titre level of 2 for cat was found in the highest concentration 10⁴ SQ-U/ml.

HR

The results of the HR method are given in Fig. 3c (cat), Fig. 4c (dog), and Fig. 5c (*Derm. pter.*). Results are expressed as mean of the two filter type eluates with standard deviation indicated. Based on the results exceeding those of the buffer by at least 2 SD, the detection limit was found to be 10 SQ-U/ml for cat and *Derm. pter.*, and 10² SQ-U/ml for dog.

Comparison between methods

The inter-assay detection limits are summarized in Table 2. In general, HR was the most sensitive assay, closely followed by RI, Mab-ELISA, and with CCIE as the most insensitive one.

DISCUSSION

The detection limits found for the assays are comparable with those of other investigators

(11, 15, 19, 21). Based on the results we decided to proceed with IgE-RI instead of IgG₄-RI for allergen systems despite the poor results of *Derm. pter.* detection. Later investigations - partly shown by the curve (dotted line) in Fig. 2b - had revealed that the selected serum pool (Dpt-0589) was far from optimal. By using a single, highly mono-specific house dust mite allergic patient the detection limit could be improved significantly. After immunoblotting, we selected serum pools containing sera with clearly specified bands being areas with major allergen but also sera with bands representing minor allergens. The disadvantage of using pooled sera is the risk of diluting single, high-titered sera and of getting unintended interference with blocking antibodies. The additional step of using rabbit catching antibodies may also decrease the sensitivity of quantifying mite allergens. Although the IgG₄-RI is faster to perform than IgE-RI (3 instead of 5 days), and requires smaller amounts of allergen for coating of the test tubes, we selected to proceed with IgE-RI because of the disputable, allergenically significant role of IgG₄ subclasses (1, 9, 14). Further, relevant IgE sera are more readily obtained. An advantage of using IgG₄-RI may be that these sera bind to a wider spectrum of proteins (18) in allergen extracts, which gives the possibility to detect potential immunogenic components in a dust or aeroallergen sample.

Investigations are now in progress to clarify the unspecific reaction found for dog assaying cat allergen, and for cat assaying mite allergen. The results of the present investigation may be due to allergenic cross-reactivity between dog and cat minor allergens (20), and the presence

of small titres of specific IgE to dog in the cat serum pool.

By using Mab-ELISA, problems of keeping a patients' serum bank are eliminated. The test is specific, when specific monoclonal or immunoabsorbed polyclonal antibodies are used as catching or secondary antibodies. The disadvantage of this highly specific test would be the risk of a negative result in measuring a sample containing only large amounts of minor allergens. Monospecific antibodies were not available for dog allergen. Therefore CCIE was chosen for the comparison with IgE-RI and HR, but the use of polyspecific antibodies limited the sensitivity of the assay. Using precoated microtitre plates, Mab-ELISA is performed within 2 days including overnight elution.

Using HR for detection of environmental allergens, it might be difficult to have access to monoallergic donors. This has been demonstrated by unspecific release of histamine to cat and *Glad. her.* in the highest concentration analysing dog allergens (Fig. 4c). Also dilution and recovery problems can be anticipated, as shown in Fig. 1a and 1b. Further, the assay has to be performed on fresh donor blood stored for no longer than 24 h. However, the test is sensitive and seems to be a potential for testing patients' environments, when the allergens causing the symptoms are unknown.

The primary aim of this study was to evaluate analysing techniques for aeroallergens sampled on glass fibre filter sheets. Using the modification described, this was accomplished, and from the results given in Table 2, one might speculate on the influence of airsampler flow rates and sampling time for identification and quanti-

Table 2.

Comparison between ranges of allergen detection limits of IgE-RI, HR, Mab-ELISA and CCIE assay. Allergen concentration (SQ-U/ml) in eluates

Assay		Cat	Dog	<i>Derm. pter.</i>
IgE-RI		5-50	10 ² -10 ³	10 ³ -10 ^{4c}
	IC ₂₀	50-5*10 ²	5*10 ² -5*10 ³	>10 ^{4c}
HR 1/2 Max	IC ₅₀	10-10 ²	10 ² -10 ³	10-10 ²
Mab-ELISA		10-10 ²	ND	10-10 ²
CCIE		ND	10 ⁴ -10 ⁵	ND

IC₂₀ and IC₅₀ corresponding to 20% and 50% inhibition. ND = not done. ^c Could be reduced significantly to 10² and 10³ SQ-U/ml by choosing another serum.

fication of aeroallergens. It has been established that indoor aeroallergens can be quantified by immunochemical analysing methods with different kinds of sampling devices, filter materials, and airflow rates (8, 11, 14, 19). These problems have not been solved by these experiments, and studies are in progress to evaluate the practical use of the analysing methods. We will continue with experimental set-ups with IgE-RI, Mab-ELISA and HR in order to quantify aeroallergens sampled "on location". However, CCIE will be excluded due to lack of sensitivity.

ACKNOWLEDGEMENTS

The valuable discussions with Joan Jensen, the technical assistance of Kirsten Ebbesen, and secretarial assistance of Anne Larsen are gratefully acknowledged. The study was supported by a grant from "Kreditforeningen Danmark".

REFERENCES

- 1 Djurup R. The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 40, 469-486, 1985.
- 2 Harboe N, Ingild A. Immunization, isolation of immunoglobulins, estimation of antibody titre. In Axelsen N, Krøll J, Wecke B (eds.): A manual of quantitative immunoelectrophoresis. *Scand J Immunol, Suppl.* 1, 161-164, 1973.
- 3 Jarolim E, Poulsen LK, Stadler BM, et al. A long term follow-up study of hyposensibilization with immunoblotting. *J Allergy Clin Immunol* 85, 996-1004, 1990.
- 4 Jensen, J, Poulsen LK, Wecke B. Indeklimaallergenerudvikling af en målemetode (Indoor allergens - development of a method of quantification). Laboratory of Medical Allergology and Ministry of Housing, Report, 27 p., 1988.
- 5 Lind P. Enzyme-linked immunosorbent assay for determination of major excrement allergens of house dust mite species *D. pteronyssinus*, *D. farinae* and *D. microceras*. *Allergy* 41, 442-451, 1986.
- 6 Lind P, Norman PS, Newton M, Löwenstein H, Schwartz B. The prevalence of indoor allergens in the Baltimore area: House dust-mite and animal-dander allergens measured by immunochemical techniques. *J Allergy Clin Immunol* 80, 541-547, 1987.
- 7 Lind P, Korsgaard J, Löwenstein H. Detection and quantitation of *Dermaphagoides* antigens in house dust by immunochemical techniques. *Allergy* 34, 319-326, 1979.
- 8 Luczynska CM, Li Y, Chapman MD, Platts-Mills TAE. Airborne concentrations and particle size distribution of allergen derived from domestic cats (*Felis domesticus*). *Am Rev Respir Dis* 141, 361-367, 1990.
- 9 Mosbech H, Djurup R, Dreborg S, Kærgaard Poulsen L, Stahl Skov P, Steringer I. Hyposensitization in asthmatics with mPEG-modified and unmodified house dust mite extract. *Allergy* 45, 130-141, 1990.
- 10 Nolte H, Storm K, Schiøtz PO. Diagnostic value of a glass fibre-based histamine analysis for allergen testing in children. *Allergy* 45, 1-11, 1990.
- 11 Platts-Mills TAE, Heymann PW, Longbottom JL, Wilkins SR. Airborne allergens associated with asthma: Particle sizes carrying dust mite and rat allergens measured with a cascade impactor. *J Allergy Clin. Immunol* 77, 857-860, 1986.
- 12 Poulsen LK, Pedersen MF, Malling H-J, Wecke B. Maxisorp RAST. A sensitive method for detection of antigen-specific human IgE in culture fluids. *Allergy* 44, 173-180, 1989.
- 13 Poulsen LK, Stahl Skov P, Mosbech H, Wecke B. Role of IgG₄ in histamine release from human basophil leukocytes. *Int Arch Allergy Appl Immunol* 86, 383-390, 1988.
- 14 Price JA, Longbottom JL. IgG antibodies in relation to exposure to laboratory-animal allergens. *J Allergy Clin Immunol* 84, 520-528, 1989.
- 15 Schwartz B, Lind P, Löwenstein H. Level of indoor allergens in dust from homes of allergic and non-allergic individuals. *Int Arch Allergy Appl Immunol* 82, 447-449, 1987.
- 16 Skov PS, Norn S, Wecke B. A new method for detecting histamine release. *Agents Actions* 14, 414-416, 1984.
- 17 Stahl Skov P, Mosbech H, Norn S, Wecke B. Sensitive glass microfibre-based histamine analysis for allergy testing in washed blood cells. *Allergy* 40, 213-218, 1985.
- 18 Søndergaard I, Poulsen LK, Østerballe O, Wecke B. A computational approach to the description of the individual immune responses. Allergen-specific IgE and IgG-subclass antibodies formed during immunotherapy. *Allergy* 46, 10-19, 1991.
- 19 Van Metre TE, Marsh DG, Franklin Adkinson N, et al. Dose of cat (*Felis domesticus*) allergen 1 (Fel d 1) that induces asthma. *J Allergy Clin Immunol* 78, 62-75, 1986.
- 20 Viander M, Valovirta E, Vanto T, Koivikko A. Cross-reactivity of cat and dog allergen extracts. RAST-inhibition studies with special reference to the allergenic activity in saliva and urine. *Int Arch Allergy Appl Immunol* 71, 252-260, 1983.
- 21 Yman L, Ponterius G, Brandt R. RAST-based allergen assay methods. International WHO-IABS symposium on standardization and control of allergens administered to man. Geneva 1974. Develop Biol Standard, Karger, Basel 29, 151-165, 1975.

Address:

C.R. Johnsen, M.D.
Allergy Unit 7542
National University Hospital
Tagensvej 20
DK-2200 Copenhagen N
Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.