

Antibodies in allergy and inflammation.

Research tools and indicators of disease.

Lars Yman

The identification of IgE and the development of the radioallergosorbent test was the initiation of a revolution in the allergy research field.

Measurement of allergen specific IgE antibodies is today an integral part of the clinical investigation of patients with suspected allergic disease. However, in addition to being a routine test for the clinical laboratory, Phadebas RAST® has served as the model for a number of modified procedures and as the basis for a wide range of applications. The aim of this presentation is to review and communicate the experience for the benefit of researchers and clinicians working in the field of allergy.

I. New developments in serum IgE measurement

The major application for IgE measurements is as an aid in investigations of suspected allergy and then primarily as a differential diagnostic tool. The development of criteria for the use of measurements of markers of disease traditionally starts with the establishment of a "normal range". In case of S-IgE this phase is not totally completed. A review of reported "normal" levels clearly shows a decreasing trend from up to more than 200 kU/l down to about 10kU/l in non-smoking healthy adults in the most recent reports. This trend is similar in all countries and is a result of improvements in test method, statistics and selection criteria.

An increased understanding of these matters opens the way for a better utilization of the diagnostic potential of IgE measurements and, in turn requires improved sensitivity of the assays not only

for pediatric use but also for measurement of adult levels. The new double antibody solid phase radioimmunoassay is an example of a sensitive method with extreme accuracy. The increased interest in determination of IgE in such as cord blood or newborns can also be covered by Phadebas IgE PRIST or by RIA procedure or by the modifications of these procedures originally designed for measurement of in vitro IgE production.

II. New developments in specific IgE antibody measurement

Laboratory tests are becoming more convenient and faster. These developments have also influenced the radioallergosorbent test. Non-radioactive one-day procedures supported by equipment facilitating the handling of the multianalyte system have been introduced. Recently also microtiter applications have appeared. Before modifying procedures or accepting new tests as being equivalent to or better than established and well documented systems any laboratory should investigate e.g. validity of the calibrator, reproducibility, true sensitivity and specificity, and correlation to reference methods. A simple investigation can give important information on the validity of the candidate procedure.

Quality control, or rather performance control is an essential part of laboratory management and is attracting increasing interest on national and international levels. The desirable interlab standardization requires access to control sera with defined and constant content. In allergy testing this means isolated IgE antibodies with defined specificity pat-

terns to a range of allergens.

Selecting species of allergens to be used for testing and treating allergic patients is a constant concern of clinicians as well as for laboratories. The number of potential allergens even within a limited environment, the complex natures of the allergen sources and the fingerprint-like specificity patterns of the individual patients are serious obstacles to a full understanding. However, data on local distribution, relations between sources and prevalence of sensitivity can be combined and form a basis for test and therapy panels.

Observations by clinicians on potential new or local sensitizers can be verified by preparation of allergen reagents on an experimental scale (Special Allergen Service).

III. Measurement of specific antibodies of various classes in allergy and related diseases.

Serological tests are historically titrations and as a consequence test results are usually expressed as titres. The introduction of the solid-phase radioimmunoassay principle and analogous enzyme immunoassays such as enzyme-linked immunosorbent assay (ELISA) provided a range of new options. However, their application to the measurement of e.g. specific IgG antibodies has not been without difficulties. The problems are primarily a consequence of the low concentration of specific antibody to be measured in the presence of a high concentration of total IgG protein. The phenomenon manifesting itself as a high non-specific binding of negative control or "normals" relative to the specific response has often been interpreted and reported in literature as a normal level or as evidence of cross reactivity between antigens. The severity of the problem is to a large extent dependent on the properties of the solid phase and can be lessened by a modification. Measurements of IgE and IgG antibodies total sets of antigens or separate antigenic components can be applied to immunotherapy follow-up or to diagnosis and therapy monitoring in various parasitic diseases such as schistosomiasis and fungi-induced aspergil-

losis. The selection of antigen is critical for the result.

IV. Antigen-specific human antibodies as reagents for qualitative and quantitative analysis of allergens.

Large panels of patients, represented by their blood serum, can be tested in sophisticated immunochemical studies using recently developed RAST based technologies. The results of such studies depend on where the samples have been collected and on proper clinical characterization of the donors. As an example, it is obvious that sera collected in Sweden should not be used for studies on ragweed allergens; a serum from a person sensitized only by birch pollen will not give full information if applied to studies on beech pollen allergens; a serum containing antibodies in high concentration to cat serum albumin may give misleading information on the presence and importance of saliva/dander components, etc.

Characterization and selection according to defined criteria are essential. Search for technologies by which IgE antibodies to separated allergenic components could be directly detected and visualized led to the blotting procedure based on transfer to CNBr-activated cellulose strips and detection by means of Beta-galactosidase labelled anti-IgE. The procedure, first presented in 1979, has since been improved and employs a range of electrophoretic procedures which can be combined with different membranes, transfer procedures and detectors. The method does not require any rabbit antisera for fixation of the allergen such as the CRIE or CEIE and the most serious drawbacks of these are therefore avoided, i.e. CIE based methods can detect only serum antibodies to components which were present in the preparation used for immunization of the rabbit and towards which the rabbit has responded. The blotting methods directly reflect the patterns of the patient sera and consequently do not suffer from these limitations.

Properly selected allergy patient sera applied as reagents in the RAST-inhibition method permits accurate measurement

of the potency of allergen preparations. Provided that adequate solid phase allergens and sera are used there assured potency correlates well with biological activity. Analysis as well as evaluation are covered by internationally agreed upon manuals and acceptance limits have been stated by several national licencing authorities.

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I:1 NEW DEVELOPMENTS IN SERUM IgE MEASUREMENT

I:1:a IgE REFERENCE VALUES (ADULTS AND CHILDREN). Criteria and calculations

Lars Yman

Adults

The use of a test for the identification of pathological cases is traditionally based on the establishment of a "normal range" for the analyte. In case of IgE such studies on healthy individuals have been reported from various parts of the world since 1967. The mean levels reported vary from about 10 to more than 200 kU/l.

The upper limit of the normal range considered as cut off between normal and allergic cases by laboratories in different countries vary from about 100kU/l to at least 700 kU/l. It is evident that the value of serum IgE measurement as a part of an allergy investigation is highly dependent on the choice of cut off level - the higher it is the less patients are likely to be subject to further investigations.

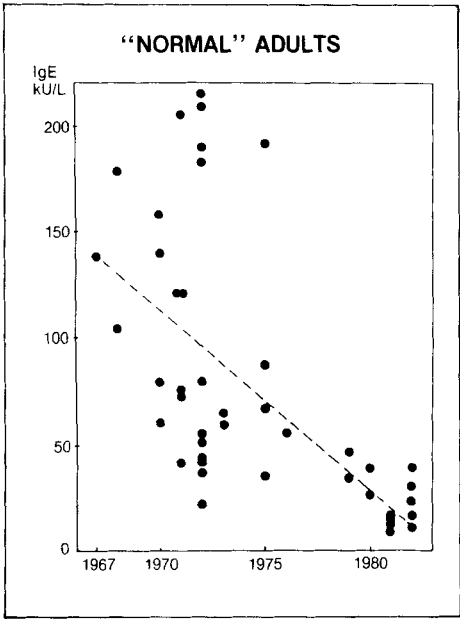


Fig. 1: A collection of reported IgE levels intended to represent the mean levels of “normal” or “healthy” or “non-atopic” groups of people. The reason for the universal downward trend can be traced to changes in

- test method
- statistical method
- selection criteria for “healthy”
- extraordinary environmental conditions
- exclusion of smokers

MEAN IgE LEVELS (kU/l)			
EFFECT OF SELECTION CRITERIA			
CLIN. HISTORY NEG.			AUTHOR
	SKIN TEST NEG.	NON SMOKERS	
36	17		BARBEE (1981)
26		12	WARREN (1982)
17	13	10	ZETTERSTRÖM (1981)
38	17		HAAHTELA (1982)
21			NYE (1975)
31			FURUKAWA (1982)

Fig. 2: Examples of the effect of tightening of selection criteria.

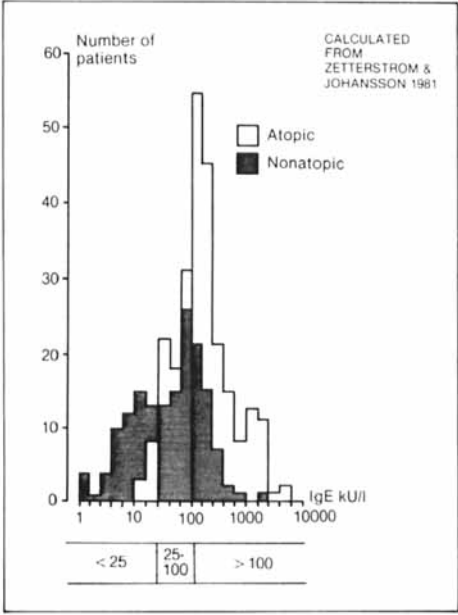


Fig 3: The distribution of IgE levels in atopic patients and non-atopic with respiratory symptoms compared to the healthy reference group.

IgE conc	25	25-100	100
n	70	125	217
% atopic	16	57	78
% non-atopic	84	43	22

80 % of the patients with levels above 100 kU/l are atopic. Only 1 % of the healthy individuals reach this level.

Children

Just as for the adults a great variety of “normal values” of more or less well-defined population samples have been reported.

Fig 4: Two studies, American and Swedish, are close enough in terms of selection criteria and results to be pooled. Log IgE versus log age showed a linear relationship. Linear regression analysis permitted calculation of the most probable level for the ages from 6 weeks to 10 years.

It is suggested that geom. mean + 1 SD is considered as “the borderline of normality” in the sense that higher levels lead to continued investigation of the cause. Levels lower than mean are unlikely to be associated with allergy.

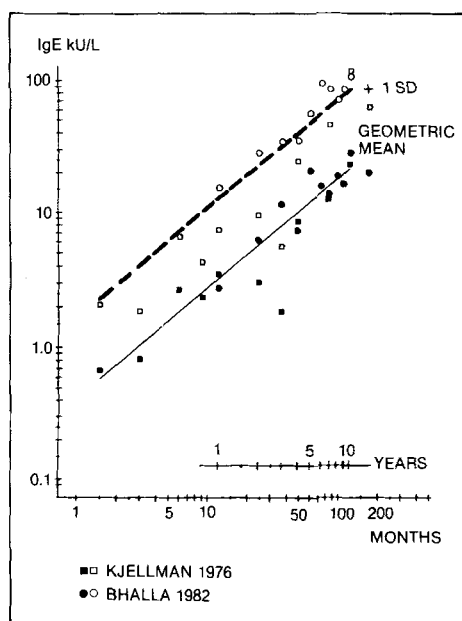


Fig 4:

I:1:b IgE MEASUREMENTS. Sensitive methods for routine and research

Anita Kober, Leif Uppström

Different measuring principles may be applied to total serum IgE determinations. The concentration of serum IgE is usually expressed as kU/l where 1 U = 2.4 ng. Normal values range down to less than 1 kU/l thus sensitive measuring methods are needed.

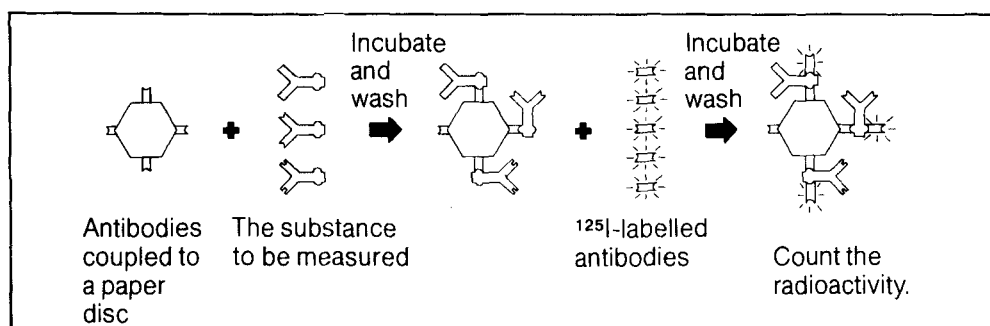


Fig 1: Phadebas IgE PRIST® test principle.

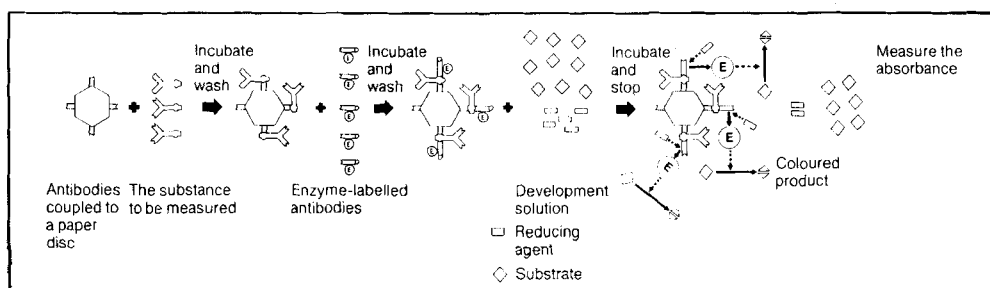


Fig 2: Phadezym IgE PRIST® test principle.

Phadebas IgE PRIST®/Phadezym IgE PRIST® are sandwich immunoassays using anti-IgE covalently coupled to paper discs as solid phase and ^{125}I or enzyme labelled anti-IgE as tracer.

Sera are normally assayed diluted 1:10 (higher dilutions possible) or undiluted giving a measuring range 0.5—1 000 kU IgE/l or above.

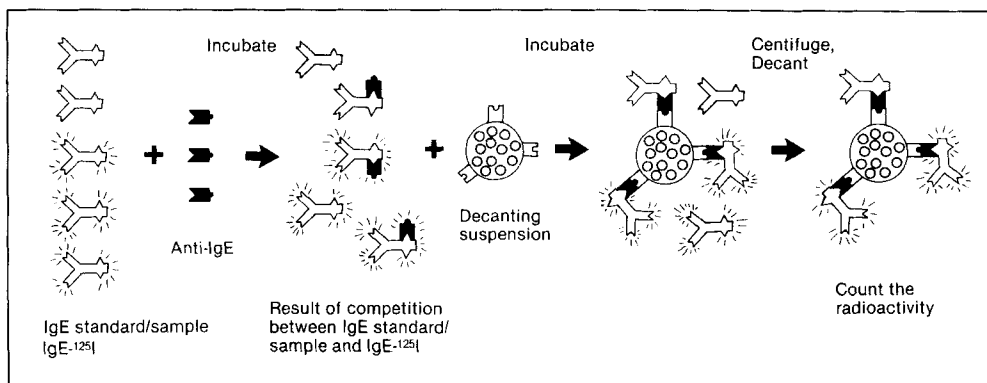


Fig 3: Pharmacia IgE RIA test principle.

The new Pharmacia IgE RIA (Fig. 3) is a solid phase double antibody radio immunoassay. IgE in the sample competes with ^{125}I -labelled IgE for binding to an

anti-IgE antibody. Separation of bound and free IgE is achieved by addition of a solid phase bound second antibody.

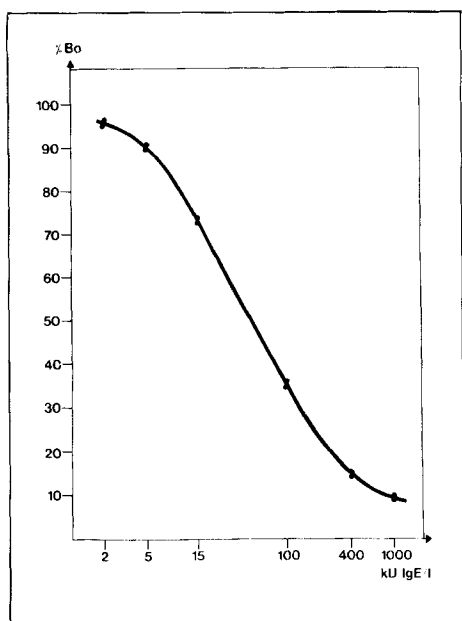


Fig 4: Pharmacia IgE RIA standard curve.

The measuring range is **2—1 000 kU/l for undiluted sera** and can be extended by dilutions of samples.

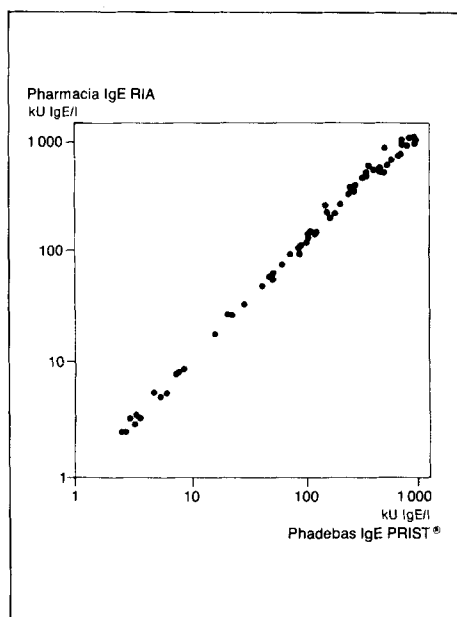
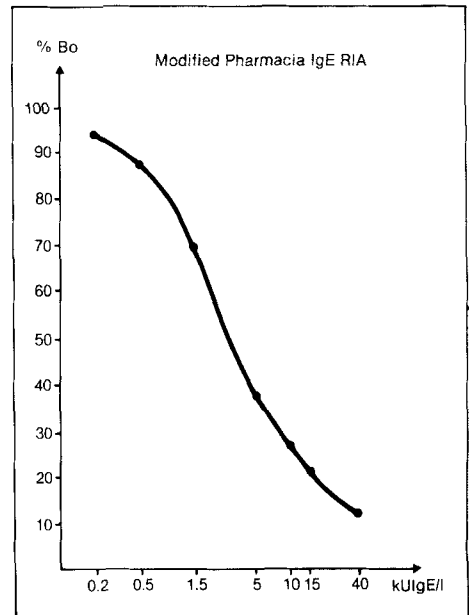
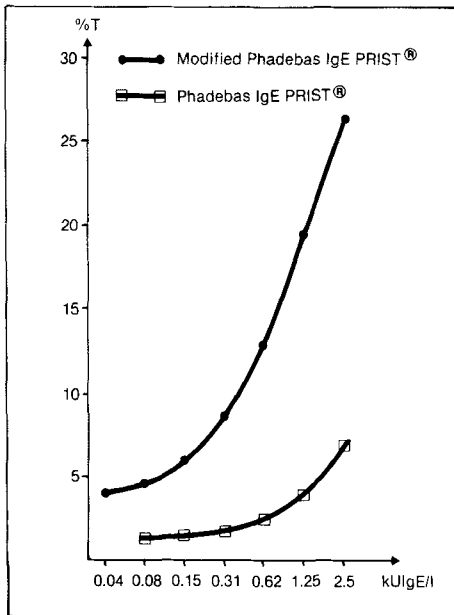


Fig 5: The correlation between values obtained with Phadebas IgE PRIST® and Pharmacia IgE RIA is good.

Figs 6, 7: For special purposes methods for measurements of very low IgE concentrations are needed. The measuring range for both Phadebas IgE PRIST® and Pharmacia IgE RIA can be extended downwards, e.g. by increasing sample volumes and incubation times.



I:2 NEW TECHNIQUES IN SPECIFIC IgE ANTIBODY MEASUREMENT

I:2:a THE MICROTITER TREND

Hasse Schröder, Kyrre Thalberg, Anette Lundberg

Among different trends in the development of laboratory tests the search for ways to obtain easier handling, shorter process times, automatic reading, and computerized data processing are important components. One means to approach these objectives would be to use the microtiter configuration in the test set-up.

Recently three different products have appeared where microtiter systems are used for assay of IgE antibodies. They are all sandwich enzyme immunoassays with basically similar procedural steps (Table 1). Allergenetics IgE FAST™ test is a within day fluorometric method while Ventrex IP Specific E™ is a double over night colorimetric procedure. Phadezym RAST® — Microtiter, a within day procedure, offers the user the possibility of both fluorometric as well as colorimetric reading.

The purpose of this investigation was to study the three microtiter methods for assay of IgE antibody. In such compari-

sons 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, batch variations.

Here we report data on the first three parameters.

Phadezym RAST® Microtiter EIA/FEIA

- Standard curves

The standard curves obtained for Phadezym RAST® Microtiter EIA/FEIA are linear and steep with a measuring range of 0.35—17.5 PRU/ml. (Phadebas RAST® standard procedure

- Correlations with Phadebas RAST® standard procedure

Positive patient sera against several allergens were run in the Phadezym RAST® EIA/FEIA procedures and the results were compared with those obtained with the standard Phadebas RAST® reference method.

Table 1. Test procedures for the three different products.

Test	Phadezym RAST® Microtiter	Allergenetics IgE FAST™ test	Ventrex IP Specific E™
Serum volume	50 µl	100 µl	200 µl diluted 5x
Allergen Matrix	paper disc in microtiter well	coated micro- titer well	coated micro- titer well
First in- cubation	1 h room temperature shaker 1200 rpm	2 h room temperature	16–20 h 2–8°C
Wash	4x quick + 10' soak	3x quick	5x quick
Conjugate	50 µl	100 µl	200 µl
Second in- cubation	2 h room temperature shaker 1200 rpm	2 h room temperature	20–24 h 2–8°C
Wash	4x quick + 10' soak	3x quick	5x quick
Substrate	EIA 200 µl / FEIA 200 µl	100 µl	200 µl
Development time	90' / 15'	30'	60'
Reading	Colorimeter / Fluorometer	Fluorometer	Colorimeter

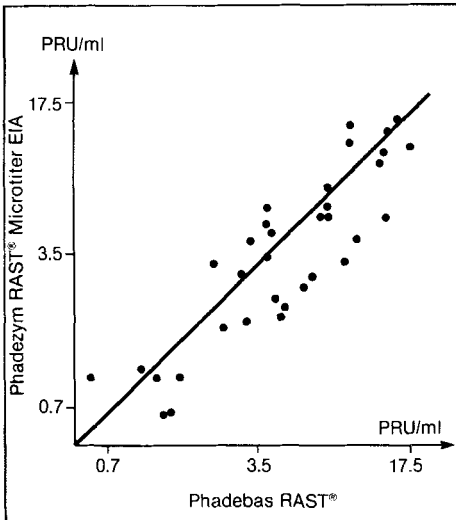


Fig 1. Correlation between Phadezym RAST® Microtiter EIA and Phadebas RAST®

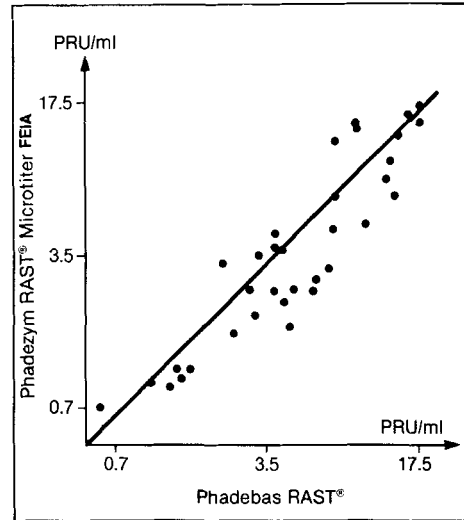


Fig 2. Correlation between Phadezym RAST® Microtiter FEIA and Phadebas RAST®

The correlations between the microtiter data and the Phadebas RAST® reference method were good (Figs 1, 2).

- Non-specific binding

The non-specific binding of negative and 1000 kU myeloma IgE/ml spiked negative serum was tested with 20 different allergens in both procedures. The results were expressed as percentage binding of the lowest calibrators. The uptake of both negative and spiked negative sera are well below half of those obtained for the lowest calibrator in both Phadezym RAST™ microtiter procedures.

Allergenetics IgE FAST™ test

- Standard curve

In any quantitative test dilutions of an unknown sample must give a slope parallel to the standard curve. We diluted the highest calibrator of Allergenetics FAST™ test with the diluent supplied with the kit. The dilutions were then assayed in Allergenetics IgE FAST™ test system and the results were compared with those obtained for the standard curve (Fig 3).

Fig 3. Standard curve and dilutions of the highest calibrator of Allergenetics IgE FAST™ test.

Assuming that the lowest calibrator indicates the lowest value acceptable by the reagents the measuring range of Allergenetics IgE FAST™ test is 2–20 "IU/ml".

We also measured the specific IgE in the calibrators of Allergenetics IgE FAST™ test by Phadebas RAST®. The measuring range as defined in this way is 1–16 PRU/ml.

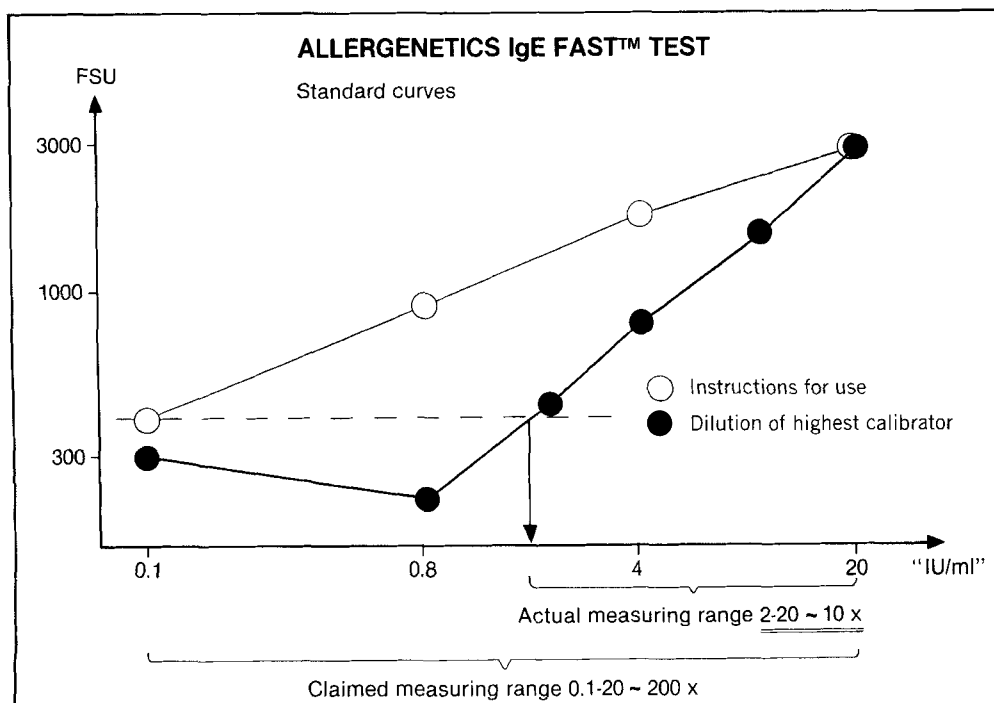
- Correlation with Phadebas RAST® standard procedure.

Positive patient sera against several allergens were assayed in Allergenetics IgE FAST™ test and the results were compared with those obtained with the Phadebas RAST® reference method.

The correlation was acceptable for sera with high titers of allergen specific IgE. For lower levels of IgE antibody a poor correlation was recorded (Fig 4).

- Non-specific binding

Non-specific binding of both negative and spiked serum was in the range of lower part of the standard curve for the moulds. The other allergens showed a low non-specific binding.



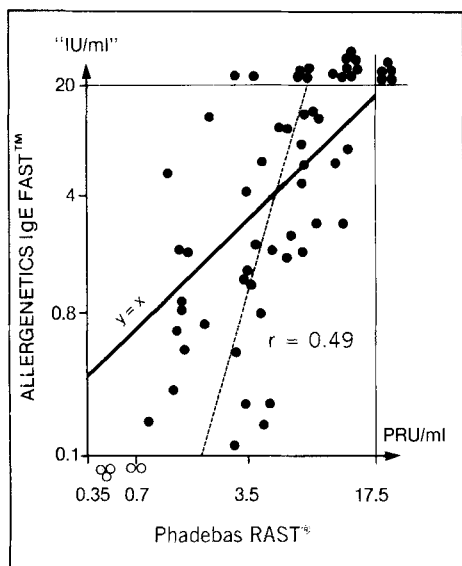


Fig 4. Correlation between Allergenetics IgE FAST™ test and Phadebas RAST®.

Ventrex IP Specific E™

• Standard curve

The sensitivity of IP Specific IgE was studied by the measurement of the concentrations of the IgE antibody in the IP Specific Specific E calibrators by Phade-

bas RAST® standard procedure. We found that the lower end of IP Specific E standard curve is at 0.6 PRU/ml. The low level zone ranged between 0.3—0.6 PRU/ml.

• Correlation with Phadebas RAST® modified procedure.

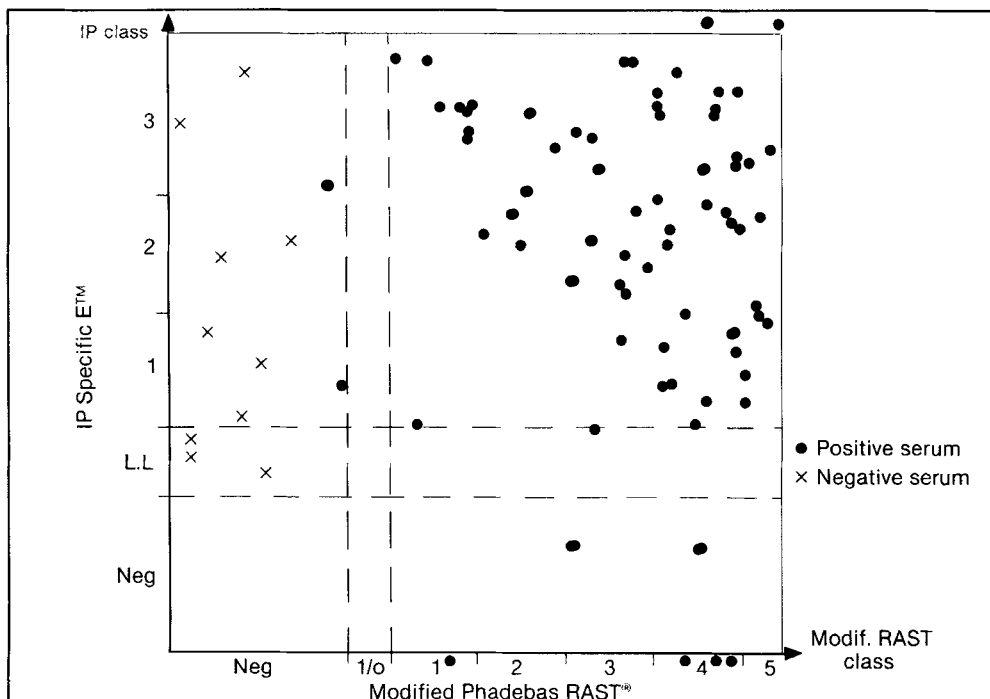
Positive patient sera against several allergens were assayed in Ventrex IP Specific E™ test and the results were compared with those obtained with the modified Phadebas RAST® method.

The correlation was random for all allergens studied (Fig 5).

• Non-specific binding

The non-specific binding of negative and 1000 kU myeloma IgE/ml spiked serum was tested. For the 10 allergens studied in no case was a negative serum scored as negative. Scores over the whole positive range were obtained (Fig 5).

Fig 5. Correlation between IP Specific E™ and modified Phadebas RAST®.



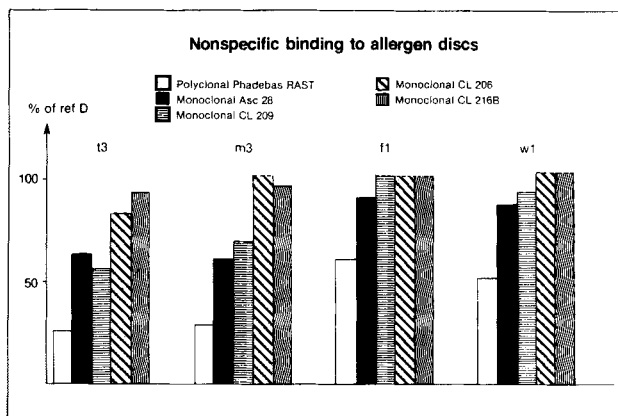
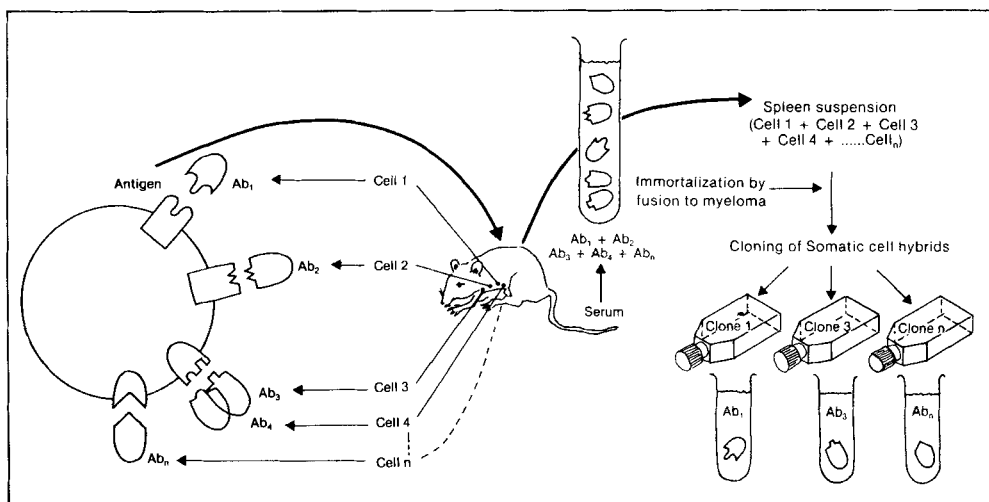
I:2:b MONOCLONAL ANTIBODIES IN IgE ASSAYS

Asta Berglund, Anita Kober, Maria Ahlberg, Eilif Persson

Expectations for monoclonal antibodies in immunoassays have been high for several reasons and especially the prospect of easy and reproducible production of large amounts of monospecific antibodies.

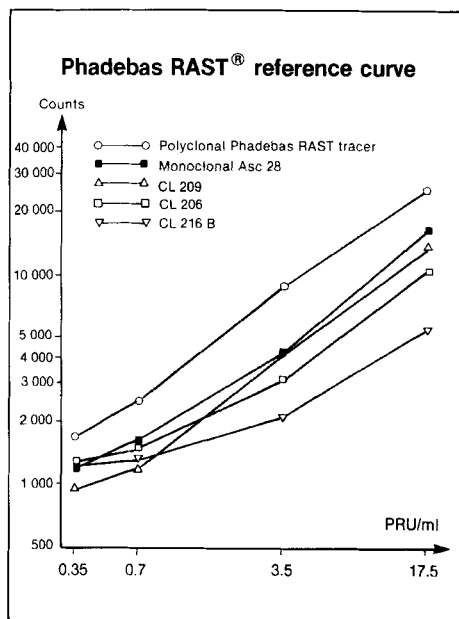
For specific IgE antibody measurements, there are strong demands for very high test sensitivity.

Several different monoclonal anti-IgE antibodies have been tested in the RAST system and compared to the Phadebas RAST® polyclonal rabbit-anti-IgE tracer. However, so far none of the tested monoclonals has been as sensitive as the polyclonal Phadebas RAST® tracer.



Non-specific binding to allergen discs is frequently higher with monoclonal antibodies.

Also in the Phadebas IgE PRIST® system the polyclonal anti-IgE tracer is superior to the monoclonal antibodies in the lower measuring range.



In order to find monoclonal antibodies of suitable equality a large number of cell lines has to be tested.

I:2:c QUALITY CONTROL IN CLINICAL LABORATORIES

Hasse Schröder, Kyrre Thalberg

Constantly reliable test results are very important for correct diagnosis and appropriate treatment. Since there are many possible sources of error associated with performing laboratory tests an internal quality control program is needed to

help prevent erroneous results from reaching the physician.

A common way to increase the reliability of laboratory data is to include control sera in the routine work.

Clinical chemistry controls are usually manufactured from pooled serum. However, the use of such products in specific IgE testing can give misleading results.

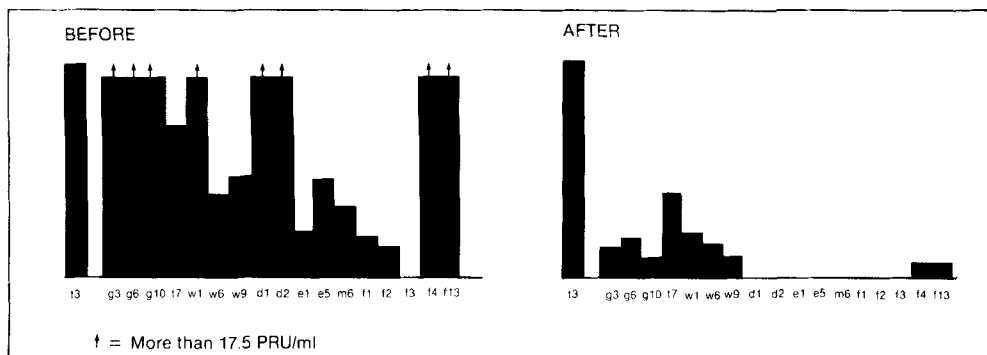


Fig 1. Specificity of a birch serum pool before and after affinity chromatography.

The pooling of serum from allergic donors may create a control with exceedingly high total IgE levels, which will interfere with the specific IgE results. Other specificity problems related to cross-reactivity between allergens can also contribute to decreased value of the control program.

An obvious way to prepare control sera with defined and constant contents of IgE antibody is to purify pooled serum from atopic patients by affinity chromatography on allergen-Sepharose®. We purified IgE antibody specific for 11 different allergens. Figure 1 shows the specificity pattern of the birch serum pool before and after affinity chromatography on a birch allergen-Sepharose® column.

Almost all non-birch specific IgE was eliminated by the process. Interesting was to see that the purified product gave a positive response when tested with oak allergen disc. Oak allergen is known to show some cross-reactivity with birch allergen.

The possibility of loss of individual allergenic specificities during affinity chromatography was investigated by means of immunoblotting and crossed enzyme immunoelectrophoretic (CEIE) techniques. We were able to demonstrate that all individual components of the ini-

tal serum pool were present in the final purified fraction.

Each purified serum was then calibrated to contain 2 PRU/ml of IgE antibody specific to the allergen stated on the vial. This value will vary somewhat between runs and between laboratories but should stay within the borders of class 2, that is 0.7—3.5 PRU/ml.

The intra- and interlab variation in Phadebas RAST® scores was studied by testing the Phadexact™ Serum Allergen-specific IgE Controls sera for timothy, short ragweed, and cat in 10 laboratories in three countries in five replicates on five occasions. The scores obtained for timothy are shown in figure 2.

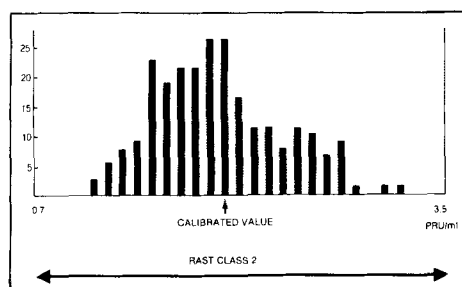


Fig 2. Distribution of results obtained by testing timothy control sera in different laboratories.

We found the within run coefficient of variation (c.v.) on the average to be 14.5 % and the between run c.v. 16 %. The individual values apart from two outliers all stayed well within the bounda-

ries of class 2 (0.7—3.5 PRU/ml). It can be concluded that Phadexact Se-Rum is excellent in the quality control of Phadebas/Phadezym RAST® procedures.

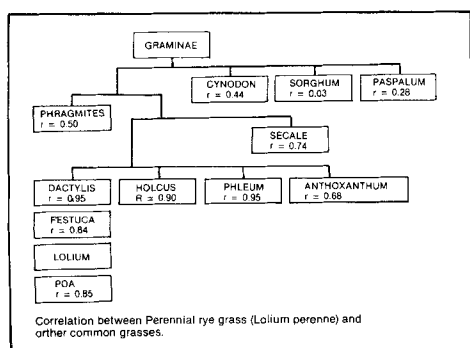
1:2:d ALLERGENS. Distribution, relations, importance

Marie-Louise Andrae and Lars Yman

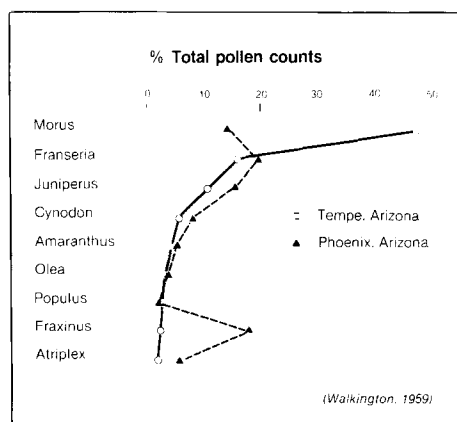
The number of potential allergen sources is practically unlimited and many regions of the world have their own local allergens. Still, it seems reasonable to state that the most troublesome allergens are universal. The house dust mites are cosmopolitans, cat and dogs are common pets in most countries, and moulds and pollen producing plants are mostly widely distributed within areas with similar climates in both hemispheres. Even so, the situation is complex and the information on the clinical relevance of most of the allergens sources is still incomplete. Gathering information on the local conditions in terms of presence of local and universal allergens is therefore a task for any clinician seeing allergic patients. Such information combined with data on cross-reactions between related species, contents of composite sources, etc. is essential for anamnesis as well as selection of tests to be performed.

Examples of useful information

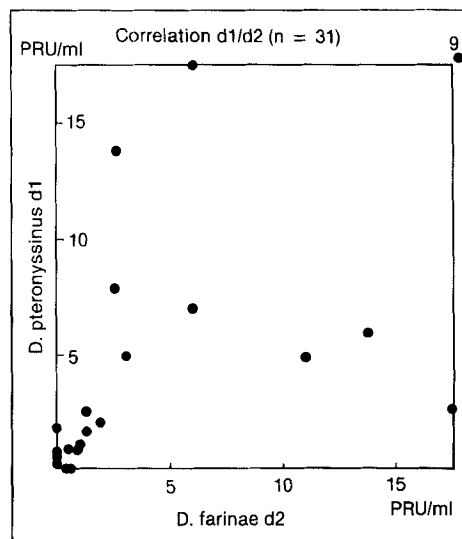
- All grasses are not crossreacting



- Pollen content of air in adjacent places may differ dramatically



- The allergen composition of the two major house dust mites (*D. pteronyssinus* and *D. farinae*) differ



- House dust mite preparations may contain horse dander allergens

- Ham may contain lactose and soy proteins

Family	Phadebas RAST®/ Phadezym RAST® code, species	Other members of the family
Plants		
Actinidiaceae	f84 kiwi fruit	
Apple	f49 apple	pear, quince
Birch	f17 hazel-nut	filbert
Buckwheat	f11 buckwheat	rhubarb
Citrus fruits	f33 orange	grapefruit, kumquat, lemon, lime tangerine
Fungi	f45 yeast (Baker's)	mushroom
Gourd	f87 melons	cantaloupe, honey dew, cassava, pumpkins, squash, cucumber
Grains	f4 wheat f5 rye f6 barley f7 oat f8 maize f9 rice f79 gluten	cane, malt, sorghum, wild rice
Lecythis	f18 Brazil nut	
Legumes	f12 pea f13 peanut f14 soya bean f15 white bean	acacia, (gum arabic), kidney bean lentil, licorice, lima bean, navy bean, senna, string bean
Lily	f47 garlic f48 onion	aloes, asparagus, chive, leek
Mustard	f89 mustard	broccoli, brussels sprouts, cabbage, cauliflower, celery cabbage collard, horseradish, kale, kohlrabi, radish, rutabaga, turnip, watercress
Palm	f36 coconut	date, sago
Parsley	f31 carrot f85 celery f86 parsley	anise, caraway, celeriac, coriander, dill, fennel, parsnip
Pedaliium	f10 sesame seed	
Plum	f20 almond	apricot, cherry, nectarine, peach, plum (prune)
Potato	f25 tomato f35 potato	chili, eggplant, green pepper, cayenne pepper
Rose	f44 strawberry	blackberry, dewberry, loganberry, raspberry, youngberry

Family	Phadebas RAST® / Phadezym RAST® code, species	Other members of the family
Animals		
Crustaceans	f23 crab f24 shrimp f80 lobster	cary fish, squid
Fish	f3 fish (cod) f40 tuna f41 salmon	anchovy, barracuda, bass, bluefish, buffalo, bullhead, butterfish, carp, catfish, caviar, chub, croaker, cusk, drum, eel, flounder, haddock, hake, hal- ibut, harvestfish, herring, mackerel, mullet, muskellunge, perch, pickerel, pike, pollack, pompano, porgy, rosefish, sardine, scrod, scup, shad, smelt, snap- per, sole, sturgeon, sucker, sunfish, swordfish, trout, weakfish, whitefish
Fowl	f1 egg white f75 egg yolk f83 chicken meat	duck, duck eggs, goose, goose eggs, grouse, guinea hen, partidge, pheasant, squab, turkey
Mammals	f2 milk f26 port f27 beef f76 α -lactalbumin f77 β -lactoglobulin f78 casein f81 cheese, cheddar type f82 cheese, mould type f88 mutton	butter, gelatin, veal, goat, (cheese, goat's milk), horse meat, rabbit, squirrel, vension
Mollusks	f37 blue mussel	abalone, clam, oyster, scallop

This table is based on data from: R K Chandra Food intolerance 1984, Elsevier Science Publishing co., inc.

- An American yellow jacket is the same as what Europeans call a common wasp (*Vespula* spp)
An American aerial yellow jacket is also called yellow hornet. *Dolichovespula arenaria* and is still what most Europeans would call a wasp

The wasp of American literature is our paper wasp (*Polistes* spp)
Our hornet (*Vespa crabro*) is invading North America under the names of European Hornet.

THE WORDS 'WASP' AND 'HORNET' IN 32 LANGUAGES

	WASP	HORNET
American	yellow jacket	hornet
Arabic	dabbour	dabbour
Bulgarian	ocá	—
Chinese	wong fong	tai wong fong
Czech	vosa	srsen
Danish	hveps	gedehams
Dutch	wesp	hoornaar
Farsi	zanbour	zanbour-e-sorkh or, zanbour-e-dodosht
Finnish	ampiainen	herhiläinen
French	grêpe	frelon
Gaelic	connsbeach	connsbeach
German	wespe	hornisse
Greek	sphex	meg-al-ee sphex
Hebrew	Tzir-ah daboor	—
Hindi	tatsiya	hara
Hungarian	darázs	lodaraze
Irish	eikveach	—
Italian	vespa	calabrone
Japanese	—	suzume-bah-chi
Latin	vespula	vespa
Malay	peñyeñgat	tebuan
Norwegian	veps	gjetehams or, geitehams
Polish	osa	szerszeń
Portugese	vespa	vespão
Romanian	viespe	gařgařn
Russian	oca	chershyen
Serbocroat	osa	strsën
Slovene	osa	srsën
Spanish	avispa	moscardón or, crabrón
Swedish	geting	bålgeting
Turkish	esek arisi	büyük esek arisi
Welsh	gwenynen	cacynen

- Some cat-sensitive patients are sensitive to the cat flea rather than the cat

I:2:e SPECIAL ALLERGEN SERVICE

Hasse Schröder, Peter Edman

The current panel of Phadebas RAST®/Phadezym RAST® allergen discs now covers more than 180 important allergens and the number is continuously increasing.

In particular geographic areas certain allergens not yet available in the allergen disc panel may be of dominating importance. However, it may also occur that a clinical observation by an allergist indicates the existence of an allergen not yet fully recognized by the scientific community.

In both these situations new allergen discs would be of considerable value.

Many years of experience from the development of Phadebas RAST®/Phadezym RAST® reagents have shown that a straight coupling of conventional diag-

nostic or therapeutic allergen extracts to activated paper discs can result in high background, suboptimal results and poor correlation with other diagnostic procedures.

As a scientific service the Research & Development Department of Pharmacia, Allergy & Diagnostics Division will undertake the preparation of allergen discs not yet included in the Phadebas RAST®/Phadezyme RAST® allergen disc panel.

The reagents thus produced will then often be used by the clinician to further explore the importance of the new allergen and might lead to increased awareness of its role in atopy.

Examples of allergens where Pharmacia made Special Allergen Discs at an early stage of investigation are cockroach, chymopapain, mosquitoes, date pollen, pork, banana and peach.

I:3 MEASUREMENT OF SPECIFIC ANTIBODIES OF VARIOUS CLASSES IN ALLERGY AND RELATED DISEASES

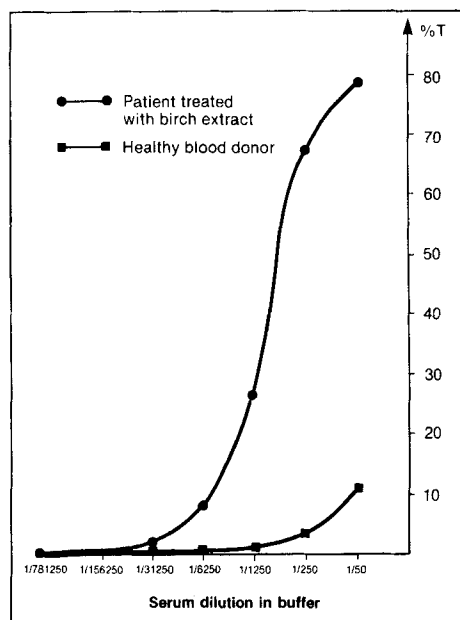
I:3:a SOLID PHASE METHODS IN SEROLOGY. Applications in allergen immunotherapy

Anita Kober, Marie Larsson, Cecilia Persson

The concentrations of allergen (antigen) specific IgG antibodies in serum is often 1 000 to 10 000 times lower than the total IgG concentration in serum. This poses special problems when measuring the small proportion of antigen-specific IgG antibodies.

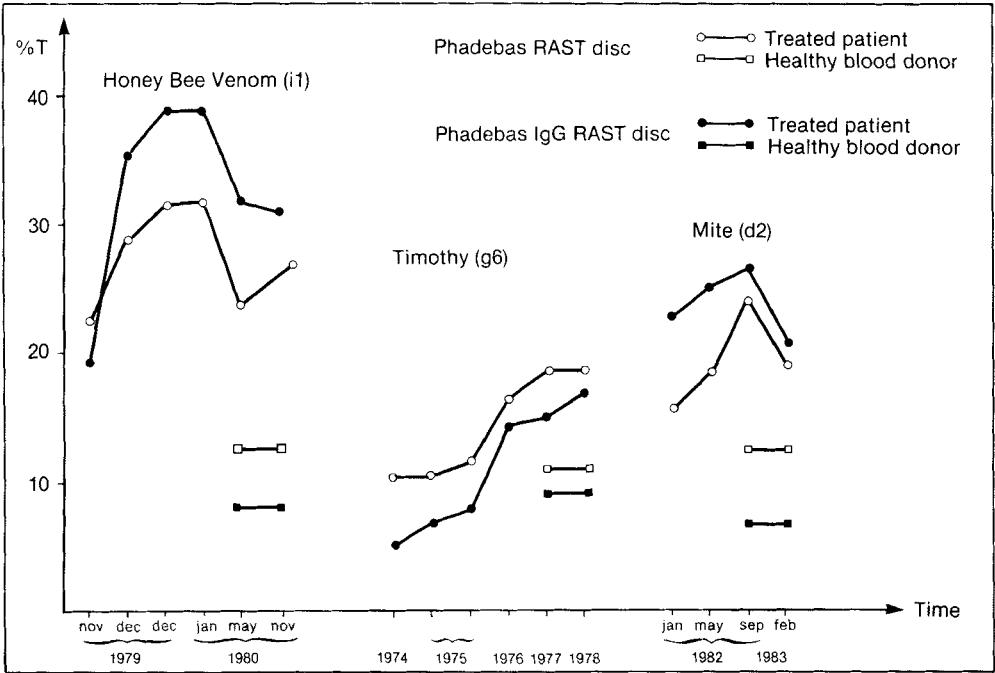
The sandwich principle with solid phase coupled antigen and a labelled (¹²⁵I or enzyme) anti-human IgG antibody is often used. In order to avoid too much interference from total IgG the serum samples are highly diluted (e.g. 1:200—1:1 000).

Different solid phases for coupling of allergen have been used for measuring specific IgG antibodies in hyposensitized allergic patients. Phadebas RAST® paper discs for specific IgE antibody measurements have been tried but are



not suitable, giving poor discrimination between patient and normal sera due to high nonspecific binding. Paper discs specially prepared for the Phadebas IgG RAST® system give better results.

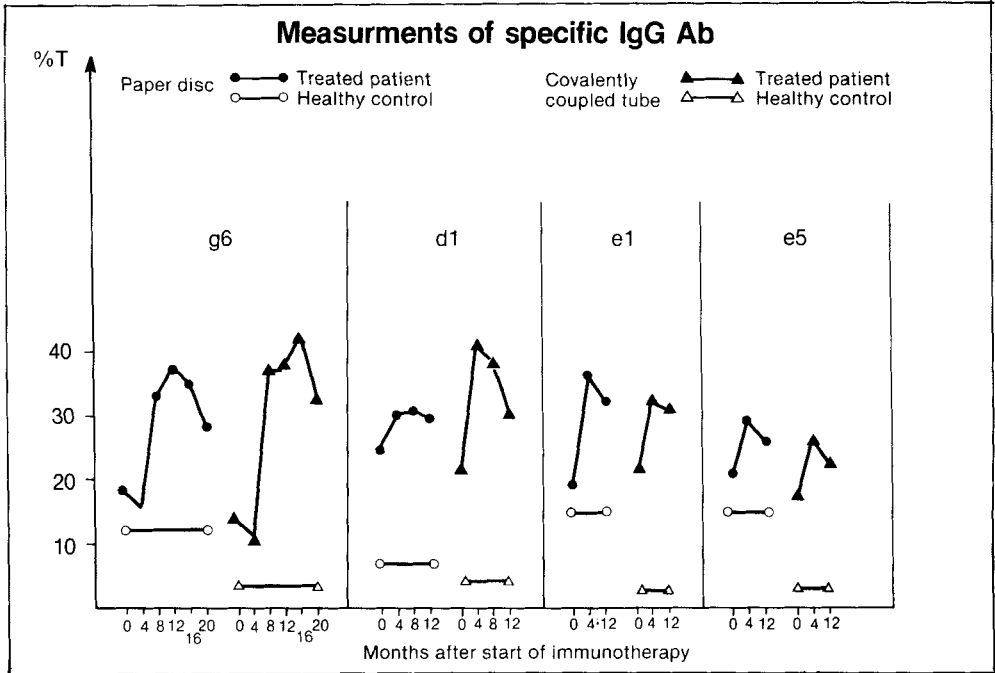
• Paper discs



The covalent coupling gives higher up-take than the frequently used passive coating although the latter method gives

satisfactory results at least for well-defined antigens.

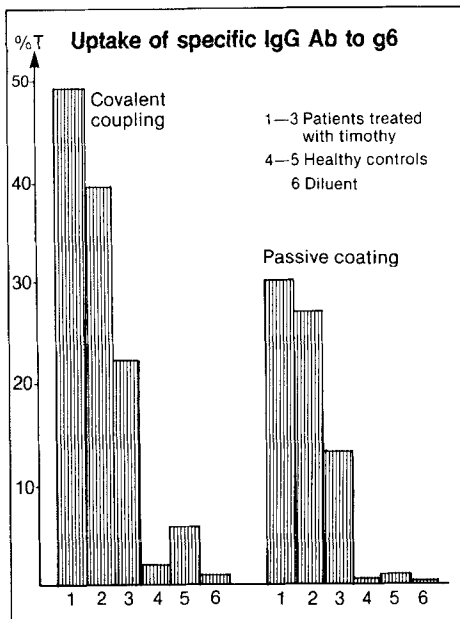
• Paper discs/and Coated tubes



An alternative solid phase with allergen covalently coupled to plastic surface (tube, microtiter well) gives a further im-

proved discrimination since nonspecific binding is lower and positive uptake is similar or higher.

• Covalent solid phases



I:3:b IgE AND IgG ANTIBODY MEASUREMENT IN PARASITOLOGY

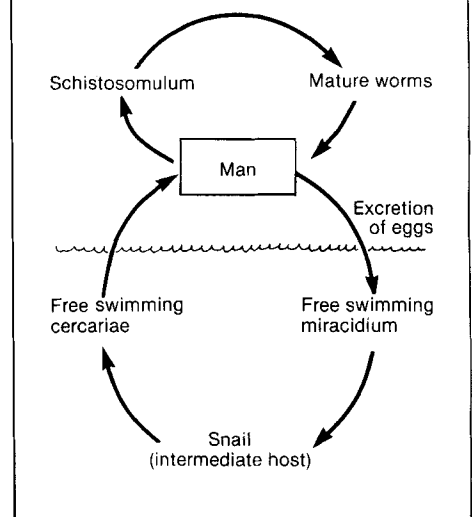
Hasse Schröder, Anders Sjölander, Marie Kullman, Gunnel Hult,* Inger Ljungström*

*Nat'l Bacteriological Laboratory, Stockholm

Schistosomiasis (bilharziosis) is a severe disease caused by infection with trematodes of the genus *Schistosoma*. The three main species which infect man are *S.mansoni*, *S.japonicum* and *S.haematobium*. *S.mansoni* occur in Africa, parts of Arabia, northern and eastern parts of South America and some Caribbean islands. *S. japonicum* is present in Japan, the Philippines, the Chinese mainland, Thailand and Indonesia. *S.haematobium* occur mainly in Africa and Middle East countries. The life-cycle of all three species is similar (Fig.1). Eggs leave man via faeces (*S.mansoni* and *S.japonicum*) or urine (*S.haematobium*). Those reaching water form free swimming miracidia that infect an appropriate snail host. During the asexual stage in snails numerous cercariae are produced and released in the water. The vertebrate host (man) is infected by penetration of mature cercariae through the skin. Immature worms, schistosomula, migrate through the tissues and vessels into lung and liver. Here they develop into adult sexually mature worms. Male

and female worms mate and migrate to their final destination according to the species. *S.mansoni* and *S.japonicum* migrate into mesenteric veins, and the eggs produced work their way through the wall of the blood vessels into the lumen of the intestine and are carried away by faeces to begin their life-cycle again. *S.haematobium* migrate to the veins of the bladder and eggs are excreted with the urine.

Fig 1. Life-cycle of *Schistosoma*



During schistosoma infection, patients are exposed to various antigens from the different life-stages. This challenge creates a complex humoral response with increased levels of mainly IgG, IgM and IgE antibody classes. The production of large amounts of circulating IgE is one of the characteristics of the immune response to most parasitic worm infections. The increase in total IgG is less marked. The purpose of this investigation was to study the human IgE and IgG response to soluble antigens of the adult worm (AWA) and eggs (SEA) of *S.mansoni*. Candidates for diagnostic antigens were selected and used for preparation of polyclonal rabbit antibodies.

Identification of antigenic components of *Schistosoma mansoni* using:

a) Crossed enzyme immuno-electrophoresis (CEIE)

Crossed enzyme immunoelectrophoresis (CEIE) was used to study the reactivity of IgE and IgG antibodies to soluble antigens from *S.mansoni* adult worm extract. Sera from 21 patients infected with *S.mansoni* and 6 negative controls were tested.

Forty-one antigens were precipitated by rabbit antibodies. Of these, 29 reacted with IgE or IgG antibodies from *S.mansoni* infected patients (Fig.2).

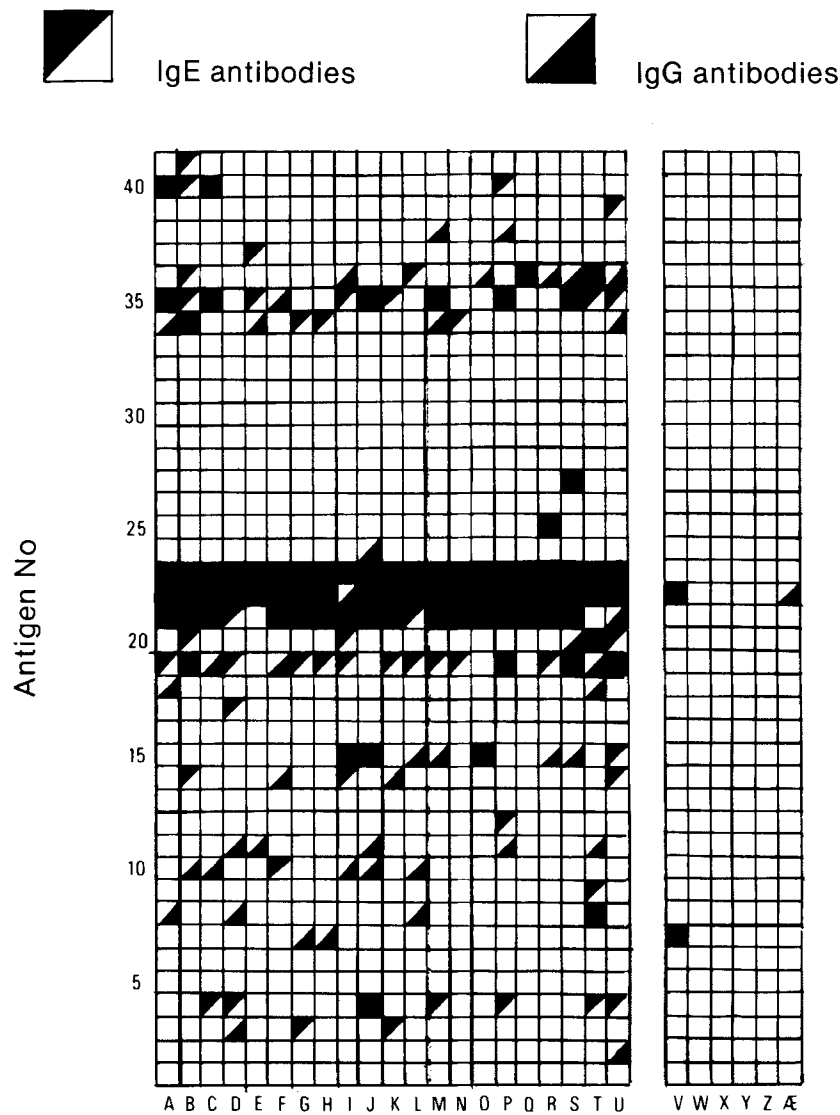


Fig 2 Patients infected with *S. mansoni*

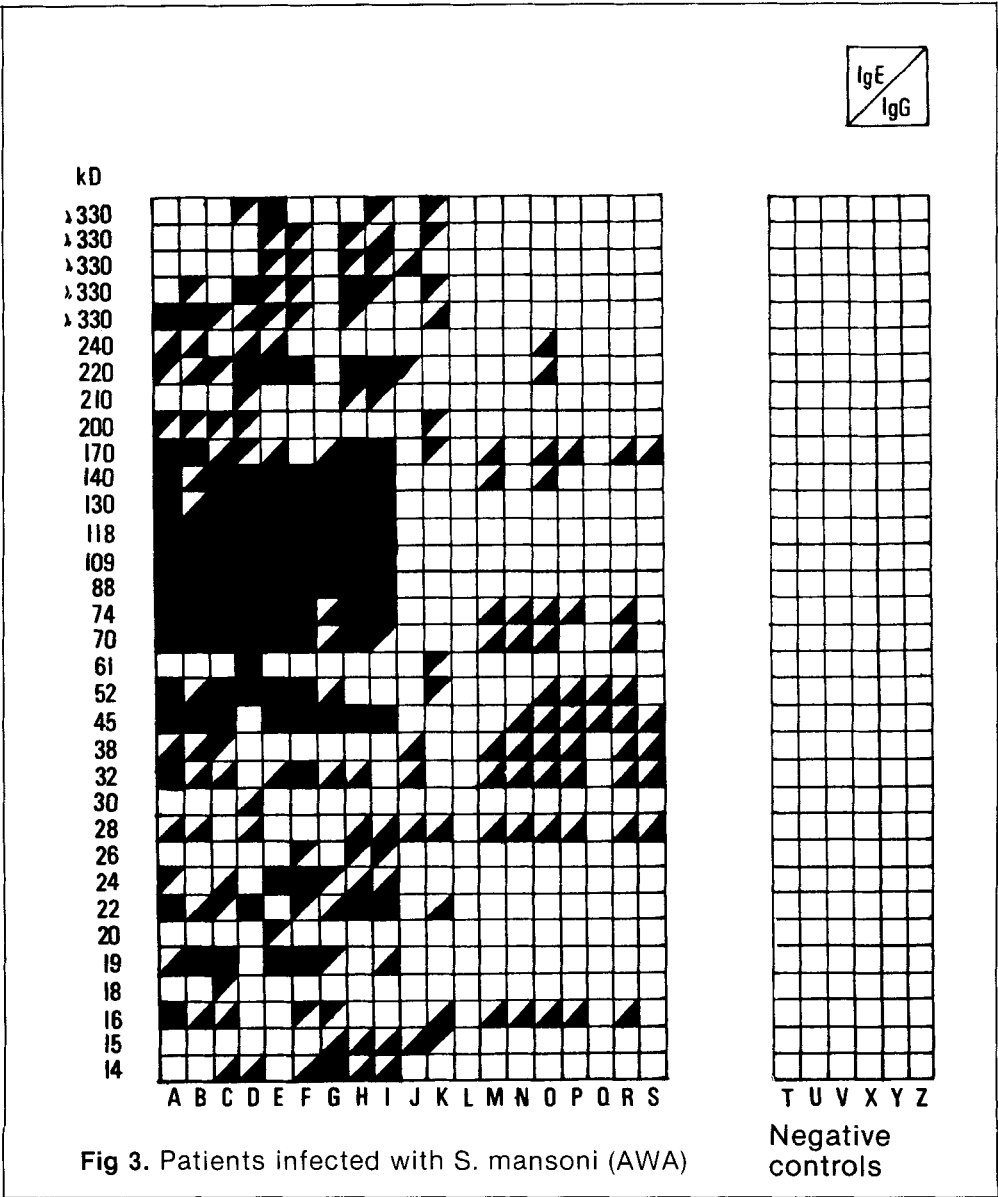
Negative controls

Three antigens, numbered 21, 22 and 23 were found which reacted with specific IgE and IgG in most patient sera. Except from these three dominating antigens, there were great differences, both between the IgE and the IgG responses, and between different sera.

b) Immunoblotting technique

Protein blotting technique based on SDS-PAGE followed by enzyme immunodetection was used for further investigation of the antigens in AWA and SEA. Sera from 19 patients with schistosomiasis were evaluated. A total of 33 antigens

were recognized in AWA (Fig.3). Eleven patients had AWA specific IgE in excess of 6 PRU/ml (Phadebas RAST®). The median number of antigens detected by IgE from these patients was 17 (11–20) and IgG 16 (14–19), respectively. The molecular weights ranged between 14 and 330 kD. Seven antigens sized 70–140 kD reacted with both IgE and IgG from most patients. The remaining 8 patients had no AWA specific IgE (<0.8 PRU/ml). Their IgG antibodies recognized 8 (2–12) antigens mainly outside the 70–140 kD range.



SEA was found to contain 21 antigens recognized by IgG only. No IgE binding was detected (Fig 4). The response was mainly directed to three groups of components with molecular weights 11–20 kD, 29–37 kD and 50–94 kD, respectively. Sera from healthy individuals showed no binding to any but two of the antigens where weak binding to a 94 kD component in SEA was found.

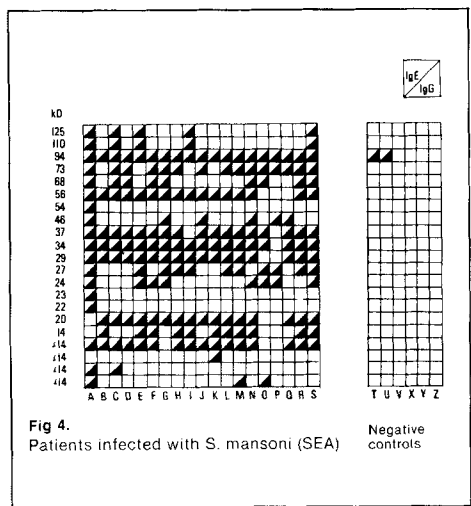


Fig 4.
Patients infected with *S. mansoni* (SEA)

Negative
controls

Preparation of polyclonal rabbit antibodies to major *S.mansoni* adult worm antigens

The three antigens (21, 22, 23) being of particular interest according to CEIE data were chosen for production of polyclonal rabbit antibodies. Following crossed immunoelectrophoresis (CIE), those precipitates containing the selected antigens were cut out with a razor blade and directly injected into lymph nodes of rabbits. The rabbit antibodies were then tested for their reactivity to *S.mansoni* adult worm extract by CIE and CEIE. CIE plates stained with Coomassie Blue revealed three precipitates correlating to the positions of antigens 21, 22 and 23. When tested with human sera in CEIE all three precipitates reacted with both IgE and IgG antibodies. By using those rabbit antibodies as precipitating antibody in CIE new series of immunization studies were performed, giving rabbits producing antibodies against antigen 23, against antigen 22, 22 and against antigens 21, 22, 23, respectively.

I:4 ANTIGEN-SPECIFIC HUMAN ANTIBODIES AS REAGENTS FOR QUALITATIVE AND QUANTITATIVE RAST

I:4:a PATIENT SERA

Per Jonsson

A

Collection

Sera are preferably collected from different regions all over the world due to the regional distribution of allergens. Some examples of suitable areas for collection are North Europe, South Europe, USA, Japan and Australia.

EXAMPLE:

Table 1 shows Phadebas RAST® results on 6 patients from the south of Sweden. They are likely to be sensitized, mainly by mugwort (*Artemisia vulgaris*), but they also show positive results against ragweed (*Ambrosia elatior*), due to cross-reactions. They are not suitable as serum reagents for ragweed, because they do not have IgE antibodies against all important components of ragweed.

Table 1	Serum No	Phadebas RAST® (PRU/ml)	
		Mugwort (w6)	Ragweed (w1)
	4 686	2.5	2.6
	5 216	> 17.5	1.6
	5 924	> 17.5	1.2
	12 696	14.2	1.5
	12 701	8.6	0.6
	13 840	4.0	1.0

C Selection

Selection of serum shall be made on the basis of the characterization.

CRITERIA FOR SELECTION:

The serum must

- have specific IgE antibodies against the allergen.
- possess a sufficiently high titer of IgE antibodies against the allergen.
- be representative as a serum reagent for the actual allergen.

A good characterization of an adequate number of sera can give you information to select a representative serum. Another useful method is to make a pool of a sufficient number of sera and in that way enrich antibodies against major components.

Additional criteria

Serum selected for RAST inhibition must also

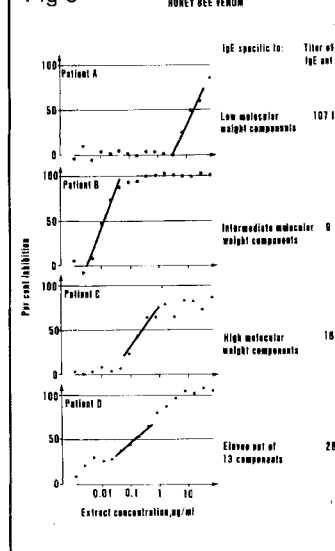
- give a sufficient slope
- give a defined plateau level
- give a low blank level
- give a representative RAST inhibition curve for the tested allergen.

EXAMPLE:

Table 2 shows good reproducibility between 6 different serum pools against timothy (*Phleum pratense*).

The effect of using different serum pools on the RAST inhibition assay of <i>Phleum pratense</i> extracts		
Serum pool No.	Relative potency (% of internal reference)	
	Extract No. 1, partly purified	Extract No. 2, crude
1342	119	189
1693	110	131
2545	91	142
2667	98	165
G I	91	117
G II	100	152
Mean	102	142
Relative s.d.	11%	17%

Fig 3



Potential difficulties

Figure 3 shows RAST inhibition assays on honey bee venom with four different sera. All sera give different types of curves. Three major types of specificities are represented by IgE antibodies specific for mellitin, phospholipase and hyaluronidase, respectively. A

B

Characterization

The serum must have appropriate concentrations of specific IgE against the important components of the allergen. Characterization of the allergen components can be performed with different techniques, for example CIE/CRIE/CEIE or immunoblotting. By making an allergogram the major components can be identified.

Figure 1 shows an immunoblotting analysis of 18 sera from *Aspergillus fumigatus* sensitized patients.

The corresponding allergogram is shown in Figure 2.

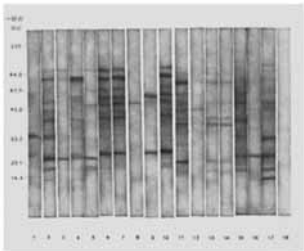


Fig 1

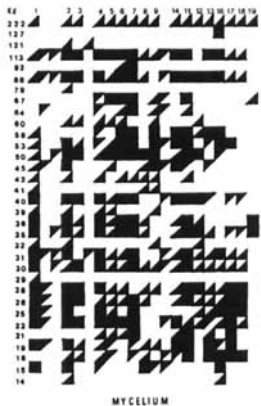
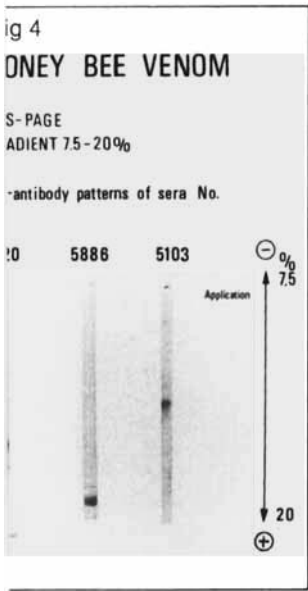


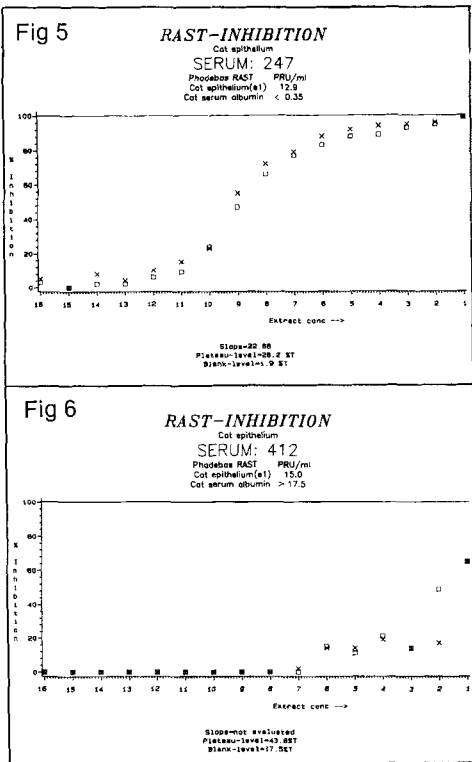
Fig 2



pool of these three sera would look very much like the fourth serum (5544), which contains antibodies to 11 out of 13 components.

Figure 4 shows the immunoblotting of sera 3120 (phospholipase), 5886 (mellitin) and 5103 (hyaluronidase).

Figures 5 and 6 show RAST inhibition assays with two different sera on the same cat epithelium extract. A serum (412), which mainly contains antibodies against cat serum albumin cannot be used for measuring of cat dander allergens. The two sera are not adjusted to a correct plateau level.



I:4:b METHODS FOR CHARACTERIZATION OF SERUM REAGENTS

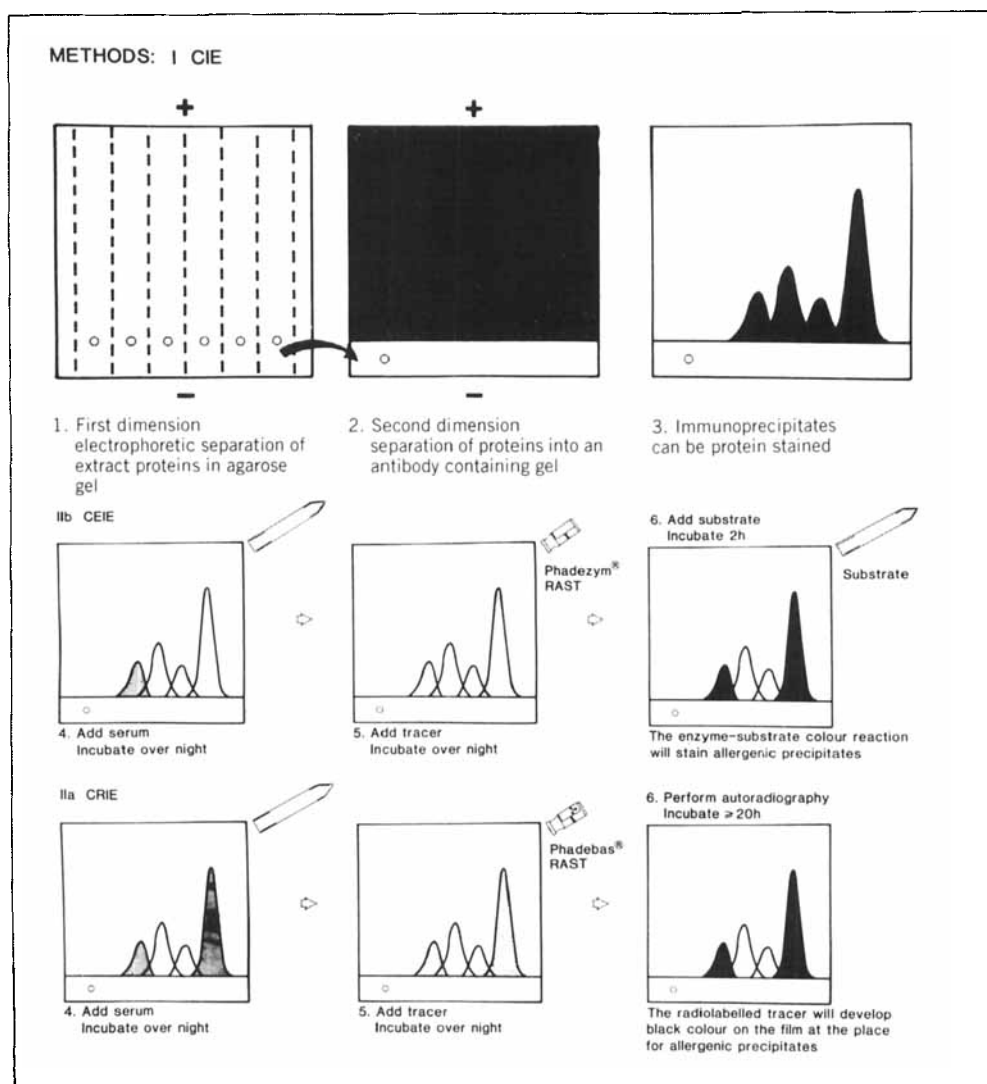
Part 1: Allergen/antibody patterns by immunoblotting and crossed immunoelectrophoresis

Wenche Rolfsen, Anders Bengtsson, Åsa Karlsson, Mats Tibell

CIE/CEIE/CRIE

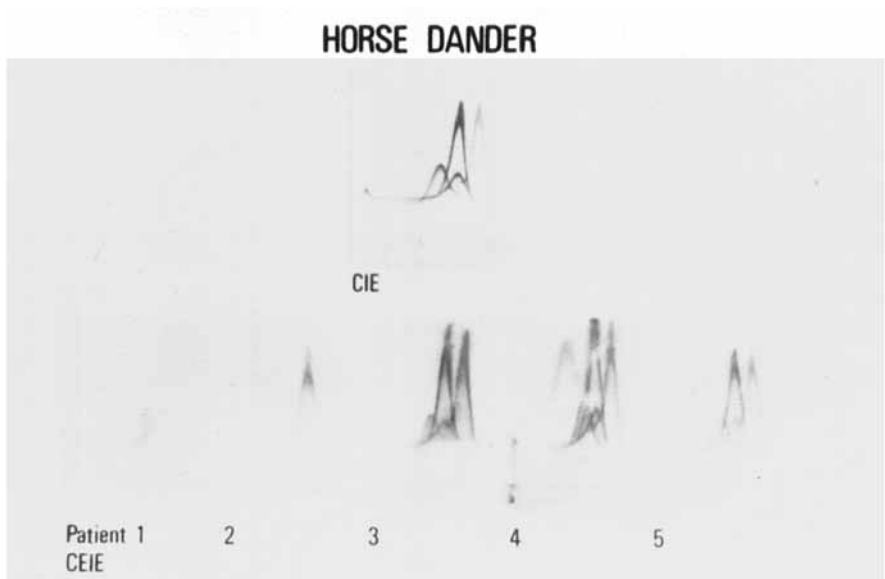
In this method allergens are first separated by charge in an agarose gelstrip.

The gelstrip is then molded next to an antibody containing gel for a second immunoelectrophoresis perpendicular to the first. The allergens move into the antibody containing gel resulting in formation of immunoprecipitates. After incubation of the CIE-plates with serum containing allergen specific antibodies the allergen-antibody complexes are detected with labelled anti-antibody.

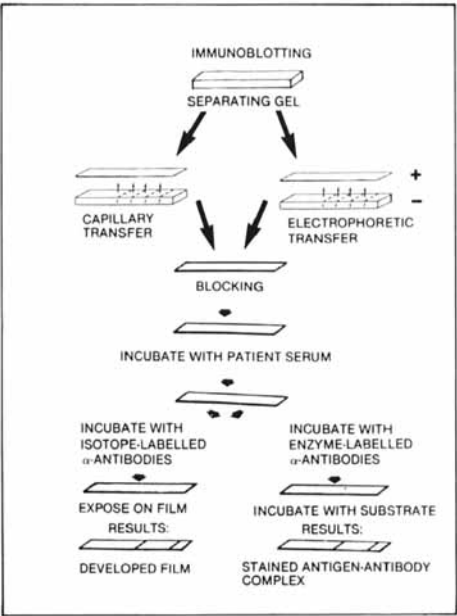


Five horse allergic patients. Allergen precipitates were detected with a Beta-

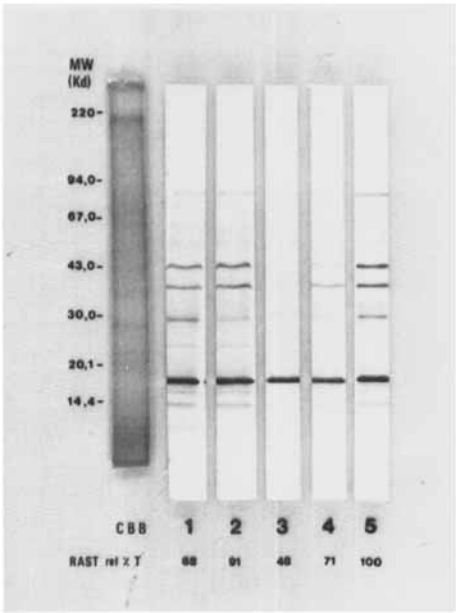
galactosidase labelled anti-IgE-antibody.



Part 2: Immunoblotting



Immunoblotting is a technique where allergens are first separated in a suitable gel, followed by electrophoretic or capillary transfer to a matrix. After incubation of the matrix with serum containing allergen specific antibodies, the bound antibodies are detected with a suitably labelled anti-antibody.



Comparison of 5 batches of Aspergillus fumigatus. Bands were detected with a Beta-galactosidase labelled anti-IgE antibody.

I:4:c ALLERGEN ASSAY RAST INHIBITION. Methodology and evaluation

Per Jonsson, Marie-Louise Andrae, Hasse Schröder, Lars Yman

RAST inhibition is used in routine quality control of raw material and extracts as well as for allergen research.

The RAST inhibition method provides a possibility to produce standardized allergen extracts.

Principle

The allergen extract reacts with a serum containing specific IgE antibodies against the tested allergen. The excess of IgE antibodies reacts with allergens, covalently coupled to a paper disc. Labelled anti-IgE antibodies are added, forming a complex. The amount of this complex B is measured. The relative potency against a reference extract is estimated by a parallel line assay method.

A RAST inhibition assay can only measure an allergen in an extract if the same allergen is represented on the solid phase and the IgE antibody reagent (the serum) contains the corresponding antibody. Furthermore, the potency measurement is clinically meaningful only if the IgE antibody reagent represents the majority of the patients in a defined group.

Characterized serum reagents together with reference extracts and reference discs form the basis for the selection of raw materials and conditions for the preparation of the Phadebas RAST® allergen discs, which are routinely used for allergen assay.

