

A new method for IgE detection in nasal mucosa

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Summary

A new method for determining IgE antibodies on mucosal surfaces has been developed with the purpose of overcoming the main problems of nasal secretion sampling and standardization. The method is based on the principle of performing the incubation of the solid-phase coupled allergen with IgE antibody directly on the mucosal surface by means of a proper applicator. About two times higher values of specific IgE have been obtained with 5 min incubation on septal mucosa, behind the internal ostium, than with 3 hr *in-vitro* incubation with native secretion. In a study of 53 children with allergic rhinitis and asthma and 10 healthy non-atopic controls the sensitivity and specificity of this new method were evident. Because of its reliability and easy execution the new method could be widely used in diagnosis of allergic disease. Further, it seems to offer a good opportunity to study the IgE-mediated reaction in the target organ more extensively.

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Introduction

The diagnosis of allergic disease is usually based on clinical history, confirmed by the detection of allergen-specific IgE in skin and/or serum. It is commonly agreed that a specific nasal, conjunctival or bronchial provocation test is the most appropriate way to reach the final diagnosis as it shows the presence of IgE antibodies in the target organ. However, as it has well-known limitations, it is not suitable for routine practice.

Experimental studies have indicated the local production of IgE in agreement with the specific provocation test even in cases with negative or weakly positive serum RAST and suggested that nasal RAST may have diagnostic value [1-3]. There are conflicting results from different studies on this question, partly due to different subject selection, but mainly due to the different technical procedures employed for the sampling and treatment of nasal secretion [4].

Problems relevant to developing a well-standardized method for IgE detection in nasal secretion have also limited the diagnostic usefulness of such measurements.

A simple, reliable test, well tolerated by patients, was needed for the study of IgE antibodies in mucosal surfaces for diagnostic and research purposes.

We have developed such a test method and have evaluated its performance in children with respiratory allergy.

Subjects and methods

The new method, i.e. nasal test (NT), is based on the principle of performing IgE antibody detection in the same conditions in which the IgE-mediated reaction occurs. For this purpose, the solid phase to which the allergen is coupled is placed on nasal mucosa. In this way the incubation of the allergen with IgE antibody is performed *in situ*, i.e. on nasal mucosa, instead of *in vitro*, in collected secretion.

Test procedure and reagents

A paper solid phase with covalently coupled allergen (Phadezym RAST, Pharmacia, Uppsala, Sweden), carefully washed in order to remove the preservatives, was employed.

A plastic applicator with a container for the solid phase was used with the aim of putting the allergen, coupled to paper on nasal septum mucosa, behind the internal ostium.

The container, made of nylon membrane (60 × 60 µm pore), allowed the contact of mucosal IgE antibodies with the allergen coupled to the paper and avoided the

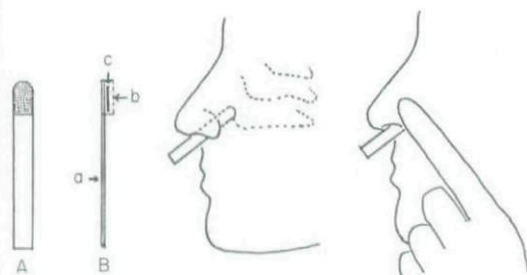


Fig. 1. The paper disc coupled to allergen is put in contact with septum nasal mucosa behind the internal ostium. A, applicator; B, cross-section of applicator (a, handle; b, container; c, substrate).

adhesion of any gross material from nasal secretion to the substrate.

The positioning of the applicator for the short time of 5 min was assured by external finger pressure (Fig. 1).

After *in-situ* incubation, the solid phase was removed from the container and put into a test tube with 50 μ l of saline solution (NaCl 0.9%) and stored at -20°C until used. After an exhaustive washing procedure, specific IgE determination was performed according to the Phadezym RAST method (Pharmacia, Uppsala, Sweden). The non-specific IgE binding on the paper substrate resulting from nasal incubation was evaluated by performing the nasal test in duplicate with uncoated discs in 10 atopic subjects. All test results were then evaluated according to the serum standard (Phadezym RAST Reference) and expressed in PRU/ml.

Test site

Altogether, 18 children affected by allergic rhinitis, with positive skin-prick test to grasses or mites, were studied in order to determine the most suitable site for NT. All tests were performed by the same investigator with a 5-min *in-situ* incubation on all occasions.

Fifteen determinations were performed on septal mucosa, above and posterior to the internal ostium (site 1) and on the opposite side, on the lateral nasal wall (site 3), by an applicator with a container on both sides, in 11 children.

Eighteen determinations were performed at two positions of the septal nasal mucosa, one behind the internal ostium (site 1) and one 5 mm lower (site 2, Fig. 2), by an applicator with two containers on the same side, one above the other, in five children.

Twenty-five determinations were performed in seven children at site 1, on both the right and left nostril (Fig. 2).

Repeated determinations in the same subject were evaluated as mean values of optical density (420 nm) for statistical purposes. Student's *t*-test for paired data, and 95% confidence limits (95% CL) were used (Table 1).

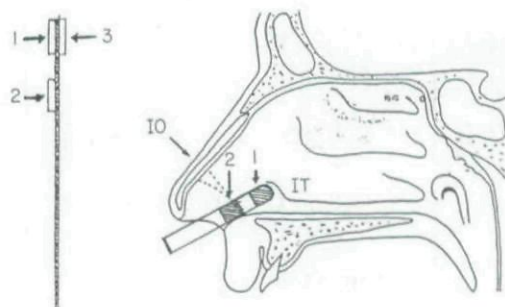


Fig. 2. Left side: positions of substrates on the applicator to be placed on septum mucosa (1–2) and on mucosa of lateral nasal wall (3). Right side: in the diagram of the nasal cavity sites 1 (1) and 2 (2) are shown; site 3 (not indicated) is in front of site 1. IO, internal ostium; IT, inferior turbinate. Site 1: on septum mucosa above and posterior to the internal ostium. Site 2: on septum mucosa below the internal ostium. Site 3: on mucosa of the lateral nasal wall, above and posterior to the internal ostium.

Table 1. Comparison between different nasal test (NT) sites using Student's *t*-test for paired data

	N	$\bar{d}$	s.d.	<i>t</i>	P
Site 1 versus site 3	11	0.155	0.164	3.129	<0.05
Site 1 versus site 2	5	0.550	0.211	5.837	<0.001
Left versus right site 1	7	-0.083	0.172	-1.278	>0.10

Specific IgE was expressed as no transformed optical density (420 nm) values.

$\bar{d}$ —mean difference.

s.d.—standard deviation of the difference.

Comparison between NT and assay in collected nasal secretion

Specific IgE was determined in 11 children by NT, i.e. performing allergen-IgE incubation on nasal mucosa *in-situ*, as well as in collected secretion (*in vitro*), according to Okuda [5].

Four NT, with *in-situ* incubations of 2, 5, 10 and 30 min, were performed in each subject at an interval of 15 min, 1 hr after having collected 0.5 ml of mucus by suction. The collected nasal secretions were incubated with allergen discs *in vitro* for the same period of time as the *in-situ* incubation, except for the 3-hr incubation which was only performed on secretion. *In-situ* and *in vitro* incubated samples were then run together, according to the RAST procedure. Median values of IgE (PRU) were calculated for each incubation time both for NT and assay in collected secretion.

Table 2. Specific IgE values obtained with different allergen-IgE antibody incubation time (*t*) on nasal mucosa (with NT) and in collected nasal secretion

Subject	Allergen	<i>t</i>	PRU/ml	
			Nasal mucosa	Secretion
B.F.	Gramin.	2 mins	2.75	0.9
		5 min	4.61	0.6
		10 min	4.7	0.35
		30 min	4.68	0.64
		3 hr	—	1.23
M.A.	<i>Dermatophagoides</i>	2 min	9.8	1.3
		5 min	16.5	1.8
		10 min	12.5	2.73
		30 min	9.9	4.03
		3 hr	—	5.3
M.M.	<i>Dermatophagoides</i>	2 min	1.49	<0.35
		5 min	2.49	<0.35
		10 min	3.48	<0.35
		30 min	2.1	<0.35
		3 hr	—	0.41
P.F.	<i>Dermatophagoides</i>	2 min	0.4	<0.35
		5 min	0.61	<0.35
		10 min	0.61	<0.35
		30 min	0.35	<0.35
		3 hr	—	0.35
P.S.	Gramin.	2 min	9.8	3.5
		5 min	>17.5	4.8
		10 min	17.5	5.2
		30 min	>17.5	5.8
		3 hr	—	7.9
B.M.	Gramin.	2 min	3.45	0.36
		5 min	6.76	0.63
		10 min	4.23	0.7
		30 min	2.8	0.58
		3 hr	—	3.2
M.F.	<i>Dermatophagoides</i>	2 min	3.5	5.13
		5 min	>17.5	6.57
		10 min	>17.5	6.8
		30 min	>17.5	7.43
		3 hr	—	8.9
P.M.	Gramin.	2 min	17.11	4.06
		5 min	>17.5	4.1
		10 min	16.27	4.15
		30 min	>17.5	7.24
		3 hr	—	9.6
L.L.	Gramin.	2 min	7.66	1.4
		5 min	10.53	1.9
		10 min	11.6	2.68
		30 min	9.83	4.06
		3 hr	—	3.54
L.E.	<i>Olea</i>	2 min	1.22	2.1
		5 min	6.86	1.8
		10 min	6.9	1.71
		30 min	7.35	1.77
		3 hr	—	2.8
S.G.	<i>Dermatophagoides</i>	2 min	2.04	0.67
		5 min	3.78	0.69
		10 min	3.5	0.69
		30 min	3.6	0.84
		3 hr	—	1.46

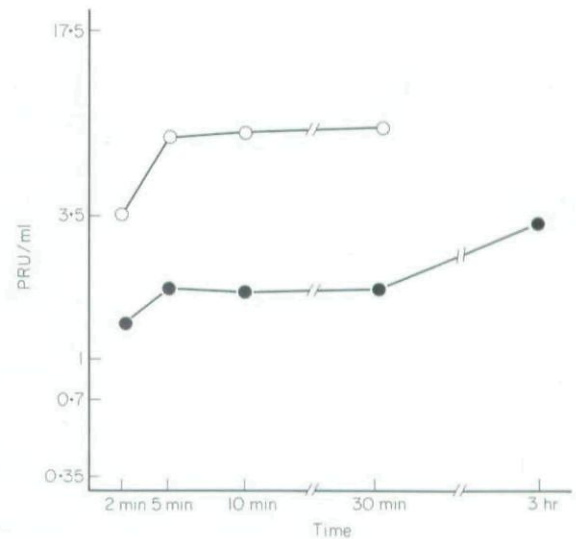


Fig. 3. Comparison of specific IgE values obtained with different incubation times, on nasal mucosa (with NT) (O) and in collected nasal secretion (●). Median values (PRU/ml).

Table 3. Reproducibility of eight replicate measurements of specific IgE with NT in six atopic children: variability of measurements (within- and between-subjects s.d.)

	Specific IgE with nasal test (OD 420 nm)
Variability of measurements	
Between-subjects s.d.	1.035
Within-subjects s.d.	0.353
<i>F</i>	8.580
<i>P</i>	0.0001

Reproducibility

The NT was repeated eight times in six children at an interval of 15 min. The tests were performed at site 1 with a 5-min incubation in all cases.

Reproducibility of NT was evaluated by one-way analysis of variance which separates between-subjects and within-subjects components of variation in the measurement (Fig. 5). Within-subjects standard deviation was also used for computation of the variation coefficient. Optical density values (420 nm) were used for the analysis.

Clinical diagnostic efficiency

NT for 81 allergens, strongly suggested by clinical history and confirmed by SPT and/or serum RAST, were

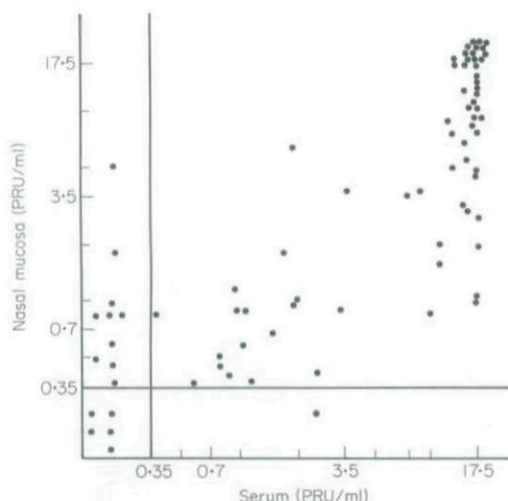


Fig. 4. Scattergram of nasal mucosa versus serum IgE to 81 allergens (PRU/ml) tested in 53 children with rhinitis and/or asthma.

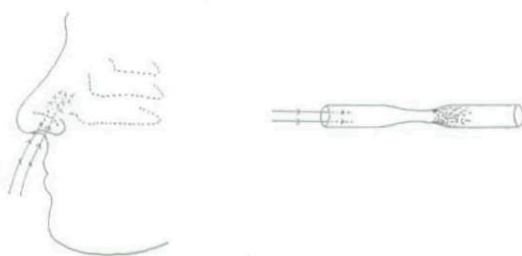


Fig. 5. Zone of mucosa (behind the internal ostium), where air flow and narrowing of the nasal cavity cause the deposition of the largest amount of allergen. Similar deposition occurs in a narrowed tube.

performed in all 53 children, aged between 2 yr and 15 yr, with rhinitis alone or with rhinitis associated with asthma, who consecutively presented to our clinic during a period of 5 months.

SPT, serum RAST and NT for *Dermatophagoides* P., *Phleum pratense* and *Parietaria* were performed in 10 non-atopic children, to examine the specificity of NT. In all cases 5 min incubation NT was performed on the septum, behind the internal ostium. SPT were performed with glycerinated extracts by Hollister Stier (Bayro-pharm, Italy). A control skin-prick test with 1% histamine was included. The allergenic reaction was evaluated in comparison with histamine. Serum-specific IgE was determined with Phadezym RAST (Pharmacia, Uppsala, Sweden).

The correlation of NT to skin-prick test and serum RAST was evaluated with Spearman's rank coefficient (r_s) for non-parametric data. To this purpose, the serum RAST and NT results were expressed as RAST class values, and the skin-prick test as 0, 1, 2, 3 and 4 levels of positivity.

Results

Comparison between NT sites

A significant difference was found between values obtained from septum (site 1) and lateral part of nostril (site 3) ($t=3.128$, $P<0.05$) (Fig. 3). Site 1 gave on the average 22.59% higher values than site 3 (95% CL=3.1–38.6%). A significant difference was also found between the position behind the internal ostium (site 1) and below (site 2) ($t=5.83$ $P<0.01$) (Fig. 3). In all cases higher values were obtained from site 1, which gave on average 50.3% higher values than site 2 (95% CL=26.39–74.26%). Mean difference of 10% (95% CL=9.2–29.4%), which was not significant, was found between the right and left nostril (Fig. 3).

The data showed that the most suitable position to perform the NT is on the septum mucosa, behind the internal ostium, i.e. in site 1.

Comparisons of results obtained with NT and assay in collected secretion

NT showed positive results of IgE antibodies in all cases with 2-min *in-situ* incubation (median value: 3.45; range: 0.4–17.11 PRU/ml).

Significantly higher values were observed with 5-min incubation (median value: 6.86; range: 0.61–>17.5 PRU/ml), while no significant increase was obtained with the 10- and 30-min *in-situ* incubations (median value: 6.9; range: 0.61–>17.5 PRU/ml, and median value: 7.35; range: 0.35–>17.5 PRU/ml, respectively) (Table 2, Fig. 3).

In collected secretion with a 2-min incubation *in-vitro* positive results were obtained in nine out of 11 cases (median value: 1.3; range: <0.35–5.13 PRU/ml).

Only a very low increase in values were observed with the 5-, 10- and 30-min *in-vitro* incubations. Median values and ranges were 1.8 (<0.35–6.57); 1.71 (<0.35–6.8) and 1.77 (<0.35–7.43) PRU/ml, respectively.

Only after a 3-hr *in-vitro* incubation were significantly higher levels of IgE antibodies found in collected secretion (median value: 3.2; range: 0.35–9.6 PRU/ml).

Positive values were also obtained in the two cases negative with up to 30-min *in-vitro* incubation (Tables 2 and 3).

A comparison of data showed significantly higher values of specific IgE with 5-min incubation *in situ* (NT) than with 3-hr *in-vitro* incubation of collected secretion (Table 2, Fig. 3).

Reproducibility

In six atopic subjects the reproducibility of eight replicate measurements of specific IgE with NT, determined by

one-way analysis of variance (Fig. 5), showed that between-subject variability, i.e. biological variability, was significantly greater than within-subject variability, i.e. measurement error ($P < 0.0001$). The coefficient of variation computed using the within-subject standard deviation was 37.6%.

Correlation of NT to SPT and serum RAST

A total of 65 out of 81 (80%) allergens examined in 53 SPT and/or serum RAST positive children were positive both with NT and serum (Fig. 4).

Five of 81 (6%) were concordantly negative with both. One allergen was positive only in serum and 10 were positive only in the nose.

The median value of serum RAST was 12.98 PRU/ml (range <0.35 – >17.5 PRU/ml) and of NT 3.5 PRU/ml (range <0.35 – >17.5 PRU/ml). The median value of 10 allergens positive only with NT (concordantly with SPT) was 0.84 PRU/ml (range 0.35–5.31 PRU/ml).

According to Spearman's test, NT showed positive correlation both with serum RAST ($r_s = 0.761$, $P < 0.01$) and skin-prick test ($r_s = 0.642$, $P < 0.01$).

Specificity of NT

A non-significant non-specific IgE binding to uncoated discs was shown by values similar to the optical density of the blank, obtained in all occasions, in the 10 atopic subjects tested.

Negative results (<0.35 PRU/ml) were obtained in all 10 non-atopic controls, for all 33 allergens tested.

Discussion

Some researchers have suggested that local antibody production occurs in nasal mucosa without being detectable either in skin or serum. They have also stated that nasal specific IgE correlates better with clinical disease and the provocation test than skin testing or serum RAST [1, 3, 6, 7].

However, the diagnostic value of measuring nasal IgE antibodies has been questioned in other studies [4], mainly because the lack of standardization does not allow comparison between the data or with serum results. Basically the study of mucosal secretions has many limitations because of sampling technique and physiologically variable concentrations of secretions.

Moreover, further dilution of these secretions, of unknown volume, is caused by the nasal washing solution and the mechanical, specific and non-specific stimulation procedure employed for collecting the necessary sample volume.

Difficulties also arise in finding absolute reference values. In fact, proteins and immunoglobulins in nasal secretion vary independently of the IgE concentrations [5].

The new method, based on incubation directly on nasal mucosa, overcomes the main problems of measurement in secretions. Allergen–IgE antibody incubation is performed *in situ*, in the nasal mucosa where the air flow deposits the largest amount of antigen, that is behind the internal ostium, the site of the first narrowing of the nasal cavity (Fig. 5). A greater quantity of antibodies was expected in this zone. In fact, we obtained significantly higher IgE antibody levels in this site of the septal mucosa than at a lower site.

Collected secretion samples are made of mucus coming from several zones of the nasal mucosa, including those where, as the results of comparison between high and low positioning of allergen coupled substrate in septal mucosa have shown, lower amounts of IgE are produced.

The greater sensitivity of this new method may also be explained by the favourable conditions in which the binding of allergen with IgE antibody occurs due to the *in-situ* incubation. The difference in the kinetics between NT and the assay in collected secretion also supports this hypothesis.

The maximum value in nasal mucosa incubated samples was reached with a 5-min incubation, in the same short time as the IgE-mediated Type I reaction which develops in the pathological process.

In collected nasal secretion on the other hand, a 3-hr incubation period was needed to reach the maximum value. We can hypothesize that the thin stratum of mucus over the mucosa has physico-chemical properties that favour the reaction of IgE molecules with allergens more than does mucus outside the nose.

The sensitivity of the method was confirmed by the results obtained in allergic subjects. The NT was concordant with SPT and/or serum RAST in the majority of the allergens tested. A better concordance of nasal test with SPT (90%) than with serum RAST (80%) was also found. Ten allergens were positive with NT, concordantly with SPT, and negative in serum; seven had higher values in nose than serum. A positive response of mucosal IgE antibody independent of serum was clearly shown by these results, confirming the local production of this immunoglobulin in agreement with other authors [8, 9].

The reliability of these data was also confirmed by negative results obtained with the new test in non-atopic controls.

In conclusion, our method has shown good sensitivity, specificity and reproducibility, and in our experience has proved to be well accepted even by pediatric subjects. It therefore seems to be an effective routine diagnostic test,

and also a very interesting method from the scientific point of view as it allows more extensive study of the IgE reaction in the target organ, combining the advantages of a specific provocation test and an antibody titration procedure.

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