

## Short communication

# Comparison of ammoniated and nonammoniated extracts in children with latex allergy

**Background:** The use of ammoniated or nonammoniated latex extracts for the diagnosis of latex allergy is still a matter of debate. The aim of our study was to compare the characteristics of the two types of extracts by immunoblotting and RAST techniques in children with ascertained latex allergy.

**Methods:** Ammoniated (AL) and nonammoniated latex (NAL) extracts were prepared and blotted on SDS-PAGE to resolve their components. Also a solid phase for RAST assays was prepared with the two extracts. The sera from 18 children (mean age 11.4 years, range 6–15 years), with ascertained latex allergy (clinical history, skin test, CAP-RAST and provocation) were used for the experiments.

**Results:** The NAL extract is resolved in many bands (5–100 kDa), whereas AL showed only few components, likely Hev b 4, 6 and 7. IgE reactivity against AL was observed only in 5/18 patients, whereas 12/18 were positive with NAL. The blotting profile against NAL was complex and the IgE recognition pattern involved different bands.

**Conclusion:** The extract obtained from NAL is able to detect specific IgE against a greater number of allergenic determinants, and therefore a greater diagnostic accuracy can be expected.

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*Hevea brasiliensis* latex is used since decades to manufacture thousands of industrial and medical products, particularly surgical gloves. However, immediate hypersensitivity reactions to natural rubber latex began to be reported starting from the 1970s, and they represent nowadays a serious and increasingly health problem in both adults and children (1, 2). Many individuals in the general population can develop latex hypersensitivity such as children playing with rubber toys, workers of the rubber industry, people using household gloves. There are also well-recognized subgroups at high risk, i.e. children with *spina bifida*, patients undergoing frequent surgical procedures and medical/paramedical personnel (3), probably due to the frequent/repeated contact with medical devices. Recent evidence indicate that the mucosal exposure to latex allergen is mainly due to aerodispersed latex proteins, carried by particles of cornstarch powder, used as lubricant in latex gloves (4). The typical symptoms of latex allergy include: urticaria, rhinitis, conjunctivitis, asthma and, less frequently, anaphylactic shock (5). In view of the possible severe manifestations, a detailed diagnosis is mandatory for appropriate prevention measures and therapeutic management.

Although it is generally accepted that the diagnosis of allergy must be based on the clinical history of symptoms associated to latex exposure, confirmative skin prick test (SPT) and/or serological assay are recommended (6).

Although the SPT test is generally considered the most reliable tool, its accuracy and reproducibility can be influenced by inadequate standardization (7). The diagnostic performance of SPT is highly dependent on the protein extract and in particular on the raw material used in its preparation. Glove eluates have been used in the past for diagnosis, but their complex preparation procedure and their considerable variability make difficult the production of commercial standardized preparations (7, 8). The use of crude latex extracts, starting from ammonium-treated latex (ammoniated latex, AL) or from latex serum (nonammoniated latex, NAL) has been proposed for the preparation of reference reagents, but there is no general consensus on which is the most reliable extract (9–11).

The aim of the present study was to compare SDS-PAGE and immunoblotting profiles of AL and NAL extracts using serum from children with well-ascertained latex allergy.

## Material and methods

### Patients

Eighteen children (nine boys, nine girls, mean age 11.4 years, age range 6–15 years) with ascertained immediate-type reactions to latex were studied. They were selected from among patients

attending the allergy units at the Departments of Paediatrics of the Universities of Palermo and Milan. The following diagnostic criteria for latex allergy were used:

- clinical history, showing correlation between symptoms and latex exposure;
- positive SPT with a commercial AL extract (Lofarma S.p.a., Milan, Italy).

A detailed history for allergic symptoms and their causes was collected in the group of children with latex allergy and past/actual atopic disorders (asthma, rhinitis, atopic dermatitis) were recorded in detail. No patient with *spina bifida* was included. All patients also underwent a provocation test with latex glove. Serum samples were collected at the first visit and total serum IgE were determined by the CAP-fluorescence enzyme immunoassay FEIA system (Pharmacia, Uppsala, Sweden). Specific IgE to latex were assayed by CAP test (Pharmacia, Uppsala, Sweden). The study was approved by the Ethic Committees of the Clinics and the parents signed a written informed consent.

### Skin test

Allergen skin tests were performed in duplicate on the forearm, with a panel of commercial allergens (Lofarma S.p.A., Milan, Italy) including: *dermatophagoides pteronyssinus*, grass mix, *parietaria judaica*, *phleum pratense*, artemisia, olive, dog and cat dander, *Alternaria* and *Cladosporium*. Latex SPT was performed with a commercial preparation from an AL extract (Lofarma, Milan, Italy). A positive control (10 mg/ml histamine hydrochloride) and a negative control (saline) were also included. According to guidelines, skin wheal size (mean of the major diameter plus its orthogonal) was measured after 15 min, and 3 mm was considered the positivity cut-off (12).

### Latex provocation test (glove test)

After washing their hands without drying, patients had to wear a latex glove (Comfit NR Latex Powdered Gloves, PT WRP Buana Multicorpora, Indonesia) on one hand and a vinyl glove (Allerderm Laboratories, Petaluma, CA, USA) on the other hand. Clinical reactions were evaluated by a physician at the onset of symptoms and 30 min after taking off the glove. Finally, the hands were washed again. The test response was evaluated as positive if immediate reaction (urticaria, rhinoconjunctivitis, Quincke's oedema) occurred (13).

### Preparation of ammoniated and nonammoniated extracts

Fresh latex of rubber tree (*H. brasiliensis*) mixed with ammonium (0.7%) was provided by the Rubber Institute of Malaysia (Kuala Lumpur, Malaysia). This ammoniated material was diluted (1 : 1, v/v) with ammonium acetate buffer, pH 6.8, and vigorously stirred for 30 min before centrifugation at 40 000 *g* at 4°C for a further 30 min. The aqueous layer containing soluble protein, once carefully separated from the rubber particles, was filtered through a 0.2- $\mu$ m membrane and subsequently dialysed across a 5-kDa cut-off membrane tube against 0.1 M ammonium carbonate and NaN<sub>3</sub> 0.03% as preservative. The protein content of this extract was 1.8 mg/ml as measured by the commercial BioRad Protein Assay Dye Reagent (Bio-Rad, Milan, Italy) using BSA as reference standard.

For the NAL, fresh latex from rubber trees was immediately frozen without ammonization and stored at -80°C. The soluble proteins were then extracted by slowly thawing the frozen latex and

centrifuging it at 40 000 *g* for 30 min. The subsequent procedure was identical to that described above. The protein content of this extract (NAL) was 10.5 mg/ml.

### SDS-PAGE and immunoblotting

Electrophoresis of AL and NAL extracts (30  $\mu$ l containing 20  $\mu$ g of protein) was carried out in a 10% polyacrilamide precast Nupage Bis-Tris gel according to manufacturer's instructions (Novex, Prodotti Gianni, Milan, Italy) at 180 mA for 1 h. The resolved proteins were transferred onto a nitrocellulose membrane (Protran BA 85, Schleicher and Schuell, Milan, Italy) according to Towbin (14). The membrane was stained with 0.1% Coomassie Brilliant Blue or saturated in Tris (0.025 M) buffered saline (TBS) containing 5% defatted dry milk, before incubation with patient's sera and control sera from nonatopic subjects. Bound specific IgE were detected by peroxidase-conjugated goat anti-human IgE serum.

### Results

Table 1 summarizes the characteristics of the allergic children enrolled in the study. All patients had positive clinical history, SPT and detectable latex-specific IgE, ranging from 2.2 to 20.1 kU/l. Despite this, three out of them had negative latex-glove test. These patients had clearly positive latex skin test; one of them had asthma and two urticaria upon latex exposure. Four children had also a history of atopic dermatitis, but at the time of the study they were in complete clinical remission. Concerning clinical reactions to latex, rhinoconjunctivitis and asthma were the most common symptoms (12/18 subjects), followed by contact urticaria (11/18). Only four patients reported a history of latex-related generalized urticaria. This latter manifestation was strongly associated with the highest level of specific IgE (Table 1). There was no significant correlation between skin test positivity with the AL commercial extract and the concentration of specific IgE in serum (Spearman's test,  $r = 0.33$ ,  $P = 0.17$ ).

SDS-PAGE profiles of AL and NAL extracts are shown in Fig. 1. NAL extract was resolved in many bands with a molecular weight ranging from 5 to more than 100 kDa (15). On the contrary, AL extract included only a few components. All patients' sera were tested in immunoblotting using both NAL and AL extracts. IgE reactivity against AL components was observed only in five out of 18 patients (Fig. 2) and in all cases such reactivity was homogeneously expressed against to 20-kDa band (likely corresponding to Hev b 6.01). The immunoblotting profile against NAL components was more complex and a major number of sera (12 out 18) resulted positive, as shown in Fig. 3. With NAL extract, the IgE recognition pattern was heterogeneous and, in addition to the 20-kDa band, a strong intensity in correspondence of other components (likely Hev b 6.02, Hev b 6.03 and Hev b 7) was observed.

Table 1. Characteristics of the latex-allergic children

Patient number	Sex	Age	Clinical symptoms* on exposure	Total IgE (kU/l)	Latex CAP (kU/l)	Latex SPT wheal (mm)	Glove test
1	M	9	CU, GU, RC	559	20.1	5 × 5	Positive
2	F	15	A, CU	640	18	7 × 5	Positive
3	M	14	CU, RC	292	4.7	6 × 6	Negative
4	M	14	A, CU, GU, RC	2500	18	20 × 10	Positive
5	F	15	A, CU, GU, RC	2440	11	5 × 4	Positive
6	M	6	CU	5007	5	6 × 5	Negative
7	F	15	A, CU, GU, RC	7400	20.1	8 × 5	Positive
8	F	10	A, RC	560	8.3	5 × 5	Positive
9	M	10	CU, RC	850	5.7	13 × 6	Positive
10	M	15	RC	918	2.9	5 × 5	Positive
11	M	13	A	182	4.7	5 × 4	Positive
12	F	10	CU	292	3.3	6 × 4	Positive
13	M	8	A	5600	2.3	7 × 7	Positive
14	F	8	A, RC	560	2.2	4 × 4	Negative
15	F	10	A, RC	890	2.9	11 × 5	Positive
16	M	9	A, CU, RC	911	8.5	7 × 5	Positive
17	F	14	A, CU	6490	11.1	8 × 5	Positive
18	F	11	A, RC	850	2.5	6 × 4	Positive

\* A, asthma; CU, contact urticaria; GU, generalized urticaria; RC, rhinoconjunctivitis.

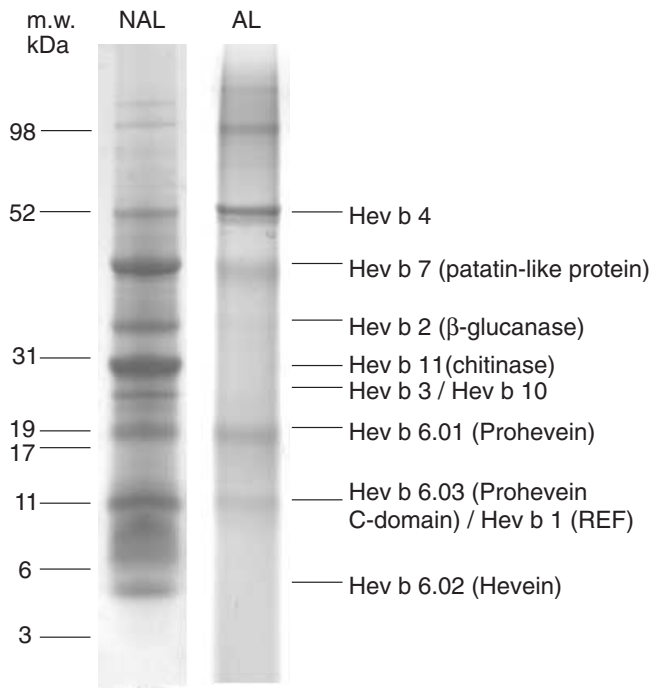


Figure 1. SDS-PAGE profile of NAL and AL extracts.

Discussion

There is an almost general consensus on the diagnosis of latex allergy by SPT and/or RAST assay with latex extracts, but there is still controversy on the most appropriate raw material (ammoniated or nonammoniated) for preparing extracts. Moreover, the real clinical role of specific IgE-binding proteins present in natural

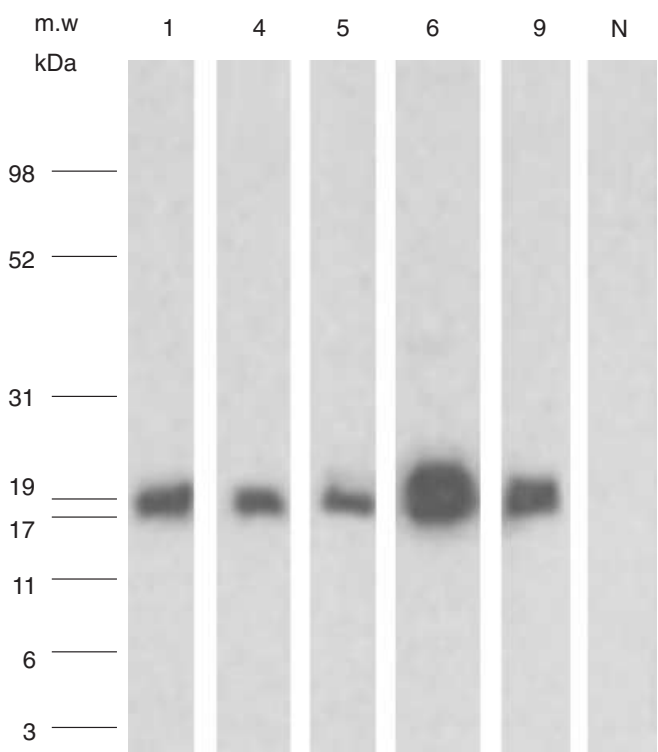


Figure 2. IgE reactivity pattern of sera from latex-sensitive patients against AL components by immunoblotting. N: pool of sera from nonallergic subject.

rubber latex is not still fully elucidated and sensitization to different groups of latex proteins seems to exist (9). Latex juice harvested from *H. brasiliensis* plants contains rubber particles and lutoids. They are suspended in an

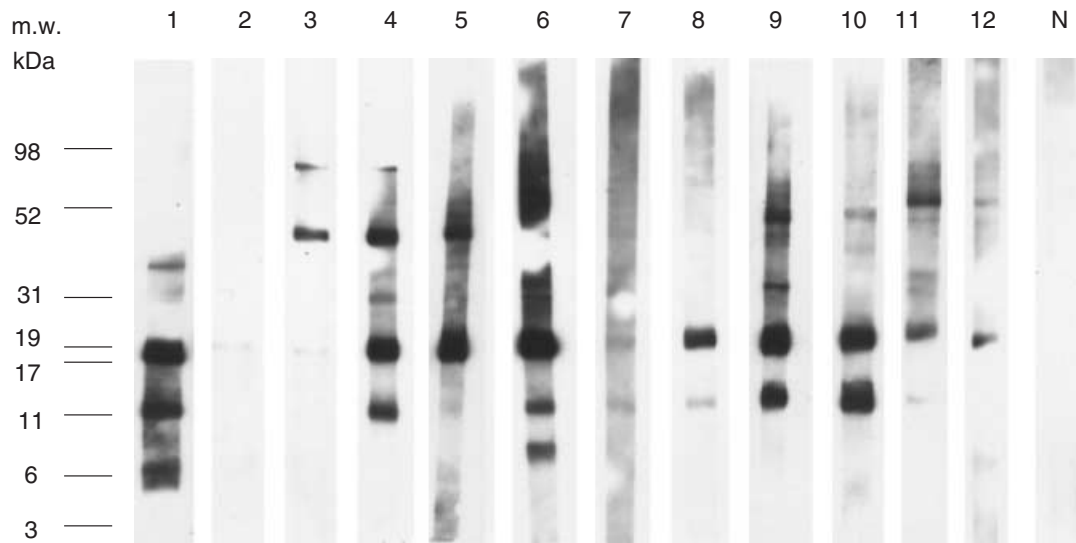


Figure 3. IgE reactivity pattern of sera from latex-sensitive patients against NAL components by immunoblotting. N: pool of sera from nonallergic subject.

aqueous liquid called C-serum, which contains Hev b 1, 3, 5, 7, 8 and 9. The luteoids contain another aqueous fluid, called B-serum, which can be easily released after breaking them by freezing and thawing. B-serum includes other allergens such as Hev b 2, Hev b 4 and Hev b 6, and a small quantity of Hev b 1 and 3 (16). The extract used in this study, called NAL, derives from a raw material constituted by B- and C-sera. In the case of AL, the fresh lymph of rubber tree is treated with ammonium, which is immediately added after harvesting.

We compared AL and NAL extracts in immunoblotting experiments, using sera from children with latex allergy. Noticeably, three children had a negative glove test despite their clear clinical allergy. This can depend in part on the glove used, since it is well known that the allergenic content is largely variable among different manufacturers (17).

Our immunoblotting data showed different results, depending on the raw material used in the preparation of the extract. This observation can have relevant diagnostic implications because the use of AL extract instead of NAL may produce false negative results (18). The relatively low IgE-binding capacity of AL extract may be due to the chemical action of ammonium. It is likely that the addition of ammonium alters somewhere the structure of latex components or destroys them or modifies relevant IgE-relevant epitopes. Moreover, Hev

b 6.02, which is 5 kDa, may be partially lost in the dialysis procedure. The immunoblotting profile of AL in our allergic children showed a low IgE binding capacity, and virtually all serum IgE bound a 20-kDa protein (likely Hev b 6.01), whereas the NAL extract detected IgE binding to other components, which might be Hev b 1, prohevein and Hev b 7. It is likely that the immunoblotting profile against NAL components may better reflect the content of proteins responsible for the type I reactions (19). In fact, allergic patients may react to 'minor' or 'major' protein determinants with different molecular weights. For instance, in the past, Hev b 7 was not considered an important allergen (20), but recently its relevance in children has been outlined (21). Indeed, the sera from six children with ascertained latex allergy (and positive provocation test) did not react against the proteins of AL and NAL extracts, suggesting that other IgE-determinants, not resolved by blotting, should exist.

Data on SDS-PAGE profile and immunoblotting of AL and NAL extracts suggest that NAL extract maybe more appropriate, at least in research setting, since it can identify sensitizations to a larger number of components of latex, although it has been shown that, *in vivo*, the efficiency of NAL is even inferior to a low-ammonium extract (22). In conclusion, our results support the diagnostic value of NAL extract as *in vitro* assay in the diagnosis of latex allergy in atopic children.

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