

Study of the allergenic fractions of latex

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Two latex (*Hevea brasiliensis*) raw materials, one from the cytosolic fraction, the other from the total natural latex have been characterised by immunoblotting techniques. The two latex fractions A and B were studied by immunoprints using eight sera from patients who were allergic to latex. They gave different profiles though there were also allergens of common molecular weights (60, 35, 30, 25, 20, 18, 15, and 10 kD). This study confirms the importance of allergic reactions to latex, that may in some patients cause anaphylactic shock.

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Prolonged and repetitive wearing of protective gloves made from the latex of *Hevea brasiliensis* is often responsible for the symptoms of immediate hypersensitization. The increased demand for medical goods based on rubber has caused an increase in the frequency of hypersensitization (6, 13, 14, 15, 16, 17).

Since the first publication on urticaria by Nutter (11), allergy to latex has been the subject of a series of articles, (7, 9, 11, 12, 18), often concerning anaphylactic shock. An IgE-dependent mechanism has been clearly shown by a number of facts:

- 1) positivity of skin tests after immediate reading,
- 2) presence of specific IgE to latex, and 3) degranulation of human basophils or histamine release by latex in allergic patients.

Several publications have described the allergenic proteins of latex.

In this study, we have attempted to identify the components of natural latex in two extracts obtained by different methods and also to ascertain their implication in allergy reactions. The analytical examination of these with sera from 8 latex-allergic pa-

tients has made it possible to establish which latex extract is most representative for use as a diagnostic extract.

Material and methods

The study comprised eight subjects aged between 14 and 45 years, who had urticaria and/or anaphylactic shock from latex gloves. Prick-tests were positive in all these subjects, as was the specific IgE by radioimmuno assay (bio Mérieux) (Table 1).

Tests showing the allergens in latex of *Hevea brasiliensis* were made on two raw materials obtained by different methods:

- The cytosolic fraction A was obtained by centrifugation of natural latex at 50 000 g for 1 h (C. d'Auzac – Laboratoire de Physiologie Végétale appliquée, Montpellier, France).

- Solution B natural latex from *Hevea brasiliensis* was obtained by the addition of 9 g/l ammonia and ultracentrifugation at 10 000 g for 1 h (latex from Malaya kindly supplied by M.J. Hetier).

Measurement of specific IgE in sera was done

Table 1. Study subjects

Subject	Sex	Age	Profession	Urticaria	Food allergy	Other sign	Prick test latex	R
LAC	M	18	–	–	–	–	+	
MEL	F	36	treatment aide	+	–	rhinitis	+	
MAT	F	40	–	+	–	rhinitis	+	
TER	M	14	–	+	banana/avocado	rhino conjunctivitis	+	
GUI	M	27	maintenance worker	+	–	–	+	
VAS	F	36	nurse	+	passion fruit	–	+	
TRO	F	15	–	–	grapes/orange/banana	–	+	
DJE	F	45	chambermaid	+	–	rhinitis	+	

* Pharmacia reagents.

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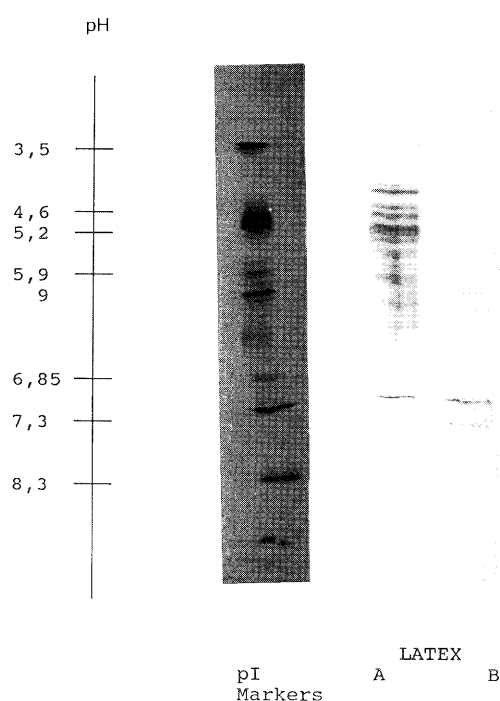


Fig. 1. IEF. Ampholine 3.5–9.5.

Table 2. Comparison of the two latex, coupling on paper discs and measuring specific IgE

Serum	Disc latex A		Disc latex B	
	Class	Units	Class	Units
GUI	IV	23	IV	18
	IV	30	IV	22
LAC	IV	42	IV	32
	IV	60	IV	25
LAN	III	9	III	6
	III	8.5	III	5.4
BOU	I	0.38	I	0.35
	II	0.46	II	0.5

the Stallerzym method (bio Mérieux – Marcy l'Etoile, France). The measurement is carried out in two stages by a sandwich technique that uses an allergen fixed to a paper disc and a monoclonal antibody. The allergens were insolubilised by coupling latex solutions to paper discs (Schleicher and Schull) that were activated by CNBr using the method of Brighton et al. (4).

The protein titre of the extracts was measured by

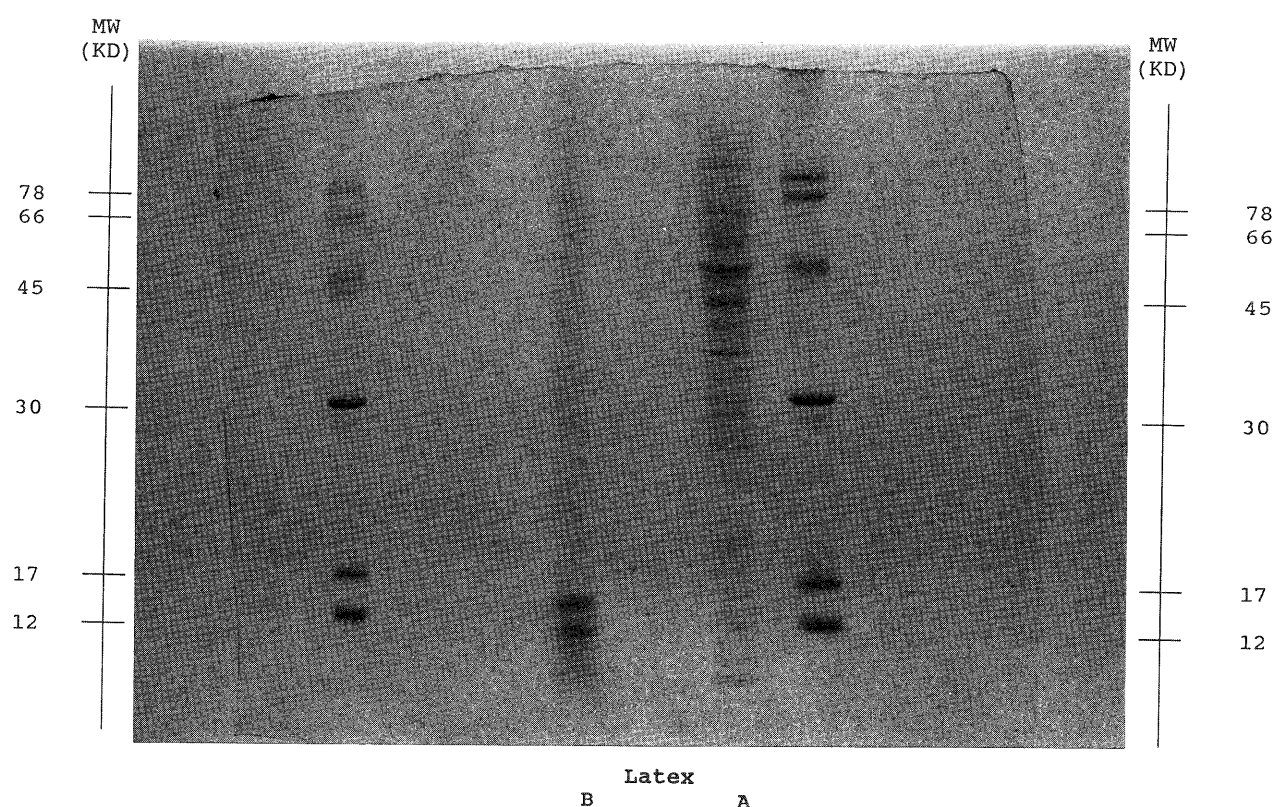


Fig. 2. SDS page. 12.5%.

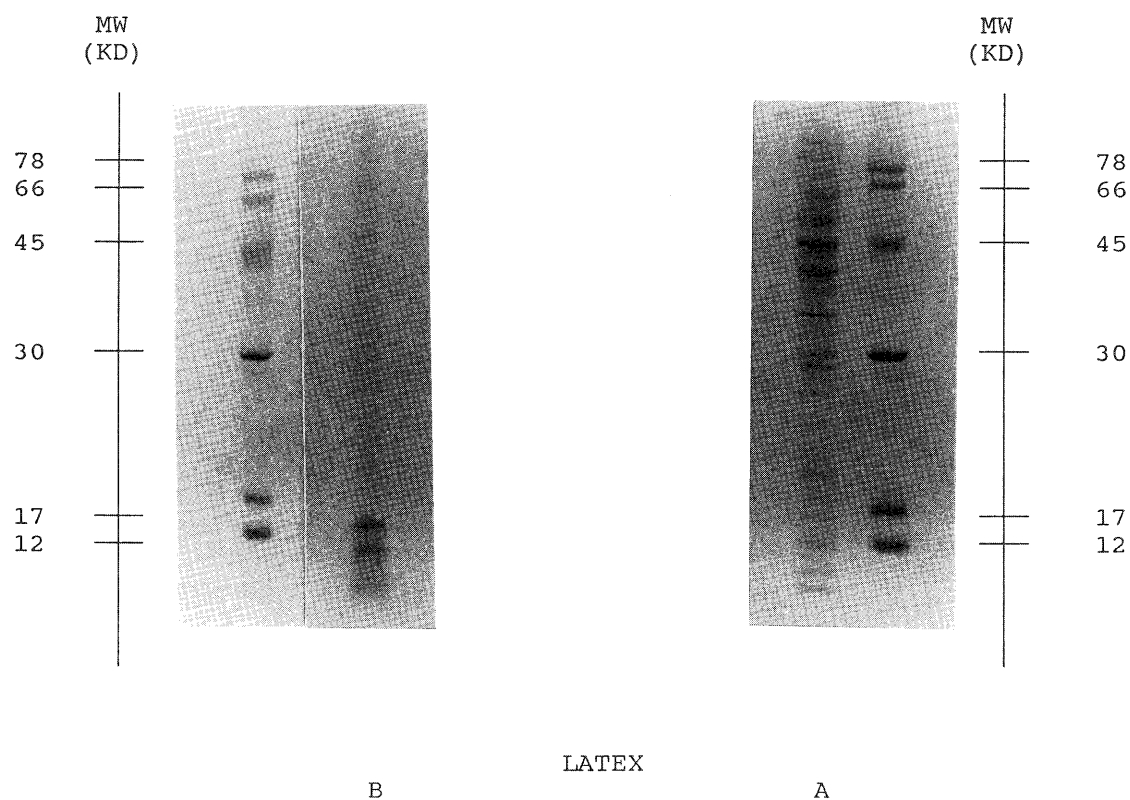


Fig. 3. Sheet of nitrocellulose after using the Transphor Electroblotting Unit.

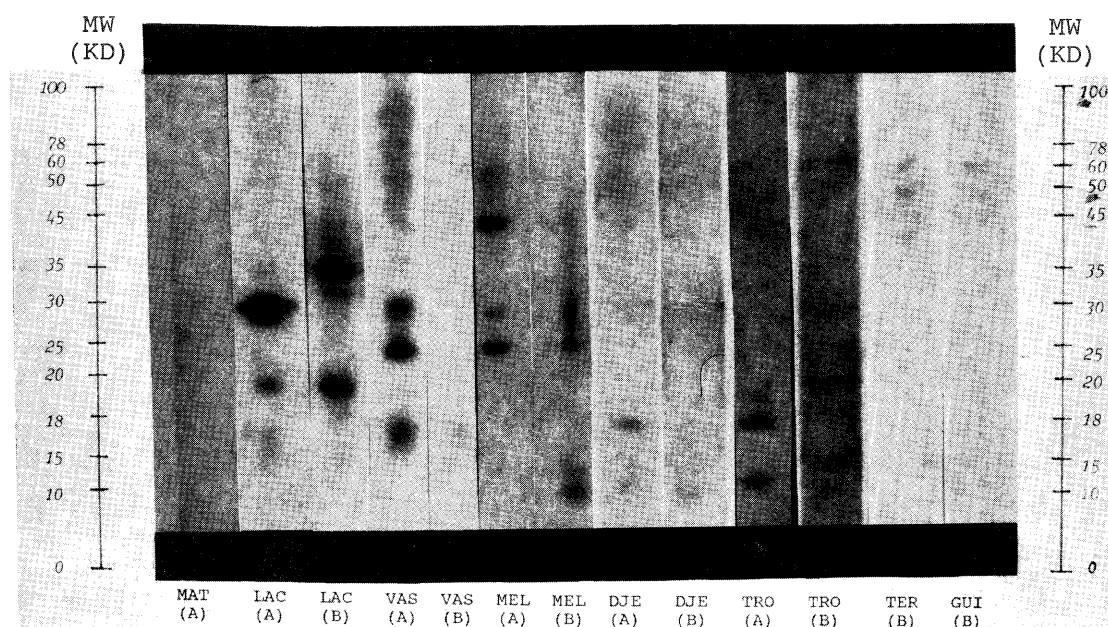


Fig. 4. Study of the fractions from two raw materials A and B, tested by means of 8 different positive sera (classes 2 to 4). The sera LAC, VAS, MEL, DJE and TRO were tested on extracts A and B. Sera TER and GUI only tested on extract B and serum MAT only on extract A.

the Bradford technique (Biorad – protein assay, Richmond, USA) using bovine serum albumin as reference.

Isoelectric focusing was carried out on 5% polyacrylamide gel, pH 3.5 to 9.5 (Pharmacia - LKB,

Uppsala Sweden) by the technique supplied by the manufacturer. Amounts of 30 μ l (45 μ g of protein) were applied.

Immunoprints were done in three stages:

1. SDS-PAGE by the method of Laemmli (8) on 12.5% polyacrylamide, using a Multiphor II (500 V at 10°C for 2 h) (Pharmacia). The latex extracts were applied at a protein concentration of 72 µg per well with 2 mercaptoethanol.
2. After migration, the proteins were transferred to a sheet of nitrocellulose (Biorad) by the method of Towbin et al. (14), using the Transphor Electroblotting Unit (Pharmacia) 60 V at 4°C for 1 h. The edges of the nitrocellulose sheets were stained with amidoblack to visualise the protein transfer and to measure the molecular weight of the latex proteins (Fig. 3).
3. The central part of the sheet was cut in strips, which were then incubated with different positive sera (Pr Leynadier – Hôpital Rothschild, Paris 12e, France) at ambient temperature, with shaking. The sera dilutions are presented in Table 3. Negative controls were performed with three samples 1:5 diluted, a normal serum and two sera from mites or grass allergic patients, with undetectable levels of latex specific IgE by RAST assay. The strips were then incubated with IgE ¹²⁵I (Stallergènes S.A., Fresnes, France), ad-

justed to 40 000 cpm/50 µl, for one night at ambient temperature without shaking.

The latex allergens were shown by autoradiography after 5 days contact with X-OMAT film (Kodak) at -70°C.

Results

Measurement of specific IgE. The discs were coupled to the two materials A and B. A comparison of the two types of discs with four sera containing specific IgE gave close results (Table 2), but with a slightly greater positivity for the discs with A. Each determination was done in duplicate.

Isoelectric focusing. The majority of the protein bands of both A and B were in the acid zone between pH 4.0 and 6.5. Although the protein concentration of the two extracts was adjusted before use, the pattern obtained by staining with Coomassie blue was less intense for latex B.

There was a basic band at pH 9.5 only for latex A (Fig. 1).

Table 3. Results of the immunoprints with two latexes A and B and 8 latex-positive sera

Extracts Latex	Positive sera							
	MAT 1	LAC 2 1:6	VAS 3 1:5	MEL 4 1:5	DJE 5 1:6	TRO 6 1:5	TER 7 1:4	GUI 8 1:9
A		Many bands of which 7 stronger 100 000	Several bands 100 000 78 000 60 000 50 000	5 bands 60 000 45 000	4 bands 2 blurred 78 000 60 000		*	*
	30 000	35 000 30 000 25 000 20 000 18 000 15 000	30 000 25 000	30 000 25 000		20 000 18 000		
			18 000	15 000	18 000	18 000		
					10 000	10 000		
B		4 bands of which 3 stronger 60 000	1 band strong	6 bands 60 000 45 000	1 band blurred	2 bands blurred	3 bands of which 2 stronger 60 000 50 000	2 bands + 1 blurred 60 000 50 000
	*	35 000 30 000 20 000		30 000 25 000				20 000
			18 000	15 000 10 000		15 000 10 000		15 000

* Sera MAT, TER, GUI were not studied.

SDS-Page. For latex A: Staining of the polyacrylamide gel by Coomassie blue showed many protein bands with molecular weights between 10 and 100 kD.

For latex B: The protein bands were less visible. Staining by Coomassie blue showed two prominent bands, with molecular weights < 17000 (Fig. 2).

Immunoprint. An allergenic protein of 30 kD was found in both A and B with several positive sera (LAC, MEL and MAT). With serum VAS this protein was found only in latex A. An allergenic protein of 10 kD was found with several sera (DJE and TRO). With the serum MEL this protein was shown only in latex B. Latex A had a protein > 78 kD, recognized by sera VAS and LAC. Latex B lacked this. Other sera with different positivity (classes 4 and 2), such as GUI and TER, appeared to show only with latex B; allergenic proteins with molecular weight > 45 kD.

For latex A, there were similarities between the immunoprints made with sera VAS, MEL and LAC. However, there were differences in the small molecular weight bands: serum LAC recognized four protein bands between 15 and 25 kD, while sera VAS and MEL recognized one (Table 3 and Fig. 4).

No bands were observed for latex B with the three control sera but it seems that these sera recognized two proteins at 18 and 10 kD in latex A (Fig. 5).

Discussion

This study of immunoprints, made on eight different positive sera, has shown distinct bands of MW 100, 78, 65, 45, 30, 25, 20, 18, 15 and 10 kD. Turjanmaa (16) described allergenic proteins from 2 kD to 70 kD. Morales (10) detected four bands 10, 24, 35 and 100 kD by immunodetection.

Two fractions of MW 30 and 10 kD have been shown with six sera from the eight tested. Results

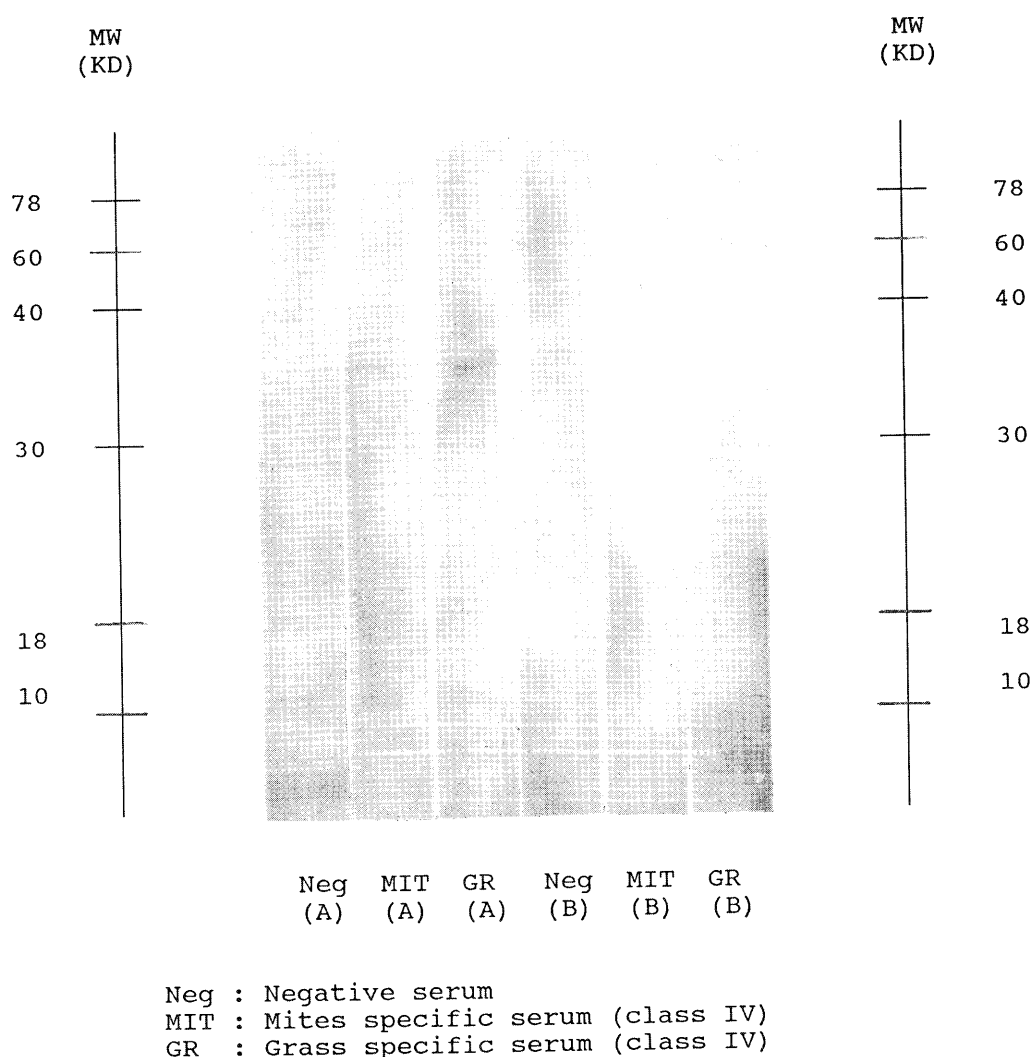


Fig. 5. Negative control for the immunoprint experiment.

with the 30 kD fraction support those of Carillo et al. (5), who also confirmed by gel filtration the presence of an allergen of 30 kD.

Axelsson (3) also confirmed this allergenic activity in a fraction of 30 kD.

The fraction of MW 10 kD found by three sera is another latex allergen. According to Archer (1), this polypeptide of 10 kD can be identified as hevein.

Morales et al. had similar results in the pH acid zone. However, extract A showed a band with basic pI (Fig. 1).

Conclusion

For all allergen extracts, mites, pollens, epithelia, the results of immunoprints depend on the choice of raw materials, the method of fractionation, and the reagents. The extract selected after these controls must be appropriate for its use (diagnosis or therapy).

The sera used did not contain the same latex-specific IgE, since when three sera from allergic subjects were tested with the same extracts the profiles obtained were not identical. Further, comparison of extracts A and B with the same sera gave different profiles.

The results of immunoprints done with latex extracts prepared by two different methods showed a large number of bands in extract B. Finally, although the strength of the bands is weaker, we have chosen this raw material for diagnostic use both *in vitro* and *in vivo*.

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