

Immunodetection of latex antigens

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Abstract

Background: Protein antigens in latex products can cause type I reactions. In the past antigens have been measured by protein assays, high-performance liquid chromatography, RAST inhibition, and skin tests. We examined the use of a mouse monoclonal antibody (CRI-C) to latex in the detection of latex antigens.

Methods: CRI-C was raised by standard techniques after immunization of a BALB/c mouse with ammoniated latex. Medical gloves were extracted and assayed with: (1) standard protein assays, (2) RAST inhibition assays with sera from health care workers allergic to latex and patients with spina bifida, (3) an ELISA with a biotinylated form of CRI-C (BiC). Reference proteins included bovine serum albumin for the protein assays and nonammoniated latex and affinity-purified C antigen for the immunoassays.

Results: Among the protein assays, the best correlation was between the Bradford and bicinchoninic acid assays. In absolute numbers the Bradford assay produced the lowest results, and OD₂₈₀ the highest. The OD₂₈₀, BCA, and Bradford methods "detected" protein in vinyl gloves. The results of RAST inhibition and BiC ELISA correlated with the protein assays. These immunoassays appeared to be more specific than the protein assays.

Conclusions: The BiC ELISA is an easy and reproducible in vitro assay of relevant latex antigen. Clinical correlation will be required for validation. (J ALLERGY CLIN IMMUNOL 1994;93:825-30.)

AL	Ammoniated latex extract
BCA	Bicinchoninic acid
BiC	Biotinylated CRI-C antibody
C-Ag	C antigen, an affinity-purified product from NAL
EDTA	Ethylenediaminetetraacetic acid
MAb	Monoclonal antibody

NAL	Nonammoniated latex extract
OD₂₈₀	Optical density at 280 nm
PBS	Phosphate-buffered saline

Peptide antigens in toys, gloves, and condoms made of natural rubber latex have been associated with type I hypersensitivity reactions.¹ The U. S. Food and Drug Administration has noted the importance of this phenomenon and is in the process of developing standards for the labeling of latex-containing medical devices and for reducing their antigen content.² Previously reported estimates of antigen content have been based on total protein estimates,³ high-performance liquid chromatography,^{4,5} RAST inhibition,^{3,6,7} and epicutaneous skin tests.³⁻⁵ We report the use of a mouse monoclonal antibody (MAb) that recognizes a major latex antigen in the detection of antigenic peptides in glove extracts.

Methods

Latex sources

Nonammoniated latex was obtained from Dr. Katrina Cornish of the U. S. Department of Agriculture. It had been collected from *Hevea brasiliensis* in Costa Rica in Tris buffer (0.1 mol/L, pH 7.5) and sodium azide (0.01%) and shipped frozen on dry ice.⁸ Ammoniated latex concentrate was obtained from Guthrie Latex (Baltimore, Md.). Seven brands of medical gloves were extracted (Table I). The gloves were obtained from previously unopened packages and were handled with vinyl gloves. All were processed with the same buffers on the same day, and none were brought into contact with known sources of latex or protein.

TABLE I. Gloves extracted and tested

Glove no.	Manufacturer	Brand	Style
1	William Schmidt*	Triflex	Orthopedic surgeon
2	William Schmidt*	Triflex	Surgeon
3	William Schmidt*	Ultraderm	
4	William Schmidt*	Triflex	Sterile examination
5	Becton-Dickinson†	Sensicare	Vinyl
6	Aladan‡	Neutraderm	
7	William Schmidt*	Triflex	Vinyl

*William Schmidt, Inc., Valencia, Calif.

†Becton-Dickinson, Rutherford, N.J.

‡Aladan Corp., Norcross, Ga

Extract preparation

Nonammoniated latex extract (NAL) and ammoniated latex extract (AL) were prepared as previously described.⁹ Gloves were extracted in phosphate-buffered saline (PBS) (pH 7.4; 1 gm latex per 5 ml buffer) at 37° C for 1 hour in sterile polypropylene tubes. The extracts were then centrifuged and filtered to remove particulate matter.

Protein assays

The protein contents of AL, NAL, and the purified antigens were estimated by absorbance of unmodified protein at 280 nm (optical density at 280 nm [OD₂₈₀]) and by the Bradford assay (Pierce Chemical Co., Rockford, Ill.), the bicinchoninic acid (BCA) assay (Pierce Chemical Co.), and the Lowry assay (Sigma Chemical Co., St. Louis, Mo.). Absorbance was compared with a known amount of bovine serum albumin for all assays.

Production of mouse hybridomas

Six- to eight-week-old BALB/c mice were injected intraperitoneally with 200 µg AL in complete Freund's adjuvant and were reinjected 2 weeks later with 200 µg AL in incomplete Freund's adjuvant. After 3 more weeks, the mouse with the highest anti-AL antibody titer was injected with 60 µg AL in sterile saline solution. Splenocytes were then fused with the myeloma cell line P3/NS1/1-Ag4-1 (ATCC, Rockville, Md.), and hybridoma cells were selected with medium containing hypoxanthine, aminopterin, and thymidine. Lines producing anti-AL antibodies were cloned at least four times by limiting dilution techniques. One IgG₁-producing hybridoma (CRI-C) was selected; the antibody was purified (>90% by sodium dodecylsulfate–polyacrylamide gel electrophoresis) from mouse ascites by ammonium sulfate precipitation and ion-exchange chromatography.

ELISA to detect latex-specific antibody

Polyvinyl chloride microtiter plates were coated with lyophilized AL or NAL (1 µg/well) dissolved in a sodium carbonate–bicarbonate buffer (0.1 mol/L, pH 9.6). Wells were washed with 0.9% NaCl containing 0.05% Tween 20 and blocked with blocking buffer. Then, 100 µl of tissue culture supernatant, mouse serum, or mouse ascites diluted in media was added and incubated at room temperature for 2 hours. Wells were washed, and anti-mouse IgG conjugated to alkaline phosphatase (Kirkegaard and Perry Laboratories, Gaithersburg, Md.), 10 µg/ml in blocking buffer, was added. After 2 hours, the wells were washed, and p-nitrophenyl phosphate (Sigma) (1 mg/ml in 0.1 mol/L glycine buffer, pH 10.4, containing 5 mmol/L MgCl₂) was added. Absorbance was measured at 414 nm with a Vmax Kinetic Microplate Reader (Molecular Devices, Menlo Park, Calif.).

Affinity purification protocol

C antigen (C-Ag) was prepared from NAL as follows. Ten milligrams of pure CRI-C was bound to cyanogen bromide–activated Sepharose 4B (Pharmacia, Uppsala, Sweden); nonreacted groups were blocked by alkaline hydrolysis. After extensive washing, 10 mg of NAL in PBS was added; the column was then washed with 10 column volumes of PBS, and bound antigen was eluted with glycine buffer (0.1 mol/L, pH 2.8). Eluted material was neutralized with one-tenth volume Tris buffer (1 mol/L, pH 8).

RAST inhibition

Serum for RAST inhibition studies was pooled from six children with spina bifida and high titers (>10%) of anti-latex IgE, as measured by AL-RAST.^{10, 11} Serum was collected after written informed consent was obtained as part of a study approved by the Institutional Review Board of Children's National Medical Center. Serum was also pooled from five health care workers with latex allergy and anti-latex IgE.

AL-RAST was performed as previously described.¹⁰ Briefly, 1 µg of AL was bound to each well in a polyvinyl chloride microtiter plate (Falcon Microtest III; Becton-Dickinson, Oxnard, Calif.), blocked with 3% bovine serum albumin in PBS with 0.05% Tween and 0.01% sodium azide ("blocking buffer"), and incubated sequentially with serum and iodine 125–labeled rabbit anti-human IgE (Pharmacia). RAST inhibition studies were performed by adding NAL, C-Ag, or glove extracts in blocking buffer to the pooled RAST-positive serum and preincubating overnight at 4° C before addition to AL-coated plates. Inhibition curves for spina bifida sera and health care worker sera were analyzed independently and compared with NAL and C-Ag standard curves,¹² the concentrations of which were estimated with the Bradford assay.

Biotinylation of CRI-C

Purified CRI-C was dialyzed into sodium borate buffer (0.1 mol/L, pH 8.8). N-hydroxysuccinimidobiotin (Sigma) was dissolved in dimethyl sulfoxide and added to the antibody at a ratio of 250 µg ester per milligram of antibody. After 4 hours, unreacted ester was hydrolyzed by the addition of 20 µl of 1 mol/L ammonium chloride per 250 µg ester, and the biotinylated antibody was dialyzed (Spectra/Por; Spectrum Medical Industries, Los Angeles, Calif.) (molecular weight cutoff = 12,000 to 14,000) into PBS.¹³

ELISA for latex antigen

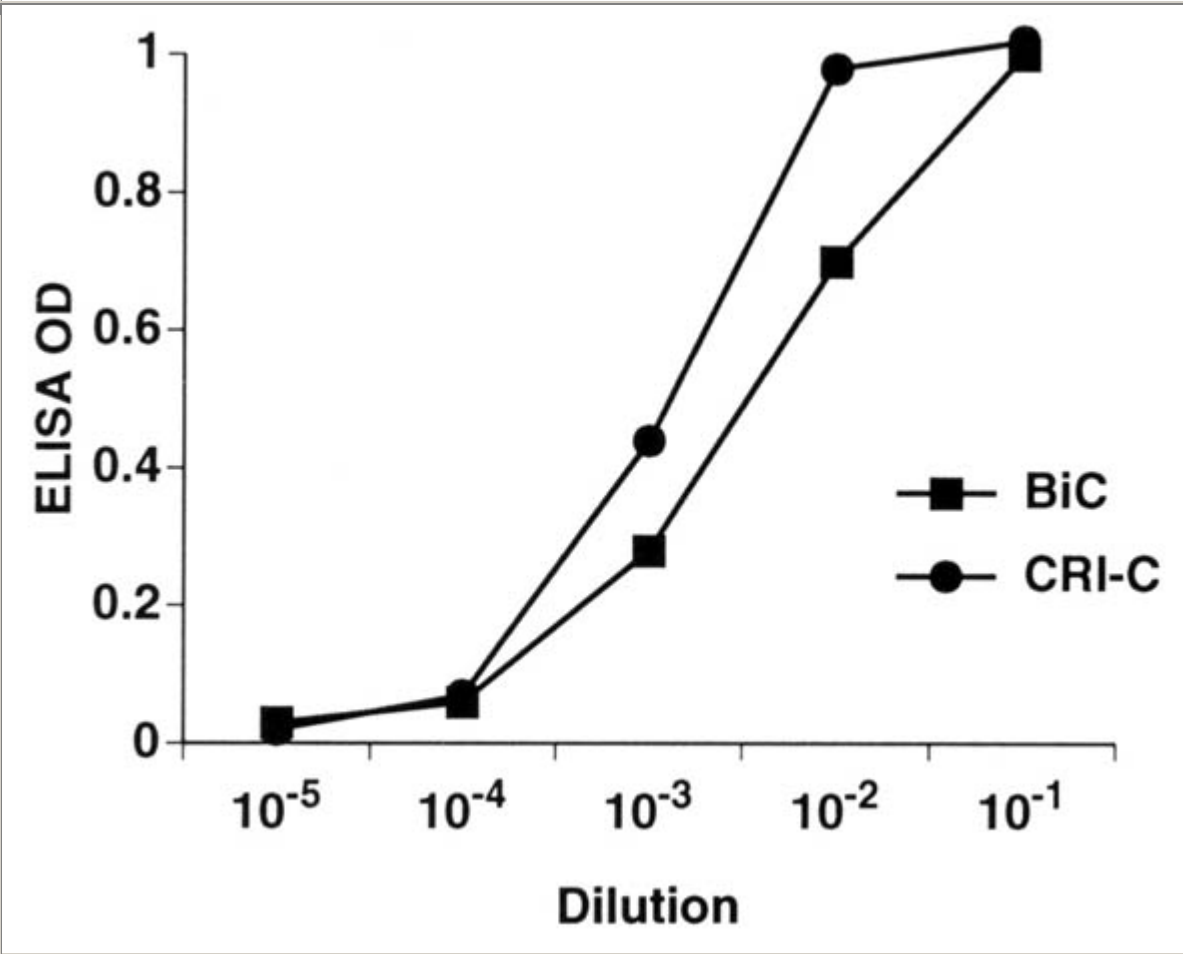
Immulon 4 plates (Dynatech, Chantilly, Va.) were coated overnight with NAL, AL, C-Ag (0.1 to 4 µg/ml), or glove extracts in sodium carbonate–bicarbonate buffer (0.1 mol/L, pH 9.6). The wells were incubated sequentially with blocking buffer, 0.5 µg of biotinylated CRI-C antibody (BiC), 0.1 µg of avidin (Pierce Chemical Co.), 0.1 µg of biotinylated alkaline phosphatase (Pierce Chemical Co.), and 0.1 mg p-nitrophenyl phosphate (Sigma) (in 0.1 mol/L glycine buffer, pH 10.4, containing 5 mmol/L MgCl₂). Absorbance was measured at 414 nm with a Vmax Kinetic Microplate Reader. Absorbance was converted to amount of C-Ag by comparison with a C-Ag standard curve, the concentration of which was estimated by the Bradford assay.

Results

Antigen binding by biotinylated antibody

MAb CRI-C was biotinylated by a standard protocol, at a ratio of 250 µg ester per milligram of antibody (12 moles of biotin per mole of IgG). Antigen-antibody binding was only slightly reduced by biotinylation, and antibody binding to AL was measurable by ELISA at concentrations as low as 10⁻⁴ mg/ml for both CRI-C and BiC (Fig. 1).

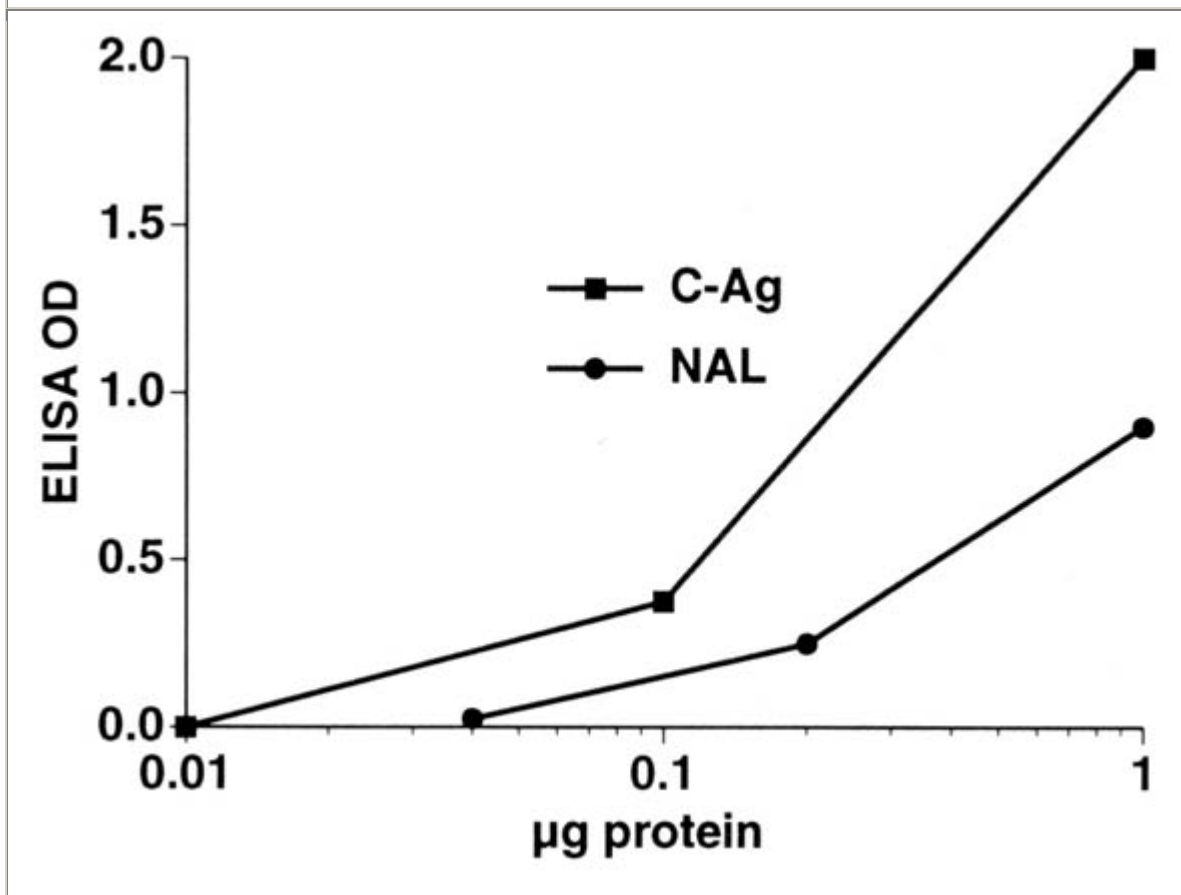
FIG. 1. ELISA to detect anti-AL antibody activity.



BiC ELISA for latex antigen

Pilot experiments with BiC from 0.1 to 2.0 μg indicated that optimal sensitivity and economy were obtained with 0.5 μg . With this quantity NAL could be detected in concentrations as low as 400 ng/ml, and C-Ag in concentrations as low as 125 ng/ml. RAST inhibition assays could detect NAL and C-Ag as low as 3 ng/ml (data not shown). When C-Ag and NAL were compared directly with the BiC ELISA in parallel portions of the curve, 0.05 μg of C-Ag produced the same absorbance at 414 nm as 0.23 μg of NAL (■Fig. 2).

FIG. 2. BiC ELISA, comparing equal amounts of NAL and C-Ag (by Bradford assay).



Glove extracts

Protein estimates in the glove extracts varied widely, depending on the method used (■Table II). All methods correlated roughly to each other. The detection of measurable protein in vinyl gloves (G5 and G7) by Bradford and BCA methods was a surprising result.

TABLE II. Protein assays of glove extracts

Glove no.	OD ₂₈₀	Protein ($\mu\text{g/ml}$)		
		Bradford assay	BCA assay	Lowry assay
1	1370	24	150	171
2	1980	23	205	214
3	1050	8	133	56

4	1020	47	280	224
5	440	8	10	<50
6	100	1	8	<50
7	720	6	22	<50

The immunologic assays for latex antigen yielded similar results (Figs. 3 and 4; Table III). The absolute RAST inhibition results varied with the serum and the reference standard used, although the only ordinal variance was between gloves 1 and 2. When spina bifida serum was used, glove 1 had more immunoreactive antigen than glove 2; the opposite was true when health care workers' serum was the antibody source.

FIG. 3. RAST inhibition assay of glove extracts, using pooled sera from (A) patients with spina bifida and positive RAST results and (B) health care workers with positive RAST results.

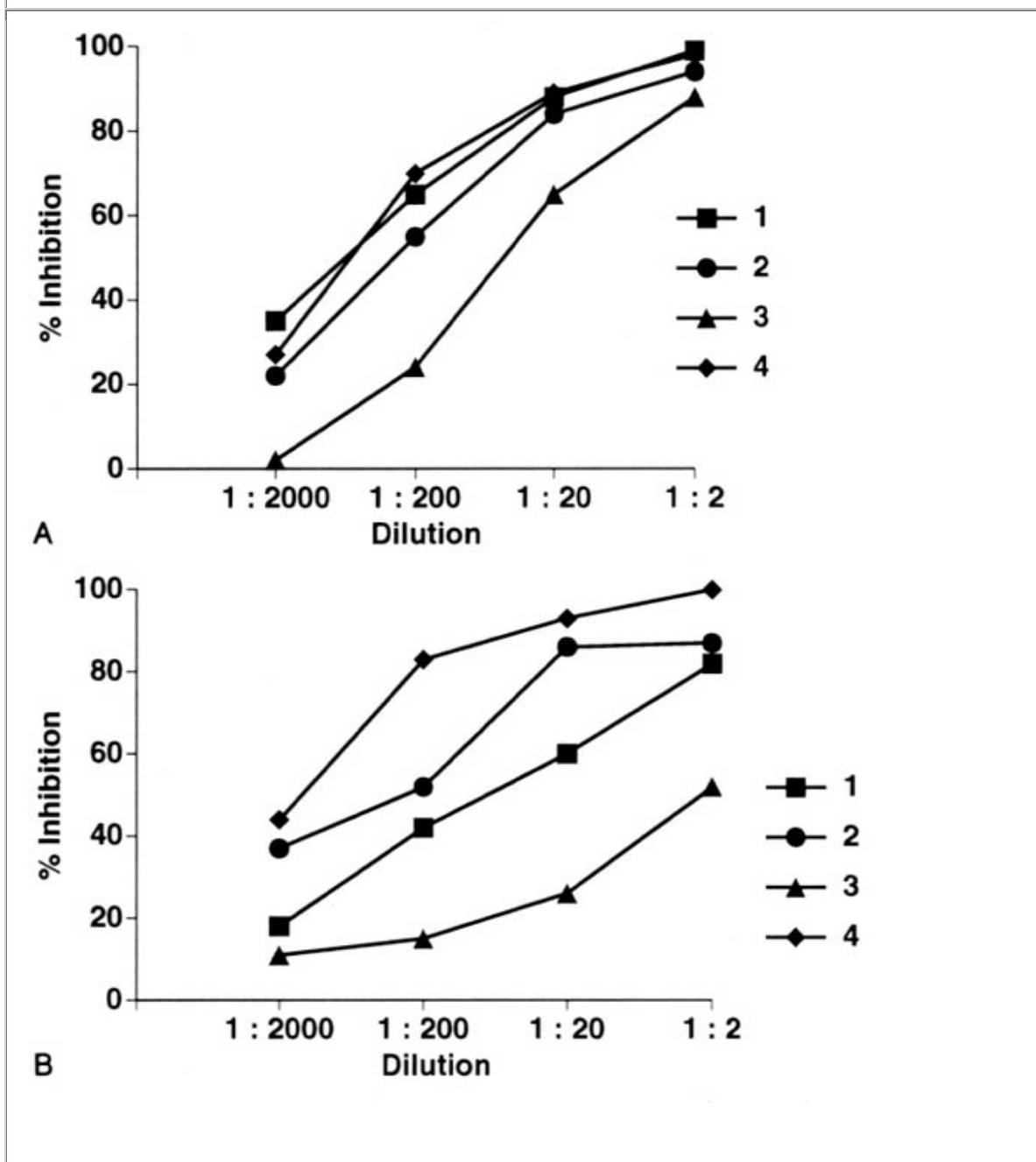


FIG. 4. BiC ELISA of glove extracts. No antigen was detected by this assay in gloves 5, 6, or 7. Antigen detected in glove 3 was on too low a scale to be depicted in this graph.

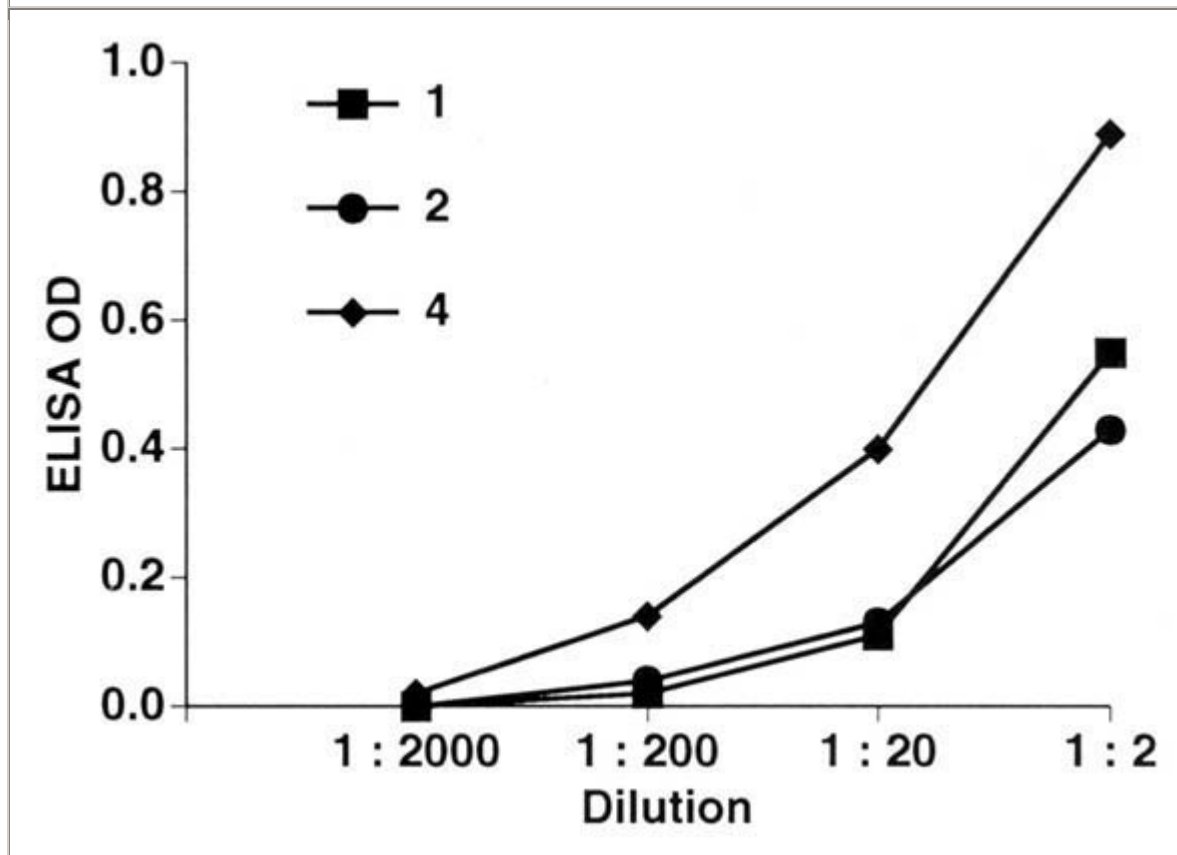


TABLE III. Immunoassays for latex antigens

Glove no.	<u>BiC</u>	<u>RI (SB)</u>		<u>RI (HCW)</u>	
	C-Ag	C-Ag	NAL	C-Ag	NAL
1	5	8.2	47.1	22.9	5.1
2	3	3.8	21.6	80	17.8
3	0.3	0.6	3.6	0.8	0.2
4	25	9.0	51.6	457.1	101.4

RI, RAST inhibition; *SB*, serum from patients with spina bifida; *HCW*, serum from health care workers.

None of the immunologic assays detected antigenic material in gloves 5, 6, and 7, in spite of the presence of measurable protein in each extract as determined by standard assays. Like the RAST inhibition with spina bifida sera, the BiC ELISA detected more antigen in glove 1 than glove 2.

Discussion

Latex allergy is a life-threatening condition, the only treatment for which is strict avoidance of exposure to latex protein antigens. The Food and Drug Administration has recommended that companies producing latex medical devices attempt to lower the total elutable protein content of their products, although no standards

have yet been set.² Both the latex industry and federal regulators have focused on total protein measurements as they begin to develop these standards. Protein assays are readily available and not technically demanding. Tomazic et al.^{14, 15} and others have worked out many of the necessary conditions for reproducible protein assays in latex product extracts. In addition, because it is widely agreed that latex antigens are proteins, it is reasonable to assume that measures that lower the measurable protein content will lower the antigen content as well. However, at least one recent report suggests that immunoreactive antigen may be present in latex products in which no protein is detectable by standard techniques.³

In this investigation we compared the measurement of latex proteins in glove extracts by absorbance at 280 nm, three standard protein assays, RAST inhibition with two different types of pooled sera, and an ELISA based on a recently developed MAb to a latex antigen. The results can be summarized as follows. Each of the four nonimmunologic protein estimates correlated well with the other protein assays. In absolute numbers the Bradford assay produced the lowest results, and the OD₂₈₀ assay produced the highest. The OD₂₈₀, BCA, and Bradford methods "detected" protein in vinyl gloves (gloves 5 and 7). The RAST inhibition and BiC ELISA correlated with the protein assays. These immunoassays were more specific than the protein assays.

Each of the protein assays is hampered by limitations of sensitivity and chemical interference. The Lowry assay can detect protein levels as low as 10 to 20 µg/ml, but common chemicals such as Tris, ammonium sulfate, ethylenediaminetetraacetic acid (EDTA) and phenols can interfere with the assay unless the protein is precipitated and redissolved before testing. The micro BCA assay is sensitive to levels as low as 0.5 µg/ml, but Tris, EDTA, and even glucose can alter the results. In contrast, the Bradford assay is unaffected by detergents, chelators, and reducing agents. Unfortunately, the binding of Coomassie Blue to proteins is highly variable. In staining procedures Boyle et al.¹⁶ found that Coomassie Blue failed to bind to a prominent immunoreactive latex peptide.

Immunoassays are less sensitive to chemical interference. Salt concentration, pH, and temperature can affect antigen-antibody interactions, as can strong reducing agents. However, Tris, detergents, EDTA, and phenols have little measurable effect at physiologic conditions.

RAST inhibition is an established method of antigen measurement,¹² which has been applied successfully to the measurement of latex antigens in previous studies.^{7, 17} Because the antigens detected by RAST inhibition are dependent on the serum used, many investigators use pooled allergic sera to minimize the effects of patient-to-patient variations in antigen specificity. However, Alenius et al.¹⁸ note that latex immunoblots with sera from a group of North Americans, 67% of whom had spina bifida, differed markedly from those with sera from a group of Finns, of whom only 13% had spina bifida. Thus even the use of pooled sera can lead to systemic errors in antigen measurement, unless efforts are made to ensure that the pool is sufficiently heterogeneous.

In this study we used two different pools of allergic sera for RAST inhibition studies, one from children with spina bifida and the other from health care workers. The results from these pools differed in the relative and absolute latex contents assigned to the glove extracts. Although the relative differences are due to the positions of the glove extract inhibition curves (■Fig. 3), the differences in the absolute content are due to the inhibition curves of the reference proteins as well (not shown).

The BiC ELISA, which is based on the antigen specificity of a mouse MAb raised to AL, was more comparable to the RAST inhibition assay in which spina bifida sera was used than that in which health care worker sera was used. This is consistent with our previous experience that C-Ag, which is the affinity-purified peptide that binds to the MAb CRI-C, is a major antigen for patients with spina bifida who are allergic to latex, but less so for latex-allergic health care workers.¹⁹ In our laboratory the BiC ELISA is less sensitive than the RAST inhibition assays but takes up to 48 hours less to complete and uses as its specific antibody an MAb with high reproducibility and unlimited supply.

The validity of the BiC ELISA, especially in studies of low-antigen latex gloves such as glove 6, will be established only by correlations with in vivo experience. The use of a single MAb may reduce the sensitivity of the assay, especially when multiple antigens are present and when the target population responds predominantly to other determinants. We plan to use the BiC ELISA, in conjunction with RAST inhibition assays, to screen the glove products purchased and used in our institution, and we will be monitoring the rate of latex-induced reactions among our patients and health care workers in an ongoing surveillance

program. We welcome the submission for testing of gloves that have been implicated in the causation of type I reactions. We also plan to evaluate these assays with skin tests in selected patients.

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Latex allergy in pediatrics

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Christopher Randolph, MD

Lipopolysaccharide augments IgG and IgE responses of mice to the latex allergen Hev b 5

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The latex allergen Hev b 5 transcript is widely distributed after subcutaneous injection in BALB/c mice of its DNA vaccine

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