

Natural rubber latex skin testing reagents: Safety and diagnostic accuracy of nonammoniated latex, ammoniated latex, and latex rubber glove extracts

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Background: Nonammoniated latex, ammoniated latex, and rubber glove extracts are the only sources of natural rubber (*Hevea brasiliensis*) latex that have potential for use as skin testing reagents in the diagnosis of latex allergy. Their diagnostic sensitivity and specificity as skin test reagents are unknown.

Objective: We conducted a phase 1/2 clinical study to examine the safety and diagnostic accuracy (sensitivity and specificity) of nonammoniated latex, ammoniated latex, and rubber glove extracts as skin test extracts to identify the most efficacious source material for future skin test reagent development.

Methods: Twenty-four adults not allergic to latex, 19 adults with hand dermatitis or pruritus, and 59 adults with a latex allergy were identified by clinical history. All provided blood and then received puncture skin tests and intradermal skin tests with nonammoniated latex, ammoniated latex, and rubber glove extracts from Malaysian *H. brasiliensis* latex by use of sequential titration. A glove provocation test and IgE anti-latex RAST were used to clarify positive history-negative skin test response and negative history-positive skin test response mismatches.

Results: All three extracts were biologically safe and sterile. After normalization to 1 mg/ml of total protein, all three extracts produced equivalent diagnostic sensitivity and specificity in puncture skin tests and intradermal skin tests at various extract concentrations. Optimal diagnostic accuracy was safely achieved at 100 µg/ml for puncture skin tests and 1 µg/ml for intradermal skin tests (e.g., nonammoniated latex: puncture skin test sensitivity 96%, specificity 100%; intradermal skin test sensitivity 93%, specificity 96%). The presence of IgE antibody in skin was highly correlated with IgE anti-latex in serum (nonammoniated latex: $r = 0.98$, $p < 0.001$; ammoniated latex: $r = 0.94$, $p < 0.001$; rubber glove extract: $r = 0.96$, $p < 0.001$). All five available subjects with a positive history, negative skin test response, and absence of IgE antibody in serum had a negative glove provocation test response, indicating no clinical evidence of latex allergy. No systemic or large local allergic reactions were observed with puncture skin tests or intradermal skin tests.

Conclusions: Equivalent diagnostic sensitivity and specificity were observed with the nonammoniated latex, ammoniated latex, and rubber glove extract skin test reagents after normalization for total protein; nonammoniated latex may be considered the reagent of choice on the basis of practical quality control and reproducibility considerations. (*J Allergy Clin Immunol* 1996;98:872-83.)

Key words: Natural rubber latex, diagnosis, skin testing, glove provocation

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Abbreviations used

AL:	Ammoniated latex allergen preparation
GLO:	Allergen extract of natural rubber latex surgical gloves
ID-ST:	Intradermal skin test
Latex:	Natural rubber latex derived from sap of <i>Hevea brasiliensis</i> trees
NAL:	Nonammoniated latex allergen preparation
P-ST:	Puncture skin test
ROC:	Receiver operating characteristics plot
ST:	Skin test

Immediate-type hypersensitivity reactions to natural rubber latex (latex) proteins have been well documented in a number of high-risk groups exposed to natural rubber products.¹⁻³ Although a variety of in vivo and in vitro methods have been proposed for use in the definitive diagnosis of latex allergy,^{4,6} their safety and diagnostic accuracy have been brought into question in the United States by the absence of Food and Drug Administration (FDA)-approved skin test (ST) reagents and provocation procedures for identifying "true" cases of latex allergy. Moreover, the absence of effective ST reagents has raised doubts about the results of reported studies on the epidemiology and natural history of latex allergy.

In designing an optimal latex ST procedure, we thought it was important to identify the best of the three potential sources of latex that would maximize diagnostic accuracy (sensitivity and specificity) and safety. We therefore designed this study to examine the diagnostic performance of a representative nonammoniated latex (NAL), ammoniated latex (AL), and glove extract (GLO), all derived from the same widely used clone of *Hevea brasiliensis* tree sap. Although each *Hevea* latex source material may have its theoretic advantages and limitations as a diagnostic reagent, qualitative and quantitative differences may exist in the allergen profile of each material on the basis of published immunochemical studies.⁷⁻⁹

In this report, we describe the results of a phase 1/2 clinical trial of NAL, AL, and GLO ST materials that were derived from *H. brasiliensis* tree, clone 600. We initially hypothesized that these three source materials, which reportedly contain different amounts and compositions of proteins, would exhibit differences in their diagnostic accuracy, operationally defined as their ability to discriminate between individuals with and without latex allergy. We chose to normalize the

extracts on the basis of total protein and to use a combination of the subject's clinical history and a glove provocation test to identify true cases. The study results disprove our working hypothesis and show that when normalized for total protein content the NAL, AL, and GLO displayed remarkably comparable diagnostic accuracy as assessed by receiver operating characteristics (ROC) plots. Moreover, results of this study identify safe extract concentrations and skin testing conditions of a proposed candidate NAL reagent that provide optimal diagnostic sensitivity and specificity. Finally, the study demonstrates that the clinical history alone is not satisfactory for identifying cases of IgE-dependent latex allergy.

METHODS

Subjects

One hundred two subjects were recruited into the study over a 1-year period from advertisements at the Johns Hopkins Asthma and Allergy Center and in Baltimore newspapers. All subjects were classified with a clinical score for latex allergy based on their clinical history by use of the criteria specified in Table 1. Subjects were excluded from the study if they were pregnant, had extensive dermatographism that precluded skin testing, or had been previously tested for latex allergy. In the safety (phase 1) study, 24 subjects (11 men, 13 women) who did not have latex allergy were evaluated (16 were not atopic, and 8 were atopic but not allergic to latex). In the diagnostic accuracy (phase 2) study, 78 subjects (8 men, 70 women) provided a history consistent with adverse reactions to natural rubber latex gloves. Of these, 19 had dermatitis and pruritus limited to the hands that occurred after hours of exposure to powdered latex gloves. Their ST data were therefore analyzed separately from that of the remaining 59 subjects, who provided a history consistent with a moderate to high probability for IgE-dependent latex allergy (Table 1). All subjects were required to refrain from taking any medications (β -blockers, antihistamines, or tricyclic antidepressants) within a week, or astemizole within 3 months, that would compromise the ST evaluation.

Latex ST reagents

NAL was prepared from crude *H. brasiliensis* (clone 600) tree sap that had been collected into sterile plastic bottles containing a nonhazardous (patented) Goodyear preservative (Akron, Ohio) (0.1 mol/L NaHCO₃, 50% wt/vol glycerol, and 3 mmol/L cysteine with no azide). Revertex (Kluang, Johor, Malaysia) provided NAL sap that was refrigerated at 4° C for 2 days until shipped over a 1-week period to the United States on ice packs. On receipt, 1 L NAL was immediately aliquoted into autoclaved 50 ml round-bottom polypropylene tubes (Nalgene, Rochester, N.Y.) and separated in an ultracentrifuge (J-21B rotor; Beckman, Fullerton, Calif.) at 10,000g for 1 hour at 4° C.

TABLE I. Dimensions of criteria for assigning clinical scores to study subjects

Clinical score	Probability of IgE-dependent latex allergy	Frequency of convincing reactions	Reaction typical of IgE	Consistency of latex reaction	Alternative explanations
0	Very low (<10%)	None	None	NA	NA
1	Low (11%-33%)	Single	Uncertain	Highly variable	>5 (many)
2	Moderate (33%-66%)	>1 and ≤ 3 (few)	Moderately convincing	Episodic	3 to 5 (several)
3	High (66%-90%)	>3 and ≤ 10 (many)	Highly convincing	Most exposures	1 to 3 (few)
4	Very high (>90%)	Always when exposed to latex	Very highly convincing	Invariable	None

NA, Not applicable.

After centrifugation, there was a 1 cm-thick white sticky latex rubber (isoprene) layer, a yellowish "latex C-serum" containing soluble allergenic proteins, and a small pellet of insoluble material. The latex C-serum was removed with a syringe-spinal needle assembly, sterile filtered (0.22 μ m, Corning filtration system; Corning, N.Y.), and frozen at -70° C in sterile Nalgene cryovials. The NAL stock contained field latex serum (pH 7.5) in a final concentration of 33% glycerol with 2 mmol/L cysteine and 0.067 mol/L NaHCO_3 . The ammoniated latex was prepared in a manner identical to the NAL except that the sap was collected in sterile bottles containing only ammonia. The final AL stock contained sterile-filtered field latex serum neutralized to pH 7.5 with a final ammonia concentration of 400 mmol/L.

A buffered saline extract of gloves was prepared from powdered latex surgeon gloves known to contain high levels of allergen by RAST inhibition.¹⁰ Multiple lots of natural rubber latex gloves from one source (manufactured from clone 600 *H. brasiliensis* latex sap; $n = 250$) were extracted with sterile phosphate-buffered saline solution (5 ml per gram of glove) that was pipetted into the intact glove. After the glove was knotted in the distal wrist area, each was put into a separate clean plastic zip-lock bag and agitated on a shaker for 16 hours at 21° C. The eluate (>90% recovery) was collected in a laminar flow hood by nicking the extended thumb of the glove over sterile 50 ml conical tubes. The eluate was separated from the donning powder by centrifugation (10 minutes, 1000g), concentrated to approximately 1.7 mg/ml in a filtration system (YM05 membrane; Amicon, Beverly, Mass.), sterile-filtered, and frozen in cryovials at -70° C.

Extract characterization and stability

Sterility of the three extracts was confirmed by the Johns Hopkins Hospital Department of Microbiology using procedures described in the Federal Register (21 CFR 610.12). Safety studies required by the FDA were performed by the Johns Hopkins Veterinary Service using the protocols described in the Federal Register (21

CFR 510.11). Immunochemical heterogeneity of the three extracts was examined by Dr. Y. Lin (Center for Biologics Evaluation and Research, FDA) using polyacrylamide gel electrophoresis methods previously described.⁸ The total protein content of each extract was measured by a micro-bicinchoninic acid assay (Pierce, Rockford, Ill.) with a bovine serum albumin standard. These protein concentrations were confirmed by Dr. V. Tomazic (FDA, Rockville, Md.) using an American Society for Testing and Materials consensus modified Lowry assay. All three latex extracts were then normalized to 1 mg/ml of total protein by using the skin test diluent (0.4% phenol-0.9% NaCl-0.03% human serum albumin; Greer Laboratories, Lenoir, N.C.). The allergen content of the normalized extracts was reevaluated by RAST inhibition¹⁰ with use of an adult serum pool that contained equal amounts of serum from 285 adult health care workers clinically allergic to latex (final IgE anti-NAL concentration 110 ng/ml), a solid-phase NAL allergosorbent, and the E5 NAL reference (100,000 allergen units[AU]/ml) provided by the FDA (CBER, Rockville, Md.).

Extract stability was examined in an initial real-time ID-ST titration study in which seven subjects highly allergic to latex received multiple ID-STs with NAL, AL, and GLO extracts at concentrations from 1 pg/ml to 10 ng/ml. Two lots of each extract source, one freshly prepared from frozen material and a second aged 67 days at 4° C, were simultaneously applied to the forearm, and wheals and erythema were measured at 15 minutes. As we reported previously,¹¹ no differences in the wheal sizes were observed with these fresh and 67-day-aged extracts in these seven subjects, indicating that the NAL, AL, and GLO extracts are stable for least 2 months when diluted to 10 pg/ml in phenol-saline-human serum albumin diluent without glycerol. On the basis of these results, all dilutions of the NAL, AL, and GLO extracts used for skin testing in this study were reprepared fresh as they approached their assigned shelf-life of 2 months.

Phase 1/2 study design

This project was conducted by using a protocol approved by the FDA under IND-4920 and the Johns Hopkins Bayview Institutional Review Board. After informed consent, a blood sample was collected for a total serum IgE measurement, a multiallergen screen (Phadiatop, Pharmacia, Akron, Ohio), and IgE anti-latex analysis.¹² Each subject completed a detailed questionnaire that evaluated the type and severity of his or her symptoms after rubber glove use, atopic and possible latex allergy history, other potential risk factors (food allergies, number of surgeries, frequency of glove use, exposure to other rubber products such as balloons and condoms), and a chronologic description of the two most recent reactions to latex gloves in terms of their exposure, rate of onset, and duration and severity of symptoms. A clinical score on a scale of 0 to 4 was assigned to the subject based on his or her history. This score is a clinical judgment of the relative probability of having IgE-dependent latex allergy by use of the criteria presented in Table I. Subjects were assigned a clinical score of 0 if they had no history of adverse reactions to the use of gloves and other rubber products. Nineteen subjects indicated one or more episodes of irritation, pruritus, or both, restricted to the hands, within hours to days after wearing powdered latex gloves, and they were assigned a low probability clinical score for latex allergy of 1. Subjects with a history of several atypical or equivocal reactions apparently caused by latex (e.g., lightheadedness, dyspnea) that were suggestive but not conclusive for latex allergy were assigned a clinical score of 2. Subjects with a history of many convincing episodes of IgE-mediated allergic reactions involving relevant organ systems, regardless of their severity and with no likely alternative explanation, were assigned a clinical probability score of 3 (high) or 4 (very high).

Progressive P-ST and ID-ST titrations were performed by using modifications of a previously described method.¹³ First, drops of NAL, AL, and GLO at 1 μ g/ml were simultaneously applied to the forearm skin, and the skin was punctured and "rocked" through the extract with a bifurcated Wyeth needle (Wyeth-Ayerst Laboratories, Radnor, Pa.). Positive ST responses were defined by an increase in mean wheal and erythema diameters (2 mm and 5 mm, respectively), over that produced by the saline control at 15 minutes. If the P-ST response was negative, subsequent P-STs were performed with all three extracts at both 100 μ g/ml and 1 mg/ml. Each P-ST set was completed before the next concentration was applied, to maximize safety. After completion of the P-STs, ID-STs were performed by administering 20 μ l intracutaneously in a tuberculin syringe at progressively increasing concentrations. If the P-ST was positive at 100 μ g/ml or 1 mg/ml, a 1 ng/ml ID-ST was performed initially. Tenfold more concentrated extracts were then administered every 15 minutes until a positive ST response with a net wheal \geq 8 mm was obtained or until the 100 μ g/ml concentration of each extract was reached.

In cases where all the P-ST results were negative at 1 mg/ml, the 1, 10, and 100 μ g/ml ID-ST doses were applied simultaneously. At 15 minutes after application, each skin test was read by measuring the mean diameter of the erythema and wheal, outlining the perimeters of each with a fine-tip rolling writer pen (Pentel, Torrance, Calif.) and transferring them onto transparent tape (Transpore 3 inch; 3M Company, Minneapolis) for a permanent record. This time-consuming sequential ST titration was performed to maximize safety.

Serologic analyses

The total serum IgE was measured in an enzyme immunoassay (IMx; Abbott Laboratories, Abbott Park, Ill.) and reported in nanograms per milliliter and as a percentile of the age-adjusted nonatopic mean.¹⁴ IgE antibodies to common aeroallergens were measured in the Phadiatop Multiscreen (Pharmacia Diagnostics) as a general screen for atopy. IgE anti-latex was measured as previously described¹¹ in a particle-based RAST with NAL, AL, and GLO proteins covalently coupled together on the allergosorbent. Results of all IgE antibody measurements were reported in nanograms per milliliter on the basis of an IgE anti-NAL reference serum pool that was calibrated in nanograms per milliliter by depletion analysis.¹⁵ The analytic sensitivity of the RAST was 1 ng/ml as determined by the concentration that produces a statistically significant response above that produced by negative control sera from 20 nonatopic subjects.

Glove provocation test

An unmasked glove provocation test was performed by using modifications of previously reported methods.^{4,6} The glove provocation test design was initially optimized with a pilot study involving a direct latex glove challenge of five subjects who had positive responses on P-ST and ID-ST for latex allergy (two men and three women; clinical score 4). All these individuals had a positive P-ST response through the powdered latex test glove used in the challenge. After informed consent, each subject was equipped with goggles and a nonlatex 3M respirator mask to prevent inhalation of latex allergen attached to cornstarch donning powder. Each subject put a high-allergen-containing powdered latex examination glove on one hand (mean latex allergen content [\pm SEM] 15,072 \pm 1448 AU per glove) and a synthetic glove on the other hand. The subjects were observed for any allergic symptoms over a 1-hour period. Only one of these five individuals had extensive pruritus and visually confirmed erythema and swelling of his hands. On the basis of the surprisingly limited number of hand reactions observed in this pilot group allergic to latex, we added a second phase to the provocation test that was used in the current study only for the evaluation of cases of positive history-negative ST response and negative history-positive ST response. This second phase involved removing the high-allergen-powdered latex glove and having the subject blow it up like a

TABLE II. Reactions observed during ST

Latex allergy status based on a clinical history	Negative clinical history for latex allergy	Hand restricted dermatitis, pruritus, and/or irritation	Positive history for latex allergy
Total number of subjects	24	19	59
Clinical score	0	1	2-4
Skin			
Generalized hives	0	0	0
Rash-irritation	0	0	1
Generalized urticaria	0	0	0
Localized pruritus	0	2	50
Wheal and erythema at skin test site	0	2	50
Eyes			
Itching	0	0	0
Tearing	0	0	0
Redness	0	0	0
Mouth, nose, throat			
Rhinitis	0	0	0
Sneezing	0	0	0
Itchy or tight throat	0	0	0
Lungs			
Asthma	0	0	0
Chest discomfort	0	0	0
Heart			
Chest pain	0	0	0
Palpitations	0	0	0
Fast pulse	0	0	0
Anaphylactic shock	0	0	0

Numbers given are numbers of subjects exhibiting a symptom during ST portion of this study.

balloon and then expell the glove powder-allergen back into his or her own face. Each subject was then observed for any evidence of allergic symptoms over a 1-hour period. The latex allergen on the glove cornstarch donning powder was confirmed by in vitro studies involving the direct binding of human IgE anti-latex and detection with radiolabeled anti-human IgE.¹⁶

In this study, only five of the nine study subjects with a positive history and negative ST response and one subject with a negative history and positive ST response were available for challenge with the following procedure. All subjects with asthma were asked to perform a peak flow measurement before (and after) glove provocation. First (without wearing goggles or a respirator), a high-allergen, powdered latex glove (same as used in the pilot study) was placed on one hand of the subject and a control synthetic glove on the other hand. The subject was observed for a 30-minute period for any adverse (allergic-type) reactions. Care was taken to minimize erythema induced by scratching the face or hands. If no allergic symptoms were observed during the initial latex glove use period, the respiratory challenge as described above was undertaken. If no allergic or asthmatic symptoms occurred within 60 minutes, the glove provocation test result was called negative.¹⁷

ROC plot and statistical analyses

Diagnostic specificity (true negative fraction: $TN/[TN + FP]$) was computed by using the ST results from the 24 subjects with a clinical score of 0. Diagnostic sensitivity (true positive fraction: $TP/[TP + FN]$) was computed by using the ST results from the 50 subjects with a positive ST response and 4 subjects with a positive history and negative ST response who were unavailable for glove provocation testing. The overall diagnostic accuracy of an ST reagent was defined by its diagnostic specificity and sensitivity at a particular reagent concentration. The 19 subjects with skin-restricted dermatitis, pruritus, and irritation and the 5 subjects with a positive history, negative ST response, and negative IgE antibody response who had a negative glove provocation test result were excluded from these analyses. ROC plots were constructed as described in the National Committee on Clinical Laboratory Standards guideline.¹⁸ The area under each ROC plot was used to compare the overall diagnostic accuracy (sensitivity and specificity) of the three ST extracts. The significance of differences observed with the multi-allergen screen for atopy and sex distribution among groups were evaluated by chi-square analysis with SPSS for Windows (SPSS

TABLE III. Demographics, serologic results, and ST results

Latex allergy status based on a clinical history	Negative clinical history for latex allergy	Hand restricted dermatitis, pruritus, and/or irritation	Positive history for latex allergy, P-ST and ID-ST negative	Positive history for latex allergy, P-ST and ID-ST positive
Total number of subjects	24	19	9	50
Sex (M/F)	11/13	2/17	2/7	4/46
Age (mean ± SEM)	36.1 ± 1.8	38.2 ± 3.2, NS	35.4 ± 3.1, NS	34.5 ± 0.9, NS
Clinical score (No. of subjects)				
0, Non-latex allergic	24	0	0	0
1, Low	0	19	0	0
2, Moderate	0	0	2	2
3, High	0	0	7	20
4, Very high	0	0	0	28
Occupation (No. of subjects)				
Nonlatex environment, clerical	12	5	0	6
Daily latex use, non-HCW	0	0	0	(multiple surgeries) 2 (florist and pantry worker)
Daily latex use, HCW (MD, RN, Laboratory)	12	14	9	42 (gloves)
Serology				
Total serum IgE (ng/ml; mean ± SEM)	226 ± 83	405 ± 172, NS	267 ± 170, NS	968 ± 290, NS
Multiallergen screen (No. positive, %)	8 (33%)	7 (37%) NS	4 (44%) NS	32 (64%) <i>p</i> = 0.01
IgE antilatax (No. positive)	1	2	1	50
IgE antilatax (ng/ml; mean ± SEM)*	1.1	1.2, 8.7	1.6	173 ± 44
P-ST†				
NAL SPT (No. positive)	0	0	0	50
AL SPT (No. positive)	0	0	0	50
GLO SPT (No. positive)	0	0	0	50
ID-ST‡				
NAL ID-ST (No. positive)	1	2	0	50
AL ID-ST (No. positive)	1	1	0	50
GLO ID-ST (No. positive)	1	1	0	50
Glove provocation test results§	1 of 1 negative	None performed	5 of 5 negative 4 not available for testing	None performed

NS, Not significant; HCW, health care worker.

*IgE anti-latex results provided only for those that had serologically positive response.

†Number of positive P-STs at highest allergen concentration tested (1 mg/ml)

‡Number of positive ID-STs at highest accepted concentration (1 µg/ml)

§Only 5 of 9 subjects with positive history, and negative ST and serologic test results were available for provocation testing. All had a negative glove provocation test result, which suggests that they have a false-positive history.

Inc., Chicago). Analysis of variance was used to assess whether there were any statistical differences in the log total serum IgE and age distribution among the study groups. Linear regression analysis was used to examine for correlations between the clinical symptom scores in subjects allergic to latex and the degree of ST positivity based on the titer or concentration of allergen required to produce a net 8 mm wheal or the level of IgE antibody in serum.

RESULTS

Safety and quality assurance testing of extracts

The NAL, AL, and GLO extracts were each shown to be safe and sterile in FDA-required and approved animal and microbiologic tests. Before normalization, total protein levels were 4.5 mg/ml (NAL, lot 394140), 12.9 mg/ml (AL, lot 490710),

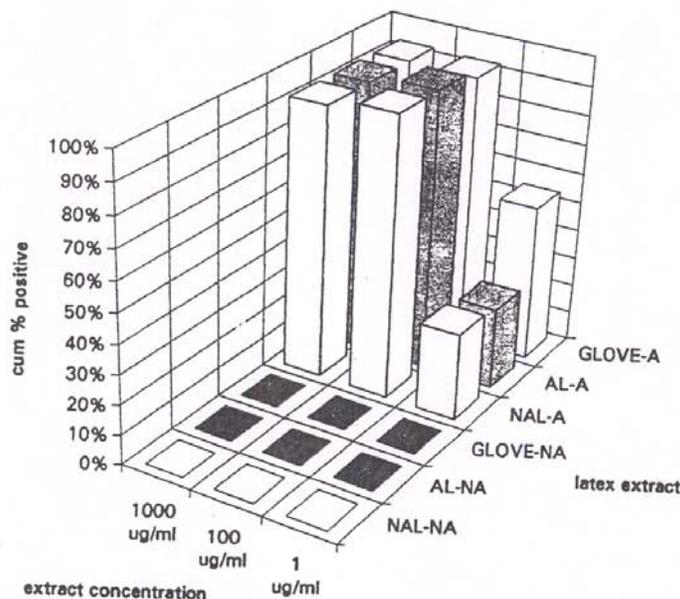


FIG. 1. Cumulative percentage of P-ST positivity of group without latex allergy (NA) ($n = 24$, clinical score = 0) and group with latex allergy (A) ($n = 54$, clinical score = 2 to 4) observed with NAL, AL, and GLO extracts at 1 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, and 1 mg/ml. Subject with food allergy who had a negative history, positive ST response, and positive IgE antibody response was included in group with no latex allergy, and four subjects with positive history, negative ST result, and negative IgE antibody response who were not available for glove provocation confirmation of their latex allergy were included in group with latex allergy.

and 1.7 mg/ml (GLO, lot 490110). These total protein levels were confirmed to be within 15% as assessed by modified Lowry method that uses a protein precipitation procedure. After normalization by dilution to 1 mg/ml, the RAST inhibition analysis measured the latex allergen content as 57,065 AU/ml (NAL), 49,532 AU/ml (AL), and 89,914 AU/ml (GLO) relative to the FDA's E5 NAL that was preassigned 100,000 AU/ml. Table II summarizes cutaneous and other reactions that were monitored in the three study groups during the skin testing portion of the study. No large local or systemic allergic reactions were observed in this study with any of the extracts in any of subjects by using the progressive skin test titration protocol.

Safety and specificity (phase 1) study

Initially, 24 subjects with no clinical evidence of latex allergy (clinical score = 0) were observed to

determine what extract concentrations would produce a false-positive P-ST and ID-ST response. Their demographics, occupations, serologic results, and ST results are summarized in Table III. Twelve were clerical workers who used latex rubber gloves intermittently, and the remaining 12 were health care workers (nurses, physicians, and laboratory personnel) who used latex gloves daily. One third of the individuals had clinical and serologic evidence of atopy including a positive response to a multiallergen IgE antibody screen.

All 24 individuals with a negative history had a negative P-ST response at 1, 100, and 1000 $\mu\text{g/ml}$ (Fig. 1). In the ID-ST evaluation, no positive reactions were observed with any of the extracts from 1 $\mu\text{g/ml}$ to 10 ng/ml (Fig. 2). At 100 ng/ml , one hospital clerk who was clinically not allergic to latex had a positive ID-ST response with all three extracts. This individual also had weakly positive

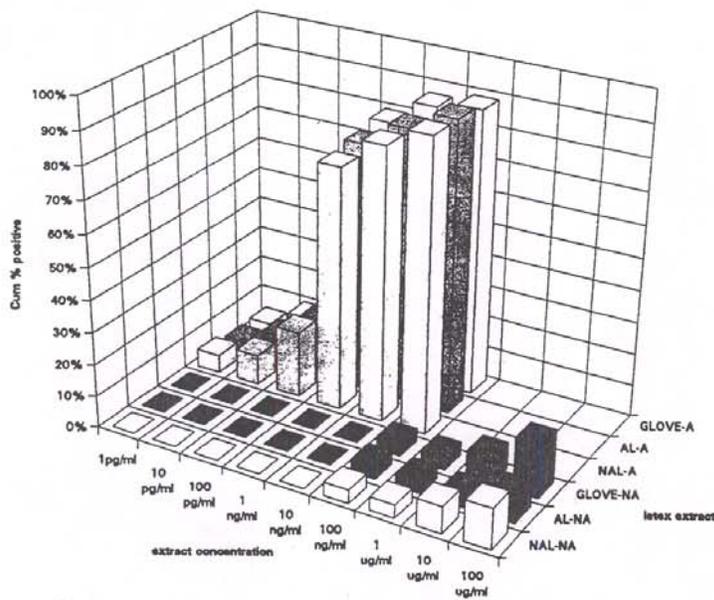


FIG. 2. Cumulative percentage of ID-ST positivity of group with no latex allergy (NA) ($n = 24$, clinical score = 0) and group with latex allergy (A) ($n = 54$, clinical score = 2 to 4) observed with NAL, AL, and GLO extracts at incremental tenfold concentrations from 1 pg/ml to 100 µg/ml. Subject with food allergy who had a negative history, positive ST response, and positive IgE antibody response was included in group with no latex allergy, and four subjects with positive history, negative ST result, and negative IgE antibody response who were not available for glove provocation confirmation of their latex allergy were included in the group with latex allergy. Group with latex allergy did not receive an ID-ST with concentrations of extracts ≥ 1 µg/ml to maximize safety.

IgE anti-latex serologic findings (1.1 ng/ml) and a negative glove provocation test result. However, this person is highly atopic and has a well-documented history and positive P-ST response to extracts of fresh avocado, bananas, and kiwi fruit. We believe that this one case represents a positive ID-ST response as a result of IgE antibody that is cross-reactive with food allergens as has been previously reported.¹⁹⁻²¹ This cross-reactive IgE antibody appears to be of little clinical significance, inasmuch as this highly atopic subject has used high-allergen powdered latex examination gloves three to four times a week over several years to transport blood to the laboratory with no apparent problems. To be conservative, we included the positive ID-ST results of this one subject who was allergic to food but clinically not allergic to latex in

the analyses as a false-positive case (Figs. 1 through 3, Table III).

Maximally achieved specificity for the ID-ST with NAL was 96% at 1 µg/ml, 92% at 10 µg/ml, and 87.5% at 100 µg/ml. The diagnostic specificities of the AL and GLO extracts were comparable to that of the NAL. The positive ID-STs observed at NAL, AL, and GLO extract concentrations above 1 µg/ml appear to be false positive because these subjects had concomitant negative serologic findings, and they could use high-allergen powdered latex gloves with no apparent clinical problems. Only two nonatopic subjects received an ID-ST with all three extracts at 1 mg/ml (data not shown). Both of these subjects had a positive ID-ST response, with a painful induration at the site of application that persisted for several days.

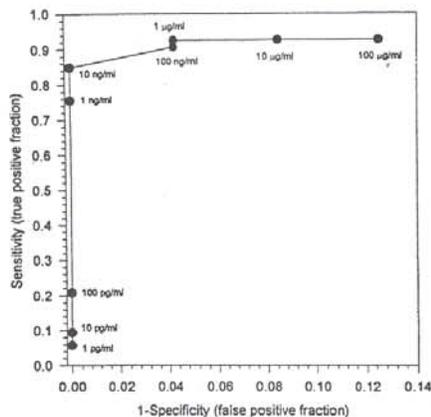


FIG. 3. ROC plots for NAL extracts used in ID-ST titration studies. "Diagnostic sensitivity" or true-positive fraction is plotted versus "1-diagnostic specificity" or false-positive fraction observed for each of nine NAL extract concentrations from 1 pg/ml to 100 µg/ml. Optimal balance of diagnostic sensitivity and specificity was achieved at an NAL ID-ST concentration of 1 µg/ml. ROC plots for AL and GLO extracts are comparable to NAL ROC curve in this figure; they have analogous areas under their curves (see Results). AL and GLO curves are therefore not individually depicted (see text for definition of populations used to compute sensitivity and specificity).

The 1 mg/ml concentration of all three extracts was therefore eliminated from any subsequent ID-ST testing. On the basis of these results, the 1 mg/ml and 1 µg/ml concentrations of the NAL, AL, and GLO extracts were identified as the highest P-ST and ID-ST doses, respectively, that could be applied while also obtaining maximal diagnostic specificity.

Diagnostic accuracy and sensitivity (phase 2) study

Of the 102 study subjects, 78 provided a clinical history of adverse reactions to natural rubber latex gloves at the time of evaluation (Table III). Their age distribution did not differ significantly from that of the 24 subjects not allergic to latex who were evaluated in the phase 1 study. The percentage of women was, however, significantly higher in the group with latex allergy as compared with the group without latex allergy ($p = 0.002$).

Nineteen of the 78 subjects with suspected latex allergy were assigned a clinical score of 1 after complaining solely of a rash, pruritus, irritation, or a combination of these, restricted to their hands

within hours of wearing latex rubber gloves (Table III). Their P-ST responses were negative at all extract concentrations. Two had a positive NAL ID-ST response with use of the 1 µg/ml threshold, and these same two had a positive IgE anti-latex response (1.2 and 8.7 ng/ml). Both of these subjects with positive ID-ST and IgE anti-latex responses had no apparent history of any allergies to cross-reactive foods.

Of the remaining 59 subjects, 9 individuals with clinical scores of 2 ($n = 2$) and 3 ($n = 7$) had negative P-ST and negative ID-ST responses to all extract concentrations below 1 µg/ml (Table III). Only 5 of these 9 subjects were available for glove provocation testing, and none of them had symptoms during the cutaneous or respiratory challenge tests. All 5 individuals had a negative latex-specific IgE response in serum. Our observations suggest that these 5 subjects provided a false-positive clinical history at the time of evaluation and are in fact not allergic to latex. Because of this observation, the ST results obtained from these 5 subjects were excluded from the cumulative positivity analyses (Figs. 1 and 2) and the sensitivity computations assessed in the ROC curve (Fig. 3). To be conservative, we included the remaining 4 subjects with positive histories and negative ST responses (only one of whom had a positive IgE antibody response) who were not available for glove provocation testing in the sensitivity computations as individuals allergic to latex with false-negative ST responses.

Of the 50 subjects with a positive P-ST and ID-ST response, 96% had clinical scores of 3 or 4. Six were clerical workers who had presumably become sensitized as a result of multiple surgeries. A florist and pantry worker were exposed to latex balloons and gloves in non-health care environments, and the remaining 42 were health care workers with extensive latex glove exposure. Interestingly, 36% of this group indicated an absence of any other allergies and had negative results on multiallergen RAST screens. All 50 had a positive IgE anti-latex response in serum with a range from 4 to 1373 ng/ml. A trend was observed for subjects with latex allergy who had progressively more severe latex allergy symptoms (e.g., clinical score 1 to 4) to have increasingly higher levels of serum IgE anti-latex ($r = 0.27$, $p = 0.02$).

The cumulative frequencies of positive P-ST and ID-ST responses in the subjects allergic to latex at the different extract concentrations are presented in Figs. 1 and 2, respectively. With a 100 µg/ml positive P-ST threshold and a 1 µg/ml positive

ID-ST threshold for all three extracts, all 50 of these subjects had positive P-ST and ID-ST results, which provided a diagnosis of "latex allergy." No glove provocation tests were performed in this group of subjects because their history and P-ST, ID-ST, and serologic results all indicated that these subjects had become sensitized to latex allergen. However, with the 4 subjects with a positive history and negative ST response included in the sensitivity computations, 96% was the maximum clinical sensitivity (TP/TP + FN) that was achieved with the NAL and GLO extracts and 90.6% with the AL.

ROC curve analyses

Sensitivity and specificity computations of the P-ST results identified 100 µg/ml as the minimal concentration of NAL, AL, and GLO extracts that maximized overall diagnostic accuracy. The ROC curve for NAL in Fig. 3 displays the diagnostic sensitivity and specificity obtained at each of the nine different concentrations used in the ID-ST study. The minimum concentration of NAL required to produce an optimal balance of sensitivity and specificity in the ID-ST is 1 µg/ml. Virtually identical areas under the ID-ST ROC curves were obtained for NAL (0.98), AL, (0.96), and GLO (0.98) extracts, which indicates that the AL and GLO extract produced equivalent diagnostic accuracy to the NAL when used as ID-ST reagents (AL and GLO ROC curves not presented). Finally, the IgE anti-latex RAST achieved a diagnostic accuracy (sensitivity 94%, specificity 96%) that was equivalent to that achieved with ID-ST for NAL and GLO extracts when 0.5 ng/ml was used as the positive-negative discriminator.

DISCUSSION

The ST is considered by many as the diagnostic method of choice for the evaluation of allergic disease.²² Therefore, the absence of a well-characterized natural rubber latex ST reagent has made routine diagnosis and research studies on the epidemiology and natural history of latex allergy difficult in the United States.²³⁻²⁴ Several nonlicensed latex ST reagents have been used to study latex allergy,^{4,6, 25-27} but their diagnostic sensitivity and specificity and in some cases safety have not been documented. The difficulty with all these studies to date has been identifying "true" cases of latex allergy in the absence of a reference method such as the ST. In this study, we used a detailed, standardized clinical history to assign a clinical score that defined the relative probability that the

subject had a latex allergy. We then performed P-ST and ID-ST titrations, starting with very dilute concentrations of protein, to maximize safety.

In cases where the clinical history and ST response were discordant, a glove provocation test was performed that included both cutaneous and respiratory challenges. In contrast to other studies that used a "glove use" test,^{4,6} we included a respiratory challenge to our provocation, after our pilot study with five subjects who had positive ST responses and latex allergy demonstrated that wearing a latex glove for up to an hour did not generally produce objective evidence of dermatitis and urticaria. Even with a careful clinical history, we incorrectly diagnosed latex allergy in 15% of the subjects. Of the five subjects with a positive history and negative ST response whom we could evaluate, all five had a negative glove provocation test response and negative serologic findings. We interpreted these results as indicating that these five subjects with a positive history, negative ST response, and negative IgE antibody result did not have an IgE-mediated latex allergy.

In addition, ST without regard to a clinical history may also lead to misdiagnosis. One of our control subjects with no history of latex allergy had a negative P-ST response but a weakly positive ID-ST response at the 100 ng/ml dose with all three extracts, and a weakly positive IgE antilatelatex (1.1 ng/ml) response. Because this subject has well-documented allergies to fresh avocado, kiwi fruit, and banana, a spurious ID-ST response appears to have resulted from cross-reactive allergens. He routinely wears high-allergen-containing powdered latex gloves to transport blood to the laboratory without difficulties, suggesting that his cross-reactive IgE antibody has little clinical significance at present. This phenomenon has also been observed by others.¹⁹⁻²¹

Safety was a major concern in this study, because Kelly et al.²⁵ had reported an 8.4% systemic reaction rate after P-ST of 107 individuals with a history of latex allergy using a multitest prick device together with a nonstandardized GLO extract and raw latex sap from India.²⁵ Of greater concern was their report that four of their subjects had anaphylaxis after P-ST. As a result, they did not even attempt ID-STs. In the present study, we used a bifurcated needle for P-ST and three extracts representing each of the potential source materials, each normalized to 1 mg/ml of total protein. Safety was addressed by performing sequential P-ST skin test titrations, applying one concentration of three extracts at 15-minute inter-

vals, and increasing the dose if the result of the previous test was negative (<2 mm wheal above the saline control). Our starting ID-ST concentration was based on the results of the previous P-ST, and our sequential titration procedure was considered safe by review committees on the basis of their experience with other allergen systems. We continued increasing the extract concentration applied intradermally until the subject's titer (net 8 mm wheal) was achieved. Following this strategy, we were able to perform sequential P-STs and ID-STs with three extracts safely in all 102 subjects without any large local wheal-and-flare reactions at the test site, or any systemic reactions (Table II).

To date, no study has directly compared the diagnostic performance of three candidate latex allergen sources to provide a basis for optimized testing and reagent development. The available ST reagents in Europe are made either from AL, which is used in the manufacturing of natural rubber latex gloves and other rubber-containing medical devices, or from a nonammoniated form of the same latex that appears to be easier to evaluate with use of immunochemical tests.⁷ Although there are no commercially available glove extract ST reagents in the United States, 15-minute extracts of a wide cross-section of latex gloves continue to be used in many offices with disregard for quality control and potency. Others perform P-STs directly through latex gloves, which admittedly can vary widely in allergen content between lots and sources or within a lot as a result of different storage conditions. Concerns about the composition of these different NAL, AL, and GLO extracts have been raised by investigators who have identified quantitative and qualitative differences in their allergen content using Western blot analysis and immunoassay inhibition tests.⁷⁻⁹ This finding would suggest that these three source materials might be expected to perform differently as diagnostic ST reagents. Our study disproves this working hypothesis and demonstrates that when these three extract sources are normalized on the basis of their total protein content (and incidentally on allergen content as well), their safety and diagnostic sensitivity and specificity are comparable. Because ammonia in the AL can cause unpredictable pH changes and losses of allergenic activity during storage, and the allergen content of different gloves lots can vary substantially depending on processes used in their manufacturing, we believe the nonammoniated form of latex sap (NAL) represents the most reproducible and stable form of the latex allergen. As such, we propose that the

NAL be considered the source material of choice for future ST reagent development. An analogous NAL reagent should also be considered for the preparation of allergen-containing reagents used in serologic methods for latex-specific IgE antibody.

One constraint to our study was the requirement from review agencies that only adults be included, for safety reasons. This restriction limited the study group almost exclusively to health care workers who have become sensitized to latex allergen through skin contact and inhalation as a result of daily occupational exposure to latex rubber gloves. In our study population, we also evaluated a florist and a pantry worker who were occupationally exposed. Children with spina bifida who are sensitized during surgeries after mucosal contact with natural rubber latex devices were not included in this study. Some evidence exists that these children may preferentially produce large amounts of IgE antibody to latex particle-associated allergens such as Hev b 1 and Hev b 3.²⁸ In contrast, adult health care workers appear to produce IgE antibody to these allergens less frequently; rather, they mount IgE immune responses to soluble latex proteins (e.g., Hev b 2 and Hev b 4) that readily adhere to the cornstarch donning powder (unpublished observations). Six adults in our study with latex allergy were presumably sensitized after multiple surgeries because they had no other identifiable risk factors. All six of these individuals had strongly positive ST responses with the three ST reagents, suggesting that both the soluble and particle-associated allergenic proteins are present in the three ST materials. The presence of both allergen groups in the latex reagents used in this study was also supported by the Western blot analyses of these extracts,⁷⁻⁹ even though it was difficult to get clear banding patterns with the AL and GLO extracts because of interference from the ammonia and glove chemicals, respectively.

In conclusion, the results of this study demonstrate that NAL, AL, and GLO extracts prepared from latex collected from clone 600 of *H. brasiliensis* trees perform equivalently well as safe and efficacious diagnostic ST materials. On the basis of sensitivity and specificity determinations of each extract at multiple concentrations, the 100 $\mu\text{g}/\text{ml}$ and 1 $\mu\text{g}/\text{ml}$ doses may be considered the minimal dose that produces the best achievable accuracy in P-ST and ID-ST, respectively. Whether the ID-ST at 1 $\mu\text{g}/\text{ml}$ is more sensitive in identifying weak latex allergy in individuals than the P-ST at 100 $\mu\text{g}/\text{ml}$ remains to be determined. On the basis of

several practical advantages of NAL associated with its consistency between lots, long-term stability, and ease of immunochemical quality control, it has been identified as the latex source of choice for commercial reagent development. The diagnostic performance of these extracts in special populations (e.g., children with spina bifida) will require further study.

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