

CHOICES

Research & Technology Supporting Your Decisions

Vol. 3 '98

In This Issue...

We'll explain the most commonly referenced assays that quantify proteins and allergens in natural rubber latex products.

In Our Next Issue...

We'll review some common synthetic polymer films used in medical gloves and their appropriate clinical usage.

Visit Our Healthcare Information Center at...

www.allegiance.net/hic

Natural Rubber Latex: Interpretation of Protein and Immunological Assay Test Results

Concerns about sensitivities associated with Natural Rubber Latex (NRL) have led to the development of a number of protein and immunological assays. These standardized tests are designed to measure the protein levels of latex products.

Three of the most commonly referenced assays are the modified Lowry, LEAP and RAST. These assays may be performed by independent labs using extracts of NRL-containing products. Results may be stated on the package label of gloves you buy. But what do these numbers mean? The following is a discussion of these commonly used test methods to help you understand and interpret the results.

The Lowry Assay

The only total protein assay currently recognized by the FDA is the modified Lowry assay (ASTM D5712-95).¹ However, this assay is relatively insensitive and is subject to interference from chemicals that are added to the gloves during production to enhance their physical properties. These chemicals may include stabilizers, antioxidants and many coagulant chemicals. Because of the lower limit of the modified Lowry assay, the lowest protein label claim currently permitted by the FDA must state that the product contains 50 micrograms or less of total water-extractable protein per gram. However, not all proteins from the rubber tree are equally antigenic (allergy generating). Some gloves can have high protein, but low allergen levels (and vice versa). Additionally, it is not known what level of protein or allergen, if any, is a "safe" level for latex-sensitized individuals. Therefore, the FDA mandates the addition of the following statement to all gloves with low-protein claims, "Caution: Safe use of this glove by or on latex sensitized individuals has not been established." Medical devices manufactured after September 30, 1998, that contain natural rubber must be labeled with statements similar to the following: "Caution: This product contains natural rubber latex which may cause allergic reactions."

Latex ELISA for Antigenic Proteins (LEAP)

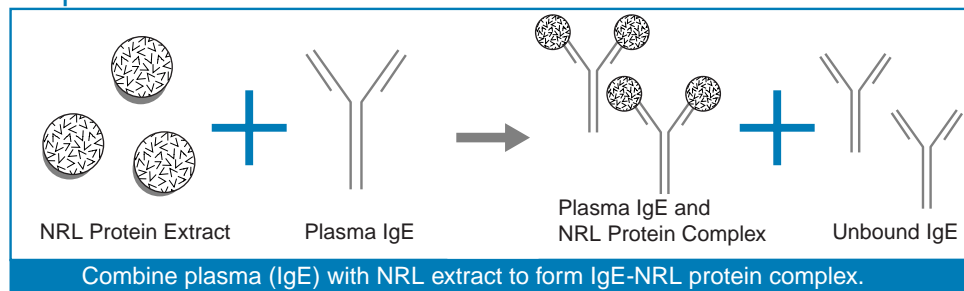
The LEAP assay is an ELISA (enzyme-linked immunosorbent assay) for natural rubber latex proteins.² This method is still in development. It has not been adopted by the American Society for Testing and Materials (ASTM) and has not been approved by the FDA. Latex allergy in humans is based on IgE antibodies reacting to specific allergenic rubber tree proteins. The LEAP assay is based on rabbit IgG antibodies reacting with all proteins isolated from a natural rubber latex-producing tree (*Hevea brasiliensis*). The results are expressed as micrograms of protein/gram of natural rubber latex product. Variations of assay results of a few micrograms between gloves from different manufacturers may not be significant, particularly if the assays for the gloves were done in different labs or at different times.

Radioallergosorbent Test – Competitive Inhibition RAST

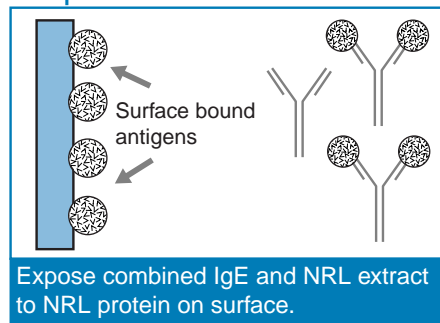
The RAST is an allergen-specific protein assay, a technique for detecting and quantifying IgE antibody in human serum samples. There are several FDA-approved RAST-type assays commercially available. The allergenic proteins are bound to a surface and then plasma is allowed to react with the allergens. If there is IgE in the plasma against that allergen, then it will bind to the surface. When used with the pooled serum of known latex-allergic individuals, this assay can measure antigenic proteins from extracts of NRL-containing products. The anti-latex IgE from the pooled sera binds to the antigenic latex proteins that are isolated from the latex-containing product. Since the IgE is already bound to the extract proteins, it can't bind to the allergenic proteins from the assay kit. Hence, the assay is competitive and results in a decrease in signal or color if the extract contains the allergens. However, the source of pooled allergic patient plasma can affect the test outcome and relevancy, since allergic individuals can react to different NRL proteins.³

Inhibition Assays for Measuring Natural Rubber Latex Antigens

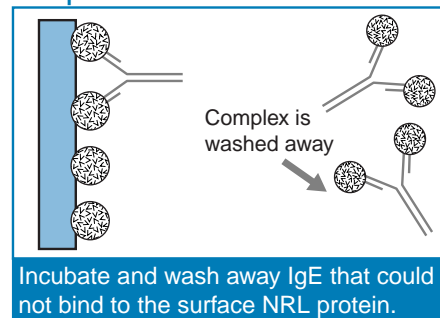
Step 1



Step 2

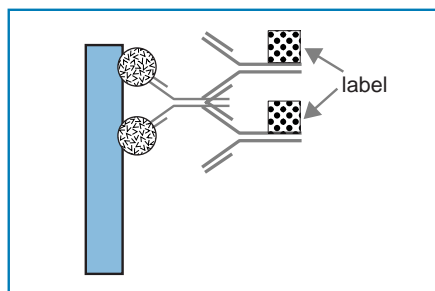


Step 3



Step 4

Incubate with labeled anti-IgE antibody, then wash away unbound labeled antibody.



Step 5

Detect and quantitate amount of label.

Radioisotope for RAST-type assay or enzyme for ELISA-type assay.

Summary of Methods

Test Method	Factor Quantified	Measurement Indicator	Test Type	Advantages	Disadvantages	Limits of Sensitivity
Modified Lowry	Total protein	Chemicals reacted with all proteins to produce a color change	Colorimetric chemical reaction	<ul style="list-style-type: none"> Commercially available reagents Relatively easy to perform Rapid results 	<ul style="list-style-type: none"> Not sensitive Many chemical interferences skew results Lacks specificity 	FDA permits label claim of $\leq 50\mu\text{g/g}$
LEAP	All latex proteins	Color change produced by enzyme-substrate interaction	ELISA	<ul style="list-style-type: none"> Specific to latex proteins Serum is plentiful No human biohazardous materials 	<ul style="list-style-type: none"> Not specific to Type I latex allergens, reacts with all latex proteins Relies on rabbit antisera, not commercially available Serum can be heterogeneous 	As low as $0.045\mu\text{g/g}$
RAST or ELISA Competitive Assays	Latex allergenic proteins	Radioisotopes (linked to allergen-antibody complexes) or enzyme-substrate color change	Competitive allergenic assay: Radio-labeled or ELISA	<ul style="list-style-type: none"> Specific to latex allergen Serum from known Type I allergic subjects Very sensitive 	<ul style="list-style-type: none"> No heterogeneity of serum pool Relative scarcity of human sera Potentially biohazardous human sera used 	Estimated at 5AU per mL

Note: The amount of latex that was extracted per mL must be known in order to make comparisons; modified Lowry and LEAP results are usually expressed as micrograms per gram of latex ($\mu\text{g/g}$); AU(allergen units)/mL for RAST.

What You Should Look For

It is important to realize that test results can vary widely. When evaluating the latex sensitivity data of various gloves, such as protein or allergen content, pay attention to the following prior to making comparative conclusions:

- the type of test
- lab personnel training and experience
- units of measurement
- serum pool
- test laboratory

For the RAST inhibition assay, modern glove products usually have results less than 100AU/mL.

Here is an example of RAST test results for various types of gloves from Allegiance:

	RAST (Allergen Units/mL)
Ultrafree™ powder-free surgical	<5
Ultraderm® surgical	<5
Triflex® standard white surgical	12
Powder-free Triflex® surgical	<5
Triflex® orthopedic surgical	32
Positive Touch™ exam	<5

Conclusion

Currently the best assay for quantifying allergenic proteins is the RAST or ELISA competitive IgE assay. The LEAP measures latex proteins and has potential for negative interferences. The modified Lowry assay measures total proteins and is the most subject to chemical interferences and variations in methodology.

References and Further Reading:

1. ASTM D5712-95 Standard Test Method for Analysis of Protein in Natural Rubber and Its Products. *Annual Book of ASTM Standards*. Volume 14.02. 1995.
2. Bollag, Daniel M., Rozycki, Michael D., Edelstein, Stuart J. *Protein Methods*, 2nd Edition, John Wiley and Sons Ltd., 1996.
3. Kenny, D.M., Challacombe, S.J. *ELISA and Other Solid Phase Immunoassays Theoretical and Practical Aspects*. John Wiley and Sons Ltd., 1988.

A Note Regarding Latex Sensitivities

Nonallergic contact dermatitis accounts for the majority of reactions in glove wearers who experience reactions, followed by Type IV delayed hypersensitivity. A Type IV reaction is a delayed-reaction contact dermatitis produced mainly by chemicals added to the latex during manufacturing. Type I hypersensitivity affects a very small percentage of glove wearers. In any case, persons with IgE-mediated clinical reactivity should use synthetic gloves, no matter how low the protein or allergen levels.