

## In this issue...

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

## 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, ELISA 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 total protein assay currently recognized by the FDA is the modified Lowry assay (ASTM D5712).<sup>1</sup> However, this assay does not measure latex allergen per se, 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 NRL must be labeled with statements similar to the following: "Caution: This product contains natural rubber latex which may cause allergic reactions."

### ELISA for antigenic proteins

The ELISA (enzyme-linked immunosorbent assay) for natural rubber latex proteins<sup>2</sup> has been adopted by the American Society for Testing and Materials (ASTM).<sup>3</sup> Latex allergy in humans is based on IgE antibodies reacting to specific allergenic rubber tree proteins. The assay for NRL proteins is based on rabbit IgG antibodies reacting with all proteins isolated from a natural rubber latex-producing tree (*Hevea brasiliensis*). Not all of these NRL proteins are necessarily allergenic to humans. The results are expressed as micrograms of protein/gram of NRL 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.<sup>3</sup> It is also difficult to standardize this assay due to the heterogeneity of the allergic response to latex allergens.

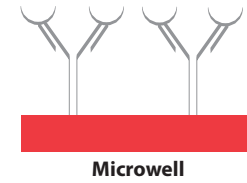
## The FITkit™ immunoenzymetric assay

An immunoenzymetric assay procedure, which quantifies specific NRL allergens, has recently been commercialized. The FITkit™ assay (FIT-Biotech, Tampere, Finland) measures four clinically relevant major latex allergens (Hev b1, Hev b3, Hev b5 and Hev b6.02) by an ELISA procedure. This assay utilizes monoclonal antibodies to capture specific Hev b proteins from a recombinant protein standard or from an unknown glove extract that is being analyzed. This assay provides a specific, sensitive and reproducible measurement of the individual NRL allergens in quantities that are expressed in nanograms per liter (Hev b5) or µg/L (Hev b1, Hev b3, Hev b6.02). Because this assay procedure is currently available through a single source, it has not yet been adopted by the American Society for Testing and Materials (ASTM) as a general procedure. There is also a question as to whether additional monoclonal antibodies should be added to the kit to cover other possible major latex allergens. No FDA guidelines or an allowable amount of specific NRL allergens have been set.

## ELISA assay for measuring individual NRL antigens (Hev b1, b3, b5, b6.02)

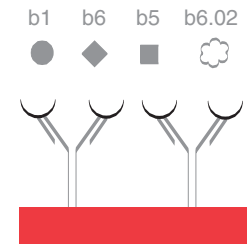
### Step 1:

**FITkit™ microwell plate precoated with mouse monoclonal anti-Hev b antibody, e.g., Hev b1.**



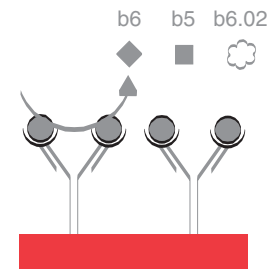
### Step 2:

**Incubate plate with extract made from NRL product. This is done in parallel with a series dilution of recombinant Hev b1 to constitute a standard curve.**



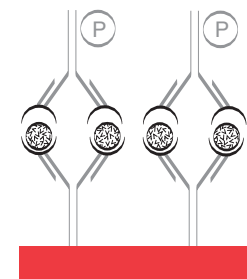
### Step 3:

**Wash plate with kit wash buffer to remove unbound NRL antigens.**



### Step 4:

**Detect bound Hev b1 by adding secondary peroxidase-conjugated monoclonal anti-Hev b1 from kit.**



### Step 5:

**Detect and quantify the amount of bound peroxidase label with substrate solution from the kit.**

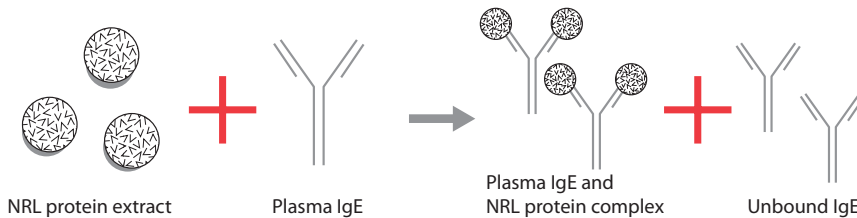
### Step 6:

**Repeat the assay on the same NRL extract with three other microwell plates that contain conjugated anti-Hev b3, b5, and b6.02.**

## Inhibition assays for measuring natural rubber latex antigens

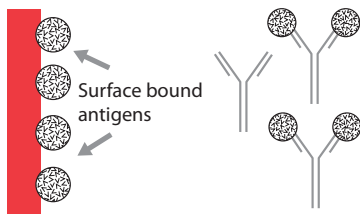
### Step 1:

**Combine NRL extract with plasma to form IgE-NRL protein complex.**



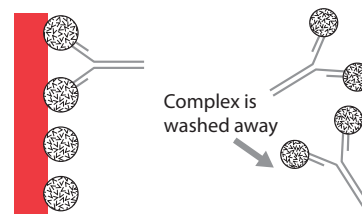
### Step 2:

**Expose combined IgE and NRL extract to NRL protein on surface.**



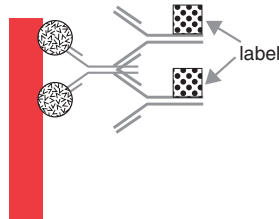
### Step 3:

**Incubate and wash away IgE that could not bind to the surface NRL protein.**



### 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 assay	Total protein	Chemical reacted with all proteins to produce a color change	Colorimetric chemical reaction	<ul style="list-style-type: none"> <li>• Commercially available reagents</li> <li>• Relatively easy to perform</li> <li>• Rapid results</li> </ul>	<ul style="list-style-type: none"> <li>• Not sensitive</li> <li>• Many chemical interferences skew results</li> <li>• Lacks specificity</li> </ul>	FDA permits label claim of $\leq 50\mu\text{g/g}$
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"> <li>• Specific to latex allergen</li> <li>• Serum from known Type I allergic subjects</li> <li>• Very sensitive</li> </ul>	<ul style="list-style-type: none"> <li>• Heterogeneity of serum pool</li> <li>• Relative scarcity of human sera</li> <li>• Potentially biohazardous human sera used</li> </ul>	Estimated at 5AU per mL
FITkit™ assay	Four specific latex allergens (Hev b1, b3, b5, b6.02)	Color change produced by enzyme-substrate interaction	ELISA	<ul style="list-style-type: none"> <li>• Commercially available reagents</li> <li>• Specific for major latex allergens</li> <li>• Standardized test</li> <li>• Very sensitive</li> <li>• Reproducible</li> </ul>	<ul style="list-style-type: none"> <li>• Does not identify all possible NRL allergens</li> <li>• Relatively expensive</li> </ul>	<ul style="list-style-type: none"> <li>• Hev b1, 10-1000<math>\mu\text{g/L}</math></li> <li>• Hev b3, 10-1000<math>\mu\text{g/L}</math></li> <li>• Hev b5, 5-100 nanograms/L</li> <li>• Hev b6.02, 5-200<math>\mu\text{g/L}</math></li> </ul>

Note: The amount of latex that was extracted per mL must be known in order to make comparisons; modified Lowry results are usually expressed as micrograms per square decimeter of latex ( $\mu\text{g}/\text{dm}^2$ ); AU (allergen units)/mL for RAST, and micrograms/gram for the FITkit™

### 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:

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

### Conclusion

Currently, the most widely used and accepted standardized assay for quantifying allergenic proteins is the RAST or ELISA competitive IgE assay. However, these assays may soon be replaced by more sensitive, specific and reproducible ELISA assays that utilize monoclonal antibody and recombinant allergen reagents to measure specific NRL allergens. The modified Lowry assay measures total proteins and is the most subject to chemical interferences and variations in methodology.

### 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 small percentage of glove wearers. In any case, people with IgE-mediated clinical reactivity should use synthetic gloves, no matter how low the protein or allergen levels.

## References and further reading

1. ASTM D5712 Standard Test Method for Analysis of Protein in Natural Rubber and Its Products. *Annual Book of ASTM Standards*. Volume 09.02. 2003.
2. Bollag, Daniel M., Rozycki, Michael D., Edelstein, Stuart J. *Protein Methods*, 2nd Edition, John Wiley and Sons Ltd., 1996.
3. ASTM D6499 Standard Test Method for Immunological Measurement of Antigenic Protein in Natural Rubber and Its Products. *Annual Book of ASTM Standards*. Volume 09.01. 2003.
4. Kenny, D.M., Challacombe, S.J. *ELISA and Other Solid Phase Immunoassays Theoretical and Practical Aspects*. John Wiley and Sons Ltd., 1988.

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