



## ELISA - Inhibition Testing of Latex Glove Protein Antigens

Residual glove protein levels can be measured by the Lowry and ELISA (Enzyme-Linked Immuno-Sorbent Assay) tests. Each of these test methods has different sensitivity and complexity, and measure either all proteins or only antigenic proteins. The ELISA-Inhibition test relies on the reaction between latex antigens and antibodies which are highly specific for natural rubber latex (NRL) proteins. The Lowry test depends on a reaction that is not as specific for latex proteins and requires a chemical reaction between apparent proteins and a chemical reagent.

### **Modified Lowry Latex Protein Test**

The Lowry test involves the reaction of latex proteins with an alkaline copper tartrate compound and the subsequent reaction of the protein-copper tartrate complex with Folin reagent, which results in a blue color detectable in a spectrophotometer <sup>1</sup>. In the ASTM modified Lowry test as it applies to the detection of latex proteins, these proteins are first precipitated in order to remove interfering, water-soluble substances, and the Lowry test is performed only after the protein precipitation and reconstitution step. The Lowry test method detects latex proteins in the microgram range. The limit of quantitation of this test was determined by ASTM to be above 70 µg/g. This test is subject to interference by chemical accelerators, such as carbamates, thiurams, benzothiazoles, and guanidines, used in the latex glove manufacturing process and phenolic chemicals naturally found in the latex itself <sup>1-3</sup>. Generally these substances increase color development resulting in an inflated or false positive protein signal. The Lowry test has been standardized as an ASTM test method D5712 for the analysis of protein in NRL and is recognized by the USA FDA for determination of protein levels in medical gloves. Manufacturers' can make protein level labeling claims of 50 µg/dm<sup>2</sup> glove surface or greater based on this test method.

### **LEAP Latex Protein Antigen Test**

LEAP, or Latex ELISA for Antigenic Proteins, measures latex proteins by using latex-specific antibodies of the rabbit to recognize them<sup>4-5</sup>. The proteins of glove extracts are diluted to various concentrations and are adsorbed to plastic wells in microtiter plates. Then a known quantity of anti-latex antiserum is added, and allowed to bind to the protein antigens. Once this antigen-antibody complex is formed, it is

reacted with a second antibody that recognizes the antigen-antibody complex. This second antibody carries a color-tag on it that turns color when activated. The color reaction product can then be detected and measured in a spectrophotometer - the more color, the more latex protein originally on the plate. This method can detect nanogram quantities of latex protein. However, drawbacks to LEAP include interference by surfactant chemicals in latex glove products, which tend to decrease the amount of glove protein binding and thus result in underdetection of protein antigens. Another drawback includes the lessened tendency of peptides or small pieces of protein to bind to the microtiter plate wells, so they are less likely to be available for detection. The detergent interference can usually be overcome by sufficient dilution of the sample protein to levels that still contain detectable protein, but do not contain enough detergent to interfere.

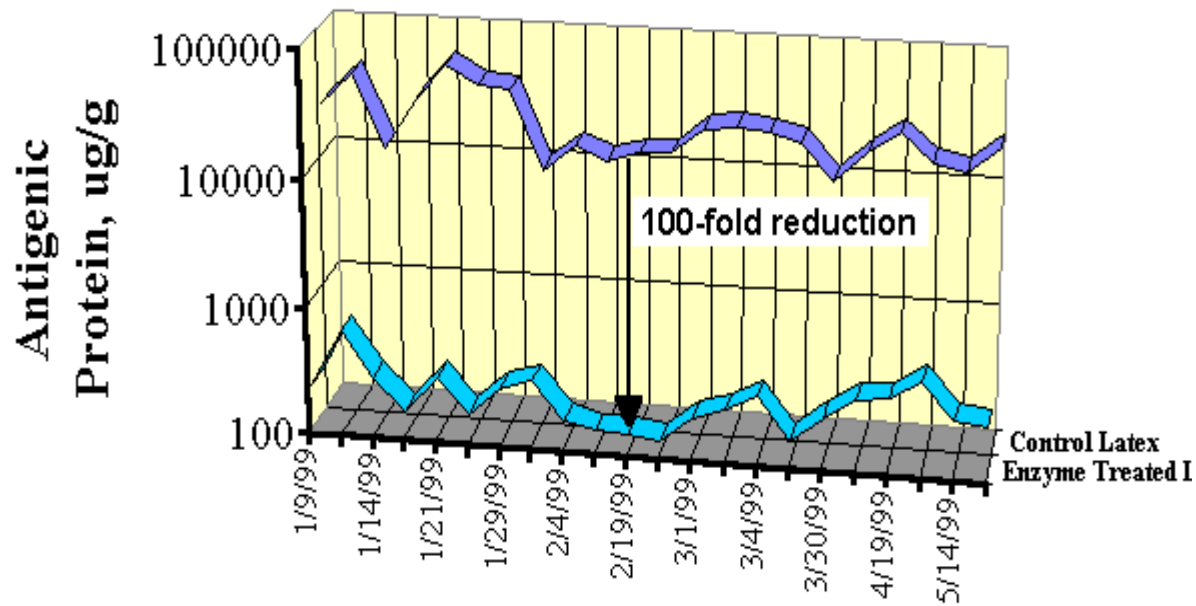
### **ELISA-Inhibition Latex Antigen Test**

The ELISA-Inhibition test for antigenic proteins, measures latex antigens by using latex-specific antibodies of the rabbit to recognize them <sup>6</sup>. ELISA-Inhibition is not the same as the LEAP test. In the ELISA-Inhibition assay, a known amount of standardized latex protein is bound or adsorbed to a solid support, e.g. plastic wells of a microtiter plate. This approach reduces the variability and surfactant interferences inherent in the LEAP assay because it relies on the binding of a standard preparation of NRL proteins to the plate and not an unknown glove extract. Then a known dilution of rabbit antiserum (latex-specific IgG antibody) is added to the plate, and mixed with one of a series of dilutions of the test glove extract. Higher levels of protein antigens in the test glove extract will inhibit the ability of the known quantity of IgG to bind with the known amount of standard latex protein on the plate. Thus the use of the term ELISA-Inhibition. The plate is then washed to remove the unbound glove extract and IgG. The amount of IgG that did bind to the microtiter plate is measured with a second anti-IgG antibody that recognizes it and produces a color. The color reaction product can then be detected and measured in a spectrophotometer: the lower the color (greater inhibition of binding), the higher the level of antigenic protein in the test glove extract that was available to prevent IgG's binding to the standard latex protein-coated plate. This method is sensitive and reproducible: nanogram quantities of latex antigens ( $> 1 \mu\text{g}/\text{dm}^2$ ) can be detected, in contrast to the microgram quantities detectable by the Lowry method.

The antigenic protein levels detected by the ELISA-Inhibition test depend on the standardization of latex protein concentrations bound to the microtiter plate, the reactivity of the anti-latex IgG rabbit antisera, and the quality of the assay

conditions. Since the immune response in rabbits can produce a fairly consistent reactivity to latex proteins, the rabbit antiserum used in the ELISA-Inhibition test is expected to be reliable in its ability to be blocked by the protein antigen in the test glove extract. The availability of standardized, uniformly reactive, pooled sera from a number of latex-immunized rabbits should allow latex antigenic protein test results to be more easily compared to one another. Currently, the ELISA-Inhibition test for latex antigenic proteins is standardized as an approved ASTM Standard Test Method. The ELISA-Inhibition test for latex antigenic protein levels in gloves should allow manufacturers to make lower protein labeling claims or claims specific to protein antigens in the future because of this tests increased specificity and sensitivity. The FDA currently does not allow manufacturers to claim protein levels below 50 micrograms per dm<sup>2</sup> of glove, due to the limit of detection of the Lowry method, but it may be possible in the future to label less than 50 micrograms of antigenic latex protein.

Allotex™ is a type of NRL that is treated with bacterial enzymes to digest the latex proteins into smaller and smaller potentially less antigenic pieces<sup>7</sup>. The Lowry test is not suited to evaluate the antigenic level of glove products made of Allotex™ since it cannot distinguish between total protein and antigenic protein, nor latex proteins of different size. The ELISA-Inhibition is a more specific and sensitive test of latex protein antigens than the Lowry test, and therefore well suited to test the antigenicity of enzyme-treated latex products. The figure below shows a 100-fold reduction the antigenic latex protein content of raw centrifuged NRL following enzyme treatment.



## Lot of Natural Rubber Latex

1. ASTM Designation D5712-99: Standard Test Method for Analysis of Protein in Natural Rubber and its Products, published September 1999.
2. Ji, TH; Interference by detergents, chelating agents, and buffers with the Lowry protein determination. Anal. Biochem. 1973;52:517-521.
3. Bensadoun, A. and Weinstein, D.; Assay of Proteins in the Presence of Interfering Materials. Anal. Biochem. 1976;70:241-250.
4. Beezhold, DH; LEAP: Latex ELISA for antigenic proteins-preliminary report. The Guthrie Journal 1992;61(2):77-81.
5. Beezhold, DH and GL Sussman; Determining the allergenic potential of latex gloves. Surgical Services Management Feb 1997;3(2):35-39.
6. ASTM: Standard Test Method for the Immunological Measurement of Antigenic Protein in Natural Rubber and Its Products, approved December 1999.
7. Perrella, F.W.; Enzyme-Treated Natural Rubber Latex: Concept to Product. Intl. Latex Conference, Akron, OH, July 1999.

**Note:** All standards referenced should be reviewed for the latest active revision level.