

Reduction of Antigenic Protein Levels in Latex Gloves after Gamma Irradiation

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Gamma irradiation is currently the method most commonly used to sterilize surgical gloves. In this study, the effect of gamma irradiation on antigenic proteins in latex gloves was examined. Protein extraction and quantitation were carried out using latex gloves before and after sterilization. Antigenic protein levels were determined by an ELISA assay specific for latex proteins (LEAP). LEAP analysis revealed a significant decrease

after gamma-irradiation sterilization. This observation may partially explain the lower levels of extractable antigenic proteins found in sterile surgical gloves compared with nonsterile examination gloves. However, gamma irradiation was less effective than autoclave sterilization in reducing protein levels. (BIOMEDICAL INSTRUMENTATION & TECHNOLOGY 1994;28:481-483)

Health care workers are at risk for developing allergic reactions to latex.¹ The majority of these reactions are delayed-type hypersensitivity reactions to chemical accelerators added to the natural rubber latex.² The most extreme cases of allergic reactions are IgE-mediated type I hypersensitivity reactions. Numerous reports have shown that residual proteins on latex medical devices cause these type I allergic reactions.³⁻⁶ Manufacturers are examining ways to reduce the levels of extractable proteins found on latex products. Evidence suggests that products with lower protein levels produce fewer allergic reactions.

One factor that has hampered efforts to reduce protein levels has been the absence of a reliable method by which to quantitate latex proteins. The colorimetric protein assays (e.g., Lowry and Bradford) are unreliable because chemical accelerators found in latex cause interferences in these assays.⁷ We have developed an ELISA method to measure residual antigenic protein levels in latex products.⁸ This assay presents fewer problems with interfering substances and is extremely sensitive. In this study we used the assay to determine the effect of sterilization on residual antigenic protein levels in latex gloves.

MATERIALS AND METHODS

The latex glove samples were weighed and cut into small pieces. Extraction of proteins was carried out in phosphate-buffered saline (PBS), pH 7.2, for 2 hours

at 37°C. Antigenic protein levels in the extracts were quantitated using the latex ELISA for antigenic proteins (LEAP) assay.⁸ In brief, the LEAP assay is carried out by adsorbing latex proteins onto 96-well polystyrene ELISA plates in 0.1 M carbonate buffer, pH 9.6, for four hours at 37°C. A purified preparation of latex protein is serially diluted on each plate to produce a standard curve (2 to 0.03 µg/ml). The standard is twofold serially diluted in duplicate. Similarly, samples containing unknown concentrations of latex proteins were also serially diluted. After the first incubation, the plates were washed once with PBS containing 0.1% Tween 20 (TPBS), pH 7.2, and unreacted sites were blocked with 10% bovine serum albumin (BSA) in TPBS for one hour. The plates were then washed once with TPBS, then incubated with 100 µl of rabbit anti-latex protein antiserum (1/5,000 dilution) overnight at 37°C. Following three washes with TPBS, the plates were reacted with 100 µl of peroxidase-labeled anti-rabbit IgG (1/1,000 dilution) for one hour. After washing five times with TPBS, a colored reaction product was produced using o-phenylenediamine (OPD, 1 mg/ml) in H₂O containing 0.1% H₂O₂. Finally, the reaction was stopped by the addition of 50 µl of 4 N sulfuric acid. The orange-colored reaction product was quantitated by reading absorbency at 492 nm using an ELISA plate reader. The concentrations of latex antigenic protein in unknown samples were determined by comparison with the standard latex protein.

RESULTS

Numerous surgical and examination gloves have been tested for extractable antigenic protein using the LEAP assay. In general, the data indicate that surgical gloves have lower levels of residual protein than do examina-

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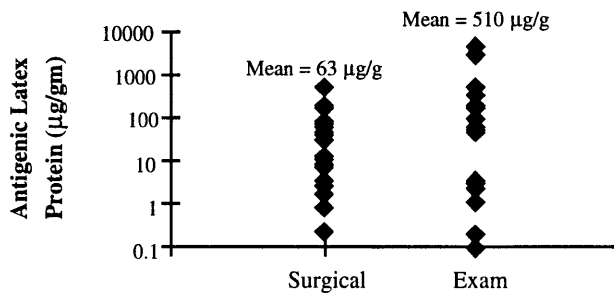


Figure 1. Antigenic latex protein levels ($\mu\text{g/g}$) of randomly chosen surgical and examination gloves.

tion gloves (Figure 1). One possible explanation for this discrepancy may be differences in the sterilization treatments these types of gloves are subjected to. To test this hypothesis, seven glove types (a single lot was used for each type) were sampled before and after sterilization (25–50 KGy). One pair of gloves at each test point was analyzed for extractable latex protein using the LEAP assay (Figure 2). The resulting data (Table 1) demonstrate a decrease in extractable protein levels after gamma-ray sterilization. Statistical analysis (paired t-test) of protein levels before and after gamma-ray sterilization indicated a significant decrease following sterilization ($p < 0.01$). One additional glove type (H) was tested before and after sterilization by autoclaving (Table 1). This treatment produced the greatest decrease in residual latex protein levels. The reductions in antigenic protein levels by sterilization for all gloves tested ranged from 17% to 90%. Interestingly, the reduction by sterilization was independent of the starting protein level, as indicated by the lack of correlation between protein levels in nonsterile gloves and percentage reductions (Figure 3). Possibly this lack of correlation was due to

gamma-ray dosage variations from lot to lot.

As shown in Table 1, certain glove types demonstrated considerable variability between gloves within the sampled pairs. This raised the possibility that the observed reduction could have been the result of glove-to-glove variability. To address this possibility, two films were cast from a single sample of liquid text. One film was further processed by gamma-ray sterilization before testing the films for extractable protein. Again, a 75% reduction due to sterilization was observed (Table 2).

DISCUSSION

Residual protein levels in latex medical gloves are known to cause type I allergic reactions.¹ Research is being conducted to elucidate the latex protein allergens and the parameters that are important in their removal from latex medical devices.⁹ Important issues include identifying the mechanisms by which latex exposure occurs, identifying the relevant protein allergens, and

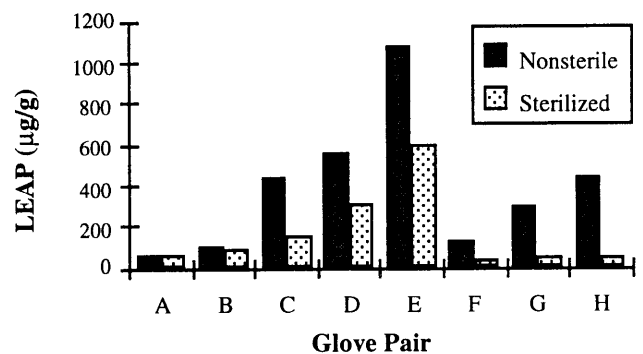


Figure 2. Mean antigenic protein values before and after sterilization for each glove pair ($\mu\text{g/g}$) by LEAP assay.

Table 1. Residual Protein Levels ($\mu\text{g/g}$) Determined by LEAP Assay for Eight Types of Gloves Sampled before and after Sterilization*

	LEAP (μg/g)						% Reduction
	Nonsterile		Sterilized		Average		
	Right	Left	Right	Left	Nonsterile	Sterilized	
Gamma-ray sterilization							
Glove type A	23.2	112	39.1	73.1	67.6	56.1	17
Glove type B	30.9	172	70.5	96.9	101	83.7	17
Glove type C	397	484	271	41	441	156	65
Glove type D	596	515	326	298	556	312	44
Glove type E	1,170	976	483	719	1,080	601	44
Glove type F	45.8	222	27.3	33.2	134	30.3	77
Glove type G	322	278	60.3	38.5	300	49.4	84
Autoclave sterilization							
Glove type H	89.6	804	30.8	56.6	447	43.7	90

* Paired t-test using the differences between nonsterile and gamma-ray-sterilized gloves yields significance at $p < 0.01$.

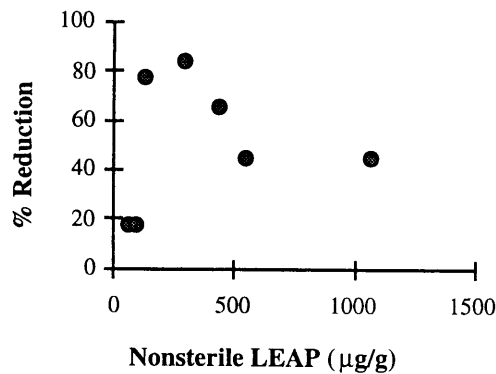


Figure 3. Calculated percentage reductions in antigenic protein levels by gamma-ray sterilization of latex gloves plotted against nonsterile values.

Table 2. LEAP Values of Nonsterile and Sterilized Latex Film

LEAP (µg/g)		% Reduction
Nonsterile	Gamma-ray-sterilized	
5,580	1,370	75

removing the offending latex allergens.¹⁰ It is generally agreed that latex protein exposure occurs by absorption through the skin, by mucous membrane contact, and by inhalation of latex protein-laden glove powders.^{1, 11, 12}

In this study, we used an ELISA technique to examine manufacturing parameters that influence extractable protein levels. We observed that sterile surgical gloves had lower levels of extractable proteins than did exami-

nation gloves. Several differences between the manufacturing processes for examination gloves and surgical gloves may account for the lower levels of latex protein in surgical gloves. It is known that leaching reduces extractable proteins,⁹ and surgical gloves undergo additional processing steps. Additionally, surgical gloves are sterilized, and the sterilization process may influence protein levels. In this study, we demonstrated a dramatic decrease (50%) in extractable antigenic latex proteins following gamma sterilization. It is not clear, however, why gamma irradiation reduces the protein levels. It is speculated that cross linking of the proteins by gamma rays may render them insoluble and less easily extracted. Alternatively, gamma rays may break peptide bonds, reducing the lengths of the peptide chains and making the smaller peptides difficult to detect. However, this is unlikely since the LEAP assay detects peptides that are 1,000 KD or less in size.

Autoclave sterilization was found to be very effective in reducing antigenic protein levels in the one sample so tested. Interestingly, the prevalent method of sterilization was changed from autoclaving to gamma irradiation during the 1960s. This change has been implicated in the adverse reactions to glove starch powders (starch granuloma).¹³ It has been shown that autoclave sterilization reduces skin-prick test positivity in comparison with gamma sterilization.¹⁴ Our limited data suggest that autoclave sterilization may be more efficient in the reduction of antigenic latex proteins and thus may produce a safer product.

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