Vytex™ Natural Rubber Latex: An Innovative Source Material for Natural Rubber Products

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BIOGRAPHICAL NOTE

Mark C. Swanson is an immunochemist of 30 years working for the Mayo Foundation, Rochester, MN. He graduated in 1978 from St. Cloud State University, St. Cloud, MN with degrees in bio-medical science and chemistry. He founded Quan-Tec-Air, Inc. in 1985. The company is dedicated to the quantification of asthmagenic bio-aerosols using specialized sampling, filtration and immunoassay techniques. The combination of air sampling expertise and de novo immunoassay design and implementation makes him a unique and valuable resource for immuno-aero-biological health hazard assessment. He is contacted frequently by industry and agencies interested in evaluating workplace bio-aerosols and their remedies.

ABSTRACT

Vytex™ natural rubber latex (NRL) is a new product designed to offer a standardized source material for the production of natural rubber products using chemically modified NRL to significantly reduce its antigenic protein content. Vytex NRL is a commercially available natural latex source that is significantly reduced in antigenic proteins and can be used in a wide array of commercial applications.

The multi patent protected method of protein removal is achieved by the introduction of aluminum hydroxide ($Al(OH)_3$) to *Hevea brasiliensis* natural rubber latex. Well known for its protein binding characteristic, treatment with $Al(OH)_3$ results in Vytex NRL, a low antigenic protein product considered desirable for manufacture of items containing NRL. Vytex NRL is easily integrated into existing manufacturing environments and processes without an additional capital equipment investment. It retains and improves upon the chemical and physical properties and provides excellent performance characteristics when compared to untreated NRL or synthetic products.

Vytex NRL is only slightly more expensive than traditional untreated NRL sources as a raw material and is competitive with, and often less expensive than, many “natural latex-free” or synthetic alternative materials and can reduce manufacturing costs for items containing NRL. Vytex NRL, like *Hevea* natural rubber latex, is naturally “green”, a renewable resource with no known human carcinogens in its natural state, unlike several of today’s synthetic alternatives.

INTRODUCTION

Hevea brasiliensis natural rubber latex as a protective material has a long history dating back to the 1800s. *Hevea* is mostly grown in Malaysia and Thailand as the crops flourishes in regions within 5 to 10 degrees of the equator and at moderate elevations[1]. Field latex, the feedstock material for Vytex NRL, is now available in several new regions around the world including India, Vietnam and Central America.

The widespread use of barrier NRL articles like gloves and condoms increased tremendously in the 1980s primarily due to the “universal precautions” policy outlined by the Centers for Disease Control and Prevention (CDC) and the Occupational Safety and Health Administration’s (OSHA) “Bloodborne Pathogens” regulation published in 1991. Natural rubber latex’s popularity and longevity can be attributed to several factors; excellent barrier properties, tensile strength, comfort, availability and positive environmental impact.

The physical properties of NRL are superior to non-latex synthetic products. ASTM requirements were modified to allow the use of non-latex synthetic materials in commercial applications. Natural rubber latex is significantly less expensive than most petroleum-based synthetic materials where the cost continues to rise and fluctuate with the rising cost of crude oil. The availability, ease of production, and the performance of NRL products continue to make natural latex the chosen material by manufacturers and users among industry and

medical professionals. Today, there are over 40,000 commercially available products made with natural rubber latex.[2]

Although the first report of an allergic reaction to latex gloves appeared in the American literature in 1933 when usage was modest,[3] the majority of latex allergic reactions were documented in the late 80s and throughout the 90s and 2000s. Published data indicate 17% of American healthcare workers and up to 73% or more of frequently exposed patients, such as those with spina bifida, were sensitized to latex proteins.[4,5]

A concern with NRL continues to be its potential implication with adverse health effects due to the antigenicity of NRL products. An instinctive means of controlling this parameter in the NRL source material is by de-proteination / protein modification. Validation of this process can be achieved by specific ASTM methods of protein quantification.

Several attempts, including new source crops, synthetic lattices and various treatment methods have been made to eliminate these problem proteins from *Hevea* NRL by biological, physical and/or chemical methods that act on proteins. One approach has been to introduce the latex articles to multiple leaching steps and chlorination. This approach does reduce the protein levels in the finished product; however it weakens the latex film thus compromising the desirable physical properties of the product.[6]

Another attempt to reduce proteins in NRL is the use of proteolytic enzymes to degrade the proteins in the latex solution. The issue with this approach is the introduction of another protein (the enzyme) to the latex, which may itself be allergenic.[6] The most logical solution is the use of low-protein latex, such as Vytex NRL since this application will more likely reduce the possibility for an allergic response in the end user of the finished product.[7] It can be theorized that if high levels of protein are not present in the raw material they cannot appear in the manufactured product.[8]

Two other non-*Hevea* NRL materials have been attempted to be commercialized in the United States; guayule rubber latex and Taraxacum kok-saghyz, also known as the Russian dandelion. These materials are reported to be higher in cost compared to natural rubber latex and presently are available only in limited quantities. *Hevea* NRL has been around for more than a century and its antigenic proteins have been thoroughly researched. The allergenic properties and economic viability of latex from the guayule & Taraxacum kok-saghyz have yet to be scrutinized. Both of these materials have their own unique set of proteins with potential allergenic behavior not yet understood.

Recently it has been shown that allergens are distributed into few protein families and posses a restricted number of biochemical functions. The allergen functions found most frequently were limited to hydrolysis of proteins, polysaccharides and lipids; binding of metal ions and lipids; storage; and cytoskeleton association. The limited number of protein families that are allergenic and the narrow functional distribution of most allergens confirm the existence of yet unknown factors that render proteins allergenic.[9] Many of these specific protein functions play into the chemistry used to manipulate them yielding a modified NRL source material.

VYTEX NATURAL RUBBER LATEX- A MODIFIED AND DEFINED NRL SOURCE

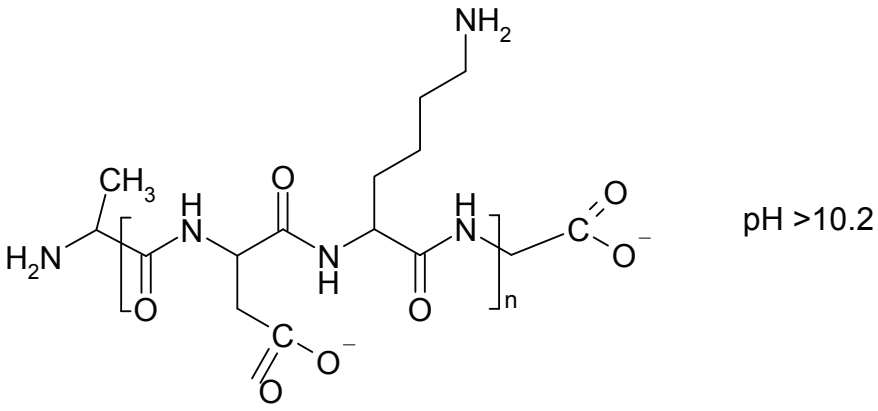
Vytex NRL is the result of rigorous research and development which began seven years ago and has resulted in two patents, with additional patent filings submitted and pending. Vytex NRL was first introduced at the International Latex Conference (ILC) in July 2005 (Charlotte, NC), followed by presentations at Rapra's Latex and Synthetic Polymer Dispersions Conference, Latex 2006 in January (Frankfurt, Germany), The Malaysian Rubber Glove Manufacturers' Association (MARGMA) in September 2006 (Kuala Lumpur, Malaysia), and at Smithers Rapra's Latex and Synthetic Polymer Dispersions Conference, Latex 2008 in January (Madrid, Spain). Additionally, Vytex NRL was featured in the Technology Trends section for Protein Issues as part of Revertex (Malaysia) Sdn Bhd presentation at ILC in August 2007 (Las Vegas, NV).

The production of Vytex NRL relies on the multi patent protected process involving the effective exchange/complexing of proteins from the field latex sap emulsion to/with $Al(OH)_3$. $Al(OH)_3$ is the most stable form of aluminum under normal conditions. Reduced forms of this metal ion will exist in charged states bonding with proteins of complementary charge which are driven toward ionic equilibria or an isoionic point. $Al(OH)_3$ is an amphoteric substance, meaning it can react as either an acid or base and readily shares electrons with proteins. Amino acids, the blocks that build the proteins are both very weak acids and very

weak bases creating the basis for both covalent and ionic bonding to $Al(OH)_3$. It is commonly used as an absorbent, emulsifier, ion exchanger or antacid.[10] Aluminum hydroxide is also used in the purification of water because it can form a jelly-like structure suspending any unwanted materials in water including bacteria.[11]

To fully describe Vytex NRL and the process employed to create Vytex NRL, it is important to examine the feedstock material, *Hevea brasiliensis*, and its fickle behavior due to its natural state. *Hevea* NRL is collected from numerous rubber plantations each with their own growing and collection characteristics which yield variable protein concentrations. Causes for the varying starting protein levels include age of the trees, seasonal differences, and the presence and levels of electrolytes including free magnesium. The structure of protein is illustrated below in Figure 1:

Figure 1



The literature clearly describes the natural rubber latex proteins' affinity for USP absorbable dusting powder. Several papers have been presented on this subject confirming this interaction. The United States Food and Drug Administration (FDA) recognized this association and recommend a limit on powder and extractable protein per glove. Allergen-laden glove powder is implicated as a major contributor to the widespread sensitization to NRL and associated workplace symptoms wherein the powder acts as a vector to spread the proteins.[12] It is this powder and protein relationship that led to trials of the inclusion of insoluble aluminum hydroxide $Al(OH)_3$ powder in the formula for Vytex NRL production. It was hypothesized by the Vystar technical team and later confirmed that under certain conditions, $Al(OH)_3$ produces protein complexes in liquid Vytex NRL solution which can be removed using existing industry practices. The removal of protein at this stage is significant as it greatly reduces the proteins availability to transfer from the oleo phase to the aqueous and back since the protein is attached to insoluble $Al(OH)_3$ as noted in Table 1. To confirm this, film samples made with Vytex NRL and *Hevea* NRL were analyzed for protein initially after processing, after a 21-day maturation period, and after 6 months to determine their protein content upon aging.

Table 1: ELISA protein results upon aging for Vytex NRL versus *Hevea* NRL

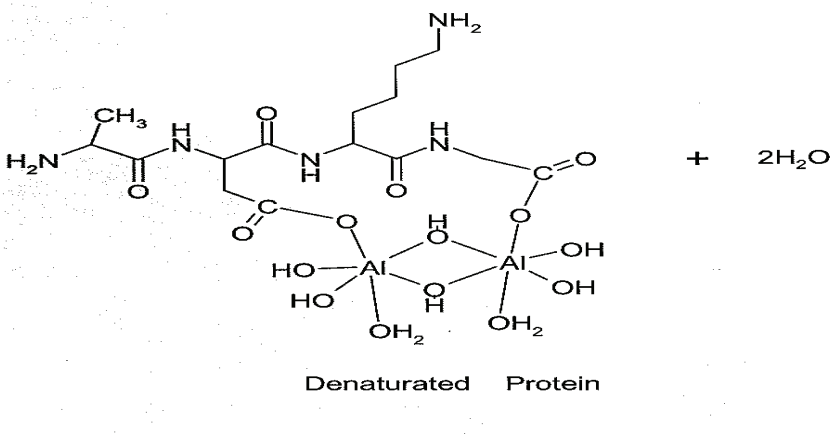
Sample ID	***ELISA ASTM D-6499-07 ug/g***	***% Reduction compared to control sample***
Vytex NRL fresh sample	2.3	28%
Vytex NRL 21-day aging	4.9	89%
Vytex NRL 6-month aging	6.4	89%
Hevea Control fresh sample	3.2	n/a
Hevea Control 21-day aging	56.3	n/a
Hevea Control 6-month aging	58.7	n/a

*All AP values given are an average of 4 "air dried" cast films.

After Vytex NRL is manufactured at the latex processor, a residual amount of $Al(OH)_3$ remains suspended in the liquid latex solution, which plays an important role in achieving low protein products especially in dipping

applications. The residual $Al(OH)_3$ is significant because inevitably there will be some protein that is covalently bonded to the rubber particle, but in the continued presence of an alkali pH (especially when compounded) the backbone of the proteins will break and the protein/peptides will go into the aqueous phase. Table 1 confirms this as a very noticeable increase is observed in the antigenic protein level of the *Hevea* control sample. The presence of $Al(OH)_3$ will complex these proteins creating an insoluble precipitate known as an organic lake. As water is removed during latex article production, an aluminum salt of the protein and $Al(OH)_3$ ions are formed displacing water as a byproduct. Once the salt is formed, the protein has lost its reaction sites.

Figure 2



The protein remains present but as part of a new molecule that is neutral and non-reactive. The digestion of the remaining protein is thermodynamically driven and well suited to achieve low protein status in typical dipping applications such as gloves and condoms. Additionally, it appears that the newly formed digested precipitate is easily washed and available for extraction using existing industry practices. To confirm this theory, we have analyzed gloves made with Vytex NRL at various stages of production and have compared the results against untreated control *Hevea* NRL glove samples.

Table 2: Protein results at various stages of production: Surgical gloves made with Vytex NRL compared to surgical gloves made with *Hevea* NRL

Sample ID	***Manufacturing Step***	***ASTM D6499-03 ug/g***	***ASTM D5712-05 ug/g***
Vytex NRL	Unleached	8.7	78
Vytex NRL	Wet-leached-1 minute @ 55°C	9.2	68
Vytex NRL	Post-leached	3.4	<42
Hevea Control	Unleached	65.7	770
Hevea Control	Wet-leached-1 minute @ 55°C	140.6	645
Hevea Control	Post-leached	103.2	795

Surgical gloves listed in Table 2 were coagulant dipped at Revertex (Malaysia) Sdn Bhd Technical Center.

Additionally, surgical gloves were made with Vytex NRL at a large commercial glove manufacturer of dipped products and were tested for protein at various stages of production. Protein results from this trial demonstrated consistent behavior to previous protein test results with surgical gloves produced with Vytex NRL achieving low levels of protein compared to the *Hevea* NRL control surgical glove samples.

Protein analyses at various stages of surgical glove production were performed to determine the protein content of gloves made with Vytex NRL in a typical glove manufacturing environment. The goal was to demonstrate that surgical gloves made with Vytex NRL would achieve low protein status (<10 ug/g of AP &

<100 ug/g of total protein) after the wet gel leaching stage. Achieving low protein status after wet gel leaching would offer glove manufacturers the potential for eliminating dry leaching and off-line post leaching for the removal of latex proteins. Eliminating these leaching processes positively impacts manufacturing costs by significantly reducing water usage, energy costs and extra material handling.

Table 3: Protein results at various stages of production

Sample ID	***Manufacturing Step***	***ASTM D6499-03 ug/g***	***ASTM D5712-05 ug/g***
Vytex NRL	Incoming (raw) latex	b.d (Below Detection)	14
Vytex NRL	After compounding	4.5	600
Vytex NRL	Post latex dipping	b.d (Below Detection)	82
Vytex NRL	Off-line processing	b.d (Below Detection)	0
Hevea Control	Incoming (raw) latex	b.d (Below Detection)	28
Hevea Control	After compounding	16.2	600
Hevea Control	Post latex dipping	19.3	396
Hevea Control	Off-line processing	b.d (Below Detection)	5

Wet-gel leaching is used primarily for the removal of excess calcium nitrate, water soluble non-rubbers such as proteins and added compounding ingredients.[13] The literature describes protein removal by wet-gel leaching to be ineffective because much of these proteins had not yet migrated to the surface when the film was heated briefly to attain the wet-gel state.[14] Since a significant amount of proteins are removed during Vytex NRL creation, the expected protein increases at various stages of glove production (as seen in the control samples in Tables 2 and 3) do not exist thus allowing manufacturers using Vytex NRL opportunities to reduce manufacturing steps.

HEVEA VS VYTEX NRL PROTEIN DETERMINATION

Vytex NRL is the only chemically modified and commercially available NRL source material and, therefore, requires close attention to detail when describing its low protein properties. Since a residual amount of modified protein remains present in Vytex NRL, it is important to precisely describe the modified nature of these proteins (peptides). Vytex NRL has been tested throughout its development primarily using the ELISA test method (ASTM D6499-03) and the Modified Lowry test method (ASTM D5712-05) to a lesser degree. All protein testing has been performed by the LEAP Testing Service, Donald Guthrie Foundation for Education Research in Sayre, PA. These test methods have unique sensitivities and specificity and measure total protein and antigenic protein, respectively.[15]

The Modified 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, resulting in a blue color read using a spectrophotometer at 700 nm.[16] The Modified Lowry test is subject to interference by chemical accelerators, such as carbamates, thiurams, benzothiazoles and guanidines, used in the production of latex gloves and phenolic chemicals naturally found in latex.[15,17,18] The Modified Lowry test has been standardized as an ASTM test method D5712-05 for the analysis of protein in NRL and is recognized by the FDA for determination of protein levels in medical gloves.

The ELISA inhibition test measures NRL antigens by using latex-specific antibodies collected from hyperimmunized rabbits.[19] This immunochemical method is much more sensitive and reproducible than the Modified Lowry test and does not suffer from the limitations of the Modified Lowry test. The FDA does not allow protein level claims below 50 micrograms per dm^2 of glove. This value has no established biological relevance but is used because of the reportable limit of detection of the Modified Lowry method.

The ELISA test is designed and performed to quantify native NRL proteins in an ammoniated state. Vytex NRL film extracts consistently yield low total protein and antigenic protein content using the ASTM methods: however reproducibility issues and divergent values were commonly observed due to protein modification. The Modified Lowry does not have the required sensitivity, and the ELISA is prone to variable protein

hydrolysis occurring during Vytex NRL formulation which causes anomalies in antigenic protein detection. It is worth noting that donning powder can create false positives and interfere with enzyme assays (ELISA) to some degree in research laboratories.[20]

While the ELISA test method demonstrates significant reduction in protein in products made with Vytex NRL, it should be noted that this method detects antigenic proteins and should not be considered as a measure of allergenic proteins or total protein per Modified Lowry. Correlation of protein/antigen levels with the level of allergenic proteins has not been fully established[21] and, therefore, in an effort to further describe Vytex NRL throughout its formulary process, we subjected several Vytex NRL unleached film extracts to direct spectrophotometric testing at 280nm. At this wavelength, absorbed proteins can be read directly from the extract. This assessment of protein has the ability to measure all proteins including any morphed proteins no longer immuno-reactive (recognized by the ELISA antibodies). The utility of this method will rely on acceptable sensitivities and freedom from interferences. Preliminary 280nm data are consistent with a downward trend in measured protein content demonstrating reasonable reproducibility.

Table 4: Spectrophotometric testing of 280nm and antigenic protein in Vytex NRL films versus *Hevea* NRL films

Sample ID	***Vytex NRL (aged for 1 month)***	***Control***
ELISA ASTM D6499-03 (ug/g)	6.3	56.3
% Decrease vs. Control	89%	n/a
O.D 280 nm (ug/g)	0.18	2.01
% Decrease vs. Control	91%	n/a

(Results provided are an average of four test film samples)

MATERIALS AND METHODS

The production of Vytex NRL is created at the latex processor which takes place primarily in Southeast Asia and the surrounding regions. This production process was easily integrated without the need for any special equipment or additional capital investment. Vystar Corporation has signed a toll manufacturing agreement with Revertex (Malaysia) Sdn Bhd and is producing Vytex NRL commercially.

During product development, several products were made with Vytex NRL and tested for performance and protein behavior. These products include examination and surgical gloves, condoms, cold seal and pressure sensitive adhesives, breather bags, latex tubing, probe covers and foam. While Vytex NRL is undergoing evaluation trials at several leading manufacturers across a wide scope of industries, it is important to examine the product development efforts that have led to the commercial introduction of Vytex NRL.

Several metric ton trials of Vytex NRL were prepared at Revertex (Malaysia) Sdn Bhd using a scale-up process ready for commercialization and distributed to select manufacturers across an array of industries. It is important to note that during product development, Vytex NRL was prepared on a commercial scale and was scrutinized against a double centrifuged *Hevea* NRL sample designated as “Control”. The double centrifuge process is a popular approach taken by suppliers of latex rubber seeking to separate latex protein from latex rubber. This method is reported to reduce proteins levels by 50% compared to single centrifuged latex.[5] The double centrifuge process yields a highly purified *Hevea* latex concentrate prepared by re-centrifuging the first centrifuged latex which has been suitably diluted. Films prepared from double centrifuged latex typically exhibit excellent clarity, low water absorption, and high dielectric properties.[22] The objective of this work is to examine and compare various types of products made with low protein Vytex NRL to products made with twice centrifuged “purified” *Hevea* under the same processing conditions.

The colloidal properties were tested on the freshly prepared Vytex NRL and the control *Hevea* NRL and then again after the lattices had matured for 21 days. The results for Vytex NRL and the control are listed in Table 5.

Table 5: Colloidal properties on fresh and aged Vytex NRL and *Hevea* NRL

Test parameter		*TSC (%)*	*NH_3 content (%)*	*VFA no.*	*Free Mg^{2+} (ppm)*	*Viscosity, cPs (sp 2/60)*	*MST (sec)*	*pH*	*Coagulum (mesh # 80/325) ppm*
Initial Field Latex		37.57	0.36	0.021	145	n/a	n/a	10.47	n/a
Vytex NRL (Commercial Scale) (fresh)	Vytex B	61.48	0.71	0.018	nil	58	480	11.05	20 / 45
	Control	61.52	0.77	0.015	nil	87.5	300	11.01	8 / 31
Vytex NRL (Commercial Scale) (after 21 days maturation)	Vytex B	61.48	0.78	0.016	nil	66	2,627	11.4	12 / 91
	Control	61.52	0.75	0.015	nil	117.5	840	10.98	12 / 19

The colloidal property results from the Vytex NRL pilot trial were deemed acceptable by industry standards, and therefore the lattices were compounded by standard additives, which are commonly used to produce surgical gloves.

SURGICAL GLOVES MADE WITH VYTEX NRL

Surgical gloves were coagulant dipped at Revertex (Malaysia) Sdn Bhd Technical Center and were evaluated in Table 6 for physical property measurements according to ASTM standards.

Table 6: Physical properties of surgical gloves made with Vytex NRL and *Hevea* NRL

Test Parameter		*Vytex NRL*	*Control*
Unaged	Modulus @ 700% (Mpa)	11.8	15.6
	Tensile Strength (Mpa)	26.9	29.1
	Elongation @ Break (%)	878	845
Aged (100°C @ 22 hrs)	Modulus @ 700%	6.5	9.5
	Tensile Strength (Mpa)	25	24.2
	Elongation @ Break (%)	941	904

The physical properties of surgical gloves made with Vytex NRL were within industry specifications. Vystar believes the reason why surgical gloves made with Vytex NRL demonstrate good stability upon aging is that the $Al(OH)_3$ removes and deactivates those molecular species vulnerable to free radical breakdown. Protein results previously listed in Table 2 appear to support this theory.

CONDOMS MADE WITH VYTEX NRL

Hevea NRL condoms are produced in very large numbers on highly automated production lines. This industry relies almost exclusively on *Hevea* natural rubber latex due to its ability to form smooth, continuous films on drying and exhibits high strength and elasticity when vulcanized and leached. A straight dipped production process is typically used for the production of condoms where a suitably shaped former is immersed in a latex mix and withdrawn, usually twice dipped, to produce a uniform layer of latex on the former. The latex deposit is dried and vulcanized before removal from the former.[23]

A suitable mixture of compounding ingredients was used for all samples to ensure colloidal stability of the latex during processing. These properties are expressed in Table 7:

Table 7: Influence of compounding ingredients on Vytex NRL and *Hevea* NRL

Test Parameter	*Control Uncompounded*	*Control Compounded*	*Vytex NRL Uncompounded*	*Vytex NRL Compounded*
pH	10.82	11.24	11.22	10.77
Viscosity (cp)	69	94	68.5	20
MST (secs)	553	128	1,412	129
TS (%)	61.5	49.4	59.8	50.4
ZOV@60min. (cp)	268	NA	56	NA

The production trial of condoms made with Vytex NRL was conducted at a large commercial manufacturer of condoms in the United States with annual capacity exceeding one billion condoms. To prepare for dipping, the latex compounds were adjusted to 50% total solids. Using a clean straight wall condom former for two straight dips for each sample, the former was dipped into the respective latex compound and dried for five minutes @125°C. Next, the former was cooled at room temperature for two minutes then dipped again into the latex compound. This dip was followed by a drying time of two minutes @125°C then the bead was rolled. After drying, the new created condom was cured for 25 minutes @125°C. The leaching was performed in hot water (93°C) for one minute. After leaching, the condoms were dried for three minutes @125°C, and then cooled at room temperature for a brief period. Next, the condoms were stripped from the former using dry starch powder. Finally, the condoms were tested for physical performance Table 8 and were packaged separately and sent to LEAP Testing Services, for ELISA and Modified Lowry protein testing with the results expressed in Table 9.

Table 8: Physical properties of condoms made with Vytex NRL and *Hevea* NRL

Test Parameter		*Vytex NRL*	*Control*
Unaged	Modulus @ 500% (Mpa)	1.4	1.5
	Tensile Strength (Mpa)	17.5	16.2
	Elongation @ Break (%)	864	846
Aged (100°C @ 22 hrs)	Modulus @ 500%	1.7	1.8
	Tensile Strength (Mpa)	18.9	13.5
	Elongation @ Break (%)	864	758

Table 8 illustrates that condoms made with Vytex NRL were 30% stronger than condoms made from *Hevea* NRL when aged and demonstrated much better resistance to aging compared to *Hevea* NRL condoms.

Table 9: ELISA and Lowry test results for condoms made with Vytex NRL versus *Hevea* NRL

Sample ID	*ASTM D6499-03 ELISA (ug/g)*	*ASTM D5712-05 Lowry (ug/g)*
Vytex NRL	<0.2	<47
Control	6.1	60

ADHESIVES MADE WITH VYTEX NRL

Natural rubber latex was the first polymer to be used to produce pressure sensitive adhesives (PSA). Natural rubber latex has inherent advantages when used in pressure sensitive and contact adhesive formulations. Natural rubber latex has a very low glass transition temperature (Tg) and also low surface energy which enables it to flow over surfaces very effectively, a key attribute of PSA. Furthermore, NRL's extremely high molecular weight gives it high internal strength preventing it from splitting during removal. The high molecular weight of NRL makes it the only material that can function as a "cold seal" contact adhesive at room

temperature. This is made possible because the low Tg and surface energy allow rubber films to flow cold very well while the mixing of the polymer chains is retarded by molecular weight.

Some disadvantages of NRL are its ability to oxidize and become embrittled, losing its tack and adhesion properties over time and its ability to "sensitize" skin as a result of allergic reactions. Oxidation is dealt with through use of anti-oxidants. Sensitization can be overcome with the use of a beginning low protein NRL such as Vytex NRL, as it is understood that if high levels of protein are not present in the raw material they cannot appear in the manufactured product.[8]

The objective of this phase of testing was to formulate cold seal (contact) and pressure sensitive adhesives using Vytex NRL and a *Hevea* natural rubber latex sample formulated for an adhesive compound and to conduct appropriate comparative testing for each sample. Testing was performed at National Polymer Laboratory, Akron, Ohio, a custom manufacturing company focusing on polymeric materials in the fields of adhesives, coatings and nanocomposites. Test results are expressed in Table 10.

During the formulary process, one particular observation regarding Vytex NRL and the *Hevea* control NRL samples was the smoothness of the Vytex NRL sample, which is attributed to improved stability. This can eliminate the need for filtering during compounding and assist in the adhesive coating process downstream.

Table 10: Physical properties of cold seal and pressure sensitive adhesives made with Vytex NRL and *Hevea* NRL

Test Parameter	***Cold Seal Control***	***Cold Seal Vytex NRL***	***PSA Control***	***PSA Vytex NRL***
Brookfield Viscosity centipoise (cps)	1,200	1,400	2,600	2,400
Coat Weight (grams per $in.^2$)	24.8	35.0	38.0	25.9
Thickness (mils)	1.0	1.4	1.5	1.0
pH	10	10	9	9
T-Peel ASTM D-1876 (Avg) units: (# / lineal in.)	1.0	0.9	n/a	n/a
(Standard Deviation) (σ) 1 min. Substrate: Face to Face*	0.4	0.2	n/a	n/a
Peel Adhesion ASTM D-3330 (Average) units: (# / lineal in.)	n/a	n/a	3.3	4.0
(Standard Deviation) (σ) 180^o Peel 1 min. Substrate: Stainless Steel	n/a	n/a	0.3	0.3
Loop Tack ASTM D-6195 (Average) units: (# / in^2.)	2.7	3.3	37.5	36.8
(Standard Deviation) (σ) Substrate: Stainless Steel	0.4	1.6	3.4	3.5
Shear (minutes) ASTM D-3654 (Average) units: (minutes)	n/a	n/a	>10,080	>10,080
Storage Modulus @-120°C (Mpa)	2,175	2,085	2,683	2,959
Onset Tg°C	-71.82	-73.66	-64.41	-64.06
Peak Tg°C	-66.20	-65.37	-53.09	-58.07

**Face to Face=Adhesive to Adhesive*

It is concluded that low protein Vytex NRL can be substituted for versions of untreated *Hevea* NRL whether treated or not in the production of cold seal and pressure sensitive adhesives without compromising the physical performance of the resultant products. The use of the low protein NRL for adhesive applications is of

particular importance because post leaching techniques commonly used for the removal of water soluble materials are not applicable for these product applications.

FOAM MADE WITH VYTEX NRL

Natural latex foam is now accepted by consumers and retailers as a premium bedding component. Due to the impact on soundness of sleep and the increased education of consumers, one-sided mattresses, meaning no flipping required, are becoming the industry standard. Latex's silky, luxurious feel and performance are separating it from other foam source materials.

There is limited information available regarding antigenic protein values in foam made with *Hevea* latex. Total latex proteins have been analyzed in *Hevea* latex foam that has been created in a similar manner to foam that has been produced with Vytex NRL. Table 11 provides a comparison for protein of foam made with Vytex NRL and foam made from *Hevea* NRL.

Table 11: ELISA and Lowry Protein Test Results for Foam made with Vytex NRL and *Hevea* NRL

Sample ID	***ASTM D6499-03 ELISA (ug/g)***	***ASTM D5712-05 Lowry (ug/g)***
Foam made with Vytex NRL	4.7	50
Foam made with *Hevea* NRL[24]	Test not performed	2,185

In addition to having significantly fewer total proteins than foam produced with *Hevea* NRL, foam produced with Vytex NRL was less odorous compared to foam produced from *Hevea* NRL. This can be contributed to Vytex NRL containing fewer biodegradable proteins. Also noteworthy is the noticeably higher opacity and whiteness of foam made with Vytex NRL.

BREATHER BAGS, TUBING AND PROBE COVERS MADE WITH VYTEX NRL

Several additional medical products have been produced with Vytex NRL. The aim of this work was to coagulant dip breather bags and tubing and to straight dip probe covers and exam gloves using Vytex NRL under similar manufacturing conditions used to produce products made with *Hevea* NRL. The protein results are illustrated in Table 12.

Table 12: ELISA and Modified Lowry test results for products made with Vytex NRL

Sample ID	***ASTM D6499-03 ELISA (ug/g)***	***ASTM D5712-05 Lowry (ug/g)***
Breather bags made with Vytex NRL	<0.2	<42
Tubing made with NRL	<0.2	<42
Probe covers made with Vytex NRL	0.4	<42
Exam gloves made with Vytex NRL	0.8	<28

CONCLUSION

Suppliers and manufacturers of natural rubber latex and its many products have long acknowledged the need and importance of reducing the antigenic properties of NRL. Recognizing this need and understanding NRL proteins' affinity for powder[25], Vystar Corporation has developed a multi patented process that introduces insoluble aluminum hydroxide powder into liquid latex that complexes and removes proteins and unwanted impurities from natural rubber latex, thus creating Vytex NRL, a low protein NRL source material. Products made with Vytex NRL have reduced antigenic protein values up to 99% over *Hevea* NRL.

Vytex NRL is produced at the latex processor level and can be easily integrated into the current processing environments without additional capital equipment investment. The protein removal and modification process that leads to Vytex NRL allows manufacturers to lower manufacturing costs with the benefit of reduced protein

levels. Reduced leaching times and resulting reductions in energy, water and material handling consumption can lead to realized cost savings.

Over 500 medical and non-medical products made with Vytex NRL have been independently tested using accredited ASTM protein test methodologies. Results show products made with Vytex NRL demonstrate excellent resistance to aging compared to control *Hevea* NRL samples due to the removal of species vulnerable to free radical breakdown. Vytex NRL can be substituted for *Hevea* NRL or synthetic alternative materials when reduced antigenic proteins and good aging characteristics are essential.

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