Clinical and Laboratory Investigation of Latex Allergy in Healthcare Workers

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature **Date**

ABSTRACT

Healthcare workers (HCW^s) wear latex gloves to protect themselves and their patients against the transmission of microbial, viral and bloodborne diseases. These individuals are primarily exposed to latex via cutaneous (direct contact) and mucocutaneous (inhalation of airborne allergens on glove powder) routes. Repeated exposure leads to the formation of circulating latex-specific IgE and subsequent sensitisation with varying clinical expression.

The airconditioning system of the Tygerberg Hospital (TBH) complex was investigated for the presence of aerosolised cornstarch glove powder and proteins. Dust samples were collected from 14 areas with different levels of latex glove usage. Dust samples were spectrophotometrically compared to a calibration graph of pure glove powder. The detection of starch and proteins in all the dust samples confirmed the presence of glove powder and possibly airborne latex allergens in the airconditioning ducts. As expected, the high exposure areas showed the highest concentrations of both starch and proteins. It is possible that other proteins than latex were involved, but the confirmed high level of protein contamination should be a cause for concern. Correlation between starch and protein levels was highly significant ($p<0.01$) in all instances.

A total of 500 questionnaires were circulated for completion by HCW^s from TBH. The response rate was 69.8%. After considering specific inclusion criteria, a study group of 152 individuals was compiled (28 males, 124 females). All subjects had current latex exposure and suffered from at least three pre-defined symptoms.

Serum was collected from all subjects and dermal fluid from 31 subjects. Total IgE and latex-specific IgE analysis were done on all serum and dermal fluid samples. Latex-specific IgE was positive (>0.35 IU/ℓ) in 23 serum and six dermal fluid samples. Skin prick tests (SPT^s) for latex were done on 59 subjects with negative serum latex-specific IgE and 34 had positive results. Twelve subjects with negative latex-specific IgE and latex SPT^s underwent patch tests with the European Standard Series, a piece of latex glove and glove powder in petrolatum. Three subjects had positive results to one or more of these allergens.

Western blot analysis for latex was done on all positive sera and dermal fluid collected from these subjects. Western blot analysis for latex proved to be more sensitive than the capRAST, because it was able to identify specific bands in samples with negative capRAST results. All subjects showed a band for *Hev b 1*, which has been confirmed as a powder-bound airborne allergen. *Hev b 6.01* is associated with HCW^s with cutaneous symptoms and this band was

recognised by 81% of the subjects. These findings confirmed that airborne and cutaneous routes are the major routes of exposure in HCW^{s}.

According to their laboratory results, subjects were divided into the following subgroups and compared statistically: Group A (serum positive, n=23), Group B (SPT positive, n=34) and Group C (negative, n=25). Group D (withdrawn, n=70) could not be used for statistical comparisons, due to incomplete results. An overall latex allergy prevalence of 38% was found. Group A differed significantly from Group B and Group C for most clinical and special investigations. Group A and B were also combined to represent all subjects with positive results (Cohort AB). The Allergy Score and Class were highly significant when Cohort AB was compared to Group C. The selection of clinical symptoms was confirmed to be relevant and work-related deterioration on any of the symptoms should bear a high index of suspicion in the evaluation of latex allergy. Numerical indices and specific symptoms showed high positive predictive values and the Allergy Score produced statistical significance in the positive subgroups when compared to the negative subgroup. Paired statistical significance was confirmed between the Allergy Score and occupational exposure (number of years, hours and pairs per week).

The areas with the highest occupational latex exposure in HCW^{s} are the face and hands. Different occupations also have different levels of exposure and two subgroups of HCW^{s} (16 laboratory technologists and 13 theatre staff) were investigated for sebum content on different facial areas and the palms and dorsal areas of both hands. Baseline measurements were done before putting on gloves. In 21 subjects follow up measurements were done following three to four hours of occupational exposure, but before washing their hands. Baseline and follow up values were compared for all the different anatomical regions. Levels on the forehead and cheeks increased over time, while the level on the nose decreased. All hand regions decreased significantly during occupational exposure, suggesting that glove powder contributes to dryness of the skin.

In conclusion, the problem posed by latex allergy will not be solved overnight and will probably remain a major occupational hazard for years to come. It is currently not possible to avoid exposure to latex, but it is imperative to institute safety measures to prevent further sensitisation in predisposed individuals and manage those already affected.

ABSTRAK

Gesondheidswerkers dra lateks handskoene om hulleself en hulle pasiënte te beskerm teen die oordrag van mikrobiale, virale en bloed-gedraagde siektes. Die lateks blootstelling vind hier hoofsaaklik plaas via kutane (direkte velkontak) en mukokutane (inaseming van lug-gedraagde allergene op hanskoen poeier) roetes. Herhaalde blootstelling veroorsaak sirkulerende lateks-spesifieke IgE en sensitisasie met variërende kliniese beelde.

Die lugreëlingstelsel van die Tygerberg hospitaalkompleks is ondersoek vir die teenwoordigheid van handskoenpoeier (stysel) en lateks proteïene. Stofmonsters is versamel in 14 areas at verskillende blootstellingsvlakke verteenwoordig het. Die stofmonsters is spektrofotometries vergelyk met 'n kalibrasiekurwe van suiwer hanskoenpoeier. Stysel en proteïene kon in all die stofmonsters aangetoon word en het die teenwoordigheid van handskoenpoeier en moontlike luggedraagde lateks proteïene in die lugreëlingstelsel bevestig. Soos verwag kon word, het die hoogste stysel en proteïen waardes in hoë blootstellingsareas voorgekom. Hoogs beduidende statistiese korrelasies ($p<0.01$) tussen die stysel en proteïenvlakke kon aangedui word in alle monsters.

'n Totaal van 500 vraelyste is gesirkuleer vir voltooiing deur TBH gesondheidswerkers, waarvan 69.8% voltooide vraelyste terugontvang is. Na evaluering van insluitingskriteria, is 'n studiegroep van 152 individue saamgestel (28 mans, 124 vrouens). Almal het huidige lateks blootstelling en ten minste three vooraf gedefinieerde simptome gerapporteer.

Serum is van die hele groep versamel en dermale vog van 31 proefpersone. Totale IgE en lateks-spesifieke IgE vlakke is op alle serum en dermale vog bepaal. Positiewe resultate (>0.35 IU/ℓ) is verkry in 23 serum en ses dermale vog monsters. Velpriktoets vir lateks is op 59 proefpersone uitgevoer en 34 daarvan het positiewe resultate opgelewer. Twaalf proefpersone met negatiewe lateks-spesifieke IgE en velpriktoets resultate het kutane plaktoetse ondergaan met die Europese Standaard Reeks, 'n stukkie lateks handskoen en handskoenpoeier in petrolatum. Drie proefpersone het positiewe resultate teen een of meer van die allergene gehad.

Westerse kladanalise vir lateks is op alle positiewe serum gedoen, asook die dermale vogte van hierdie proefpersone. Westerse kladanalise vir lateks blyk baie meer sensitief te wees as die capRAST, aangesien dit spesifieke bande kon identifiseer in monsters capRAST resultate. Alle monsters het 'n band getoon vir *Hev b 1*, 'n poeier-gebinde, luggedraade allergeen. *Hev b 6.01* is geassosieer met gesondheidswerkers met velsimptome en hierdie band is gevind in 81% van

die monsters. Hierdie resultate bevestig dat die belangrikste blootstelling aan lateks in gesondheidswerkers deur die vel en inaseming plaasvind.

Proefpersone is in die volgende three groepe verdeel volgens laboratorium resultate en statisties vergelyk: Groep A (positiewe serum, n=23), Groep B (positiewe velpriktoetse, n=34) en Groep C (negatief, n=25). Groep D (onttrek, n=70) kon nie vir betekenisvolle statistiese vergelykings aangewend word nie, as gevolg van onvolledige resultate. 'n Finale lateks allergie prevalensie van 38% is gevind. Groep A het hoogs beduidend verskil van Groep B en C vir die meeste van die kliniese en spesiale laboratoriumondersoeke. Groep A en B is gekombineer om alle proefpersone in te sluit met positiewe resultate (Kohort AB). Die Allergie Telling en Klas van Kohort AB was hoogs beduidend in vergelyking met Groep C. Die gekose simptome is bevestig as relevant en enige werksverwante verergering van simptome moet met 'n hoë mate van agterdog bejeën word in lateks allergie. Numeriese indekse en spesifieke simptome het hoë positiewe voorspellingswaardes gelewer en die Allergie Telling was hoogs beduidend in the positiewe subgroep in vergelyking met die negatiewe subgroep. Gepaarde statistiese beduidenheid is ook gevind tussen die Allergie Telling en beroepsblootstelling (jare van blootstelling, uur en paar handskoene per week).

Die meeste beroepsblootstelling aan lateks in gesondheidswerkers vind plaas op die hande en gesig. Verskillende beroepe het ook verskillende blootstellingsvlakke en two subgroepe gesondheidswerkers (16 laboratorium tegnoloë en 13 teater personeel) is ondersoek vir die sebumgehalte op veskillende areas van die gesig en hande. Basislynvlakke is gemeet voordat handskoene aangetrek is en in 21 gevalle is opvolgvlakke gemeet na three to four uur beroepsblootstelling, maar voor die hande gewas is. Basislyn en opvolgvlakke is met mekaar vergelyk vir al die anatomiese areas. Die voorkop en wange het 'n toename in sebumgehalte getoon, terwyl dié van die neus afgeneem het. Al die areas op die hande toon 'n hoogs beduidende afname tydens beroepsblootstelling, wat impliseer dat hanskoenpoeier moontlik bydra tot droogheid van die vel.

In samevatting, die lateks allergie probleem sal nie oornag opgelos word nie en sal waarskynlik 'n belangrike beroepsrisiko bly vir die aansienlike toekoms. Totale vermyding van lateks is tans onmoontlik en daarom is dit van uiterste belang om voorsorgmaatreëls in plek te stel om verdere sensitisasie in blootgestelde individue te verhoed en die wat reeds geaffekteer is, effektief te hanteer.

DEDICATION

To Johan and Elané, with love,
and in loving memory of my father, Corrie –
my role model and best friend.

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ABBREVIATIONS

AA	amino acid
Abs	absorbance
ACD	allergic contact dermatitis
AIDS	acquired immunodeficiency syndrome
APC	antigen-presenting cell
B-cell	bone marrow-derived lymphocyte
BSA	bovine serum albumin
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FEIA	fluoroenzymeimmunoassay
GM-CSF	granulocyte-macrophage stimulating factor
GSH	Groote Schuur Hospital
HCW	healthcare worker
HEP	histamine equivalent prick
HIV	human immunodeficiency virus
ICD	irritant contact dermatitis
Ig	immunoglobulin
IL	interleukin
IFN	interferon
IPP	isopentenyl pyrophosphate
IT	immunotherapy
IUIS	International Union of Immunological Societies
kD	kilodalton
LC	Langerhans cell
LEAP	latex ELISA for antigenic protein
LT	leukotriene
Me	median
MHC	major-histocompatibility-complex
MW	molecular weight
NRL	natural rubber latex
OR	operating room
PAF	platelet-activating factor
PAGE	polyacrylamide gel electrophoresis
PG	prostaglandin

pI	isoelectric point
PPD	N-isopropyl-N-phenyl-4-phenylenediamine
PPV	positive predictive value
p-value	probability value
r	recombinant
RAST	radio allergosorbent test
REF	rubber elongation factor
RSA	Republic of South Africa
SB	spina bifida
SC	stratum corneum
SCF	stem cell factor
SCH	stratum corneum hydration
SCIT	subcutaneous immunotherapy
SD	standard deviation
SDS	sodium dodecyl sulphate
SLIT	sublingual immunotherapy
SPT	skin prick test
TBH	Tygerberg Hospital
TCA	trichloroacetic acid
T-cell	thymus-derived lymphocyte
TEWL	transepidermal water loss
TNF	tumour necrosis factor
UK	United Kingdom
USA	United States of America
WHO	World Health Organisation

CHAPTER 1:
LITERATURE REVIEW

1.1. INTRODUCTION

Natural rubber latex (NRL) and compounds with rubbery properties are linear, high polymers with molecular weights (MW^s) of up to 100 kilodalton (kD). Most rubbers contain reactive double bonds, which allow the molecules to cross-link and polymerise. However, these double bonds also open rubber to attack and destruction by heat, light, oxygen and ozone (Cronin, 1980).

Archaeologists have found that latex items were used in ancient Mesoamerica as early as 1600 BC. The oldest rubber articles consisted of twelve solid rubber balls that ranged from 13-30 cm in diameter with a weight of 0.5-7.0 kg. Radiocarbon dating confirmed the two oldest balls to be from 1600 BC. Additional rubber artefacts included wooden tool handles wrapped with rubber, human figurines, casts of human hands, a hollow human head, and a stone tool hafted with a rubber band. These early rubber artefacts were made with latex from the *Castilla elastica* tree, which was mixed with the juice from a morning glory vine (*Ipomoea alba*) to improve quality (Ownby, 2002).

In 1813 Adam Elias von Siebold suggested that surgeons use swine or horse bladders as gloves during delivery of infants to infected women to reduce the risk of transmission. Between 1840 and 1842, Sir Thomas Watson advised the invention of gloves to protect the hands of physicians. Thomas Hancock patented rubber gloves in 1830. However, the instability of rubber remained a problem for years (Randolph, 2001; Ownby, 2002).

The first widespread use of NRL products in the medical field followed the production of dentures. Charles Goodyear patented the manufacturing process in 1851 (Meade *et al*, 2002). By 1852 rubber gloves were listed in a French surgical catalogue and the first patent for rubber surgical gloves was granted to T Forster in 1878 (Ownby, 2002). In 1890 the Goodyear Rubber Company produced their first pair of NRL gloves to protect the hands of surgeons and assistants against the irritation of phenolic antiseptics and mercurial salts (Ellis, 1990; Ellis, 1997). The breast surgeon, Dr William Stuart Halstead was one of a number of pioneers in the United States of America (USA) and Europe to experiment with this new invention (Ellis, 1990; Walls, 1996; Zerin *et al*, 1996; Turner, 1997;

Warshaw, 1998). In 1897 Zoege von Manteuffel of Dorpat suggested that all members of a surgical team wear rubber gloves to prevent transmission of infection. It took until approximately 1900 AD before surgical gloves were commonly used (Ownby, 2002). NRL has become the primary ingredient of gloves since the 1920s (Ellis, 1997). The comfort, barrier and tactile properties of powdered NRL gloves have been thought to be ideal at the time (Sussman & Beezhold, 1995).

It was generally accepted that rubber was not allergenic and sensitivity to rubber gloves was limited to housewives, wearing it for protection during housework (Cronin, 1980). However, extensive research during the past two decades has contradicted these statements. NRL glove allergy is currently acknowledged as a major occupational problem among healthcare workers (HCW^s) (Turjanmaa *et al*, 1996; De Beer & Cilliers, 2001). NRL gloves were also recognised as a potential etiological agent of occupational asthma since the beginning of the 1990s (Tarlo *et al*, 1990; Marcos *et al*, 1991).

1.2. RUBBER PRODUCTION

1.2.1. The Rubber Tree

NRL is produced by approximately 2 000 different plant species. More than 99% of commercial NRL originates from the *Hevea brasiliensis* tree of the family *Euphorbiaceae* (Cronin, 1980; Jaeger *et al*, 1992; Czuppon *et al*, 1993; Pumphrey, 1994; Kurup & Fink, 2001; Sussman *et al*, 2002). It grows in humid climates at temperatures between 20°C and 28°C. The trees exhibit efficient wound repair mechanisms to deal with invading micro-organisms, insects and fungi. This mechanism seals the wounds and inhibiting micro-organism growth (Sussman *et al*, 2002).

An average tree takes six to eight years to reach harvesting maturity and yields enough latex to manufacture approximately ten pairs of gloves per week. Tapping is done every two to three days by shaving a thin strip of bark from the bottom of the original cut ***(Figure 1.1)***. When the cuttings reach the ground, the bark is permitted to renew itself before a new tapping panel is started (De Beer & Cilliers, 2001).

A tube-like network of laticifers beneath the surface contains proteins involved in polyisoprene biosynthesis, latex coagulation and the plant's defence system. The

Figure 1.1: Tapping of the *Hevea brasiliensis* tree

A diagonal cut angled downward is made through the bark, 30-50% of the circumference of the trunk. The latex flows upwards into the incision and finally coagulates within the cut ends of the laticiferous ducts, sealing the wound. About 30 mℓ of latex per tapping is collected.

cytoplasm consists of 65% water, 30-33% spherical rubber particles (Cronin, 1980; Pumphrey, 1994; Siler & Cornish, 1995; Randolph, 2001), 2% resin and 1-1.8% proteins (Jaeger *et al*, 1992; Potter, 1998a; Potter, 1998b; Kurup & Fink, 2001). The protein concentration of liquid concentrate is reported to be 16.53 mg/mℓ (Levy *et al*, 1992). The rubber particles have a modal diameter of 400 nm (Pumphrey, 1994) and contain 25-45% polymerised cis-1,4-polyisoprene. It is coated with a layer of hydrophilic materials, allergenic soluble and particle-bound proteins, lipids and phospholipids. However, some of the proteins are very hydrophobic with limited solubility (Siler & Cornish, 1995). The cytoplasm contains nuclei, mitochondria, lutoids (Pumphrey, 1994), lipids, cofactors (Arreguin *et al*, 1988) and inorganic constituents, e.g. calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc (Kurup & Fink, 2001; Sussman *et al*, 2002).

1.2.2. Manufacturing Processes

NRL products are produced from two major types of raw material, i.e. liquid concentrate and solid dry rubber. Only 10-20% of NRL is tapped for the preparation of liquid concentrate. Its inherent high elasticity makes it ideal for the manufacturing of gloves (Turner, 1997; Meade *et al*, 2002). Dry rubber is coagulated, creped, crumbled and washed extensively and then thoroughly dried at above 100°C in the form of solid bales and sheets. Dry rubber is used for the production of tyres, hoses, belts, gaskets, etc (Meade *et al*, 2002; Yip & Cacioli, 2002).

During the manufacturing process, up to 200 chemicals are added to the concentrate to provide strength, stretch and durability to the finished product (Fisher, 1997; Turner, 1997; De Beer & Cilliers, 2001). A **preservative** (usually ammonium hydroxide, formaldehyde, sodium sulphate or zinc oxide) is added immediately after collection to prevent autocoagulation, deterioration and bacterial contamination. Ammonia (0.7%) has a pH of 11.4 and hydrolyses the NRL proteins, causing conformational changes in relevant peptides (Slater & Chhabra, 1992; Beezhold & Beck, 1992; Beezhold *et al*, 1994b; Akasawa *et al*, 1996; Toraason *et al*, 2000). The addition of formic or acetic acid reduces the pH to less than four. **Vulcanisation** or curing is done by heating the latex emulsion in the presence of sulphur, selenium, tellurium, peroxides or 4,4'-dithiomorpholine (Jaeger *et al*, 1992; Pumphrey, 1994; Warshaw, 1998). **Accelerators** (amines, benzothiazoles, carbamates, guanidines, hydroxy-toluene, mercaptobenzothiazoles, tetramethylthiuram disulfide, thioureas, thiurams) act as catalysts and control the rate, uniformity and completeness of vulcanisation (Spaner *et al*, 1989; Cohen *et al*, 1998; Randolph, 2001). **Antioxidants** (amines, butylated hydroxytoluene, hydroquinone, monobenzyl ether, paraphenylenediamine, phenol derivatives, quinolines, thiocarbamates, zinc oxide) and **antiozonants** prevent oxidation and ozone degradation of unsaturated isoprene bonds, which are highly reactive with oxygen (Jaeger *et al*, 1992; Warshaw, 1998; Randolph, 2001). **Anticoagulants** convert the emulsion to a 60% liquid and 40% solid phase. **Peptisers** (thio-ß-naphthol) are used to melt the colloid latex gels, while **fillers** (barium sulphate, calcium carbonate, carbon black, kaolin, magnesium carbonate) may be added to stiffen the final rubber product. **Softeners** (oils, fatty acids, pine tar, waxes) are added to enhance elasticity. **Pigments** (zinc oxide, lithopone) are used to provide the colour of the final product (Cronin, 1980).

These additives may constitute up to 5% of the final product and can act as haptens to cause mainly type IV hypersensitivity reactions (Cronin, 1980; Akasawa *et al*, 1996; Axelsson *et al*, 1987; Spaner *et al*, 1989; Jaeger *et al*, 1992; Pumphrey, 1994; Gibbon *et al*, 2001). Manufacturers and consumers are thus exposed to a mixture of residual chemicals and hydrolysed proteins, including water-soluble latex proteins of between < 2 µm and about 10 µm (Beezhold & Beck, 1992; Oh *et al*, 1999; Toraason *et al*, 2000).

Most NRL gloves are manufactured by a dipping method. Moulds are made of porcelain, metal or very hard plastic, which are mounted on a rack and slowly dipped into the NRL in the presence or absence of a destabilising chemical. The product is then washed to remove the excess chemicals before it is vulcanised in ovens at 100-130°C, or 140-160°C in the case of dry rubber products (Moneret-Vautrin *et al*, 1993). Leaching is done after vulcanisation. Although the native NRL proteins are aggregated and fragmented, the allergenic epitopes remain unchanged (Hunt *et al*, 2002). Before removing the gloves from the moulds, they are dipped in a slurry tank where cornstarch powder is applied and a large part of the allergens deposited on the inner surface of the gloves (Yip & Cacioli, 2002). Although most allergens adsorb to the cornstarch powder, some remain as dried soluble proteins on the inner surfaces (Hunt *et al*, 2002). The cornstarch powder is not allergenic, but other contaminants and preservatives in the starch (e.g. ethylene oxide, casein, etc) may cause allergic reactions in sensitised individuals (Potter, 1998a; Potter,1998b).

1.3. LATEX ALLERGENS

The relation between immunoglobulin (Ig) E-binding potential of a protein and clinical symptoms is influenced by physical properties (e.g. solubility, stability, size and the compactness of the overall protein fold) and immunologic properties (e.g. affinity and epitope valence). An allergen is regarded as a major allergen if it is responsible for > 20% of the allergenic reactivity in > 20% of the sensitised patients. Therefore, removal of a truly major allergen from an extract should have a noticeable effect on the overall reactivity of the extract. Allergenicity of a product is determined by the level and route of exposure, digestibility in the case of food allergens, and size and solubility in the case of airborne allergens (Aalberse, 2000). The amount of allergenic proteins produced by latex trees can vary up to 25-fold between batches of latex. This can be

attributed to seasonal variation, moisture, temperature, fertiliser, chemical and soil composition (Hamilton *et al*, 1998; Sussman *et al*, 2002).

NRL proteins comprise less than 10% of the dry weight of latex (Randolph, 2001). Of the more than 240 separate polypeptides that can be discerned by two-dimensional electrophoresis in latex concentrate, only 57 are reportedly allergenic (Slater & Chhabra, 1992; Czuppon *et al*, 1993; Kelly *et al*, 1994a; Raulf-Heimsoth *et al*, 1996; Weissman & Lewis, 2002). Ammoniated latex is the source material for most medical NRL products causing allergic reactions (Slater & Chhabra, 1992). Proteins capable of inducing anaphylaxis are water-soluble, thermostable and trypsin-sensitive or glycoproteins (Jaeger *et al*, 1992). Accurate measurement of the proteins in medical products has proven to be difficult. Although proteins can be isolated from the end-use products, routine protein assays are often too insensitive to measure protein levels in extracts from these products (Beezhold, 1993). If the sensitising product contains different epitopes than the material used in the determining assays, false negative results may occur (Kelly *et al*, 1994a).

The specificity of the allergens binding to IgE or thymus-derived lymphocytes (T-cell) depends on the amino acid (AA) sequence of the allergens. The T-cell epitopes are linear and major-histocompatibility-complex (MHC) class II restricted, while the bone marrow-derived lymphocyte (B-cell) epitopes are usually conformational, rarely linear, and not MHC controlled. However, some linear B-cell epitopes may adequately block IgE binding and cross-linking of allergens on the surface of basophils and mast cells (Kurup & Fink, 2001). Immunologically, NRL proteins differ from commonly encountered allergens. Some new allergic epitopes may be created during processing (Mäkinen-Kiljunen *et al*, 1992; Ownby, 1993; De Beer & Cilliers, 2001), or denaturation may expose epitopes that are cryptic to the native molecules (Beezhold, 1993; Beezhold *et al*, 1994b). Most NRL proteins are not tightly bound to the rubber, but are water-soluble and rapidly (within five minutes) leach out of the product, although complete extraction may take up to 18 hours or more. The majority of NRL proteins have acidic pH between 4.0 and 6.5 (Beezhold, 1993).

A number of NRL proteins (*Hev b 1* to *Hev b 13*) have received designation as allergens ***(Table 1.1)***. All, except for *Hev b 4*, have been cloned as recombinant (r) proteins (Yip *et al*, 2000; Nieto *et al*, 2002).

Table 1.1: Registered natural rubber latex allergens

Allergen names are designated by the International Union of Immunological Societies (IUIS)

Name	Trivial Name	Physiological role	pI	MW	AA
Hev b 1	rubber elongation factor	rubber biosynthesis	4.9	14.59	137
Hev b 2	ß-1,3-glucanase	defence-related protein	4.9,5.5,9 9.3,9.5,10	36	N/A
Hev b 3	small rubber-particle protein	rubber biosynthesis defence-related protein	4.8	22.3	204
Hev b 4	microhelix complex, cyanogenic glucosidase	defence-related protein	4.5	50-57, 100-110	N/A
Hev b 5	acidic latex protein	fusion protein	3.5	16	N/A
Hev b 6.01	prohevein (hevein preprotein)	defence-related protein, latex coagulation	5.6	20	187
Hev b 6.02	mature hevein	defence-related protein, latex coagulation	4.9	4.7	43
Hev b 6.03	prohevein C-terminal domain	defence-related protein, latex coagulation	6.4,7.0, 7.4	14	138
Hev b 7	patatin homologue	defence-related protein inhibits rubber biosynthesis	4.8	46	N/A
Hev b 8	latex profilin	structural protein	4.9	13.9	N/A
Hev b 9	latex enolase	glycolysis, gluconeogenesis	5.6,6.4	47.6	445
Hev b 10	manganese-superoxide dismutase	protects against oxygen-related radicals	6.3	22.9	N/A
Hev b 11	class I chitinase	plant-pathogen interaction	5.1	33	N/A
Hev b 13	esterase	unknown		43	N/A
Hevamine A & B		antifungal, latex coagulation		29.5	N/A
Prenyltransferase		rubber elongation		38	N/A

pI = isoelectric point; MW = molecular weight in kilodalton; AA = amino acids; N/A = not available

(Turjanmaa *et al*, 1988a; Light & Dennis, 1989; Slater & Chhabra, 1992; Slater *et al*, 1996; Posch *et al*, 1997; Kostyal *et al*, 1998; De Beer *et al*, 1999; Sowka *et al*, 1999; De Beer, 2000; Wagner *et al*, 2000; De Beer & Cilliers, 2001; Kurup & Fink, 2001; Rihs *et al*, 2001; Sussman *et al*, 2002; Bernstein *et al*, 2003).

Fresh NRL can be separated by centrifugation into three main fractions. The white upper layer consists of rubber particles and includes *Hev b 1* and *Hev b 3.* An aqueous middle layer contains the cytoplasm from the latex vessels and includes *Hev b 5, Hev b 7* and *Hev b 8,* and the bottom fraction contains lutoids, *Hev b 2, Hev b 4, Hev b 6.01, Hev b 6.02, Hev b 6.03,* hevamine, *Hev b 11* and class II chitinases (Yeang *et al*, 1996; Kostyal *et al*, 1998; Kurup & Fink, 2001). Generally, *Hev b 1* and *Hev b 3* are highly associated with spina bifida (SB) patients with latex allergy who have repeated mucosal contact with latex. These proteins are particle-bound and less soluble than other latex antigens. *Hev b 2, Hev b 4, Hev b 6* and *Hev b 7* are associated with adult HCW^s, where most exposure takes place via cutaneous and respiratory routes (Poley & Slater, 2000, Woolhiser *et al*, 2000; Randolph, 2001; Weissman & Lewis, 2002). *Hev b* 5 is an important allergen in both HCW^s and children with SB (Poley & Slater, 2000).

1.3.1. Rubber Elongation Factor (*Hev b 1*)

Rubber elongation factor (REF) was the first well-characterised NRL allergen (Ownby, 1993). It is a spherical, homotetrameric molecule with a calculated MW of 58.4 kD present in stoichiometric amounts relative to the number of growing rubber molecules (Dennis *et al*, 1989a; Sussman *et al*, 2002). The coherent interaction of the four subunits of 14.59 kD each must be non-covalent, due to the total absence of disulphide bridges (Czuppon *et al*, 1993; Posch *et al*, 1997). It is a water-insoluble protein, tightly bound to large (> 350 nm in diameter) rubber particles (Kurup & Fink, 2001). REF has an isoelectric point (pI) of 4.9, a hydrated specific volume of 0.93 mℓ/g and a diameter of 35.1 Å (Sussman *et al*, 2002). For reference purposes, rubber has a MW of approximately 500 kD and a specific volume of 1.1 mℓ/g (Dennis & Light & Dennis, 1989b). REF has a length of 137 AA^s, but lacks cysteine, methionine, histidine and tryptophan (Dennis *et al*, 1989a; Ownby, 1993; Sussman *et al*, 2002). The most allergenic portions of *Hev b* 1 are the peptides with AA^s 31-64 and 124-134 (Yman & Lundberg, 1997). The NH_2 terminus is highly charged and contains only acidic residues (five of the first twelve AA^s). It constitutes 10-60% of the total protein, but is absent in the supernatant fluid of centrifuged rubber particles (Dennis & Light, 1989b; Sussman *et al*, 2002).

REF plays a role in rubber elongation in docking and positioning prenyltransferase on the rubber molecule (Dennis *et al*, 1989a; Meade *et al*, 2002). Such docking involves a

reorientation of the binding sites for isopentenyl pyrophosphate (IPP) and the allylic primer. Additionally, REF positions and protects the growing pyrophosphate ends on the molecule (Dennis *et al*, 1989a; Czuppon *et al*, 1993). REF and prenyltransferase work together to synthesise the isoprene chains (Beezhold *et al*, 1994b; Randolph, 2001). It is not known in which part of the *Hevea* plant REF is synthesised. Whether REF is present in leaves in newly formed laticifers or due to synthesis in leaf or carbium cells prior to secretion into latex is not known (Dennis *et al*, 1989a).

It is a major allergen in children with SB (Alenius *et al*, 1994; Lu *et al*, 1995; Alenius *et al*, 1996; Turjanmaa *et al*, 1996; Yeang *et al*, 1996; De Silva *et al*, 2000; Kurup *et al*, 2000; Poley & Slater, 2000; Yip *et al*, 2000). In many cases REF is the monosensitiser in SB patients (Chen *et al*, 1997), but is only recognised by 50% of sensitised HCW^{s} (Posch *et al*, 1997; Sussman *et al*, 2002). However, it was found as a powder-bound, airborne allergen after glove donning (Raulf-Heimsoth *et al*, 1996). It may therefore increase sensitisation in HCW^{s}, where airborne allergens are most important in sensitisation.

1.3.2. ß-1,3-Glucanase (*Hev b 2*)

Plant β-1,3-glucanases are monomers with a MW between 25 and 35 kD. Most are endoglucanases with the potential to partially degrade fungal cell walls by hydrolysing the β-1,3-glucan fibres of the growing hyphae of filamentous fungi (Breiteneder & Ebner, 2000; Sussman *et al*, 2002). Their main function is to defend the plants against fungal infection (Meade *et al*, 2002).

Hev b 2 is a 36 kD defence-related protein located in the lutoids and shows high homology to several plant endo-1,3-ß-glucosidases (Posch *et al*, 1997; Kurup & Fink, 2001). Several isozymes of *Hev b 2* exist, both acidic (pI 4.9, 5.5) and basic (pI 9, 9.3, 9.5, 10) isoforms (Posch *et al*, 1997; Sussman *et al*, 2002). Cross-reactions to banana, potato and tomato exist and significantly increased amounts are expressed in bananas during ripening (Breiteneder & Ebner, 2000).

It is considered to be a significant NRL allergen with IgE-binding capacity and T-cell stimulation properties (Alenius *et al*, 1994). It has increased allergenic reactivity in children with SB (Czuppon *et al*, 1993; Beezhold *et al*, 1994b; Kostyal *et al*, 1998) and HCW^{s} (Yip *et al*, 2000; Randolph, 2001; Sussman *et al*, 2002).

1.3.3. Small Rubber Particle Protein (*Hev b 3*)

The 22.3 kD *Hev b 3* is a small (< 70 nm) insoluble protein associated with rubber particles with a pI of 4.8 (Yman & Lundberg, 1997; Oh *et al*, 1999; Kurup & Fink, 2001; Sussman *et al*, 2002). It has 204 AA^{s}, plays a role in plant defence mechanisms and rubber biosynthesis, by synthesising long-chain polyisoprene (Yeang *et al*, 1996; Bohle *et al*, 2000; Meade *et al*, 2002; Sussman *et al*, 2002). *Hev b 3* shares 47% sequence identity with *Hev b 1* on the AA level (Posch *et al*, 1997; Oh *et al*, 1999; Sussman *et al*, 2002). These proteins also possess significant structural similarity, consequently leading to IgE cross-reactivity (Bohle *et al*, 2000; Yip *et al*, 2000).

It is a common allergen in children with SB (Alenius *et al*, 1994; Yeang *et al*, 1996; Oh *et al*, 1999; De Silva *et al*, 2000; Kurup *et al*, 2000), but shows weaker and less frequent reactivity in HCW^{s} (Lu *et al*, 1995; Sussman *et al*, 2002). It may also be associated with anaphylactic reactions, because sera from other patient groups with anaphylactic reactions to NRL reacted with the purified allergen (Lu *et al*, 1995).

1.3.4. Microhelix Protein Complex (*Hev b 4*)

Hev b 4 is an acidic (pI 4.5) defence-related protein, isolated from the lutoids and shown to be a component of the microhelix protein component (Kurup & Fink, 2001). It has a MW of 50-57 kD in the reduced and 100-110 kD in the unreduced form (Posch *et al*, 1997; Sussman *et al*, 2002). The function of this protein is still unknown (Meade *et al*, 2002).

Both HCW^{s} and SB patients show IgE-binding to *Hev b 4* (Sussman *et al*, 2002).

1.3.5. Acidic Latex Protein (*Hev b 5*)

Hev b 5 is a proline-rich structural protein in the cytoplasm with a MW of 16 kD and pI of 3.5 (Yman & Lundberg, 1997; Kurup 2001). It retains its allergenicity, even after autoclaving (Sussman *et al*, 2002). Apart from being a structural protein, no other functions are known (Meade *et al*, 2002). Although the predicted MW is 16-17 kD, this protein migrates at around 24 kD on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (De Silva *et al*, 2000). It has a high degree of homology to proteins in acidic kiwi fruit and potato, especially on nucleotide and deduced protein sequence level (Slater *et al*, 1996; Posch *et al*, 1997; Yman & Lundberg, 1997; Kostyal *et al*,

1998; Yip *et al*, 2000; Sussman *et al*, 2002). The epitopes in kiwi protein appear in regions of limited homology, questioning the likelihood that this protein is responsible for cross-reactivity with kiwi (Toraason *et al*, 2000). However, the AA sequence of *Hev b 5* shows 46% identity to another acidic protein in kiwi and this may provide a molecular explanation for the high frequency of fruit allergies in latex allergic patients (De Silva *et al*, 2000).

It is a highly acidic protein and is recognised by 92% of HCW^s and 56-65% of SB patients with latex allergy. It is a major allergen and demonstrates strong T-cell response in both groups (Slater *et al*, 1996; Posch *et al*, 1997; Kostyal *et al*, 1998; De Silva *et al*, 2000; Yip *et al*, 2000; Sussman *et al*, 2002).

1.3.6. Hevein *(Hev b 6)*

1.3.6.1. Prohevein (*Hev b 6.01*)

Prohevein is probably the most studied allergen in latex (Meade *et al*, 2002). Located in the lutoids, it is synthesised as a 20 kD preprotein of hevein with a pI of 5.6 and IgE-binding capacity (Posch *et al*, 1997; Kurup & Fink, 2001; Sussman *et al*, 2002). It is 187 AA^s long and is post-translationally processed into two allergenic fragments, the 4.7 kD (43 AA^s) amino-terminal hevein (*Hev b 6.02*) and the 14 kD (138 AA^s) carboxyl-terminal prohevein (*Hev b 6.03*) (Beezhold *et al*, 1994a; Beezhold *et al*, 1994b; Posch *et al*, 1997; Sussman *et al*, 2002). The IgE-binding capacity of prohevein in latex is mostly attributed to its N-terminal domain, the 4.7 kD hevein (Blanco *et al*, 1999).

Hev b 6 is responsible for much of the cross-reactivity to fruits (Toraason *et al*, 2000). Structural homologies have been demonstrated between prohevein and other plant-chitin-binding proteins and cereal lectins (Blanco *et al*, 1999). Tobacco contains a 21 kD prohevein-like allergen that reacts with serum IgE from patients with latex allergy (Hänninen *et al*, 2000; Kurup & Fink, 2001).

It is the most frequently recognised NRL allergen among adult patients presenting primarily with cutaneous symptoms (Alenius *et al*, 1994; Slater & Trybul, 1994; Lu *et al*, 1995; Alenius *et al*, 1996; Turjanmaa *et al*, 1996). However, this sensitisation is not only limited to adult patients and almost 80% of children with latex allergy tested in Finland was also affected by this allergen (Alenius *et al*, 1996).

1.3.6.2. Mature Hevein (*Hev b 6.02*)

Hevein synthesised as prohevein (20 kD, 204 AA^s), which is post-translationally processed into the 4.7 kD amino-terminal hevein and a 14 kD carboxyl-terminal C-domain (Beezhold *et al*, 1994a; Alenius *et al*, 1996; Chen *et al*, 1997; Kostyal *et al*, 1998; Yip *et al*, 2000; Sussman *et al*, 2002). The mature hevein molecule has a pI of 4.9 and contains 43 AA^s. Pure crystalline hevein was the first isolated NRL protein in rubber trees and is one of the most abundant proteins in the lutoid bodies (Chen *et al*, 1997; Kurup & Fink, 2001). It is released when damaged cells interact with a 22 kD receptor glycoprotein in the envelope around the rubber particles, causing the coagulation of latex (Alenius *et al*, 1996; Yip *et al*, 2000; Randolph, 2001). It also has four disulphide bonds, is rich in cysteine and glycine and contains 5% sulphur, but no carbohydrates (Sussman *et al*, 2002).

The solubility of pure hevein in water at 0°C is less than 1%, but it increases rapidly in the presence of natural salts, such as ammonium sulphate solutions (Archer, 1960). It is a major wound-repair and chitin-binding protein, which inhibits the growth of several chitin-containing fungi at the wound site (Pumphrey, 1994; Breiteneder & Ebner, 2000). Hevein shares a great deal of AA homology with the wound-induced proteins from potato, avocado, banana and chestnut (Posch *et al*, 1997; Breiteneder & Ebner, 2000). When expressed in tomatoes, it increases the resistance of the plant to fungal pathogens (Kostyal *et al*, 1998). Structural homology also exists with allergens from ragweed, wheat germ agglutinin, endochitinases and other cereal lectins (Beezhold *et al*, 1994a; Posch *et al*, 1997; Sussman *et al*, 2002).

The major IgE binding component in hevein has been mapped to the N-terminal region of the protein (Beezhold *et al*, 1994a; Alenius *et al*, 1996; Kostyal *et al*, 1998; Yip *et al*, 2000; Sussman *et al*, 2002). It is the most important allergen eluting from high-allergen medical gloves (Palosuo *et al*, 1998) and a major allergen for HCW^s (Beezhold *et al*, 1994b; Yip *et al*, 2000), but not for children with SB (Alenius *et al*, 1994; Lu *et al*, 1995; Alenius *et al*, 1996; Chen *et al*, 1997; Posch *et al*, 1997; Turjanmaa *et al*, 1997).

1.3.6.3. Prohevein C-Terminal Domain (*Hev b 6.03*)

Prohevein carboxyl-terminal domain is a 14 kD defence-related protein located in the lutoids (Kurup & Fink, 2001) with pI values of 6.4, 7.0 and 7.4 (Sussman *et al*, 2002). It has

138 AA^s and is involved in latex coagulation together with *Hev b 6.01* and *Hev 6.02* (Beezhold *et al*, 1994a; Beezhold *et al*, 1994b; Posch *et al*, 1997).

1.3.7. Patatin-like Allergen (*Hev b 7*)

Hev b 7 has a MW of 46 kD and a pI of 4.8, which is very close to the determined pI of patatin (4.75) (Kostyal *et al*, 1998; Sussman *et al*, 2002). It is a cytosolic protein with esterase activity and inhibits rubber biosynthesis by interfering with the incorporation of isopentenyl diphosphate into rubber (Kurup & Fink, 2001; Sussman *et al*, 2002). It has structural homology to patatin, a phospholipase tuber storage protein found in plant families, e.g. potato and tomato (Kostyal *et al*, 1998; Yip *et al*, 2000; Meade *et al*, 2002). It may have a dual role as a somatic storage protein and an enzyme involved in host resistance by inhibiting larval growth of certain pest insects (Posch *et al*, 1997; Sussman *et al*, 2002). Although 15 epitopes have been identified, little evidence was found of cross-reactivity between potato and patatin (Toraason *et al*, 2000).

To date four isoforms of *Hev b 7* have been published that differ in their AA sequence by at least seven AA^s. In addition, one of the peptide sequences determined for the purified natural *Hev b 7* did not completely match the published AA sequences, indicating the existence of still other isoforms. This could explain why *Hev b 7* and the homologues do not contribute to cross-reactivity in the latex-fruit syndrome (Sowka *et al*, 1999). A previously unidentified peptide sequence was found in the 14 kD and 110 kD proteins (Pumphrey, 1994). The sequences obtained for the 46 kD and 110 kD proteins were identical, suggesting that the 110 kD molecule may possibly be a preprotein or dimer of incomplete dissociation of the 46 kD protein. The N-terminal sequence obtained from these proteins was unique, indicating this is a previously unrecognised NRL protein (Beezhold *et al*, 1994b).

Hev b 7 is one of the newest members of a growing list of cloned NRL-derived proteins shown to elicit an allergic response. This is an important allergen for latex-allergic adults (Yip *et al*, 2000) and to a lesser extent SB patients (Sussman *et al*, 2002).

1.3.8. Profilin (*Hev b 8)*

Hev b 8 is a member of the profilin family, well-known pan-allergens in many plant species and currently recognised as ubiquitous cross-reactive plant allergens

(Breiteneder & Ebner, 2000; Toraason *et al*, 2000; Yip *et al*, 2000; Meade *et al*, 2002). It is a 13.9 kD protein located in the cytoplasm with a pI of 4.9 and is an actin-regulating protein (Turjanmaa *et al*, 1996; Kurup & Fink, 2001; Sussman *et al*, 2002). Profilin has negligible importance in NRL extracts as significant allergen, because it could not be demonstrated NRL glove extracts (Turjanmaa *et al*, 1996; Nieto *et al*, 2002).

Profilin is an important cross-reacting allergen in several plant sources, e.g. tree, grass and weeds, pollens, ragweed, fresh fruit, vegetables, banana and celery (Nieto *et al*, 2002). This protein may be responsible for the latex-fruit cross-reactions, although not extensively (Heese *et al*, 1997; Kurup & Fink, 2001; Toraason *et al*, 2000; Sussman *et al*, 2002). Patients sensitised to pollen profilins characteristically react to a broad range of inhalant and nutritive allergen sources (Breiteneder & Ebner, 2000).

It is not a major allergen in latex allergy, as only about 3% of latex allergic HCW^{s} reacted against the recombinant isoform and only two out of 17 SB patients had IgE antibodies directed against r*Hev b 8* (Yip *et al*, 2000). However, 100% of SB patients and 88% of latex allergic adults showed sensitisation to the purified profilin. The reason for the discrepancies may be twofold. The MW of *Hev b 1* (14.6 kD) is in the same range as profilin and may complicate distinguishing between the two allergens. It could also be due to the extremely low profilin content in latex sources, which borders on the threshold of allergen exposure levels (Nieto *et al*, 2002). Crude antigens contain relevant and non-relevant proteins and their use may produce variable results and interpretations (Kurup *et al*, 2000).

1.3.9. Latex enolase (*Hev b 9)*

Hev b 9 is a cytoplasmic latex enolase protein with 445 AA^{s}, a calculated MW of 47.6 kD and pI values of 5.6 and 6.4 (Kurup & Fink, 2001). Enolase is a highly expressed key enzyme of glycolysis and gluconeogenesis in eukaryotic and prokaryotic cells and converts glycerate 2-phosphate to phosphoenolpyruvate and *vice versa* (Wagner *et al*, 2000; Meade *et al*, 2002). It shows very high homology to *Ricinus communis* enolase, tomato enolase and *Cladosporium* enolase (Kurup & Fink, 2001; Sussman *et al*, 2002).

Although studies so far have failed to demonstrate *Hev b 9* in latex allergic patients (Kurup & Fink, 2001), its *in vitro* cross-reactivity with *Hev b 10* to mold allergens, indicate a possible latex-mold syndrome (Wagner *et al*, 2000; Sussman *et al*, 2002).

1.3.10. Manganese-Superoxide Dismutase (*Hev b 10)*

Hev b 10 is a manganese-superoxide dismutase protein with a MW of 22.9 kD and pI of 6.3. It is found in the mitochondria and protects against the toxicity of oxygen-related radicals (Sussman *et al*, 2002). It has been detected in a number of fungi, bacteria and *Aspergillus fumigatus* and is regarded as a major allergen (Kurup & Fink, 2001). Like *Hev b 9*, *in vitro* cross-reactivity can be demonstrated, but clinical cross-reactivity between latex and molds has not been reported (Meade *et al*, 2002).

The allergenicity has not been studied to any depth, but it is not regarded as an important latex allergen. Only two of 20 SB patients and none of 20 latex allergic HCW^s showed specific IgE antibodies to r*Hev b 10* (Rihs *et al*, 2001).

1.3.11. Class I Chitinase *(Hev b 11)*

Hev b 11 is a type I NRL chitinase. It has a MW of 33 kD and a pI of 5.1. Chitinases and lysozymes constitute about 25% of the lutoid proteins. The chitin-binding domain has 58% homology with N-terminal hevein and probably also share epitopes with similar allergens from avocado and banana (Meade *et al*, 2002). Their main function is plant-pathogen interaction and it is considered a minor allergen in latex allergy (Sussman *et al*, 2002).

The immunohistochemical basis of the latex-fruit syndrome seems to be the sensitisation to a 30 kD allergen, which has been confirmed in NRL, avocado and banana by immunoblotting techniques. The allergenicity of class I chitinases seems to be the pan-allergens responsible for this syndrome, as its allergenicity has been demonstrated in more than 50% of a population with latex and banana allergy. Class II chitinases are very close to class I with more than 60% of identical residues in the catalytic domain, but lack the N-terminal hevein-like domain (Blanco *et al*, 1999).

1.3.12. Esterase *(Hev b 13)*

Hev b 13 is a newly described latex allergen. It has a MW of 43 kD and was identified as an early nodule specific protein of legumes. *Hev b 13* appears to be a major *in vivo* allergen in HCW^s with latex allergy (Bernstein *et al*, 2003).

1.3.13. Other

1.3.13.1. Hevamine

In 1976 Archer isolated hevamine A and B from NRL lutoids. In 1983 Tata demonstrated that both are bifunctional lysozymes / chitinases and with antifungal activity (Jekel *et al*, 1991; Pumphrey, 1994; Posch *et al*, 1997; Randolph, 2001). Hevamine has been crystallised for X-ray diffraction studies and described in 1990 (Jekel *et al*, 1991). Hevamine A is a 29.5 kD, trypsin-sensitive, basic protein and plays a role in plugging the latex vessels and cessation of latex flow (Jekel *et al*, 1991; Beezhold *et al*, 1994a; Beezhold *et al*, 1994b). It catalyses the cleavage of the β-1,4-glycosidic bonds of chitin, as well as the sugar moiety of peptidoglycan (Sussman *et al*, 2002).

Hevamine is most frequently recognised by latex allergic patients with SB, but not HCW^s (De Beer, 2000).

1.3.13.2. Prenyltransferase

Cis-prenyltransferase was first sequenced in 1989 and is a hydrophobic membrane-bound enzyme (Light & Dennis, 1989). It generates multimers of the isoprene subunit, resulting in a polyisoprene chain several thousand isoprene units in length (Light & Dennis, 1989; Randolph, 2001; Sussman *et al*, 2002). It is found both free in the cytosol and in association with rubber particles, and together with REF, plays a role in the elongation of polyisoprene chains (Dennis *et al*, 1989a; Light & Dennis, 1989). It is a dimer with a monomeric MW of 38 kD and is stabilised by thiols. Purified prenyltransferase and deproteinated rubber particles constitute 40-60% of the biosynthetic activity of whole NRL in samples matched for rubber content. Like other prenyltransferases, this enzyme requires a divalent cation (Mg^{2+}) to catalyse thousands of 1-4 *cis* condensations (Z-oligomerisation) of IPP (the prenyl acceptor) to rubber (the prenyl donor) before random termination occurs (Light & Dennis, 1989).

1.4. LATEX GLOVES

The most important reason why HCW^s wear gloves is to protect themselves and their patients against the transmission of microbial, viral and bloodborne diseases, such as pyogenic organisms, mycobacteria, fungi, spirochaetal diseases, parasites, hepatitis

and human immunodeficiency virus (HIV) (Heese *et al*, 1991; Committee Report, 1993; Cohen *et al*, 1998). In 1987, the USA Center for Disease Control adopted the following "Universal Precautions for Prevention of Viral Transmission in the Health Care Setting" (Grzybowski *et al*, 1996; Cohen *et al*, 1998; Bowyer, 1999a):

> *"All healthcare workers should routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or other body fluids of any patient is anticipated. Gloves should be worn for touching blood and body fluids, mucous membranes or non-intact skin of all patients; for handling items or surfaces soiled with blood or body fluids, and for performing venepuncture and other vascular-access procedures."*

This was followed by the Occupational Safety and Health Administration bloodborne pathogen standard in 1992 (Garabrant & Schweitzer, 2002). Virtually all persons who work in medical laboratories, phlebotomy and intravenous services, nursing, respiratory therapy, radiology and dental clinics, as well as emergency medical, fire and police personnel started wearing protective gloves and eye protection when they performed venepuncture, attended trauma-injured patients or when they were exposed to soiled dressings, human blood, or tissue products (Bubak *et al*, 1992). Because products manufactured from NRL are impermeable to transmissible viruses, especially HIV and hepatitis, the demand for NRL gloves increased more than eight-fold between 1987 and 1988 (Randolph, 2001).

Before the adoption of this universal precautions, an estimated 300 million gloves were used annually (D'Epiro, 1996). The use of gloves in the USA increased from twelve billion pairs used in 1987 to 20 billion pairs in 1996 (Meade *et al*, 2002) and 200 billion pairs used in 1997 (Toraason *et al*, 2000). Glove importation to the USA increased from one billion in 1987, to eight billion in 1988 and 21 billion in 1996 (Hayes *et al*, 2000).

Originally gloves were sterilised by boiling and donned wet over wet hands. With the introduction of dry sterilization, a dusting powder consisting of *Lycopodium* species (moss spores), talcum powder or a mixture of the two (Ellis, 1990), was used to prevent the gloves from sticking to the moulds and to facilitate ease of application (Potter, 1998a; Potter, 1998b). Talcum powder is finely pulverised mineral talc, consisting of a combination of hydrous magnesium silicate (chemically pure talc), calcium magnesium carbonate, calcium magnesium silicate and traces of other related substances (Ellis,

1990; Ellis, 1997). Calcium carbonate is also often used as a mould-releasing agent in the manufacturing process (Beezhold & Beck, 1992).

Initially, talcum powder proved to be inert and caused few problems (Beezhold & Beck, 1992). However, in 1917 the first talc granuloma (*pseudotuberculoma silicoticum*) was diagnosed and more than 50 cases appeared by 1947. By the early 1940s, the dangers of talc were well recognised. The superiority of cornstarch powder (amylose and amylopectin) cross-linked with phosphorus oxychloride or epichlorhydrin and mixed with 1.5% magnesium oxide and 1.5% tricalcium phosphate was reported (Fisher, 1987; Baur, 2003). This mixture remains in use today, despite several reports of starch-induced peritonitis and intraperitoneal granulomas (Ellis, 1990).

To counteract this problem, autoclave sterilisation of powdered gloves was replaced by gamma sterilisation. A rat model showed that autoclaved starch was almost totally absorbed from the peritoneal cavity within a period of 48 hours, while irradiated starch was still not fully absorbed after 70 days. Scanning electron microscopic studies on autoclaved starch showed that the surface of the granules was pitted and cracked, while irradiated starch showed a smooth surface. Autoclave sterilisation significantly degraded the proteins and reduced adhesion and granuloma formation (Beezhold & Beck, 1992). Irradiation, on the other hand, does not damage the granule sufficiently to lead to early absorption (Ellis, 1990).

Complete removal of the allergenic proteins from gloves is necessary to limit or prevent allergic reactions, but it is very difficult to achieve. Chlorination, which is widely used in the production of powder-free gloves, diminishes water-soluble proteins, but has a detrimental effect on the physical properties of the latex (Randolph, 2001). Conventional washing of powdered gloves in saline solution fails to remove all starch particles. Cleansing with 10 mℓ of povidone-iodine followed by rinsing in sterile water, reduced the median number of starch granules per glove from 2 720 granules per mm^2 to 0. Although this technique seems effective, it is rather bothersome to staff and quite expensive (Ellis, 1990). It is common practice in many hospitals in Hong Kong and China to re-use sterile latex gloves by autoclaving. The process of repeated washing, autoclaving and re-sterilisation remove significant amounts of latex antigens from the gloves and reduce their allergenic potential. This could explain the relatively low

incidence of latex allergy in these countries (3.3%) compared to 2-12% in Western populations (Leung *et al*, 1997; Meade *et al*, 2002).

1.5. HISTORY OF LATEX ALLERGY

In 1927 Stern reported a case with recurring urticaria and laryngeal oedema from a rubber dental prosthesis (Downing, 1933; Fuchs, 1994; Nieto *et al*, 1996; Konrad *et al*, 1997; Warshaw, 1998; Randolph, 2001; Meade *et al*, 2002; Ownby, 2002). A second case report by Grimm described asthma caused by a rubber-coated electric cable, which warmed up when the wire was electrified. Results of provocation tests conducted under partial control in both cases, were positive and the symptoms recurred after re-exposure (Fuchs, 1994).

During 1931 to 1932, at least seven cases with irritant and delayed contact dermatitis were reported to a particular type of rubber glove. The USA standardised patch test method was done by applying a piece of the rubber glove to a shaven wrist. Following exposure for 24 hours, a marked dermatitis reaction was evident. Cellophane and two other types of gloves served as controls, but showed no reaction after 24 hours. The same type of dermatitis reaction was induced by application of the same rubber glove in two other patients without clinical sensitisation. No reaction had appeared after 24 hours, but after 72 hours there was a marked reaction with intense itching that persisted for almost three weeks. After this brand of gloves was removed from use, no further reports of dermatitis were documented (Downing, 1933; Ownby, 2002).

The problem was only mentioned again in an allergy handbook by K Hansen in 1957 (Fuchs, 1994; Ownby, 2002). In 1966, two women presented with pruritus vulvae and 33 men with dermatitis caused rubber condoms. Condom allergy clinically presents with dermatitis of the pubis, scrotum and groin, and may also include vulvitis from other contraceptive agents, such as spermicidal jellies, creams, foams, diaphragms and lubricants (Taylor, 1986).

The first clear description of immediate-type hypersensitivity was published in 1979 (Nutter, 1979). This case involved an European housewife with chronic atopic dermatitis who presented with IgE-mediated contact urticaria to NRL gloves (Ranta & Ownby, 2004). This is often incorrectly quoted as the first case of latex allergy in history (Nutter, 1979; Wrangsjö *et al*, 1986; Turjanmaa, 1987; Turjanmaa *et al*, 1988a; Slater & Chhabra, 1992; Kurup

et al, 1993; Moneret-Vautrin *et al*, 1993; Zerin *et al*, 1996). The first latex anaphylaxis was reported by Dr Kristina Turjanmaa in 1984 (Ownby, 2002) and the first death caused by latex allergy was published in the medical literature in 1989 (Grzybowski *et al*, 1996). Latex allergy in the USA was first mentioned in 1988 (Spaner *et al*, 1989; Taylor *et al*, 1989; Cornish & Brichta, 2002) and the first case in the Republic of South Africa (RSA) was diagnosed at Groote Schuur Hospital (GSH), Cape Town, in 1993 (Marais *et al*, 1997; Potter, 2001a; Potter *et al*, 2001b).

By 1994 latex allergy affected at least 17 million adults in the general USA population, 10-40% of HCW^{s} and up to 60% of individuals exposed to multiple surgical procedures, such as children with SB (Cornish & Brichta, 2002). Between 1989 and 1995 a twelve-fold increase in the incidence of latex allergy in Europe was documented and a disproportional increase in type I reactions to NRL overshadowed the decrease in type IV reactions since 1992 (Heese *et al*, 1997).

Increasing interest in latex allergy is evident from the number of publications on latex allergy found per year. It increased from one in 1979, to 85 in 1987 (Newsom & Shaw, 1997) to an average of 130-160 per year in the late 1990s (Ownby, 2002). The main reason for this increase follows confirmation that latex allergy existed (Ownby, 2002). Other factors include (Taylor *et al*, 1999):

1. the increased frequency and duration of exposure mainly as a result of HIV;
2. changes in the manufacturing processes;
3. increased recognition of latex allergy and improved diagnostic techniques;
4. increased awareness among individuals routinely exposed to NRL; and
5. a true increase of latex allergy parallel to increased atopy and sensitivity to many allergens in Western populations.

The acquired immunodeficiency syndrome (AIDS) epidemic compelled HCW^{s} to spend most of their working hours wearing NRL gloves and changing them up to 30 times a day. Political conflict in Liberia, a major world supplier of NRL, led to a severe world-wide shortage of rubber. This caused marginal producers and new and inexperienced glove manufacturers to release inadequately washed and cured products. The leaching process to remove excess proteins or debris had been abbreviated from six months to six weeks or even totally eliminated (Cornish & Brichta, 2002). Phytohormones had also

been used on rubber plantations to stimulate higher yield and protein concentration. The end result was a sacrifice of quality for quantity (Slater & Trybul, 1994; Walls, 1996; Randolph 2001).

Numerous single case reports underline the problem of sensitisation to a whole range of non-medical NRL products, e.g. condoms, toy balloons, household gloves and baby pacifiers ***(Table 1.2)*** (Alenius *et al,* 1996). For example, an eleven-month-old baby presented with a persistent cough since the age of three months. Extensive investigation confirmed latex allergy. The only NRL contact was continuous exposure to the pacifier (Venuta *et al*, 1999). A female patient experienced anaphylactic symptoms at a hair salon within minutes following contact with a NRL adhesive used for the application of hair extensions. Another patient experienced contact urticaria of the lips from contact with a chocolate bar wrapper containing NRL. NRL differs from butyl- or petroleum-based synthetic rubbers, including latex house paints, which do not pose any hazard to latex-sensitive individuals (Wakelin & White, 2002).

1.6. THE IMMUNE SYSTEM AND LATEX ALLERGY

The skin represents the largest organ of the human body. In addition to its structural functions, a specific immunological environment has developed in the skin. The theory of the skin immune system was introduced in 1987 (Bos, 1997). It consists of professional immune cells (macrophages, neutrophils, dendritic cells and lymphocytes) and non-professional immune cells (keratinocytes and sebocytes). The immune system consists of innate and adaptive immunity that utilise two very different mechanisms for host defence (Delves & Roitt, 2000a; Janeway *et al*, 2001; Koreck *et al*, 2003).

1.6.1. Innate Immune Response

The innate system provides early defence against pathogen attack and alerts the immune system that pathogen invasion has begun. It lacks immunologic memory (Jackson & Cerio, 1988). Dendritic cells, including Langerhans cells (LC^s), become activated and act as antigen-presenting cells (APC^s) when pattern-recognition surface receptors recognise distinctive pathogen-associated molecular patterns on micro-organism surfaces. The antigen is processed intracellularly and is presented to T-cells by MHC molecules on dendritic cell surfaces (Delves & Roitt, 2000a).

Table 1.2: Sources of natural rubber latex

Exposure to latex is not limited to medical environments and medical devices. Several household items also contain latex and contact to these items can provoke allergic reactions in sensitised individuals.

Medical	Household
Anaesthetic masks	Baby bottle nipples
Bandages	Balloons
Blood pressure cuffs	Bandages
Breathing bags / circuits	Carpet backing
Bulb syringes / Syringe stoppers	Condoms
Catheters / Foley catheters	Diapers
Cervical dilators	Diaphragms
Colostomy pouches / Urine bags	Dishwashing gloves
Dental devices / Orthodontic elastics	Douche bulbs
Electrode pads	Elastic in clothes
Endotracheal tubes / Feeding tubes	Erasers
Enema retention cuff	Eye droppers
Epidural catheter injection adapter	Hot water bottles
Esophageal dilator and protective cover	Koosh balls
Eyedropper masks	Paints (waterproofing)
Gloves / Face masks / Straps	Raincoats
Haemodialysers	Rubber bands
Hot water bottles / Warming blankets	Rubber toys
Implants / Teeth protectors	Rubber grips (rackets, bicycles, tools)
Injection adaptors / IV injection ports	Shoes
Instrument mats / Rubber sheeting	Sports equipment
Intra-aortic balloon	Swimming goggles / masks
Nasal airways	
Stethoscope tubing / Tourniquets	
Ultrasound covers	
Ventilator bellows and tubing	
Wheelchairs	
Wound drains	

Signaling takes place through the Toll pathway and germline-encoded receptors that are expressed on a wide variety of cells, e.g. macrophages, epithelial cells and neutrophils. The mannose receptor is found on macrophages, but not on monocytes and neutrophils, while the CD_{14} receptor is found predominantly on monocytes and macrophages. Inflammation at the site of infection is initiated by macrophages producing inflammatory mediators that include prostaglandins (PG^s), leukotrienes (LT^s) and platelet-activating factor (PAF), followed by cytokines and chemokines (Delves & Roitt, 2000a; Koreck *et al*, 2003). Alternatively, the inflammatory response is triggered through activation of the complement cascade (Janeway *et al*, 2001).

1.6.2. Adaptive Immune Response

The adaptive immune response is highly specific for a particular pathogen. Although the innate response does not alter on repeated exposure to a given infectious agent, the adaptive response improves with each successive encounter with the same pathogen (Male, 2001). Adaptive immune responses can be classified into three groups, depending on the principal effector cell (Brostoff *et al*, 1991):

- B cells (antibody response, e.g. type I latex allergy)
- CD_8^+ T cells (anti-viral cytotoxicity)
- Macrophages and other myeloid cells (cell-mediated immunity, e.g. type IV latex allergy)

The adaptive immune response involves both cellular and humoral mechanisms, characterised by specific immunological memory (Jackson & Cerio, 1988). Lymphocytes are capable of producing about 10^{15} different B-cell antibody and T-cell receptor variable regions (Delves & Roitt, 2000a). Certain memory T-cells remember the anatomical site where they first encountered an antigen and are recruited back to the skin during inflammation. T-cells that have never been activated by an antigen are called native T-cells and efficiently migrate from blood into lymph nodes and return to blood through efferent lymphatics (Robert & Kupper, 1999). Both T-cell and B-cell receptors have binding sites of only 600-1 700 Å and recognise only a small part of the antigen, the antigenic epitope (Delves & Roitt, 2000a).

The humoral immune response is initiated when a specific antigen binds to the lymphocyte surface. Macrophages present the antigen to the B-cell, which interacts

with the T-cell, replicates and differentiates into a plasma cell (Dahl, 1981c). Some microbial antigens can activate B-cells without the help of T-cells and are called T-cell independent antigens. However, most antigens require help from CD_4 T-cells and are called T-cell dependent antigens. When these antigens are bound to B-cell receptors, they are internalised and processed by the B-cell into short peptides, which are brought to the cell surface by MHC class II molecules. Once the immune system is stimulated by an immunogenic epitope, additional epitopes on the antigen may be drawn into the response as a result of the general upregulation of antigen processing and presentation. This effect, referred to as epitope spreading, may spill over to other antigens (intermolecular spreading). In some autoimmune diseases, e.g. systemic lupus erythematosus, a structural complex of several independent molecules present in the nucleosome may provoke a broad spectrum of autoantibodies (Delves & Roitt, 2000b; Janeway *et al*, 2001).

1.6.3. Complement Activation

The complement system consists of about 20-30 plasma proteins that react with one another to opsonisise pathogens and induce a series of inflammatory responses (Male, 2001). The complement components (C_1 through C_9 plus B, D and P) act on one another sequentially and form the complement cascade. The cascade begins by the binding of C_1q to an antigen-antibody complex or of C_3 to a bacterial or other membrane surface (without the assistance of an antibody). The binding of C_1 initiates the classical pathway and the direct binding of C_3 initiates the alternative pathway (Melvold, 1993). The classical pathway can also be activated during an adaptive immune response by the binding of C_1q to the antibody : antigen complexes. This is thus a key link between the effector mechanisms of innate and adaptive immunity (Janeway *et al*, 2001).

The alternative pathway does not depend on a pathogen-binding protein for its initiation; instead it is initiated through the spontaneous hydrolysis of the thioester bond in C_3 to form C_3b. These molecules coat the pathogen surface and C_3a initiates local inflammatory responses. Complement activation leads to the binding of large numbers of C_3b to C_3 convertase, resulting in the formation of C_5 convertase and the subsequent release of C_3a and C_5a. The small complement fragments C_3a, C_4a and C_5a act on specific receptors to produce local inflammatory responses. These fragments are

known as anaphylotoxins, because production of large amounts induces a generalized circulatory collapse and anaphylactic shock (Yarfitz & Brinkley, 2001; Janeway *et al*, 2001).

C_3a is a chemotactic factor for human mast cells and eosinophils and induces the release of histamine and other vasoactive mediators (Schwartz, 1990). C_5a has a chemotactic effect on granulocytes, monocytes and macrophages, all of which have receptors for C_5a. C_5a concentration is not a good parameter to follow complement activation, because it binds rapidly to the C_5a receptor and, in contrast to C_3a, is cleared from plasma within minutes (Furebring *et al*, 2002). Human skin mast cells express CD_{88}, the receptor for C_5a, but those in the lung do not (Werfel *et al*, 1997; Sabroe *et al*, 2002).

1.6.4. Mast Cells

Mast cells are derived from CD_{34}^+ haemopoietic progenitor cells and are usually round, oval or spindle-shaped, with a round nuclei and diameter of 13.2 ± 0.2 µm (Kambe *et al*, 2001; Prussin & Metcalfe, 2003). The cytoplasm contains membrane-bound granules and lipid bodies (Wedemeyer *et al*, 2000b). Except for the small number of mast cells resident in the bone marrow, maturation typically occurs in the peripheral tissues (Williams & Galli, 2000; Artuc *et al*, 2002). Human mast cells are concentrated in the subepidermis (Burland & Mills, 1982). They are richly distributed in the deeper region of the central nervous system, in upper and lower respiratory epithelium, bronchial lumen, gastrointestinal mucosa and submucosa, bone marrow and the skin. Mast cells enlarge as they increase in age (Wasserman, 1993).

Mast cell numbers in skin are approximately 10 000 / mm^3 and mast cells isolated from human lung, skin, lymphoid tissue and small intestine contain approximately 3-8 pg histamine per cell (Greaves *et al*, 1972; Metcalfe *et al*, 1997; Prussin & Metcalfe, 2003). Histamine is formed from the decarboxylation of the AA histidine in the Golgi apparatus. It is found in most tissues of the body, but is present in high concentrations in the skin and lungs (Burland & Mills, 1982; Rang *et al*, 1995). Histamine and PG D_2 cause dilatation and increased permeability of blood vessels and are largely responsible for the clinical manifestations of allergic reactions, such as rhinitis, asthma and urticaria (Yarfitz & Brinkley, 2001).

Stem cell factor (SCF) is the major growth factor and acts on the cell-surface receptor, c-Kit (Wedemeyer & Galli, 2000a; Williams & Galli, 2000; Kambe *et al*, 2001; Boyce, 2003).

SCF maintains mast cell viability and promotes maturation (Metcalfe *et al*, 1997). The c-Kit receptor is restricted to only a few cell types in the skin, such as mast cells and melanocytes and is expressed early in mast cell differentiation together with other cytokine receptors (Welker *et al*, 2000). These other cytokine receptors are lost during progenitor cell maturation, whereas c-Kit expression is maintained (Bühring *et al*, 1999). Mast cells express FcεRI, the high affinity receptor for the Ig superfamily, on their surface and are activated when allergens dimerise and cross-link pre-formed IgE bound to these receptors ***(Figure 1.2)***.

Human mast cells can bind with high affinity in excess of 1×10^5 IgE molecules to specific Fc receptors on their cell surface (Church *et al*, 1982; Hide *et al*, 1993). Dermal mast cells, however, rarely seem to be fully sensitised with IgE (Sabroe *et al*, 2002).

Two types of human mast cells have been identified based on different compositional and ultrastructural properties as well as T-cell dependency. These are referred to as MC_T (tryptase-positive and chymase-negative, mucosal-like) and MC_{TC} (tryptase-positive and chymase-positive, connective tissue like) cells on the basis of their distinctive neutral protease compositions. Mast cells in healthy-looking human skin are mainly MC_{TC} cells (Irani *et al*, 1989; Saarinen *et al*, 2001). Activation can be elicited both by immunologic (IgE or substance P) secretagogues, and non-immunologic (opiates, aspirin and non-steroidal anti-inflammatory) secretagogues (Beltrani, 1999).

Activation leads to mast cells degranulation within seconds and release inflammatory mediators (chymase, eicosanoids, histamine, tryptase and other neutral proteases), proteinases (carboxypeptidase A and small amounts of sulphatases and exoglycosidases), chemokines and additional cytokines (such as interleukin (IL)-4 and IL-13) (Kirshenbaum *et al*, 1999). In addition, newly generated lipid mediators, such as PG D_2, LT C_4, and PAF are liberated. PG D_2 and LT C_4 are released only on activation via IgE (Koppert *et al*, 2001). This leads to a T_{H2} response. Immediate reaction caused by mast cell degranulation is followed by a more sustained inflammation, the late phase reaction that involves recruitment of other effector cells, e.g. T_{H2} lymphocytes, eosinophils and basophils (Janeway *et al*, 2001).

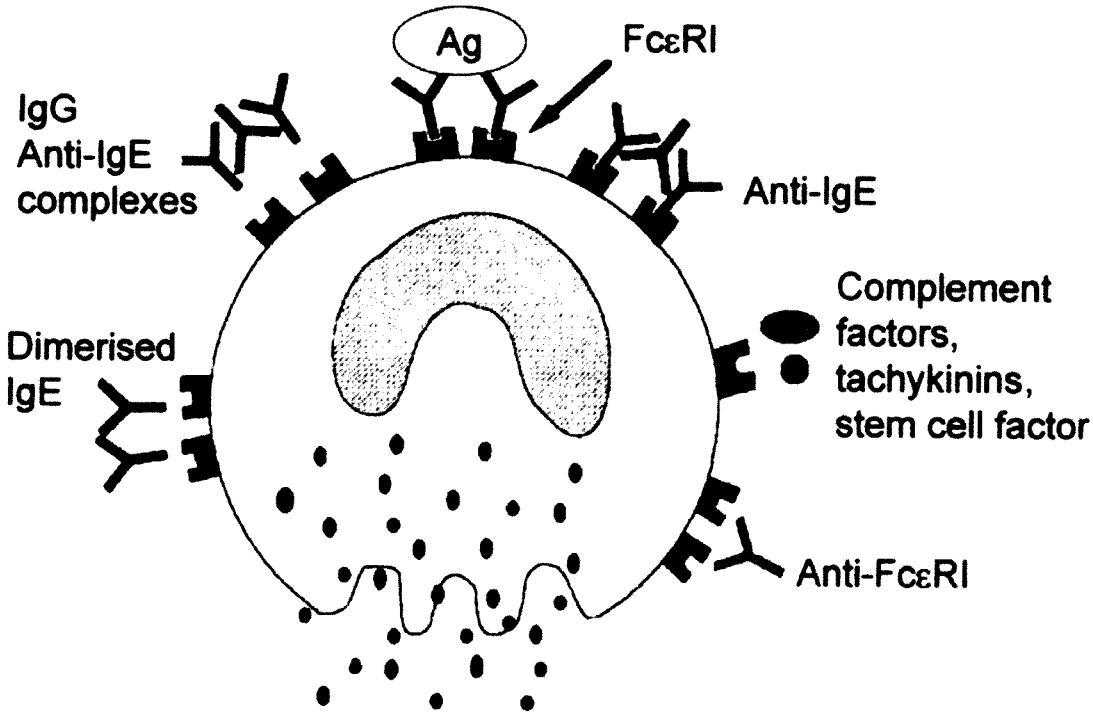


Figure 1.2: Mast cell activation caused by FcεRI cross-linking

When an antigen combines with specific IgE, it is dimerised (Hide *et al*, 1993). The binding of the α-chain of FcεRI occurs via the Fc region of IgE in a 1:1 ratio (Metcalfe *et al*, 1997; Prussin & Metcalfe, 2003). The FcεRI is itself cross-linked and this leads to mast cell or basophil activation. IgG anti-IgE causes similar cross-linking and mast cell activation (Greaves 2000).

Histamine in the blood appears maximally by 30 minutes after allergen challenge and tryptase by 60 minutes (Schwartz, 1989). Maximal histamine release is obtained when 10% of the receptors are aggregated and a detectable response is achieved when ± 100 of 3×10^5 receptors per cell are aggregated (Metcalfe *et al*, 1997). H_1 mediates bronchoconstriction, vasoconstriction and oedema formation. Stimulation of pulmonary H_2 receptors plays an important modulatory role, causing bronchodilation, inhibiting the further release of mediators (histamine, PG^s and lysosomal enzymes) and influencing the pulmonary vascular responses (Eyre & Chand, 1982). H_1 receptors are coupled to the phosphatidylinositol hydrolysis pathway with histamine binding resulting in the mobilisation of intracellular Ca^{2+}. H_2 receptors are coupled to the adenyl cyclase system leading to increased cAMP production (Fitzsimons *et al*, 1997).

Both tryptase and histamine are present in secretory granules of mast cells and are therefore released simultaneously. The different time course with which they appear in the circulation and blister fluid after allergen-mediated simulation of tissue mast cells reflects their unequal rates of diffusion from tissue to fluids (Schwartz, 1990).

1.6.5. Basophils

Basophils are also derived from bone marrow, but unlike mast cells, differentiate into white cells that circulate in the peripheral blood from where they can be recruited into the tissues (Carman, 1994). The number of basophils in human blood is relatively constant, about 40 basophils / mm^3 blood. They form less than 3% of the total leucocytes and have an estimated half-life of only six hours (Beaven, 1982). Mediators stored preformed in the cytoplasmic granules of basophils include chondroitin sulphates, proteases and histamine. Basophils express FcεRI on their surface and when they are activated by cytokines or antigens, release histamine, IL-4 and IL-13. They express little or no c-Kit, cell-associated tryptase, chymase or carboxypeptidase A (Wedemeyer & Galli, 2000a; Janeway *et al*, 2001).

Mast cells and basophils share certain morphological and biochemical properties, e.g. both possess receptors for IgE, C_3a and C_5a (Plaut & Lichtenstein, 1982). However, they differ from each other in terms of mediator production, surface receptor expression, and response to distinct stimuli (Bühring *et al*, 1999). Basophils possess a polylobed nucleus and differ form mast cells in their relatively smooth cell surface and granule morphology, which is larger and less structured than that of the mast cell (Wasserman, 1993).

1.6.6. Eosinophils

The eosinophil has a long association with allergic disease of the lung and other tissues. Eosinophil chemotaxis occurs in response to many stimuli, including antigen-antibody complexes, complement, PG^s and histamine (Eyre & Chand, 1982). Eosinophils are also involved in immune responses against large parasites and are capable of killing them by direct contact (Melvold, 1993).

On activation, eosinophils release highly toxic granule proteins and free radicals, which can kill micro-organisms and parasites, but can also cause significant tissue damage in allergic reactions. It also induces the synthesis of chemical mediators, such as PG^s, LT^s

and cytokines, which amplifies the inflammatory response by activating epithelial cells and recruiting and activating more eosinophils and leukocytes. Eosinophil degranulation releases major basic protein, which in turn causes degranulation of mast cells and basophils. Eosinophils express FcεRI only when they are activated (Janeway *et al*, 2001).

1.6.7. Langerhans Cells

LC^s form part of the macrophage-monocyte system and are derived from the bone marrow. They are present as dendritic cells in all parts of the epidermis, but more in the upper spinosum layer, in lymphoid tissue and at sites with closest contact with external environments (Cumberbatch *et al*, 2000). They have dark nuclei, a pale or clear cytoplasm and are characterised by a unique cytoplasmic organelle, known as the Birbeck granules. Other dendritic cells in the dermis lack the Birbeck granules. Depending on the anatomical location, the density of LC^s ranges between 490 and 1 000 / mm^2 and they account for about 2% of the epidermal cell population (Seité *et al*, 2003). Their surface receptors are similar to that of macrophages (receptors for Ig Fc and complement C_3) and show high expression of CD_1a and MHC class I and II molecules. By 1992 the IgE-binding capacity of epidermal LC^s via the low affinity IgE receptor FcεRII was described (Novak *et al*, 2003).

LC^s are the most potent APC^s of the skin immune system (Saloga & Knop, 2000). During the sensitisation phase of a cutaneous hypersensitivity response, LC^s take up the antigen, which is degraded by adhering MHC class II molecules and proteolytic enzymes. E-cadherin, which mediates LC-keratinocyte adhesion, is downregulated and induces the detachment of LC^s from neighbouring keratinocytes. The secretion of matrix metalloproteinases dendritic cells digest extracellular matrices and facilitates their crossing of the basement membrane and migration through the collagen thicket. When they gain access to draining lymphatic vessels in the dermis, they initiate a primary immune response by activating T-cells and producing memory T-cells. While migrating, LC^s mature as dendritic cells and are characterised by lower amounts of Birbeck granules in the cytoplasm with subsequent higher expression of MHC Class II molecules on the plasma membrane (Janeway *et al*, 2001; Stoitzner *et al*, 2002; Stoitzner *et al*, 2003).

LC^s do not possess an innate ability to distinguish between an antigenic challenge (which warrants signal transduction to peripheral lymphoid tissue) and other changes in the local environment, which does not necessarily need an immunogenic stimulus. They are primed to deliver samples of the cutaneous antigenic environment to lymph nodes at times of local tissue damage or disruption sufficient to induce upregulation of pro-inflammatory cytokines (Cumberbatch *et al*, 2000). Their capacity as APC^s is important in several forms of delayed-type hypersensitivity reactions, including contact hypersensitivity and rejection of skin grafts (Seité *et al*, 2003). The late-phase reaction is dependent on the interaction of allergen with cells bearing surface-bound, allergen-specific IgE (Ying *et al*, 1998; Beltrani, 1999). Preferential uptake and presentation of IgE-bound allergens by epidermal LC^s via FcεRI is an important mechanism of cutaneous inflammation in atopic dermatitis. LC-like dendritic cells derived from monocytes of atopic dermatitis patients carry high levels of FcεRI and low levels of CD_{23} or FcεRII, while monocytes from healthy subjects express only low levels of FcεRI (Reich *et al*, 2001).

Acute disruption of the epidermal barrier by tape stripping has been reported to increase LC expression of MHC II and CD_{86} and induce a T_{H2}-dominant cytokine response (Woolhiser *et al*, 2000). Similar disruption by acetone treatment can cause a significant increase in epidermal LC density within 24 hours. A German study showed that open application of patch test allergens following acetone pre-treatment resulted in strong allergic reactions and a 2.4-fold increase in LC density. Epidermal proliferation also showed a six-fold increase and strong bullous reactions. This increased LC density was also accompanied by an enhanced response in allergic contact dermatitis (Proksch & Brasch, 1997).

Occlusion is an effective way of enhancing the penetration of many substances through the skin barrier and may be regarded as a very mild stimulus to the epidermis. It causes cellular changes in epidermal cells and the barrier capacity of the SC (Mikulowska, 1990; Emilson *et al*, 1993). It also increases mitotic activity, cause epidermal thickening and induce LC^s to move to a more central position in the epidermis. It induces a transient response in the skin compatible with an inflammatory reaction and can even enhance wound healing (Emilson *et al*, 1993). In occluded guinea-pig skin, LC^s had a more dendritic appearance than normal skin. Cells had larger vacuoles than

normal, dilatated endoplasmic reticulum and a more villous cell membrane (Lindberg & Forslind, 1981). Occluded rat skin showed that CO_2 emission increased during the first three hours and then remained constant for the next 24 hours. Human skin showed a widening of intercellular spaces within the first three hours. This was accompanied with by intracellular vacuolisation in keratinocytes, formation of intercellular debris and spongiosis. The number of LC^s also increased (Lindberg *et al*, 1982). All these signs can be ascribed to enhanced activation. LC^s are therefore not only immunocompetent cells, but also seem to be alerted in presumably non-immunoreactive situations as well (Mikulowska, 1990).

1.6.8. Immunoglobulin E

In 1967 IgE was confirmed to be the reagin in serum that mediates immediate-type wheal and flare reactions (Greaves *et al*, 1972; Zeiss & Pruzansky, 1993; Prussin & Metcalfe, 2003). IgE is produced when IL-4 acts on IgM-IgD-bearing resting B lymphocytes. The effect is enhanced by T cells, CD_4 and CD23. IFN-γ, which suppresses IgE synthesis, acts on this same point. This leads to the formation of precursor B lymphocytes. During terminal differentiation of IgE B cells to plasma cells producing IgE, IL-5, IL-6 and IgE potentiating factor enhances and IgE suppressor factor inhibits the process (Zeiss & Pruzansky, 1993). It has a size of approximately 190 kD and circulates the blood as a monomer. It shows no transplacental transfer, and unlike other Ig^s, does not activate complement by the classic pathway. IgE is thermo-labile and will not sensitise after it has been heated to 56°C for several hours. Its concentration is highly age-dependent and it constitutes approximately 0.0005% of the total serum Ig^s in adults. Approximately 51% of total body IgE is estimated to exist in the intravascular compartment (Zeiss & Pruzansky, 1993; Hamilton & Adkinson, 2003; Prussin & Metcalfe, 2003). The mean total circulating IgE was found to be 3.3 µg/kg/day. The rate of production is approximately 2.3 µg/kg/day. IgE has a very short half-life, with an intravascular survival of only 2.3 days. The half-life in the skin was found to be between eight and 14 days (Zeiss & Pruzansky, 1993; Platts-Mills, 2001).

FcεRI has a binding constant in the 10^{-9}-10^{-10} M range for its ligand, IgE (Beaven, 1982; Metcalfe *et al*, 1997; Wedi *et al*, 2000). It is expressed in large numbers in a tetrameric ($\alpha\beta\gamma_2$) form (an IgE-binding α-subunit, a tetraspan β subunit and two identical disulfide-linked γ subunits) and in smaller numbers in a trimeric form ($\alpha\gamma_2$) on eosinophils,

monocytes, macrophages, dendritic and LCs. The α- and γ-chains each cross the cell membrane with a single helical segment, while the β-chain spans the bilayer four times. The extracellular domain of the α-chain binds IgE with high affinity, and the γ-chain transduces signals via two immunoreceptor tyrosine-based activation motifs in its cytoplasmic tail. The β-chain acts as an amplifier of signalling downstream of the receptor. In addition, the β-chain augments the maturation of the α-chain and its intracellular trafficking to the cell surface, leading to increased surface expression. In rodents all three subunits must be present for efficient cell surface expression, while human cells can express FcεRI function in the absence of the β-chain (Metcalfe *et al*, 1997; Wedemeyer & Galli, 2000a ; Williams & Galli, 2000; Wilson *et al*, 2000; Wurzburg & Jardetzky, 2001; Novak *et al*, 2003; Prussin & Metcalfe, 2003; Takagi *et al*, 2003). Although other cell populations, such as LCs, can bind IgE through the FcεRI receptors in human skin, it has been shown that non-lesional skin from healthy patients contains few IgE^+ cells, all of them located in the dermis (Brazís *et al*, 2002).

IgE is produced by plasma cells in lymph nodes (adenoids, tonsils, bronchial, peritoneal and plasma cells) and is tightly bound to the mast cell surface through FcεRI. Signalling is initiated by the activation of a myristylated (membrane-associated) Src-related protein tyrosine kinase, Lyn, following FcεRI cross-linking. After signalling, cross-linked FcεRI are endocytosed through coated pits and transported to the endosomal system for degradation (Wilson *et al*, 2001). It seems likely that presenting an antigen transmucosally at very low doses favours the activation of T_{H2} cells over T_{H1} cells. IgE antibody production requires T_{H2} cells that produce IL-4 and IL-13 (Janeway *et al*, 2001).

IgE-receptor density reflects circulating levels of the antibody (Benyon *et al*, 1987) and FcεRIα expression correlates with serum IgE levels (Wurzburg & Jardetzky, 2001). However, it does not correlate with FcεRI levels on peripheral blood-derived monocytes and eosinophils. Serum IgE levels correlate well with the *in vivo* number of IgE molecules per basophil, suggesting a strong positive correlation between total serum and cell-bound IgE on human basophils. The affinity of IgE receptors on human basophils ranges from 7.1×10^8 to 2.8×10^{10} M (Malveaux *et al*, 1978). Total IgE levels are influenced by age, genetic predisposition, ethnicity, immune status, season of the year and some disease processes. Increased serum IgE levels are found in parasitic

diseases, infections, cutaneous diseases, such as atopic dermatitis and neoplastic conditions, and immunodeficiency diseases (Prussin & Metcalfe, 2003).

During sensitisation the Fab (antibody fragment) on an IgE antibody binds to the allergen and the Fc fragment binds to IgE receptors on mast cells and basophils. On re-exposure to the allergen, the free arm of the Y-shaped IgE molecule binds to the allergen. If two IgE molecules are engaged, a bridge is formed between them and the two receptors are brought closer together. Aggregation of receptors activates the mast cell, which releases allergic mediators, such as histamine, PAF, LT^s and cytokines (Carman, 1994). Recombinant allergens are able to induce histamine release from basophils in allergic donors, but allergen fragments that cannot bind IgE are unable to elicit mediator secretion (Hauswirth *et al*, 2002). Histamine release induced by anti-IgE is slow (five to six minutes) and completely dependent on the presence of an extracellular source of calcium (Lowman *et al*, 1988). The high affinity binding of IgE by FcεRI and subsequent stabilisation upregulate FcεRI on mast cells and basophils even as serum IgE decreases. The memory produced this way persisted *in vivo* for as long as 56 days, despite the finding that serum levels of injected IgE had become undetectable by day six. This mechanism of memory formation and maintenance is totally different from the immunological memory of T and B cells (Kubo *et al*, 2003).

1.7. CLINICAL LATEX ALLERGY

An individual who has an allergic reaction elicits immune responses to environmental or other antigens, causing inflammation and damage to the body. Most often, the actual allergens are relatively harmless, and it is the immune response that causes the damage. There are four types of allergic reactions, as classified by Gell and Coombs (Janeway *et al*, 2001; Platts-Mills, 2001; Kemp & Lockey, 2002):

Type I IgE-dependent immediate reaction,
Type II cytotoxic IgG or IgM dependent reaction,
Type III involves the immune complexes and is dependent on IgG and IgM, and
Type IV delayed T-lymphocyte dependent reaction.

Type I-III humoral responses elicit immediate reactions (two minutes to eight hours), while symptoms of a cell-mediated response (type IV) can be delayed for days following

antigen exposure (24-72 hours). Each type involves distinct mechanisms, cells, mediator molecules, initiation time and clinical manifestations (Yarfitz & Brinkley, 2001).

Latex allergy is caused by repeated exposure to NRL proteins (Alenius *et al*, 1994; Beezhold *et al*, 1994b; Yip *et al*, 2000). It may clinically manifest as irritant contact dermatitis (ICD), immediate-type I allergic reactions, eczematous allergic contact dermatitis (ACD), or a combination of two or more (Fisher, 1997; Ranta & Ownby, 2004). In a German study of 39 HCW^{s}, delayed-type allergy accounted for 82% of occupational latex allergy, whereas type I only accounted for 33%. Although it was a small study, the results suggest that ACD is more common in HCW^{s} and that some HCW^{s} experience both type I and IV hypersensitivity reactions (Heese, 1991; Ranta & Ownby, 2004). The different reactions are compared in ***Table 1.3***.

1.7.1. Immediate Type I Latex Allergy

Type I or immediate hypersensitivity is characterised by the production of IgE antibodies against foreign proteins that are commonly present in the environment (Platts-Mills, 2001).

Type I allergy has been divided into four clinical stages, first described by Maibach and Johnson in 1975 (Turner, 1997; Warshaw, 1998):

Stage 1: localised urticaria where there is a contact wheal-and-flare response

Stage 2: generalised urticaria, with dilation of capillaries and relaxation of smooth muscles due to histamine release

Stage 3: urticaria and bronchial asthma, sneezing, coughs, nasal congestion and conjunctivitis

Stage 4: urticaria with anaphylaxis when there is hypotension, shock, dyspnoea, possibly leading to death

Latex allergy is a typical occupational, immunologically-mediated immediate type I reaction (Levy *et al*, 1992; Bowyer, 1999b). Unlike toxins, allergens such as NRL, elicit their adverse effects in two stages. The first sensitising stage is characterised by a relatively extended time period between the stimulus and the detection of antibodies (Virella, 1993). It induces a humoral response, generating antibody-secreting plasma

Table 1.3: Clinical manifestation of latex allergy

NRL gloves can cause different allergic reactions. Consideration of the different variables should enable proper distinction and accurate diagnosis (Ranta & Ownby, 2004)

Variable	Irritant	Type I	Type IV
Aetiology	Occlusion, maceration	IgE-mediated	T-cell mediated
Require previous sensitisation	No	Yes	Yes
Onset of reaction	Gradual	Acute: within 30 mins	Subacute / delayed: 24 – 48 hrs > exposure, can be 8 hrs – 5 days
Characteristics	Dryness, scaling, cracking, erythema of hands	Urticaria, angioedema, pruritus, allergic rhinitis, asthma, anaphylaxis	Erythema, scaling, vesiculation
Triggers / exacerbants	Frequent hand washing, harsh chemicals, cold winter weather	NRL proteins	Chemicals e.g. accelerators and antioxidants
Location of reaction	Localised	Localised / generalised	Localised

cells and memory cells able to provoke a more powerful response on re-exposure (Yarfitz & Brinkley, 2001). In the second stage repeated exposure to the allergen elicits the effector response. The threshold levels for the two stages are different (Poley & Slater, 2000).

The enhanced secondary response needs a low threshold dose of the immunogen and has a shorter lag phase than the primary response. The antibody concentration rises sooner, peak concentrations are higher and high levels of antibody persist for longer periods of time (Virella, 1993, Cornish & Brichta, 2002). It is characterised by the cross-linking and degranulation of mast cells and basophils, with the subsequent release of inflammatory mediators (Castells & Schwartz, 1988; Schwartz, 1990; Shalit *et al*, 1990, Chambeyron *et al*, 1992; Cohen *et al*, 1998, Vanderhaeghe & Bouic, 1999). These mediators increase vascular permeability, vasodilatation and bronchoconstriction and present

clinically as urticaria, hypotension and asthma (Alenius *et al*, 1991; Levy *et al*, 1992; Kelly, 1996).

Type I latex allergy is mainly caused by NRL proteins remaining on the surface of NRL products, e.g. gloves (Turjanmaa *et al*, 1988a; Spaner *et al*, 1989; Taylor *et al*, 1989; Tarlo *et al*, 1990; Heese *et al*, 1991; Beezhold *et al*, 1994a; Slater 1994; D'Epiro, 1995; Hadjiliadis *et al*, 1996; Nieto *et al*, 1996; Taylor & Praditsuwan, 1996; Pretorius, 1999). Typical symptoms occur within a few minutes up to an hour of exposure (Turjanmaa & Reunala, 1988b; Taylor & Praditsuwan, 1996; Fisher, 1997). It usually disappears within hours, but may take up to 24 hours to clear. Type I late-phase reactions are mediated by low-affinity receptors and peak six to twelve hours after exposure (Jackson & Cerio, 1988; Metcalfe *et al*, 1997; Warshaw, 1998; Wrangsjö *et al*, 2001).

IgE response against NRL proteins varies considerably in individuals with latex allergy (Alenius *et al*, 1994). It depends on the load of constant allergen exposure, features of transepidermal penetration and individual immunologic response. Individuals may develop allergic manifestations within a year of exposure to high concentrations of latex proteins. A person can progress from being asymptomatic, through a phase of developing ACD to rubber chemicals (Wrangsjö *et al*, 1986), to localised contact urticaria, with a gradual progression to respiratory symptoms and anaphylaxis. Others quickly go from being asymptomatic to developing quite severe bronchospasm and rhinoconjunctivitis without developing any skin lesions (Potter 1998a; Potter, 1998b). About 25-50% of latex allergic individuals are asymptomatic and the first symptom may be a life-threatening reaction (Marcos, 1997; De Beer, 2000; Brathwaite *et al*, 2001; De Beer & Cilliers, 2001).

Skin manifestations in latex allergy are mostly limited to the dorsal surface of the hands, metacarphalangeal joints, thenar, hypothenar, wrist and distal areas of the forearms. These regions are continuously exposed to latex proteins within NRL gloves. The occlusion caused by the wearing of gloves subsequently leads to LC activation. Localised erythema, eczema, pruritus and urticarial wheals are the most frequent symptoms (Beuers *et al*, 1990; Pecquet *et al*, 1990; Warshaw, 1998; Holme & Lever, 1999; Pretorius, 1999). Extension beyond the contact site, generalised cutaneous symptoms and other IgE-mediated clinical diseases, such as asthma and bronchospasm, may also occur (Beuers *et al*, 1990; Heese *et al*, 1991; Hamilton *et al*, 2002; Weissman & Lewis, 2002).

Once the specific IgE molecules reach the circulation via haematogenous spread, systemic involvement may arise. Laryngeal oedema may cause severe upper respiratory obstruction, which may progress to asphyxia, hypotension and cardiovascular collapse (Heese *et al*, 1997). If the gastrointestinal system is involved, nausea, vomiting or abdominal cramps may occur. Respiratory or cardiac system involvement is characterised by angioedema, bronchial asthma, rhinoconjunctivitis, hypotension or anaphylaxis (Fisher, 1997).

Mucosal swelling occurs typically after oral, vaginal, rectal or other mucosal contact with NRL products, e.g. balloons, catheters, gloves and condoms (Levy *et al*, 1992; Turjanmaa *et al*, 1996). NRL urethral catheters can induce urethritis and urethral strictures in some patients. These strictures cause poor blood flow and the release of toxic substance from the catheters, leading to catheter toxicity. The toxins accumulate in the surrounding tissue and produce major antigenic complexes, tissue damage and scarring. NRL catheters activate complement substantially more than non-NRL catheters (Garred *et al*, 1990; Moneret-Vautrin *et al*, 1993).

Anaphylaxis can occur via any method of exposure, but especially during medical and dental procedures due to contact with mucosal and vascular membranes (Axelsson *et al*, 1987; Spaner *et al*, 1989; Belsito, 1990; Beuers *et al*, 1990; Tarlo *et al*, 1990; Pequet, 1990; Alenius *et al*, 1991; Laurent *et al*, 1992; Kurup *et al*, 1993; Bowyer, 1999b). The respiratory symptoms and shock are due to a massive local release of histamine and other mediators following adsorption and systemic distribution of the antigens (Baur & Jäger, 1990). NRL-containing devices may cause other localised allergic manifestations intraoperatively, such as angioedema after application of an anaesthetic mask, bronchospasm after tracheal intubation, and oedema of the larynx after removal of an intubation cannula (Moneret-Vautrin *et al*, 1993).

Anaphylaxis in latex allergic persons may occur once every 13.6 exposures (Kelly *et al*, 1994b). Death, usually due to organ damage sustained during anaphylaxis, can occur within minutes, or even many days after anaphylaxis (Kelly *et al*, 1994a; Manjra, 1994). Whether pulmonary or cardiovascular symptoms will predominate during anaphylaxis is difficult to predict. In some individuals, prodromal symptoms include sneezing, conjunctivitis, generalised pruritus or urticaria, whereas in others the onset of anaphylaxis may be precipitous. The risk of fatality is partially determined by the

rapidity with which the NRL allergen penetrates into the body. Anaphylaxis can be fatal even with timely therapy (Randolph, 2001).

It is still controversial if a clear relationship exists between latex-specific IgE levels and severity of symptoms. While some studies report a positive correlation (Jaeger *et al*, 1992), others could not find any clear relationship (Hadjiliadis *et al*, 1996). Inhaled allergens show an agreement of up to 75% between SPT results and symptoms, although it is much lower with ingested allergens (Jackson & Cerio, 1988).

1.7.2. Delayed Type IV Latex Allergy

Type IV reactions are mediated by antigen-specific effector T-cells. It can be elicited by either CD_4 or CD_8 T-cells, depending on the pathway by which the antigen is processed. Typical antigens are highly reactive small molecules that can easily penetrate intact skin. They then react with self proteins, creating protein-hapten complexes that are recognised by T-cells as foreign antigens. Sensitisation takes ten to 14 days in humans (Britton, 2001). During the sensitisation phase, LC^s process the antigen and migrate to regional lymph nodes. T-cells are activated and stimulate the production of memory T-cells, which end up in the dermis. In the elicitation phase, the antigen is presented to the memory T-cells in the dermis, with the release of T-cell cytokines, such as interferon (IFN)-γ and IL-17 (Zerin *et al*, 1996; Fisher, 1997). Stimulated epidermal keratinocytes release cytokines (IL-1, IL-6, TNF-α and granulocyte-macrophage stimulating factor [GM-CSF]) and chemokines (IL-8 and interferon-inducible proteins 9 and 10). These cells enhance the inflammatory response by inducing migration of monocytes into the lesion, maturation of macrophages and attraction of more T-cells (Janeway *et al*, 2001).

ICD, also known as *non-immunologic contact urticaria*, occurs without any previous sensitisation. It is not an allergic condition and the effects are usually reversible (Bowyer, 1999b). It is caused by different chemicals found in NRL or other glove components that promote a non-antibody-mediated release of vasoactive mediators, such as histamine, PG^s, LT^s or substance P (Heese *et al*, 1991; Cohen *et al*, 1998). ICD is a result of local irritation, necrosis and elimination of superficial epidermal cells (Randolph, 2001). It presents clinically as erythema, pruritus and oedema, followed by lichenified, crusted papules on the exposed areas (Cohen *et al*, 1998). It affects 25-40% of regular glove wearers (Bowyer, 1999b). Prolonged and repeated NRL exposure is

aggravated by sweating and rubbing due to glove occlusion, leading to papular and ulcerative lesions (Heese *et al*, 1991; Cohen *et al*, 1998). An individual with ICD is at higher risk of developing IgE-mediated sensitivity. Irritants diminish the natural barrier of the skin, permitting more allergens to penetrate from the gloves into the body (Belsito, 1990; Fullerton *et al*, 1996; Randolph, 2001).

Unlike ICD, ACD directly involves the interaction of the *immune system* and depends on the patient's genetic susceptibility (Bubak *et al*, 1992). The offending antigen is introduced epicutaneously through intact skin. The sensitising agents are typically unstable reactive molecules that can form complexes with host proteins (Robert & Kupper, 1999). NRL-related ACD is caused by T-cell mediated sensitisation to rubber additives, usually residual low MW chemical additives and accelerators, e.g. thiurams, carbamates, amines and benzothiazoles (Toraason *et al*, 2000; Kurup & Fink, 2001). Once a patient is sensitised, subsequent challenges from the same allergen will cause a type IV reaction within a few minutes up to an hour after exposure (Taylor & Praditsuwan, 1996; Fisher, 1997; Warshaw, 1998). It may also develop up to five days after exposure (Potter, 1996b; Cohen *et al*, 1998). The reaction is usually confined to the site of contact, but can spread peripherally and reaches a maximum after 24-48 hours. It is characterised by erythematous, vesicular and eczematous skin lesions, and can be associated with oedema and pruritus (Bowyer, 1999b). Patients with ACD alone seldom have systemic symptoms (Potter, 1996b).

Distinction between ACD and ICD is difficult. When a patch test result is strongly positive, it is usually possible to differentiate the sharply demarcated, bullous or pustular irritant reaction from the spreading eczematous reaction to an allergen. When a primary irritant is applied to the skin and occluded, minimal itching, erythema and slight infiltration will usually be restricted to the occluded area. Substances to which a patient has a cellular response tend to give a markedly pruritic, infiltrated, papular or vesicular reaction, which extends beyond the rim of the occluding disc. However, there is no morphological way of distinguishing a weak irritant patch test from a weak allergic test (Fisher, 1986; Veien, 1992). Histochemical identification of cutaneous cellular inflow following exposure could be used to identify ACD / ICD. Microcutaneous perfusion could shed light on the inflammatory mediator cascade to identify ACD and ICD.

With continued exposure, skin lesions become chronic and develop a lichenified, crusted and thickened appearance (Sussman & Beezhold, 1995). Lesions disappear gradually if exposure is discontinued. The frequent simultaneous positive patch tests to thiurams and chemically related carbamates could be a result of coincidental sensitisation and cross-reactions (Heese *et al*, 1991). A large prevalence study in Hong Kong showed that 31% of their subjects suffered from glove dermatitis, compared to 16-22% in Australia and 40% in France (Leung *et al*, 1997). A German study confirmed that 2.6% of their patients were sensitised to at least one rubber chemical (Gutgesell *et al*, 2000). A multicenter study conducted by the British Contact Dermatitis Group reported a delayed-type NRL hypersensitivity in about 1% of their dermatology population and found it particularly problematic in a proportion of patients with hand eczema. Patients with positive patch tests should therefore be investigated for contact urticaria or type I latex allergy (Sommer *et al*, 2002).

Protein contact dermatitis and urticaria are common in occupational dermatology. The increasing number of eliciting environmental agents includes food proteins, e.g. fish, egg, flour, meat, vegetables, fruits and spices, and also allergens from animals, e.g. hair, saliva and placenta. Occupational contact urticaria is relatively common in veterinary surgeons working with animals (Turjanmaa & Reunala, 1988b), but occurs more frequently in persons handling fish or meat, e.g. fish-factory workers, bakers, butchers, cooks and slaughterhouse workers. Protein contact dermatitis may appear with or without preceding urticaria and involves both immunological and non-immunological stimuli (Reiche, 2002). NRL allergy may also manifest as protein contact dermatitis on the hands of HCW^{s} and NRL glove users (Alenius *et al*, 2002).

1.8. PATIENTS AND PEOPLE AT RISK

Although the presence of latex-specific IgE is not equivalent to the clinical latex allergy in patients, the seroprevalence of latex-specific IgE in a population is directly proportional to the risk of latex allergy in that population (Poley & Slater, 2000). Various studies have shown that increasing exposure increases the risk of sensitisation (Weissman & Lewis, 2002; Ranta & Ownby, 2004). Definite risk factors have also been identified and confirmed (De Beer & Cilliers, 2001; Warshaw, 1998) ***(Table 1.4)***.

Table 1.4: Risk factors for the development of latex allergy

The following risk factors have been identified and confirmed by various studies. The common denominator in most of these instances is the exposure to latex proteins.

Occupational exposure to NRL
Healthcare workers
Rubber industry workers
Janitorial workers
Food handlers
Multiple surgical procedures
Spina bifida
Congenital abnormalities, e.g. myelodysplasia, genitourinary abnormalities
Frequent mucosal exposure to NRL products
Dental
Contraceptive, e.g. male and female condoms
Daily urinary catheterisation
Manual faecal disimpactation
Age of first exposure and total exposure
Pre-existing hand eczema
Atopy
Female gender
Fruit allergy

Anyone may become sensitised to NRL through repeated contact to NRL objects, but specific groups appear to be at a higher risk than the general population to develop latex allergy. Unfortunately, despite the identification of many high-risk groups and contributing factors, consistency still lacks in the literature as to the historical and demographic factors that actually increase risk (Lebenbom-Mansour *et al*, 1997).

1.8.1. Healthcare Workers

During the mid 1980s HCW[s] were reported to have an overall 2.9% sensitisation to latex. In 1992 reports increased to 10% and by 1995, the USA incidence had reached 17% (Kelly, 1995). At the Hamilton Hospital in Ontario, Canada, latex allergy was present in 12.1% of HCW[s] (Potter *et al*, 2001b). Prevalence rates as high as 45% confirmed by skin prick tests (SPT[s]), 40% by radioallergosorbent test (RAST) and 35% by scratch tests were reported in a Croatian study (Lipozencic *et al*, 1998). The prevalence of 3.3% found in HCW[s] in a Hong Kong hospital was low compared to the 5-10% in Western populations. The 6.8% SPT positivity was comparable to reports of 2.6-10.7% in Western populations (Leung *et al*, 1997). Clinical latex allergy is lower and prevalence rates of 1.6%, 4.5% and 4.7% have been reported (Hunt *et al*, 2002). However, the annual incidence of newly sensitised individuals is estimated to be between 1% and 2.5% (Garabrant & Schweitzer, 2002; Ranta & Ownby, 2004).

Reported prevalence rates in the RSA are 5-17% at GSH (Potter, 1996a; Marais *et al*, 1997; Potter *et al*, 2001b), 7% at Red Cross Hospital (Potter *et al*, 2001b), 30% at Tygerberg Hospital (TBH) (De Beer, 2000) and 11-24% at the Blood Transfusion Service (Pretorius, 1999). A small study at the Institute for Medical Research in Johannesburg, found an overall prevalence of 2.7% (Potter *et al*, 2001b).

A United Kingdom (UK) study found the most important risk factors to be frequent changing of gloves (Turjanmaa, 1987) and to work as an operating room (OR) nurse (Smedley *et al*, 1999). Some studies agree that OR personnel are at increased risk of sensitisation (Turjanmaa, 1987), while others found them to be the lowest risk group among all nursing specialities (Grzybowski *et al*, 1996). Other studies found the highest risk among laboratory workers, although a few only found a moderate to relatively low risk in this group (Garabrant & Schweitzer, 2002). A prevalence of 7% was found among Finnish OR personnel and in France and Canada it ranged between 9% and 10% for HCW[s] in general (Potter *et al*, 2001b). Approximately 7.5% surgeons, 5.5-10.7% nurses, 6-7% surgical personnel and 13.7-38% dental staff are affected by latex allergy (Bowyer, 1999b; Garabrant & Schweitzer, 2002).

Currently over 9.9 million people are employed by the healthcare industry in the USA. Some 2.9-12.1% of these are estimated to be allergic to latex (Meade *et al*, 2002). HCW[s]

often specifically develop IgE immune responses to soluble NRL proteins (*Hev b 2* and *Hev b 4*) that readily adhere to cornstarch donning powder and are inhaled (Hamilton & Adkinson, 1996).

A possible relationship exists between the duration of occupational exposure and latex sensitisation. A Dutch study showed a decreasing risk of sensitisation (both latex-specific IgE and SPT responses) with increasing duration of exposure to latex gloves. Similarly, a Swiss and Finnish study reported that the duration of exposure to latex gloves was higher among non-sensitised subjects than among latex-sensitised subjects (Konrad *et al*, 1997). Four other studies have found only weakly positive associations between the duration of latex glove use and sensitisation. One study found a significantly increasing trend in the prevalence of positive SPT responses among dental students as they progressed through four years of dental school. However, the poor participation in this study questions the validity of these results (Garabrant & Schweitzer, 2002).

A large randomised study of Swedish dentists showed a high response rate of 88% and the findings should be valid for all Swedish dentists. Females and younger dentists used gloves more frequently than older males. This pattern has also been observed in other studies in Sweden, England and Wales (Wrangsjö *et al*, 2001). Dental students use gloves substantially from their first year onwards and a Canadian Dental Faculty showed a 10% prevalence among third-year students in 1995. After switching to low-protein powder-free gloves, a follow-up study was done in 2000. The prevalence rates were significantly reduced to 3% in their study group and 0% in first and second year students, who were not exposed to powder at all (Saary *et al*, 2002).

1.8.2. Other Occupational Exposure

Workers in the rubber industry are continuously exposed to NRL. Subsequent latex allergy poses major occupational problems in these workers (Sussman *et al*, 2002). The reported incidence of asthma in this group ranges from 6-11% (Tarlo *et al*, 1990; Sri-Akajunt *et al*, 2000; Woolhiser, 2000). A Malaysian study found positive SPT^s to NRL glove extract in 2.4% of their group (Pumphrey, 1994). These results are inconclusive, due to the small number of subjects evaluated (Kurup & Fink, 2001).

Other occupations with exposure to NRL and reported latex allergy include food handlers, glove manufacturers (10.9%), doll manufacturers (9%) and greenhouse workers (5%) (Randolph, 2001). Occupational latex allergy has also emerged among hairdressers and IgE-mediated (type I) latex allergy seems to be more prevalent cell-mediated (type IV) latex allergy (Sajjachareonpong *et al*, 2002; Nettis *et al*, 2003).

Workers in the textile industry and elasticised ribbon-manufacturing plant have also been affected by latex allergy. Sewing the elasticised ribbons induce a fine dust of cloth and latex that could be inhaled and sensitise individuals (Weytjens *et al*, 1999).

1.8.3. Spina Bifida and Other Congenital Abnormalities

The reported incidence of NRL allergy in SB patients ranges from 18-70% (Walls, 1996; Hayes *et al*, 2000; Woolhiser *et al*, 2000; Kurup 2001; Poley & Slater, 2000). Patients preferentially produce large amounts of IgE antibodies to rubber particle-associated allergens, such as *Hev b 1* and *Hev b 3* (Hamilton & Adkinson, 1996; Yeang *et al*, 1996).

Most patients with SB undergo two surgical procedures within the first week of their lives and repeatedly thereafter. This early and repeated mucosal and visceral exposure to potent NRL allergens results in high incidences of sensitisation (Slater & Trybul, 1994; D'Epiro, 1995; Degenhardt *et al*, 2001). Frequency is more important than duration of exposure to induce sensitisation (Patriarca *et al*, 2002a). Children at risk include those with congenital abnormalities, myelomeningocele or SB, myelodysplasia, multiple previous surgical procedures, urogenital abnormalities and malformations, e.g. cloacal malformations, exstrophy-epispadias complex and imperforate anus, vetriculoperitoneal shunts and neurological impairment, e.g. cerebral palsy (Bubak *et al*, 1992; Kurup *et al*, 1993; Zerin *et al*, 1996; Poley & Slater, 2000; Randolph, 2001; Sussman *et al*, 2002).

A control group was compared to children with and without SB. The latter group had undergone multiple surgical procedures. Latex sensitivity was absent in the control group, but confirmed in 59% of the SB group and 55% of the non-SB group. This study confirmed repeated exposure as an independent risk factor (Porri *et al*, 1997).

Patients with SB have a risk of OR latex-related anaphylaxis of 500 times greater than that of the general population (Kelly *et al*, 1994a). In contrast with other reports where latex-related anaphylaxis occurred between 40 and 225 minutes into a procedure, each

anaphylactic reaction in a study of children with SB occurred during anaesthesia induction and before surgical incision (Kelly *et al*, 1994b).

Not only the number, but also the type of surgical procedures seems to influence the risk of developing latex allergy. Contact on the meningi or the contribution of SB-specific susceptibility factors may have a strong sensitising potential (Bohle 2000; Degenhardt 2001).

Limited information is available on the mean age when sensitisation occurs, but the youngest patient in one study of children with SB was 14 months old (Venuta *et al*, 1999). A multivariate study of 100 consecutive SB patients in Spain showed that the rate of sensitisation increased with increasing age, but the results were inconclusive, because the number of diagnostic and therapeutic procedures also increased, as well as in the absolute levels of total serum IgE (Nieto *et al*, 1996).

In a large survey of 1 523 paediatric general surgical operations, patients with more than eight surgical procedures had a significantly higher risk of developing clinical latex allergy. All sensitised patients had at least one surgical procedure during the first year of their lives (Degenhardt *et al*, 2001). In a Germany study only 0.9% of children with up to two surgical interventions and 34.1% with three or more procedures were sensitised to latex. The median number of surgical interventions was one in the non-sensitised group and three in the sensitised group. Averages of 2.8 and 7.3 surgical procedures were found for adults and children with latex allergy respectively (Theissen *et al*, 1997). Another study found no latex-specific IgE antibodies in SB children with one surgical procedure, while in 39% of patients with two to six procedures were sensitised. An 80% prevalence was found in patients with seven or more surgical procedures (Chen *et al*, 1997; Hayes *et al*, 2000) and nine or more was found to be a significant independent risk factor in the developing of latex allergy (Kelly *et al*, 1994b).

A study on latex allergic children attending a Finnish Dermatology clinic failed to show any correlation between latex allergy and multiple surgical interventions. The number of children who had not undergone previous surgery was three times higher than those who had a history of multiple surgery. A common denominator in these two groups was the presence of atopy in 97% of the first group and 83% of the latter group (Ylitalo *et al*,

1997). As few as two surgical procedures has been shown to be a risk factor for clinical latex allergy in atopic children (Bernardini *et al*, 2003).

1.8.4. Atopy and Atopic Dermatitis

The term "atopy" has ambiguous meaning in medical literature. Some reports use the term to describe a patient having positive SPT results to major aeroallergens (Turjanmaa, 1987; Levy *et al*, 1992; Levy *et al*, 2000; Nettis *et al*, 2003). For others, the term atopy refers to an individual complying with the Hanifin & Rajka (1980), or more refined Mar & Marks (1999) criteria for atopic dermatitis according to the UK Working Party Diagnostic Criteria for Atopic Dermatitis ***(Table 1.5)*** (Williams *et al*, 1994).

Skin susceptibility differs greatly between individuals with active and inactive atopic dermatitis. Atopic skin becomes more susceptible to irritants with simultaneous exposure to clinical relevant aeroallergens (Löffler & Effendy, 1999). The SC is considered to be the major barrier between topical antigens and the dermal immune system, but is frequently compromised due to dermatitis (Woolhiser *et al*, 2000).

Atopic dermatitis can be distinguished from other dermatoses by just six features (Williams *et al*, 1994):

i) history of flexural involvement;

ii) onset under the age of two years;

iii) history of an itchy rash;

iv) personal history of asthma;

v) history of a dry skin; and

vi) visible flexural dermatitis.

Atopy is a strong and consistent risk factor for latex sensitisation and is present in 60-80% of latex allergic patients (Wrangsjö *et al*, 1986; Turjanmaa, 1987; Turjanmaa & Reunala, 1988b; Beuers *et al*, 1990; Pecquet *et al*, 1990; Heese *et al*, 1991; Niggeman, 1997; Levy *et al*, 2000; Garabrant *et al*, 2001; Garabrant & Schweitzer, 2002). Similarly, a 1% prevalence of latex allergy was reported in the general population of Canada, Finland and France was, compared to 9% in the atopic population (Charous *et al*, 2002). Nurses with at least one atopic disease (hay fever, chronic sinusitis, asthma, hand and

Table 1.5: UK Working Party diagnostic criteria for atopic dermatitis

Different sets of primary and secondary criteria are available for the diagnosis of atopic dermatitis. The concise UK Working party criteria for atopic dermatitis were used in this study (Williams *et al*, 1994).

To be diagnosed with atopic dermatitis, a person must have:

1. An itchy skin condition (or parental report of scratching or rubbing in a child)

Plus three or more of:

1. History of involvement of the skin creases such as folds of elbows, behind the knees, front or ankles or neck
2. A personal history of asthma or hay fever (or history of atopic disease in a first-degree relative in those under the age of four years)
3. A history of generally dry skin in the past year
4. Visible flexural dermatitis (or dermatitis involving the cheeks or forehead and outer limbs in children under the age of four years)
5. Onset under the age of two years (not used if the child is younger than four)

generalised eczema) were at least three times more likely to have latex-specific IgE antibodies. Nurses with at least one positive SPT to inhalant allergens, were also significantly more likely to be seropositive (Grzybowski *et al*, 1996).

Although atopy and frequent exposure are independent risk factors for latex allergy, a combination of these two factors considerably increases the risk of sensitisation (Moneret-Vautrin *et al*, 1993; Randolph, 2001). Atopic patients sensitise faster, and target organs respond to slightly lower levels of specific IgE (Marcos *et al*, 1991; Holme & Lever, 1999).

Atopic individuals often suffer from irritant hand eczema and the continuous use of NRL gloves is an important predisposing factor for the development of latex allergy (Nutter, 1979; Wrangsjö *et al*, 1986; Axelsson *et al*, 1987; Turjanmaa, 1987; Jaeger *et al*, 1992; Taylor & Praditsuwan, 1996; Taylor *et al*, 1999; Ranta & Ownby, 2004). A Finnish survey found hand eczema in 60% of latex allergic persons and only 17.4% of non-sensitised persons (Turjanmaa, 1987; Levy *et al*, 1992). A Swedish study showed that clinically confirmed hand eczema more than doubled the risk of glove dermatitis (Wrangsjö *et al*, 2001). Skin

inflammation has an adjuvant effect on the sensitisation process by facilitating rapid absorption of NRL proteins (Axelsson *et al*, 1987; Fuchs, 1994; Hunt *et al*, 1995; Warshaw, 1998; Gibbon *et al*, 2001). Excessive washing, hand occlusion and the use of irritants should be avoided in ICD (Toraason *et al*, 2000).

An increasingly common practice is to apply protective hand creams before donning gloves. This actually increases the amount of latex protein transferred from gloves to the hands of the wearer (McCaskell, 1995; Bowyer, 1999a; Taylor *et al*, 1999; Wakelin & White, 1999). Water-soluble NRL proteins are absorbed by the barrier cream and kept in direct cutaneous contact for longer periods. Enhanced exposure leads to higher percutaneous absorption and increased allergic reactions (Lodén, 1995; McCaskell, 1995; De Beer *et al*, 1999; De Beer, 2000; De Beer & Cilliers, 2001; Smith Pease, 2002). Although some barrier creams have been shown to lower the average flux rates of tritiated water through fresh and frozen skin (Van der Bijl *et al*, 2000), not all of these preparations are capable of producing adequate protection. When compounds with higher MW^s were tested at 20°C and 37°C, a significant increase in flux rates was found at 37°C, which is also comparable to body temperature (Van der Bijl *et al*, 2002). Therefore, all barrier creams should be used judiciously, because petroleum-based products can deteriorate NRL and compromise glove quality (Beezhold, 1994).

NRL proteins are capable of penetrating both intact and abraded skin (Hayes *et al*, 2000; Weissman & Lewis, 2002). Percutaneous studies showed that after 24 hours of exposure to topically applied NRL proteins, 1.2-2.2% either penetrated into or through intact skin. Removal of the SC significantly increased the penetration to approximately 30%. Proteins ranging from 3-26 kD were able to penetrate the epidermis and the upper section of the dermis (Hayes *et al*, 2000).

1.8.5. Other

Limited data is available on the prevalence of latex allergy in the general population or non-occupationally exposed groups. Estimated prevalence rates range between < 0.12% and 6.5% (Walls, 1996; Warshaw, 1998; Hayes *et al*, 2000; Poley & Slater, 2000; Galobardes *et* al, 2001; Meade *et al*, 2002). Incidences of 6.4% in volunteer blood donors (Weiss, 1995; Ownby *et al*, 1996) and 6.7% in ambulatory surgical patients (Lebenbom-Mansour *et al*, 1997) have been published in the late 1990s. However, an estimated

0.8% incidence in the general population with no identified risk factors has also been reported (Lyttle, 1994). Clinical latex allergy was present in 2.6% of German allergological patients (Ruëff *et al*, 1998), while 1.8% of subjects not intensely exposed to NRL had clinical symptoms (Moneret-Vautrin *et al*, 1993). It would be inaccurate to extrapolate these figures to the general population, because both groups presented with some allergic manifestations at Allergology departments and were additionally subjected to SPT^s to latex. The 6.4-7.9% found in blood donors is representative of latex allergy in the general population (Weiss, 1995; Ownby *et al*, 1996; Ruëff *et al*, 1998; Ownby, 2002). Differences in reported latex allergy prevalence rates world-wide could be ascribed to different, and often non-standardised, diagnostic techniques and study populations (De Beer, 2000).

In 1997; an estimated 12% intraoperative anaphylactic reactions were associated with latex allergy (Lebenbom-Mansour *et al*, 1997). Direct mucous contact with NRL gloves can induce anaphylactic shock, even without surgery (Warshaw, 1998). Anaphylaxis have occurred during vaginal examination (Axelsson *et al*, 1987; Levy *et al*, 1992), vaginal delivery and after caesarean sections (Laurent *et al*, 1992). There is no doubt that dermatomucosal or serous membrane contact with NRL in latex-sensitive persons carries with it a relatively high risk of death (Moneret-Vautrin *et al*, 1993).

Several studies have reported a lack of association between latex allergy and age (Page *et al*, 2000). However, the age of first exposure seems to be crucial in the development of latex allergy. If the first exposure takes place at an age where the immune system is still immature, the chances of sensitisation will increase dramatically (Chen *et al*, 1997; Mazón *et al*, 1997).

Raised IgE levels and latex allergy prevalence rates have been reported in children with severe burns. This could be explained by the fact that latex gloves used to manage these patients come in contact with extended areas of damaged skin. However, a German study involving 15 children in the intensive care unit for severe burns could not find a special risk of sensitisation and allergy to latex (Niggeman *et al*, 2000).

1.8.6. Ethnic Group / Gender

Racial differences relate to the group that a person belongs to as a result of a mix of physical features (e.g. skin colour, hair texture), ancestry, geographical origins, genetic

endowment, culture and socio-economic status. Race is traditionally considered as being defined by others, whereas ethnicity is self-defined. The differences between ethnicity and race are gradually being eroded and are now so intertwined that consideration of one necessarily requires consideration of the other (Lillie-Blanton & La Veist, 1996; Scully & Bedi, 2000). For the purpose of this study, the terms race and ethnic group will be used synonymously.

Non-white race has been observed to be a risk factor for latex sensitisation in a large study of nurses (Grzybowski *et al*, 1996), among ambulatory surgery patients (Lebenbom-Mansour *et al*, 1997), among blood donors in the general population (Ownby *et al*, 1996) and in a group of patients with SB (Kelly *et al*, 1994b).

Male gender was also a recognised risk factor in some studies (Ownby *et al*, 1996; Lebenbom-Mansour *et al*, 1997; Garabrant *et al*, 2001), while other studies have found a female predominance or no difference at all (Page *et al*, 2000). A female predominance of 3:1 has been found in a review of 145 cases of systemic latex allergy. This can be attributed to the fact that more females are employed in high risk occupations, e.g. nursing (Warshaw, 1998), but the effect of female hormones plays an additional role. Estradiol enhances histamine release with a much stronger wheal-and-flare reaction following SPT^s during ovulation when oestrogen levels are at their peak, than in the rest of the menstrual cycle. Male controls showed no variation (Kalogeromitros *et al*, 1995). A study done on Swedish dentists confirmed this trend. Females reported significantly more glove intolerance than males (Wrangsjö *et al*, 2001).

A study of UK blood donors found 4% of all donors had positive latex-specific IgE levels, while younger males had a 8.2% positive rate. The overall prevalence was 4.6% in males and 3.3% in females. Higher prevalence rates occurred during summer, with 8.4% in males and 6.0% in females. The male-to-female ratio in the blood donors was the opposite of that in the general population (Ownby *et al*, 1996).

1.8.7. Food Allergies

It is unclear if latex allergy predisposes a person to specific food allergies or vice versa (Lavaud *et al*, 1995; Potter, 1996a). Up to 76% of individuals with clinical latex allergy are sensitised to plant-derived foods and a large majority of fruit-allergic patients are sensitised to latex. A French study found sensitisation to many foods in latex allergic

patients were much more related to coexisting atopy, except in cases of banana, avocado, kiwi, papaya and chestnut. Sensitisation to foods other than these might be a reflection of pollen sensitivity, and not latex sensitivity (Levy *et al*, 2000). Results from Detroit showed individuals with clinical allergy to kiwi fruit to be more than 14 times as likely to also be sensitive to NRL (Lebenbom-Mansour *et al*, 1997).

Cross-reactivity between fruit and latex may be due to ethylene, a gas used to hasten commercial ripening. When forced to ripen quickly under high ethylene concentrations, plants produce allergenic wound-repair proteins that are similar to wound-repair proteins made by *H.brasiliensis* (Warshaw, 1998).

1.9. ROUTES OF SENSITISATION

The route of exposure to an antigen is considered to be crucial in determining which type of immunological response (IgE vs IgG) is induced (Beezhold & Beck, 1992). Even if the immune system is already primed before birth, allergen exposure after birth is likely to be of great importance for the induction, continuation, differentiation and the outcome of the allergic immune responses in the long run (Saloga & Knop, 2000). Direct mucosal and parenteral exposure to latex poses the greatest risk of anaphylaxis (Poley & Slater, 2000). Exposure to NRL proteins can take place via the following routes (Sussman & Beezhold, 1995; Brehler *et al*, 1997; Turner, 1997):

- **cutaneous:** gloves, masks, adhesives, catheters, drains, ileostomy bags
- **mucosal membranes:** products used in dentistry and anaesthesia
- **inhalation:** aerosolisation of glove powder
- **parenteral:** NRL products used in surgery
- **intravascular:** products stored in syringes with rubber stoppers

Sensitisation to allergens displays varying results (Saloga & Knop, 2000):

- no immune response;
- short-lived and terminated immune responses;
- induction of anergy;
- induction of regulatory / suppressor cells with various functions and life-spans;
- apoptosis of allergen-specific T-cells;
- induction of long-lived T_{H1} or T_{H2} immune responses;

- production of inflammatory mediators that influence the immune and non-immunologic systems;
- induction of antibodies against allergens with varying fine specificities;
- production of different Ig classes, especially IgE;
- receptor binding by antibodies, e.g. FcεRI on basophils, mast cells and APC^s, leading to degranulation of basophils and mast cells, inflammation and tissue remodeling.

The major exposure routes in HCW^s are inhalation / mucocutaneous and direct skin contact (Alenius *et al*, 1991; De Beer, 2000; Leynadier *et al*, 2000; Toraason *et al*, 2000; Reiter, 2002). Other factors contribute to the variation and severity of the reaction and include protein source (e.g. glove or other product), NRL type (e.g. low ammoniated, high ammoniated) and individual immune responses (Baur *et al*, 1997).

The use of latex gloves by food handlers is a potential route of inadvertent NRL exposure. Food is prepared with the use of latex gloves and contaminated by latex proteins. These proteins are present as "hidden ingredients" in food and can cause severe reactions, such as anaphylaxis, in sensitised patients (Bernardini *et al*, 2002). Although accidental exposure through ingestion is common, transmission of allergens through kissing is also possible. Even a brief kiss from well-meaning relatives who have just eaten the source of the allergen can trigger a systemic reaction in an allergic individual (Hallett & Teuber, 2002b). Severe drug-induced angioedema was induced in a 45-year-old woman after a kiss from her husband who ingested a becampicillin tablet two hours earlier (Fischer & Gronenberg, 2002). Peanuts, walnuts and tree nuts are often the cause of severe allergic reactions induced by food. Another patient was kissed on the cheek by his mother just after she tasted pea soup and a large wheal immediately developed at the site of the kiss. Some patients had reactions even after their partners brushed their teeth (Hallett *et al*, 2002a).

Particles impacting in the nasopharynx can be swallowed and enter the gastrointestinal tract. Mucous membranes of the gastrointestinal and urogenital tracts can be exposed to NRL allergens by direct contact with indwelling NRL devices, e.g. catheters. Internal exposure can occur via the use of NRL gloves during surgical procedures and internally placed NRL devices, e.g. wound drains (Weissman & Lewis, 2002). Animal studies with indirect immunoblotting showed that mice sensitised intratracheally or topically with NRL

produced IgE antibodies against *Hev b 6, Hev b 2* and *Hev b 4*, whereas those sensitised subcutaneously produced IgE antibodies against *Hev b 1* and *Hev b 3* (Lehto *et al*, 2003).

Body sweat inside gloves may enhance the water-solubility of the NRL proteins and may facilitate cutaneous penetration (Beezhold, 1994; Reiter, 2002), although sweat is not essential (McCaskell, 1995). Fingerprint assays demonstrated that latex proteins are transferred from the glove to the skin immediately upon contact. This rapid reaction suggests that protein allergens are released upon contact and body sweat or prolonged exposure is not required to liberate the NRL proteins from surgical gloves. Powder-free gloves had much lower levels of transferred proteins (Beezhold, 1994). A conscientious hand washing technique should be adhered to after glove removal to ensure that all powder and proteins left on the skin are removed (Smith *et al*, 1993).

Occlusion is recognised as an enhancer of percutaneous absorption. An increased flux across occluded skin is often found for lipophilic substances, but less frequently for highly lipophilic substances (Makki *et al*, 1996). Studies investigating percutaneous penetration are usually conducted under occlusion. Occlusion leads to the entrapment of water, which would normally be lost to the surrounding environment. This increased hydration causes a consequent swelling of corneocytes, an uptake of water in the intercellular skin lipids and the elevation of skin surface temperature from 32°C to 37°C (Taylor *et al*, 2002a). Wearing of gloves for prolonged periods could result in similar physiological changes.

1.9.1. Skin Structure

The skin protects man from the environment. It weighs an average of 4 kg and covers an area of 2 m^2 (Lodén, 1995). The skin consists of various layers, each with a particular task, including the outermost SC, viable epidermis, dermis, and the subcutaneous tissue ***(Figure 1.3)***. The SC is approximately 10-20 µm thick and has an acidic pH between 4 and 6.5 (Yosipovitch *et al*, 1998; Kalia *et al*, 2000). It consists of many layers of cells with the corneocytes being imbedded into lipids (Engström *et al*, 2000). Intercellular lipid membranes are in a solid (impermeable) or liquid crystalline (permeable) phase. Membrane phospholipids depend on the proportion of solid phase lipids, and a

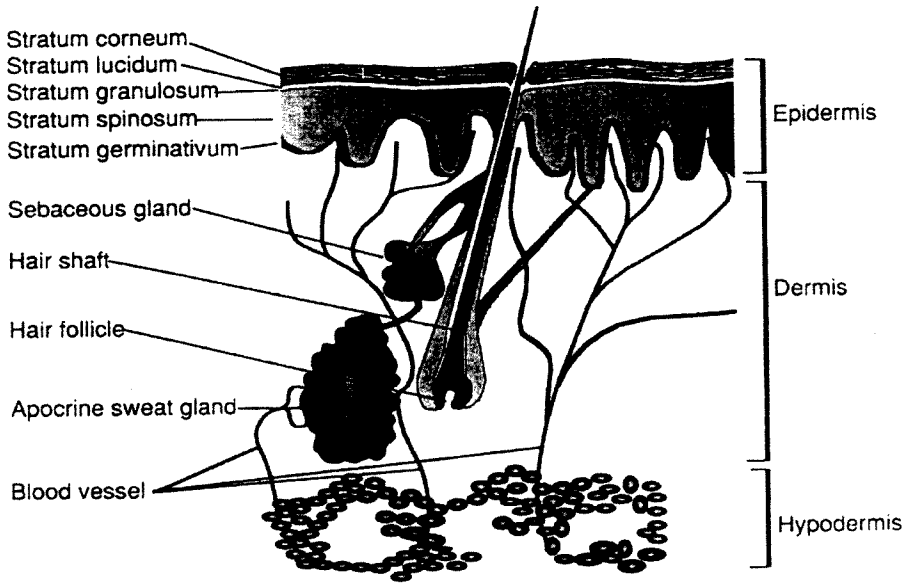


Figure 1.3: Cross-sectional view of the skin

The epidermis consists of different layers that renew the skin and produce melanin. The dermis is responsible for the elastic properties of the skin. Collagen and elastic fibres stabilise the skin and prevent it from being torn. The hypodermis is the bottom layer of the skin and contains fat cells, nutrients, blood vessels, sebaceous glands, sweat glands and arrector pili muscles for the hair. It therefore serves as a depot and protects against physical injuries (Morimoto *et al*, 1994).

"percolation threshold" (i.e. the proportion of solid phase lipid required to prevent continuous diffusion) has been observed (Kitson & Thewalt, 2000).

The SC protects the body against invasion by harmful exogenous agents, such as micro-organisms. Water loss is prevented by about 20 tightly stacked layers of corneocytes and intercellular lipids (Kalia *et al*, 2000). The superficial portion of the SC can easily dehydrate as a result of exogenous factors or pathology, while the deeper portion is always well hydrated, because of its structural closeness to the water saturated viable epidermis (Tagami, 1994). It contains hydrophilic and hydrophobic domains that prevent pronounced hydrophilic and hydrophobic compounds from entering or leaving the body (Engström *et al*, 2000). Ceramides that account for 50% of the SC, are the result of keratinocyte differentiation and are one of the major lamellar phase-forming lipids (Smith Pease, 2002). Cholesterol makes up approximately 25% and

fatty acids 10% of the SC lipid mass (Wertz, 2000). A normal healthy SC is not totally impermeable and many topically applied, moderately lipophilic compounds with low MW^{s} (< 500 dalton) can penetrate the skin readily *in vivo* and *in vitro* (Smith Pease, 2002).

The corneocytes are continuously replaced from the stratum germinativum to form the SC (Morimoto *et al*, 1994). They form important carriers of melanin and are of major importance in the innate immune system and inflammatory cascade. The process where cells are transformed into keratin, become flatter and eventually die, takes between 52 and 75 days in healthy skin. The dead cells are then released from the surface singly or as small scales (Lodén, 1995).

The dermis is responsible for the elastic properties of the skin and also contains sensory cells. All sensations (pain, itching, temperature, etc) are transferred from the receptors via nerves to the brain as electric impulses. High content of chondroitin and hyaluronic acid provides a liquid depot, which nourishes the epidermal cells. Fat cells are the main component of the hypodermis or subcutis and serve as protection against mechanical trauma. The volume, thickness of the layer and arrangement of the cells are constitutionally and hormonally regulated. Subcutaneous fat cells are rare on the nose, eyelids and outer ear (CK Electronic, 2001).

Lipids are synthesised at a constant rate in sebaceous glands and secreted onto the skin surface an average eight days after synthesis (Downing *et al*, 1981). General skin condition is defined by a combination of surface texture, colour and physiological properties, e.g. hydration, sebum content and surface acidity. The presence of an adequate amount of water in the SC, or SC hydration (SCH), is important for general appearance of a soft and smooth skin. Sebum, which is secreted by the sebaceous glands together with other epidermal lipids, helps maintain hydration of the skin by providing a protective layer on the skin surface to prevent water loss. Skin lipids and AA^{s} contribute to surface acidity that protects the skin from pathogens. These skin characteristics are affected by endogenous and environmental factors, such as ageing, atopy, exposure to sunlight, chemicals and chemical damage (Boelsma *et al*, 2003). Irradiation of the skin, e.g. PUVA, causes disintegration of the SC lipids. A regeneration process is induced, leading to an extrusion of lipid-filled lamellar bodies into the intercellular space and an increase in the SC lipids (Löffler *et al*, 2002).

1.9.2. Skin Barrier

"Shall I compare thee to a rubber sheet
Protecting man from toxins, drought, and heat,
Where mortal bricks and mortar meet
To build a multilayered wall so neat,
That with thou living membranes can't compete
Or rather – to a horny piece of meat
That makes Sara wrap look quite obsolete" – H Boddé *(Rees, 1999)*

Skin barrier function depends on the presence of a unique mixture of lipids in the intercellular spaces of the SC. As epidermal cells move from the basement membrane outward through the epidermis and towards the skin surface, they undergo differentiation. One major aspect is the accumulation of lipids, packaged in the lamellar granule. Towards the end of the differentiation process, the contents of the lamellar granule is discharged into the intercellular space (Wertz, 2000). Sphingolipids, free sterols and phospholipids play a key role in barrier function and are essential to prevent excessive water loss. A SC containing 20-35% water exhibits the softness and pliability of normal SC. If the epidermal barrier is defective, e.g. in atopic dermatitis, or has been damaged by exogenous substances, signals must be transmitted to the intracellular space to initiate repair. The major physiological signal is transepidermal water loss (TEWL) and as little as 1% TEWL is sufficient to initiate lipid synthesis. Remoisturisation of the skin occurs in four steps (Draelos, 2000):

- initiation of barrier repair
- alteration of surface cutaneous moisture partition coefficient
- onset of dermal-epidermal moisture diffusion
- synthesis of intercellular lipids.

The MW and lipophilicity of applied substances are important factors in determining its percutaneous penetration. The ability of peptides to penetrate normal skin is more than a thousand times lower than that of e.g. formaldehyde (Lodén, 1995). The total lipid content of the SC, degree of SCH, corneocyte size and thickness of the SC are all contributing factors to differences in skin permeability (Lodén, 1995). However, a dysfunctional skin barrier enhances percutaneous absorption of environmental haptens or large (> 500 dalton) antigens (Smith Pease, 2002). Immune responses induced by

topical sensitisation with haptens occur with a T_{H2} dominant cytokine profile composed of suppressed T_{H1} and enhanced T_{H2} cytokine responses. When these haptens are inhaled or absorbed, a T_{H2}-dominant immune response is induced (Kondo *et al*, 1998).

Bioengineering methods, such as TEWL, SCH or resistance, skin surface pH and skin temperature in different anatomical sites can be used to assess skin barrier function. Several studies on neonates investigated skin maturation and function and compared it to adult skin (Wilhelm *et al*, 1991; Denda & Tsuchiya, 2000b; Muramatsu *et al*, 1987; Schwindt *et al*, 1998). The epidermis of a full-term infant is morphologically indistinguishable from that of adults (Yosipovitch *et al*, 1998). However, during the first two days of life, the SC barrier is still adapting to extrauterine life and measurements of most anatomical sites show significant changes. After five days of daily washing, the skin lipid-profile from an infant is reported to be similar to that of adult skin (Yosipovitch *et al*, 2000).

1.9.2.1. Transepidermal Water Loss

TEWL represents the water evaporation rate from the skin surface ($g/m^2/hr$) and reflects the integrity of the SC. In healthy skin TEWL and SCH remain directly proportional. Following damage to the skin or barrier function, a dissociation appears and in the case of dry skin, TEWL increases and SCH decreases (Ozawa & Takahashi, 1994). An inverse relationship between corneocyte size and TEWL has also been confirmed (Wilhelm *et al*, 1991). Age-related differences in TEWL on most anatomical regions have been identified with significantly lower values in the elderly (Cua *et al*, 1990; Wilhelm *et al*, 1991; Denda & Tsuchiya, 2000b). However, adult patients with atopic dermatitis show increased TEWL values (Eberlein-König *et al*, 2000).

Typical basal values of TEWL in healthy adults are in the order of 5-10 $g/m^2/hr$. Perturbation of the barrier, either by physical disruption, chemical attack or because of disease, can severely compromise the role of the SC (Kalia *et al*, 2000). Twice daily water exposure did not influence the barrier function of healthy volunteers. However, glove occlusion resulted in increased TEWL and visible irritant skin reactions (Ramsing & Agner, 1997). Irritant exposure on the hands of patients with active atopic dermatitis have also resulted in higher TEWL values (Löffler & Effendy, 1999).

For accurate TEWL measurement, individuals should rest for 15-30 minutes before measurements, with the skin at the measuring site left uncovered. Simultaneous

measurement of skin temperature is recommended, and TEWL under direct light sources are not recommended. Only TEWL values from the same anatomical area are expected to be comparable (Serup, 1994).

1.9.2.2. Stratum Corneum Hydration

Water in the SC is associated with the hydrophilic parts of the intercellular lipids and with keratin fibres within the corneocytes. The fibrous elements in the corneocytes have hydrophilic properties and also contain a water soluble fraction, which enhances the water holding capacity (Lodén, 1995).

SCH, skin surface electrical capacitance or resistance readings for one-second provide a measure of baseline surface hydration. Continuous readings for twelve-second periods measure the changing hydration of the skin surface occluded by the meter probe and provide an indirect measure of transepidermal water movement. Continuous SCH measurement is positively correlated with TEWL (Okah *et al*, 1995).

Electrical skin resistance is exclusively located within the SC. It is directly related to the thickness of the SC and a thin SC has a lower resistance than in those areas where the SC is thick (Muramatsu *et al*, 1987). Therefore, gradual removal of SC (e.g. by tape stripping or scrubbing) will cause a proportional decrease in SCH. Scrubbing is routinely done by HCW^s before performing surgical procedures and the effect can be compared to about three tape strippings (Lodén, 1995). SCH is closely related to the content of free AA^s in the SC and absorbs water from the environment instantaneously to provide hygroscopicity to the SC (Ozawa & Takahashi, 1994). Exposure to low humidity induces profound changes in epidermal proliferation and function and usually result in lower levels of SCH (Denda, 2000a). The SC has an inherent water-retaining capacity and absorbed water is lost rather slowly (Tagami, 1994). Individuals who regularly used moisturisers, showed decreased SCH values on the hands during a period when no moisturisers were used, but water exposure alone will not influence the hydration state of the SC (Ramsing & Agner, 1997).

Perspiration decreases resistance of the skin by serving as an added parallel high resistance in the electrical circuit and by moistening the skin (Muramatsu *et al*, 1987). Even slight perspiration greatly influences the skin surface hydration state, which is often the case on the facial and palmo-plantar skin (Tagami, 1994). However, cornstarch

powder inside gloves causes dryness of the skin and can lead to a reduction in the barrier properties of the skin (Pretorius & Bester, 2000). In 1968 already, it was found that powdering of the SC destroyed the lipid membranes and made the skin more susceptible to drying out effects (Lodén, 1995).

1.9.2.3. Skin Surface pH

Skin pH is determined by water-soluble substances in the SC, the secretion of perspiration and sebum, as well as exuded carbonic acid. The average pH ranges between 5.4 and 5.9 and has a protective function. It differs slightly between adult males and females and shows regional and temporal variation (Gfatter *et al*, 1997). Intact skin exhibits an ability to recover from pH changes, even when exposed to highly alkaline materials (Ertel, 2000). High pH is frequently associated with high TEWL and low SCH (Dikstein & Zlotogorski, 1994; Yosipovitch *et al*, 1998). However, decreased skin surface pH is correlated with increased SCH and sebum content values (Boelsma 2003).

The skin pH pattern observed during the newborn period is related to the presence of vernix caseosa, which has an average pH of 7.4 (Behrendt & Green, 1958). However, the SC acidifies during the first week of life (Yosipovitch *et al*, 2000). Infants with healthy skin show an average pH of 6.6 ± 0.25. Newborns and small infants have higher skin surface pH and lower free fatty acid content than adults (Gfatter *et al*, 1997). Dryness of the skin, e.g. in atopic dermatitis, increases skin pH values. Primary school children without eczema showed a mean pH value of 5.18 on the volar forearm. A similar group with atopic eczema produced a mean pH of 5.23 in uninvolved skin and 5.54 in involved skin. Healthy controls showed a mean pH of 4.86 (Eberlein-König *et al*, 2000).

Adult males and females showed similar values on the forehead and cheek. The pH is constant until the age of 80 and then increases slightly in both areas. Average adult skin surface pH is 4.0-5.5 on the forehead and 4.3-5.9 on the cheek and both values are correlated by the equation: *pH on the cheek = 1.1 + 0.8 pH on the forehead* (Dikstein & Zlotogorski, 1994).

1.9.2.4. Sebum Content

Sebaceous glands produce lipids that contribute to maintaining the skin's hydration state. The lipid composition varies in different regions of the skin. The distribution of

ceramides also appears to be gender- and age-related (Lodén, 1995). Unsaturated fatty acids prevent growth of bacteria on the skin and contribute to skin acidity (Boelsma *et al*, 2003). Structural lipids of sebaceous origin and SC lipids from lamellar bodies, control the water retaining-capacity of the skin. Cleansing agents and washing may influence the hydration state by dissolving fat from the surface. An Austrian study evaluating the effect of soap in infants recorded a mean basal sebum value of 4.34 $\mu g/cm^2$. The control group (tap water without detergent) showed a decrease of only 0.93 $\mu g/cm^2$, while the use of alkaline soap decreased the value by 4.81 $\mu g/cm^2$. No statistically significant difference was found between the two groups using detergents with an acidic pH (Gfatter *et al*, 1997).

Petrolatum has the ability to accelerate the initial stages of barrier repair. Topically applied petrolatum affects the lipid bilayer structure by permeating the SC and localising in the intercellular spaces. It also forms a semi occlusive barrier on skin and may reduce moisture loss by up to 50% (Ertel, 2000).

SC lipid content decreases with increasing age in humans. In a study done at the University of California between groups of 14 young adults (23-29 years) and 15 aged individuals (60-80 years), the casual sebum content showed a great anatomic variation with more than 100-fold differences among regions. Low sebum values were recorded on the thigh, ankle and volar forearm, as opposed to sebum-rich areas, such as the post auricular region and forehead (Wilhelm *et al*, 1991).

1.9.3. Environmental Factors

Skin barrier homeostasis is affected by systemic and environmental factors (Okah *et al*, 1995; Denda & Tsuchiya, 2000b). The following factors influence the skin barrier function of unmodified skin in humans:

1.9.3.1. Age

The effect of age on skin barrier function is surprisingly small. The epidermal development *in utero* is complete at 34 weeks gestation. Even babies born at 30-32 weeks gestational age have a competent barrier, comparable to that of adults (Kalia *et al*, 1998). At birth, the human term newborn has developed mechanisms for successful transition from an aquatic to terrestrial environment. The development of the

hydrophobic the vernix caseosa in the last trimester of gestation and SC formation lead to a relatively water-impermeable barrier (Okah *et al*, 1995). The skin of newborns and small infants differs from adult skin in that the connection between the dermis and epidermis is weaker, the permeability of the SC is higher and melanin production is decreased. Until puberty the sebaceous glands have no function (Gfatter *et al*, 1997).

The barrier in the elderly becomes fragile and its recovery becomes delayed with aging (Denda, 2000a). This is probably because of significantly decreased lipid levels found with increasing age. In general, young adults show higher SC hydration levels than neonates, children and elderly people (Wilhelm 1991; Tagami, 1994; Manuskiatti *et al*, 1998; Benfeldt, 1999; Denda & Tsuchiya, 2000b; Boelsma *et al*, 2003).

The visible changes in aged skin are noted primarily in exposed skin and represent extrinsic age-related effects. Xerosis could be the result of a decreased barrier function of the SC (Aisen *et al*, 1997).

1.9.3.2. Gender

Gender could influence disease severity, rather than disease prevalence by impacting on the barrier function. Although there are no known gender-related differences in permeability barrier function in adults, exogenous estrogens accelerate and androgens delay barrier ontogenesis *in utero*. Male foetuses demonstrate slower barrier development than female littermates. However, adult females are not known to exhibit superior barrier function to males (Kao *et al*, 2001).

Most studies fail to demonstrate a difference in TEWL, SCH, skin surface pH and casual sebum content between males and females (Cua *et al*, 1990; Wilhelm *et al*, 1991). Significantly lower pH values were found in males than in females. In the group over 50 years, sebum content tends decrease in women and increase in men with increasing age. These differences may be caused by the known age-related hormonal fluctuations in both men and women and the effects of hormones on sebaceous gland activity (Boelsma *et al*, 2003).

Female skin seems to be more prone to irritation. The mean TEWL increase after exposure to sodium laurel sulphate was larger in women than in males. These studies further showed a variation in skin susceptibility relating to the menstrual cycle.

Reactivity is increased on day one of the menstrual cycle compared to days nine to eleven. Estradiol suppresses cellular immune response, possibly causing an alteration in the responsiveness of the regulatory cells of the cellular immune system (Agner *et al*, 1991; Benfeldt, 1999). Results on the variation in sebum excretion during the menstrual cycle are conflicting. One study could did not find any cyclic variation in sebum production, while another study showed a decrease in the sebum excretion rate in the premenstrual phase and a few days after ovulation (Agner *et al*, 1991).

1.9.3.3. Ethnic Group

Microscopic examination of skin from different ethnic groups shows no substantial differences. The SC is equally thick in black and white skin, although black skin contains more cell layers (Berardesca & Maibach, 2003). Dark skin shows a more resistant barrier after tape stripping of the SC, regardless of race as such (Benfeldt, 1999). Darker skin also recovers faster after barrier damage induced by tape stripping (Berardesca & Maibach, 2003).

Normal-appearing white cadaver skin showed greater percutaneous absorption of various substances than black skin under similar conditions (Taylor, 2002b). Despite other conflicting results, black skin shows a more efficient epidermal barrier, possibly due to the protective role of melanin (Okah *et al*, 1995; Singh *et al*, 2000). The biosynthesis of melanin occurs within the melanosome, an exocrine cell present in the basal layer of the epidermis. The size and aggregation of melanosomes within the melanocyte and keratinocyte vary according to skin colour, but the number does not differ between ethnic groups. Melanosomes in black skin are distributed throughout the entire epidermis, including the stratum basale, granulosum, lucidum and corneum. In fair skin only a few melanosomes are present in the stratum basale and malphigian layer (Taylor, 2002b).

White subjects were found to be more easily sensitised to a variety of contact allergens than black subjects. Black subjects require a significantly longer exposure to develop irritant reactions (Taylor, 2002b; Berardesca & Maibach, 2003). Especially formaldehyde and formaldehyde-releasing preservatives show higher sensitisation rates in white patients. These biocides are found in a number of industrial settings, but are primarily

allergenic when used in cosmetics and personal care products, such as moisturisers and shampoos (DeLeo *et al*, 2002).

Baseline TEWL values in different ethnic groups were found to be in the rank order: Caucasian > Asian > Hispanic > Black. Apart from a more sensitive TEWL response, the SC of darker skin also shows increased intercellular cohesion, higher ceramide content and SCH than white skin (Berardesca, 1994; Singh *et al*, 2000; Berardesca & Maibach, 2003).

Skin resistance, which is correlated with greater eccrine gland activity, is higher in black than white subjects. Black skin also shows higher sebum values, due to larger sebaceous glands (Taylor, 2002b).

1.9.3.4. Anatomical Site

The regional variation in the content and composition of the intercellular lipid are some of the main factors influencing the rate of permeation across the human SC barrier at various sites (Elias *et al*, 1981; Benfeldt, 1999). The mean thickness and number of cell layers of the SC and the regional distribution of hair follicles and eccrine sweat glands may also play a role. Most of the sebaceous glands are found on the T-zone of the face, scalp and upper part of the back and chest (CK Electronic, 2001). The SC of the extremities is significantly thicker than that of the abdomen and the back, arm and thigh show lower penetration rates than abdominal skin (Schwindt *et al*, 1998).

Baseline TEWL varies with the following rank order: palm > sole > forehead = post auricular skin = nail = dorsum of hand > forearm = upper arm = thigh = chest = abdomen = back (Benfeldt, 1999). The calf and forearm show similar characteristics in terms of TEWL, SCH and desquamation and are lower than those from the crow's-foot site (Black *et al*, 2000). In elderly patients the TEWL rank order is: palm > forehead > ankle > post auricular with the lowest found on the volar forearm. SCH correlates significantly with desquamation (Manuskiatti *et al*, 1998) and is represented by: post auricular > upper back > lower back > forehead with the lowest value on the dorsal forearm (Cua *et al*, 1990).

In the first study of regional variation (Feldmann & Maibach, 1967) scrotal skin showed 42 times more absorption of ^{14}C hydrocortisone than the forearm. A direct correlation was

demonstrated between TEWL and percutaneous absorption after skin stripping on the arm, abdomen, post auricular area and forehead (Benfeldt, 1999). Apparent absorption was increased in areas where follicles are larger and numerous (e.g. forehead and scalp) and decreased where the SC is thicker (e.g. the foot). Structural differences may be responsible for the increased absorption in hairy areas, although follicular absorption may be greater than transepidermal absorption. This study found the order of absorption to be scrotum > jaw > forehead > axilla > scalp > back > dorsal forearm > ventral forearm > palm > lateral ankle > plantar foot arch (Feldmann & Maibach, 1967).

1.9.3.5. Other Factors

Time-dependent variation (marked reduction between 20:00 and 23:00) with circadian reactivity rhythms and differences in anatomical sites of TEWL, surface pH and skin temperature have been reported (Denda & Tsuchiya, 2000b). Skin permeability is higher in the evening and night than in the morning (Yosipovitch *et al*, 1998). This variation may reflect alterations in sweat gland activity, accompanying by the known diurnal variation in core temperature (Benfeldt, 1999).

The *season* in which skin susceptibility is tested influences the outcome. The proportion of ceramides, cholesterol and fatty acids are significantly lower in winter and spring than in summer. Especially the ceramide esterified to linoleic acid is decreased in winter (Lodén, 1995). Accordingly, healthy volunteers displayed increased susceptibility in winter, while atopic dermatitis patients showed reduced barrier function and SCH during winter (Löffler & Effendy, 1999). Dermatitis-like conditions all tend to worsen during the winter season, when humidity is low (Denda & Tsuchiya, 2000b). Patch tests with sodium laurel sulphate in 18 volunteers showed increased TEWL in winter compared to summer months (Agner & Serup, 1989). The decreased lipid content in the SC in winter or low outdoor temperature and low relative humidity in the winter could be responsible (Benfeldt, 1999). Skin temperature may also be higher in summer, resulting in higher TEWL values, thus counteracting the influence of the changes in humidity (Agner & Serup, 1989; Black *et al*, 2000). The seasonal variation in the lipid composition may indicate that the SC is functionally inferior in winter. This could further explain the known susceptibility to skin xerosis and faulty desquamation at this time of the year (Lodén, 1995).

Psychological stress stimulates increased production of glucocorticoids, which in turn adversely affects permeability barrier homeostasis (Denda, 2000a). Co-administration of tranquillisers is able to block this stress-induced deterioration in barrier function. Sustained psychological stress is associated with alterations in both humoral and cellular responses. It affects the autonomous nervous system production of norepinephrine, which has a direct effect on β2-adrenergic receptors on lymphocytes, and initiates a T_{H1} shift to T_{H2} with a subsequent change in cytokine production. The final result is a decreased cellular and increased humoral immunity (Cilliers, 2002). In a study done in medical, dental and pharmacy students, a strong correlation was found between increased stress levels and barrier recovery rates during examination periods, with a definite reduction in psychological stress during a subsequent vacation period. Stress-induced release of neuroimmune substances can adversely influence cutaneous homeostasis through activation of immunologic / inflammatory processes in the deeper skin layers and increasing systemic glucocorticoid levels (Garg *et al*, 2001).

1.10. AIRBORNE ALLERGENS

The first case report of latex allergy purely via an airborne route was reported in 1990. This nurse initially experienced only local discomfort when wearing NRL gloves, but very soon progressed to severe respiratory symptoms. She also developed immediate asthmatic reactions when she was not wearing gloves. Latex SPT^s induced a wheal of 7 x 15 mm (Baur & Jäger, 1990; Lagier *et al*, 1990).

A common misconception is that the major cause of type I latex allergy is cornstarch powder, the dry donning lubricant used in gloves. Cornstarch is a heavily cross-linked carbohydrate with particle sizes of 1-3 µm in diameter that can be transported into the respiratory tract (Baur *et al*, 1993; Pumphrey, 1994; Sri-Akajunt *et al*, 2000; Weissman & Lewis, 2002). It is an extremely rare sensitiser and rarely the primary allergen (Bubak *et al*, 1992; Potter, 1998a; Potter, 1998b; Hamilton *et al*, 2002). It can, however, worsen existing dermatitis by mechanical irritation, act as a vector or carrier for airborne NRL proteins or disrupt the skin barrier function by absorbing moisture and drying of the skin (Beezhold *et al*, 1994a; Turner, 1997; Hamilton *et al*, 2002).

Glove powder treated with soluble NRL proteins show specific biochemical properties. Western blot analysis confirmed that NRL proteins bind to cornstarch powder, but not to

calcium carbonate powder. Powder that was not treated with NRL proteins also showed no immunoreactive proteins (Beezhold & Beck, 1992). Talc irreversibly binds latex allergens, while the allergen link with cornstarch is unstable, resulting in much higher concentrations of airborne NRL allergens (Baur, 2003).

Airborne allergens are primarily generated by active glove use (Charous *et al*, 2000). It inoculates surgical tissue and contaminate suture material, instruments, drapes or sponges in OR units (Beezhold, 1994). Unless shielded by protective gear, the ocular and nasal mucosal surfaces are fully exposed to airborne allergens, irrespective of the size. Particles > 10 µm are unable to penetrate below the level of the glottis and are deposited mainly in the nose, pharynx and upper airways, while particles measuring 5-10 µm are deposited in larger bronchi on the mucosal surfaces of the trachea and large airways.

Small particles of < 5 µm in diameter penetrate the smaller airways in the peripheral regions of the lungs ***(Figure 1.4)*** (Tarlo & Purdham, 2002). More than 80% of NRL allergens in a laboratory were associated with particles of > 7µm and only 13% were associated with smaller particles (< 4 µm) (Swanson *et al*, 1994; Meade *et al*, 2002).

The eyes and nose have therefore higher levels of exposure to airborne allergens than the lungs and nasal symptoms usually precede the development of lower respiratory tract symptoms by months or years (Fish, 2002).

NRL aeroallergens cause rhinitis, conjunctivitis and bronchial asthma in pre-sensitised HCW^s (Baur & Jäger, 1990; Lagier 1990; Turjanmaa *et al*, 1990; Marcos *et al*, 1991; Jaeger *et al*, 1992; Mäkinen-Kiljunen *et al*, 1992; Baur *et al*, 1993; Czuppon *et al*, 1993; Swanson *et al*, 1994; Warshaw, 1998; Wakelin & White, 1999). It is currently the fourth most common cause of occupational asthma reported to the Surveillance of Work-related Occupational Respiratory Disease surveillance program (Vandenplas *et al*, 2002).

Protein aeroallergen concentration appears to correlate with the amount of glove protein and cornstarch powder (Baur & Jäger, 1990; Swanson *et al*, 1994). Powdered examination gloves contain on average more than 600 µg of latex allergen per gram of glove (Poley & Slater, 2000). Other studies showed 0.2-4 000 µg of proteins per glove (Hayes *et al*, 2000). Powdered gloves produced extractable allergen levels of < 5-12 100 µg/mℓ,

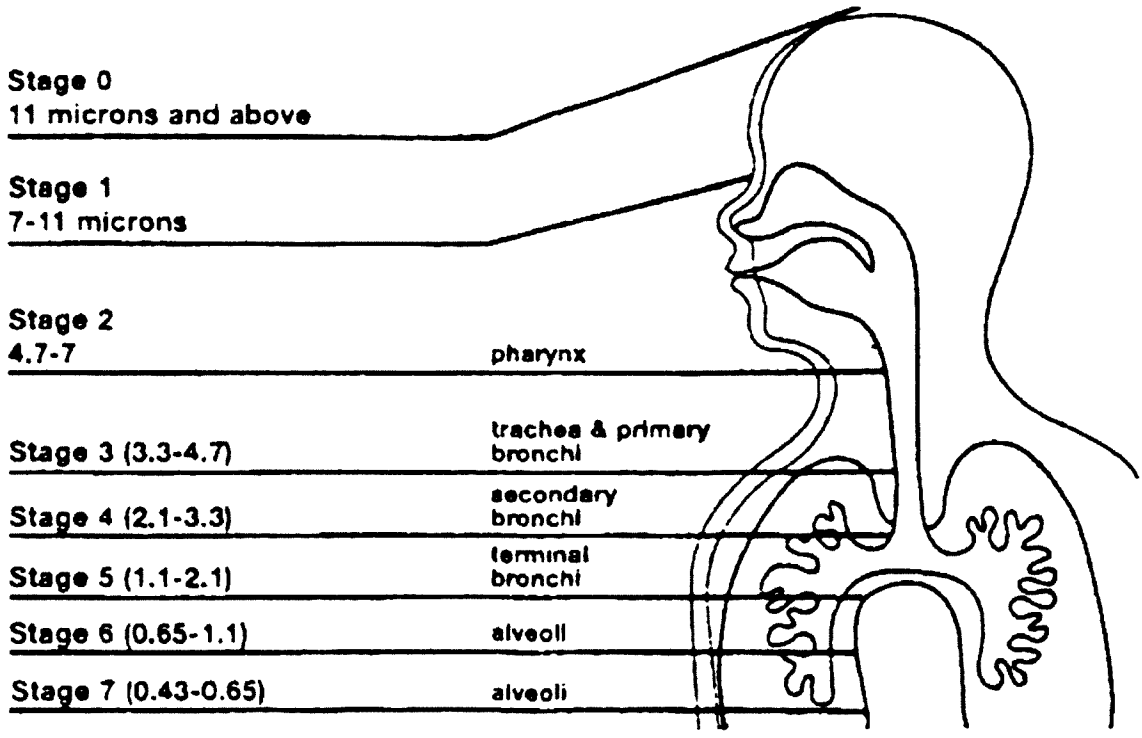


Figure 1.4: Airborne allergen sizes captured by an Anderson Sampler

The Anderson Sampler is able to enumerate viable fungal spores. Air passes through a series of sieve-like plates, each containing 400 holes and representing a different stage. As the air moves from plate to plate, the diameter of the holes decreases. Larger particles are retained by the upper plates and the smaller ones by successive lower plates. Growth medium is placed beneath each plate and the spores that pass through the holes fall onto the agar. Here they form colonies, which can be counted. Each stage of particle capture corresponds to a stage of particle size captured in the human respiratory system (Gutman & Bush, 1993; Newsom & Shaw, 1997).

while powder-free gloves ranged from < 5-61 µg/mℓ (Yunginger *et al*, 1994). Powdered gloves released between 18 843 ± 1 885 and 53 ± 2 586 small airborne particles (0.5-5 µm), while non-powdered gloves only released 230 ± 43 and 557 ± 73 (Chabane & Leynadier *et al*, 2000).

Other factors than particle size and concentration also influence inhaled particle deposition. Rhinitis or nasal abnormalities lead to mouth-breathing with loss of nasal

filter and increased lower airway deposition. Subjects with airflow limitation and smokers have greater lung deposition of fine (1 µm) particles (Tarlo & Purdham, 2002).

Once the major source of NRL aeroallergens is removed by the use of powder-free or synthetic gloves, specific IgE levels should drop below the detection limit (Allmers *et al*, 1998). A clear and consistent dose response exist between NRL aeroallergens levels and symptoms in sensitised persons. When allergen levels measured by personal and volumetric samplers were less than 0.4 ng/mm^3, symptoms were absent, and when levels were greater than 0.6 ng/m^3, symptoms commenced. An aeroallergen level of 0.6 ng/m^3 was therefore suggested as threshold level at which allergic reactions are expected to occur in sensitised individuals (Poley & Slater, 2000; Toraason *et al*, 2000; Randolph, 2001; Garabrant & Schweitzer, 2002; Hunt *et al*, 2002; Weissman & Lewis, 2002). Latex-related anaphylaxis and asthma can be associated with allergen levels of 39-311 ng/m^3 (Baur *et al*, 1998).

Latex particle concentrations can exceed 200 µg/m^3 in areas where powdered gloves are used (Poley & Slater, 2000). Different studies reported the following NRL aeroallergen concentrations: 111 ± 25 ng/m^3 (Hamilton & Brown, 2000; Hunt *et al*, 2002), 13-121 ng/m^3 (Levy *et al*, 1992), 2-138 ng/m^3, 13 -208 ng/m^3 (Swanson; 1994; Brehler 1997; Hunt 2002), 39-311 ng/m^3 (Tarlo *et al*, 1994), or even higher (Heese *et al*, 1997). These levels are distinctly higher than in laboratories where powder-free gloves were used (< 0.02 ng/m^3) (Tarlo *et al*, 1994).

One of the highest personal breathing zone concentrations (419 ± 292 ng/m^3) was measured for an anaesthesiologist (Hamilton & Brown, 2000; Hunt *et al*, 2002). Other personal breathing zone concentrations in high glove-usage areas ranged from 8-974 ng/m^3, while in areas where gloves were seldom or never used, it only ranged from 0.3-1.8 ng/m^3 (Levy *et al*, 1992; Swanson *et al*, 1994; Brehler *et al*, 1997; Hunt 2002). The latex aeroallergen levels were strongly correlated with the total number of gloves used on designated high allergen glove use days. Latex aeroallergen levels during low allergen glove use days (0.1-3.5 ng/m^3) were significantly lower than on high allergen glove use days (2.2-56.4 ng/m^3), but not significantly different from that on non-surgery days (0.1-3.6 ng/m^3) (Heilman *et al*, 1996).

An Australian study measured the dose of allergenic latex particles inhaled by HCW[s] during routine tasks and found that all subjects inhaled *Hev b 5* bearing particles. The number of particles inhaled while wearing standard, powdered gloves was 23.8-fold higher than not wearing gloves and 9.7-fold higher than when wearing non-powdered gloves (Mitakakis *et al*, 2002). Almost all collected particles had a diameter between 5 µm and 12 µm. Another study found most of the particles carrying *Hev b 1* to be 10-20 µm in diameter and confirmed that 95% of these particles were cornstarch granules (Poulos *et al*, 2002).

A series of UK surveys confirmed a statistical difference in the level of starch contamination between a psychiatric ward with no glove usage, compared to the trauma department where powder-free gloves were used. Further analysis showed that the non-ventilated intensive care ward was significantly worse than other clinical units (Newsom & Shaw, 1997). An Anderson sampler used in another study showed that almost 75% of the allergens were carried on particles > 14 µm, and only about 20% on particles that could be expected to remain suspended in the air stream to be deposited in the lungs. This is consistent with the more frequent occurrence of upper airway symptoms in the eyes and nose as compared with symptoms of asthma. According to the results from these studies, few symptoms occurred at breathing zone levels below 10 ng/m^3 and that levels approaching 1 000 ng/m^3 resulted in severe asthma. Challenge tests found that higher protein values (100-1 000 ng/m^3) were required to provoke mild symptoms. It was recommended to consider values below 10 ng/m^3 as "low", 10-50 ng/m^3 as "moderate" and more than 50 ng/m^3 as "high" (Hunt *et al*, 2002). Surface contamination levels and exposure limits were specified for dust contamination as well. The greater the concentration on the surfaces, the greater the inhalation exposure will be when the dust becomes airborne ***(Table 1.6)*** (Reiter, 2002).

Minimal amounts of latex allergen were found in the dust samples from the passive ventilation duct of a non-forced air heated building at the Mayo Clinic. In contrast, samples of carpets and fabric upholstery from work area chairs contained significant amounts of latex allergen (up to 370 ng/m^3) and can be regarded as allergen repositories capable of sustaining elevated aeroallergen concentration, even after removal of the primary source. During non-working hours when gloves were not used,

Table 1.6: Recommended exposure limits for latex exposure

Challenge tests have confirmed a relationship between symptoms and latex allergen concentration. Increasing concentrations elicit more severe symptoms in sensitised individuals (Hunt *et al*, 2002).

Relative Risk	Airborne exposure	Dust contamination
Low	< 10 ng/m^3	< 10 µg/m^3
Moderate	10-50 ng/m^3	10-100 µg/m^3
High	> 50 ng/m^3	> 100 µg/m^3

aeroallergen concentrations became undetectable in non-carpeted rooms. However, carpet dust samples obtained after more than ten months after powder-free intervention still contained notable amounts of latex allergen. The carpeting only appeared to retard rather than prevent aerosolisation of the latex (Charous *et al*, 2000).

In the process from tapping and manufacture of latex gloves through to the use, a UK epidemiological study found the highest aeroallergen exposure to be in Thailand manufacturing factories (7.3 µg/m^3), followed by moderate exposure in rubber plantation workers (2.36 µg/m^3). UK hospitals showed aeroallergens values of only 0.46 µg/m^3 (Sri-Akajunt *et al*, 2000). In another NRL glove factory, no airborne latex allergens were detectable in the production area of synthetic gloves, which was well isolated from the section producing latex gloves. The highest concentration of aeroallergens was found in the production and vulcanisation areas, followed by the technical laboratory, where the quality of newly produced gloves was to be approved. A considerable lower aeroallergen load was found in the section where latex concentrate was formulated for production. Fine dust was collected from the glove production area floor and showed extremely high NRL allergen concentrations (Baur, 2003). Generally, at the point where the gloves are removed from the formers the concentration can be as high as 20 000 ng/m^3 (Hunt *et al*, 2002).

When air sampling was done in a room with 30 helium filled balloons, no allergen was detectable when the balloons were quietly present. After they were batted around, the allergen level rose to 4 ng/m^3 and after they were popped, it rose further to 20 ng/m^3 (Hunt *et al*, 2002). Latex condoms, baby pacifiers and other medical items produced

extractable allergen levels ranging between < 5-50 µg/mℓ only, while in the levels in toy balloons were comparable to powdered gloves (4 700 µg/mℓ) (Yunginger *et al*, 1994).

Rubber tyres are not a significant source of allergen, if they are a source at all (Hunt *et al*, 2002). Tyre treads made from blends of crumbled natural and synthetic rubbers contain 200-fold less latex per unit of protein than latex gloves. A German study could not find a consistent association between exposure to road traffic in a large city and allergic latex sensitisation in children and concluded that traffic exposure was not a risk factor for the development of latex allergy in children (Hirsch *et al*, 2000).

1.11. CROSS-REACTIVITIES

Cross-reactivity is largely determined by structural aspects, as cross-reactive proteins share structural features. All cross-reactive proteins have a similar fold, but proteins with a similar fold are not necessarily cross-reactive. At least 70% AA identity is required for cross-reactivity (Aalberse, 2000). However, AA sequence similarity alone is not sufficient to predict cross-reactivity. Four different isoforms of the major allergen of hazel pollen, *Cor a 1*, differ in IgE binding, although these isoforms have AA identities of 96% to 99% with each other. They also differ in their ability to induce activation of allergen-specific T-cell clones (Sowka *et al*, 1999).

One of the first reported cases of latex-fruit cross-reactivity described a nurse with confirmed latex allergy, who had an allergic reaction to banana (M'Raihi *et al*, 1991). More patients with latex allergy subsequently presented with allergies to avocado, kiwi and chestnut. Clinically, these patients often have perioral itching and local urticaria, and occasionally life-threatening, food-induced anaphylactic shock (Levy *et al*, 1992; Sussman & Beezhold, 1995).

The "latex-fruit syndrome" involves cross-reactions of latex allergy and a number of phytogenetically distant plants, mainly fruit and nuts. The most common cross-reactions are with avocado, banana, chestnut, fig, kiwi, mango, melon, papaya, passion fruit, peach, pineapple and tomato ***(Table 1.7)***. Cross-reactions have also been reported for barley, bluegrass, grass, legumes, potato, pollens, ragweed, spinach, sweet pepper, wheat and the weeping fig (Añibarro *et al*, 1993; Heese *et al*, 1997; Maillard *et al*, 1999; Toraason *et al*, 2000; Randolph, 2001; Ranta & Ownby, 2004).

Table 1.7: Food associated with the "latex-fruit syndrome"

It involves cross-reactions between latex allergy and a number of phytogenetically distant plants, mainly fruit and nuts. The cross-reactivity is presumably related to the common epitopes or shared plant pan-allergens (De Beer 2001; Randolph 2001; Alvarado *et al*, 2002).

High	Moderate	Low / Undetermined	
avocado	apple	pear	peach
banana	carrot	plum	cherry
chestnut	celery	pineapple	strawberry
passion fruit	kiwi fruit	fig	grape
	(raw) potato	apricot	nectarine
	papaya	mugwort	wheat
	tomato	mango	rye
	melon	grass	ragweed
		hazelnut	walnut
		peanut	soybean

Less common sources of cross-reactivity are apple, apricot, beet, celery, cherry, citrus fruits, coconut, condurango bark, dill, grape, loquat, oregano, peanut, peppers, pineapple and sage. This cross-reactivity is presumably related to the common epitopes or shared plant pan-allergens of profilin, patatin, plant stress proteins (*Win 1* and *2*) and *Bet 1* and *2* (Wagner *et al*, 2000; Randolph, 2001; Alvarado *et al*, 2002). Alternatively, class I chitinases, prohevein, hevein, carbohydrates and defence-related proteins have all been implicated in contributing towards cross-reactions. Some of these significant proteins have enzymatic activities and include hydrolases, enolases and proteases (Patriarca *et al*, 2002a ; Kurup & Fink, 2001).

Common electrophoretic protein bands or homologous AA sequences have been found in avocado, banana (Lavaud *et al*, 1995; Pastorello *et al*, 1996), chestnut (Añibarro *et al*, 1993), citrus fruit, guava, pear, strawberries (Lavaud *et al*, 1995) and potato (Heese *et al*, 1997). Cross-reactive allergens in banana appear at several MW between 23 and 47 kD and in avocado between 27 and 91 kD (Poley & Slater, 2000).

The major allergen in kiwi fruit (*Actinidia chinensis*) is a 30 kD profilin. It probably cross-reacts with *Hev b 8*, which is also present in banana, birch pollen, cherries, guava, mandarin orange, peach and strawberry (Lavaud *et al*, 1995; Pastorello *et al*, 1996). Similarly, *Hev b* 5 is strongly homologous to the complementary DNA sequence in kiwi fruit and patatin contains a region with strong homology to *Hev b* 7. Both these may contribute towards the reported cross-reactions (Poley & Slater, 2000).

Peeling of raw potatoes caused oculonasal symptoms, wheezing and contact urticaria in adults with seasonal allergic rhinitis. In other cases, it precipitated various allergic reactions, including rhinoconjunctivitis, wheezing, contact urticaria and flares of atopic dermatitis. Allergy to raw potato is usually associated with oral allergy syndrome. Only a few cases of allergic reactions to cooked potato have been reported. The allergens in potato appear to be heat-labile and raw potato contains several allergens, which are not present in potato starch or flour (De Beer, 2000; Beausoleil *et al*, 2001; De Beer & Cilliers, 2001).

Ethylene, which is used to hasten fruit ripening, induces the expression of class I chitinases. However, plant class I chitinases loose their allergenic activity, IgE-binding capacity and *in vivo* reactivity by heating. This could explain why mainly fresh fruits are associated with the latex-fruit syndrome, whereas plant materials that are cooked before being consumed, even those possessing class I chitinases, are rarely related to latex allergy (Sánchez-Monge *et al*, 2000).

1.12. *IN VIVO* AND *IN VITRO* DIAGNOSTIC TECHNIQUES

Effective management of a person with a type I latex allergy begins with a proper diagnosis that includes a complete clinical history, physical examination and patient suspicion (Hamilton *et al*, 1998; Hamilton & Adkinson, 2003). Most patients are aware of local or respiratory symptoms when wearing gloves or improvement of these symptoms during holidays. Any discomfort after a visit to the dentist or gynaecologist should prompt an evaluation for latex allergy (Warshaw, 1998). History alone may not identify all latex allergic individuals and one of several confirmatory tests should be used to detect allergen-specific IgE in the skin or blood (Lebenbom-Mansour *et al*, 1997; Hamilton & Adkinson, 2003).

The detection of specific IgE antibodies establishes that sensitisation has occurred, but does not always indicate the presence of an allergic disease. Food allergy is often followed by sensitisation to common environmental allergens and sensitisation to aeroallergens usually precedes symptomatic inhalant allergies by several years (Ricci *et al*, 2003). Patients who have latex-specific IgE antibodies without clinical reactivity may have cross-reactive antibodies of no significance (Poley & Slater, 2000).

Different diagnostic approaches for specific purposes are summarised in ***Table 1.8*** (Warshaw, 1998; De Beer & Cilliers, 2001). None of the existing *in vivo* or *in vitro* diagnostic methods are 100% diagnostically accurate, mainly because of the variety of NRL allergens (Sussman & Beezhold, 1995; Lebenbom-Mansour *et al*, 1997; Palczynski 2000; De Beer & Cilliers, 2001). Misdiagnosis may result from over-reliance on clinical history and failure to perform objective tests (Ranta & Ownby, 2004). Most investigators agree that the best screening method for latex allergy remains the SPT, provided that it is performed with standardised allergen extracts and in a safe environment under controlled conditions (Turjanmaa *et al*, 1996).

An important factor to bear in mind when comparing published results is that many studies use in-house or self-prepared allergen extracts (Nieto *et al*, 1996). Even with a careful clinical history, patients can be incorrectly diagnosed with latex allergy, yielding negative latex-specific IgE and SPT results (Hamilton & Adkinson, 1996). Due to the high sensitivity and specificity of SPT, latex-specific IgE detectable by RAST can only be demonstrated in 50-90% of individuals with positive SPT^{s} (Randolph, 2001). Therefore, set against clinical information, a combination of at least two or more methods should be used for diagnosis (De Beer, 2000; De Beer & Cilliers, 2001).

1.12.1. Questionnaire (*in vivo*)

The questionnaire is designed to capture demographic data, current and previous exposure to NRL, other risk factors, e.g. atopy, surgical procedures, specific food allergies and symptoms. This is a cheap and easy diagnostic aid with a reasonably high sensitivity (De Beer, 2000; De Beer & Cilliers, 2001). Due to low specificity and low positive predictive value (PPV), this should not be used alone (Lebenbom-Mansour *et al*, 1997).

Table 1.8: Diagnostic tests for latex allergy

Different diagnostic approaches are usually used for specific purposes, but none of the methods is 100% accurate. It is therefore recommended that a combination of the available tests are used for diagnosis (De Beer, 2000; Palczynski 2000; De Beer & Cilliers, 2001).

Research	Clinical
Basophil histamine release test	Intradermal / inhalation tests
Cross and rocket immunoelectrophoresis	Latex allergosorbent assay (AlaSTAT)
Cytometric assay	Open and closed patch tests
ELISA	RAST (capRAST)
Flow cytometry	Rub test
Immunoblots	SPT
Radioimmunoassay	Scratch chamber test
Reverse enzyme immunoassay	Latex-specific antibody assays

1.12.2. Skin Prick Test (*in vivo*)

Antigens are commercially available or in-house extracts can be made by dissolving glove powder in saline. A drop of antigen solution is placed on the forearm and the epidermis is pricked with a sterile lancet to a depth of exactly 1 mm. The remaining solution is gently wiped away and the reaction is read within 15 minutes. Saline serves as a negative control. A wheal-and-flare reaction of more than 2 mm is regarded as positive (Chapel & Haene, 1993; Warshaw, 1998). It is estimated that this method introduces a volume of 10^{-6} mℓ of test solution into the epidermis (Lessof, 1981).

The European Academy of Allergy and Clinical Immunology guidelines recommend the following method to confirm a positive SPT. The longest diameter of the wheal and the diameter perpendicular to it are measured and the average calculated. A score of 2+ indicates an average diameter half that of the wheal induced by 10 mg/mℓ histamine (positive control), 3+ is when the average diameter is equal to the wheal induced by histamine, and 4+ is when the average diameter is double to the wheal induced by the

histamine prick. Wheal responses of 2+ or greater are considered as positive (Bernardini *et al*, 2003).

This method has a 2% risk of anaphylaxis (Bowyer, 1999b). Patients should not be subjected to SPT^s if they have systemic symptoms, bronchospasm or anaphylactic reactions with NRL contact, but it would be relatively safe to perform a SPT following a negative latex-specific IgE (De Beer, 2000; Brathwaite *et al*, 2001; De Beer & Cilliers, 2001; Degenhardt *et al*, 2001; Potter *et al*, 2001b). Other disadvantages include a high rate of false positive results in highly dermographic patients, as well as non-specific irritant reactions, especially in atopic patients who often have positive SPT^s to multiple antigens (Williams *et al*, 1992; Chapel & Haene, 1993). It may be poorly tolerated in infants and children (Holme & Lever, 1999). False negative SPT results might be due to desensitisation caused by recent contact with the allergen or the presence of blocking IgG antibodies interfering with IgE-dependent responses. Or the levels of allergen-specific, basophil-bound IgE may be too low to mediate an allergic reaction (Hauswirth *et al*, 2002).

This method is widely used and is relatively cheap to perform (Warshaw, 1998). It has a high sensitivity and specificity, but like all tests for specific reactivity, the tests are only as good as the purity and antigenicity of the reagents used (Chapel & Haene, 1993). As the SPT uses fewer allergens than the scratch test, a negative SPT should be followed up by a scratch test to confirm the diagnosis (Konrad *et al*, 1997).

1.12.3. Intradermal Test (*in vivo*)

Intradermal testing is similar to the SPT ***(Figure 1.5)***, except that the antigen is more highly diluted in saline and injected directly into the dermis of the patient's lower arm (Chapel & Haene, 1993; Warshaw, 1998; Bowyer, 1999b). The volume of injection ranges from 0.01-0.02 mℓ of the test extract (Lessof, 1981).

The intradermal test is more sensitive than SPT and although it gives reproducible results, the risk of anaphylaxis is higher than any of the previous methods (Bowyer, 1999b). It is a painful procedure with a low specificity and high rate of false positive results (Warshaw, 1998). It is also often difficult to interpret results in patients with severe dermatitis or eczema (Bowyer, 1999b).

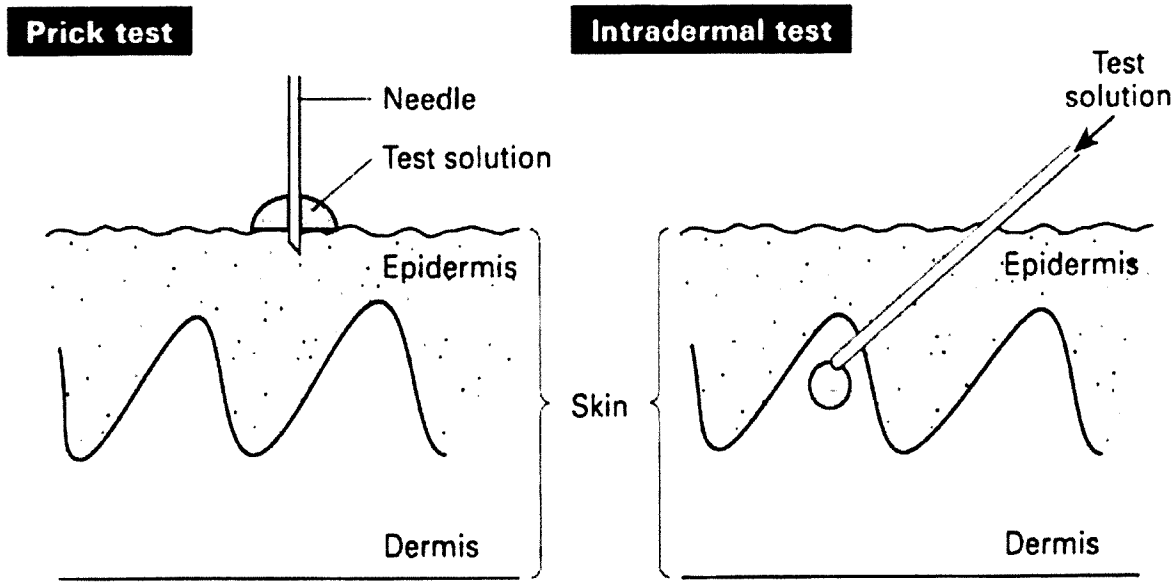


Figure 1.5: Difference between a SPT and intradermal test
With the SPT the antigen is introduced into the epidermis to a depth of exactly 1 mm, while highly diluted antigen is injected directly into the dermis with an intradermal test.

1.12.4. Scratch Chamber Test (*in vivo*)

A scratch of 6 mm is made on the volar aspect of the arm with a sterile lancet. A piece of NRL glove is moistened with saline and applied over the scratch under occlusion for 15 to 20 minutes. A wheal-and-flare reaction of more than 2 mm within 30 minutes is regarded as positive (Chapel & Haene, 1993; Warshaw, 1998).

This method has a relatively high sensitivity, but also a high risk of anaphylaxis and a high rate of false positive reactions (Chapel & Haene, 1993; Warshaw, 1998).

1.12.5. Use / Rub Test (*in vivo*)

With a use test a NRL glove is applied directly to a wet hand for 15 minutes. A vinyl glove is used as control. In the case of the rub test or repeated open application test a piece of glove, glove extract or latex fluid is repeatedly rubbed onto the volar aspect of the forearm over a period of time (Chase, 1976; Warshaw, 1998). An open test may be preferable for irritant substances. Eczematous reactions after seven days may be

interpreted as an allergy, but the rate of false positive and negative reactions is unknown (Fullerton *et al*, 1996).

Both methods are relatively safe, but anaphylaxis can occur in highly sensitised patients. Results are reliable in patients with allergic skin diseases, but may be negative in patients with respiratory symptoms (Warshaw, 1998; Palczynski *et al*, 2000).

1.12.6. Inhalation / Provocation Test (*in vivo*)

For inhalation tests the patient handles NRL gloves and spirometry is done at 15-30 minute intervals. With nasal provocation tests a latex solution on a cotton swab is applied to the nasal mucosa for five minutes. The eyes, nose and bronchi are simultaneously challenged (Warshaw, 1998; Palczynski *et al*, 2000).

These methods have a high sensitivity and specificity and can accurately identify patients with occupational asthma (Palczynski *et al*, 2000). The disadvantages include risk of anaphylaxis and it requires a highly allergenic brand of gloves (Turjanmaa *et al*, 1996; Warshaw, 1998; Palczynski *et al*, 2000). Provocation test results remain difficult to compare. Protocols differ with regard to the latex product (glove source and type) used, the duration of rubber product application and subject exposure, and the method used in scoring individual symptoms (Hamilton *et al*, 2002).

1.12.7. Latex-Specific IgE (*in vitro*)

The RAST and enzyme-liked immunosorbent assay (ELISA) are automated methods measuring an antigen-antibody reaction. The RAST is performed by incubating the patient's serum with a solid-phase support (i.e. a paper disc) coupled to a specific antigen. Antigen-specific IgE, if present in serum, attaches to this bound antigen. After washing to remove non-specific IgE, the antigen-coated disc is incubated with radiolabeled anti-IgE. The amount of radioactive anti-IgE attached to the patient's antigen-specific IgE on the disc provides a measure of the specific IgE content of the serum (Atkins & Metcalfe, 1987; Chapel & Haene, 1993; Warshaw, 1998). The original RAST, developed in 1966, defined a positive result as bound radioactivity two to five times greater than that of a negative control assay (Williams *et al*, 1992).

The ELISA or AlaSTAT latex-specific IgE uses an enzyme, such as alkaline phosphatase or horseradish peroxide, instead of a radioisotope that is attached to the

anti-IgE (Weiss, 1995). The amount of anti-IgE bound to antigen-specific IgE is then proportional to the bound enzyme activity (Atkins & Metcalfe, 1987).

This is by far the safest method for the patient with a high sensitivity and specificity (Weiss, 1995; Bowyer, 1999b; De Beer, 2000; De Beer & Cilliers, 2001). The method is expensive, time-consuming and not as sensitive as SPT^s (Moneret-Vautrin *et al*, 1993; Bowyer, 1999b). Some investigators feel that the specific IgE assay is not sufficient to establish the diagnosis (Leynadier *et al*, 2000). In patients with milder symptoms, false negative results sometimes occur (Turjanmaa *et al*, 1988a). Follow-up SPT^s can be used to confirm the diagnosis (De Beer, 2000; De Beer & Cilliers, 2001).

1.12.8. Cytometric Assays / Basophil Histamine Release Test (*in vitro*)

This method involves multicolour flow cytometric analysis of activated basophils. When basophils are activated with specific allergen that is recognised by FcεRI-bound IgE, it secretes and generates quantifiable bioactive mediators, and also upregulate the expression of markers, such as CD_{45} and CD_{63}. Whole blood is incubated with IL-3 within three hours after collection. Basophils are subsequently pre-activated and mediator release or CD_{63} expression is increased. Cells are stained with monoclonal biotinylated antibodies to human IgE and monoclonal phycoerythrin-conjugated antihuman CD_{63}. A series of washing and lysing of cells follow. After centrifugation another wash is performed and green fluorescence and side scatter are then employed to gate at least 500 activated basophils that express a high density of surface IgE. The final value is deducted from the amount of basophilic expression of CD_{63} (Warshaw, 1998; Ebo *et al*, 2002).

It is a very highly sensitive (93%) and specific (91-100%) test for conditions, such as latex allergy (Ebo *et al*, 2002; Sanz *et al*, 2002). It also carries no risk of anaphylaxis and the sensitivity rate is comparable with SPT^s (Warshaw, 1998). Cross-reactivity due to so-called pan-allergens and carbohydrate determinants (antigenic structures shared by taxonomically distant allergenic sources) often interfere with final results (Ebo *et al*, 2002). The test is very expensive and time-consuming, which makes it unsuitable for routine analysis, but of value in research techniques (Warshaw, 1998).

1.12.9. Patch Test (*in vivo*)

This test is used to diagnose a delayed type IV hypersensitivity reaction. Several panels of common antigens are commercially available. The antigens are placed in a aluminium Finn chamber and applied to intact skin under occlusion to posterior trunkal area. After 48 hours the patches are removed and results are read after 48 and 96 hours (Dahl, 1981b; De Beer, 2000; De Beer & Cilliers, 2001).

Positive allergic reactions exhibit erythema, oedema, vesiculation and in extreme reactions pustule formation and ulceration. Positive reactions are frequently pruritic, but severe reactions may be painful. The International Contact Dermatitis Research Group has suggested the following quantification method (Dahl, 1981b):

?+ doubtful reaction
\+ weak (non-vesicular reaction)
++ strong (oedematous or vesicular reaction)
+++ extreme reaction (ulcerative or bullous)

This is a very simple procedure, but it is sometimes difficult to distinguish between irritant and allergic responses (Jackson & Cerio, 1988). A true allergic reaction persists for several days. Irritation skin reactions usually subside within a few hours. When differentiation between the two reactions is not clear, the area should be examined the following day (Slavin, 1993). Patch tests are of limited use in the diagnosis of latex allergy, because it tests for the additives in NRL products and not for NRL proteins (De Beer, 2000; De Beer & Cilliers, 2001). Patch tests with purified NRL proteins carry the possibility of anaphylaxis.

1.13. SUCTION BLISTER FORMATION AND DERMAL FLUID

Suction blister fluid harvesting has been an established method for over 30 years (Benfeldt, 1999). Application of prolonged suction to the skin surface causes separation of the epidermis from the dermis along the lamina lucida. Blisters can also be induced by application of irritating compounds, e.g. cantharidine, which results in an inflammatory exudate with a higher albumin content than suction blister fluid. The fluid drawn into this newly formed compartment can be sampled and analysed for the content of inflammatory mediators, inflammatory cells or other components (Kiistala,

1968; Benfeldt, 1999). Different studies have shown the release of histamine, bradykinin, PG E_2 and PG $F_{2\alpha}$ (Cilliers, 1993), serine proteinases, proteoglycans, tryptase, LT C_4 and LT D_4 in dermal fluid during inflammatory skin reactions (Deleuran *et al*, 1991). Predominantly histamine and PG D_2 were obtained from skin blisters after antigen challenge (Lawrence *et al*, 1987).

Lipid analysis showed that all major classes of lipids present in serum (triglycerides, cholesterolesters and phospholipids) were also present in blister fluid. The blister fluid / serum concentration ratio of proteins was dependent on the logarithm of their MW. This ratio dropped sharply from 0.90 to 0.29 for a MW lying between 6.6 kD (insulin) and 60 kD (albumin). The concentration of high MW substances seems to be determined mainly by passive diffusion, while the flow of low MW substances into blister fluid obeys the law of diffusion (Vermeer *et al*, 1979).

Blister fluid tryptase levels are elevated in patients with urticaria, but not atopic dermatitis or psoriasis (Brockow *et al*, 2002). Histamine levels are increased in blister fluid of patients with atopic dermatitis (Cilliers, 1993), cold and chronic urticaria. In some patients histamine levels are even higher in blisters raised over urticarial wheals (Dahl, 1981a). Tryptase levels were also found to be highest in dermal fluid of active lesions, whereas tryptase could not be detected in the serum of these patients. The release of histamine and tryptase seems to correlate closely, although it has been shown that ongoing antigen stimulation leads to a continuous low, but significant release of histamine lasting up to six hours, whereas the level of tryptase falls to insignificant levels by that time. Histamine is metabolised and tryptase neutralised rapidly. Tryptase has a half-life of only approximately two hours, and this suggests that the tryptase detected in skin originated from the skin mast cells through local release (Deleuran *et al*, 1991). Tryptase levels in blister fluid and plasma have been shown to correlate with dermal mast cell numbers, suggesting that the skin is a major production site for plasma tryptase. However, soluble SCF in the blister fluid correlated neither with mast cell numbers in the dermis, nor skin blister total tryptase levels (Castells & Schwartz, 1988).

1.14. MANAGEMENT

Since the beginning of the 1980s, latex allergy increased dramatically world-wide (White, 1997). The main therapeutic approach in latex-sensitised people is to avoid latex

exposure (Potter, 1998a; Potter, 1998b). This is a balance between disease prophylaxis, lifestyle options and livelihood (Poley & Slater, 2000).

Primary latex exposure prevention for the non-sensitised is a more recent concept and involves minimizing or eliminating latex contact for individuals of known risk groups from the outset. *Secondary latex prevention* for the latex allergic subject involves screening to identify asymptomatic individuals or known symptomatic patients (Poley & Slater, 2000).

Once the diagnosis is confirmed, the degree of impairment must be determined. The physician must then inform (with the patient's consent) the employer and risk managers regarding the diagnosis and most effective interventions. The employer must also be educated about latex allergy and made aware of potential unfavourable clinical outcomes (Bernstein, 2002). Patient education with regard to intervention, available treatment and the latex-fruit syndrome remains an important priority (Zerin *et al*, 1996). The use of Medic Alert discs, EpiPen or Anaguard autoinjectors should be encouraged (Randolph, 2001). Milder symptoms can be prevented or controlled by the administration of antihistamines and steroids (Patriarca *et al*, 2002a).

Condoms and latex gloves are essential in reducing the risk of sexually or blood-transmitted diseases. Glove quality should be regulated by restricting the amount of extractable proteins (Potter, 2001a). The use of alternative products is controversial, because other materials are more expensive and may have different mechanical characteristics (Patriarca *et al*, 2002a; Patriarca *et al*, 2002b).

There is an emerging global consensus that it would be medically negligent to perform an open procedure wearing starch powdered gloves (White, 1997). A powder-free policy has been adopted in the UK, Canada, Scandinavia and Germany to limit unnecessary exposure and sensitisation (Potter, 2001a). Complete removal of powdered latex gloves has resulted in undetectable levels of airborne latex particles and latex allergic HCW^{s} have been able to return to their workplace. This has also significantly reduced individual symptoms, some even reverting to undetectable latex-specific IgE on follow-up after creating a latex-free environment (Wrangsjö *et al*, 1986, Sussman & Beezhold, 1995; Allmers *et al*, 1998; Brathwaite *et al*, 2001; Weissman & Lewis, 2002; Ranta & Ownby, 2004). Even though latex sensitisation may be reduced by avoidance measures, it does

not eliminate the risk for resensitisation with re-exposure (Hamilton & Brown, 2000; Ranta & Ownby, 2004).

Individuals with confirmed latex allergy should inform their medical and dental practitioners before any procedure involving contact with mucosal surfaces or the abdominal cavity are performed. Clinical staff must be fully informed of the problems associated with latex allergy and the necessary precautions for dealing with allergic individuals (Wakelin & White, 1999). Identification by Medic Alert discs could prompt correct management in cases of comatose latex allergic patients.

There is some controversy regarding at what stage a latex allergic patient with a perceived reaction should use their epinephrine or adrenaline and when to go to the emergency room. The usual advice to patients is that the epinephrine or adrenaline is for "first aid" only and that they should go immediately to the nearest emergency medical facility for further treatment. However, several case reports and a survey of UK casualty and emergency medical staff, has shown a high degree of ignorance regarding the management of all types of latex allergy. The patient may actually be at greater risk of prolonging or worsening of reactions through hospital attendance. The changed policy for severe latex allergic patients recommends that emergency attendance is avoided unless self-treatment is ineffective. Patients are encouraged to use their epinephrine or adrenaline early, accompanied by oral prednisolone (20 mg) or intramuscular hydrocortisone sodium succinate (100 mg). Provided there is good response, hospital attendance is not required. Failure to respond to 2 x 0.3 mg epinephrine subcutaneously, 1 mg epinephrine by nebuliser, or recurrence of symptoms, warrants a transfer to a hospital. This has significantly improved patient confidence and re-established the patients' control over their disease (Spickett *et al*, 2002).

Latex allergic patients often report allergic symptoms or anaphylaxis when exposed to medical or other products they believe contained latex. Fear of recurrent adverse or anaphylactic reactions could lead to an association between perceived exposure and allergic symptoms. Conditioned reflex and / or anxiety reactions similar to those reported after exposure to actual latex may follow, even in the complete absence of latex (McCamish & Golden, 2002). The American Academy of Allergy and Immunology has published guidelines for providing care to persons with latex allergy. A flow chart

for management of individuals with possible latex allergy is shown in ***Figure 1.6*** (Sussman & Beezhold, 1995).

1.14.1. Occupational Health and Safety Act

NRL is defined as a substance hazardous to health by the Control of Substances Hazardous to Health Regulations in 1994 and employers have a legal obligation to take measures to ensure the safety of their employees (Wakelin & White, 1999). In the RSA substances causing allergic reactions are notifiable to the Commissioner for Occupational Diseases. The employee's rights are protected by the Occupational Health and Safety Act No 85 of 1993, under which the employer must provide, as far as reasonably practical, a working environment which is safe and without risk to the health of the employee. However, voluntarily subjecting oneself to further exposure could seriously prejudice any action brought to a court of law. Once diagnosed, a latex allergic HCW may seek compensation from the Compensation for Occupational Injuries and Diseases Act No 130 of 1993 (previously Workmen's Compensation Act, 1941) and should be managed and followed up by the diagnosing physician accordingly law (Potter, 1998a; Potter, 1998b; Potter, 1998c; Potter, 2001a).

Occupational latex allergy incurs direct and indirect cost. The direct costs entail the purchase of powder-free or latex-free gloves, the substitution of other hospital equipment and the cost of installing air filtration / laminar flow changing stations. The indirect costs are worker-related and include job relocation, job change without retraining, or retraining and re-education for non-clinical placement (Taylor, 1997).

The potential cost of ignoring latex allergy (litigation, retraining, compensation, re-structuring of hospital environments, etc) are much higher than those incurred by changing a healthcare institution to low allergy powder-free gloves. Since latex allergy is not as clearly defined as a "loss of limb" or other overt function, the Workmen's Compensation System has been reluctant to classify HCW^{s} as disabled. Consequently, there are very few examples of HCW^{s} receiving disability payments for latex allergy. Employees who lose their jobs also lose their health insurance and workmen's disability payments. These are powerful incentives not to rock the boat and risk becoming unemployable due to latex allergy (Taylor, 1997). Follow up studies in Sweden show that

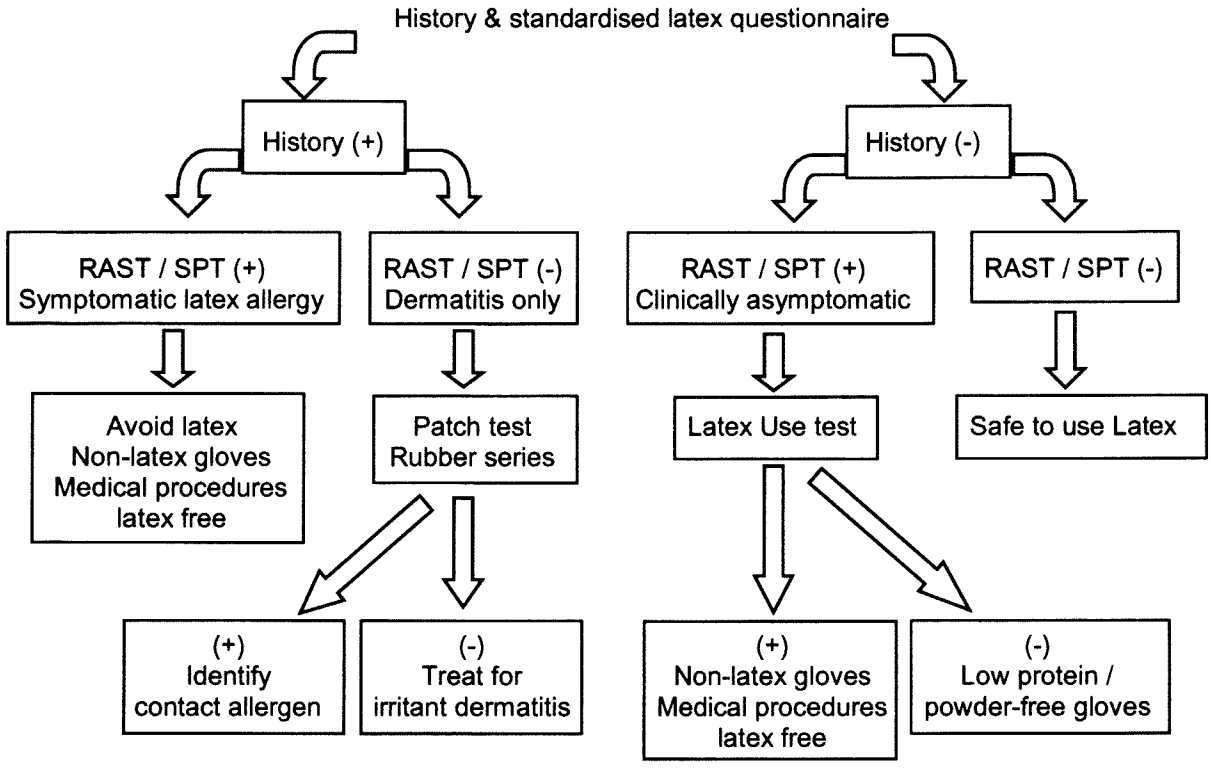


Figure 1.6: Flow chart for latex allergy diagnosis and management

The American Academy of Allergy and Immunology recommends these guidelines for the proper diagnosis and management of individuals with possible latex allergy. Even gloves with extremely low levels of extractable protein can provoke reactions in sensitised individuals (White, 1997).

up to 40% of HCW^{s} with latex allergy resigned, changed jobs or took early retirement after they have been diagnosed with latex allergy (Taylor *et al*, 1999). However, a follow up study at the Tampere University Hospital in Finland three years after converting to low allergen latex gloves, showed that none of these patients had changed work because of latex allergy and that hand eczema diminished significantly (Turjanmaa *et al*, 2002). A Belgian study did not find a significant difference in the severity of asthma between subjects who had been completely removed from their workplace and those who had been transferred to lower exposure. Compared with complete removal, reduction was associated with a substantially lower socio-economic impact (Vandenplas *et al*, 2002).

Conflicting reports exist as to whether intervention measures, particularly glove purchasing patterns, are cost-effective. It has been estimated that to convert an entire USA dental school to powder-free latex gloves would cost almost $14 000 per year, a

32% increase. By contrast, when powdered high allergen gloves were substituted by low allergen gloves at the Mayo Clinic in 1993, a cost saving of about $200 000 per year was possible (Hunt *et al*, 2002). A study done in Georgia concluded that if >1% of workers become fully or >2% partially disabled and seeked full compensation, it would be cost-effective for a tertiary-care facility to switch to latex-free gloves (Ranta & Ownby, 2004).

The role of premedication in the management of latex allergic patients who require surgery, is questionable (Poley & Slater, 2000). Prophylactic medications include corticosteroids, H_1 and H_2 antihistamines and adrenergic agents. However, there have been reports of allergic reactions despite pre-treatment (Lebenbom-Mansour *et al*, 1997). Such practices of prophylaxis could mask an allergic response, leaving staff unaware that latex allergy or perhaps another allergy had occurred, and the patient more sensitised to future contacts with the offending allergen (Bowyer, 1999a). It has also been shown that it is not very effective in the prevention of anaphylactic reactions to latex or radio contrast material (Lieberman, 2002).

1.14.2. Anaphylaxis

Anaphylaxis due to immediate hypersensitivity can occur after interaction between a specific antigen and homocytotrophic antibody. This reaction is rapid and often dramatic and unanticipated. Death may occur through airway obstruction or irreversible vascular collapse. Anaphylactoid reactions are clinically similar to anaphylaxis. They are not mediated by antigen-antibody interaction, but result from substances acting directly on mast cells and basophils, causing the systemic release of histamine and other pharmacological mediators (McGrath, 1993).

It is essential that prevention of anaphylaxis should at all times be the basis of management in high risk patients. Each subsequent reaction in IgE-mediated anaphylaxis is more severe and serious (Manjra, 1994). Symptoms include angioedema, bronchospasm, cardiac arrhythmias, diffuse erythema, hyperperistalsis, pruritus and urticaria. The most serious symptoms are laryngeal oedema, lower airways obstruction and hypotension, while milder symptoms, such as nausea, vomiting, light-headedness, headache, feeling of impending doom and unconsciousness, may also occur. Anaphylaxis is classified as severe by a systolic blood pressure of < 90 mmHg, a

respiratory rate of ≥ 25 breaths per minute and / or a Glasgow Coma Scale score < 15 (Kay, 2001a; Kemp & Lockey, 2002).

The most common agents causing intraoperative anaphylaxis are muscle relaxants (McGrath, 1993). Latex accounts for a significant number of intraoperative anaphylactic reactions and the incidence of latex-related intraoperative anaphylaxis is increasing. Latex containing devices have been implicated in over 1 100 anaphylactic reports to the FDA that resulted in at least 15 deaths between 1988 and 1992 (Slater *et al*, 1996). In 1993 an estimated incidence of latex anaphylaxis in Australia was found to be between 1 : 10 000 and 1 : 20 000 and a French study reported an incidence of 1 : 13 000 surgical procedures with a mortality rate of 6% in 1999 (Lieberman, 2002).

Cardiovascular collapse appears to be more common during surgical than non-surgical anaphylaxis. Surgical anaphylaxis occurs mostly during the induction procedure when muscle relaxants, sedatives and opiates are administered. Latex anaphylaxis occurs during maintenance anaesthesia, usually after a delay of 30-290 minutes (Volcheck & Li 1994; Lieberman, 2002). The most frequently associated surgical procedures are obstetric and gynaecological procedures in about 50%, followed by abdominal surgery in 20% of cases and orthopaedic surgery in 10% (Lieberman, 2002). Anaphylaxis is followed by the release of high levels of tryptase within one to two hours of clinical anaphylaxis (Kelly, 1993; Castells & Schwartz, 1988). Seven hours after a patient had suffered intraoperative anaphylaxis, serum tryptase levels measured 18.8 ng/mℓ, returned to 7.7 ng/mℓ 19 hours after the episode and were back to normal (< 5 ng/mℓ) four months after full recovery (Volcheck 1994).

Treatment of anaphylaxis involves ensuring oxygenation and perfusion, prevention of mediator release and counteracting the effect of circulating mediators by the use of adrenaline, oxygen and isotonic fluids. Antihistamines may be added to reduce angioedema, urticaria and pruritus (Manjra, 1994). Since an anaphylactic reaction may become biphasic with recurrence within eight to twelve hours, close observation is warranted after a reaction. Corticosteroids and H_1 and H_2 antagonists are indicated for use with inhaled ß-blocking agents if the patient has bronchospasm. If a patient is on a ß-blocking agent (e.g. propranolol) the response to standard doses of adrenaline may be incomplete. Higher doses of adrenaline and the use of glucagon may overcome this incomplete response (Castells & Schwartz, 1988).

Intramuscular injection into the thigh (vastus lateralis) in adults in superior to intramuscular or subcutaneous injection into the arm (deltoid). A study of adult diabetic patients showed that the tissue depth of the subcutaneous layer of the lateral thigh in women was on average 16.4 mm, compared to 6.3 mm in men. The length of the EpiPen needle is 14.29 mm and therefore not long enough to ensure intramuscular injection in many women and some men. Epinephrine has a rapid onset of action and is effective via intramuscular and subcutaneous routes (Kemp & Lockey, 2002; Chowdhury & Meyer, 2002).

1.14.3. Latex-Free vs Hypoallergenic

The alternative for latex gloves in latex allergic HCW^{s} is plastic, vinyl or nitrile gloves. The Department of Health and Medical Services Directorate of the USA classified plastic gloves as ill-fitting and unsuitable for protection, and vinyl gloves to be of inferior quality. Compared to vinyl gloves, NRL gloves have lower rates of perforation and better strength, elasticity, tactile sensitivity, comfort, fit, barrier properties and durability (Yip & Cacioli, 2002; Ranta & Ownby, 2004). The lattice network in NRL allows small puncture holes to reseal automatically and NRL gloves are therefore nine times more effective in protection against viral leaks than vinyl gloves (Bowyer, 1999a; Charous *et al*, 2002). Other non-latex gloves, such as nitrile rubber, tactylon, black copolymer and vinyl plastisols, are more expensive than vinyl gloves (Bowyer, 1999a).

Nitrile is a synthetic polymer that exhibits rubber-like characteristics when vulcanised. However, some differences make this polymer unique. Instead of polyisoprene, the backbone of the nitrile polymer is composed of the three monomers, acrylonitrile, butadiene and carboxylic acid. Acrylonitrile provides permeation resistance characteristics to a wide variety of fluids. Nitrile polymers are especially resistant to hydrocarbon oils and fats and display superior barrier properties to a wide range of chemicals. The butadiene component contributes to the softness, flexibility and feel of the glove. Through vulcanisation, butadiene enhances the rubber-like or elastic quality of the glove (Welker *et al*, 1998). Although the currently available synthetic gloves may be protein-free, they are not allergy-free. Instances of type IV and even type I allergic reactions caused by residual chemicals have been reported. In addition, NRL gloves are acceptably biodegradable (Ranta & Ownby, 2004). This reduces the problem with

most synthetic gloves that emit hazardous and toxic substances during incineration (Yip & Cacioli, 2002).

The process of chlorination is used to produce powder-free latex articles. This can reduce the tackiness of latex film surfaces without the addition of lubricating powders. Polymer coating provides an alternative way to reduce surface tackiness of powder-free latex gloves. The inside of glove surfaces are coated with a polymer, e.g. hydrogel, acrylic polyurethane, silicone polymer, a polymer blend or polyhydroxythyl-methacylate to reduce contact with latex. A dual approach is often adopted by coating the inside and chlorinating the outside of the glove (Yip & Cacioli, 2002). However, different studies have reported immediate-type cutaneous reactions to these gloves (Baur *et al*, 1997; Ranta & Ownby, 2004).

The term "hypoallergenic" is inconsistent and misleading with respect to NRL containing products. The "Source to Surgery" publication of June 1993 states that the term "hypoallergenic" refers to any manufacturer's gloves or device, which passes a specific evaluation test. However, measurable allergen levels were found in almost 50% of hypoallergenic gloves tested (Yunginger 1994). The use of the possible alternative term "low irritant" is suggested, because not all persons with irritant or ACD are allergic to NRL. Hypoallergenic gloves often solve the problems of these patients, as they do not necessarily need to use latex-free gloves (Smith *et al*, 1993). In 1997; the FDA instructed manufacturers to remove the hypoallergenic claim from the labeling of all NRL-containing devices, because hypoallergic is neither latex-free nor latex-safe (Cohen *et al*, 1998).

1.14.4. Guayule Latex

Guayule (*Parthenium argentatum*), a Mexican desert shrub (Cornish & Brichta, 2002), and kok-saghyz, a Russian dandelion, have previously been used as commercial sources of rubber (Pumphrey, 1994). Intensive research had been conducted on guayule as an alternative rubber source to *hevea*. Yulex Corporation was granted a license by the USA Department of Agriculture for the exclusive right to commercialise guayule latex products. The crop matures in 18 months and regrows after harvesting. Only 10% of the plant produces rubber and achieves ± 46% solid content, compared to ± 60% of *hevea* latex (Cornish & Brichta, 2002; Lobeck, 2003).

Only 16 g of latex is needed to make a pair of gloves, less than 2 g for a condom and 1 µg for a catheter (Lobeck, 2003). Guayule rubber particles have a specific gravity of 0.94-0.96 and contain only about 0.6% proteins, compared to 1% in *hevea* latex. However, if high protein levels are allowed to remain in the guayule latex products, they are capable of inducing an allergy of their own (Cornish & Brichta, 2002).

Gloves and condoms made of guayule latex were filled with a phi X174 solution and centrifuged. This is a test virus smaller than bacteria, HIV, hepatitis B and herpes simplex. After an hour of centrifugation, the virus had failed to pass through the glove or condom into a buffer solution. Guayule latex also seems to have a longer shelf life than conventional rubber and is harder to pierce (Walker, 1999).

1.14.5. Desensitisation / Immunotherapy

Specific immunotherapy (IT) consists of administering increasing concentrations of allergen extracts over time. IgG "blocking" antibodies compete with IgE for allergen and prevent the aggregation of IgE complexes and the α-chain of FcεRI-α on mast cells. The steric conformation is altered and IgE binds to APC^s. A shift from the production of T_{H2}-type cytokines (IL-4 and IL-5) to T_{H1}-type cytokines (IFN-γ and IL-12) is induced (Kay, 2001b). Subcutaneous IT (SCIT) also redirects the lymphocyte response towards the T_{H1}-type and reduces cytokine production (IL-4, IL-5 and IL-13). SCIT modifies the natural history of allergic disease and prevents the onset of new sensitisations for up to three to five years after discontinuation. It modulates an allergen-specific T lymphocyte response *in vitro* and increases the IgG4 : IgE ratio *in vivo* (Di Rienzo *et al*, 2003). However, conventional specific IT can cause general, and potentially fatal, anaphylaxis, particularly during the induction phase (Kay, 2001b).

Since SPT^s with crude latex extracts have induced anaphylactic episodes, IT was not possible until recently (Slater *et al*, 1996). Controlled, double-blinded studies confirmed the efficacy and safety of allergen-specific IT and sublingual IT (SLIT) in respiratory allergy (Pereira *et al*, 1999; Nucera *et al*, 2001; Patriarca *et al*, 2002b; Di Rienzo *et al*, 2003). Desensitisation in latex allergy should theoretically occur through the same routes as sensitisation, i.e. cutaneous, mucous membranes, parenteral contact or inhalation (Patriarca *et al*, 2002a). However, like venom IT, it has a high risk of systemic reactions. In contrast to common aeroallergens, latex SCIT has shown an increased prevalence of

systemic reactions (Pereira *et al*, 1999; Leynadier *et al*, 2000; Nucera *et al*, 2001; Meade *et al*, 2002). Two alternative methods (percutaneous and SLIT) proved to be safer and more effective, even though further studies on larger groups of patients are needed (Meade *et al*, 2002; Patriarca *et al*, 2002a).

A preliminary trial in France demonstrated the clinical benefit of latex immunotherapy. Seventeen patients with latex skin allergy and rhinitis were included in this study. Treatment started with a two day course of rush IT in hospital. When compared to controls, symptom and global medication scores in the latex-treated group were markedly decreased after twelve months. Most injections were well tolerated, but several adverse effects, including hypotension, urticaria, wheezing and pharyngeal oedema, were observed during the maintenance phase (Leynadier *et al*, 2000). Rush SLIT is performed by administering increasing doses of latex extracts under the tongue for three minutes every 20 minutes. Patients were hospitalised for the four-day duration of the therapy. After the desensitisation period, a maintenance dose is taken sublingually three times a week for three months. In an Italian study, a rush SLIT protocol for latex was successfully completed in twelve patients without side-effects. After three months all patients showed significant improvement in symptoms and no significant variation in symptom scores from controls. Only two patients manifested symptoms during the maintenance phase, but did not require therapy. In three patients the treatment failed, but their latex allergy was less severe than before desensitisation. The advantage of this method is the autoadministration during the maintenance phase (Patriarca *et al*, 2002a). The treatment proved to be safe even in patients with occupational latex-induced asthma. It should be noted, though, that these patients did not suffer from severe systemic symptoms, only cutaneous or mild respiratory symptoms (Patriarca *et al*, 2002b).

CHAPTER 2:
MATERIALS AND METHODS

2.1. OBJECTIVES

1. Several studies have been able to confirm the presence of NRL proteins on glove powder. The NRL proteins adhere to glove powder, which becomes aerosolised and introduced to the respiratory and cutaneous systems of HCW^s and patients. Airborne latex allergens are well documented in personal breathing zones and surface contamination has also been evaluated in high glove usage areas. Limited evidence has been published on latex contamination in airconditioning ducts (Kelly *et al*, 2000). This study will investigate the airconditioning system for the presence of glove powder and proteins in the extraction ducts of areas with different glove usage. The functionality of the airconditioning filters will be assessed by investigating dust in the return ducts.

2. Individuals with possible latex allergy will be assessed by using history (questionnaire), clinical (atopy, symptoms, calculation of the Allergy Score) and laboratory (RAST, SPT, Western blot) investigations. A representative prevalence rate of latex allergy in high risk areas (theatres and laboratories) TBH will be established. Due to financial constraints, no control group will be investigated. Historical reference will be made to the control group investigated during a previous study at the same institution (De Beer, 2000).

   Latex-specific IgE concentration in dermal fluid of symptomatic subjects will be determined and compared to their serum values to assess different compartments in the sensitisation process. This might also shed light on the phenomenon why a large proportion of individuals have positive latex SPT^s, while the latex-specific IgE results are negative.

3. Four subgroups (Group A = positive latex-specific IgE; Group B = negative latex-specific IgE and positive latex SPT^s; Group C = negative latex-specific IgE and negative latex SPT^s; and Group D = negative latex-specific IgE with no follow up) will be compiled for comparison and statistical analysis in an

effort to establish a risk profile for each group. Where possible, further investigation of Group C with the use of skin patch tests will be done to investigate a possible type IV hypersensitivity to glove additive allergens and latex gloves and powder.

Western blot analyses for latex will be done on the serum and dermal fluid of subjects where the latex-specific IgE was > 0.35 IU/ℓ to identify specific latex protein bands. Serum and dermal fluid results per subject will also be compared. In cases where a subject had a positive serum, but negative dermal fluid latex-specific IgE, the dermal fluid will also be included for Western blot for comparison.

Airborne NRL allergens are capable of attaching to the superficial film of the skin (Fisher, 1997). The presence of sebum and / or sweat on the skin surface will theoretically enhance percutaneous absorption by maintaining direct contact between the epidermis and water soluble NRL proteins. However, it has also been suggested in the literature that glove powder inside gloves absorbs moisture and causes dryness of the hands (Lodén, 1995; Pretorius & Bester, 2000). This will also decrease barrier properties of the skin and enhance percutaneous absorption. Different anatomical sites with high occupational exposure to latex via direct contact or airborne allergens (hands and face) will be evaluated for sebum content. Baseline values will be compared to assess anatomical variability among different regions. These sites will be re-evaluated after three to four hours to assess change induced by occupational exposure. Baseline and follow up measurements for all regions will be compared statistically between specific subgroups with high occupational exposure (laboratory and theatre staff).

2.2. ETHICAL ASPECTS

The project was registered with the Ethics Committee of the University of Stellenbosch (Research Committee C). This Committee adheres to the following guidelines in the evaluation and monitoring of research protocols:

- Declaration of Helsinki

- Guidelines on Ethics of the South African Medical Research Council
- International Guidelines: Council for International Organisations of Medical Sciences (CIOMS)
- International Conference on Harmonisation Guidelines for Good Clinical Practice
- Guidelines for Good Practice in the conduct of Clinical Trials in Human Participants in South Africa of the Department of Health, RSA
- Applicable RSA legislation

The Committee is accredited with the Office for Human Research Protections (OHPR) of the Federal Department of Health and Human Services, USA, under the following registration code: IORG 0001263, IRB 00001681 and FWA 00001372.

Project Number 98/046 was assigned to the study. The protocol, including informed consent forms, was compiled exactly to the specifications and instructions of this Committee.

Permission was obtained from the Management of TBH, Provincial Administration of the Western Cape and the Dean of the Faculty of Health Sciences of the University of Stellenbosch at Tygerberg Campus to conduct the research on these premises.

2.3. AIRCONDITIONING DUCTS

2.3.1. Sample Collection

Fourteen dust samples were collected from the airconditioning ducts in different areas of the TBH complex to represent different levels of latex exposure.

Four samples were taken from the grids of the extraction ducts in the Virology laboratory, with a high volume of daily latex glove usage. Different locations within the Virology department were selected, because the volume of glove use varies among the individual areas, e.g. staff doing specimen reception and serology use less gloves than those doing virus isolation. Further samples were collected at the Gynaecology, Paediatric surgery and Emergency theatres at TBH. One sample was taken from the grid of the extraction duct and one from the return duct of each of these theatres. These ten samples represent areas with high daily latex glove usage.

One sample was taken from the grid of the extraction duct in the Dermatology Laboratory, where latex glove usage was terminated six years ago. However, an adjacent laboratory uses latex gloves on a daily basis and this sample represents an area with low, rather than no latex exposure. A single sample was also obtained from the extraction duct in the airconditioning control room servicing the Dean's offices where no gloves are used and this area represents no latex exposure.

Two samples were taken from the airconditioning control room servicing the Virology laboratory; one from the extraction duct before the filter and one from the return duct after the air had passed through the filter.

The dust samples were numbered as follows:

1: Virology Laboratory – Sample reception extraction duct grid

2: Virology Laboratory – Routine laboratory extraction duct grid

3: Virology Laboratory – Serology laboratory extraction duct grid

4: Virology Laboratory – Virus isolation laboratory extraction duct grid

5: Gynaecology Theatre – Extraction duct grid

6: Gynaecology Theatre – Return duct grid

7: Paediatric Surgery Theatre – Extraction duct grid

8: Paediatric Surgery Theatre – Return duct grid

9: Emergency Theatre – Extraction duct grid

10: Emergency Theatre – Return duct grid

11: Dermatology Laboratory – Extraction duct grid

12: Control Room (Dean's Office) – Extraction duct

13: Control Room (Virology Laboratory) – Extraction duct

14: Control Room (Virology Laboratory) – Return duct

2.3.2. Reagents

2.3.2.1. 10X SDS PAGE Running Buffer

Trizma base (Sigma, T-1503)	30.3 g
Glycine (AnalaR, 10119)	144 g

10% SDS (UniLAB, 582 36 00)	100 mℓ

Make up to 1 ℓ with distilled water and store at room temperature

2.3.2.2. Bradford Solution

Coomassie brilliant blue G-250 (Sigma, B-0770)	250 mg
95% Ethanol (Merck, 983)	125 mℓ
Phosphoric acid (Merck, 573)	250 mℓ

Made up to 500 mℓ with distilled water, filter and store at 4°C

2.3.2.3. Lysis Buffer

10X Tris-Buffered Saline (Bio-RAD, 170-6435) pH 7.4

Just before use, add

Phenylmethylsulphonyl Fluoride (Sigma, P-7626)	10 µℓ/mℓ
Aprotinin (Sigma, A6279)	1 µg/mℓ

Store at room temperature

2.3.3. Determination of the Starch Content

Airborne latex allergens are associated with glove powder throughout the literature. The glove powder was therefore scraped off three pairs of sterile surgical latex gloves currently used at TBH (Examtex®, Union Drug, Pty Ltd) and a stock solution was prepared by dissolving 125 mg of the starch glove powder in 25 mℓ distilled water with a final concentration of 5 mg/mℓ. Starch powder calibration standards were prepared in increasing concentrations to create a linear calibration curve, according to Beer's Law ***(Table 2.1)***.

All test samples were prepared as solutions of 5 mg/mℓ in distilled water and mixed for 20 – 30 seconds on a Vortex-Genie mixer (Scientific Industries, Inc. New York, USA). Fresh tubes were numbered and used for the transfer of 1 mℓ from each tube (test samples and calibration standards). Immediately after that 1 mℓ of Lugol solution (Sigma, L-6146), containing 6% KI and 4% iodine crystals, and 5 mℓ distilled water were added. All tubes were mixed for 20-30 seconds on a vortex.

Table 2.1: Starch calibration standards

Calibration standards with increasing concentrations were prepared from a 5 mg/mℓ stock solution to construct a calibration curve. The mathematical equation generated by the graph was used to calculate the starch content of individual dust samples.

Tube	Stock Solution	Distilled Water	Concentration
A1	0 mℓ	16 mℓ	0 mg/16 mℓ
B1	1 mℓ	15 mℓ	5 mg/16 mℓ
C1	2 mℓ	14 mℓ	10 mg/16 mℓ
D1	4 mℓ	12 mℓ	20 mg/16 mℓ
E1	6 mℓ	10 mℓ	30 mg/16 mℓ
F1	8 mℓ	8 mℓ	40 mg/16 mℓ

The wavelength of a Perkin-Elmer Lambda 5 UV/VIS Spectrophotometer was adjusted to 620 nm and the function setting was set to Absorbance (Abs) mode. The content of tube A was used as the blank to zero the spectrophotometer. The absorbance of the calibration standards was read against the blank and a calibration curve was constructed, using Microsoft® Excel software. A trend line was fitted to the graph of the calibration standards.

Test samples were read exactly the same as the calibration standards and individual absorbance values recorded. The mathematical equation representing the graph was applied to the absorbance values of the test samples and the concentration of starch was calculated and converted to a concentration of mg/mℓ for each test sample.

2.3.4. Protein Precipitation of Samples

Trichloroacetic acid (TCA) (Merck, 807) was used to precipitate the protein in the remaining test sample solutions. Equal volumes of each test sample and 20% TCA (1 mℓ each) was added together and mixed for 20 – 30 seconds on a vortex. These samples were then incubated on ice for 30 minutes and centrifuged at 4°C for 15 minutes at 4 500 r.p.m. in a MSE Mistral 1 000® centrifuge.

The supernatant fluid was removed and discarded. Subsequently, 300 µℓ of cold acetone (AnalaR, 10003) was added to each tube. Test samples were again mixed well on a vortex and centrifuged for five minutes at 4°C. The supernatant was removed once again and the pellet was left to dry. All test samples were resuspended in SDS PAGE running buffer and mixed for 20-30 seconds on a vortex.

2.3.5. Determination of Protein Concentration

Bradford solution was diluted 1 : 5 in distilled water and filtered. It is important to note that the filtered solution should be brown, not blue. A stock solution was prepared by adding 100 µℓ of Bovine serum albumin (BSA) (Sigma, A-2153) stock to 400 µℓ fresh lysis buffer with a concentration of 1 mg/mℓ. Calibration standards were prepared in increasing concentrations to create a linear calibration curve according to Beer's Law ***(Table 2.2)***.

Table 2.2: Bradford calibration standards

Calibration standards with increasing concentrations were prepared from Bradford solution to construct a calibration curve. The mathematical equation generated by the graph was used to calculate the protein content of individual dust samples.

Tube	BSA (µℓ)	Lysis buffer (µℓ)	Bradford (mℓ)	Concentration
A2	0 µℓ	20 µℓ	1 mℓ	0 µg/20 µℓ
B2	2 µℓ	18 µℓ	1 mℓ	2 µg/20 µℓ
C2	4 µℓ	16 µℓ	1 mℓ	4 µg/20 µℓ
D2	6 µℓ	14 µℓ	1 mℓ	6 µg/20 µℓ
E2	8 µℓ	12 µℓ	1 mℓ	8 µg/20 µℓ
F2	10 µℓ	10 µℓ	1 mℓ	10 µg/20 µℓ
G2	12 µℓ	8 µℓ	1 mℓ	12 µg/20 µℓ
H2	14 µℓ	6 µℓ	1 mℓ	14 µg/20 µℓ
I2	16 µℓ	4 µℓ	1 mℓ	16 µg/20 µℓ
J2	18 µℓ	2 µℓ	1 mℓ	18 µg/20 µℓ
K2	20 µℓ	0 µℓ	1 mℓ	20 µg/20 µℓ

The content of Tube A2 was used as the blank to zero the spectrophotometer. The absorbance of the calibration standards was read against the blank and a calibration curve was constructed, using Microsoft® Excel software. A trend line was fitted to the graph of the calibration standards.

All test samples were diluted 20 µℓ in 1 mℓ of undiluted Bradford solution, mixed for 20-30 seconds on a vortex and left to stand for five minutes. The absorbance of all test samples was read at 595 nm in the Abs mode according to protocol. The mathematical equation representing the graph was applied to the absorbance values of the test samples and the protein concentration was calculated and converted to a final protein concentration of mg/mℓ for each test sample.

2.4. PATIENT SELECTION

2.4.1. Study Population

A previous study done at TBH during 1997-2000 (De Beer *et al*, 1999; De Beer, 2000; De Beer & Cilliers, 2001) identified laboratories and theatres as areas with high latex exposure, due to the number of latex gloves used on a daily basis. A questionnaire ***(Figure 2.1)*** was compiled and circulated among 500 people working in these high exposure areas at TBH, Stellenbosch University Faculty of Health Sciences at Tygerberg Campus and the Western Province Blood Transfusion Service laboratory in TBH. Completed questionnaires were evaluated for possible follow up.

The questionnaire captured demographic data, current job description, past / present latex exposure, other risk factors (multiple surgical procedures, specific food allergies, personal or family history of atopy, anaphylactic reactions), as well as all surgical procedures and possible side-effects. The literature suggests that latex allergy only became problematic after the well-known case report of a housewife with latex allergy was published in 1979 (Nutter, 1979). Therefore, for statistical purposes the total number of surgical procedures was divided into the number of procedures before and after 1980.

Questionnaire - Latex Allergy

Date: _____ **Patient Name:** _____

Date of Birth: _____ **R/S:** _____

Work phone no: _____ **TBH Folder Number:** _____

Age: _____ Sex: _____ Occupation: _____

Department: _____ Short job description: _____

Do you use latex **GLOVES?** _____ How many years? _____ Are you allergic to latex? _____

How many hours per week: _____ How many pairs of gloves per week? _____

ATOPY (Eczema / Asthma / Hay fever / Itchy skin): Personal: _____ Family: _____

ALLERGIES: Drugs _____

Food _____

Other _____

Previous **OPERATIONS (general anaesthesia / epidural / spinal block):** _____

Name all procedures and year: _____

_____

_____

Side effects from anaesthesia: _____

CURRENT SYMPTOMS:	**Absent**	**Mild**	**Moderate**	**Severe**	**Better outside work**	**Duration of symptoms**
Conjunctivitis (red / itchy eyes):	☐	☐	☐	☐	☐	_____
Nasal congestion (blocked nose / hay fever):	☐	☐	☐	☐	☐	_____
Rhinitis (hay fever):	☐	☐	☐	☐	☐	_____
Shortness of breath:	☐	☐	☐	☐	☐	_____
Bronchospasm (asthma / tight chest):	☐	☐	☐	☐	☐	_____
Urticaria (hives):	☐	☐	☐	☐	☐	_____
Angioedema (severe swelling of face):	☐	☐	☐	☐	☐	_____
Anaphylactic shock:	☐	☐	☐	☐	☐	_____
Pruritus (itch):	☐	☐	☐	☐	☐	_____
Skin rash:	☐	☐	☐	☐	☐	_____
Hand eczema:	☐	☐	☐	☐	☐	_____
Other: _____	☐	☐	☐	☐	☐	_____

I understand that all the above information will be managed confidentially and I hereby give consent that this information may be used for research purposes without compromising my privacy.

Signed: _____ Date: _____

Figure 2.1: Latex Allergy Questionnaire

Demographic, clinical and other relevant information was captured and recorded into the patient database.

The listed symptoms were chosen after extensive review of the literature and statistical confirmation of the relevance obtained during a previous latex allergy study done at TBH (De Beer *et al*, 1999; De Beer, 2000; De Beer & Cilliers, 2001). Symptoms cover all three routes of exposure, namely aeroallergen reactions (conjunctivitis, nasal congestion, rhinitis, shortness of breath and bronchospasm), systemic involvement (angioedema and anaphylactic shock) and skin contact (urticaria, pruritus, skin rash and hand eczema).

An ethically approved and legally acceptable informed consent clause was signed and dated by all subjects on completion of the questionnaire. This represented the subject's voluntary participation and commitment from the research team to confidential management of all information. Before performing any clinical procedure, a second informed consent form ***(Figure 2.2)***, detailing all additional procedures, was signed and dated by subjects. It was countersigned by the investigator and a witness in the presence of the subject.

2.4.2. Study Group

The study population consisted of HCW^{s} who had frequent exposure to / or direct contact with latex products or aeroallergens (doctors, medical laboratory technologists, nursing staff, pathologists) and who responded to advertisements. Individuals who reported a history of skin or respiratory symptoms as a result of latex exposure were invited to complete a questionnaire. Individuals who complied with any one of the following pre-defined criteria on the questionnaire were followed up to be included in the Study Group:

- suspected or confirmed latex allergy
- work-related symptoms
- two or more severe symptoms
- four or more moderate symptoms
- food allergies to avocado, banana, carrot, kiwi, mango, potato or nuts
- bronchospasm or anaphylactic reactions with latex contact

Latex Allergy Study
Informed Consent

I, the undersigned, ... (full name)
(address) ..... confirm that:

1. I was invited to take part in a Latex Allergy Study, conducted by the Department of Dermatology, University of Stellenbosch and Tygerberg Hospital.
2. I was informed that:
   - 2.1. Repeated exposure to latex (e.g. surgical gloves and other medical equipment) can result in the development of a specific allergic reaction due to specific antibodies which form in the blood
   - 2.2. My personal risk profile will be determined by means of:
     - 2.2.1. completion of a questionnaire
     - 2.2.2. special laboratory investigations (marked items only):
       - ❒ Total IgE and latex-specific IgE (10 mℓ clotted blood)
       - ❒ Latex skin prick tests on volar aspect of forearm for diagnosis in cases of negative in vitro latex-specific IgE results
       - ❒ Patch tests with European Standard (23 allergens), as well as latex glove and glove powder
       - ❒ Vacuum chamber collection of dermal fluid of abdominal skin over 1½ to 2 hours
   - 2.3. I will at all times be handled with latex-free gloves and in an latex-free environment
   - 2.4. No procedure will be carried out without the availability of proper emergency equipment and highly trained medical personnel
3. All information supplied will be managed strictly confidentially, but may be used for possible publication purposes. I will only be identified by means of an identification code.
4. If any allergic or other medical condition is identified during this examination, I will be informed immediately to plan treatment or management thereof.
5. I may refuse to participate in the study and such refusal will not in any way influence my current / future treatment at this institution.
6. Participation in this study will not imply any additional costs for myself.
7. If I have any problems or queries, I can contact **Prof Jacques Cilliers** at tel **938 5433 / 938 4067 / 082 929 0572** or **Corena de Beer** at tel **938 9453 / 082 826 0453.**
8. I have read and understand this consent form. My questions have been answered and I voluntarily consent to participate.

Signed at ..... on this ..... day of .....20...

Volunteer: .....

Investigator: .....

Witness: .....

Figure 2.2: Informed consent form

Before any procedure was performed, subjects were asked to mark the applicable box and date and sign the form to give consent towards the procedure. The investigator and a witness then signed the form.

Subjects were approached individually and followed up for further investigation. A total of 152 subjects were included in the Study Group. Due to financial constraints, investigation of a control group was not repeated. A previous study confirmed the role of exposure in the development of latex allergy, as well as the statistical significance of the listed symptoms in the experimental group (De Beer, 2000).

An Allergy Score was introduced during a previous study at TBH. A highly significant difference was found between the experimental and control groups (De Beer, 2000). The number and severity of symptoms listed on the questionnaire were used to calculate an individual's Allergy Score. The following formula was used and a maximum score of 33 was possible:

Allergy Score = (1 x total mild) + (2 x total moderate) + (3 x total severe)

Different classes were also assigned to individual subjects. Class 0 included subjects who thought they were not allergic to latex. Class 1 represented those who thought or knew that they were allergic to latex and Class 2 represented those who were unsure.

2.4.3. Specimen Collection

The evacuated-tube system or closed collection system was the method of choice for blood-specimen collection for this study. The antecubital fossa was cleaned with sterile Webcol® alcohol preps, saturated with 70% isopropyl alcohol, and allowed to air dry. A Vacutainer PrecisionGlide™ needle (0.8 x 38 mm, 21 gauge) was used for venepuncture in the median antecubital vein. Two 5 mℓ BD Vacutainer™ SST tubes were filled with blood and inverted a few times to ensure sufficient mixture with the SST clot activator inside the tubes. The tubes were labelled in the presence of the subject with the subject's name and date of collection. Subject numbers were assigned to samples starting from A001 to A152 in order of the collection date and written on the subject's questionnaire.

Samples were left to clot at room temperature (± 24°C) for 15-20 minutes and then centrifuged for ten minutes at 4 500 r.p.m. in a MSE Mistral 1 000® centrifuge. Serum was transferred to two 3.5 mℓ sterile Greiner® cryovials, labelled with the subject number and date of collection, and stored at -10°C until the day of analysis.

2.4.4. Total IgE and Latex-Specific IgE

The Pharmacia CAP System™ IgE Fluoroenzymeimmunoassay (FEIA) was used for quantitative measurement of circulating total serum IgE (code 10-9125-01) and latex-specific IgE (code 10-9395-01) in all 152 subjects (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). One of the two samples from each subject was thawed at room temperature on the morning of analysis and both assays were performed simultaneously using the same serum sample. Procedures were carried out at room temperature according to the manufacturer's instructions. All tests were done in duplicate. The only difference between the procedures for total IgE and latex-specific IgE is the antibodies used in the manufacturing of the ImmunoCAPs. Mouse anti-IgE was used for total IgE and rabbit anti-IgE for latex-specific IgE.

The total IgE (code 10-9394-01) and specific IgE (code 10-9213-01) standards are calibrated by the manufacturer against the 2nd International Reference Preparation 75/502 of human serum IgE from World Health Organisation (WHO).

In short, 50 µℓ of undiluted standards, controls (total IgE code10-9267-01, specific IgE code 10-9266-01) and serum was incubated with anti-IgE (mouse and rabbit monoclonal antibodies for total serum IgE and latex-specific IgE respectively), covalently coupled to immunoCAPs ***(Figure 2.3)***, for 30 ± 5 minutes (sample incubation). After washing with UniCAP washing solution (8.6 mℓ 2.9% Kathon CG + 40 mℓ 0.05% Kathon CG in 5 ℓ distilled water, code 10-9293-01), 50 µℓ of enzyme anti-IgE (mouse / rabbit monoclonal antibodies coupled to β-galactosidase) was added to each sample and incubated for 150 ± 10 minutes to form a complex (conjugate incubation). All unbound enzyme anti-IgE was then washed away with washing solution and the bound complex was incubated with 50 µℓ development solution (0.01% 4-methylumbelliferyl-β-D-galactoside) for 10 ± 1 minutes (development incubation). The reaction was terminated by adding 400 µℓ of stop solution (4% sodium carbonate) and allowed two to three minutes for the eluation process to complete, the fluorescence of the eluate was measured in a FluoroCount 96™ microplate reader. The fluorescence was directly proportional to the concentration of the IgE or specific IgE in the sample.

2.4.5. Skin Prick Tests

Subjects with negative *in vitro* latex-specific IgE results (< 0.35 IU/ℓ), but who experienced work-related symptoms or reported more than two severe or four moderate symptoms on the questionnaire, were subjected to latex SPT^s under controlled conditions. Informed consent was signed by the subject and countersigned by the investigator and a witness before SPT^s were done. The medial forearm was cleaned with sterile Webcol® alcohol preps, saturated with 70% isopropyl alcohol, and allowed to air dry. A single drop each of commercially available Soluprick® SQ solution (ALK-Abelló, Denmark, code 960) ***(Figure 2.4)***, a positive control (10 mg/mℓ histamine dihydrochloride, ALK-Abelló, 091) and negative control (50% saline in glycerine, ALK-Abelló, 090) were applied to the medial forearm. The skin was then punctured with a sterile ALK Lancet (ALK Abelló, 0543) and the results read 10-15 minutes after application.

The kit contains three extracts of partly purified latex allergen. The negative and positive controls and the 1 histamine equivalent prick (HEP) solution (1 : 1 000 dilution, 3.2 µg/mℓ) were applied first. If the 1 HEP solution elicited no reaction within ten minutes, the 10 HEP solution (1 : 100 dilution, 32.0 µg/mℓ) was applied and when there was no reaction after another ten minutes, the 100 HEP solution (1 : 10 dilution, 320.0 µg/mℓ) was applied. It is imperative to start with the highest diluted solution to prevent any untoward reactions against latex. The average diameter of each wheal was established by measuring the longest diameter and the diameter perpendicular to it. If the average measurement of the wheal caused by the latex allergen solution was more than 2 mm greater than that caused by the negative control, the test was regarded as positive. If any of the three solutions caused a wheal and flare reaction, no further testing was done and the subject was regarded as being allergic to latex.

Due to the potential danger of anaphylactic shock when performing latex SPT^s on sensitised individuals, a number of special safety precautions were adhered to during active exposure to latex SPT extracts.

- The latex-free Koeberg Theatre (Ground floor, TBH) was prepared for stabilising and further management in case of any anaphylactic reaction due to exposure to latex SPT extracts.

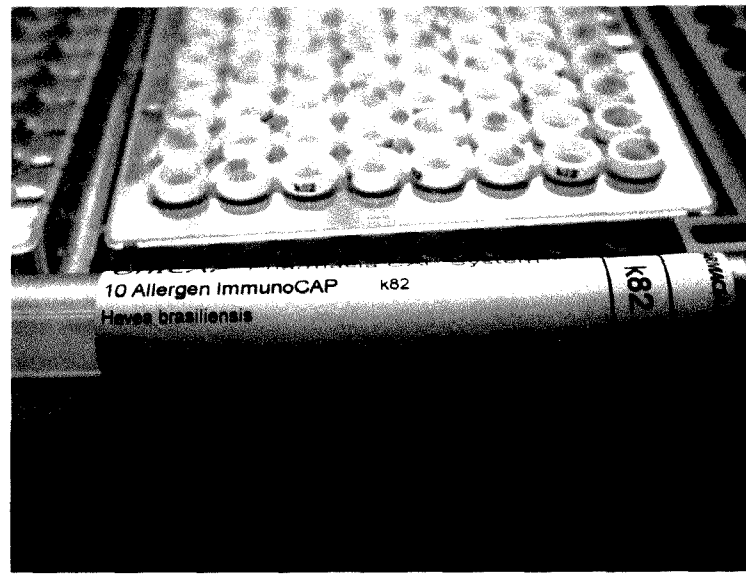


Figure 2.3: ***Hevea brasiliensis*** **latex (k82) immunoCAPs**

Specific anti-IgE is coupled covalently to a cellulose carrier in the ImmunoCAP. The serum binds to the anti-IgE and forms a complex with enzyme-labelled antibodies. After a series of incubation and washing steps the fluorescence of the eluate is measured to calculate specific IgE concentration in the serum.

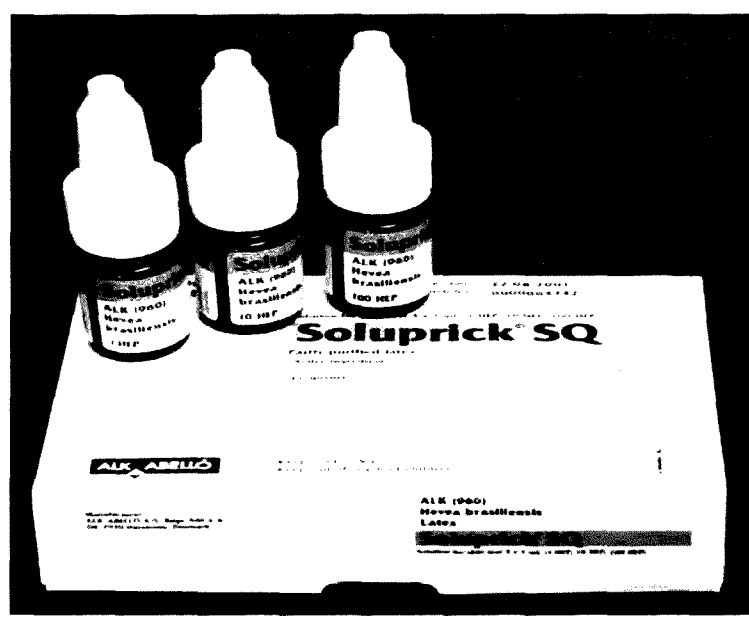


Figure 2.4: **Soluprick SQ® prick test allergens**

The kit contains partly purified latex extracts in three different dilutions (1 HEP, 10 HEP and 100 HEP) and the lowest concentration (1 : 1 000) is applied first to minimise adverse reactions to active latex. Only when the result is negative, the higher solutions (1 : 100 and 1 : 10) should be applied.

- A standard emergency trolley was available at all times at the testing site. All latex-containing items were replaced with latex-free alternatives.
- A Dermatology consultant or registrar was present and an Internal Medicine physician was on standby during testing.
- Subjects were closely monitored for any signs of discomfort during testing, e.g. couching, wheezing, faintness and pallor.
- SPT^{s} were carried out according to the manufacturer's specifications.

2.4.6. Western Blot

The measurement of allergen-specific IgE has become a standard method for use in the diagnosis of allergic disease. The Western blot method was developed in 1967 (Wide *et al*, 1967) and has been improved significantly over the past years by incorporating new technology (Weiss, 1997). It was designed as a qualitative measure of allergen-specific IgE and is most commonly used to confirm specific IgE tests. Pooled serum from allergic patients is used to visualise specific allergens in complex mixtures, such as serum. The proteins and glycoproteins in the pooled serum are separated according to their MW with SDS-PAGE and transferred to nitrocellulose membranes. The allergens on the membrane can then be probed with IgE from patient samples. Each IgE reactive component appears as a band corresponding to the molecular mass that can be analytically determined.

The AlaSTAT® AlaBLOT™ Specific IgE method (Diagnostic Products Corporation®, Los Angeles) was used. During manufacturing, allergen extracts are electrophoresed in 4-20% gradient polyacrylamide gels and the resolved extract components are electrotransferred to nitrocellulose membranes. These membranes are provided in the AlaBLOT™ kits as membrane strips of 5 x 100 mm.

Western blot analysis for latex was done on all sera with latex-specific IgE levels of more than 0.35 IU/ℓ. Where dermal fluid was collected from these subjects, it was also analysed, regardless of the dermal fluid latex-specific IgE value. The procedure was carried out at room temperature according to the manufacturer's instructions.

In short, the wells of an AlaBLOT™ reagent tray were numbered according to the specimen numbers. Membrane strips were placed face up in the wells, moistened with

1 mℓ of wash solution (concentrated buffered saline solution with surfactants) and incubated for five to ten minutes ***(Figure 2.5)***. The strips must be placed face up to ensure optimal binding of the serum to the allergen extracts on the strips. After aspirating the wash solution, the nitrocellulose membrane strips were incubated with 50 µℓ sample (serum or dermal fluid) and 450 µℓ sample diluent (buffered protein solution) for two hours. During this time latex-specific IgE in the samples recognised and bound to determinants in the allergenic extracts and was immobilised on the nitrocellulose membrane strips. All wells were washed three times with 1 mℓ wash solution for five to ten minutes per wash and aspirated dry.

An alkaline phosphatase-labelled murine monoclonal anti-IgE antibody (0.5 mℓ) was then added to the nitrocellulose membrane strips and incubated for 30 minutes. This second antibody was immobilised at sites where IgE was present on the membranes. The free antibody was aspirated from the well and the strips were washed three times with 1 mℓ wash solution for five to ten minutes per wash and aspirated dry. To visualise the allergenic determinants recognised by a subject's specific IgE, 0.5 mℓ alkaline phosphatase substrate solution (5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium in a stabilising buffer) was added and the strips were left to develop colour for 15 minutes. To stop the colour development, the strips were washed three times with 1 mℓ distilled water for five minutes per wash. The strips were then air dried on a paper towel.

The MW of a determinant could be estimated by comparing its relative mobility to the relative mobilities of protein markers of known MW. A formula has been devised by die manufacturing company by using MW markers ranging from 14 kD to 200 kD. However, the package insert warns against the estimation of a MW less than 14 kD, because the mobility of the smaller proteins was not in the linear range of the plot used to calculate the formula.

The MW^s of determinant bands were calculated by measuring the distance in millimetres between the centre of the determinant band and the outside edge of the dye front. The base -10 logarithm of the determinants' MW in kD *(log MW)* from the measured distance *(d)* was then calculated, using the following lot-specific formula printed on the strip module's label ***(Figure 2.6)***: **Log MW = 0.027 x d + 0.91**

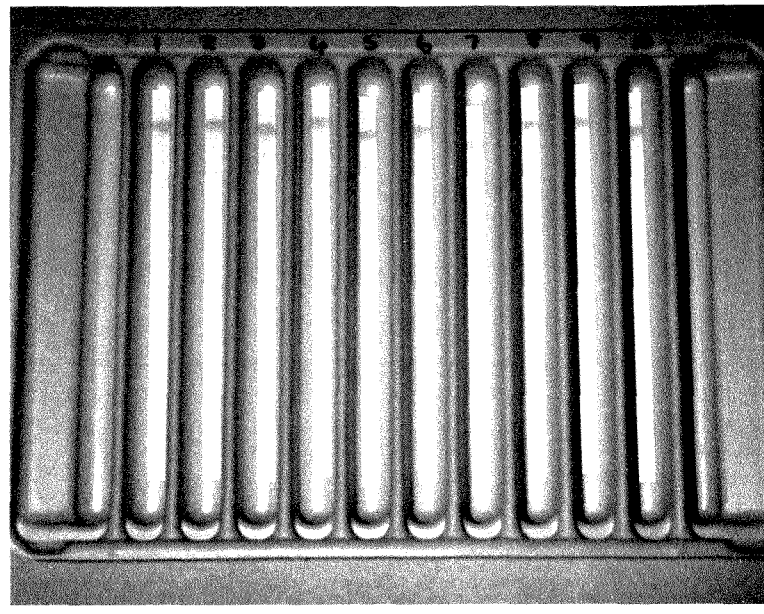


Figure 2.5: AlaSTAT® AlaBLOT™ reagent tray

Membrane strips were moistened in the reagent tray before sample incubation. The pink dye front indicated the upper side of the strips. Individual wells were numbered with the corresponding sample number and all reagents were added to the strips in the wells.

Figure 2.6: AlaSTAT® AlaBLOT™ allergen nitrocellulose membrane strips

During manufacture a calibration curve of protein standards is used to develop the MW estimation formula. Each allergen strip module has a unique lot-specific formula to calculate the MW of individual samples.

2.4.7. Dermal Fluid Collection

Subjects already enrolled in the study were approached at a later stage to obtain dermal fluid collection for further analysis. Informed consent forms were amended and signed before collection of the dermal fluid. The existing subject number was used for dermal fluid, followed by a "B", e.g. A001B.

Vacuum chambers were custom-manufactured from metacryle acid polymer (Cilliers, 1993). The round chamber was 50 mm in diameter and 50 mm in height with a replaceable dome-shaped base with 5 x 5 mm round holes ***(Figure 2.7)***. Silicone tubing connected the chamber to an Eschmann® High Vacuum pump ***(Figure 2.8)***. The chambers were immersed in Instrubac Blue™ solution (benzyl ammonium chloride, hexabutyldistannoxane and quaternary compound, but gluteraldehyde-free) (BAC Products, Pty, Ltd) for 30 minutes before use. The chambers and bases were then thoroughly rinsed with distilled water to prevent contact or irritant dermatitis. Four vacuum chambers were connected to the vacuum pump by silicone tubes. The remaining two tubes were clamped to restrict all air flow ***(Figure 2.9)***.

The abdominal area was disinfected with Dismed D-Germ™ antiseptic hand rub (0.5 g chlorhexidine and 70 mℓ n-propyl alcohol / 100 mℓ) (Dismed Parma, Pty, Ltd) and left to air dry. Dermal fluid was also collected from the medial forearm in only one subject (A030). A controlled vacuum of –45 kPa was sustained for a period of 90-120 minutes. The epidermis separated from the dermis and formed artificial 5 mm vesicles filled with extravascular dermal fluid ***(Figure 2.10)***. After suction was gradually reduced and finally terminated, the chambers were carefully removed to minimise damage to the vesicle walls. All dermal fluid was extracted from each vesicle, using a 5 mℓ Promex® sterile syringe and 23 gauge Microlance® sterile needle ***(Figure 2.11)***. It was then divided into two separate cryovials for storage at -10°C until the day of analysis. A total of 500-1 000 µℓ dermal fluid was collected per subject. Within 24 hours, the vesicle areas were dry and after seven days only mild scabs and slight hyperpigmentation remained ***(Figure 2.12)***. Within a month of the procedure, the skin had healed completely.

Total IgE and latex-specific IgE determinations were done on all dermal fluid specimens according to the same procedures described previously.

Figure 2.7: Vacuum chamber

The dome-shaped base is replaceable and fits the chamber perfectly. The holes facilitate vesicle formation by separation of the epidermis from the dermis when a constant vacuum is applied to the test area. Dermal fluid from the vesicles was collected for further analysis.

Figure 2.8: Eschmann® High Vacuum pump

Vacuum chambers were connected to the vacuum pump and a constant vacuum of –45 kPa was applied for 90 – 120 minutes to induce vesicle formation for dermal fluid collection.

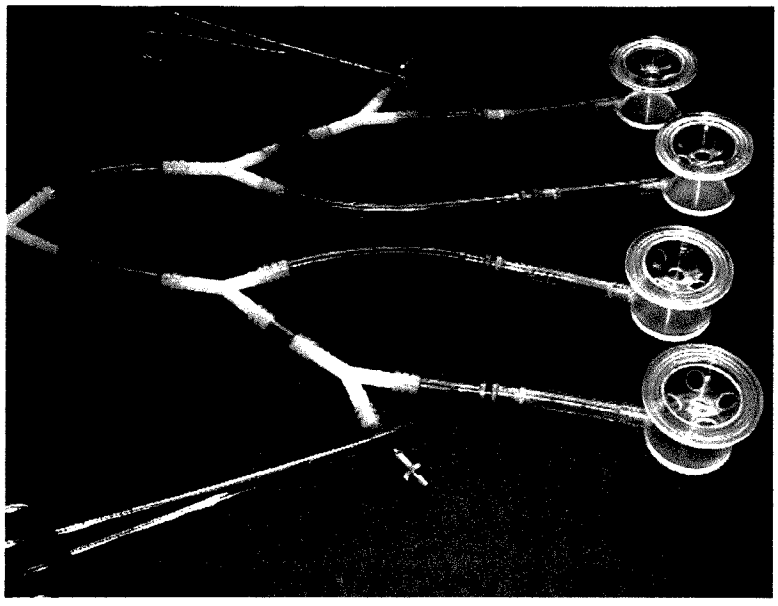


Figure 2.9: Vacuum chambers connected to the vacuum pump

The abdominal area was disinfected before application of the vacuum chambers. Only four chambers were used due to the limited abdominal skin area of subjects. By clamping off the outermost tubes, an equal vacuum was induced in the four remaining chambers.

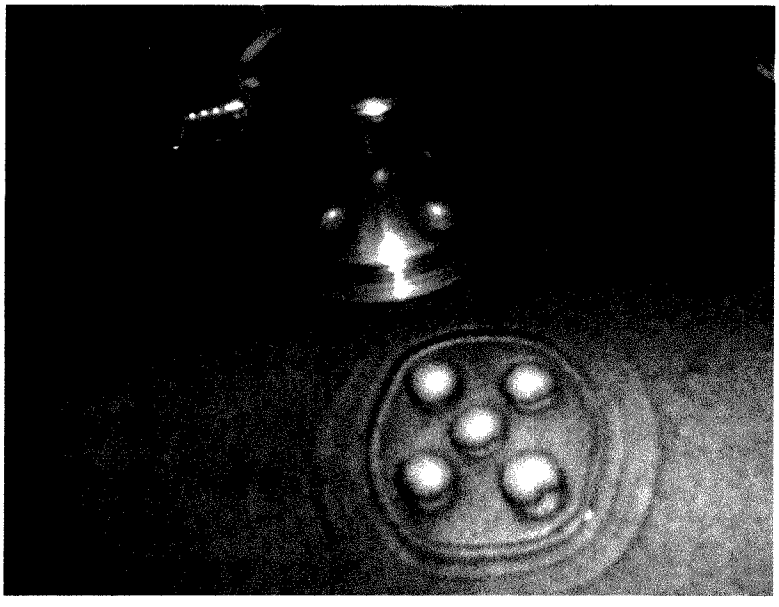


Figure 2.10: Vesicles after two hours of vacuum

The vacuum caused the dome-shaped base to be in close contact with the skin for the duration of the collection procedure. The inner dome holes allowed separation of the epidermis from the dermis resulting in the formation of vesicles filled with dermal fluid.

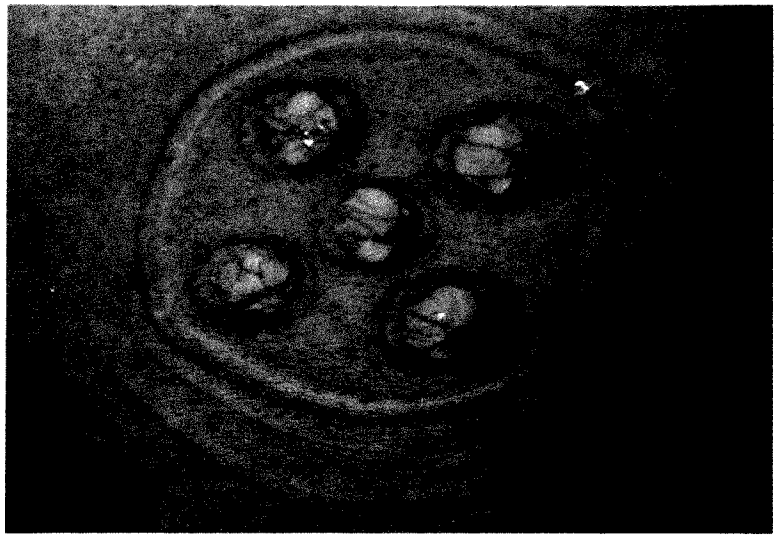


Figure 2.11: Empty vesicles after extraction of extravascular dermal fluid

Dermal fluid was withdrawn from the vesicles with a sterile syringe. Most subjects experienced only local sensitivity immediately after extraction and the area was covered with gauze to prevent chafing from clothes.

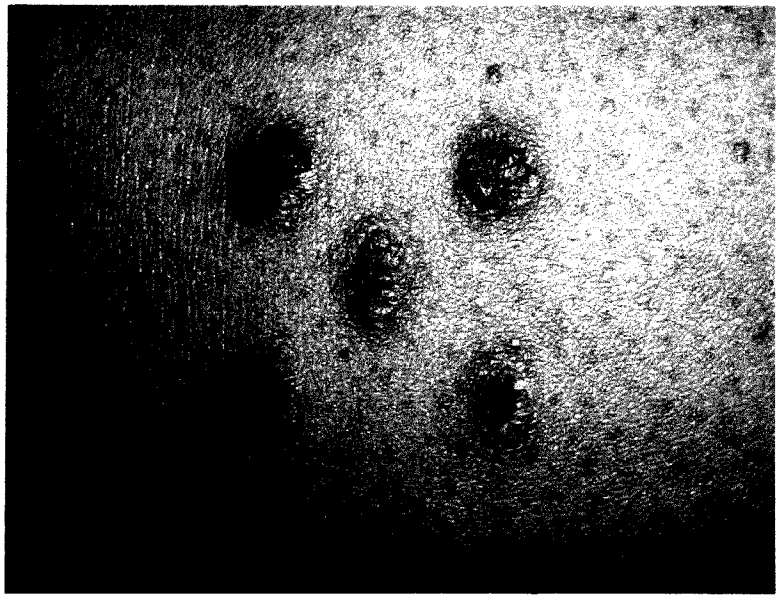


Figure 2.12: Healing crusts seven days after harvest of dermal fluid

Vesicles were resolved and crusts formed within a week after collection. Within a month all lesions were completely healed and only slight hyperpigmentation was evident.

2.4.8. Patch Tests

After informed consent was signed, cutaneous patch tests were done on selected subjects with negative latex-specific IgE and SPT results to investigate a possible type IV hypersensitivity reaction to latex. The European Standard patch test series (Chemotechnique Diagnostics®, Sweden, S-1000), a piece of latex glove and 1% glove powder in petrolatum were used. Allergens were placed in additive free polyethylene plastic IQ chambers (Chemotechnique Diagnostics®, Sweden, IQ-100) on a hypoallergenic non-woven adhesive tape and applied to skin under additional occlusion (Albupore, Smith & Nephew™) to the posterior trunkal area. After 48 hours the patches were removed and results were read after 48 and 96 hours.

The series consists of the following 23 allergens:

1.	Potassium Dichromate	0.5% in petrolatum
2.	4-Phenylenediamine base	1% in petrolatum
3.	Thiuram Mix	1% in petrolatum
4.	Neomycin Sulphate	20% in petrolatum
5.	Cobalt Chloride	1% in petrolatum
6.	Benzocaine	5% in petrolatum
7.	Nickel sulphate	5% in petrolatum
8.	Clioquinol (Chinoform, Vioform)	5% in petrolatum
9.	Colophony	20% in petrolatum
10.	Paraben Mix	16% in petrolatum
11.	N-Isopropyl-N-phenyl-4-phenylenediamine	0.1% in petrolatum
12.	Wool Alcohols	30% in petrolatum
13.	Mercapto Mix	1% in petrolatum
14.	Epoxy Resin	1% in petrolatum
15.	Balsam of Peru	25% in petrolatum
16.	4-tert-Butylphenolformaldehyde Resin	1% in petrolatum
17.	Mercaptobenzothiazole	2% in petrolatum
18.	Formaldehyde	1% in water
19.	Fragrance Mix	8% in petrolatum
20.	Sesquiterpene Lactone Mix	0.1% in petrolatum
21.	Quaternium-15	1% in petrolatum
22.	Primin	0.01% in petrolatum
23.	CI+Me-isothiazolinone	0.01% in water

2.4.9. Sebum Measurement

Two of the cutaneous areas with most obvious occupational exposure in HCW^s are the face and hands. Some latex proteins are lipophilic and water-soluble and sweat and / or sebum would theoretically enhance penetration of these proteins by the skin. To investigate the effect of wearing gloves and being in contact with airborne allergens on skin sebum values, these anatomical areas were selected to be analysed for sebum content before and after occupational exposure. A data capture form was compiled and used to record the different values ***(Figure 2.13)***.

Only theatre and laboratory personnel were recruited for this investigation. Baseline measurements were done before donning the first pair of gloves for the day. Where possible, follow-up measurements were done after removing the gloves three to four hours later, but before washing their hands. The exact time of measurement and work location were recorded on each subject's data capture form.

Sebum content was evaluated photometrically with a Sebumeter SM810® (Courage & Khazaka, Germany). The cassette contains a matt synthetic tape of 0.1 mm thickness. The measuring head of the cassette exposes a 64 mm^2 measuring section of the tape ***(Figure 2.14)***, which is placed directly on the skin where it absorbs surface sebum. Measurement is based on the principle of a grease spot photometer, where high lipid levels cause a greater transparency of the measuring tape (Wilhelm *et al*, 1991). The Sebumeter SM810® was calibrated by the manufacturing company in Germany before it was used for these measurements.

Before measurement, the tape was advanced to an unused section and inserted into the aperture to zero the device. On removal of the cassette, a countdown of 30 seconds began, controlled by a clock in the device. The cassette was held in close contact with the measuring area for the full 30 seconds to obtain reliable results. The measuring head of the cassette was then re-inserted into the aperture and an internal photocell measured the transparency of the tape ***(Figure 2.15)***. The light transmission represented the sebum content on the surface of the measuring area. A built-in microprocessor calculated the result, which was shown on the display in mg sebum/cm^2 of the skin and recorded on the data capture form.

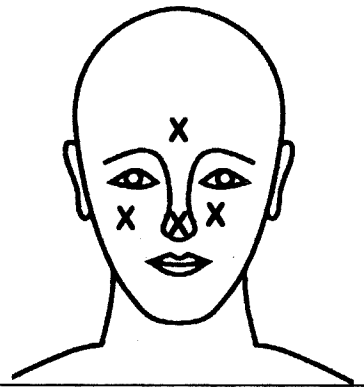
Patient Name: .....

Project Number: .....

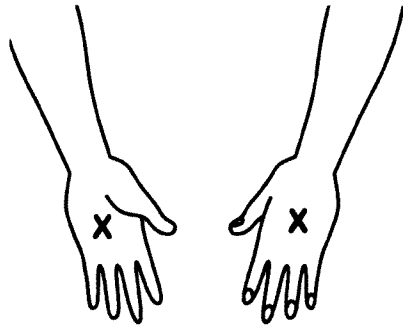
Occupation: **Laboratory** ..... **Theatre** .....

Date of Birth: ..... **Sex:** ..... **Race:** .....

Date: .....



	08:00	13:00
Forehead	.....	.....
(L) Cheek	.....	.....
(R) Cheek	.....	.....
Nose	.....	.....



	08:00	13:00
(L) Palm	.....	.....
(L) Dorsum	.....	.....
(R) Palm	.....	.....
(R) Dorsum	.....	.....

Figure 2.13: Sebum content data capture form

Specific anatomical areas were selected to be analysed. "X" indicated the areas. Measurement was done before commencement of daily duties and where possible, again after three to four hours of occupational exposure. The exact time when measurement was done was recorded on the form.

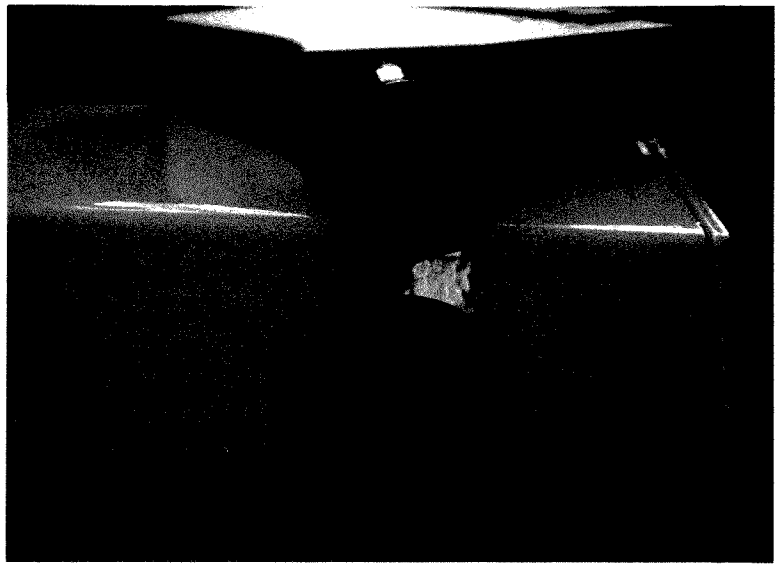


Figure 2.14: The photocell of the Sebumeter SM810™

Measurement is based on the principle of a grease spot photometer. The cassette contains a matt synthetic tape of 0.1 mm thickness and exposes a 64 mm^2 measuring section for skin sebum analysis. The tape was placed directly on the skin for 30 seconds where it absorbed sebum on the skin surface.

Figure 2.15: Measurement of skin sebum content

High lipid levels cause greater transparency of the measuring tape. The cassette was inserted into the aperture where a grease spot photometer or photocell measured the transparency of the measuring tape. The light transmission represented the sebum content on the surface of the measuring area and the result was displayed in mg sebum/cm^2.

2.5. STATISTICAL ANALYSIS

The following four subgroups were identified within the Study Group according to pre-defined criteria and compared statistically (descriptive statistics, Odds and Risk Ratios, Chi-Square, Mann-Whitney U or Wilcoxon Rank-Sum tests for difference in medians, t-tests, contingency table analyses and analysis of variance).

- **Group A**: 23 selected, consenting subjects with positive serum latex-specific IgE (> 0.35 KU/ℓ);
- **Group B**: 34 selected, consenting subjects with negative serum latex-specific IgE and positive cutaneous SPT^s to latex;
- **Group C**: 25 selected, consenting subjects with negative serum latex-specific IgE and negative cutaneous SPT^s to latex; and
- **Group D**: 70 consenting subjects with negative serum latex-specific IgE, but who were lost to follow up. No further procedures were done on this group.

The basic significance level of $p=0.05$ was used for all tests. Confidence intervals of 95% was used and $p<0.05$ was regarded as statistically significant and $p<0.01$ as highly significant.

CHAPTER 3:
ENVIRONMENTAL CONTAMINATION

3.1. INTRODUCTION

Cornstarch powder is used as the dry donning lubricant in the manufacturing of gloves. It has a high affinity for extractable latex proteins (Bowyer, 1999a), which can migrate from the gloves, attach to the starch particles and become airborne (Fisher, 1987; Pumphrey, 1994). These airborne NRL allergens cause major respiratory allergy among exposed workers and is currently the fourth most common cause of occupational asthma reported to the Surveillance of work-related Occupational Respiratory Disease program (Vandenplas *et al*, 2002).

The role of glove powder in the aerosolisation of NRL allergens and the development of subsequent respiratory symptoms has been confirmed by various studies (Baur *et al*, 1998). Recent evidence has shown that latex allergens can be distributed via airconditioning ducts and cause re-contamination of the air. Studies done at the Mayo Clinic, Minnesota, showed that detectable latex allergen levels of up to 1.58-170.23 µg/g of dust were present in both air supply and return ducts of a hospital where powdered latex gloves were use on a daily basis. No allergens were detected in the ducts of a hospital where non-latex gloves were used for the previous six years (Kelly *et al*, 2000). Similarly, another study found latex allergen concentration of 2 µg/g of dust in the ventilation ducts (Charous *et al*, 2000). An Australian study further confirmed that subjects wearing powdered latex gloves inhaled 26 times more latex allergen than those wearing powder-free and vinyl gloves (Poulos *et al*, 2002).

At the time of the study at TBH powdered latex gloves were routinely used. Only once a staff member was diagnosed with latex allergy, either by RAST or SPT, latex-free gloves were issued for that individual and powder-free gloves for the immediate co-workers. This investigation attempted to assess the airconditioning system of the TBH complex with regard to possible glove powder contamination. Samples were also obtained from the airconditioning control room to evaluate if the filters were able to capture and remove the glove powder and proteins from the air before recirculating it into the building.

3.2. METHODS AND RESULTS

3.2.1. Specimen Collection

The airconditioning system of the TBH complex was installed when the hospital was built in the 1970s. Inside air is extracted from the passages and rooms from a duct opening approximately 30 cm from the floor. This air is passed through two 65% and one 95% filter, after which one third of the volume is replaced with outside air. The mixture is then resupplied through duct openings situated in the roof or very high on the walls. The ducts are not cleaned and the filters are not replaced on a regular basis. Each filter has a manometer which indicates when the filters need cleaning or replacing. This is done by the Maintenance department and depending on the area serviced, the period varies between two and four years. The filters in the areas from where the different dust samples were collected were at least two years old.

The samples represented different levels of exposure according to the number of powdered latex gloves worn on a daily basis and were as follows:

High Exposure:

1: Virology Laboratory – Sample reception extraction duct grid
2: Virology Laboratory – Routine laboratory extraction duct grid
3: Virology Laboratory – Serology laboratory extraction duct grid
4: Virology Laboratory – Virus isolation laboratory extraction duct grid
5: Gynaecology Theatre – Extraction duct grid
6: Gynaecology Theatre – Return duct grid
7: Paediatric Surgery Theatre – Extraction duct grid
8: Paediatric Surgery Theatre – Return duct grid
9: Emergency Theatre – Extraction duct grid
10: Emergency Theatre – Return duct grid

Low Exposure:

11: Dermatology Laboratory – Extraction duct grid

No Exposure:

12: Control Room (Dean's Office) – Extraction duct

Evaluation of Control room filters:

13: Control Room (Virology Laboratory) – Extraction duct

14: Control Room (Virology Laboratory) – Return duct

3.2.2. Starch Concentration of Dust Samples

Glove powder consists of cornstarch and the presence of starch in the dust samples would implicate that airborne glove powder settled in the dust in the airconditioning system.

A stock solution with a concentration of 5 mg starch per mℓ distilled water was prepared from glove powder. Standard solutions of starch concentrations ranging from 0 mg/mℓ to 40 mg/mℓ were prepared from the stock solution to construct a calibration curve (see Table 2.1). The spectrophotometer was set to zero with Tube A1 (distilled water) and the absorbance of each calibration standard was read at 620 nm and recorded ***(Table 3.1)***.

A linear graph was obtained for the absorbance values of the calibration standards and a trend line was fitted to the graph ***(Figure 3.1)***. An R^2 value of 0.9954 was calculated for the calibration curve. The mathematical equation produced by the trend line *(y = 0.035x – 0.0505)* was applied to the absorbance value *(y)* for each test sample. The starch concentration *(x)* of each test sample was calculated and converted to a final concentration of mg/mℓ ***(Table 3.2)***.

Table 3.1: Absorbance values of starch calibration standards

A stock solution with a concentration of 5 mg/mℓ starch was prepared from glove powder. Standard solutions with increasing concentrations ranging form 5 mg/16 mℓ to 40 mg/16 mℓ were prepared from the stock solution to construct a calibration curve (see Table 2.1). The absorbance of each calibrating standard was spectrophotometrically read against distilled water at 620 nm.

Tube	Concentration (mg/16 m)	Absorbance (620 nm)
A1	0 mg/16 mℓ	0.000
B1	5 mg/16 mℓ	0.105
C1	10 mg/16 mℓ	0.290
D1	20 mg/16 mℓ	0.603
E1	30 mg/16 mℓ	0.986
F1	40 mg/16 mℓ	1.387

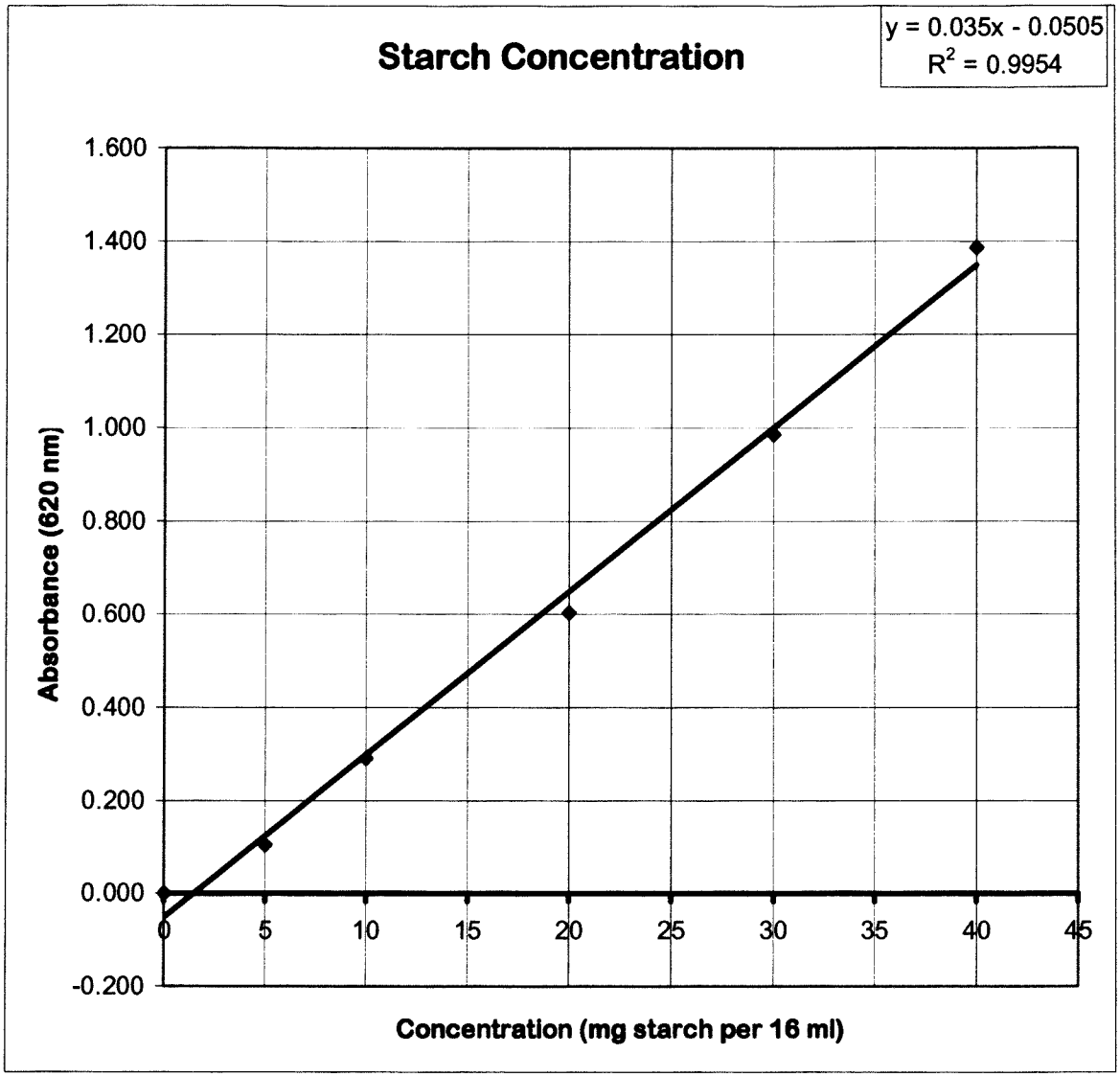


Figure 3.1: Starch calibration standards

A linear graph was created from the absorbance values of the calibration standards. A trend line was then fitted to the graph that produced the equation that was used to calculate the concentration of the test samples (see Table 3.2).

Table 3.2: Calculated starch concentration of test samples

Dust samples collected from the airconditioning system were prepared as 5 mg/mℓ solutions in distilled water. The absorbance of each test sample was spectrophotometrically read at 620 nm. The equation produced by the starch calibration curve (see Figure 3.1) was used to calculate the final starch concentration of the test samples.

Test Sample	Absorbance (620 nm)	Concentration (µg/mℓ)
High exposure		
1 (Virology Sample reception)	0.870	1643.750
2 (Virology Routine laboratory)	1.079	2016.938
3 (Virology Serology laboratory)	0.821	1556.250
4 (Virology Virus isolation laboratory)	2.999	5445.563
5 (Gynaecology Theatre out)	2.240	4090.188
6 (Gynaecology Theatre in)	1.012	1897.313
7 (Paediatrics Theatre out)	0.828	1569.750
8 (Paediatrics Theatre in)	0.696	1333.063
9 (Emergency Theatre out)	1.374	2543.750
10 (Emergency Theatre in)	0.648	1247.313
Low Exposure		
11 (Dermatology)	0.130	322.313
No Exposure		
12 (Dean's Office)	0.075	224.125
Control room filters		
13 (Control room out)	0.270	572.313
14 (Control room in)	0.116	297.313

Out = extraction duct; In = return duct

3.2.3. Protein Concentration of Dust Samples

Latex proteins adhere to the cornstarch particles and become airborne with the glove powder. The dust samples were further investigated for the presence of proteins. If this could be confirmed, it would be reasonable to accept that these proteins could be responsible for the latex-associated respiratory symptoms in sensitised individuals.

The proteins in the dust samples were precipitated with 20% TCA and acetone. After a series of centrifugation, the pellet was resuspended in SDS PAGE running buffer and mixed well.

Calibration standards were prepared from Bradford solution in concentrations that ranged from 0 µg/20 µℓ to 20 µg/µℓ (see Table 2.2). The spectrophotometer was set to zero with the content of Tube A2 (0 µg/20 µℓ) and the absorbance of each calibration standard was read at 595 nm and recorded ***(Table 3.3)***.

A linear graph was created with the absorbance values of the calibration standards and a trend line was fitted to the graph ***(Figure 3.2)***. An R^2 value of 0.9862 was calculated for the curve.

The absorbance of the test samples was spectrophotometrically read at 595 nm, using the content of Tube A2 as the blank. By applying the mathematical equation produced by the graph *(y = 0.1368x – 0.09)*, the absorbance value *(y)* for each sample was calculated as protein concentration (µg) per 20 µℓ water *(x)* and converted to a final concentration of µg/mℓ ***(Table 3.4)***.

A highly significant correlation ($p<0.01$) was found between the concentrations of starch and proteins in all dust samples collected from the airconditioning ducts ***(Figure 3.3)***.

Table 3.3: Absorbance values of Bradford calibration standards

Calibration standards were prepared from Bradford solution in increasing concentrations that ranged from 0 µg/20 µℓ to 20 µg/µℓ to construct a calibration curve (see Table 2.2). The absorbance of each calibration standard was spectrophotometrically read against the blank (A2) at 595 nm.

Tube	Concentration (µg/20 µℓ)	Absorbance (595 nm)
A2	0 µg/20 µℓ	0.000
B2	2 µg/20 µℓ	0.355
C2	4 µg/20 µℓ	0.678
D2	6 µg/20 µℓ	0.898
E2	8 µg/20 µℓ	1.280
F2	10 µg/20 µℓ	1.544
G2	12 µg/20 µℓ	1.780
H2	14 µg/20 µℓ	1.934
I2	16 µg/20 µℓ	2.070
J2	18 µg/20 µℓ	2.736
K2	20 µg/20 µℓ	2.766

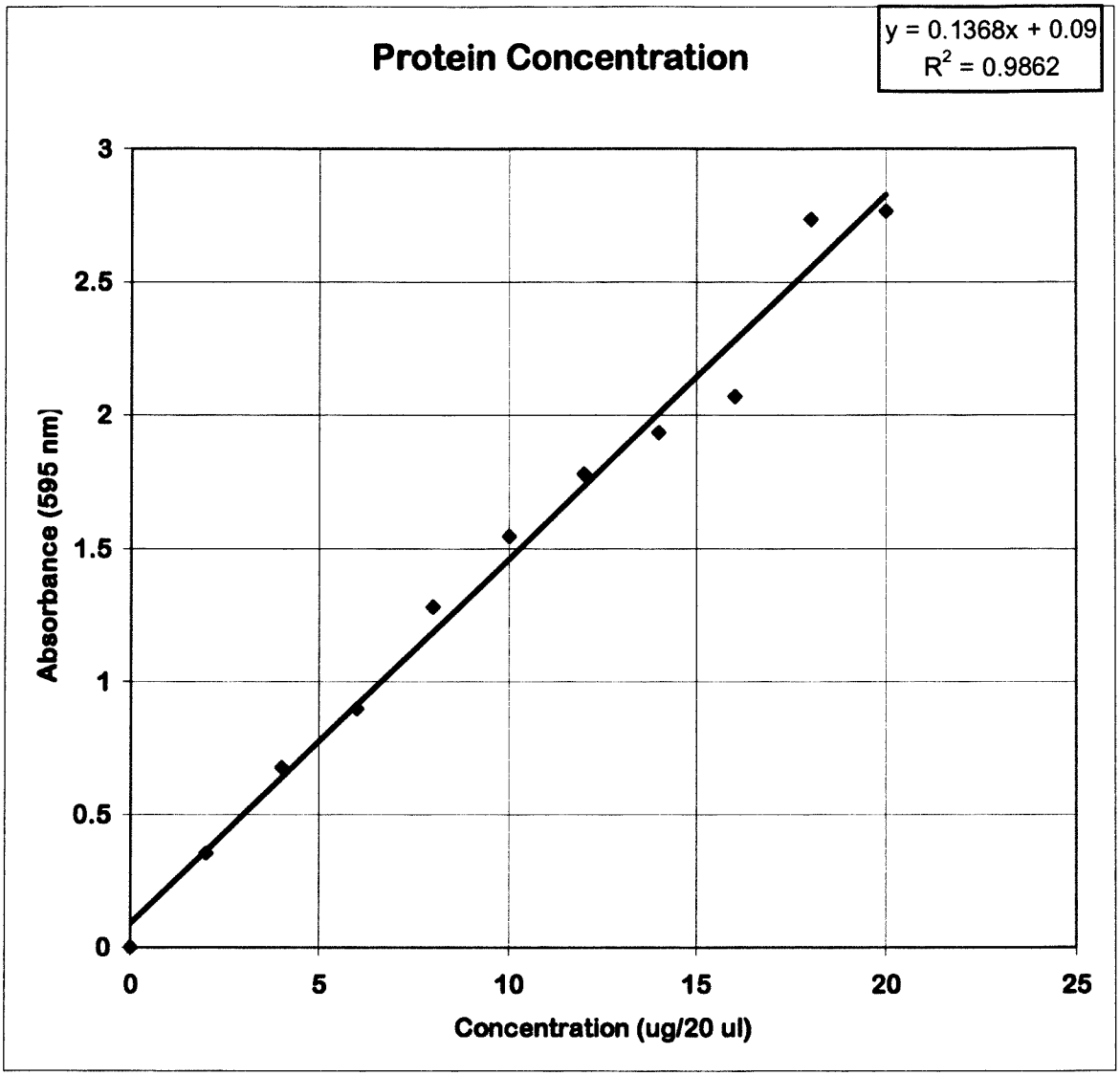


Figure 3.2: Bradford calibration standards

A linear graph was created from the absorbance values of the calibrating standards. A trend line was then fitted to the graph that produced the equation that was used to calculate the concentration of the test samples (see Table 3.4).

Table 3.4: Calculated protein concentrations of test samples

Dust samples collected from the airconditioning system were prepared as 5 mg/mℓ solutions in distilled water. The absorbance of each test sample was spectrophotometrically read at 595 nm. The equation produced by the Bradford protein calibration curve (see Figure 3.2) was used to calculate the final protein concentration of the test samples.

Test Sample	Absorbance (620 nm)	Concentration (µg/mℓ)
High exposure		
1 (Virology Sample reception)	0.183	33.991
2 (Virology Routine laboratory)	0.280	69.444
3 (Virology Serology laboratory)	0.389	109.284
4 (Virology Virus isolation laboratory)	0.673	213.085
5 (Gynaecology Theatre out)	0.482	143.275
6 (Gynaecology Theatre in)	0.266	64.327
7 (Paediatrics Theatre out)	0.240	54.825
8 (Paediatrics Theatre in)	0.204	41.667
9 (Emergency Theatre out)	0.256	60.673
10 (Emergency Theatre in)	0.192	37.281
Low Exposure		
11 (Dermatology)	0.149	21.564
No Exposure		
12 (Dean's Office)	0.118	10.234
Control room filters		
13 (Control room out)	0.175	31.067
14 (Control room in)	0.166	27.778

Out = extraction duct; In = return duct

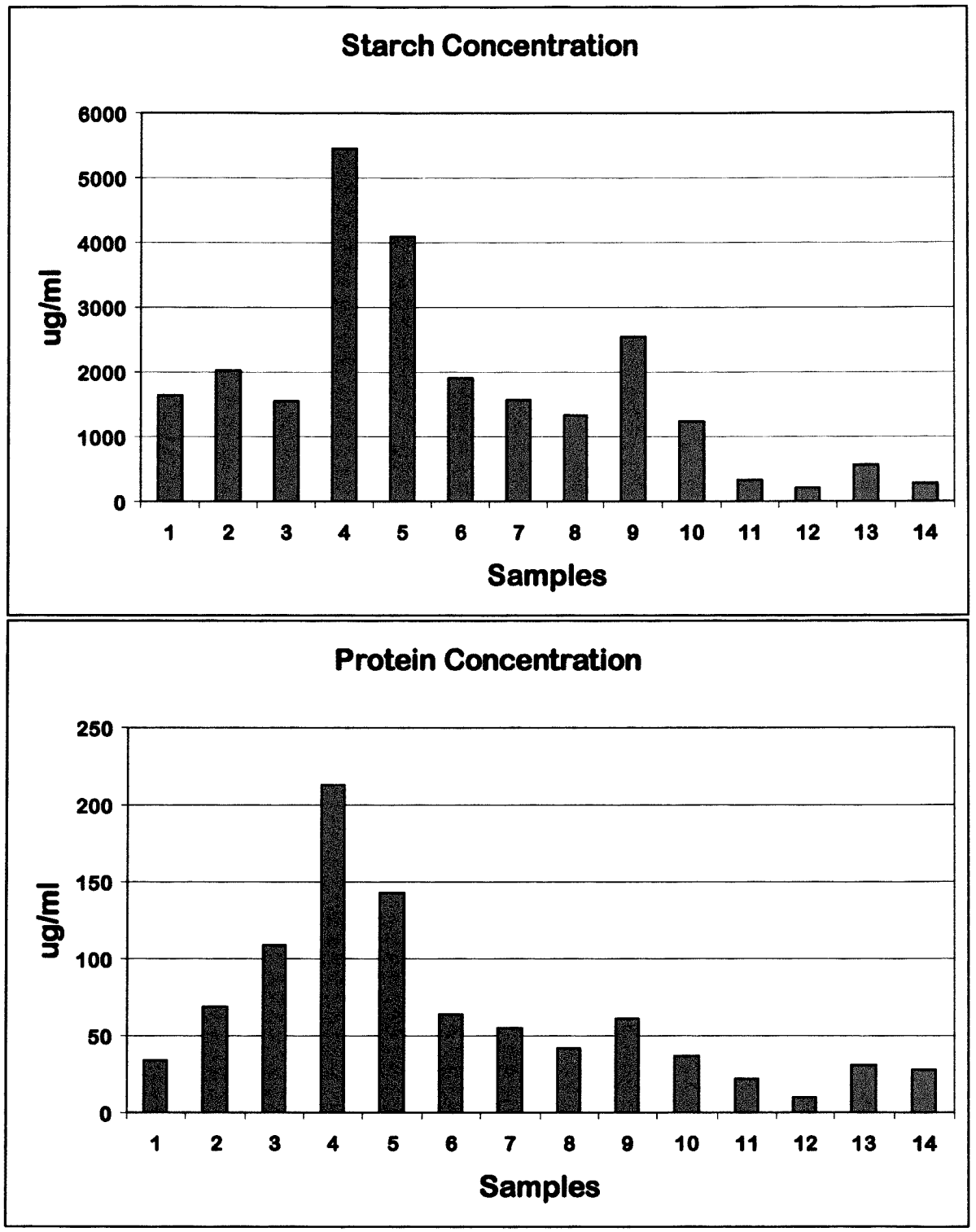


Figure 3.3: Starch and protein concentration of test samples

The columns represent dust samples as described in section 3.2.1. Similar distribution of the starch and protein concentrations was found in all test samples collected from airconditioning ducts. A highly significant correlation ($p<0.01$) was found between the starch and protein concentration in the dust samples.

3.3. DISCUSSION

The Guthrie Research Institute in the USA was contacted in an effort to have the dust samples analysed with the Latex ELISA for Antigenic Proteins (LEAP) assay. However, the cost at that stage was $250 per sample and it was not financially possible. These samples were then sent to the Department of Immunology at GSH for Western blot analysis, but the laboratory did not have any success with the method and could not provide any results. In electronic correspondence with the Malaysian Rubber Board (Mr Mohmad Rouyan bin Haji Baker and Dr Hasma Hashim) it was suggested that the starch be quantitatively determined, since the intensity of the blue iodine test is proportional to the starch concentration. However, protein concentrations were also determined on the samples in order to strengthen the assumption that latex proteins were possibly present in the dust samples.

The current study was then able to confirm the presence of both starch and proteins in all the samples collected from the air supply and return ducts from the airconditioning system at the TBH complex.

Without exception, the areas with the highest glove usage showed the highest concentrations for both starch and proteins. In both instances, the highest levels were measured in the Virology Laboratory where virus isolation was done. The potential infective nature of work done in this area necessitates high glove usage. The next highest values were measured in the extraction duct in the Gynaecology Theatre. The samples collected from the other high exposure areas produced comparable results for both variables.

Although the samples collected from the return ducts still showed high starch and protein concentrations, it was slightly lower than those found in the extraction ducts. The airconditioning filters reduced the amount of starch and protein in the dust, but failed to eliminate it. The filters in the various theatres only managed a reduction in starch and protein concentrations of 53.61% and 55.10% in the Gynaecology Theatre, 15.08% and 24.00% in the Paediatric Surgery Theatre and 50.97% and 38.55% in the Emergency Theatre, respectively. No clear or consistent trend could be identified in the reduction rates of starch or proteins. It was also not possible to explain the relatively

low reduction rates of the Paediatric Surgery Theatre compared to the other theatres investigated.

Although the concentrations were lower than those measured in high exposure areas, the areas with low and no latex exposure both showed traces of starch and proteins. The Dermatology Laboratory showed relatively low levels for both starch and proteins. The use of latex gloves in this area had been terminated six years previously, but an adjacent laboratory still used latex gloves. Therefore, this was considered as an area with low latex exposure. Nobody used latex gloves in the immediate vicinity of the Dean's Office and exposure should therefore theoretically be absent. Although this sample produced the lowest starch and protein concentrations of all tested, it still contained detectable starch and protein levels.

The high concentration of starch and protein still present in the samples from the return ducts suggested that the airconditioning filtering system was inadequate in eliminating glove powder before returning it to the work areas. This hypothesis was confirmed by the results of the filter in the control room servicing the Virology Laboratory that only managed a reduction of 48.05% in the starch concentration and an even lower 10.59% in protein concentration in the air recirculated back into the building. However, these proteins may not exclusively be NRL proteins, but may also include other contaminants, such as proteins, viruses and possibly bacteria.

An interesting finding is the marked decrease in starch and protein concentrations from the extraction ducts in the different sections of the Virology Laboratory to the control room filter that services this area. The airconditioning system is a closed system and theoretically all extracted particles should reach the control room to be filtered before the air is recirculated into the building. This strongly suggests that the protein-laden glove powder settles somewhere in the ducts before it reaches the control room. When this settled dust is disturbed in any way thereafter, it can become airborne again and cause re-contamination of the area.

In conclusion, airborne glove powder is a potential major problem at this institution. Throughout the literature, glove powder is associated with and has been confirmed to contain NRL allergens. Therefore, some of the proteins found in the ducts might have been NRL, even though it was not confirmed. Regardless of the nature of the proteins,

the airconditioning system was found to be contaminated by proteins. These allergens may potentially be recirculated into the building. Only a small number of ducts were evaluated and all of these were contaminated by glove powder. This could also apply to the entire airconditioning system. Distribution of airborne glove powder was not only limited to work areas with high glove usage, but also to areas where gloves are seldom or never worn. Contamination either took place through normal airflow in the passages, or was recirculated into the building via the airconditioning system.

The inability of the air handling units to completely filter recirculated air has severe implications for the safe return of latex sensitive workers to buildings where powdered latex examination gloves are utilised (Kelly *et al*, 2000). Merely eliminating powdered gloves will not solve the problem, because of the possibility of recontamination by recirculation of the settled dust. In addition to eliminating the use of powdered gloves, an adequate filter system should be installed and all airconditioning ducts should be thoroughly cleaned or completely replaced to effectively prevent latex-associated symptoms in sensitised HCW^{s}.

The use of latex is ubiquitous throughout the healthcare industry and numerous medical devices are made of, or contain NRL (see also Table 1.2). However, latex gloves pose the biggest problem. NRL proteins are able to leach from the glove and become airborne with glove powder. It may also attach to the skin and penetrate intact or diseased skin, resulting in allergic reactions. The use of latex gloves should ideally be avoided throughout institutions. This should minimise the risk for serious adverse allergic reactions to sensitised HCW^{s} and patients. However, total elimination is currently not feasible, as alternative materials are either expensive or not suitable as replacements.

CHAPTER 4:
STUDY GROUP DATA

4.1. INTRODUCTION

HCW^s are at major risk of developing latex allergy, mainly due to prolonged daily exposure to high doses of NRL in gloves and aeroallergens on glove powder (De Beer, 2000; Sussman *et al*, 2002). It is unclear whether cumulative, peak or current exposure is the most important determinant of sensitisation. However, latex-specific IgE levels are significantly influenced if measured during a period of active latex exposure (Wrangsjö *et al*, 1986, Allmers *et al*, 1998; Brathwaite *et al*, 2001; Weissman & Lewis, 2002).

Atopy is a strong and consistent risk factor for the development of latex allergy (Wrangsjö *et al*, 1986; Turjanmaa, 1987; Turjanmaa & Reunala, 1988b; Beuers *et al*, 1990; Pecquet *et al*, 1990; Heese *et al*, 1991; Niggeman, 1997; Levy *et al*, 2000; Garabrant *et al*, 2001; Garabrant & Schweitzer, 2002). Although atopy and frequent exposure are independent risk factors for latex allergy, a combination of these two factors considerably increases the risk of clinical latex allergy (Moneret-Vautrin *et al*, 1993; Randolph, 2001).

Reported prevalence rates for latex allergy in the RSA range between 2.7% and 30% (Potter, 1996a; Marais *et al*, 1997; De Beer *et al*, 1999; Pretorius, 1999; Potter *et al*, 2001b). None of the existing diagnostic methods for latex allergy are 100% diagnostically accurate and a combination of two or more is recommended (Sussman & Beezhold, 1995; Palczynski 2000; De Beer & Cilliers, 2001). There is global consensus that the best routine screening method for latex allergy is the SPT, provided that it is performed in a safe environment under controlled conditions (Turjanmaa *et al*, 1996).

Harvesting and analysis of dermal fluid from suction blisters have been performed for more than 30 years (Benfeldt, 1999). A wide range of inflammatory mediators, cells and other components are found in the extravascular compartment (Kiistala, 1968; Benfeldt, 1999). Dermal fluid analysis confirmed the presence of histamine, bradykinin, PG E_2, PG $F_2\alpha$ (Cilliers, 1993), PG D_2 (Lawrence *et al*, 1987), serine proteinases, proteoglycans, tryptase, LT C_4 and LT D_4 (Dahl, 1981a; Deleuran *et al*, 1991), as well as triglycerides, cholesterolesters and phospholipids (Vermeer *et al*, 1979).

Animal studies have shown that when NRL sensitisation took place intratracheally or topically, specific IgE antibodies against *Hev b 2, Hev b 4* and *Hev b 6* were raised, whereas subcutaneous sensitisation produced IgE antibodies against *Hev b 1* and *Hev b 3* (Lehto *et al*, 2003). In HCW^{s}, the skin and respiratory system are most commonly affected by latex allergy (De Beer *et al*, 1999; De Beer, 2000; De Beer & Cilliers, 2001). The IgE immune responses are frequently directed against the soluble NRL proteins, *Hev b 2* and *Hev b 4* (Hamilton & Adkinson, 1996). Skin lipids contribute to maintaining the skin in a hydrated state and sebum and sweat on the skin surface will therefore favour percutaneous absorption of soluble NRL proteins, e.g. *Hev b 6.01*, to hydrophobic NRL proteins, e.g. REF (Boelsma *et al*, 2003; Lehto *et al*, 2003).

In this chapter clinical findings and results from the Study Group will be reported and discussed in terms of demographic data, symptoms, special laboratory investigations and sebum content.

4.2. METHODS AND RESULTS

A previous study done at TBH during 1997-2000 identified laboratories and theatres as areas with high occupational latex exposure (De Beer, 2000). These areas were targeted for placing volunteer recruitment advertisements. Advertisements were also placed on notice boards in the Faculty of Heath Sciences and Dentistry.

4.2.1. Compilation of the Study Group

A total of 500 questionnaires was circulated to staff members at TBH and 349 completed questionnaires were returned for evaluation. This represents a response rate of 69.8%.

The symptoms listed on the questionnaire cover the three major routes of exposure:

- aeroallergens (conjunctivitis, nasal congestion, rhinitis, shortness of breath and bronchospasm),
- systemic involvement (angioedema and anaphylactic shock) and
- skin contact (urticaria, pruritus, skin rash and hand eczema)

Subjects who complied with one of the following pre-defined criteria were followed up for inclusion in the Study Group:

- suspected or confirmed latex allergy / work-related symptoms
- ≥ two severe symptoms / ≥ four moderate symptoms
- food allergies to avocado, banana, carrot, kiwi, mango, potato or nuts
- bronchospasm or anaphylactic reactions following latex contact

The Study Group consisted of 152 consenting HCW^{s}; 28 males and 124 females. Their mean age was 34.0 ± 7.7 (Me 33.5, range 19-59) years. The majority of subjects were Caucasian (54.6%), followed by Coloured (42.1%), Indian (2.0%) and African (1.3%). According to the Human Resources Statistics of TBH, the gender distribution of the Study Group was an acceptable reflection of the study population (TBH HCW^{s}). The ratio of Caucasian to Coloured individuals was also acceptable and representative.

4.2.2. Numerical Indices

4.2.2.1. Class

Subjects were assigned to different Classes according to their own suspicion of latex allergy. Class 0 included subjects who thought they were not allergic to latex (7.9%), Class 1 included those who suspected or knew that they were allergic to latex (38.8%) and Class 2 included all subjects who were unsure of their status (53.3%).

4.2.2.2. Allergy Score

An Allergy Score was calculated for each subject by using the total number and severity of individual symptoms and the following formula (De Beer, 2000):

Allergy Score = (1 x total mild) + (2 x total moderate) + (3 x total severe)

A maximum Allergy Score of 33 per subject was possible. A mean Allergy Score of 11.4 ± 6.3 (range 1-29) was generated by the Study Group.

All symptoms were present in up to 73.7% of the Study Group ***(Table 4.1)***. Symptoms were work-related in 1.3-48.0%, indicating a relatively high level of discomfort in patients exposed to latex gloves and glove powder.

Table 4.1: Frequency and severity of symptoms recorded by the Study Group

Subjects scored each individual symptom according to its severity and indicated if they thought it was work-related. The total number and severity of individual symptoms were used to calculate the Allergy Score.

Symptom	Total		Mild		Moderate		Severe		Work-related	
	n	%	n	%	n	%	n	%	n	%
Conjunctivitis	91	59.9	19	12.5	37	24.3	35	23.0	52	34.2
Nasal congestion	111	73.0	15	9.9	48	31.6	48	31.6	58	38.2
Rhinitis	105	69.1	15	9.9	44	29.0	46	30.3	48	31.6
Shortness of breath	49	32.2	21	13.8	18	11.8	10	6.6	17	11.2
Bronchospasm	55	36.2	23	15.1	18	11.8	14	9.2	22	14.5
Urticaria	39	25.7	14	9.2	11	7.2	14	9.2	18	11.8
Angioedema	30	19.7	9	5.9	10	6.6	11	7.2	14	9.2
Anaphylaxis	8	5.3	2	1.3	2	1.3	4	2.6	2	1.3
Pruritus	112	73.7	16	10.5	38	25.0	58	38.2	73	48.0
Skin rash	106	69.7	17	11.2	41	27.0	48	31.6	68	44.7
Hand eczema	80	52.6	18	11.8	20	13.2	42	27.6	54	35.5

Ten subjects (6.6%) reported just a single symptom, while two (1.3%) subjects reported all eleven symptoms. The most number of symptoms per subject reported by the Study Group was five in 29 subjects (19.1%). The frequency distribution of the total number of symptoms was five (19.1%), six (13.2%), two (11.8%), eight (11.8%), three (10.5%), four (9.2%), seven (9.2%), one (6.6%), ten (5.3%), nine (2.0%) and eleven (1.3%), respectively.

The symptom most frequently reported was pruritus (73.7%), followed by nasal congestion (73.0%), skin rash (69.7%) and rhinitis (69.1%), respectively. Pruritus also showed the highest association with work-related symptoms (48.0%), followed by skin rash (44.7%) and nasal congestion (38.2%), respectively. Additional conditions reported by the Study Group were psoriasis in two (1.3%) subjects and porphyria and scombroid syndrome (allergy to multiple fish species, including mackerel, tuna, marlin and swordfish) in a single subject each (0.7%).

4.2.3. Occupational Exposure

The majority of subjects recruited were nursing staff (40.1%), followed by laboratory staff (33.6%) and doctors (15.1%), respectively. Other occupations included dentistry (7.2%), scientific (2.0%), radiography (1.3%) and audiology staff (0.7%) ***(Table 4.2)***. At the time of testing, all subjects reported current occupational latex exposure by daily glove usage.

The group had a mean of 10.2 ± 6.9 (range 0.2-30.0) years of total exposure to latex gloves. Their mean glove usage was 26.0 ± 14.7 (range 1.0-80.0) hours per week and the number of gloves worn was 20.8 ± 16.9 (range 1.0-100.0) pairs per week. The number of years of exposure most frequently indicated by the Study Group was 10 and 20 years in 9.2% of subjects, followed by five years of exposure in 6.6% and four and 18 years in 5.9% each. Other reported frequencies ranged between 0.7% and 5.3% of subjects.

Table 4.2: Occupations represented by the Study Group

The study population included HCW[s] who wear latex gloves on a daily basis. Sampling was done primarily in theatres and laboratories of TBH, as these areas were previously confirmed to have high latex exposure. The distribution of the Study Group was therefore acceptable as representative of the study population.

Occupation / Department	n	%
Audiology	1	0.7
Dentistry	11	7.2
Doctors	23	15.1
Laboratory staff	51	33.6
Nursing staff	61	40.1
Radiography	2	1.3
Scientists	3	2.0

4.2.4. Surgical Exposure

Subjects were asked to report all previous surgical procedures on the questionnaire and 124 (81.6%) individuals reported a total between one and 19 procedures. The landmark case of latex allergy was published in 1979 (Nutter, 1979) and the number of procedures before and after 1980 was therefore reported separately. Before 1980, a total of 67 procedures (mean 0.4 ± 1.0, range 0-8) were reported by 43 subjects and after 1980, a total of 309 surgical procedures (mean 2.0 ± 2.3, range 0-19) by 112 subjects.

An absence of surgical procedures was recorded by 109 (71.7%) and 40 (26.3%) subjects before and after 1980, respectively. Single and two procedures were reported most frequently before (21.7% and 15.8%, respectively) and after 1980 (24.3% and 15.8%, respectively). The highest number of procedures in both groups (eight and 19, respectively) was reported by only one subject (0.7%).

A total of 37 subjects (24.3%) reported specific events following their procedures. The most frequent events were nausea in 16 subjects (10.5%), followed by skin manifestations in six (4.0%), hypotension in four (2.6%) and anaphylactic shock in three subjects (2.0%). Other symptoms included anxiety, asthma, nosebleeds and sleepiness.

4.2.5. Atopy

Atopy has been shown to predispose an individual to the development of latex allergy. The importance of food allergies in latex allergic individuals has also been confirmed (De Beer *et al*, 1999; De Beer, 2000; De Beer & Cilliers, 2001). Atopic diseases and specific allergies present in the Study Group is summarised in ***Table 4.3***.

For the purpose of this study, self-reported or personal atopy was defined as eczema, asthma, hay fever or an itchy skin. Personal atopy was present in 75.7% of the Study Group. A single atopic condition was reported by 35.5%, two conditions by 26.3%, three conditions by 6.6% and all four conditions by 7.2%. Family history of atopy was reported by 53.3%. A combination of personal and family history of atopy was present in 46.1% of subjects, and 82.9% reported either a personal or family history of atopy. Only 17.1% did not have a personal or family history of atopy.

Table 4.3: Atopic diseases and specific allergies reported by the Study Group

The existence of atopic disease and other allergies provide an idea of an individual's overall allergic profile.

		n	%
Eczema		46	30.3
Asthma		30	19.7
Hay fever		84	55.3
Itchy skin		48	31.6
Family history of atopy		81	53.3
Allergies:	Drugs / Chemicals	37	24.3
	Penicillin	*8*	*5.3*
	Sulphonamides	*5*	*3.3*
	Aspirin, Amoxycillin, Tetracycline	*3*	*2.0*
	Cidex, Diclofenac sodium, Dentistry Material, Homeopathic preparations, Ibuprofen, Iodine, Laxatives, Morphine, Nickel, Paracetamol, Preservatives, Prochlorperazine, Propoxyphene hydrochloride, Pyrazinamide, Scoline	*1*	*0.7*
	Food	38	25.0
	Fish	*6*	*4.0*
	Dairy, Nuts	*3*	*2.0*
	Latex-fruit syndrome	*3*	*2.0*
	Avocado, Paw-paw, Pineapple	*2*	*1.3*
	Banana, Carrot, Cheese, Flour, Garlic, Kiwi fruit, Lactose, Melon, Oil, Orange, Papain, Potato, Sesame, Spices, Tomato, Watermelon	*1*	*0.7*
	Other	39	25.7
	Cats	*9*	*5.9*
	Grass, House dust mite	*8*	*5.3*
	Pollen	*6*	*4.0*
	Dogs	*4*	*2.6*
	Bee sting, Cosmetics, Insects, Plaster	*3*	*2.0*
	Cigarette smoke, Detergents, Glue, Pantyhose, Plants	*2*	*1.3*
	Condoms	*1*	*0.66*

An interesting observation was that although eczema, hay fever and an itchy skin as part of personal atopy were reported by 30.3%, 55.3% and 31.6%, respectively, a higher percentage of the same subjects reported hand eczema (52.6%), rhinitis (69.1%) and pruritus (73.7%) as current symptoms.

Allergy to penicillin was most frequently reported (5.3% of the group), while allergies to other pharmaceutical drugs were fairly uncommon. A wide spectrum of food allergies was present. Of note is the confirmed latex-fruit syndrome in 2.0% of the group and allergies to food known to cross-react with latex, e.g. avocado, banana, carrot, kiwi fruit, melon, paw-paw, pineapple and potato (1.3%).

Allergies to different inhalants were also reported, e.g. grass (5.3%), house dust mite (5.3%), pollen (4.0%) and animal dander (cats in 5.9% and dogs in 2.6%, respectively). A single subject (0.7%) admitted to having experienced repeated allergic reactions to latex condoms.

4.2.6. Total IgE and Latex-specific IgE

In vivo latex-specific IgE was selected as a first line diagnostic tool, because it renders quantitative results and is regarded as the safest for the patient (Weiss, 1995; Bowyer, 1999b; De Beer, 2000; De Beer & Cilliers, 2001). Total serum IgE and latex-specific IgE were done according to the CapRAST method.

Total IgE and latex-specific IgE levels were determined on 152 serum and 31 dermal fluid specimens. Mean total IgE levels were 146.89 ± 268.74 IU/ℓ (range 1.00-1817.00 IU/ℓ) in serum and 55.12 ± 107.46 IU/ℓ (range 1.00-431.00 IU/ℓ) in dermal fluid.

Different ethnic groups have different normal values for total serum IgE. According to these normal values, 20 (24.1%) Caucasian subjects had total serum IgE values above the upper normal limit of 100 IU/ℓ and 14 (21.9%) Coloured subjects had total serum IgE values above the upper normal limit of 200 IU/ℓ. None of the subjects in the Indian and Black ethnic groups had elevated total serum IgE values (> 500 IU/ℓ). Four subjects (two Caucasian and two Coloured) had values of > 1 000 IU/ℓ and a further two subjects had elevated values of 947 IU/ℓ and 993 IU/ℓ respectively.

Mean latex-specific IgE levels were 2.39 ± 2.76 IU/ℓ (range <0.35-10.35 IU/ℓ) in serum and 1.42 ± 1.38 IU/ℓ (range <0.35-4.00 IU/ℓ) in dermal fluid. Positive serum latex-specific IgE values (> 0.35 IU/ℓ) were recorded in 23/152 (15.1%) subjects, while 6/31 (19.4%) dermal fluid specimens yielded positive latex-specific IgE results.

4.2.7. Skin Prick Tests

Often individuals with negative latex-specific IgE values have positive latex SPT^s, especially when symptoms are limited to the contact site. It is generally accepted that a person who has a positive SPT to latex, will also have detectable latex-specific IgE values. It is relatively safe to perform SPT^s to latex when the serum latex-specific IgE is negative, as systemic reactions have not been reported in such cases.

An example of a positive SPT to latex is presented as ***Figure 4.1***.

Follow up SPT^s for latex were done on 59 subjects and were positive in 34 (57.6%) and negative in 25 (42.4%). These figures represent 22.4% and 16.5% of entire the Study Group respectively.

A total of 70 (46.1%) subjects were lost to follow up after the initial venepuncture. The reasons were withdrawal from the study in 52 (74.3%) subjects, resignation or relocation to other healthcare institutions in 15 (21.4%) and pregnancy in three (4.3%). These figures represent 34.2%, 9.9% and 2.0% of the Study Group respectively.

4.2.8. Western Blot

The AlaBLOT® AlaSTAT™ Specific IgE method was used to determine Western blot bands for latex. This method is designed for qualitative determinations of specific IgE results. The determinant bands of each strip were measured in millimetres from the dye front *(d)* and the MW^s calculated by using the following formula provided with the reagent kit:

$$\textit{Log MW} = 0.027 \times d + 0.91$$

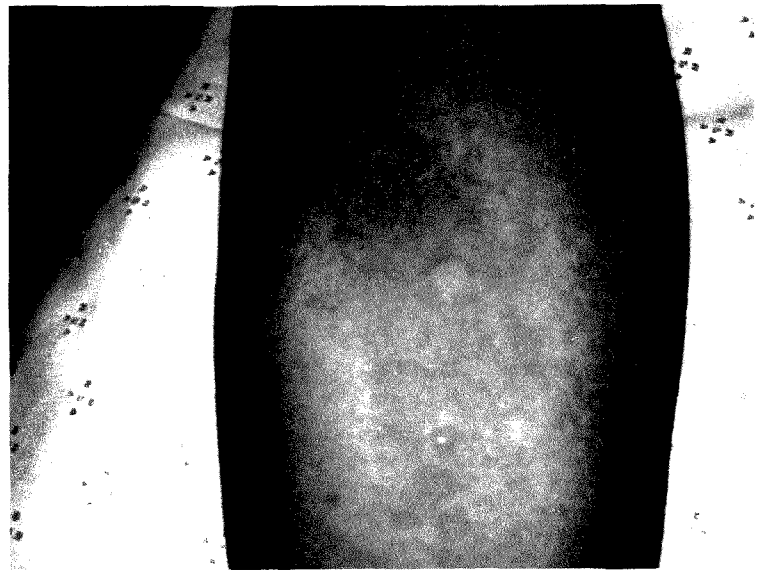


Figure 4.1: Results from latex SPTs

No reaction was caused by the negative control (-), while the positive control (+) and 1 HEP latex allergen induced wheal and flare reactions. No further testing was done in this case, because the wheal caused by the 1 HEP solution was sufficient to be regarded as a positive result.

Western blot analysis was done on all sera with positive latex-specific IgE values (n = 23), as well as dermal fluid from these subjects (n = 14), regardless of the latex-specific IgE result. Although eight of these dermal fluid specimens yielded negative latex-specific IgE results, all specimens showed Western blot bands.

Results are listed in ***Table 4.4***. The presence of bands on the allergen strips indicated the presence of specific IgE, but the intensity of the bands did not correlate with the specific IgE value. Very faint bands were present on certain membrane strips (e.g. 6, 8, 10 and 11 in ***Figure 4.2***), while others showed very dark and definite bands. Without exception, all the bands present in dermal fluid of individuals were also present in their serum.

Hev b 1 (14.6 kD) has been confirmed as a powder-bound, airborne allergen, but only in about 50% of HCW^{s} allergic to latex (Poulos *et al*, 2002). All subjects tested in the current study recognised *Hev b 1* both in their serum and dermal fluid.

Hev b 2 (36 kD) and *Hev b 4* (50-57 kD, 100-110 kD) are considered significant allergens in latex allergic HCW^{s} (Randolph, 2001; Sussman *et al*, 2002). The current study confirmed *Hev b 2* in twelve specimens (32.4%). Eight specimens (21.6%) showed a 56 kD band, which may be the reduced form of *Hev b 4*. A further six (16.2%) showed a 110 kD band, suggesting that it is the unreduced form of *Hev b 4*. Only three (8.1%) specimens contained both bands.

Hev b 3 (22.3 kD) shows less frequent reactivity in HCW^{s}, but suggest an association with anaphylactic reactions (Lu *et al*, 1995). This allergen was recognised by five specimens (13.5%) in this study, of which three had a history of latex-related anaphylaxis.

Hev b 5 (24 kD), *Hev b 7* (46 kD) and *Hev b 13* (43 kD) are regarded as major allergens in HCW^{s} (Yip *et al*, 2000; Sussman *et al*, 2002; Bernstein *et al*, 2003). *Hev b 5* was recognised by 20 specimens (54.1%). However, *Hev b 7* was not found to be a major allergen in this study, as only one (2.7%) specimen showed reactivity to it. *Hev b 13* was confirmed in twelve specimens (32.4%). Hevamine (30 kD) has been reported to show infrequent association with HCW^{s} (Carillo, 1986; Axelsson *et al*, 1987; Warshaw, 1998). In this study, reactivity to hevamine was confirmed in ten specimens (27.0%).

Table 4.4: Calculated sizes of latex allergens present in serum and dermal fluid

Western blot analysis was used to confirm the presence of latex-specific IgE in all specimens tested. The formula utilised the distance of the determinant bands from the dye front on the allergen strips and a constant value to calculate the MW^s of the individual allergens.

Nr	RAST (IU/ℓ)	Calculated MW (kD) of latex allergen bands													
A004	0.72	14.6	20												
A004B	<0.35	14.6													
A005	1.35	14.6	20	22											
A005B	<0.35	14.6	20	22		26			41						
A008	1.14	14.6	20		24		30								
A008B	<0.35	14.6	20		24										
A009	1.52	14.6	20		24			36	41	43				70	110
A009B	<0.35	14.6													
A011	1.41	14.6	20		24	26	30	36		43			56		
A016	0.42	14.6	20				30								
A021	6.74	14.6	20			26	30	36		43			56		
A021B	0.74	14.6	20		24	26									
A022	0.59	14.6	20		24	26				43					
A030	2.93	14.6	20	22	24	26	30	36	41	43	46	50	56	70	110
A030A	<0.35	14.6	20												
A030B	0.59	14.6	20							43					
A036	0.68	14.6	20		24	26									
A036B	<0.35	14.6	20		24	26									
A043	0.69	14.6	20			26									
A043B	<0.35	14.6				26									
A046	0.62	14.6	20	22	24	26	30	36	41	43		50	56	70	
A051	10.35	14.6	20		24		30	36		43		50	56	70	110
A051B	4.00	14.6	20		24	26	30	36		43		50			
A065	3.23	14.6	20		24	26		36	41	43					110
A065B	0.45	14.6	20		24	26		36	41			50			110
A070	4.97	14.6	20		24	26									
A070B	1.92	14.6	20		24	26									
A085	0.40	14.6	20			26									
A099	0.43	14.6	20			26									
A099B	<0.35	14.6													
A127	0.59	14.6	20			26									
A139	2.60	14.6	20	22		26									
A142	0.88	14.6	20		24	26		36	41			50	56		
A145	2.72	14.6	20		24	26	30			43		50	56		
A150	1.16	14.6					30	36				50	56		110
A151	8.71	14.6			24	26		36		43					
A151B	1.06	14.6			24	26									

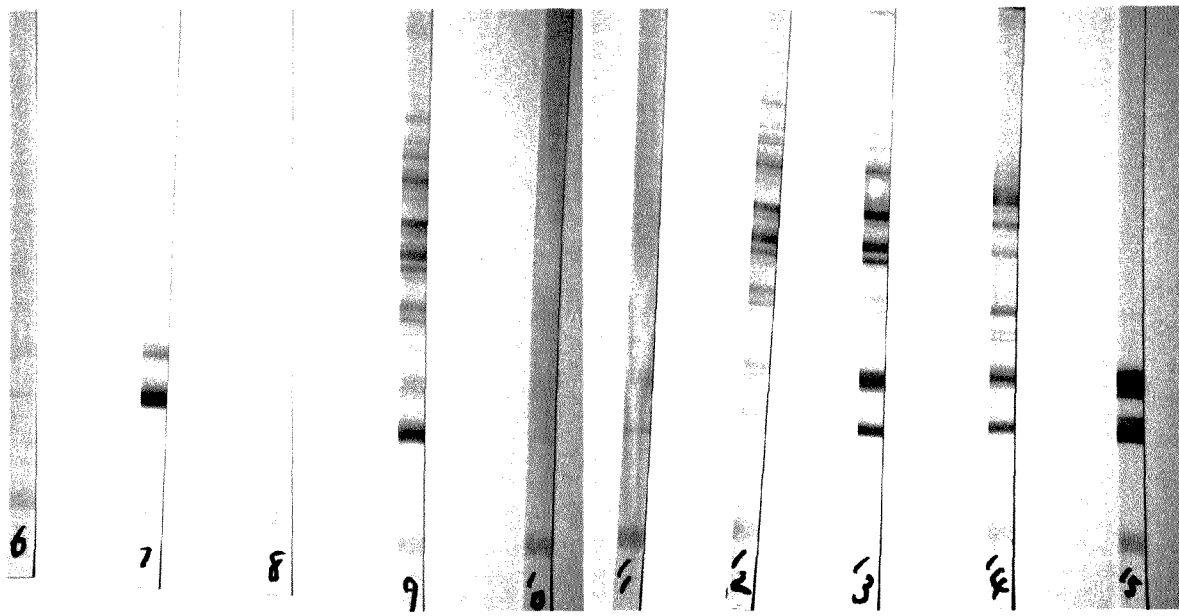


Figure 4.2: Positive latex-specific Western blot strips

The intensity of stained bands on the blot did not correlate with the latex-specific serum values. For example, the latex-specific IgE value for number 15 was 4.97 IU/ℓ, compared to 10.35 IU/ℓ for number 13 and 6.74 IU/ℓ for number 7. The presence of bands on the allergen strips merely indicated that the sample tested contained latex-specific IgE.

Hevein is the allergen most frequently recognised by HCW^s with cutaneous symptoms (Slater & Trybul, 1994; Alenius *et al*, 1996; Turjanmaa *et al*, 1996). Thirty specimens (81.1%) showed a 20 kD band that is consistent with *Hev b 6.01*. No band corresponding with *Hev b 6.03* (14 kD) could be identified in any of the specimens and the presence of *Hev b 6.02* (4.7 kD) could not be confirmed, due to the inability of the method to identify molecules smaller than 14 kD.

The 26 kD allergen found in the current study also seems to be a major allergen with 64.9% reactivity, but the size could not be matched with a known latex allergen. It could be speculated that the bands that appeared at 50 kD were associated with the 56 kD *Hev b 4*, although six of the eight specimens had bands for both MW^s. Additional unknown allergens with MW^s of 26 kD, 41 kD, 50 kD and 70 kD were found in 24, seven, eight and four specimens respectively.

No evidence of *Hev b 8, Hev b 9, Hev b 10* or *Hev b 11* could be found. Only small percentages of patients in previous studies have shown reactivity to these allergens (Yip *et al*, 2000; Kurup & Fink, 2001; Rihs *et al*, 2001; Sussman *et al*, 2002).

An interesting, and at present unexplained, observation was that dermal fluid collected form different anatomical areas also showed different results. Dermal fluid was collected from the volar forearm (A030A) and abdominal area (A030B) in subject A030. Clinical abdominal cutaneous involvement in latex allergy is unusual and the abdomen is unlikely to be routinely or directly exposed to NRL. However, the area was chosen because it could accommodate the four vacuum chambers with ease, whereas the area on the arm was limited. The relatively flat surface area of the abdomen also facilitated a closed vacuum system, which was a problem on the arm. The dermal fluid latex-specific IgE values were < 0.35 IU/ℓ on the arm and 0.39 IU/ℓ on the abdomen, respectively. Western blot analysis confirmed the presence of *Hev b 1* and *Hev b 6.01* in both specimens. However, the abdominal dermal fluid had an additional band for *Hev b 13*. The reason for this discrepancy is not known. Serum from this subject included all three proteins.

4.2.9. Patch Tests

Since 1992 increasing reports of delayed-type IV hypersensitivity to NRL with, or without type I hypersensitivity, appeared. In a prospective study on type IV allergy to

NRL, the UK Contact Dermatitis Group found it to be a real problem for patients with hand eczema. They recommended patch testing with ammonia-preserved NRL and that these patients be investigated for NRL contact urticaria (Sommer *et al*, 2002).

Only twelve subjects (7.9%) with negative latex-specific IgE and latex SPT^{s} consented to cutaneous patch tests with the Standard European Series (see section 2.3.8.), a piece of latex glove and 1% glove powder in petrolatum. Five (3.3%) subjects reported positive results to patch tests done previously (two nickel sulphate, two cidex and one perfume). This was not repeated or confirmed by the current study. ACD to cosmetics and nickel sulphate was reported by two (1.3%) subjects each and paraben mix by a single (0.7%) subject. Only three subjects showed positive results after 48 hours and all reactions were still present after 96 hours. This confirmed that the reactions were due to ACD and not irritant contact dermatitis.

All three subjects showed a positive patch test to thiuram mix (1% in petrolatum). A UK study found a statistically significant increase in the incidence of thiuram allergy in HCW^{s} with hand dermatitis between 1989 and 1995 (Gibbon *et al*, 2001). Thiurams are primarily used as accelerators in the production of rubber. It is present in various articles made of rubber, e.g. balloons, catheters, elastic bandages, stethoscopes, etc. but also in disinfectants, germicides, insecticides, adhesives, soaps, shampoos and as an anti-alcohol treatment. Cross-reactions are possible with carbamates and N-isopropyl-N-phenyl-4-phenylenediamine (PPD). The thiurams were the most common sensitisers among rubber components tested by the USA Contact Dermatitis Group. Of the individuals tested, 7.7% were sensitive to thiuram mix (Marks & DeLeo, 1997).

The first subject reacted to thiuram mix, benzocaine (5% in petrolatum), PPD (0.1% in petrolatum), formaldehyde (1% in water), nickel sulphate (5% in petrolatum), wool alcohols (30% in petrolatum), latex glove and glove powder (1% in petrolatum). Benzocaine is a local anaesthetic present in a wide variety of topical and oral preparations. Benzocaine is known to cross-react with PPD. Apart from a main ingredient in black and grey rubber products, PPD is also used in hair and other dyes, earphones, facemasks, elasticised clothing and photographic materials. The prevalence of positive patch tests to benzocaine and PPD is approximately 2.2% and 2.1% respectively. Formaldehyde is present in cosmetics, household products,

industrial sources, paint, rubber and resins. The prevalence of positive formaldehyde patch tests is about 7.8%. Nickel sensitivity is usually acquired non-occupationally from jewellery and other metal objects on clothing, e.g. wire support for lingerie. It also occurs in medical and dental instruments. Between 4.5% and 5.8% of the general population is sensitive to nickel and patch test clinics show up to 30-40% sensitivity. This figure is most probably related to ear piercing, as metal pins used for piercing release nickel in varying amounts, thus allowing exposure to the antigen. Wool alcohol is the alcohol fraction of wool wax and a 2.9% prevalence of positive patch tests is found. It is used in topical medications, cosmetics, polish, wax and wire insulators (Marks & DeLeo, 1997).

The second subject had positive reactions to thiuram mix and paraben mix (16% in petrolatum – 4% each of methyl-4-hydroxybenzoate, ethyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate and butyl-4-hydroxybenzoate). Parabens are most commonly used as preservatives in cosmetics. The USA Contact Dermatitis Group found a 2.3% positive rate for paraben mix in their patients (Marks & DeLeo, 1997).

The third subject produced positive results to thiuram mix and cobalt chloride (0.5% in petrolatum). Exposure to cobalt chloride includes jewellery, metal components of clothes, e.g. buttons, snaps, clasps and buckles, hair dye, antiperspirants, joint replacements and dental appliances (Marks & DeLeo, 1997).

4.2.10. Sebum Content

A variety of methods for the determination of sebum have been published over the past century. This includes different methods of scraping, washing and extraction. Absorbance onto paper was used as early as 1866. Later cigarette paper was applied to the forehead for three hours and the extracted lipid from the paper was weighed. This method was later simplified by using a direct gravimetric evaluation of the absorbent paper. The photometric method was first introduced in 1970. Light transmission through opalescent grass increased when the rough surface was coated with fat following direct contact to the forehead (Serup, 1991). The Sebumeter SM 810™ is based on this principle, except that the glass has been replaced by a matt synthetic tape (CK Electronic, 2001).

Sebum content has been reported to show great anatomical variability among regions. A few studies have attempted to establish normal values for sebum content in different anatomical areas. Low sebum levels were reported for the ankle, forearm and thigh, while sebum-rich areas included the forehead and post-auricular area. However, a difference was also found between three measuring sites on the forehead. This may be based on the fact that the number and size of sebaceous glands in the region of the lateral forehead are markedly lower than in the center (Wilhelm *et al*, 1991). Normal volunteers produced values of 100-200 $\mu g/cm^2$ on the forehead, 70-180 $\mu g/cm^2$ on the cheeks and > 6 $\mu g/cm^2$ on the arms and hands (CK Electronic, 2001). Young healthy adults (25-30 years) showed mean sebum values of 3.4 ± 0.7 $\mu g/cm^2$ on the palms, 2.2 ± 0.9 $\mu g/cm^2$ on the dorsal area of the hand and 137.9 ± 19.9 $\mu g/cm^2$ on the forehead (Wilhelm *et al*, 1991). Another group of healthy individuals (35-38 years) showed median values of 197.3 $\mu g/cm^2$ and 6.3 $\mu g/cm^2$ for the forehead and dorsum of the hand respectively (Sator *et al*, 2003). Baseline sebum levels of 273.2 ± 132.8 $\mu g/cm^2$ on the forehead of patients with acne have been reported (Serup, 1991).

A Sebumeter SM810™ was used to measure sebum values of specific anatomical areas with high exposure to latex. Only laboratory and theatre staff were included in this investigation. Informed consent was signed by all subjects before conducting the measurements and a total of 400 individual sebum measurements were done. Baseline values were measured in 29 subjects. Sixteen individuals (55.2%) were working in a laboratory and 13 were nursing staff (44.8%) from different theatres. In 21 cases (72.4%) it was possible to measure follow-up sebum levels after three to four hours of occupational exposure to latex gloves. Eleven (68.8%) of the laboratory workers and ten (76.9%) of the theatre staff were followed up. Descriptive statistics of baseline and follow up values are summarised in ***Table 4.5***.

The forehead and both cheeks showed increased values over time, although it was not found to be statistically significant. The nose showed a 12.8% decreased value at follow up, but it was not statistically significant when compared to the mean baseline value.

Although the baseline values were slightly higher than those published, significantly decreased values were found for all follow up values on both hands.

Table 4.5: Baseline and follow up sebum values for the Study Group

Specific anatomic areas with high occupational exposure to latex were selected for sebum content evaluation. Baseline measurements were done early in the morning before putting on gloves and where possible, follow up measurements were done after taking off the gloves, but before washing of hands. Increased follow up values were found for most facial regions, while the nose and hands showed a reduction in mean values.

Anatomical region	Baseline (n = 29)	Follow up (n = 21)	% Change	p-value
Forehead	126.79 ± 70.03	149.19 ± 77.10	17.7%	0.30
Left cheek	61.24 ± 61.73	64.24 ± 56.48	4.9%	0.86
Right cheek	62.34 ± 52.68	63.95 ± 55.82	1.7%	0.92
Nose	91.45 ± 69.22	79.71 ± 46.45	-12.8%	0.48
Left palm	5.62 ± 6.34	2.05 ± 2.09	-63.4%	<0.01
Left dorsum	3.52 ± 4.29	0.43 ± 0.75	-87.8%	<0.01
Right palm	7.24 ± 10.40	2.86 ± 5.34	-60.5%	0.06
Right dorsum	3.48 ± 5.30	0.76 ± 1.09	-78.2%	0.01

4.3. DISCUSSION

This was the second study done at TBH to investigate latex allergy among HCW^s. A preliminary study was done in 1999-2000 where a control group (administration personnel) was compared to an experimental group of HCW^s (De Beer, 2000). Occupational exposure was then confirmed as one of the major contributors in the development of latex allergy among HCW^s. Due to financial constraints it was decided not to include a control group in the current study. Subject selection was based on the findings of the preliminary study with regard to inclusion criteria. The current study was also characterised by a relatively large sample size compared to previous research conducted on the same study population (i.e. HCW^s at TBH).

No intervention took place to eliminate latex gloves and glove powder from the work place following the preliminary study. TBH did not change the glove purchasing policy and the same brand of gloves was still in use. Only individuals identified in the previous study with latex allergy were accommodated with latex-free gloves for personal use. No individual from the previous study was included in the current study and it could therefore be accepted that the level of exposure in the current Study Group was comparable to those of individuals from the preliminary study. All data collected was considered to be new and relevant.

The current study could not confirm any significant differences between ethnic group, gender or age for any of the parameters. However, the only valid comparison would be between Caucasians and Coloureds due to inadequate numbers of Black and Indian subjects. Although the literature suggests that a black skin has a more effective barrier than lighter skin (Berardesca, 1994; Okah *et al*, 1995; Benfeldt, 1999), this could not be confirmed or contradicted due to the small number of dark skinned individuals included in the Study Group. Similarly, the uneven distribution between male and female subjects in the Study Group made it impossible to draw definite conclusions with regard to the influence of gender in latex allergy.

The selection of individual symptoms was confirmed to be relevant in individuals with possible latex allergy, as demonstrated by the high incidences of reported work-related symptoms. The absence of these symptoms in the control group investigated previously (De Beer *et al*, 1999; De Beer, 2000; De Beer & Cilliers, 2001) ranged between

68% and 100% and hand eczema, conjunctivitis and pruritus displayed statistical significance when compared to the experimental group. More important was the statistical significance of all symptoms, except nasal congestion and rhinitis, when the latex allergic group was compared to the group with negative results. The frequency of the symptoms in the current study ranged between 5.3% and 73.7%. These symptoms could therefore be used to form the basis of a clinical profile in the diagnostic approach of a person with suspected latex allergy.

An Allergy Score was formulated by utilising the number and severity of symptoms and proved to be a very useful indicator when evaluating individuals with possible latex allergy. This numeric scoring system was also used retrospectively during the preliminary TBH study (De Beer, 2000) and was validated by the current study. It would be possible to use the Allergy Score during the screening process in an effort to select individuals for follow up and confirmatory laboratory investigations. Although it might not be foolproof, this method may be a cost-effective aid in the diagnosis of latex allergy. Further research is needed to establish and verify a "normal value" for the Allergy Score, where an increased value most probably would indicate a positive laboratory or skin test.

Other research used self-administered questionnaires to evaluate the relationship between subjective complaints or self-reported disease and the results of a medical examination. Most studies found definite relations between objective medical screening and the subjective or self-reported complaints from patients (Brasche *et al*, 2001). The subjective perception of subjects enrolled in the current study was assessed when they had to indicate on the Questionnaire if they were allergic to latex. Although reported clinical symptoms were not confirmed by medical examinations, it was accepted as present, because all subjects had some kind of medical education and background. A high association between subjective perception of latex allergy and confirmation by means of SPT^{s} or latex-specific IgE analysis was found.

A discrepancy has been found between lower reported figures for eczema, asthma, hay fever and an itchy skin as part of personal atopy, and higher incidences for eczema, asthma, rhinitis and pruritus as part of current symptoms. It is possible that subjects did not perceive these particular symptoms as atopy, but as allergic manifestations caused by glove usage. Most subjects presented with the symptoms only after they

encountered occupational exposure to latex gloves and glove powder. It is striking that the more severe manifestations of allergy (anaphylaxis and bronchospasm) were not associated with latex allergy by the subjects, whereas skin symptoms were.

The current study found a latex allergy prevalence of 15.1% and 22.4% with latex-specific IgE determination and SPT^{s} respectively. An overall prevalence of 37.5% was found for the Study Group. This figure is higher than the 20.8% overall prevalence found in a previous study done at this institution that included a control group. However, when the prevalence was calculated for the experimental group with high latex exposure only, a prevalence of 30.7% was found (De Beer, 2000). The current figure could also have been influenced by selection or participation bias, because recruitment was done in personnel with a high risk of latex allergy and inclusion of individuals was based on the presence of at least two pre-defined apparent latex-related symptoms. Work-related symptoms in 1.3-48.0% of cases indicated a relatively high level of discomfort in individuals exposed to latex gloves and glove powder. Reporting bias could be responsible for the high incidence of work-related symptoms, where local symptoms were not necessarily associated with sensitisation.

It was not possible to categorically differentiate between latex sensitivity obtained through occupational and surgical exposure. Much research had been done in children with SB correlating the number of surgical procedures with latex-specific IgE levels (Kelly *et al*, 1994b; Chen *et al*, 1997; Theissen *et al*, 1997; Hayes *et al*, 2000; Bernardini *et al*, 2003). However, the same association can not be made for HCW^{s}, because they are frequently exposed to other sources of latex. More and more of the individual latex allergens are found in both these risk groups and it is currently not possible to differentiate between latex sensitivity caused by internal (i.e. surgical) exposure compared to cutaneous and respiratory exposure (i.e. occupational exposure). The current study could not find a clear association between surgical procedures (before or after 1980) and latex allergy in the Study Group.

Due to financial constraints no testing was done for cross-reactive food reactions. However, clinical history suggested latex-fruit syndrome in three subjects, while single reports of clinical allergy were noted for foods known to cross-react with latex (avocado, banana, carrot, melon and paw-paw). All three subjects who reported latex-fruit syndrome were confirmed with positive latex-specific IgE and most of the single reports

also occurred either in subjects with positive latex-specific IgE or latex SPT[s]. Although these food allergies were not diagnostically confirmed, one can assume the existence of clinical cross-reactions in these subjects.

An allergen is regarded as a major allergen if it is responsible for > 20% of the allergenic reactivity in > 20% of the sensitised patients (Aalberse, 2000). Major allergens associated with mucosal exposure are *Hev b 1* (Raulf-Heimsoth *et al*, 1996; Poulos *et al*, 2002), *Hev b 3* (Lu *et al*, 1995; Sussman *et al*, 2002) and *Hev b 5*, while cutaneous and respiratory exposure more often lead to sensitivity to *Hev b 2* (Yip *et al*, 2000; Randolph, 2001; Sussman *et al*, 2002), *Hev b 4* (Sussman *et al*, 2002), *Hev b 5* (Slater *et al*, 1996; Posch *et al*, 1997; Kostyal *et al*, 1998; De Silva *et al*, 2000; Yip *et al*, 2000; Sussman *et al*, 2002), *Hev b 6* (Alenius *et al*, 1994; Slater & Trybul, 1994; Lu *et al*, 1995; Alenius *et al*, 1996; Turjanmaa *et al*, 1996) and *Hev b 7* (Poley & Slater, 2000; Yip *et al*, 2000; Randolph, 2001; Weissman & Lewis, 2002).

The following allergens were all confirmed to be major allergens present in more than 20% of samples in the current study: *Hev b 1* (100.0%), *Hev b 2* (32.4%), *Hev b* 4 (21.6%), *Hev b 5* (54.1%), *Hev b 6.01* (81.1%), *Hev b 13* (32.4%) and Hevamine (27.0%), respectively.

The most important latex allergen in this study was *Hev b 1* that showed 100% reactivity. Several studies have confirmed *Hev b 1* to be an airborne, powder-bound allergen and this finding correlated well with the route of exposure to latex in the subjects of the current study. Together with the finding of glove powder and latex proteins in the airconditioning system of the TBH complex, it can be concluded that one of the major routes of exposure to latex proteins in the current study population was airborne allergens carried on glove powder.

The presence of *Hev b 3* in subjects with a history of anaphylactic reactions is also in agreement with published results of its association with anaphylaxis (Lu *et al*, 1995). Overall, most findings were in keeping with published results where the main routes of exposure were cutaneous and respiratory. Contrary to published reports (Yip *et al*, 2000), the allergenicity of *Hev b 7* could not be confirmed in more than one specimen.

In the past, positive patch tests to NRL have been interpreted as either allergic or irritant contact dermatitis by different groups (Wakelin & White, 1999). Others have contributed

the results to additives, such as benzothiazoles, carbamates and thiurams (Tarlo *et al*, 1990; Toraason *et al*, 2000). Proper diagnosis may be difficult, because in some patients, type I and IV hypersensitivity may coexist. NRL can also provoke contact urticaria and present as protein contact dermatitis. Patch testing with glove pieces should be interpreted with caution, as ingredients of glove powder, e.g. epichlorhydrin and sorbic acid, may cause delayed type hypersensitivity (Warshaw, 1998).

A UK study found a statistically significant increase in the incidence of thiuram allergy in HCW^{s} with hand dermatitis between 1989 and 1995 (Gibbon *et al*, 2001). Cross-reactions are possible with N-isopropyl-N-phenyl-4-phenylenediamine (PPD) and carbamates. The current study identified a 25.0% (3/12) sensitivity to thiuram mix, but the incidence may not be clinically relevant due to the small number tested. Sensitisation in these three subjects most probably occurred through direct contact with gloves. The first subject with multiple positive patch test results worked as a nursing assistant in the TBH Faculty of Dentistry. Because she also reacted to a piece of latex glove and glove powder, it would be reasonably safe to conclude that her sensitivity was most probably caused by occupational exposure, cross-reactions and to a lesser extent, personal use of cosmetics. She reported severe and work-related hand eczema. Although she was not atopic and had a very low total serum IgE value (< 2 IU/ℓ), her Allergy Score was 24, indicating that she suffered from several of the symptoms associated with the use of latex.

The second subject who reacted to thiuram mix and parabens was an OR nurse with a total serum IgE of 363.00 IU/ℓ and an Allergy Score of 18. Her symptoms were both respiratory and cutaneous and she also reported severe and work-related hand eczema. Despite the slightly elevated total serum IgE, she was not atopic. In this case, cosmetics and hand eczema seemed to be the only causative agents for sensitisation.

A third subject reacted to thiuram mix and cobalt chloride. Although she had a personal and family history of atopy, her total serum IgE was only 20.0 IU/ℓ and her Allergy Score was 11. Her symptoms were mainly respiratory in nature and it was not possible to conclusively identify the cause of her sensitisation.

Latex proteins may attach themselves to the superficial film of sebum or sweat on the surface of the skin (Fisher, 1997). Sebum content was measured on the forehead, both

cheeks and nose, because of continuous exposure to airborne allergens via glove powder. While in the OR, theatre staff wore masks that shielded the nose and cheek areas from direct contact to airborne allergens, while the forehead stayed exposed. None of the subjects had visible or active acne and the baseline values obtained from the Study Group were comparable to normal volunteers.

Baseline sebum values obtained in the current study were comparable or slightly lower than those reported in the literature (Serup, 1991; Wilhelm *et al*, 1991; CK Electronic, 2001; Sator *et al*, 2003).

Sebaceous glands produce lipids that contribute to maintaining the hydration state and water retaining capacity of the skin (Gfatter *et al*, 1997; Boelsma *et al*, 2003). It has further been proven that removal of the SC causes a proportional decrease in the general hydration of the skin (Ozawa & Takahashi, 1994). Perspiration also decreases resistance of the skin by serving as an added parallel high resistance in the electrical circuit (Muramatsu *et al*, 1987). Lower skin sebum levels could therefore be associated with lower hydration of the SC.

The fairly common presence of hand eczema implies a decreased skin barrier function in the current subject group. This is possibly the reason why baseline sebum values on the dorsal areas of the hands were lower compared to published results. However, baseline values measured on the palms of subjects were higher than healthy volunteer groups from the literature. It is possible that hand eczema does not have the same impact on the palms as on the dorsal areas.

Follow up measurements were done to evaluate fluctuation in sebum values over a period of active exposure to gloves and glove powder. As was expected, the sebum values for the exposed forehead and both cheeks increased during occupational exposure. However, the mean value measured on the nose decreased over the same period of time. This phenomenon could possibly be attributed to the facemasks absorbing the surface sebum over time.

Follow up measurements of the hands were done after gloves were taken off, but before the subjects washed their hands. Sebum values should therefore reflect a true reflection of the sebum content and hydration state of the hands inside the gloves. It has been suggested that cornstarch powder inside gloves can cause dryness of the skin

and lead to a reduction in the barrier properties of the skin (Lodén, 1995; Pretorius & Bester, 2000). This trend was confirmed by the results of the current study that showed highly significantly ($p<0.01$) decreased follow up values for both palmar and dorsal areas of both hands. Palmar values declined by more than 60% and dorsal values by approximately 80%.

More meaningful discussion and conclusions with regard to sebum values in the current study is included in Chapter 5, where subgroups from theatre and laboratories will be compared.

CHAPTER 5:
SUBGROUP DATA

5.1. INTRODUCTION

HCW^s use latex gloves on a daily basis to prevent transmission of microbial and viral infections to and from patients. Latex proteins in most surgical gloves are readily extractable and are absorbed when in direct contact with the skin or mucous membranes of patients and HCW^s. Repeated exposure to these proteins causes sensitisation, the formation of circulating latex-specific IgE antibodies and clinical latex allergy.

Latex allergy in HCW^s manifests as type I immediate or type IV delayed type hypersensitivity. Direct skin contact usually leads to type IV contact dermatitis, where the reaction is largely limited to the site of direct contact, i.e. the hands. However, type I latex allergy can be triggered by cutaneous, percutaneous, mucosal and parenteral contact. Systemic anaphylactic reactions usually occur after mucosal or parenteral exposure (Cohen *et al*, 1998).

Atopy and frequent exposure are independent risk factors for the development of latex allergy, but a combination of the two increases the risk by up to nine to 19-fold (Warshaw, 1998). Atopic individuals often suffer from hand eczema secondary to occupational duties and use gloves for hand protection. Continuous exposure to latex gloves through a disrupted skin barrier is an important predisposing factor for the development of latex allergy.

IgE response to NRL varies considerably in different individuals (Alenius *et al*, 1994). A detailed history and thorough clinical examination are therefore essential for proper diagnosis. Risk factors and a history of immediate symptoms should guide the physician in the choice of diagnostic procedure. Negative results in the presence of persistent symptoms should be verified with other diagnostic methods. SPT^s remain the gold standard in diagnostic testing for latex allergy, but it is unwise in cases with a high risk of systemic reactions. SPT^s often identify latex allergic individuals without circulating latex-specific IgE antibodies, but these individuals usually develop latex-specific IgE antibodies if exposure to latex persists (Potter, 1998a; Potter, 1998b).

In this chapter clinical findings and results from the different subgroups of the Study Group will be reported and discussed in terms of demographic data, symptoms, special laboratory investigations and sebum content in an attempt to find clinically relevant discriminants between such groups.

5.2. METHODS AND RESULTS

5.2.1. Compilation of Subgroups

Latex-specific IgE was determined in all sera according to the Pharmacia CAP system™ (see section 2.3.4.). Where possible, subjects with negative latex-specific IgE results were followed up for latex SPT^s with Soluprick SQ® solution (see section 2.3.5.). The Study Group was divided into four subgroups according to serum latex-specific IgE and latex SPT results. Statistical comparison among the subgroups was done by using Chi-square statistics, Analysis of Variance, Kruskal-Wallis One-Way ANOVA on Rank, Mann-Whitney U Test and Turkey-Kramer Multiple-Comparison Test. Confidence intervals of 95% were used for all tests and p-values of less than 0.05 were considered statistically significant, while p-values of less than 0.01 were considered highly significant.

5.2.1.1. Group A

This subgroup included 23 consenting subjects (five males, 18 females) with positive (> 0.35 KU/ℓ) serum latex-specific IgE results. This subgroup will also be referred to as the *"Serum [+]"* group.

The mean age of 33.8 ± 6.9 (Me 33, range 20-51) years corresponded well (p=0.92) with the Study Group (mean 34.0 ± 7.7 years), but the proportional male to female ratio of 1 : 3.6 was slightly lower than the 1 : 4.4 found in the Study Group. The median of 33.0 years was also very similar to the 33.5 years of the Study Group.

The largest proportion of this subgroup consisted of Caucasian subjects (65.2%), followed by Coloured subjects (30.4%) and a single Indian (4.4%) subject. No African individuals showed positive latex-specific IgE results. Although not statistically significant, this subgroup contained marginally more laboratory (43.5%) than nursing staff (34.8%) than the entire Study Group.

5.2.1.2. Group B

This subgroup consisted of 34 consenting subjects (four males, 30 females) with negative (< 0.35 KU/ℓ) serum latex-specific IgE and positive latex SPT^s. This subgroup will also be referred to as the *"SPT [+]"* group.

The mean age (36.1 ± 7.8, Me 36, range 23-54 years) and proportional male to female ratio (1 : 7.4) were higher than the Study Group. A very similar distribution was found between Coloured (50.0%) and Caucasian (47.1%) subjects. Only one Indian and no African subject were included in this subgroup. The majority of subjects were nursing staff (55.9%).

For selected statistical comparisons, data from Group A and Group B was combined to represent all latex allergic individuals in the Study Group. It will be referred to as *Cohort AB*.

5.2.1.3. Group C

This subgroup consisted of 25 subjects (five males, 20 females) with negative serum latex-specific IgE and negative latex SPT^s. This subgroup will also be referred to as the *"Negative"* group.

The mean age (35.8 ± 8.4, Me 35, range 22-59 years) was only slightly higher than the Study Group, but the proportional male to female ratio of 1 : 4.0 corresponded well. Caucasian (52.0%) and Coloured (48.0%) subjects were the only ethnic groups included in this subgroup. The vast majority of subjects were nursing staff (64.0%).

5.2.1.4. Group D

The remaining 70 subjects from the Study Group were included in this subgroup. They all had negative serum latex-specific IgE, but were lost to follow up and no further procedures were done. A conclusive diagnosis could not be made in this subgroup, because negative serum latex-specific IgE results could not be followed up with latex SPT^s. The relevance of statistical differences from other groups may therefore be questionable and will not be reported. This subgroup will also be referred to as the *"Withdrawn"* group.

The mean age (32.4 ± 7.4, Me 31, range 19-57 years) was slightly lower than the Study Group, but the proportional male to female ratio (1 : 4.0) corresponded well. The ethnic distribution corresponded very well with the Study Group and was represented by Caucasian (55.7%), Coloured (40.0%), African (2.9%) and Indian (1.4%) subjects. The majority was laboratory staff (45.7%), followed by nursing staff (25.7%) and doctors (17.1%).

5.2.1.5. Statistical Analysis

The occupational distribution within Group A showed a highly significant difference from Group B ($p<0.01$) and Group C ($p<0.01$). Significantly more laboratory than nursing staff was included in Group A. Group B and Group C showed similar distribution of slightly more nursing than laboratory staff, but did not differ significantly from each other. However, when compared to Cohort AB, Group C had significantly more nursing staff than laboratory staff ($p<0.01$). Comparative descriptive statistics for demographic data of the individual subgroups are summarised in ***Table 5.1***.

5.2.2. Numerical Indices

5.2.2.1. Class

For statistical comparison, subjects were assigned to different Classes according to their personal suspicion of latex allergy. Class 0 included all subjects with a negative suspicion of latex allergy, Class 1 indicated a positive suspicion and all those who were not sure, were assigned to Class 2. The Study Group comprised 59 subjects in Class 1 (38.8%), twelve in Class 0 (7.9%) and 81 in Class 2 (53.3%), respectively.

Group A: Of the 23 subjects confirmed with latex allergy by means of positive serum latex-specific IgE results, 13 (56.5%) subjects were assigned to Class 1, a single (4.4%) to Class 0 and nine (39.1%) to Class 2, respectively.

Group B: The 34 subjects confirmed with latex allergy by means of positive SPT^s, included 16 (47.1%) Class 1, a single (2.9%) Class 0 and 17 (50.0%) Class 2 subjects.

Table 5.1: Demographic data per subgroup

All demographic parameters of the individual subgroups corresponded well with the Study Group and no statistically significant differences were found between the subgroups, or when compared to the Study Group.

	Group A Serum [+]	Group B SPT [+]	Group C Negative	Group D Withdrawn
n	23	34	25	70
M : F	5 : 18	4 : 30	5 : 20	14 : 56
Age (years):				
Mean	33.8	36.1	35.8	32.4
SD	6.9	7.8	8.4	7.4
Range	31.0	31.0	37.0	38.0
Median	33.0	36.0	35.0	31.0
Ethnic Distribution:				
African	0	0	0	2
Caucasian	15	16	13	39
Coloured	7	17	12	28
Indian	1	1	0	1
Occupation:				
Audiology	1	0	0	0
Dentistry	0	4	2	5
Doctors	4	3	4	12
Laboratory staff	10	6	3	32
Nursing Staff	8	19	16	18
Radiography	0	1	0	1
Scientists	0	1	0	2

To calculate the PPV for patient suspicion of latex allergy (Class 1), all subjects who were unsure (Class 2) or had a negative suspicion of latex allergy (Class 0), were regarded as negative. The PPV of Class 1 within Group A (Serum [+]) was 22.0% and in Group B (SPT /) 27.1%, respectively. The PPV to confirm latex allergy by either one of the methods (Cohort AB) was 49.2%.

Group C: Twenty-five subjects could not be confirmed with latex allergy and were regarded as negative. In this subgroup four (16.0%) subjects were assigned to Class 1, two (8.0%) to Class 0 and 19 (76.0%) to Class 2, respectively.

Group D: This subgroup consisted of 26 (37.1%) subjects in Class 1, eight (11.4%) in Class 0 and 36 (51.4%) in Class 2, respectively. No further statistical analysis was possible in this subgroup.

Statistical analysis: The distribution of Class within Group A was significantly different from the Study Group ($p=0.03$) and Group C ($p<0.01$), respectively. In comparison, significantly more subjects in Group A were assigned to Class 1. Group B showed a very equal distribution between Class 1 and 2 and did not differ significantly from any other subgroup. Group C was highly significantly different from Group A ($p<0.01$), Group B ($p<0.01$) and Cohort AB ($p<0.01$), respectively, with significantly more subjects assigned to Class 2.

5.2.2.2. Allergy Score

Subjects scored the presence and severity of their symptoms on the Questionnaire and indicated if they experienced any work-related deterioration ***(Table 5.2)***. An Allergy Score was calculated for each individual according to the number and severity of their symptoms.

By applying the following formula, a mean Allergy Score for the Study Group of 11.4 ± 6.3 (Me 10.5, range 1-29) was calculated:

Allergy Score = (1 x total mild) + (2 x total moderate) + (3 x total severe)

Group A: The mean Allergy Score was 17.3 ± 5.8 (Me 18, range 2-29). The 25% and 75% quartile values were 14.5 and 19.0, respectively. Comparison of the mean Allergy Scores for the different subgroups is presented as ***Figure 5.1***.

Table 5.2: Frequency of symptoms per subgroup

The list of symptoms were selected after extensive review of the literature and represent all three major routes of exposure to latex, i.e. respiratory, systemic and cutaneous contact. An overall trend was found that the occurrences of symptoms in Group A > Group B > Group C.

	Group A Serum [+]		Group B SPT [+]		Group C Negative		Group D Withdrawn	
	n	%	n	%	n	%	n	%
	(n=23)		(n=34)		(n=25)		(n=70)	
Conjunctivitis	20	87.0	18	52.9	12	48.0	41	58.6
Work-related	17	73.9	9	26.5	6	24.0	20	28.6
Nasal congestion	20	87.0	21	61.8	17	68.0	53	75.7
Work-related	12	60.0	12	35.3	11	44.0	23	32.9
Rhinitis	21	91.3	23	67.7	13	52.0	48	68.6
Work-related	11	47.8	10	29.4	9	36.0	18	25.7
Shortness of breath	10	43.5	12	35.3	10	40.0	17	24.3
Work-related	3	13.0	5	14.7	6	24.0	3	4.3
Bronchospasm	11	47.8	13	56.5	10	40.0	21	30.0
Work-related	6	26.1	5	14.7	5	20.0	6	8.6
Urticaria	12	60.0	5	14.7	3	12.0	19	27.1
Work-related	9	39.1	4	11.8	2	8.0	3	4.3
Angioedema	11	47.8	5	14.7	3	12.0	11	15.7
Work-related	7	30.4	3	8.8	3	12.0	1	1.4
Anaphylaxis	4	17.4	1	2.9	0	0.0	3	4.3
Work-related	1	4.4	1	2.9	0	0.0	0	0.0
Pruritus	20	87.0	26	76.5	18	72.0	48	68.6
Work-related	14	60.9	16	47.1	15	60.0	28	40.0
Skin rash	21	91.3	22	64.7	16	64.0	47	67.1
Work-related	15	65.2	15	44.1	12	48.0	26	37.1
Hand eczema	16	69.6	19	55.9	13	52.0	32	45.7
Work-related	12	60.0	12	35.3	12	48.0	18	25.7

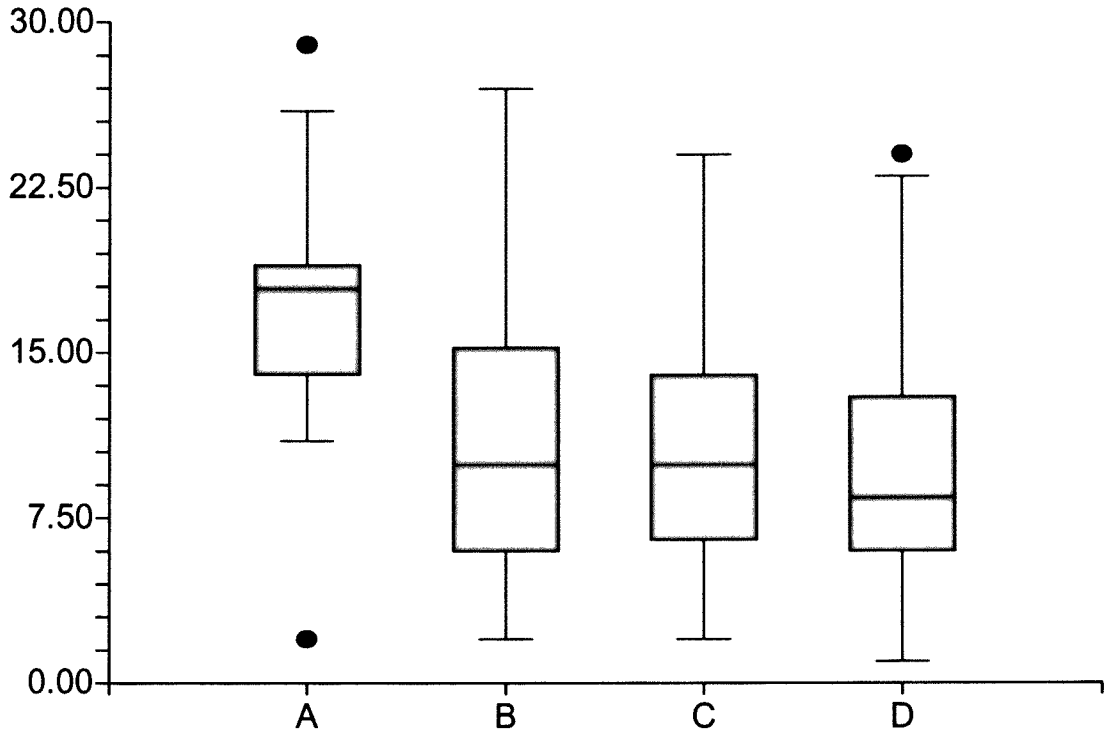


Figure 5.1: Box and whisker plot for the Allergy Scores per subgroup

[Allergy Score = (1 x mild) + (2 x moderate) + (3 x severe)]

Only three outliers occurred in the different subgroups. In Group A (Serum [+]) the minimum and maximum values were outliers, while in Group D (Withdrawn) the only outlier was the maximum value. The difference in the Allergy Score of Group A compared to the other subgroups was highly significant ($p<0.01$).

Group B: The mean Allergy Score was 11.1 ± 6.2 (Me 10, range 2-27). The 25% and 75% quartile values were 6.0 and 15.0, respectively. All these values were slightly lower than the Study Group, but not statistically significant.

Group C: The mean Allergy Score was 10.9 ± 6.0 (Me 10, range 2-24). The 25% and 75% quartile values were 5.0 and 13.0, respectively. All these values were lower than the Study Group, but not statistically significant.

Group D: The mean Allergy Score was 9.9 ± 5.7 (Me 8.5, range 1-24). The 25% and 75% quartile values were 6.0 and 13.0, respectively. The mean and median values were markedly lower than the Study Group.

Statistical analysis: The Allergy Score from Group A was significantly higher than the Study Group ($p<0.01$), Group B ($p<0.01$) and Group C ($p<0.01$), respectively. The Allergy Score from Cohort AB was also significantly ($p=0.03$) higher that that of Group C.

Multiple regression, combined reports and Chi-Square statistics found the following symptoms to be statistically significant in subjects with latex allergy (Cohort AB):

- Conjunctivitis ($p=0.03$)
- Work-related Conjunctivitis ($p<0.01$)
- Rhinitis ($p=0.03$)
- Work-related Shortness of Breath ($p=0.05$)
- Urticaria ($p<0.01$)
- Work-related Urticaria ($p<0.01$)
- Angioedema ($p<0.01$)
- Work-related Angioedema ($p<0.01$)
- Anaphylaxis ($p=0.03$)

Similarly, multiple regression identified the following statistically significant individual symptoms within Group A:

- Severe Conjunctivitis ($p=0.01$)
- Moderate Rhinitis ($p=0.03$)
- Severe Urticaria ($p<0.01$)
- Severe Skin Rash ($p=0.03$)

When comparing Cohort AB (all positive subjects) to Group C (all negative subjects), the following PPV^S were found:

➢ Conjunctivitis	76.0%
Work-related conjunctivitis	81.3%
➢ Nasal Congestion	70.7%
Work-related nasal congestion	68.6%
➢ Rhinitis	77.2%
Work-related rhinitis	70.0%
➢ Shortness of Breath	71.0%
Work-related shortness of breath	57.1%
➢ Bronchospasm	72.7%
Work-related bronchospasm	68.8%
➢ Urticaria	85.0%
Work-related urticaria	86.7%
➢ Angioedema	88.9%
Work-related angioedema	83.3%
➢ Anaphylaxis	100.0%
Work-related anaphylaxis	100.0%
➢ Pruritus	73.0%
Work-related pruritus	66.7%
➢ Skin Rash	74.1%
Work-related skin rash	71.4%
➢ Hand Eczema	74.5%
Work-related hand eczema	68.6%

These symptoms are valuable in cases of suspected latex allergy, but should not be evaluated in isolation. PPV^s of >75.0% for symptoms and >80% for work-related symptoms confirmed the statistical significance found within Cohort AB (*vide supra*).

5.2.3. Exposure

Exposure in HCW^s occurs occupationally by wearing latex gloves on a daily basis or by surgical procedures. Occupational exposure was recorded as total number of years exposed to latex gloves, number of hours and pairs per week ***(Table 5.3)***.

Table 5.3: Occupational and surgical exposure per subgroup

Individuals can be sensitised through occupational and surgical exposure to latex gloves. The role of occupational exposure in the development of latex allergy in HCW[s] has been confirmed statistically (De Beer, 2000). Surgical exposure leads to high sensitisation rates in individuals with multiple surgical procedures, e.g. children with spina bifida. The total number of surgical procedures in this study was divided into the number before and after 1980 to coincide with Nutter's landmark latex allergy case report (1979).

		Group A Serum [+]	Group B SPT [+]	Group C Negative	Group D Withdrawn
		(n=23)	(n=34)	(n=25)	(n=70)
Occupational exposure to latex gloves:					
Number of years:	Mean	9.6 ± 5.8	10.9 ± 7.4	11.4 ± 8.5	9.7 ± 6.3
	Median	10	10.5	12	9
	Range	0.5 – 21	1 - 25	0.2 - 30	0.7 - 20
Hours per week:	Mean	22.6 ± 15.6	27.8 ± 14.7	29.5 ± 13.8	25.1 ± 14.7
	Median	25	38.8	40	30
	Range	1 – 40	1 – 40	6 – 40	5 - 80
Pairs per week:	Mean	19.4 ± 17.0	19.9 ± 12.4	23.9 ± 22.8	20.6 ± 16.6
	Median	15	20	20	15
	Range	1 – 80	1 – 50	3 – 100	3 - 100
Number of surgical procedures:					
< 1980		18	10	6	33
	Mean	0.8 ± 1.8	0.3 ± 0.5	0.2 ± 0.5	0.5 ± 0.9
	Median	0	0	0	0
	Range	0 – 8	0 – 1	0 – 2	0 - 5
> 1980		76	69	35	129
	Mean	3.3 ± 4.1	2.0 ± 2.2	1.4 ± 1.4	1.8 ± 1.8
	Median	3	1	1	2
	Range	0 – 19	0 – 10	0 – 4	0 - 9
Side effects		5	11	2	19

5.2.3.1. Occupational Exposure

Occupational exposure in the Study Group reached means of 10.2 ± 6.9 (Me 10, range 0.2-30.0) years of exposure, 26.0 ± 14.7 (Me 30, range 1-80) hours per week and 20.8 ± 16.9 (Me 15, range 1-100) pairs of gloves per week, respectively.

Group A: The mean values for all three variables were slightly higher than the Study Group. The median values for the number of years and pairs per week were the same as the Study Group, but lower for hours per week.

Group B: The mean values for number of years and hours per week were slightly higher than the Study Group, while the pairs per week were lower. The median values for all three categories were higher than the Study Group and Group A.

Group C: The mean and median values were higher than the Study Group, Group A and Group B.

Group D: The mean values were slightly lower than that of the Study Group, Group A, Group B and Group C. Median values were comparable to Group A.

Statistical analysis: No statistical differences for any of the variables could be demonstrated among any individual subgroups or when compared to the Study Group. However, within the individual subgroups, the following paired statistics produced statistical significance:

- Group A, Allergy Score & Number of Years ($p<0.01$)
- Group B, Allergy Score & Hours per Week ($p<0.01$)
- Group B, Allergy Score & Pairs per Week ($p<0.01$)
- Cohort AB, Allergy Score & Number of Years ($p=0.01$)
- Cohort AB, Allergy Score & Hours per Week ($p<0.01$)
- Cohort AB, Allergy Score & Pairs per Week ($p=0.01$)

5.2.3.2. Surgical Exposure

Before 1980, 43 subjects in the Study Group reported a total of 67 (mean 0.4 ± 1.0, Me 0, range 0-8) surgical procedures. A total of 309 procedures (mean 2.0 ± 2.3, Me 1, range 0-19) were reported by 112 subjects after 1980.

Group A: Seven (30.4%) subjects reported a total of 18 surgical procedures before 1980 and 20 (87.0%) subjects listed a total of 76 procedures after 1980. Mean values for both periods were higher than the Study Group. The subjects who reported the most procedures before (n = 8) and after (n = 19) 1980 in the Study Group, were both in this subgroup. Post-surgery side-effects of note included anaphylaxis in three (13.0%) subjects and single occurrences (4.4%) for asthma and nosebleeds.

Group B: Ten (29.4%) subjects reported ten surgical procedures before 1980, while a total of 69 procedures were listed by 25 (73.5%) subjects after 1980. The mean value before 1980 was lower than the Study Group, while the mean value after 1980 was the same with a slightly lower SD. Post-surgery side-effects in this group included nausea in seven (20.6%) subjects, skin manifestations in two (5.9%) and hypotension in a single (2.9%) subject.

Group C: Five (20.0%) subjects reported six surgical procedures before 1980, while 35 procedures were listed by 16 (64.0%) subjects after 1980. The mean values for all three variables were slightly lower than the Study Group. The median value for the number of years was slightly lower than the Study Group, while the hours and pairs per week were the same as the Study Group. Nausea was the only post-surgery side-effect reported in two (8.0%) subjects.

Group D: Twenty-one (30.0%) subjects reported a total of 33 surgical procedures before 1980, while 129 procedures were listed by 51 (72.9%) subjects after 1980. Side-effects of note from anaesthesia included nausea in seven (10.0%), skin manifestations in four (5.7%) and hypotension in three (4.3%) subjects, respectively.

Statistical analysis: No statistical differences could be demonstrated among the subgroups for the period before 1980. However, Group A underwent significantly more procedures after 1980 than Group C (p=0.04). Cohort AB also showed similar statistical significance for this period when compared to Group C (p=0.03).

5.2.4. Atopy

Personal atopy was present in 75.6% of the Study Group, while family history of atopy was reported by 53.3%. A combination of personal and family history of atopy was present in 46.1% of subjects, and 82.9% had either a personal or family history of atopy.

The distribution of atopic diseases and specific allergies per subgroup is summarised in ***Table 5.4***. The frequency of atopic diseases per subgroup is presented as ***Figure 5.2***.

Group A: Only two (8.7%) subjects did not report any personal or family history of atopy. In twelve (52.2%) subjects both were present, while only the remaining nine (39.1%) subjects reported personal atopy only. In six (26.1%) subjects all four atopy criteria were present, while a single (4.4%) subject presented with three criteria. Six (26.1%) and eight (34.8%) subjects reported two and one of the criteria, respectively.

Drug allergies comprised two for tetracyclines and one each for penicillin and aspirin. All three subjects in the Study Group who reported allergy to diclofenac sodium and confirmed latex-fruit syndrome were in this group. Only one subject reported fish allergy. House dust mite, grass and cats were the most prominent other allergies in four subjects each, followed by dogs in three and pollen in one subject.

Group B: In this group eight (23.5%) subjects had no personal or family history of atopy. Fourteen (41.2%) subjects had both, and personal or family history only was reported by eight (11.8%) and four (11.8%), respectively. A single (2.9%) subject reported all four atopy criteria, while two (5.9%) subjects presented with three criteria. Nine (26.5%) and ten (29.4%) subjects reported two and one of the criteria, respectively.

Drug allergies included penicillin and amoxycillin in two subjects each and only one aspirin allergy. Two subjects each reported allergies to fish and nuts. Allergy to cats was present in three subjects, and grass and pollen in two each.

Table 5.4: Atopic diseases and other allergies per subgroup

Atopy was characterised as eczema, asthma, hay fever and an itchy skin. Personal atopy was recorded in high percentages in all individual subgroups. Family history of atopy was present in approximately 50% of the subgroups. The highest incidence of a combination of personal and family history of atopy was found in Group A (Serum [+]).

	Group A Serum [+]		Group B SPT [+]		Group C Negative		Group D Withdrawn	
	n	%	n	%	n	%	n	%
	(n=23)		(n=34)		(n=25)		(n=70)	
Personal Atopy	21	91.3	22	64.7	18	72.0	54	77.1
Eczema	11	47.8	8	23.5	6	24.0	21	30.0
Asthma	7	30.4	4	11.8	7	28.0	12	17.1
Hay fever	17	60.9	17	50.0	12	48.0	38	54.3
Itchy skin	12	52.2	9	26.5	5	20.0	22	31.4
Family History of atopy	12	52.2	18	52.9	13	52.0	38	54.3
Either Personal or Family history of atopy	21	91.3	26	76.5	20	80.0	59	84.3
Both Personal and Family history of atopy	12	52.2	14	41.2	11	44.0	33	47.1
Other Allergies:	16	69.6	17	50.0	5	20.0	25	35.7
Drugs	8	34.8	11	32.4	1	4.0	12	17.1
Food	6	26.1	6	17.7	3	12.0	8	11.4
Other	10	43.5	10	29.4	2	8.0	17	24.3

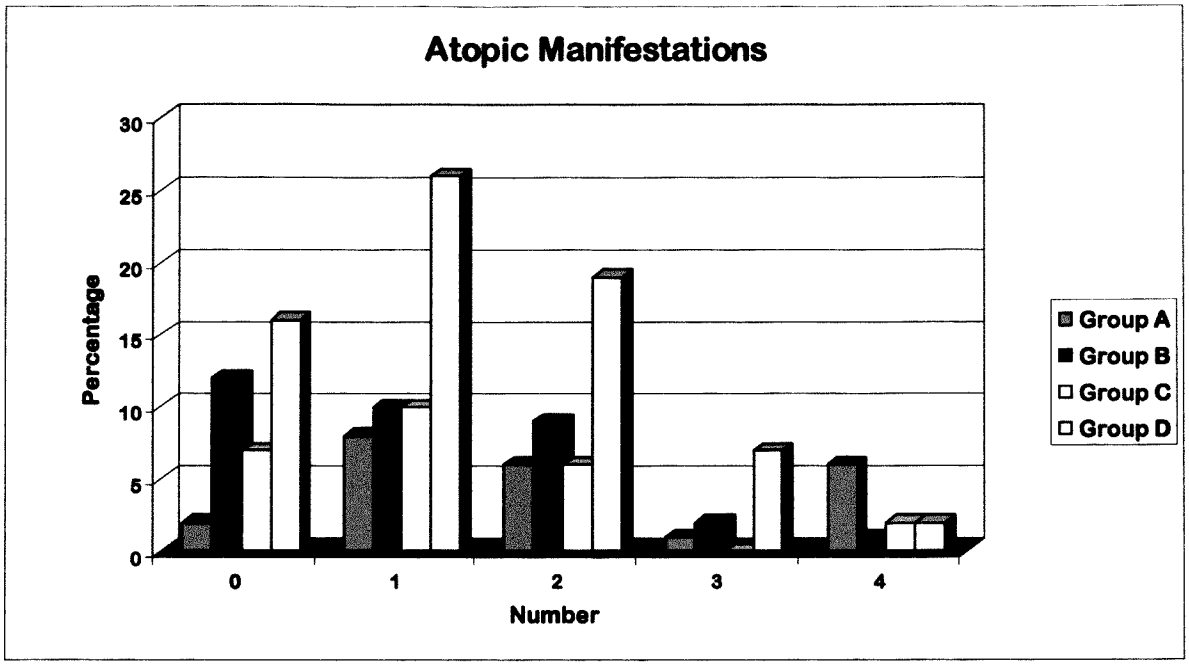


Figure 5.2: Frequency of atopic diseases per subgroup

Group A (Serum [+]) differed significantly from the Study Group ($p<0.05$), Group B (SPT [+]) ($p<0.05$) and Group D (Withdrawn) ($p<0.01$) with regard to the number of atopic diseases reported by the subjects. No significant difference could be demonstrated between Group A and Group D (Withdrawn) or between any of the other subgroups.

Group C: Seven (28.0%) subjects in this group had no personal or family history of atopy. Both personal and family history of atopy was present in eleven (44.0%) subjects. Seven (28.0%) and two (8.0%) subjects, respectively, reported either personal or family history. Two (8.0%) subjects reported all four atopy criteria, while there was none that reported three symptoms. Six (24.0%) and ten (40.0%) subjects reported two and one of the criteria, respectively.

The only drug allergy of note was a single subject who reported allergy to sulphonamides. Two subjects reported dairy allergy and one fish allergy. Allergy to house dust mite and bee sting was also present in one subject each.

Group D: Eleven (15.7%) subjects in this group had no personal or family history of atopy, 34 (48.6%) had both and 21 (30.0%) and four (5.7%), respectively, reported either personal or family history. Two (2.9%) subjects reported all four atopy criteria, while seven (10.0%) reported three symptoms. Twenty (28.6%) and 26 (37.1%) subjects reported two and one of the criteria, respectively.

Five penicillin allergies were reported, followed by four sulphonamides and one each for aspirin, amoxycillin and tetracyclines. Fish allergy was the most prominent food allergy in two subjects, followed by dairy and nuts allergy in one subject each. Allergies to house dust mite and pollen were present in three subjects each, followed by grass and cats in two each and dogs in a single subject each. The only subject who reported condom allergy was in this group.

Statistical analysis: The presence of any of the atopic diseases in Group A was significantly more than Group B ($p=0.02$), but only markedly more than the Study Group ($p=0.09$) and Group C ($p=0.09$), respectively. No statistical differences could be demonstrated for Group B. No other statistical differences could be confirmed in any of the other groups or when compared to the Study Group.

When the number of atopic diseases was compared between the groups, Group A showed significantly more than the Study Group ($p=0.05$), Group B ($p=0.04$) and Group D ($p<0.01$), respectively. The other groups did not differ from each other or when compared to the Study Group. Cohort AB was also not statistically different from Group C, Group D or the Study Group.

5.2.5. Total IgE

Total IgE levels were determined on 152 serum and 31 dermal fluid specimens. Mean values for the Study Group were 146.89 ± 268.74 (Me 46.90, range 1.00-1 817.00) IU/ℓ for total serum IgE and 55.12 ± 107.46 (Me 12.18, range 1.00-431.00) IU/ℓ for total dermal fluid IgE. Comparative statistics for the subgroups are summarised in ***Table 5.5***.

Group A: The mean serum IgE value was 211.29 ± 285.19 (Me 102.00, range 17.20-1 093.50) IU/ℓ. The 25% and 75% quartile values were 50.64 IU/ℓ and 219.75 IU/ℓ, respectively. The high standard deviation and mean values, which were markedly higher than the median, indicated a very flat curve and skew distribution. Values within the 25-50% interval (50.65-102.00 IU/ℓ) were grouped closer together than those in the 50-75% interval (102.00-219.75 IU/ℓ). However, the mean was only slightly lower than the 75% quartile, indicating extremely high and widely distributed values in the upper 25% interval (219.75-1 093.00 IU/ℓ).

The mean dermal fluid IgE value was 68.52 ± 121.34 (Me 23.83, range 1.00-431.00) IU/ℓ. The 25% and 75% quartile values were 10.24 IU/ℓ and 52.80 IU/ℓ, respectively. These values also showed a very skew distribution, with the mean almost half the SD and more than twice the median. The lower 25-75% of values (10.24-52.80 IU/ℓ) were grouped closely together, while the upper 25% (52.80-431.00 IU/ℓ) showed a very wide distribution. In this instance, the mean was also higher than the 75% quartile value, indicating extremely high values in the upper 25% interval.

Group B: The mean serum IgE value was 134.74 ± 265.20 (Me 39.84, range 4.55-1 366.50) IU/ℓ. The 25% and 75% quartile values were 16.83 IU/ℓ and 107.70 IU/ℓ, respectively. This group also displayed very skew distribution, with the mean value half of the SD and almost three times higher than the median. The mean was also higher than the 75% quartile value, indicating extremely high values in the upper 25% interval (107.70-1 366.50 IU/ℓ). Values were grouped closely together within the 25-50% interval (16.83-39.84 IU/ℓ).

Table 5.5: Total IgE and latex-specific IgE values per subgroup

Group A (Serum [+]) showed higher values for all variables compared to the other subgroups. In all cases the SD values were much higher than the means, resulting in flat curves and wide distribution of values. In most cases the extremely high maximum values resulted in mean values, which were markedly higher than the median values. Group A differed significantly from Groups B (SPT [+]), Group C (Negative) and Group D (Withdrawn).

	Group A Serum [+]	**Group B SPT [+]**	**Group C Negative**	**Group D Withdrawn**
Serum:				
n	23	34	25	70
Total IgE (IU/ℓ)				
Mean	211.29	134.74	151.00	130.24
SD	285.19	265.20	268.73	267.55
Median	102.00	39.84	34.50	38.00
Range	17.20-1 093.50	4.55-1 366.50	1.00-1 210.00	2.99-1 817.00
Latex-specific IgE (IU/ℓ)				
Mean	2.39	< 0.35	< 0.35	< 0.35
SD	2.76	N/A	N/A	N/A
Median	1.16	N/A	N/A	N/A
Range	0.40 – 10.35	N/A	N/A	N/A
Dermal Fluid:				
n	13	12	5	1
Total IgE (IU/ℓ)				
Mean	68.52	29.87	91.04	17.55
SD	121.34	35.84	187.85	N/A
Median	23.83	11.88	8.52	N/A
Range	1.00-431.00	1.00-109.00	1.00-427.00	N/A
Latex-specific IgE (IU/ℓ)				
Mean	1.42	< 0.35	< 0.35	< 0.35
SD	1.38	N/A	N/A	N/A
Median	0.83	N/A	N/A	N/A
Range	0.45 – 4.00	N/A	N/A	N/A

The mean dermal fluid IgE value was 29.87 ± 35.84 (Me 11.88, range 1.00-109.00) IU/ℓ. The 25% and 75% quartile values were 8.02 IU/ℓ and 40.96 IU/ℓ, respectively. The mean and SD were closer together, indicating a smaller distribution and higher curve, but the median was only about a third of the mean. Values in the 25-50% interval (8.02-11.88 IU/ℓ) were grouped closely together, while the upper 25% interval (40.96-109.00 IU/ℓ) again showed a wider distribution.

Group C: The mean serum IgE value was 151.00 ± 268.73 (Me 34.50, range 1.00-1 210.00) IU/ℓ. The 25% and 75% quartile values were 9.79 IU/ℓ and 144.75 IU/ℓ, respectively. The mean and SD values were also closer together than Group A, but the mean was higher than the 75% quartile and more than four times the median. Values were grouped closely together within the 25-75% interval (9.79-144.75 IU/ℓ), while the upper 25% interval (144.75-1 2120.00 IU/ℓ) again showed extremely high values with a very wide distribution.

The mean dermal fluid IgE value was 91.04 ± 187.85 (Me 8.52, range 1.00-427.00) IU/ℓ. The 25% and 75% quartile values were 7.08 IU/ℓ and 11.60 IU/ℓ, respectively. Only five values were available in this subgroup, questioning the validity of statistical analysis of these figures. However, four of the values were normally distributed below the 75% interval (< 11.60 IU/ℓ). The maximum value was extremely high and caused the mean and SD to be very high compared to the other values.

Group D: The mean serum IgE value was 130.24 ± 267.55 (Me 38.00, range 2.99-1 817.00) IU/ℓ. The 25% and 75% quartile values were 17.48 IU/ℓ and 125.35 IU/ℓ, respectively. This subgroup also showed a mean of only half the SD and a median of about a third of the mean. The mean was also higher than the 75% quartile, once again indicating a very wide and skew distribution. The majority of the values were grouped closely together within the 25-50% interval (17.48-38.00 IU/ℓ), while the upper 75% interval contained widely distributed and extremely high values.

Only one dermal fluid was analysed in this group and the results can therefore not be used for statistical analysis.

Statistical analysis: All the subgroups showed a very flat curve and wide distribution for total IgE in both serum and dermal fluid. There was an overall tendency of closely grouped values in the 25-50% interval. The maximum values in all subgroups resulted in high mean and even higher SD values. The total serum IgE values and total dermal fluid IgE values showed no statistical differences among the subgroups.

The Allergy Score correlated highly significantly with total serum IgE levels in all subgroups ($p<0.01$). Paired statistical significance was also confirmed between serum and dermal fluid total IgE in Group A ($p=0.04$), as well as in Group B ($p=0.03$). This was not the case for Group C and calculation was not possible for Group D, because there was only one dermal fluid value.

5.2.6. Latex-specific IgE

Latex-specific IgE levels were determined on 152 serum and 31 dermal fluid specimens, respectively. Only Group A had measurable serum and dermal fluid latex-specific IgE levels and the statistics were therefore identical to the Study Group.

The mean serum latex-specific IgE value was 2.39 ± 2.76 (Me 1.16, range < 0.35-10.35) IU/ℓ. The 25% and 75% quartile values were 0.65 IU/ℓ and 2.83 IU/ℓ, respectively. Although the median was only half of the mean, the values were very evenly distributed with six lower than the 25% quartile and six higher than the 75% quartile.

The mean dermal fluid latex-specific IgE value was 1.42 ± 1.38 (Me 0.83, range < 0.35-4.00) IU/ℓ. The 25% and 75% quartile values were 0.50 IU/ℓ and 1.71 IU/ℓ, respectively. Seven samples yielded negative results (< 0.35 IU/ℓ), two were below the 25% quartile, two were within the 25-75% interval and two were higher than the 75% quartile, respectively.

Statistical analysis: No correlation ($p=0.25$) was evident between serum and dermal fluid latex-specific IgE values within Group A.

5.2.7. Sebum Content

Different healthcare professions experience different levels of exposure to latex gloves and glove powder. Laboratory staff usually work in open laboratories where a free flow of air is present in the laboratories and passages. These individuals also tend to move around, work in different areas and share working space with only a limited number of other individuals. It seems therefore that laboratory staff are exposed to less concentrated loads of airborne latex allergens compared to theatre staff. The latter group spend several hours per day in an enclosed environment, usually around or in close contact with quite a number of other individuals during surgical procedures. These HCW^{s} stay stationary for most of the time and all doors and windows are closed for the duration of the surgical procedure. It has been shown that active glove use generates airborne allergens (Charous *et al*, 2000) and that one of the highest concentrations measured was found in the personal breathing zone of an anaesthesiologist (Hamilton & Brown, 2000; Hunt *et al*, 2002). It could be assumed that a similar exposure load also applies to surgeons and nursing staff in a surgical team. The hydration state of the hand regions should also theoretically differ due to different glove routines adhered to by laboratory and theatre staff. Theatre staff don their gloves to wet hands after a thorough scrubbing routine, whereas laboratory staff generally put on their gloves on dry hands without prior scrubbing or washing.

Two different subgroups were therefore identified and investigated to compare their sebum content before and after occupational exposure. Three males and 13 females were included in the Laboratory Subgroup. Their mean age was 34.4 ± 10.2 (Me 35, range 21-52) years and the group included nine Caucasian, four Coloured and three Black individuals. The Theatre Subgroup consisted of one male and twelve females. Their mean age was 33.3 ± 7.0 (Me 32, range 25-49) years and the group included three Caucasian and ten Coloured individuals. No statistical differences could be demonstrated between the subgroups for any of the demographic parameters.

Values obtained at baseline and follow up (three to four hours later) are summarised in ***Table 5.6***. For each of the individual anatomical regions, the difference between the baseline and follow up values was calculated as a percentage change. Comparison was also done to evaluate possible statistical significance between the values.

Table 5.6: Comparison between baseline and follow up sebum values

Follow up sebum measurement was done in 21 subjects three to four hours after baseline measurements. Mean baseline values were compared to follow up values for each anatomical area within the individual subgroups. The percentage change in the values of each parameter was also calculated. The Laboratory Subgroup showed increased values for most facial regions, while the nose and hands showed a decline. Except for the left cheek, the Theatre Subgroup showed decreased values for all other parameters. See also Figure 5.3.

Anatomical region	Baseline	Follow up	% Change	p-value
Laboratory Subgroup	**(n = 16)**	**(n = 11)**		
Forehead	108.00 ± 72.84	153.18 ± 78.48	41.83%	0.10
Left cheek	52.31 ± 54.54	56.09 ± 35.28	7.23%	0.82
Right cheek	48.31 ± 43.12	57.09 ± 41.88	18.17%	0.56
Nose	80.00 ± 75.63	62.00 ± 47.72	-22.50%	0.43
Left palm	5.88 ± 5.95	1.36 ± 1.75	-76.87%	<0.01
Left dorsum	3.31 ± 5.30	0.36 ± 0.50	-89.12%	0.04
Right palm	4.19 ± 4.43	1.27 ± 1.35	-69.69%	0.02
Right dorsum	2.56 ± 2.58	0.45 ± 0.69	-82.42%	<0.01
Theatre Subgroup	**(n = 13)**	**(n = 10)**		
Forehead	149.92 ± 61.33	144.80 ± 79.53	-3.42%	0.86
Left cheek	72.23 ± 70.25	73.20 ± 74.40	1.34%	0.97
Right cheek	79.62 ± 59.71	71.50 ± 69.67	-10.20%	0.75
Nose	105.54 ± 60.34	99.20 ± 38.20	-6.01%	0.75
Left palm	5.31 ± 7.02	2.80 ± 2.25	-47.27%	0.24
Left dorsum	3.77 ± 2.77	0.50 ± 0.97	-86.74%	<0.01
Right palm	11.00 ± 14.15	4.60 ± 7.41	-58.18%	0.17
Right dorsum	4.62 ± 7.40	1.10 ± 1.37	-76.19%	0.12

Comparing the subgroups per anatomical area produced no statistical significant correlations for any of the baseline values (n = 29). Comparison of follow up values (n = 21) showed statistically higher values for the nose in the Theatre Subgroup than the Laboratory Subgroup (p=0.03). This could possibly be ascribed to the occlusion effect of the facemask worn by theatre staff during procedures.

Comparison of baseline and follow up values was only done on data where follow up values were available (n = 21). Comparing these values for the same anatomical area showed significant decreases for the left palm, left dorsum, right palm and right dorsum in the Laboratory subgroup ($p<0.01$, $p=0.05$, $p=0.02$ and $p<0.01$, respectively). Only the left dorsum value decreased significantly ($p<0.01$) in the Theatre Subgroup. Most facial regions in the Laboratory Subgroup showed increased values at follow up, while only the left cheek in the Theatre Subgroup increased in value. The reason for these unilateral differences is unknown. The mean percentage change for both subgroups is presented as ***Figure 5.3***. The forehead represented the only significant change when comparing the two subgroups (p=0.05).

Time-dependent variation with circadian reactivity rhythms and differences in TEWL, surface pH and skin temperature has been reported and suggests a higher permeability at night than in the morning (Yosipovitch *et al*, 1998; Denda & Tsuchiya, 2000b). To investigate if sebum content also varies with time, measurements were done in specific timeframes, i.e. 07:00-09:00 (1st timeframe), 09:00-11:00 (2nd timeframe) and 11:00-13:00 (3rd timeframe) ***(Table 5.7)***.

When comparing the two subgroups, the forehead (p=0.01) and right cheek ($p<0.01$) showed significantly higher values in the Theatre Subgroup than the Laboratory Subgroup during the 1st timeframe. In eight cases, the first measurement took place during the 2nd timeframe. However, measurement was done before commencement of work and was regarded as a true reflection of baseline values. It could again be speculated that the closed environment inside theatre could be responsible for the increased values. No other significant correlations were found between the subgroups for any of the other anatomical areas or timeframes.

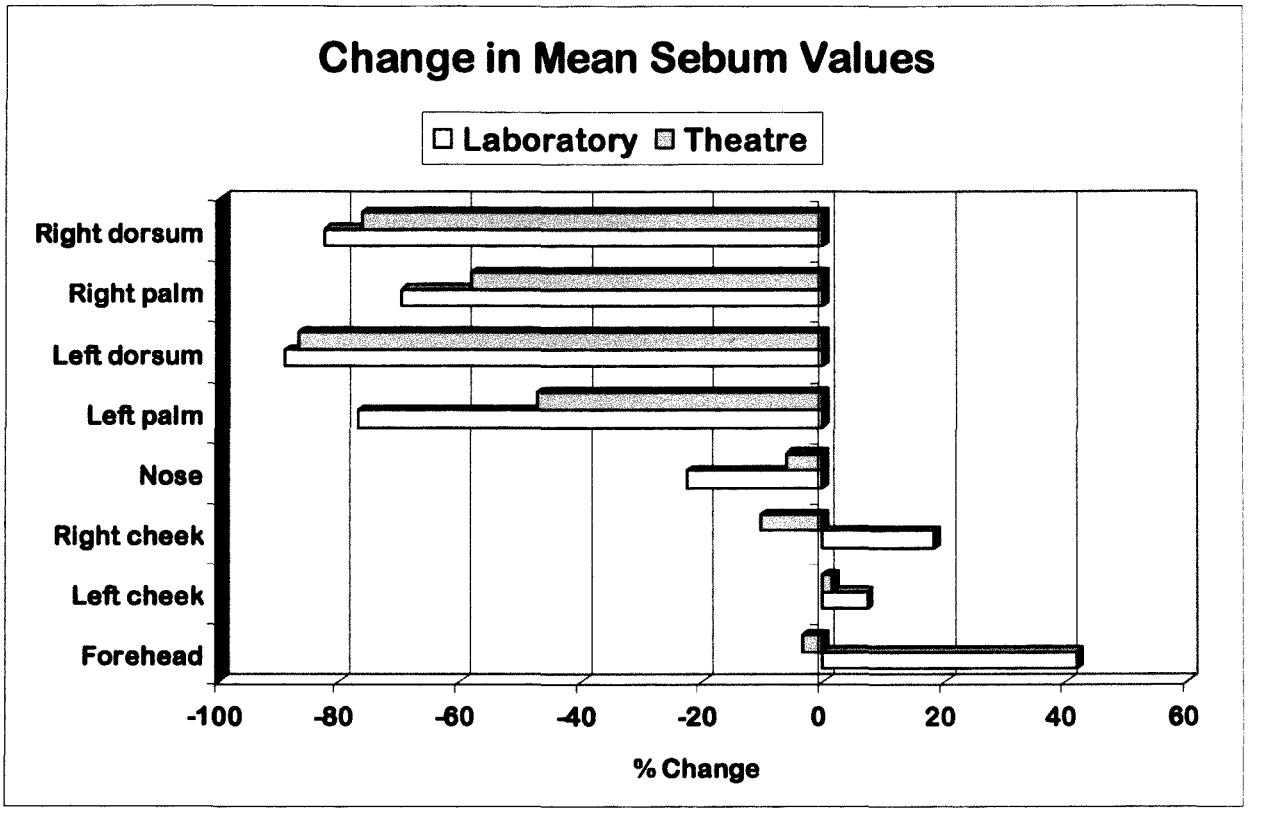


Figure 5.3: Comparison of change between baseline and follow up sebum values
Baseline measurements were done early in the morning before putting on gloves and where possible, follow up measurements were done in 21 subjects after taking off the gloves after three to four hours, but before washing the hands. This graph represents baseline and follow up values only where follow up was possible. The dorsal and palmar areas of both hands showed pronounced decreased values over time. Except for the nose, other facial areas showed a tendency to increase over time.

Table 5.7: Comparison of anatomical regions per timeframe between subgroups
Individual sebum measurements were divided and the different timeframes were compared between the subgroups. The only significant differences were found for the forehead and right cheek for the timeframe 07:00-09:00.

	Laboratory	Theatre	p-value
07:00 – 09:00 (n)	**(11)**	**(8)**	
Forehead	83.55 ± 58.35	165.38 ± 60.18	0.01
Left Cheek	41.18 ± 56.30	99.38 ± 77.81	0.09
Right Cheek	27.91 ± 20.94	105.88 ± 61.37	<0.01
Nose	64.45 ± 65.06	104.50 ± 53.25	0.16
Left Palm	4.64 ± 4.11	4.13 ± 3.98	0.79
Left Dorsum	4.36 ± 6.09	5.25 ± 2.55	0.67
Right Palm	3.82 ± 3.43	5.25 ± 2.55	0.31
Right Dorsum	3.09 ± 2.70	6.88 ± 8.85	0.28
09:00 – 11:00 (n)	**(10)**	**(4)**	
Forehead	140.60 ± 66.82	120.25 ± 69.12	0.64
Left Cheek	57.10 ± 45.24	28.50 ± 22.49	0.15
Right Cheek	63.60 ± 50.81	38.25 ± 26.25	0.25
Nose	78.80 ± 74.24	106.75 ± 89.10	0.61
Left Palm	4.60 ± 7.24	8.25 ± 11.95	0.61
Left Dorsum	0.60 ± 1.26	1.50 ± 0.58	0.09
Right Palm	2.80 ± 4.96	17.50 ± 21.61	0.27
Right Dorsum	0.90 ± 1.52	0.75 ± 0.50	0.79
11:00 – 13:00 (n)	**(6)**	**(11)**	
Forehead	181.33 ± 89.37	144.82 ± 75.45	0.42
Left Cheek	71.67 ± 25.53	69.27 ± 71.77	0.92
Right Cheek	76.33 ± 37.48	68.18 ± 67.00	0.75
Nose	77.50 ± 59.14	100.09 ± 36.36	0.42
Left Palm	2.00 ± 2.10	2.82 ± 2.14	0.46
Left Dorsum	0.50 ± 0.55	0.55 ± 0.93	0.89
Right Palm	1.83 ± 1.60	7.00 ± 10.62	0.14
Right Dorsum	0.50 ± 0.84	1.18 ± 1.33	0.22

Different timeframes within the same subgroup were then compared, irrespective if it was baseline or follow up values. The right dorsum value in the Laboratory Subgroup was significantly lower ($p=0.03$) in the 1st than 2nd timeframe. The 2nd timeframe also showed significantly lower values for the forehead ($p=0.05$), right cheek ($p=0.03$) and right dorsum ($p=0.01$) than the 3rd timeframe.

Conversely, significantly higher values were recorded in the Theatre Subgroup in the 1st compared to the 2nd timeframe for the left cheek ($p=0.04$), right cheek ($p=0.03$) and left dorsum ($p<0.01$). The left dorsum also showed statistically significant decline from the 1st to the 2nd timeframe ($p<0.01$) and again from the 2nd to the 3rd timeframe ($p=0.04$). Both subgroups displayed this decline over time in values measured on the hands. It is possible that it could be a result of the drying capacity of the glove powder, as suggested in the literature (Pretorius & Bester, 2000).

It was possible to identify specific differences among specific timeframes in most of the anatomical regions, as well as between the different subgroups. However, the scientific significance of these findings is elusive. An overall tendency of increasing facial values over time was identified in the Laboratory Subgroup. The Theatre Subgroup showed the highest values in the 1st timeframe, with a sharp decline to the 2nd timeframe and partial recovery during the 3rd timeframe. This is a very interesting phenomenon, which lacks scientific explanation at present, but warrants further investigation.

A box and whisker plot for the forehead region is presented as ***Figure 5.4*** to illustrate a highly significant difference between the two subgroups in the 1st timeframe ($p<0.01$).

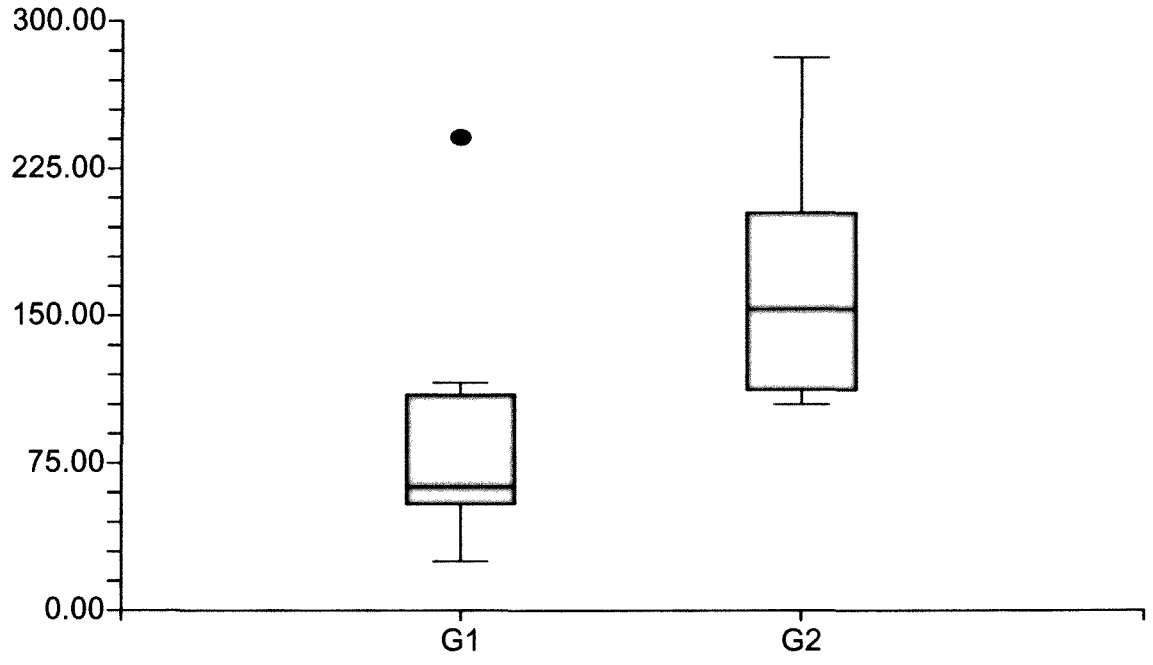


Figure 5.4: Box and whisker plot for the forehead in the timeframe 07:00 – 09:00

G1 (Laboratory Subgroup) displayed a very skewed distribution with a much lower median (64.00) than mean (83.55) value. Only one outlier (241.00) occurred in this group. G2 (Theatre Subgroup) displayed a normal distribution with a mean value of 165.38 and median of 154.50. The medians of the two groups differed significantly and produced a statistically significant correlation between the groups (p=0.01).

5.3. DISCUSSION

It was possible to identify three unique subgroups within the Study Group. All subjects were tested for *in vitro* type I latex allergy. This could be confirmed in 15.1% of patients. A further 22.4% of subjects had negative latex-specific IgE, but positive latex SPT^s. The third subgroup had both negative *in vitro* and SPT results.

An interesting finding was that the individuals with positive serum results (Group A) were significantly more likely to be laboratory than nursing staff. In contrast, subjects with negative latex-specific IgE results (Group B and C) were more likely to be nursing than laboratory staff. Statistical comparison failed to identify any significant difference between Group A and D, which probably mitigates the effect of the large dropout rate. This difference could not be attributed to the route or duration of exposure between these two professions and no other correlation could be found to clarify this finding. This kind of comparison has not been reported in the literature and individual reports did not offer any plausible explanation.

Numerical indices used in this study proved to be useful aids in evaluating possible latex allergy. As expected, the majority of subjects with positive results (latex-specific IgE or SPT^s) correctly predicted their diagnosis (Class 1). Similarly, the majority of subjects with negative results were assigned to Class 2, because they suspected that they were not allergic to latex. In all subgroups, Class 0 constituted such an insignificant percentage of subjects that it would be safe to regard these individuals as negative or Class 2.

The Allergy Score has only been used in one previous study (De Beer, 2000). A highly significant difference ($p<0.01$) was found between a latex allergic group and a non-allergic group. The former group included subjects with positive serum and SPT results and was therefore comparable to Cohort AB in the current study. However, the mean Allergy Score in Cohort AB of the current study was markedly higher than the positive group from the previous study (13.6 vs 9.2). The mean Allergy Scores from all subgroups were higher than that of the previous positive group. It is possible that volunteer bias could be a contributing factor in this finding. Inclusion in the current Study Group was based on the presence of two or more moderate to severe symptoms, which were used to calculate the Allergy Score. No normal value for the Allergy Score

exists at present, but a definite differentiation was evident among the scores from subjects with positive latex-specific IgE, positive SPT^s and negative results.

Although these numerical indices do not render conclusive and independent results, the relatively high PPV of Class 1 and high significant value of the Allergy Score in Group A could be utilised as part of a structured and cost-effective approach in the diagnosis and management of individuals with possible latex allergy. The current 49.2% PPV for Class 1 corresponds very well with the previous PPV of 49.3% for a similar study group. In the previous group a combination of anaphylaxis and bronchospasm showed the highest PPV (85.7%), followed by anaphylaxis (66.7%) and a combination of suspicion (Class 1) and more than three symptoms (57.5%), respectively. However, the prevalence rates for the respective criteria were all relatively low (2.5%, 3.3% and 17.0%, respectively) and this may question the validity of the predictions (De Beer & Cilliers, 2003).

The relevance and validity of symptoms selected for calculation of the Allergy Score were confirmed with PPV^s ranging from 70-100%. The statistically significant symptoms confirmed in Group A represented both respiratory and cutaneous routes of exposure. This is in agreement with literature reports that HCW^s are exposed to NRL proteins via both routes (Alenius *et al*, 1991; De Beer, 2000; Leynadier *et al*, 2000; Toraason *et al*, 2000; Reiter, 2002). It was not possible to differentiate between individuals with positive serum and SPT results in terms of the presence or severity of specific symptoms. Most HCW^s are primarily sensitised via the cutaneous route and initially present with positive latex SPT^s. With repeated exposure, however, respiratory and systemic sensitisation follow and individuals usually progress to present with positive latex-specific IgE results. The current symptoms confirmed in the latter group included conjunctivitis and rhinitis (respiratory exposure), as well as urticaria and skin rash (cutaneous exposure). When the group was extended to include individuals with positive SPT^s, additional symptoms showed significance (shortness of breath, angioedema and anaphylaxis). However, these symptoms are not exclusively associated with cutaneous sensitisation. Although it was not statistically confirmed, the overall tendency in symptom frequency was Serum [+] > SPT [+] > Negative for most of the individual symptoms. The significant Allergy Score in Group A confirms this trend. The frequency for symptoms in Cohort AB was also far higher than Group C for all symptoms. Due to the relatively small sample

size and diversity of symptom distribution a threshold number of symptoms in latex allergy could not be established. However, work-related deterioration of any of the symptoms should be regarded with a high index of suspicion.

The Allergy Score produced paired statistical significance with all occupational exposure variables within all positive groups (Group A, Group B and Cohort AB). Group C showed complete independence between the Allergy Score and all occupational exposure variables. Negative results did not seem to be influenced by occupational exposure as measured by the current parameters. The paired correlations produced by the positive groups confirmed the importance of exposure as a contributing factor in the development of latex allergy. This is in agreement with previous studies that included control groups, where exposure was statistically confirmed to be the major contributing factor (De Beer, 2000; Galobardes *et al*, 2001).

No clear correlation could be confirmed between surgical exposure before 1980 and latex allergy status in any of the subgroups. Latex allergy became more prominent after the well-known case report of latex allergy was published in 1979 (Nutter, 1979). The statistically higher number of procedures after 1980 in Group A and Cohort AB compared to Group C identified a similar tendency. Exposure through personal surgery seems to contribute in the formation of circulating latex-specific IgE, although the exact number is still controversial. The literature suggests that not only the number, but also the type of procedure has an effect on the development of latex allergy (Bohle *et al*, 2000; Degenhardt *et al*, 2001). This study recorded only the total number of procedures. Latex has been implicated in anaphylactic reactions since the late 1980^{s} and an increasing number of deaths has been reported (Slater *et al*, 1996; Lebenbom-Mansour *et al*, 1997; Lieberman, 2002). In the present study, Group A included three individuals who reported anaphylaxis as one of their previous symptoms.

Atopy has long been proven as a strong and consistent risk factor for latex sensitisation and is reported in 60-80% of latex allergic patients in the literature (Wrangsjö *et al*, 1986; Turjanmaa, 1987; Turjanmaa & Reunala, 1988b; Beuers *et al*, 1990; Pecquet *et al*, 1990; Heese *et al*, 1991; Niggeman, 1997; Levy *et al*, 2000; Garabrant *et al*, 2001; Garabrant & Schweitzer, 2002). In complete agreement with the literature, the current study confirmed personal atopy in 76% of subjects and personal or family history in 83%. Group A showed statistical significance for all atopy parameters, despite a lack of significance for total

serum IgE. It would therefore be safe to disregard the value of the total serum IgE in cases with latex allergy, although it was included in the initial diagnostic criteria for atopic dermatitis (Hanifin & Rajka, 1980). The prevalence of eczema and hand eczema did not show the expected significance, although both parameters showed the highest prevalence in subjects with positive serum results. The lack of statistical significance could be attributed to a relatively high occurrence in all subgroups.

As stated earlier, the total serum or dermal fluid IgE did not contribute statistically to the overall differentiation among the subgroups. The relatively high normal values for total serum IgE further makes meaningful interpretation inconclusive. An interesting finding was the highly significant correlation between the Allergy Score and total serum IgE in all subgroups. This phenomenon is difficult to explain, but the wide range and high SD values of total serum IgE values in all subgroups resulted in a very flat distribution curve. All subgroups showed a dense population of values, including the median values, within the 25-50% quartiles. In all four subgroups the SD values were higher than the means and the maximum values were > 1 000 IU/ℓ. It is therefore possible that the extreme high values were responsible for the lack of correlation.

The paired statistical significance found between serum and dermal fluid IgE values should be interpreted with caution, due to rather limited sample size in both cases. However, it is possible to be a valid finding, as the rest of the statistical results support this possibility. More research will be needed to investigate the validity of this hypothesis.

This study was able to identify interesting differences in sebum values between theatre and laboratory staff. Theatre staff displayed higher overall facial sebum values than laboratory staff. The most plausible explanation for these increased values is the relatively closed working environment of theatre staff. Increased sweat production and even the occlusive effect of the facemasks could also play a role. The decrease in forehead values of theatre staff could speculatively be due to the absorbing nature of the theatre masks and caps worn by all staff during surgical procedures.

To test this hypothesis, the same individuals could be evaluated inside theatre and again in an outpatient or ward environment at corresponding times. This could discriminate between environmental and personal variables, such as the number of

sebaceous glands, which differs greatly from person to person. Although differences between the left and right cheeks were statistically different, this could be purely coincidental, as no physiologically or scientifically sound explanation could be offered for this observation.

The results obtained from the hands of both groups are in keeping with the literature that suggests that glove powder can cause dryness inside gloves by absorbing skin sebum or sweat (Lodén, 1995; Pretorius & Bester, 2000). The Laboratory Subgroup displayed significantly decreased values for all hand regions at follow up. Although all values were decreased at follow up in the Laboratory Subgroup, the Theatre Subgroup only had significant decreased values for the left dorsum. Once again, the reason for this difference is unclear. The pre-donning scrub routine should theoretically affect all hand regions or at least the two dorsal regions equally and this is probably a spurious result.

Further research is needed in an attempt to shed light on the apparent conflicting findings with regard to sebum content of the skin. Specific anatomical areas should be targeted for investigation in bigger groups to obtain more consistent and reproducible results and identify possible trends in specific groups. Sebum content is not a reliable reflection of the skin barrier function and TEWL should rather be used, as it reflects on the integrity of the SC (Wilhelm et al, 1991; Ozawa & Takahashi, 1995; Schwindt et al, 1998; Denda, 2000a; Draelos, 2000). However, it was valuable for the purpose of this study to evaluate the baseline presence of sebum on the skin surface and demonstrate an increase or decline over time.

CHAPTER 6:
DISCUSSION

This study attempted a global assessment of latex allergy in HCW[s] and ranged from environmental exposure by latex glove powder, to the clinical profile of affected or symptomatic individuals, through to the physical effects latex gloves and airborne allergens have on the individual's skin.

6.1. ENVIRONMENTAL CONTAMINATION

The current study was able to identify a rather comprehensive and wide ranging contamination by glove powder in the airconditioning system at the TBH complex. Both starch and proteins were confirmed in all samples collected from the air supply and return ducts from the airconditioning system. It was possible to confirm the presence of glove powder in all the dust samples. Glove powder is associated with airborne latex allergens throughout the literature and although not proven, it would be safe to assume that these proteins were most probably latex. Even though it was not confirmed to be NRL proteins by immunoblotting, inhibition or other immunological assays, contamination by some unknown protein source was sufficiently proven in the extraction and return ducts. The redistribution and repeated inhalation of these contaminants will undoubtedly result in sensitisation of susceptible individuals in the long run. The exact nature of the proteins is irrelevant, due to the inherent ability of any protein to elicit allergic reactions in sensitised individuals.

Further research could attempt to identify these proteins immunologically. The LEAP assay quantitates antigenic latex proteins in latex product extracts using an indirect ELISA and could be utilised for this purpose. Possible association or correlation should be investigated with specific antibodies found in the serum of affected staff members, e.g. by Western blot analysis.

Elimination of latex contamination in healthcare facilities will undoubtedly prove to be difficult and expensive. Although gloves and glove powder are the major culprits in distributing latex allergens, a vast number of other medical devices also contain latex. However, proteins do not leach as easily from hard rubber or latex devices as from latex gloves, but a sensitised individual is capable of having an allergic reaction even from

hard latex. Furthermore, contamination is not limited to airconditioning ducts, but furniture, clothing, curtains, carpeting, etc, can also be inoculated by latex allergens (Charous *et al*, 2000). Even after an extended period of dormancy, and as soon as these settled allergens are disturbed, they can become airborne again and induce allergic sensitisation and elicit reactions in previously sensitised individuals (Kelly *et al*, 2000).

A definite and essential first step in rectifying this problem would be the elimination of all powdered gloves from use. An acceptable alternative must be found to replace latex, e.g. guayule latex, synthetic or nitrile gloves. It is imperative that the quality of the substitute be comparable to that of latex to provide sufficient protection against viruses and other pathogens, e.g. HIV, hepatitis, microbes, etc. It would be a dream come true if all latex gloves could be phased out and completely replaced by latex-free gloves. However, this is currently not a viable or cost-effective option. GSH and Red Cross Children's Hospital in Cape Town have successfully and completely converted to powder-free gloves and TBH should actually follow suit. Additionally, all airconditioning ducts should be thoroughly cleaned and all filters replaced at regular intervals. The permissible particle size of the filters should be reconsidered to capture as much and small as possible proteins capable of inducing allergies. It is also important that this cleaning process should be done in a relatively short time, to prevent re-contamination from old dirty ducts.

An interesting prospect may involve the electrostatic charge of the working environment. Hydrogen bonds in proteins, which are also present in latex proteins, are primarily electrostatic in origin (Blaber, 1998). Although the net electrostatic charge of glove powder and NRL proteins have not been determined, it could theoretically be utilised to create a repulsion force between proteins and working surfaces, e.g. walls, airconditioning ducts, etc. Filters could also be remodelled or replaced with a double filtering system, which can be galvanically charged to limit redistribution via the airconditioning system. Ultimately, when proteins do not adhere to surfaces, airconditioning systems should be able to remove more airborne allergens from the rooms and ducts. However, more intensive research is needed to make this a viable concept.

6.2. LATEX ALLERGY PREVALENCE

An important finding was a rising incidence in latex allergy among TBH HCW^{s} since 2000. The prevalence increased from 31% to 38% in individuals with occupational exposure. The literature confirmed an annual increase in sensitisation of 1.0-2.5% (Garabrant & Schweitzer, 2002; Ranta & Ownby, 2004). Since the previous TBH study, no intervention with regard to glove substitution or purchasing took place. This is still the highest latex allergy prevalence reported in the RSA and is only comparable to Croatian figures of 35-40% (Lipozencic *et al*, 1998).

During 2002 an average of 4 256 individuals were employed at TBH of which approximately 2 634 were healthcare professionals. When the current prevalence rate is extrapolated to the total number of HCW^{s} at TBH, an estimated 988 employees might be affected by clinical latex allergy. The actual cost of latex allergy for the employer has not been established in the RSA, but at the current increasing rate, it could cost the country dearly, not only in monetary terms. Unfortunately, the highest skilled healthcare professionals have the highest and most intensive exposure, e.g. anaesthetists, laboratory technologists, nursing sisters, pathologists, surgeons, etc. These individuals usually do not have another option than to resign or resort to another profession with less or no latex exposure.

6.3. CLINICAL EVALUATION

Three unique patient groups could be confirmed on clinical grounds and laboratory results. All subjects experienced a number of the pre-defined symptoms, but only 15% could be confirmed to have circulating latex-specific IgE. Unfortunately, 46% of the initial study group were lost to follow up due to a variety of reasons. The remaining subjects were divided into groups with positive and negative latex SPT results. Specific differences had been identified among individuals with positive serum, positive skin and negative serum and skin results. As expected, individuals with positive serum results differ on almost every clinical and laboratory parameter from the other groups. In many instances, features of individuals with positive serum and positive skin results overlapped. However, it remains extremely difficult to predict the differentiation point in the immunological profiles of these individuals at present. Different scientific approaches could possibly be utilised to create a scientifically sound forecast of when

sensitisation and symptoms will commence. However, this will require regular invasive investigation of subjects exposed to latex from a zero exposure level, e.g. skin biopsies.

The results from the current study have been able to confirm a number of clinical and laboratory characteristics in an individual with latex allergy. All the pre-defined symptoms seem to be relevant and different permutations of these symptoms could be useful in predicting latex allergy. Work-related deterioration in any of the symptoms should undoubtedly warrant an investigation. The current study relied on the subject's personal opinion for the presence and severity of the symptoms. Because the study was conducted among healthcare professionals with some previous medical education, their opinions were regarded as a true reflection of the clinical condition. It would be invaluable in the diagnostic approach to confirm the presence and severity of symptoms by means of lung functions, skin biopsies and other relevant diagnostic procedures. Mast cells can be exposed to a monoclonal anti-human IgE marker to react with the epsilon chain of allergic patients. This should enable visualisation of the bound and available IgE receptors on the mast cell surface. A true numerical index representing the level of sensitisation could then be compiled from standardised *in vitro* criteria. Although the current Allergy Score produced a significant prediction value, it was subjected to the perception of subjects, which can vary considerably and may not necessarily be a true reflection of the clinical picture.

In summary, the following characteristics were confirmed by the current study to be relevant in individuals with positive serum or SPT results:

- occupational exposure on a regular basis
- personal suspicion of latex allergy
- increased Allergy Score
- work-related deterioration of symptoms
- conjunctivitis / rhinitis / urticaria / angioedema / anaphylaxis
- multiple surgical procedures after 1980
- personal history of atopy

Further research is necessary to identify a cut-off or upper limit value for the Allergy Score with regard to latex allergy. The number of surgical procedures in a population of

adult HCW^{s} associated with the risk of developing latex allergy also needs to be established. Specific permutations with symptoms and atopic diseases need to be assessed to identify the highest probabilities for the development of latex allergy. A previous attempt had been made to try and compile a predictive profile for latex allergy, but the sample size was relatively small and more research is needed to conclusively validate the findings (De Beer & Cilliers, 2003). It needs to be emphasised that no single method exists to identify all individuals at risk of developing latex allergy.

A predictive test based on a standardised questionnaire would be very useful as a screening tool, as latex-specific IgE and SPT^{s} are expensive and require specialised services. In order to conform to the RSA Primary Healthcare Plan a simple and cost-effective algorithm needs to be established to enable general practitioners or district surgeons in rural areas to accurately predict and / or diagnose susceptible or sensitised HCW^{s} and patients.

6.4. SPECIAL INVESTIGATIONS

Total serum IgE did not contribute significantly as an indicator of latex allergy, although a statistical correlation was found with the Allergy Score. The significance of this association is presently unknown, because the Allergy Score is calculated from specific symptoms that could also be related to atopy or other IgE-mediated diseases. It was possible to identify a statistical correlation between the total IgE values in serum and dermal fluid of subjects with positive serum and SPT results. This finding can be of use in future studies or diagnosis, especially where the serum latex-specific IgE is negative. However, the method of collecting dermal fluid is a long and cumbersome procedure, which needs specialised equipment. There is also not much difference between the cost of performing total dermal fluid IgE and serum latex-specific IgE. The SPT is much cheaper and more readily available and should be considered first.

Regarding confirmation of sensitisation against latex by special laboratory investigations, Western blot analysis proved to be far more sensitive than latex-specific IgE. Even though selected dermal fluid specimens yielded negative latex-specific IgE values, Western blot analysis confirmed at least one band in every specimen. Retrospectively, if Western blot analysis had been done on serum or dermal fluid of subjects with negative latex-specific IgE results, a much higher positive rate would have

been possible. It would also have been interesting to be able to correlate positive SPT results with Western blot bands in dermal fluid collected at the site of the SPT reaction. Due to financial constraints this was not possible in the current study, due to financial considerations. Results from the dermal fluid specimens collected at different anatomical areas from one subject produced different bands. This might be of benefit in future studies to identify a specific route of sensitisation, provided that this result is repeatable in a greater number of subjects.

Important findings were the high incidence of sensitisation to *Hev b 1* and *Hev b 6.01*, which had been confirmed to be the major allergens involved in sensitisation via airborne and cutaneous routes respectively. It was confirmed by the results of the current study that most subjects were sensitised to proteins via direct contact with gloves or inhalation of glove powder. It is possible that previous surgical procedures might have contributed in selected subjects, but differentiation was not possible with the current results. At least seven known major allergens could be confirmed. The previously unidentified bands found in the current study warrant further investigation and possible AA sequencing.

Most publications suggest that individuals with glove-related symptoms who have negative serum and SPT results, most probably suffer from a type IV hypersensitivity reaction to additives in the gloves (Toraason *et al*, 2000; Kurup & Fink, 2001). Only a very small number of subjects were evaluated by means of patch tests and only one showed a true type IV reaction to a piece of latex glove and glove powder applied with the standard patch test allergens. The reaction was confirmed to be allergic and not irritant when it was still present 96 hours after application and 48 hours after the patches were removed. Irritant reactions tend to subside when the contact allergen is removed. However, thiuram mix was present in all three subjects with positive patch test results. After step-by-step evaluation of all possible triggers, occupational exposure seemed to be the main culprit in all the cases. Thiurams are primarily used as accelerators in the production of rubber and are present in various rubber articles, e.g. balloons, catheters, elastic bandages, stethoscopes, etc. The significance of the results might be questionable, due to the small number of subjects tested.

6.5. SEBUM CONTENT

This was the first study to assess the value of skin sebum content in a population of adult HCW[s]. The findings were useful and identified a number of new research opportunities. The general perception that sweat production inside gloves has a positive effect on the solubility and percutaneous absorption of latex proteins, could not be confirmed by the findings of the current study (Beezhold, 1994; Reiter, 2002). In accordance with a limited number of publications, glove powder was shown to cause an overall dryness inside the gloves (Beezhold *et al*, 1994a; Lodén, 1995; Turner, 1997; Pretorius & Bester, 2000; Hamilton *et al*, 2002). The relatively low surface sebum levels further suggest that the proteins that cross the skin barrier, do not necessarily depend on sweat or other moisture inside the gloves to liberate them (McCaskell, 1995). An *in vitro* model is available to measure the percutaneous penetration of NRL proteins (Hayes *et al*, 2000). It would be interesting to identify the MW[s] of the specific proteins capable of crossing intact and disrupted skin barrier under these circumstances. Theoretically, the proteins in the smaller MW range should be able to cross the barrier without much interference.

The different sebum profiles of laboratory and theatre staff need to be further investigated and clarified. The role of the working environment, e.g. theatre vs laboratory, also needs verification and clarification. The role of scrubbing procedures before donning gloves should also receive attention. Possible correlation between sebum content and TEWL or SCH might contribute to a more comprehensive understanding of the micro-environment inside the gloves of a HCW.

Hand eczema has repeatedly been implicated in disruption of the skin barrier (Nutter, 1979; Wrangsjö *et al*, 1986; Axelsson *et al*, 1987; Turjanmaa, 1987; Levy *et al*, 1992; Jaeger *et al*, 1992; Taylor & Praditsuwan, 1996; Taylor *et al*, 1999; Wrangsjö *et al*, 2001). For the current study, visible hand eczema was regarded as synonymous to a disrupted skin barrier. The purpose of this part of the study was not to evaluate the skin barrier function *per se*, but to assess the physical effect gloves and glove powder has on the skin. True evaluation of the skin barrier function should include measurement of TEWL, SCH and skin surface pH.

6.6. CONCLUSION

In conclusion, latex allergy will probably remain a major occupational problem for years to come. Different protocols for latex IT are currently being investigated, but it remains a dangerous modality for individuals with severe latex allergy. It is imperative to prevent sensitisation of more individuals by avoiding or limiting exposure to NRL proteins. It is extremely difficult, because of the ubiquity of latex, especially in the medical environment, but also the high frequency of cross-reacting food. Additionally, the current global AIDS epidemic encourages the conscientious use of gloves (and condoms) for personal protection. The high cost and limited availability of latex-free alternatives currently worsen an already complicated situation, especially for individuals in rural areas.

The main therapeutic approach should concentrate on proper education of individual at risk, timeous diagnosis and / or accurate diagnosis with proper further management. Much emphasis is placed in Primary Healthcare in the RSA and where possible, it is attempted to take healthcare to the people. Rural and urban healthcare facilities are likely to be equally influenced by latex allergy. However, rural areas might lack the expertise on specialised and fairly uncommon conditions, such as latex allergy. It is therefore important to limit exposure and sensitisation, but also provide a structured and proven algorithm for identifying and diagnosing latex allergy in both HCW^{s} and patients.

With the identification of more infectious and contagious agents, the need for personal protection is likely to remain or increase. It is imperative that these protective measures are safe for the user and the adverse effects are kept to a minimum. Protective gloves and condoms should not only be able to prevent transmission of diseases, the user should be able to have complete confidence in its qualities and trust it with his life.

6.7. FUTURE RESEARCH OPPORTUNITIES

- Validation of the Allergy Score and other numerical indices in latex allergic individuals compared to a valid control group from the general population to establish normal ranges and / or threshold values
- Follow up of SPT negative individuals with repeat SPT^s or latex-specific IgE to determine true sensitisation at a later stage
- Compilation of a predictive profile for latex allergy on clinical signs and symptoms, and laboratory values
- Investigation of IgE in the skin, both bound to the mast cell and circulating in the extravascular compartment
- Comparison of dermal fluid values for specific IgE and / or Western blot analysis from different anatomical areas to define the role direct contact has on positive results
- Comparison of sebum values from different anatomical areas in latex-exposed and a control group from the general population
- Immunological Identification of possible latex proteins in airconditioning ducts (LEAP, RAST inhibition or Western blot assays)
- Evaluation of the percutaneous penetration of natural latex proteins by using radiolabeled I^{125} and a continuous flow-through diffusion apparatus (Hayes *et al*, 2000 and Van der Bijl *et al*, 2000)
- Investigating the influence of electrostatic charges of the walls or inside airconditioning ducts on the adherence affinity of latex proteins
- The efficacy of percutaneous IT and SLIT in a large group of latex allergic patients

CHAPTER 7:
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