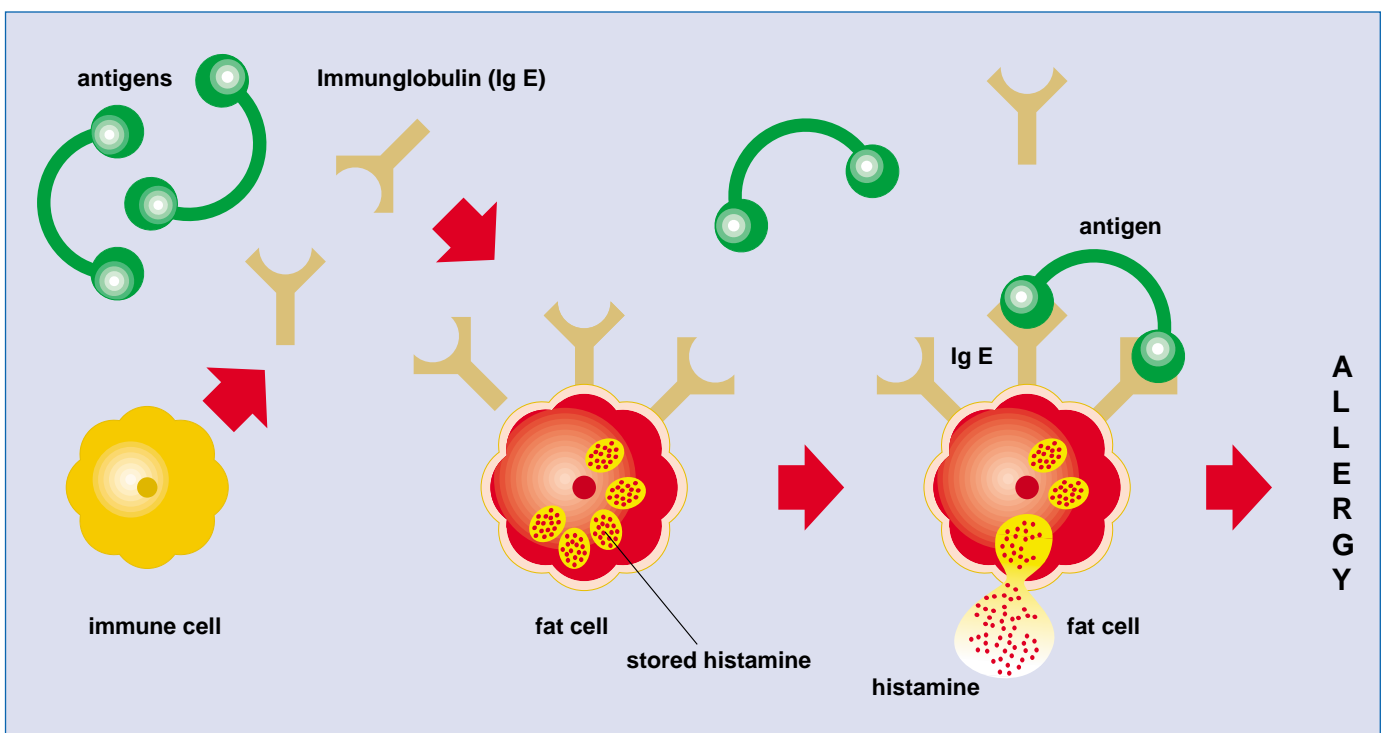


Latex allergy with medical gloves – Diagnosis and protein determination

For medical gloves the subject of latex allergies continues to be a burning issue. European Standard EN 1441 imposes on manufacturers a duty to carry out a risk analysis, which also relates to the allergenic potency of a glove under prEN 455-3. Although the methods for determining proteins in latex gloves are described in this standard, other methods are repeatedly applied. The user is very often uninformed on how the different procedures should be evaluated. SEMPERMED informs.



Antigens penetrating into the body will give rise to the formation of immune cells of specific antibodies (immunoglobulin). These antibodies deposit on and sensitize the fat cells. Upon subsequent contact, the fat cells can now catch "their" antigens. This leads to the emission of histamine – which runs rampant in the case of an allergic person.

Reactions often described under the general heading of latex allergy need to be differentiated between pseudo-allergic (irritating) and allergic reactions. While irritating reactions can be traced back to physical (powder particles) and chemical damage (pH-value, occlusion) of the skin, immunological overreactions to glove constituents are the cause of true allergies. In the case of allergic reactions to medical gloves, a differentiation is made between type I and type IV allergies.

Type IV allergies (cell-transmitted) are as a rule the result of chemicals which are used in the manufacturing process of gloves. The cause of type IV allergies is the group of accelerators (vulcanisation accelerators), with thiurames being the main source. Many chemicals are present both in latex gloves and non-latex gloves. Type IV reactions can therefore occur in gloves made from synthetic latex as well as in gloves made from natural latex.

Type I allergies (IgE-transmitted) [IgE = immunoglobulin or antibodies of class E] are caused by the proteins contained in the latex milk. At present, it is only possible to reduce, but not to eliminate, these latex proteins in the finished glove.

Today, we wish to introduce to you the best-known methods for the **diagnosis** of a latex allergy and – in a separate chapter – the most important methods for determining the **allergenic potency of latex gloves**.

Diagnosis

In the diagnostic clarification of allergic reactions to latex gloves, a separation is made in principle between

Method for diagnostic clarification of allergic reactions to medical gloves

	Type IV	Type I
In-vivo	Epicutaneous test	Prick-Test Scratch test Intracutaneous test Rubbing test Provocation test dermal – glove wear test inhaling – with powdered gloves
In-vitro	Lymphocyte transformation test	Determining specific IgE e.g. RAST CAR FEIA Stimulation test e.g. histamine release

Table 1 (no claim is made as to the completeness of this list)

Diagnosis of a type IV allergy

Recognition of symptoms

Clinically, type IV allergy manifests itself as allergic contact eczema which appears between 24 – 48 hours after contact with the allergen and shows a crescendo-like progression. Further characteristics of the allergic contact eczema comprise (in addition to itching erythema) blisters, vesiculae and scaling of a particularly spreading form, i.e. skin effects which go beyond the contact lateral of the allergen (e.g. the latex glove). In this way it can clearly be separated from irritating skin symptoms.

In-vivo-test

Epicutaneous test

This test is carried out on normal skin, free from



Epicutaneous test: Applying test substances by means of test plasters

inflammation, on the back. The test substances are contained in small aluminium chambers (e.g. Finn chamber) and applied to the skin by means of generally available epicutaneous test plasters.

The test substance then remains in contact with the skin for 48 hours. The reaction is recorded after 24 or 48 hours, and again after 72 hours after initial application on the back. The interpretation is carried out in accordance with the recommendations of ICDRG (International Contact Dermatitis Research Group). An allergic reaction is present if the skin irritations remain for at least 48 hours. If it is suspected that the allergy is caused by medical gloves, all important rubbercontent agents and a piece of latex glove are tested in addition to the standard series (the most important type IV allergens).

In-vitro-test

The lymphocyte transformation test is used for in-vitro testing for type IV allergies, where the lymphocyte growth through the allergen is stimulated in the blood of the patient. This very expensive and time-consuming test for diagnosis is however only applied in exceptional cases.

Diagnosis of a type I allergy

Recognition of symptoms

Unlike type IV allergies, the clinical symptoms of type I allergies usually manifest themselves within 5 to 30 minutes after allergen contact. For this reason, this

reaction is also described as “immediate-type allergy”. The type I allergy comprises 4 stages (as per Krogh and Maibach), which can be observed in a classical manner in the case of a latex allergy.

Stage I	Localised contact urticaria
Stage II	Generalized urticaria, incl. Lid oedema
Stage III	Urticaria and mucous membrane symptoms (asthma bronchial allergy, rhinoconjunctivitis, orolaryngeal and gastrointestinal symptoms)

Table 2

In-vivo-test

In-vivo tests are in the main, skin tests where allergens penetrate into the fat cells in the connective tissue of the corium, which releases an IgE-transmitted immediate-type reaction. In this connection, standardisation of the various methods is a problem, as the test result depends on several factors: inter alia, on the allergen quantity in the test extract applied and on the protein types which have sensitized the patient.

Prick test

For the prick test the allergenic substance is applied to the skin in the forearm bend. The skin is subsequently briefly pricked through the substance and elevated with the aid of a fine needle or a lancet, but no bleeding should occur. After 20 to 40 minutes the test reaction is recorded in accordance with defined rules.



Prick test

Evaluation	Quaddel-size (mm)	Erythem-size (mm)
0	0	< 3
+	2 – 3	3 – 5
++	3	6 – 10
+++	4 – 6	11 – 20
++++	> 6 (pseudopodia)	> 20

Table 3: Evaluation of prick test reactions as per Ring (1988)



Scratch test

Scratch test

Here about 5 mm of the skin is scratched with a lancet (no bleeding) prior to applying the substance containing the allergen; readings are the same as for the prick test.



Intracutaneous test

Intracutaneous test

0.02 to 0.05 ml of a heavily diluted allergen solution (depending upon substance) are intracutaneously injected and the reactions are evaluated in a similar manner as for the prick test after 20 to 40 minutes. The intracutaneous test is however rarely used for diagnosing latex allergies.

Rubbing test

Where there is a risk of strong allergic reactions (anaphylactic shock) the test allergen is only lightly rubbed into the skin. The reactions are assessed in a similar manner as for the prick test. In case of a negative result, it is however necessary to follow this up with a prick test.

Provocation test

For a provocation test, the patient is brought into contact with the allergy-releasing material under the best possible realistic conditions. For a latex-glove allergy, the options are the glove-wearing test and the inhaling provocation test.

Glove-wearing test

For the glove-wearing test, the gloves (or even just one glove finger) are pulled over the hand, made damp with water, and worn for 30 minutes. The reactions are subsequently assessed similarly to the prick test. This method is primarily used if the past history of the patient clearly points to a latex allergy, but where no specific IgE antibodies have been found in the blood.

Inhaling provocation test

For the inhaling provocation test, function of the upper and lower breathing passages is tested on the patient in a lung function chamber under allergen provocation. In the case of gloves, this is usually done with the powder of the latex gloves used, which, as is known, transports the allergenic latex proteins. The reactions of the lung (spirometry, whole-body plethysmography, rhinomanometry) are recorded continuously.

NOTE:

All in-vivo tests for type I allergens must only be carried out by experienced doctors under the relevant emergency procedures, as these tests can – although rarely do – lead to anaphylactic reactions. Patients and assisting staff must be comprehensively informed on the possible risks.

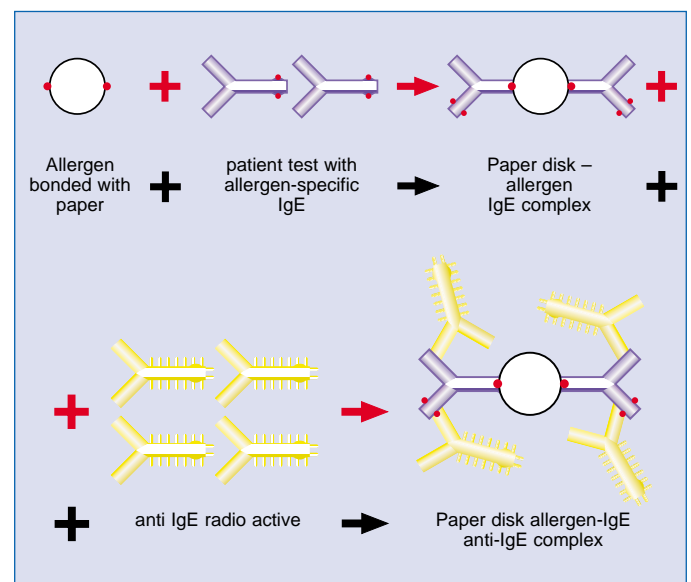
In-vitro test

For in-vitro tests the determination of specific IgE (immunoglobulin or antibodies of Class E) and various stimulation tests are available.

Determining allergen-specific IgE antibodies **RAST (Radio-Allergo-Sorbent Test)**

The RAST (Radio-Allergo-Sorbent Test) developed by Messrs. Pharmacia (Uppsala) was the first method for determining allergen-specific IgE antibodies. In the meantime (after expiry of patent rights) a whole series of other, equally valid methods have come into existence, all of which proceed along the same basic principles.

The word RAST has however become synonymous for determining allergen-specific IgE, even if the measurement of antibodies is nowadays no longer carried out by radio-active marking. With all these procedures, an allergen is initially bonded on to a fixed phase (paper disk, polystyrene vessel or polystyrene balls). The patient serum is then added which leads to the attaching of suitable IgE antibodies to the fixed allergen. After washing off the serum, the IgE molecules, which are now also fixed, can be detected with a correspondingly marked anti-human IgE antibody. These anti-human IgE antibodies are obtained by injecting human antibodies into test animals. If these detection antibodies are radioactively marked, the quantity of bonded IgE antibodies can be determined in a gamma counter.

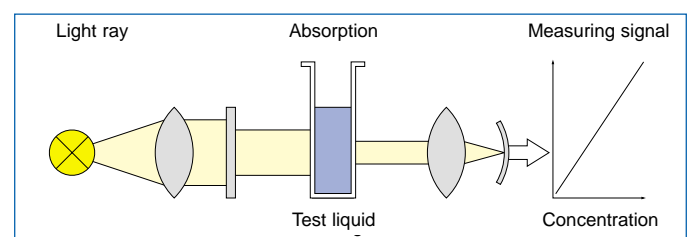


RAST (Radio-Allergo-Sorbent Test)

If the detection antibodies were marked by an enzyme, an enzymatic reaction will follow. For this a solution is added after the enzyme-marked anti-human IgE antibodies were added, which generates a colorant or a fluorescent colorant. The colour concentration can be measured with the aid of a photometer.

Photometry

The basic principle of the photometric determination of the concentration: a light ray of defined intensity hits



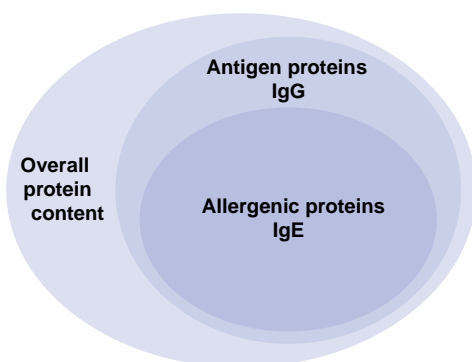
the test liquid, leading to a weakening of this light ray, which is then measured. The concentration of the test liquid can be determined by means of a calibration curve.

Stimulation test (e.g. histamine release test)

For this test procedure, the blood cells (either in the original blood or in the isolated lymphocytes) are incubated with various concentrations of the allergen. This stimulates the B lymphocytes which bring the corresponding IgE molecule to the surface, into a reaction, typical for a type I allergy. This includes, for example, the release of histamine and the new synthesis and release of prostaglandins. These agents (mediators) can then be measured and give information on the strength of the allergic reaction. Such procedures are very time-consuming and expensive. They are therefore used in special cases only.

Allergen Potency of Latex Gloves

With the methods available for this purpose, it is important to differentiate between ascertaining the overall protein content and the allergen content of latex gloves. The method described below uses the various characteristics of proteins in order to ascertain the allergen potency of latex gloves



All allergens are antigens, but not all antigens are allergenic

The method for determining the overall protein content ascertains all soluble proteins contained in the latex glove, i.e. including those which do not originate from the latex milk, but which are added by some manufacturers (e.g. casein).

At present, 240 different latex proteins have been identified in latex milk, the basic material for latex gloves. The

primary difference is their molecular weight which ranges from 2 to 200 kilo-Dalton (kD). This multitude of proteins is however found only in latex milk as raw material and not in the extracts from latex gloves. In these extracts there are only very few of the proteins which were originally present, but there is a multitude of peptides with a molecular weight of <10 kD. These need to be looked upon as the derivative products of the original proteins. It can be assumed that, in contrast with the latex milk, in gloves all latex proteins are of allergological relevance. It has meanwhile been possible to identify some allergens and to analyse their primary structure.

Table 4

allergen	mol. weight (kD)	amino acids number
Prohevein	20	187
Hevein (N-terminal)*	4,7	43
Hevein (C-terminal)*	14	138
REF**	14,6	137
Hevamine	29,6	273
Prenyltransferasis	38	

*resulting from matured prohevein

**rubber elongation factor,

Plays an important role as allergen inter alia with spina-bifida children.

kD: Kilo Dalton = molecular weight

Partially known in their primary structure are also a 27 kD, a 36 kD and a 100 – 110 kD protein.

Methods for determining the allergenic potency of latex gloves:

	Methods	Unit
Chemical analytical methods (overall protein content)	-Lowry -HPLC -Bradford	µg/g** µg/g
Immunological methods (allergen content)	-RAST inhibition -ELISA inhibition -LEAP	AU*/ml AU/ml µg/g

** microgramme protein per gramme Latex

*AU=arbitrary units (not standardized)

Table 5 (no claim is made as to the completeness of this list)

Why is it so difficult to measure the proteins in a latex glove?

Conventional methods for measuring proteins have been designed to determine proteins as main components of a bio-chemical specimen. Latex gloves contain only traces of proteins (ppm, parts per million), which

must be measured in the presence of a series of chemicals, which however interfere with many colorimetric methods. The European Union has therefore financed a project, which is designed to investigate the measurement of proteins and vulcanization accelerators in latex gloves and the allergological relevance of the test results. This project has been carried out at the Dermatology Clinics in Erlangen and Copenhagen and at ÖIBW in Vienna.

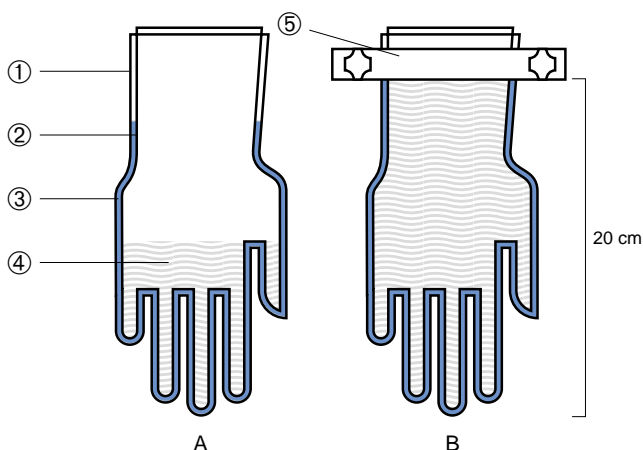
The results show that the modified Lowry method leads to acceptable results which correlate well with the clinic (prick test).

Extraction procedure

However, of equal importance with the measuring procedure, is the extraction procedure by which the proteins are released from the gloves. In the above-mentioned European project, a method for this has been developed which was incorporated in the European Standard (prEN 455-3). For this a buffer must be used, as powdered gloves – because they sometimes contain magnesium oxide – often reach very high pH values (up to 10,5 pH) in unbuffered extracts. International (CEN, ASTM, ISO) agreement was reached on a buffer of pH 7.4.

prEN 455-3

An extraction liquid is placed between two gloves (one inserted in the other). The inner glove is subsequently filled with a colour solution. Over a period of 2 hours at a defined temperature all water-soluble proteins are



1 outer glove, 2 inner glove, 3 extract buffer, 4 colour solution, 5 glove clamp

released from the inside of the outer glove and the outside of the second glove.

Afterwards the colour solution is removed and the extract remaining between the gloves is determined by the methods described below.

Advantage:

This extraction procedure has the advantage that only the glove surface is extracted, that very little fluid suffices for extraction purposes and that it allows a good simulation of wearing gloves. As the inner glove is filled with a coloured solution (to locate any holes) this ensures an optimum contact of the extraction solution over the entire surface.

ASTM D 5712

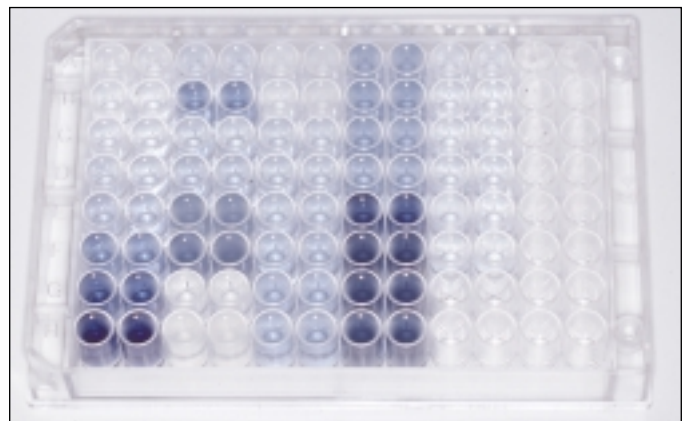
In the USA, gloves are initially cut into small pieces in order to obtain the glove extract and subsequently extracted in a watery solution under specified conditions.

Chemical analytical methods

With these methods the total protein content is ascertained from the extracts of gloves from natural latex.

The modified Lowry Method

This colorimetric method is based on the reaction of copper ions with the proteins and the folins reagents to a blue colourant. The blueing is characteristic and its light absorption is measured with a spectro photometer at a wave length of 600-750 nm (see under Photometry).



Measuring proteins by means of modified Lowry in microtiter plates

In order to be able to measure the existing traces of protein, they must first be precipitated by the addition of acids and subsequently dissolved in a small volume in order to become concentrated. This precipitation phase also effects a partial cleansing of other substances which hinder the Lowry method.

Advantage:

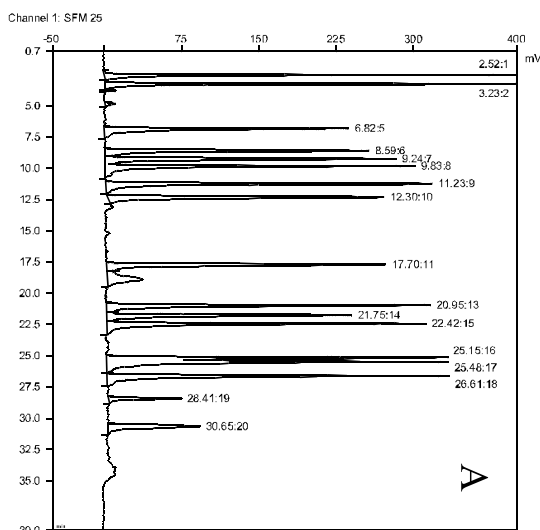
This method for measuring proteins in gloves is prescribed in the European Standard as well as in ASTM and ISO. The method is relatively easy to carry out and is also suitable for routine monitoring during the production process. It furthermore shows an acceptable correlation to the clinical data of prick tests.

Disadvantage:

There are unfortunately a number of chemicals which can interfere with this colour reaction. It has however been shown during the past two years that the presence of interference substances has become less common.

Amino acid analysis by means of HPLC (High Pressure Liquid Chromatography)

With this method the proteins are reduced to the individual amino acids with the aid of hydrochloric acid. These amino acids are then separated with the aid of HPLC and quantitatively determined. The sum total of the individual amino acids equals the protein concentration.



Each peak corresponds to one amino acid. The concentrations can be determined via the peak surface area.

Advantage:

This method has the advantage that it is completely independent of the structure of the proteins and not disturbed by chemical additives. In the above-mentioned European research project amino acid determination showed the best correlation to the prick tests.

Disadvantage:

Unfortunately this complex method is very time-consuming and expensive so that it cannot be used as a routine method.

Immunological Methods

There is also a series of immunological methods for determining latex proteins. With the aid of these methods it is possible to determine allergenic proteins in a more specific manner than with total protein analysis. It has so far been possible to prove the existence of more than 50 different proteins with allergenic characteristics in latex milk. In addition to some proteins, a large number of protein fragments containing the allergenic epitopy (molecule areas, which effect bonding with IgE molecules) are found in the extracts from latex gloves. Because of this large number of allergenically effective proteins and peptides, it is not yet possible to standardise and establish immunological methods in such a way that in all laboratories worldwide the same allergens can be determined in comparable concentrations.

RAST inhibition (Radio Allergo Sorbent Test)

ELISA inhibition (Enzyme-Linked Immuno Sorbent Assay)

With these procedures the methods for determining allergenic-specific IgE antibodies (as described earlier) are employed for measuring latex proteins. A pool from many serums of latex-allergic patients is incubated with the latex extracts (for the production of latex extract see Extraction Procedure). Here the latex allergens from the glove extract bond with the suitable IgE antibodies from the serum. During the subsequent measurement of latex-specific IgE antibodies by means of the methods described earlier (e.g. RAST), the antibodies which have already bonded are no longer recognized and therefore cannot be measured. From the reduction of the specific IgE compared against a specimen which was not treated with glove extract, the concentration of allergens in the extract can be calculated via standard curves.

An allergen solution from the latex milk and/or glove extracts are used as standard. These procedures are suitable for measuring dependent on three components, which are not capable of being standardised at present. These components are:

– **The allergen coupled to the fixed phase.**

It must be ensured that all allergologically effective proteins and peptides which can be present in gloves are effectively bonded to the fixed phase. The allergens which are commercially available differ from manufacturer to manufacturer.

– **The standard for establishing calibration curves.**

The standard must contain all allergologically relevant proteins and peptides.

– **The serum pool.**

The mixture from patient serum must contain IgE molecules in sufficient numbers compared with all relevant proteins and peptides.

Advantage:

Both RAST inhibition and ELISA inhibition are extremely sensitive test methods for ascertaining allergenic latex proteins.

Disadvantage:

Both methods are at present dependent on human serum as the source for IgE antibodies. Furthermore they are time- and money- consuming and therefore unsuitable for routine use in the production process.

LEAP (Latex ELISA for Antigenic Protein)

This procedure developed by Beezhold is an enzyme-immunological method for determining latex proteins. With this procedure the latex proteins from the glove extract are bonded onto the surface of polystyrol micro-

titer plates and subsequently incubated with rabbit- anti-latex antibodies. These antibodies are from rabbits which have been immunised with cleansed latex proteins, and therefore develop IgG antibodies. The quantity of the bonded rabbit anti-latex protein antibodies is made "visible" in that a second antibody (goat anti-rabbit-IgG antibody) is added which reacts with the rabbit antibodies. This second antibody is marked with an enzyme which reacts with discolouration as a result of the addition of a suitable substrate. The colour development is measured by means of spectrophotometry.

Advantage:

This test method is very sensitive. The exclusive use of animal serums frees from dependence on human serums.

Disadvantage:

This test does not measure the allergenic latex proteins, but only those proteins which have caused an immunity reaction in the rabbit (antigen latex proteins). It has the further disadvantage that proteins and peptides with a molecular weight of <10.000 D, are bonded only incompletely to the surface of polystyrol. Only an incomplete ascertainment is therefore possible of the proteins in glove extracts, in which these small molecules form the major part of allergenic proteins.

Brief Glossary of Terms

Antigen:	Foreign matter (e.g. bacteria) which generates antibodies in the human body
Allergen:	Matter which causes an allergy in persons with a corresponding disposition
Antibody:	An antibody formed in the blood serum – as reaction against the penetration of antigens
FEIA:	Fluorescence Enzyme Immuno Assay
Inhibition:	Inhibition, restriction or reduction of chemical processes through inhibitors
Peptide:	Short-chained protein
Casein:	Protein from cow's milk

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