Latex allergy up-dated

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Finland

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Adverse reactions to gloves

Reaction	Mechanism	Typical presentation	Target
Irritant	Non-immune	Dry, crusted lesions	Unknown
Type I (immediate)	IgE-mediated	Contact urticaria	Latex proteins
Type IV (delayed)	Cell mediated	Allergic contact dermatitis	Added chemicals

Latex Allergy

- Immediate hypersensitivity to **natural rubber latex (NRL)** has become increasingly common during the last twenty years and is currently one of the most frequently encountered occupational diseases among the health care workers (HCW)
- Type I NRL allergy is caused by allergenic **proteins** eluting form manufactured rubber products

Milestones in Studies on Latex Allergy

- Contact urticaria from household gloves (Nutter 1979)
- Contact urticaria from surgical gloves (Förström 1980)
- NRL allergy shown to be IgE-mediated disease by Prausnitz-Kustner test (Köpman and Hannuksela 1983)
- Latex-induced anaphylaxis (Turjanmaa et al 1984)
- NRL allergy recognized in the U.S. around 1989 (Slater)
- Cross-reactivity: “Latex-fruit syndrome” (M’Raihi et al 1991, Blanco et al 1994)
- First latex allergen (Rubber elongation factor or REF, Hev b1) characterized at molecular level (Czuppon et al, 1993)
- The importance of prohevein/hevein as a major allergen recognized (Alenius et al 1995, 1996)

Content

- Clinical picture, prevalence and risk groups
- Diagnosis
- Allergens, cross-reactions, allergen standardization
- Prevention
- Conclusions

Symptoms of Latex Allergy

- Vary from mild local reactions to severe systemic reactions
- The most frequently reported manifestation is ***contact urticaria***
- Systemic reactions, e.g. anaphylactic shock, are usually connected to exposure through mucous membranes
- Allergic rhinitis and asthma can occur after inhalation of latex allergens

Main Risk Groups of Type I Latex Allergy

- Health care workers (HCW)
- People with hand dermatitis
- Children with *spina bifida* or other congenital anomalies with histories of multiple surgeries at an early age
- People with allergy to certain tropical fruits (esp. banana, avocado and kiwi)
- Atopic individuals. Note: Almost half of normal young people in the westernised countries are today atopic!

  This implies that 40-50% of normal people react positively in skin prick tests to one or several various environmental allergens. Atopic constitution alone does not allow to stigmatise individuals belonging into a major latex allergy risk group

Prevalence of Latex Allergy in the Mid 1990s Based on Skin Prick Testing

❑ General population	< 1 %
❑ Health care workers	3 - 11 %
❑ Operating room personnel	8 - 10 %
❑ Children with spina bifida	32 - 51 %

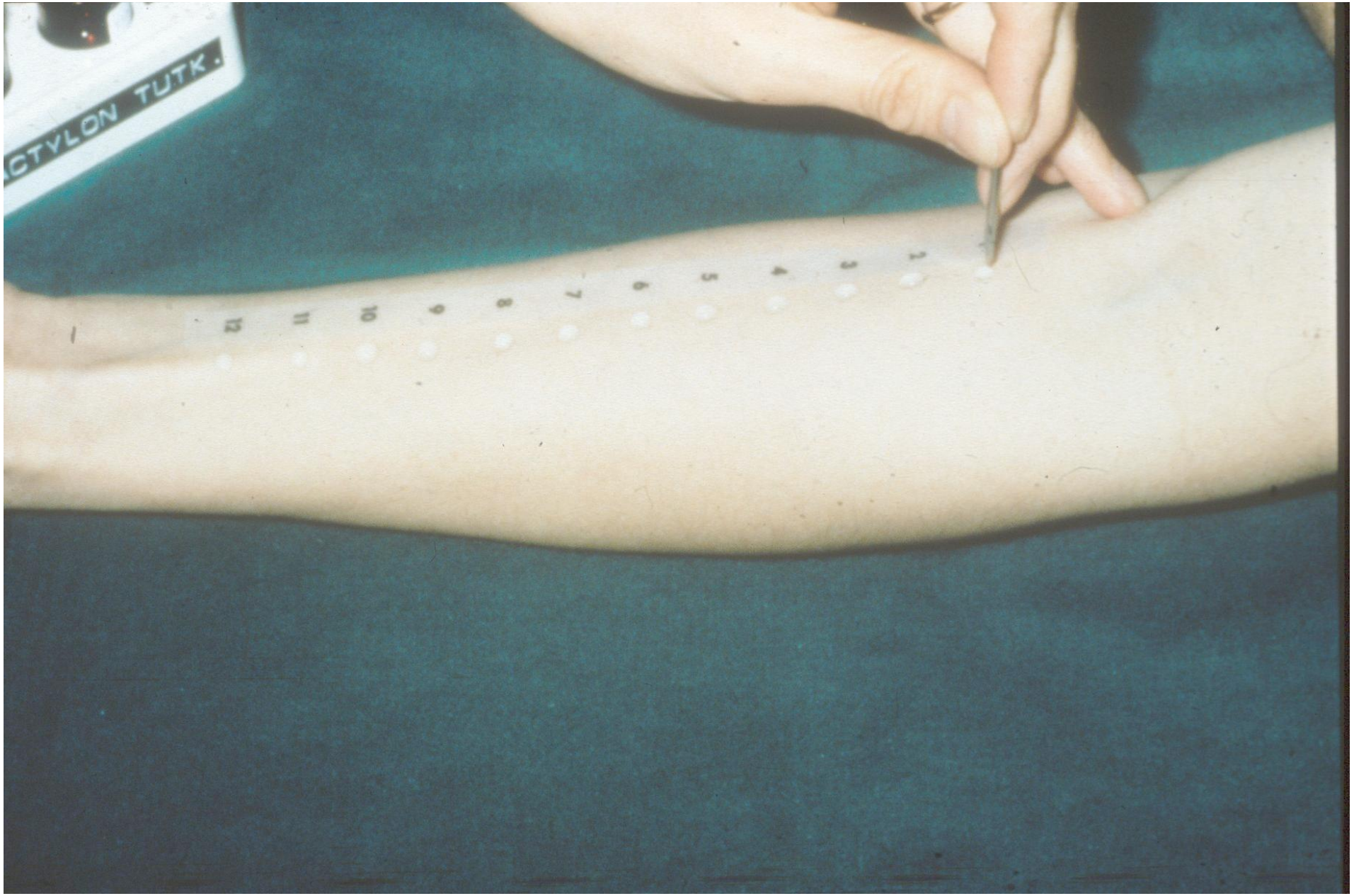
Turjanmaa K., et al., "Natural Rubber Latex Allergy – Review. Allergy 51: 593-602, 1996

Examples of low NRL allergy prevalence among HCWs (reports from countries/centres where the use of low allergen/low protein gloves has been enforced)

Study	Prevalence
Turjanmaa et al, J Allergy Clin Immunol 2002 (Finland)	1.0% (6/603)
Crippa et al, Med Lav (Italian) 2004 (Italy)	1.3% (37/1557)
Zeiss et al, Ann Allergy Asthma Immunol 2003 (USA)	0.6% (25/4339)
Chowdhury & Statham, Br J Dermatol 2003 (UK)	0.56% (11/1959)
Poole & Nagendran, Occup Med 2001 (UK)	0.5% (17/3500)

Diagnosis of NRL allergy

Method	Comment
Clinical history	Will not identify all allergic patients
Skin prick testing	Rapid and safe; commercial allergens available; sensitivity >90%
Glove use or challenge tests (skin, lungs)	Highly allergenic glove brand required; potentially dangerous; emergency treatment facilities needed
In vitro tests (RAST, AlaSTAT, etc.)	Commercial tests available; sensitivities and specifities variable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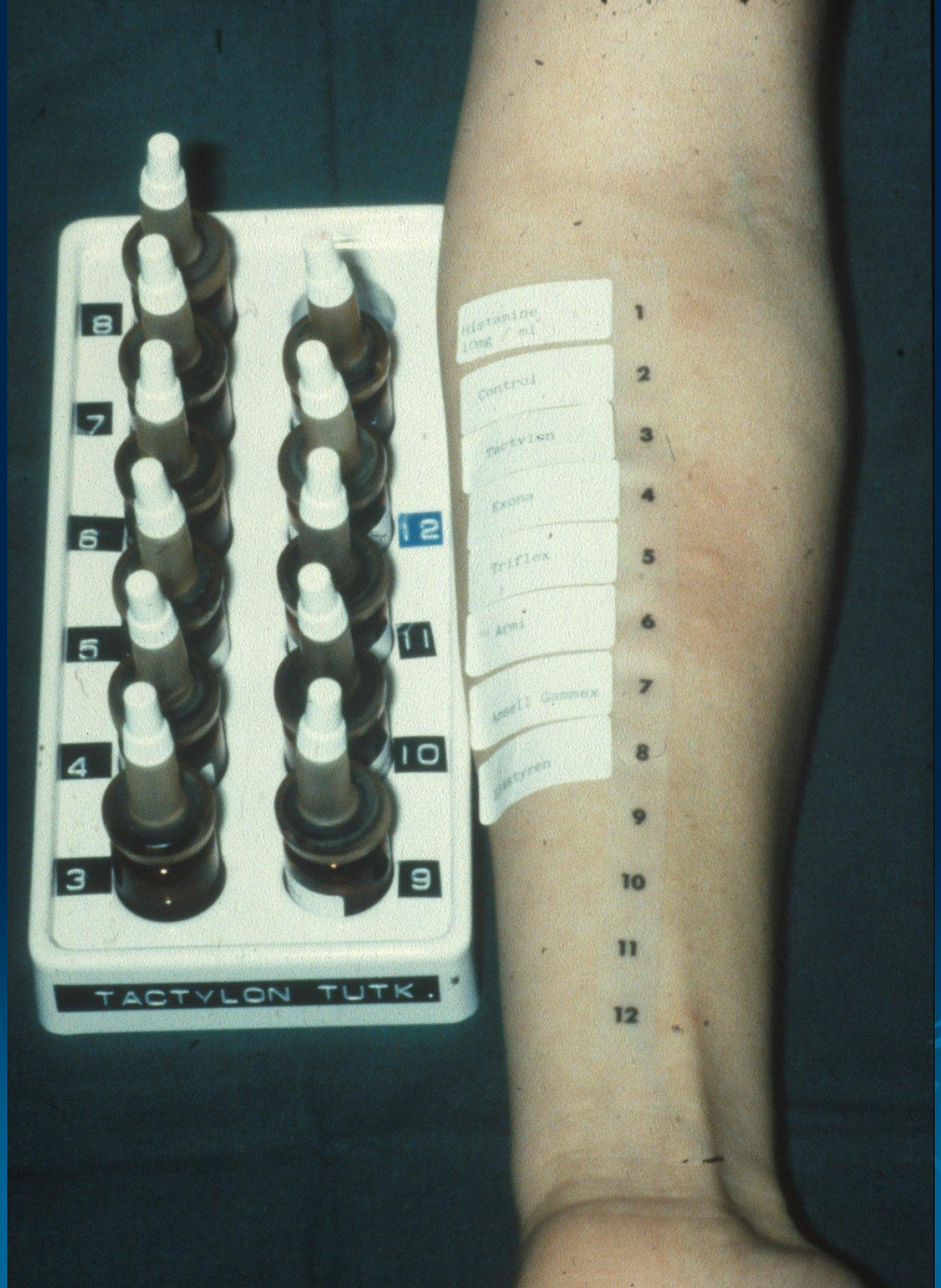
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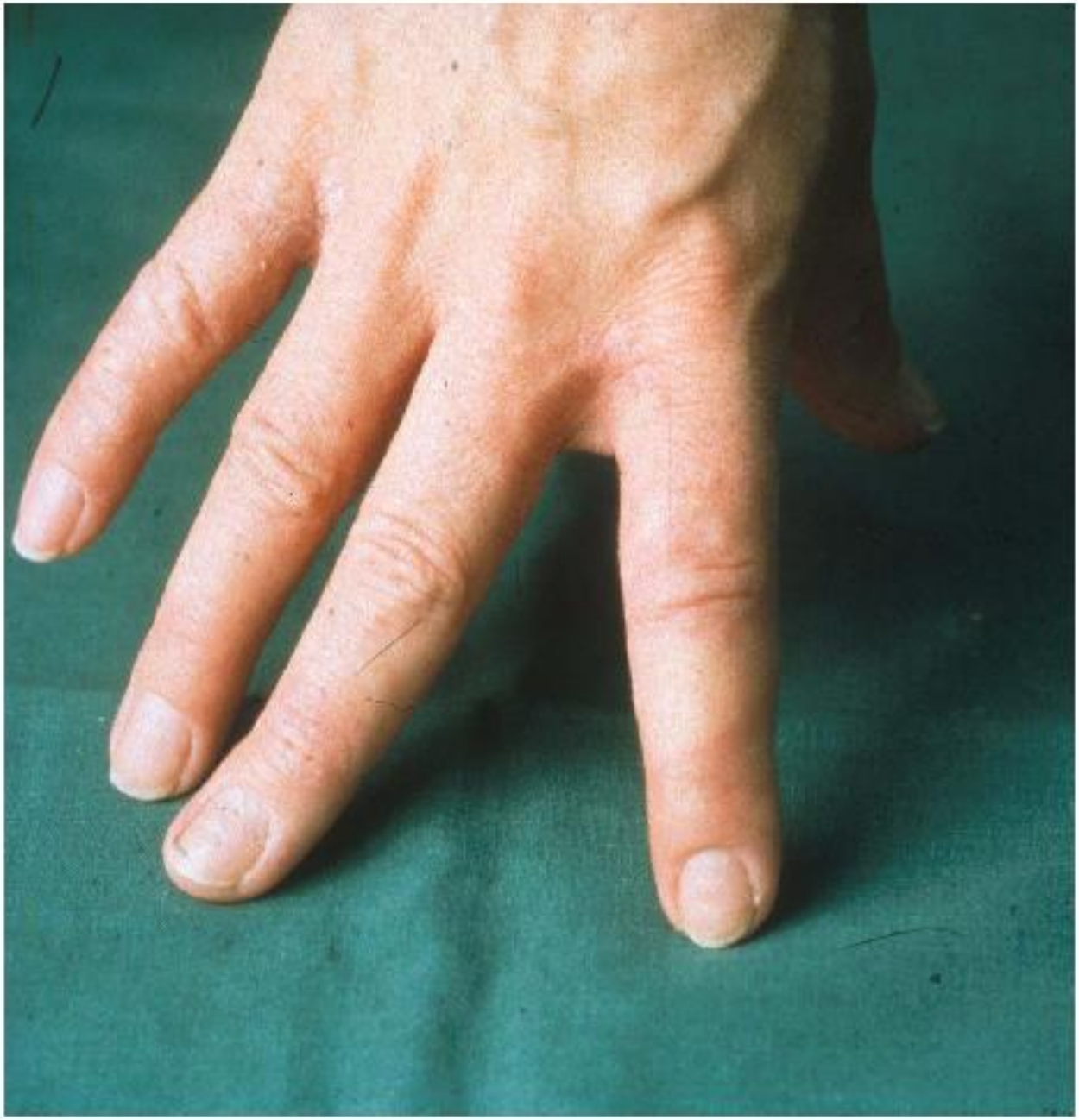


What is the difference between "sensitisation" to latex and "latex allergy"

Sensitisation: Positive IgE result in a latex serological test or latex skin prick test but no compatible histories or symptoms on exposure to latex products

Allergy (Clinical allergy): Positive IgE test results and, upon exposure to latex products, typical allergic symptoms, such as contact urticaria, rhinitis or asthma,

Latex of the Rubber Tree
(Hevea brasiliensis)

34% polyisoprene (rubber)

1% - 2% proteins

0.4% fatty acids

1% resins

0.6% ash

1.4% sugar

60.0% water

Latex Allergens

- Liquid latex of the rubber tree, *Hevea brasiliensis*, contains a large selection of plant proteins, **many** of which belong to so-called ***stress (defense) proteins***
- The WHO/IUIS Allergen Nomenclature Sub-Committee currently lists 13 latex allergens characterized at the molecular level (www.allergen.org)

Latex Allergens, Nomenclature 2008

- Hev b 1 (Rubber elongation factor; REF)
- Hev b 2 (Endoglucanase)
- Hev b 3 (23-27 kDa rubber particle protein)
- Hev b 4 (Microhelix protein complex)
- **Hev b 5 (Acidic 16 kDa protein)**
- **Hev b 6.01 (Prohevein, hevein preprotein)**
- **Hev b 6.02 (Hevein, mature hevein)**
- Hev b 7 (Patatin-like Hevea protein)
- Hev b 8 (Hevea profilin)
- Hev b 9 (Hevea enolase)
- Hev b 10 (Manganese superoxide dismutase)
- Hev b 11 (Hevea endochitinase)
- Hev b 12 Hevea Lipid transfer protein (LTP)
- Hev b 13 (Esterase) Early nodulin specific protein

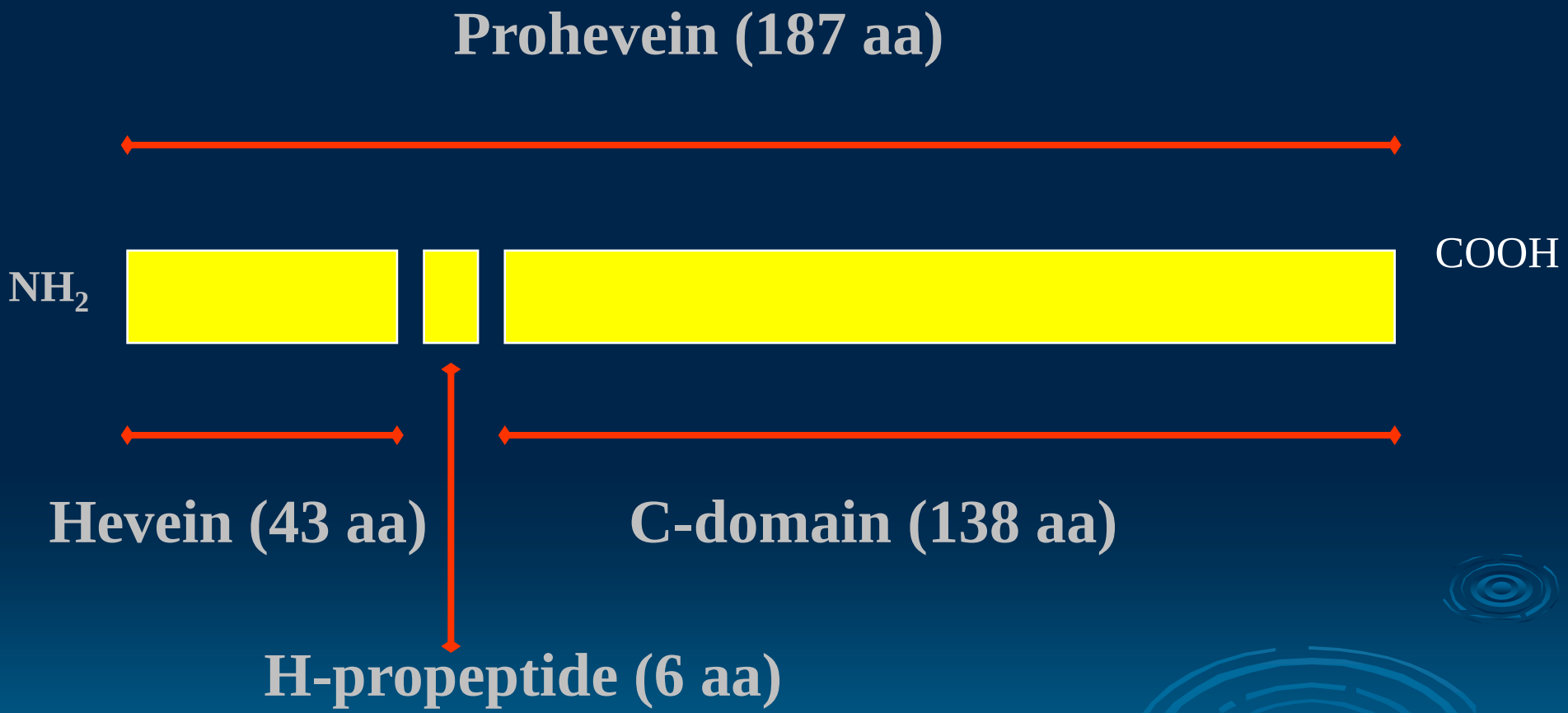
Allergens Demonstrated in Extracts of Latex Gloves

- **Hev b 1** (Czuppon et al 1993, Raulf-Heimsoth et al 2001, Palosuo et al 2002)
- **Hev b 3** (Lu et al 1995, Palosuo et al 2002)
- **Hev b 5** (Akasawa et al 1996, Sutherland et al 2002, Palosuo et al 2002)
- **Hev b 6.02** (Alenius et al 1996, Palosuo et al 2002)
- Hev b 2 (Yeang et al 2004)
- Hev b 13 (Yeang et al 2004, Lee et al 2006)

Hevein (Hev b 6.02) is the most important NRL allergen, abundantly present in virtually all high allergen gloves

Hev b 5 is the second important allergen for latex-allergic adults

Molecular Composition of Prohevein, a Major Latex Allergen



General Conclusions on Latex Allergens

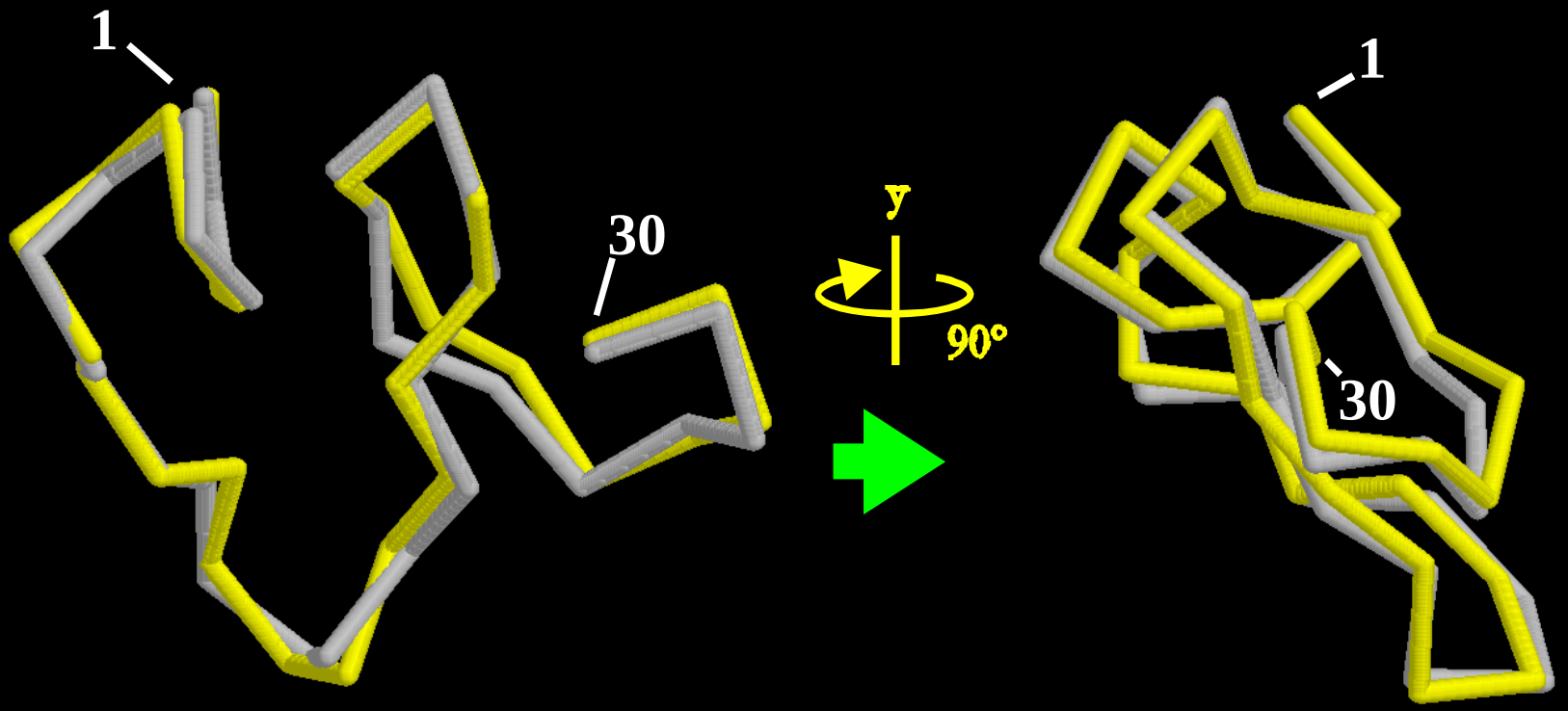
- At present, it appears that most (if not all) clinically relevant latex allergens have been identified and characterized
- Some 4-6 latex allergens may be responsible for the majority of the clinical symptoms of latex allergy
- When the significance of “new” allergens is assessed, their purity should be rigorously controlled

Cross-Reactions

Latex - Fruit -Syndrome

- Due to alleged allergen cross-reactions, latex-allergic patients frequently (up to 50%) show allergy to various fruits, such as banana, avocado, chestnut, and kiwi
- A considerable part of the cross-reactivity between latex and various fruits is based on structural homology between hevein and hevein-like domains of plant class I chitinases

Structural homology between hevein, major latex allergen, and hevein-like protein from banana



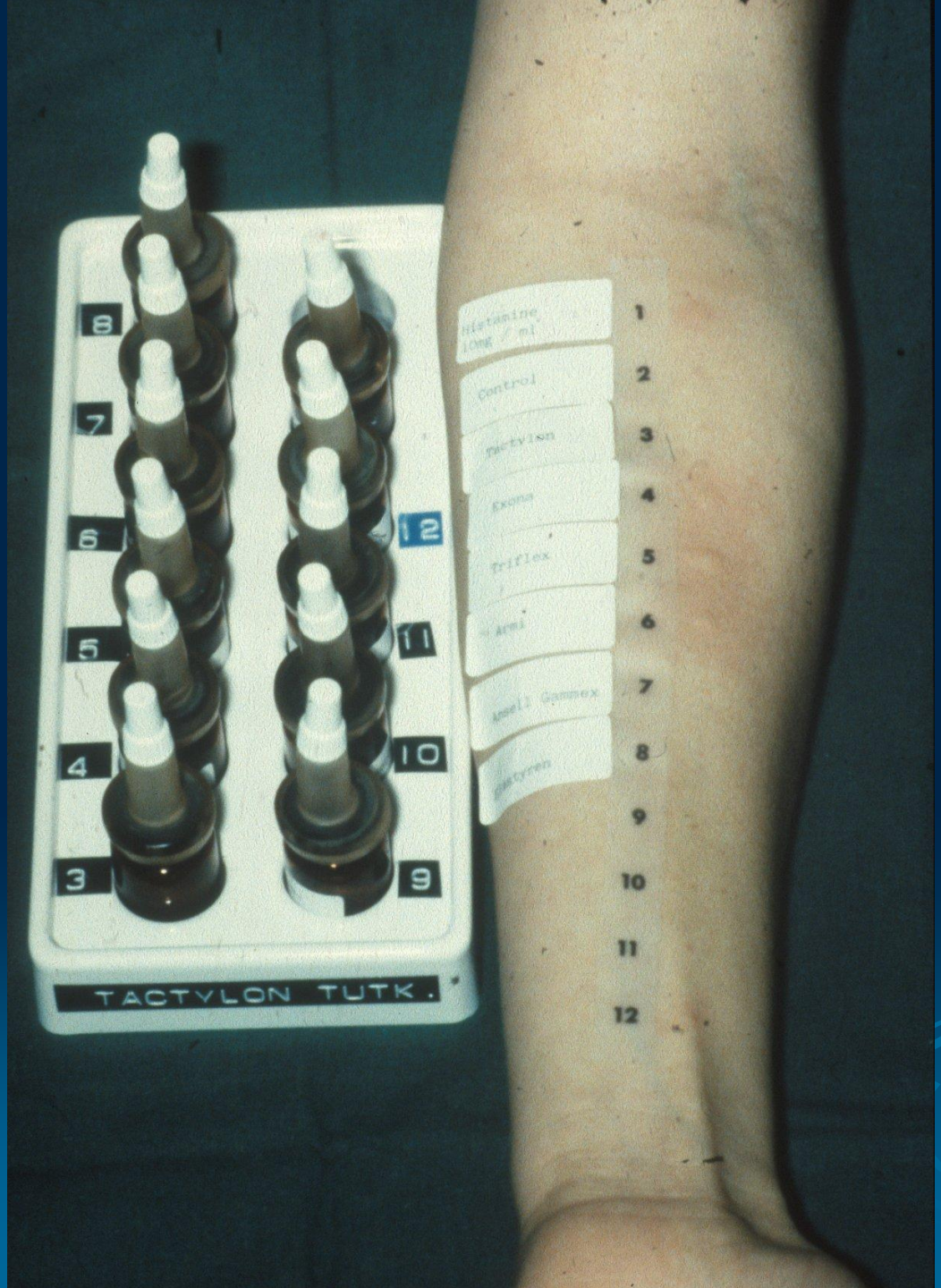
hevein

N-terminus of 32 kD banana protein

Allergens in Latex Gloves

Available Methods to Measure Allergenic Proteins in Extracts of Latex Gloves

- Skin prick tests in voluntary latex allergic patients
- IgE -immunoblotting
- RAST-inhibition and IgE ELISA-inhibition methods
- *In situ* localisation of allergens by immunogold electron microscopy
- ***Capture EIA methods with monoclonal antibodies to specific allergenic proteins***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Triflex
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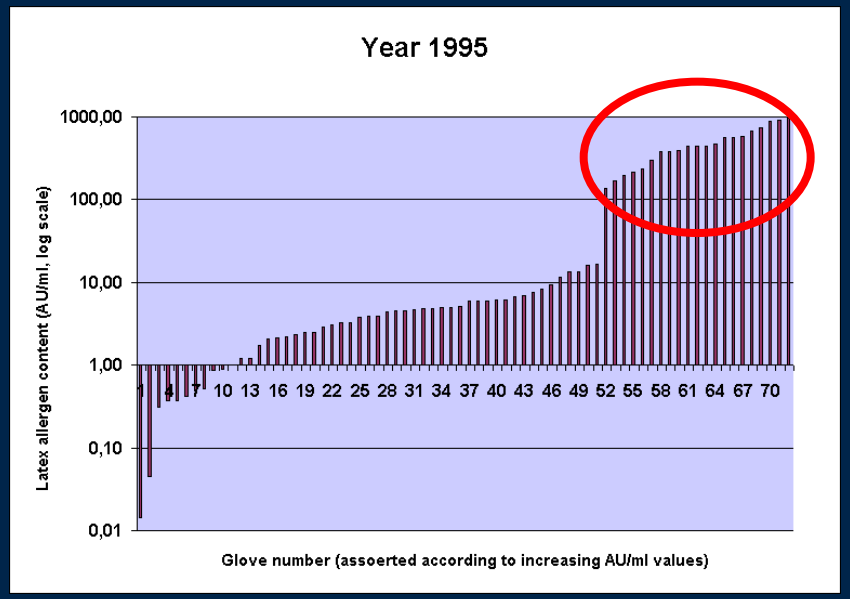
Natural rubber allergen content of latex gloves

A market surveillance study 2003

TLT-info 1/2003

www.nam.fi/english/publications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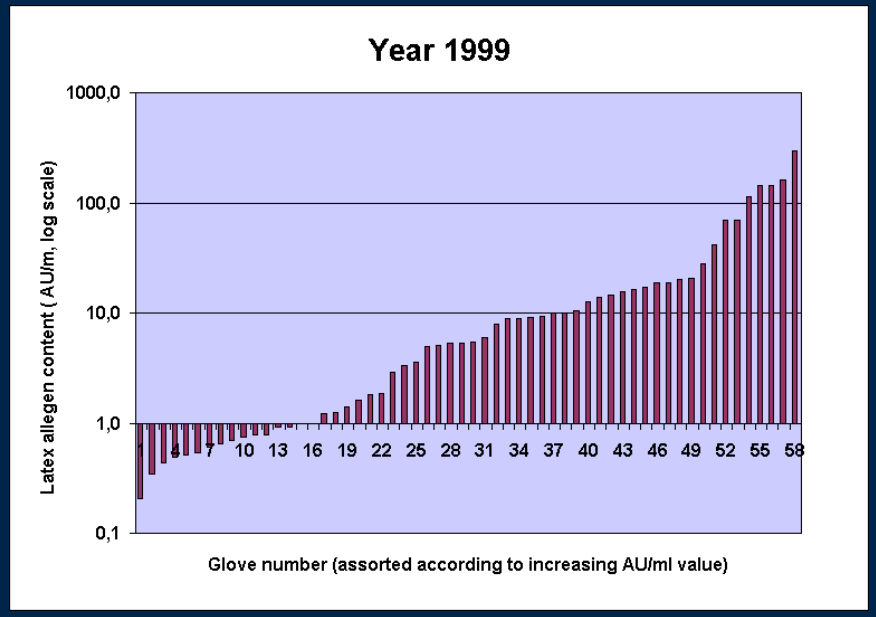
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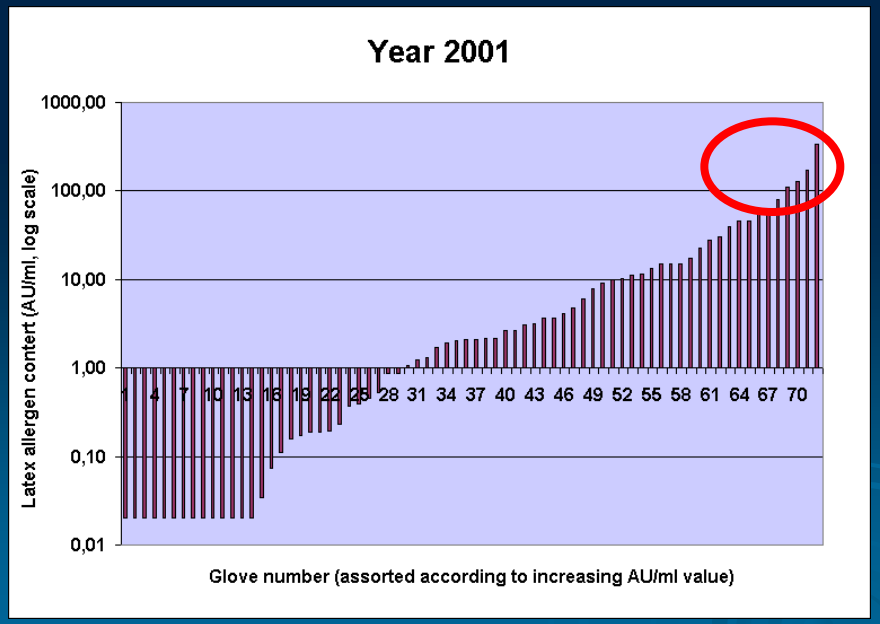
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Latex allergen content (AU/ml, log scale)
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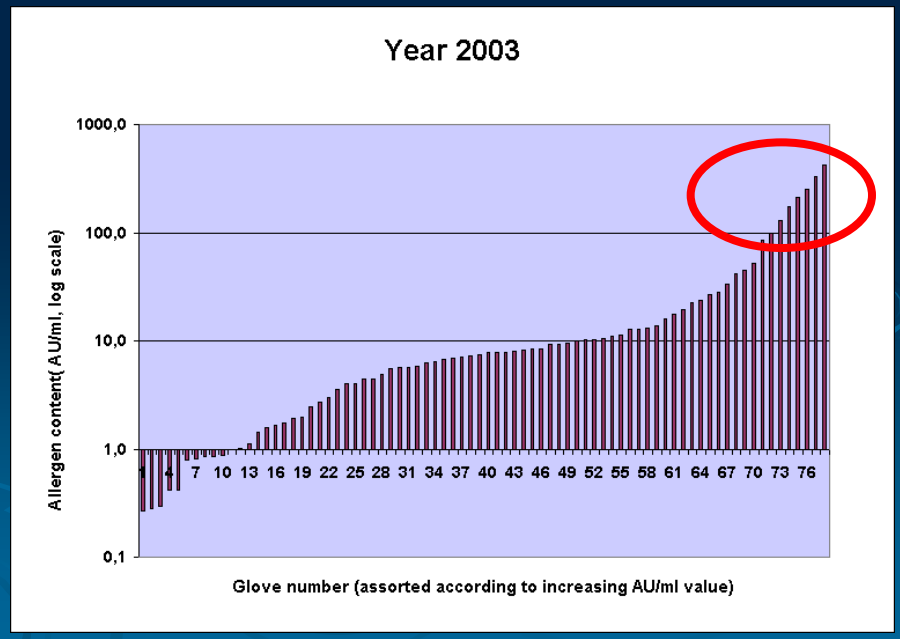
Glove number (assorted according to increasing AU/ml value)


Year 2003
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Allergen content(AU/ml, log scale)
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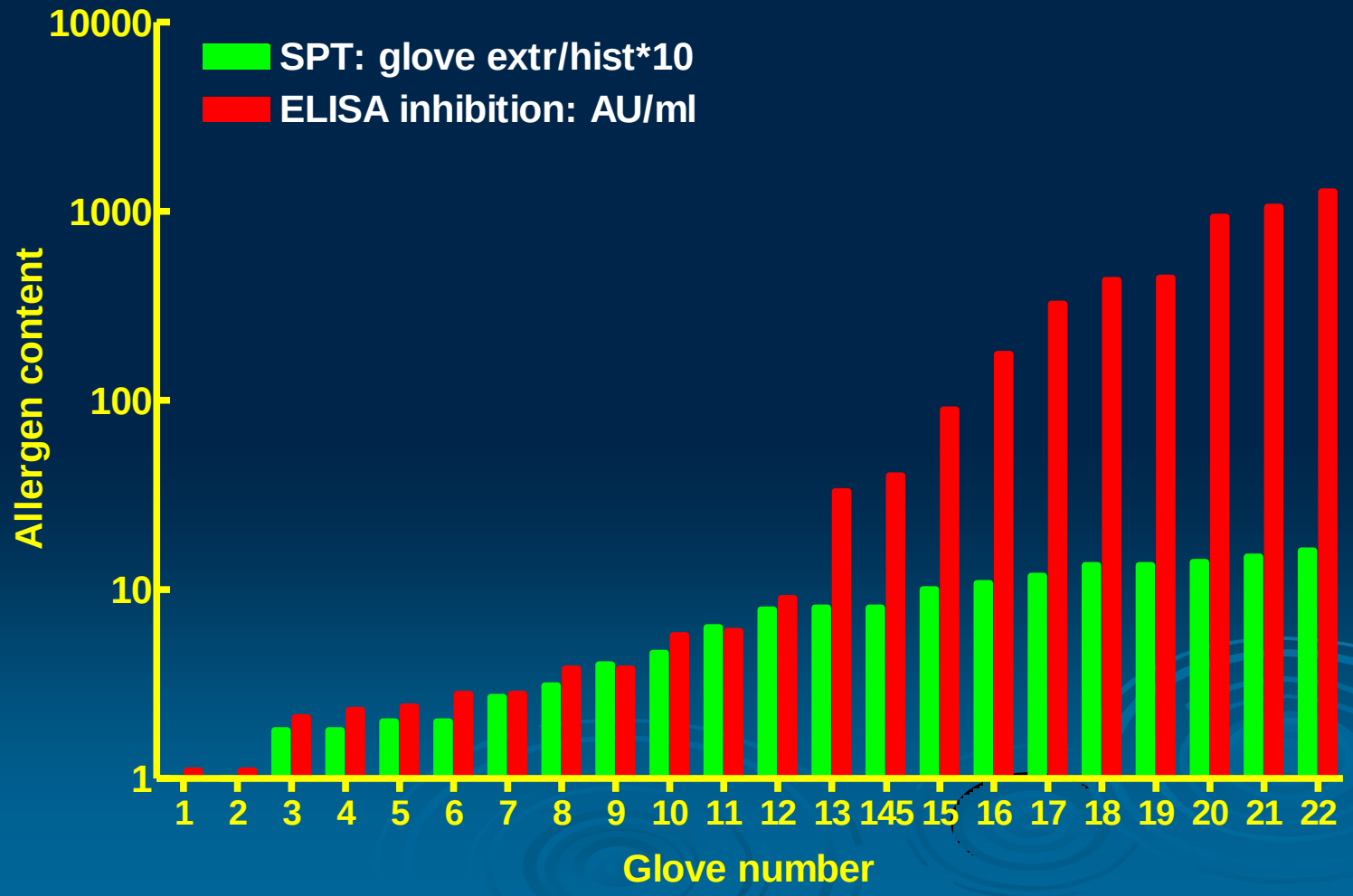
Glove number (assorted according to increasing AU/ml value)

SPT reactivity and total allergen level by IgE ELISA inhibition in 22 gloves (1994)

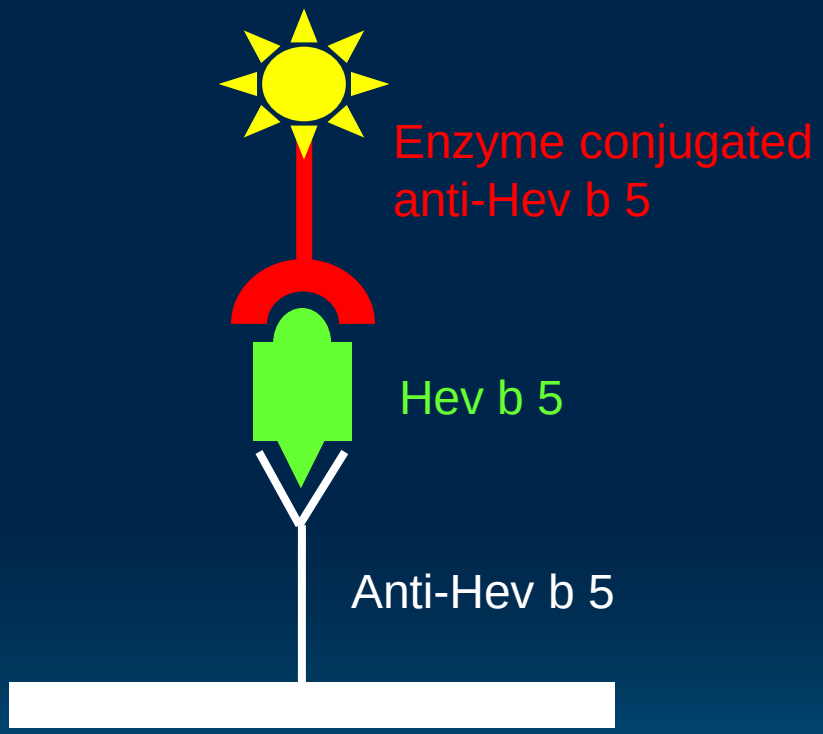


Capture-EIAs for the Measurement of Latex Allergens

- Capture EIAs were developed by FITBiotech* to quantify the levels of four allergens, Hev b1, Hev b3, Hev b5 and Hev b6.02 in extracts of latex products
- Monoclonal and polyclonal antibodies and respective purified recombinant or native allergens were used as the key reagents
- Kits were launched in December 2001

**Principal investigator in FITBiotech: Tytti Kärkkäinen*

Capture-EIAs) for the Measurement of Latex Allergens*

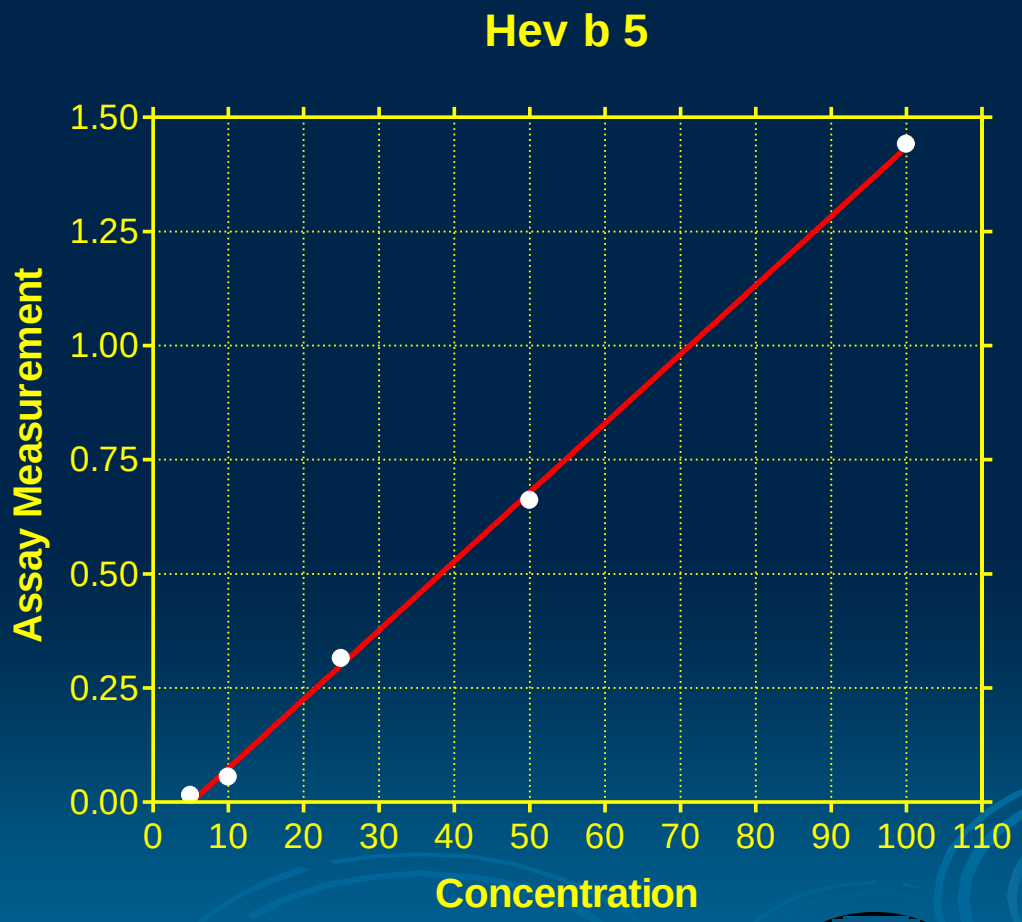


Monoclonal antibodies and recombinant allergens required

*) IEMA (Immunoenzymetric assay)

Example

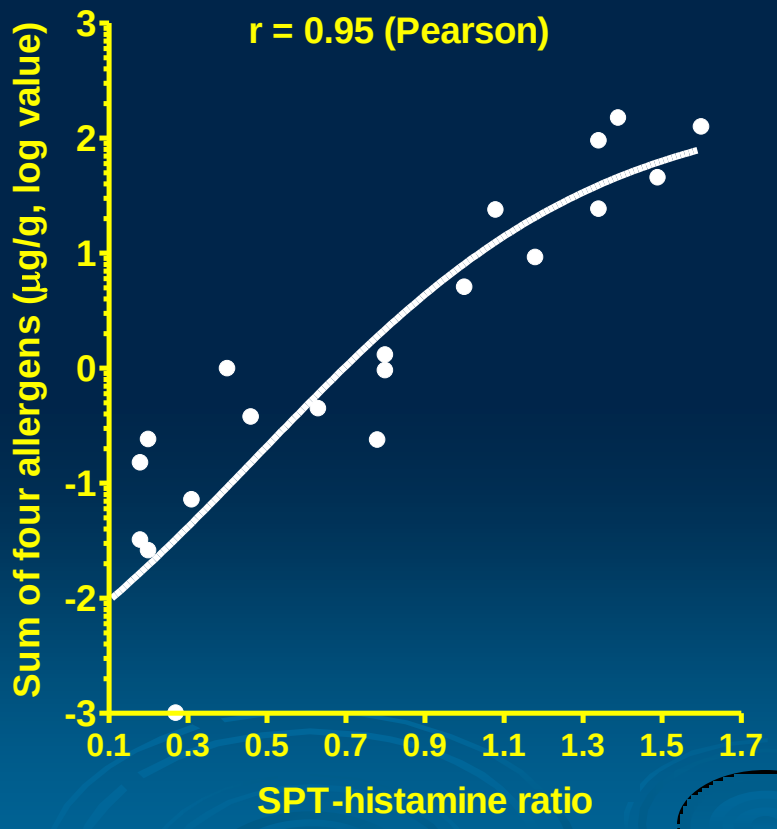
Hev b 5 standard curve



Measurement of Specific Allergen Levels of Latex Gloves

22 latex gloves, collected in 1994-95 in Finland, and previously analyzed for allergen activity by SPT and ELISA-inhibition, (Palosuo T et al. Allergy 1998;53:59-67), were examined for the presence of the four specific latex allergens

Correlation between the sum of four allergens in 22 glove extracts and SPT reactivity in 20 latex allergic patients



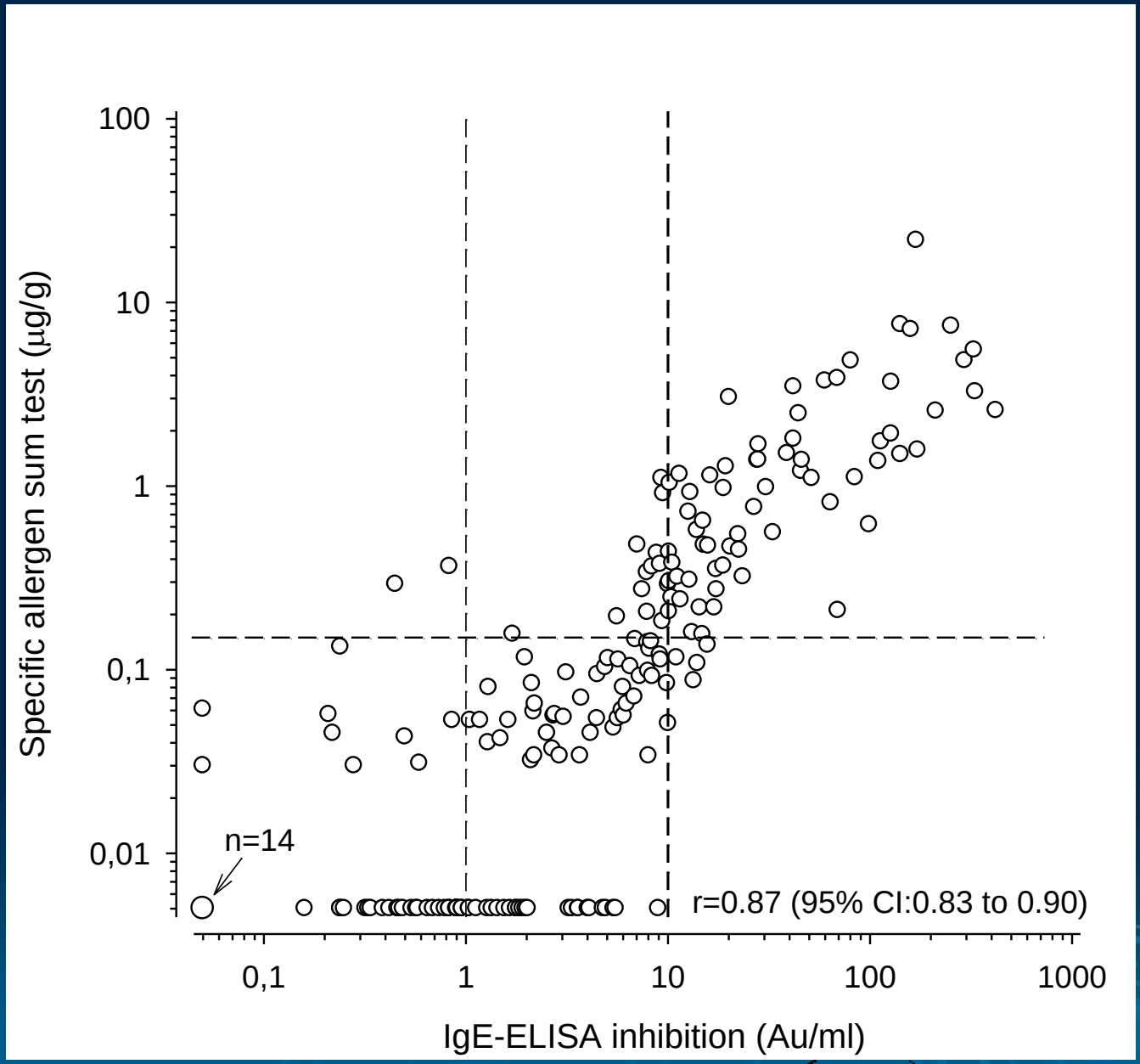
Palosuo T et al, Methods 2002, 27:52-58

Latex allergy: The sum quantity of four major allergens shows the allergenic potential of medical gloves

Timo Palosuo, Hely Reinikka-Railo, Hannu Kautiainen, Harri Alenius, Nisse Kalkkinen, Markku Kulomaa, Timo Reunala, Kristiina Turjanmaa

National Public Health Institute, Helsinki
National Agency of Medicines, Helsinki
Rheumatism Foundation Hospital, Heinola,
Institute of Biotechnology, University of Helsinki
Department of Dermatology, Tampere University and University Hospital
Finnish Institute of Occupational Health, Helsinki
Department of Biological and Environmental Science, University of Jyväskylä

Allergy 2007:62:781-786



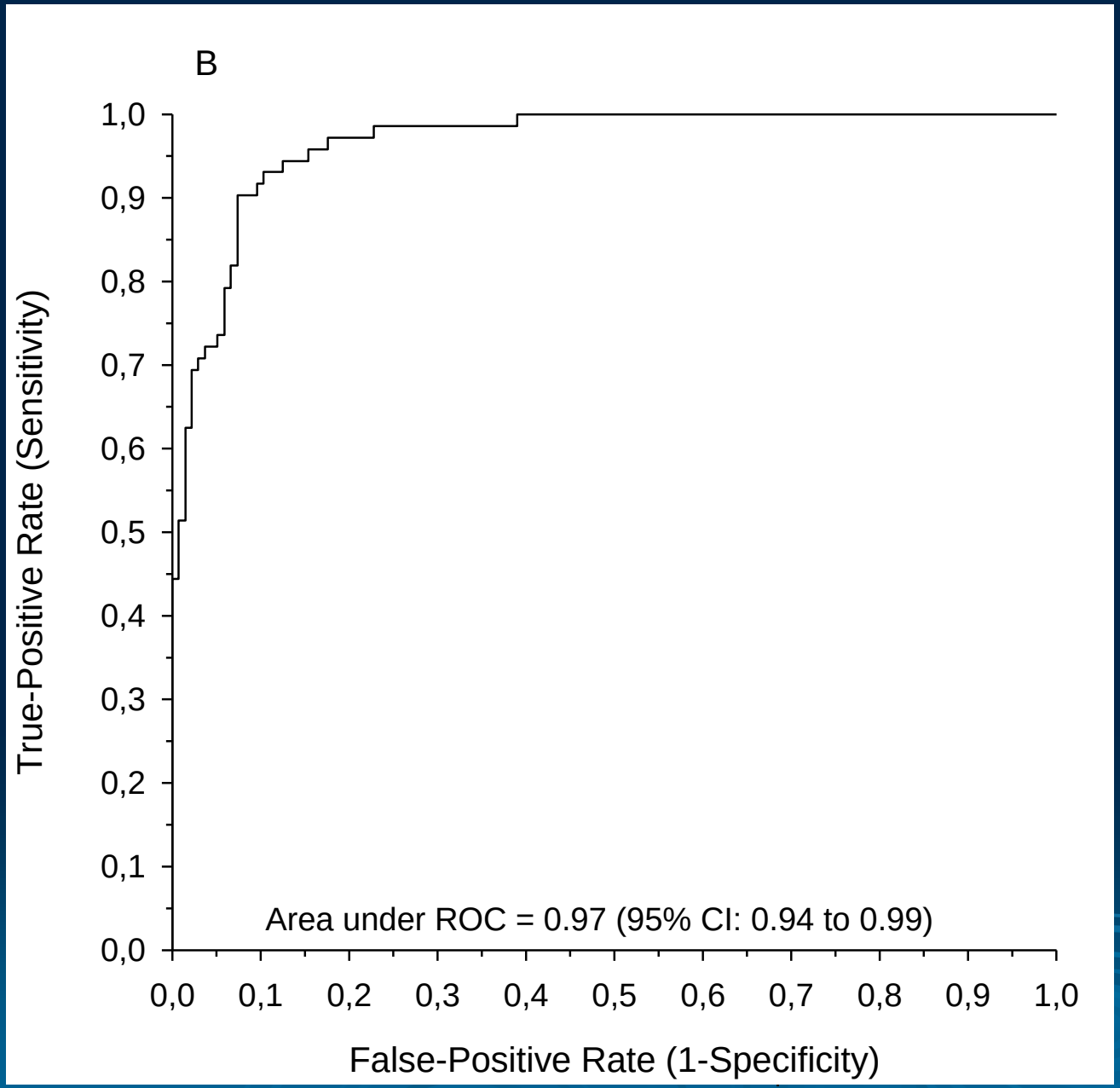
Palosuo T et al, Allergy 2007

Specific allergen sum test

IgE-ELISA inhibition	Cut-off point	Sensitivity	Specificity	PPV†	NPV‡	LR+*
<10 AU/ml	<0.03	1.00 (0.95 to 1.00)	0.49 (0.41 to 0.58)	0.51 (0.43 to 0.60)	1.00 (0.95 to 1.00)	1.97 (1.68 to 2.34)
	<0.15	0.93 (0.85 to 0.98)	0.90 (0.83 to 0.94)	0.83 (0.73 to 0.90)	0.96 (0.91 to 0.99)	9.04 (5.60 to 14.98)
	<0.30	0.40 (0.29 to 0.52)	0.94 (0.89 to 0.97)	0.78 (0.62 to 0.90)	0.75 (0.68 to 0.81)	6.85 (3.38 to 14.06)
	<1.15	0.42 (0.30 to 0.54)	1.00 (0.97 to 1.00)	1.00 (0.88 to 1.00)	0.76 (0.69 to 0.82)	NA

† Positive predictive value.
‡ Negative predictive value.
* Likelihood ratio for positive result. (ratio of the chance of a positive result if the glove is allergenic (“sick”) to the chance of a positive result if glove is not allergenic (“sick”)

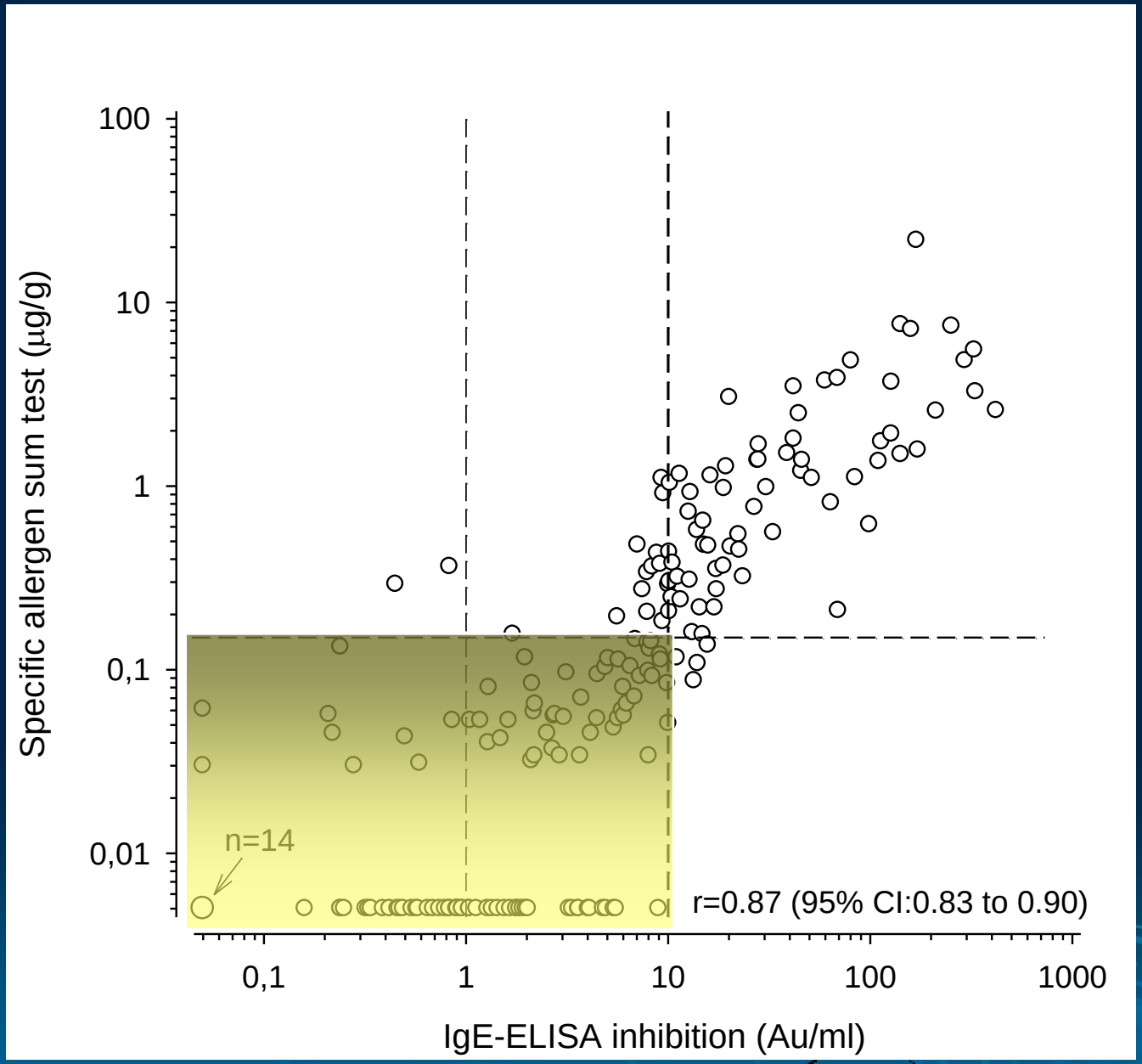
Palosuo T et al, Allergy 2007



Palosuo T et al, Allergy 2007

Main conclusion of the four latex allergen sum test

When the sum of the four allergens is below 0.15 µg/g, the latex glove is highly likely to be “low allergenic”: the result is comparable to that seen in human IgE inhibition or skin prick testing



Palosuo T et al, Allergy 2007

The American Society of Testing and Materials (ASTM) has adopted the immunoenzymetric assay (IEMA) methods as a new ASTM Standard (August 2008)*

* **Designation D 7427-08**

Standard Test Method for Immunological Measurement of Four Principal Allergenic Proteins (Hev b1, 3, 5 and 6.02) in Natural Rubber and its Products Derived from Latex

Total protein assays (1)

- Total extractable protein determination by Modified Lowry's technique has been widely used for the indirect (rough) estimation of allergen content in NRL products

- The amount of extractable protein, especially in the high concentration range, has correlated relatively well with the allergen content of NRL gloves measured by skin prick test (SPT) or human IgE-based assays

Total protein assays (2)

- Total protein methods do not discriminate between allergenic and non-allergenic proteins

- At low protein concentrations the Lowry assay is of insufficient sensitivity *(e.g., Audo C, et al, Med Sci Monit 2004;10:PI 81-86)*

- Available standards for measuring leachable proteins: CEN: prEN 455-3, ASTM: D5712, D6499-03

Prevention

Principles of Prevention

- ***Primary prevention*: prevent sensitization (lower the allergen levels in latex products and avoid unnecessary exposure)**

- ***Secondary prevention*: prevent clinical allergy (in the already sensitized subject): avoid exposure to allergenic products)**

Can exposure to latex allergens be lowered?

- Yes, by controlling the levels of extractable allergens in manufactured products
- Ideally, no allergens should be detectable in latex products
- Problem: it has not been possible to establish any safety limits

Prevalence of Latex Allergy in Glove Using Hospital Personnel at Tampere University Hospital, Tampere, Finland

1987:	**15/512**	**(2.9%)**
1999:	**6/603**	**(1.0%)**

During the follow-up only low allergen gloves were accepted in the hospital

Turjanmaa K et al, J Allergy Clin Immunol 2002

When is it mandatory to use non-latex gloves in medical work?

- When choosing gloves for HCWs with symptomatic, properly diagnosed latex allergy (and sometimes to their co-workers)
- When gloves are selected for HCWs to be used in surgical or invasive procedures on latex-allergic patients
- Always when treating children with spina bifida or other congenital anomalies requiring multiple surgical procedures

"Safety" issue in relation to the four principal latex allergens (1)

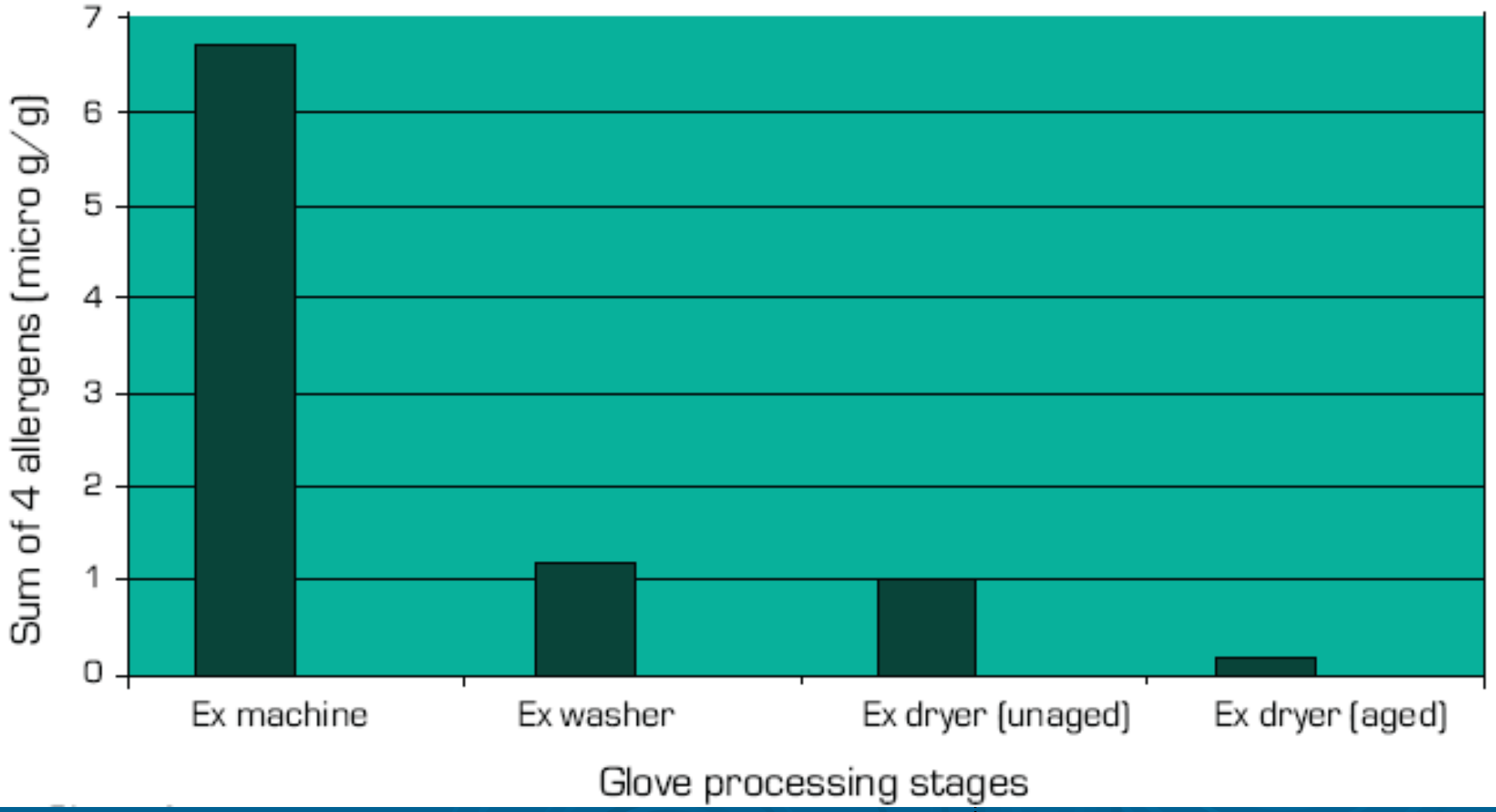
- Absolute safety referring to potential allergy risk cannot be guaranteed to results from any assay (even if all known allergens are tested), since:

- In principle, any protein can be allergenic and currently unknown allergens may be present in the investigated product, and, there is wide individual variation in peoples' immune reactivity and immune responses

"Safety" issue in relation to the four principal latex allergens (2)

- The four allergen sum test seems to provide today the most reliable means to ensure the lowest practicable latex allergen levels (ALARP principle) in NRL products
- Low allergen concentrations (sum < 0.15 µg/g) enable the selection of gloves that for most practical purposes can be considered as "virtually safe" .

Effects of post-processing stages on allergen content of gloves



FIT BIOTECH

CERTIFICATE OF ANALYSIS

FITkit TESTING SERVICES

Customer: Berner

Sample/s: Ansell Gammex PF Hydrasoft, powder free latex surgical gloves with moisturiser

Lot No: 0504450404

FIT code: GL 5149

Arrival date: 14.06.2005

Test Results

Hev b 1 µg/g	Hev b 3 µg/g	Hev b 5 µg/g	Hev b 6.02 µg/g	Total µg/g
ND (not detected)	ND	ND	ND	ND

17th of June 2005

Ioana Stanescu, VP Operations
Quality & Regulatory Affairs

Note: For research use only. Not for diagnostic purposes.

Report QA05154

FIT Biotech Oyj Plc.
Lenkkeilijänkatu 10
33520 Tampere
Finland
Tel. +358 3 3138 7000
Fax +358 3 3138 7050
www.fitbiotech.com

BUSINESS IDENTITY CODE FI0538 118 5-1

Conclusions (1)

- Diagnosis of NRL allergy is still often problematic, especially in the USA, due to the lack of standardized skin prick test reagent, and lack of high allergenic gloves for challenge test

- The number of new cases of IgE-mediated (Type 1) NRL allergy is decreasing in westernized countries: the peak of the ”epidemic” appears to be passed

- Some 4-6 latex allergens may be responsible for the majority of the clinical symptoms of latex allergy

Conclusions (2)

- Allergenic potential of latex gloves can today be measured with remarkable accuracy (high specificity and sensitivity) by means of a recently standardised IEMA method (ASTM D7427).
- Low allergen latex gloves (four allergen sum < 0.15 µg/g) pose minimal allergy risks to the general population and even to HCWs regularly exposed to gloves in occupational settings
- Low allergen gloves are made by several rubber manufacturers and are readily available

Conclusions (3)

- Knowledge about allergen contents in gloves on the market seems to have a marked impact on glove selecting policies

- Primary prevention of latex allergy among health care workers is effective in many countries where health authorities inform users and regulate glove quality

- For specific immunotherapy, promises are seen in modified “hypoallergenic” proteins or polypeptides

Acknowledgements

- Timo Palosuo
- Harri Alenius
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- Kari Airenne
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