

No conflicts of interest

# Diagnosis of allergic contact dermatitis in 2013

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# Diagnosis of allergic contact dermatitis in 2013

- Clinical diagnosis
- Human skin testing
- In vitro testing



# Diagnosis of allergic contact dermatitis in 2013

- Clinical diagnosis
- Human skin testing  
→ patch testing
- In vitro testing

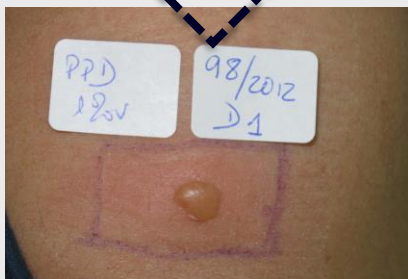
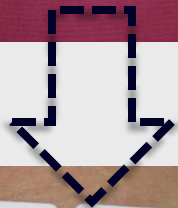
# Clinical diagnosis

- Typical ACD – acute eczema
- “atypical” ACD
  - different patterns of contact hypersensitivity
- ACD mixed with related dermatitis
- ACD complicating other dermatosis





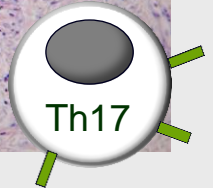
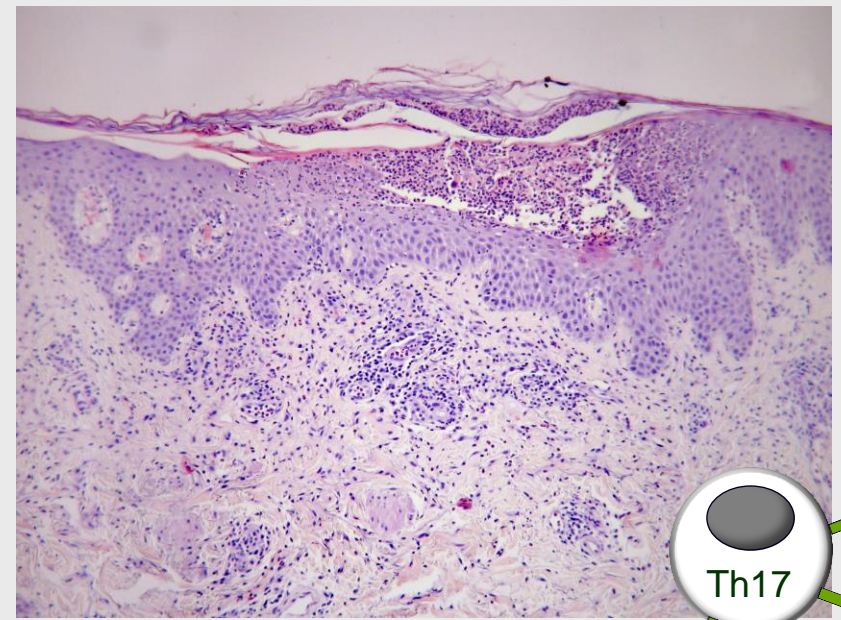
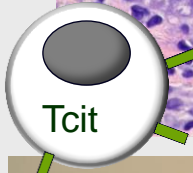
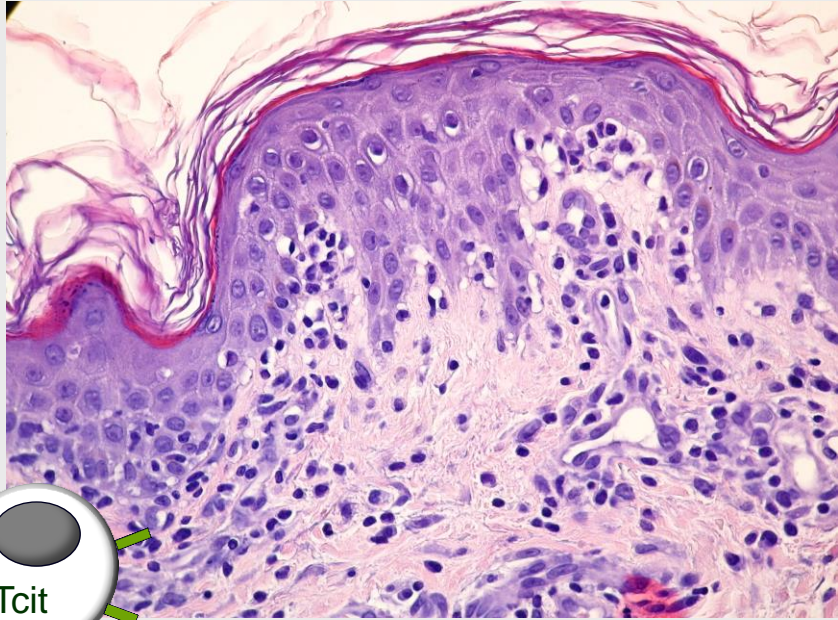
# Clinical diagnosis



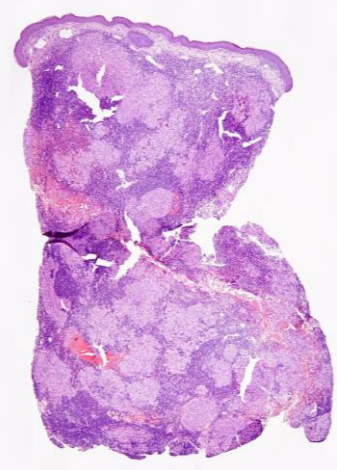
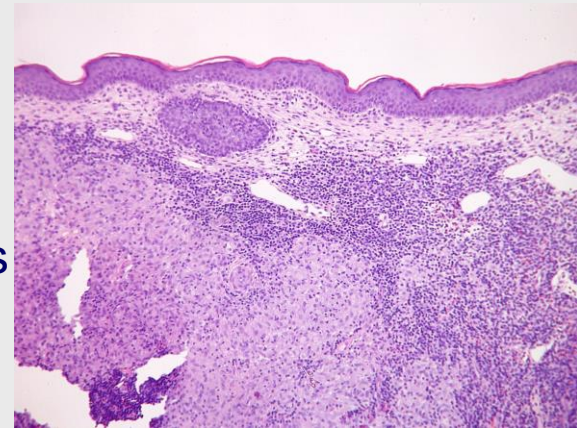
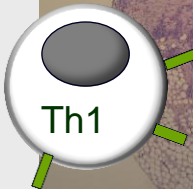
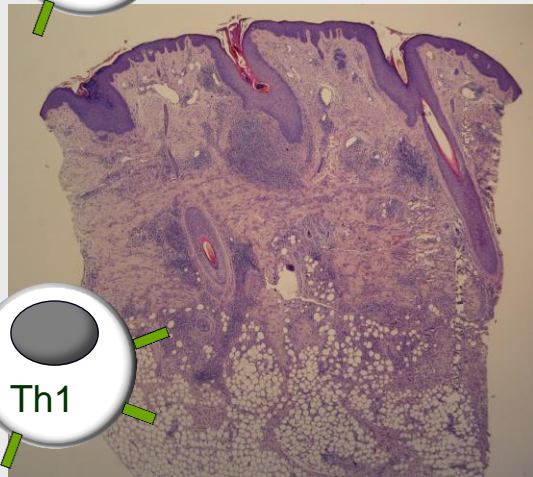
Different patterns of allergic contact reactions



# Contact eczema



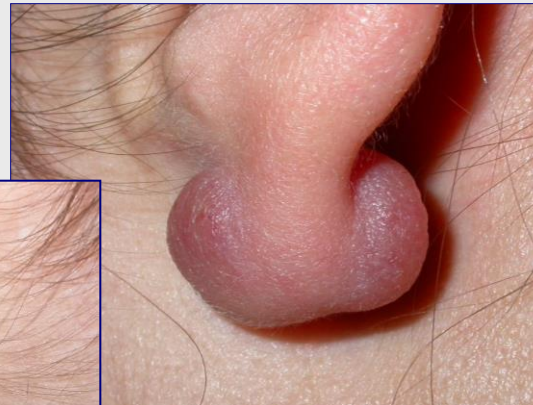
Cytotoxic  
Pustular  
Lymphomatoid  
Granulomatous  
Lichenoid





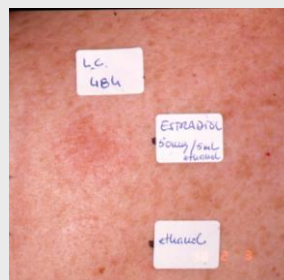
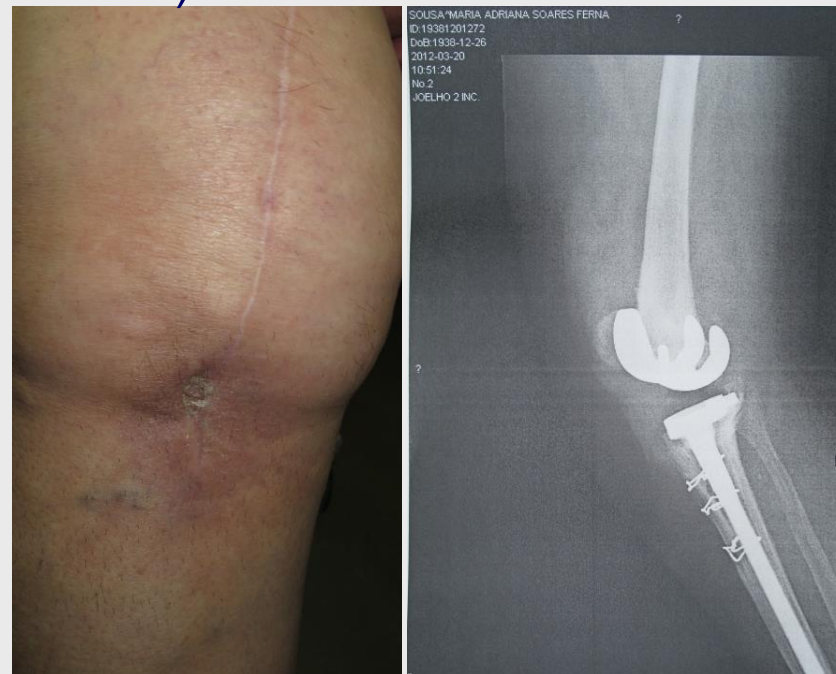
# “atypical” ACD

- Granulomatous reaction from palladium





# “atypical” ACD (from inside)







ACD is not only eczema ...

ACD has many “faces” ...

**Fernando Pessoa**

Álvaro de Campos

Ricardo Reis

Alberto Caeiro

Bernardo Soares

....

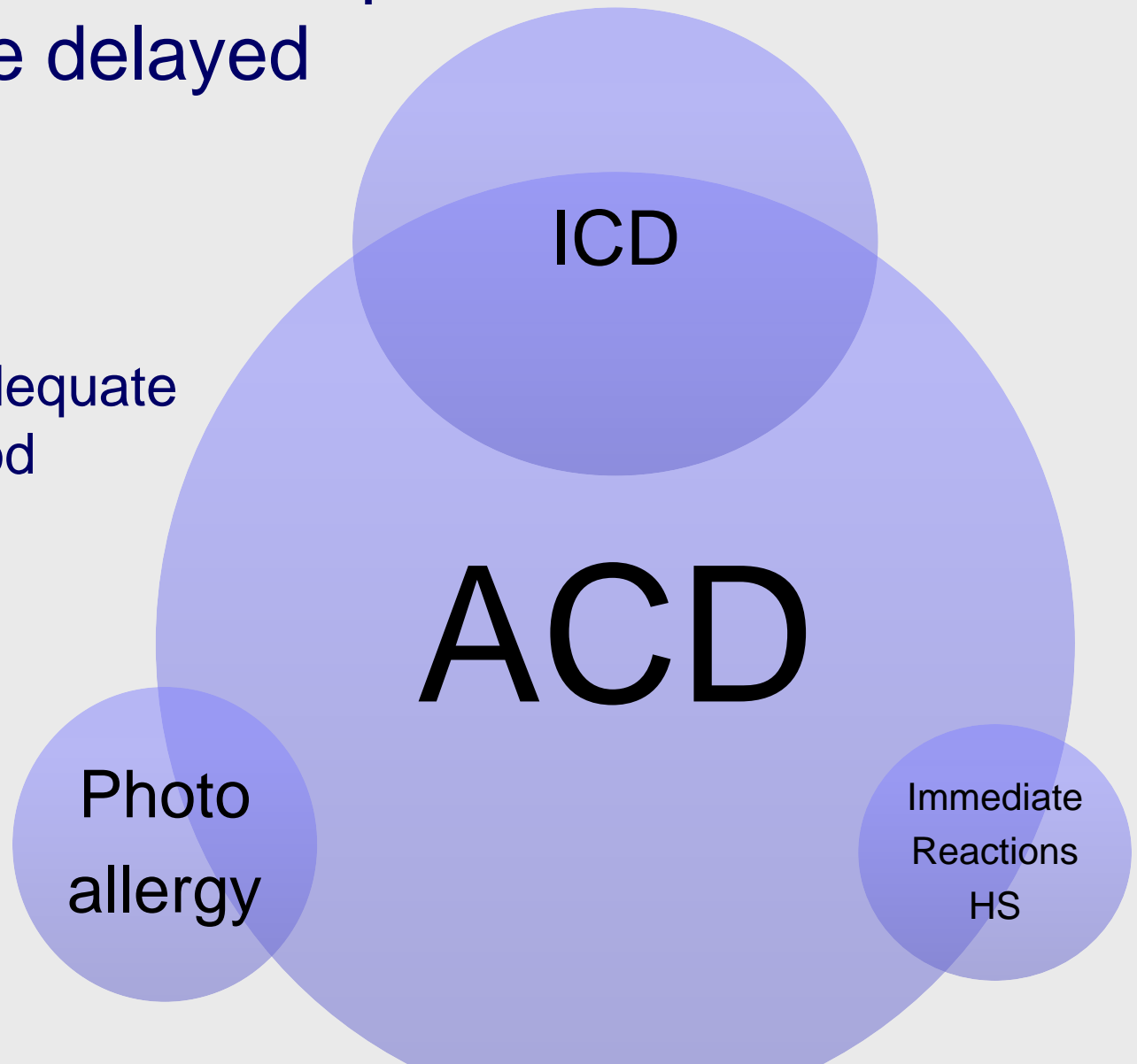
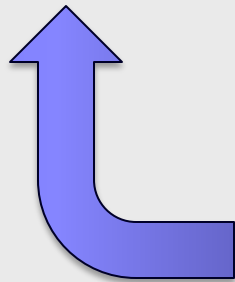


ACD is more than  
delayed HS to contact sensitizers



ACD may be more complex than a simple delayed HS reaction

Choice of most adequate skin testing method



# Skin testing - methods

## ■ Type of skin testing



### Photoallergy

### Photo-patch tests

Duplicate set of tests  
Irradiate 1 set D2  
UVA - 5J/cm<sup>2</sup> (PUVA)  
Compare results



Photoallergic contact dermatitis from benzydamine presenting mainly as lip dermatitis. MM Canelas, JC Cardoso, M Gonçalo, A Figueiredo. Contact Dermatitis 2010;63:85-8

# Skin testing - methods

## ■ Type of skin testing



**Hand dermatitis**  
**Protein contact dermatitis**

Prick testing  
Prick-prick testing  
Immediate reading



# Clinical diagnosis

- Typical ACD – acute eczema
- “atypical” ACD
  - different patterns of contact hypersensitivity
- ACD mixed with related dermatitis
- ACD complicating other dermatitis
  - Atopic dermatitis
  - Hand dermatitis
  - Stasis dermatitis
  - Chronic actinic dermatitis (Ph+PhA+ACD) ....

# Diagnosis of ACD in 2013

## Clinical diagnosis

ACD  
?

Allergen  
exposure ?

Skin  
testing

# Diagnosis of ACD in 2013

**Skin test**

**Patch  
testing**

**ROAT  
Use tests**

**Allergens**

**Baseline  
series**

**Other series**  
Other allergens  
Patients' own products

# Patch testing

## Centenary technique

### 1896 - Joseph Jadassohn

« Funktionelle Hautprüfung »

grey mercury ointment

5 cm<sup>2</sup> + plaster/24h

drug eruption from iv Hg in treatment for syphilis

*Breslau / Graz*

### 1929 – Bruno Bloch

Basel ...Zurich

1cm<sup>2</sup>, linen, 24h, grading system

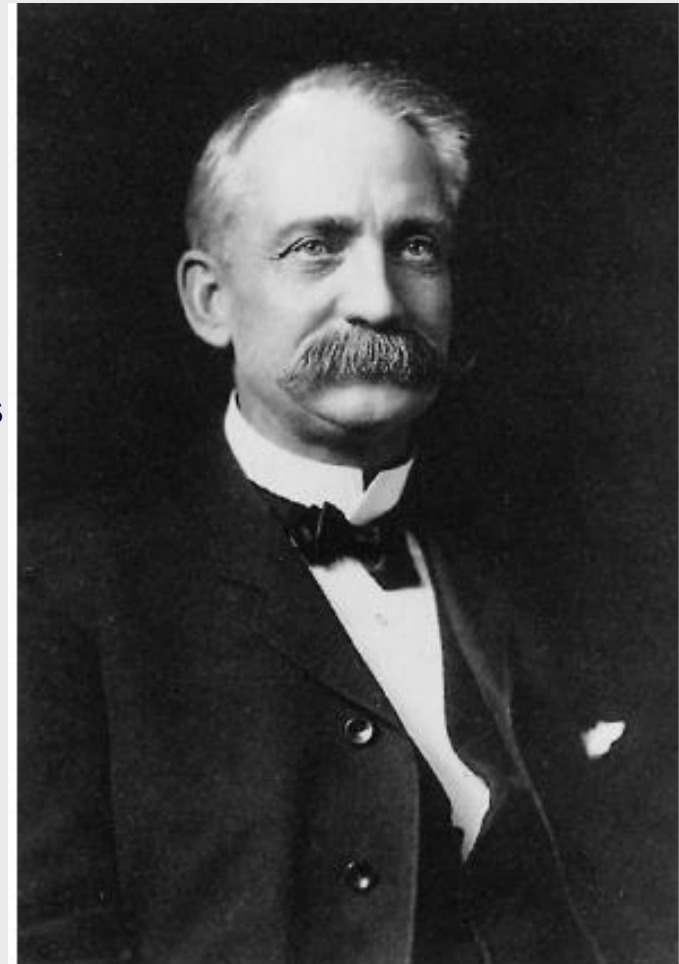
CD and systemic CD

baseline series

Primin, arnica, Hg, formaldehyde,

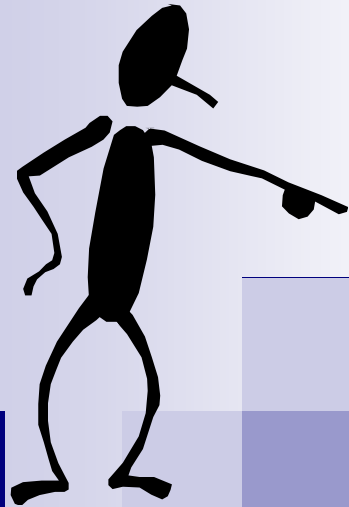
turpentine, iodoform, quinine, naphthalene

.... **Marion Sulzberger**



Josef Jadassohn (1863--1936)





# Patch testing ... in 2013

## → **Diagnosis of ACD**

- ... other delayed hypersensitivity reactions
- ... investigation immune mechanisms



# Patch testing – objectives

**False negative**

**Positive relevant PT**

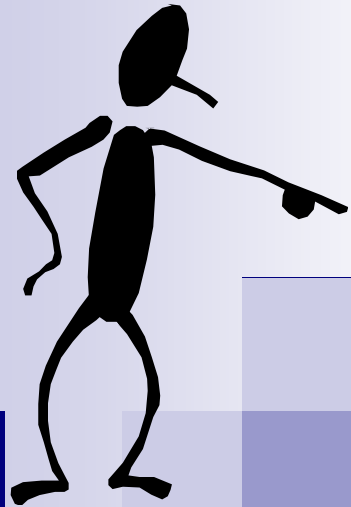
**False + Irritation**

**Exuberant reactions**

**Active sensitization**

**“Non-relevant allergens”**





# Patch testing ... in 2013

→ Standardization

# Patch testing – standardized technique

## ■ Procedures

- Duration and site of application (48h/back)
- Amount of allergens to apply
- Reading times

## ■ Material

- Chambers for testing allergens (pet/vehicles)
- > 500 commercialized allergens (as drugs)
- Pre-prepared allergens (TrueTest)

## ■ Validated guidelines reading / scoring

## ■ Scoring for relevance



# Patch testing - material

## ■ Quantity - Dose

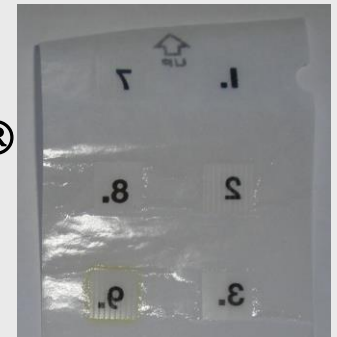


### Vaseline

- 5-7mm ribbon
- 20 mg for Finn Camber®
- 30 mg large IQ chambers®

### Liquid – pipetting

- 15 microlitres for Finn® chamber®
- 20 microlitres for van der Bend®
- 25 microlitres for large IQ chambers®



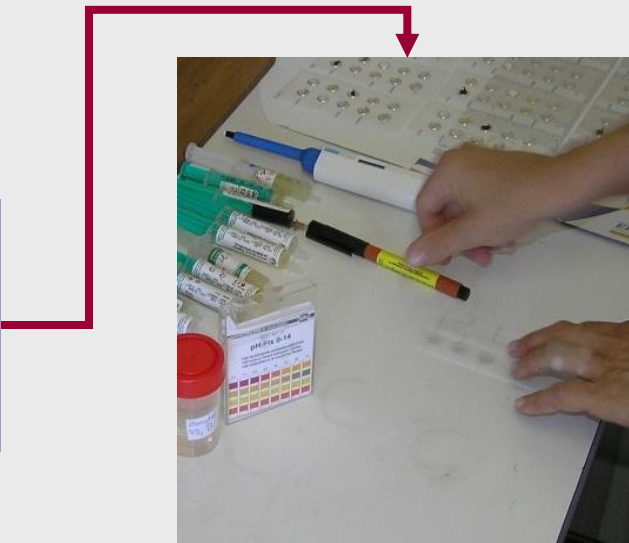
Limited number allergens



# Patch testing - material

- **Quantity of application**  
allergens with narrow margin  
between irritation/allergy
- Formaldehyde (1 → 2%)
- MI/MCI and MI (500-2000 ppm)
- MDBGN

Micro-pipetting  
15 - 17µl  
Finn chambers





# Patch testing – Reading

- Day2 and D4 ... D7  
2 readings between D2 and D7
- Grading reactions - ICDRG guidelines  
(-, +?; +, ++, +++, IR)  
positive: – erythema + papules infiltration whole test area





# Patch testing

## Interpretation / **relevance**

- Current relevance v. past relevance
  - 0-non-traced; 1-doubtful; 2-possible; 3-likely

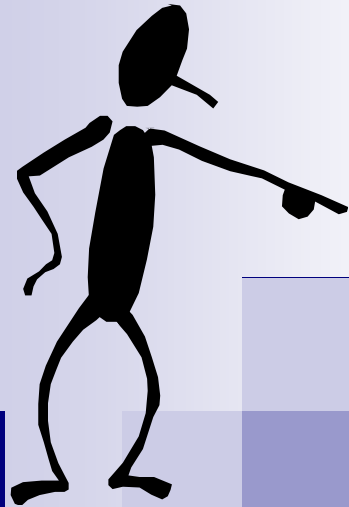
Lachapelle J-M. A proposed relevance scoring for positive allergic patch test reactions: practical implications and limitations. *Contact Dermatitis* 1997;36:39-43.

## ■ COADEX

- C-current relevance
- O-old or past relevance
- A-actively sensitized
- D-relevance not known
- E-exposed
- X-cross-reaction

Bourke J, Coulson I, English J. British Association of Dermatologists' Guidelines for care of contact dermatitis. *Br J Dermatol* 2001; 145:877–85





# Patch testing ... in 2013

- Material
- Procedures

**→ PITFALLS**

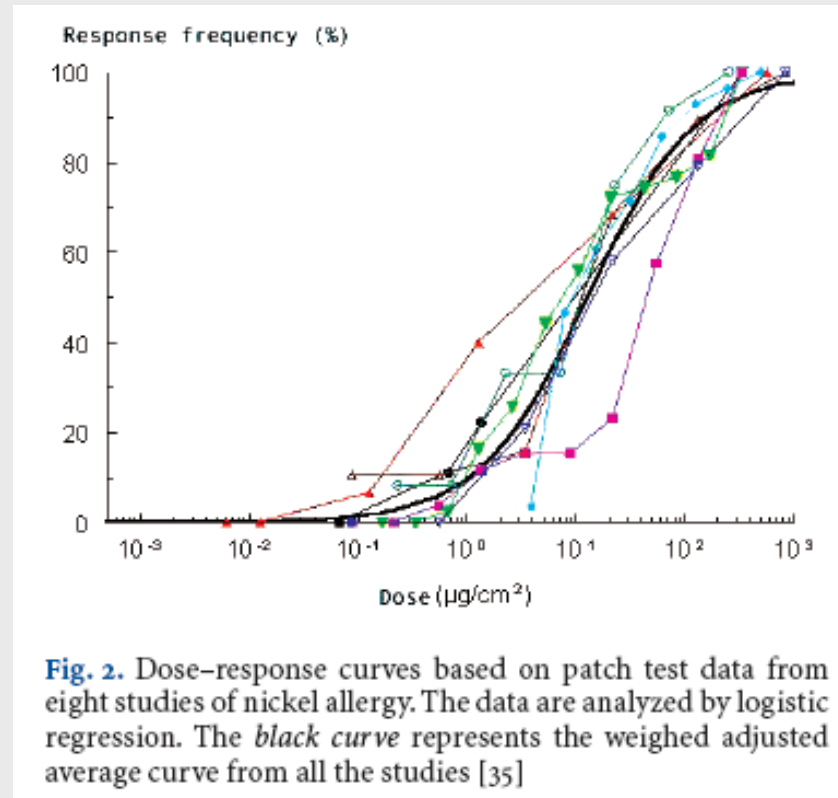


False neg  
Positive IR  
Active  
sensitization

# Patch testing - material

- Many thousands of allergens  
haptens, prohaptens ... standardization !!
  - Eliciting dose (Dose/unit skin area)
  
- Quality of the preparations  
(mixes and “natural allergens”)
  
- Quantity of allergen (dispersion, degradation)

# Patch testing - material



- Adequate **eliciting dose** of PT allergen/**surface area** weight/cm<sup>2</sup> - number of molecules/cm<sup>2</sup>

# Patch testing - material

- **QUANTITY – allergen preparations**
- Incorrect dispersion of the allergen in PT material ...
- Unexpected low dose  
on the material for PT  
material really applied on the skin

# Patch testing - material

## ■ Allergen degradation in syringe

*Contact Dermatitis 2004: 51: 73–78  
Printed in Denmark. All rights reserved*

*Copyright © Blackwell Munksgaard 2004*

CONTACT DERMATITIS

1000 x less than labelled allergen concentration

### **Poor correlation between stated and found concentrations of diphenylmethane-4,4'-diisocyanate (4,4'-MDI) in petrolatum patch-test preparations**

MALIN FRICK<sup>1</sup>, ERIK ZIMERSON<sup>1</sup>, DANIEL KARLSSON<sup>2</sup>, ÅSA MARAND<sup>2</sup>, GUNNAR SKARPING<sup>2</sup>, MARLÉNE ISAKSSON<sup>1</sup> AND MAGNUS BRUZE<sup>1</sup>

<sup>1</sup>Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö and <sup>2</sup>Work Environment Chemistry, Stockholm University, Håssleholm, Sweden

Polymerisation of 4,4'-MDI in petrolatum – more unstable than 2,4-TDI or 1,2-HDI

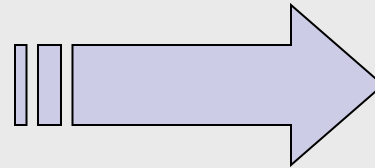


# Patch testing - material

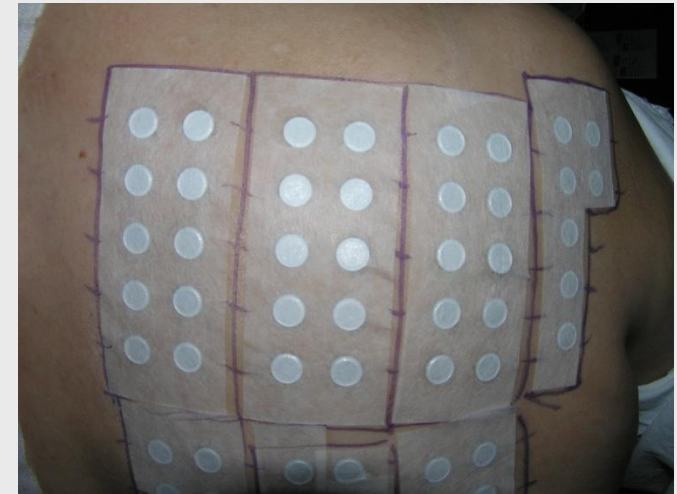
## ■ Allergen degradation before application



**EVAPORATION  
OXIDATION**



**No preparation  
In advance**



### ➤ **Variable evaporation / oxidation on air exposure**

- temperature (freeze); - humidity; - light (UV) ...

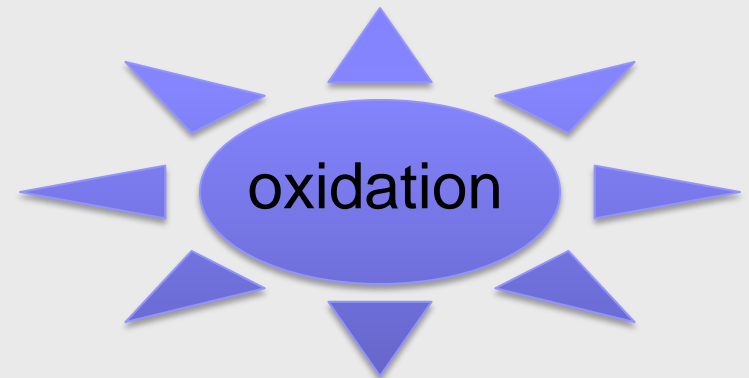
Methyl methacrylate (0% at D2); HEMA, TREGDA, EGDMA (0% D8)



# Patch testing - material

## ■ **Fragrances – variable evaporation**

- Lyral – persists long time in the PT
  - > 95% at D9 – 5°C
  - 70% at D9 – ambient temperature
  
- Citronellol – > 25% lost in PT at 24h (ambient temperature)  
*(Gilpin SJ, HuiX, Maibach HI. Dermatitis 2009)*
  
- Terpenes from essential oils - slow reduction in the PT  
(geraniol, linanlool, linalyl acetate)  
*(A.T. Karhlberg and col.)*





# Patch testing - material

- Quantity/quality → bioavailability of the allergen in the epidermis (Kt/DCs ... T cells)
  - Vehicles – inadequate
  - Testing salt for metals



Minoxidil in propylene glycol



Acyclovir ...





# Patch testing - methods

## ■ Bioavailability of allergen in PT

best salt to test

### ■ METALS - Ni, Co, Cr, Hg, gold

**Palladium (Na tetrachloropalladate), titanium ? ....**



# Patch testing - material

## ■ QUALITY of ALLERGEN

- Mixtures of allergens
- “Natural” allergens – distinct origins/distinct chemicals
  - Plant allergens
  - Essential oils
- Purity of allergens ( $\neq$  real life)
  - Allergen purity is necessary for investigation
  - Not always the best for diagnostic purposes
    - Impurities can be the real allergen in ACD



# Allergen "impurities"

## Tinosorb M<sup>®</sup> and Decylglucoside



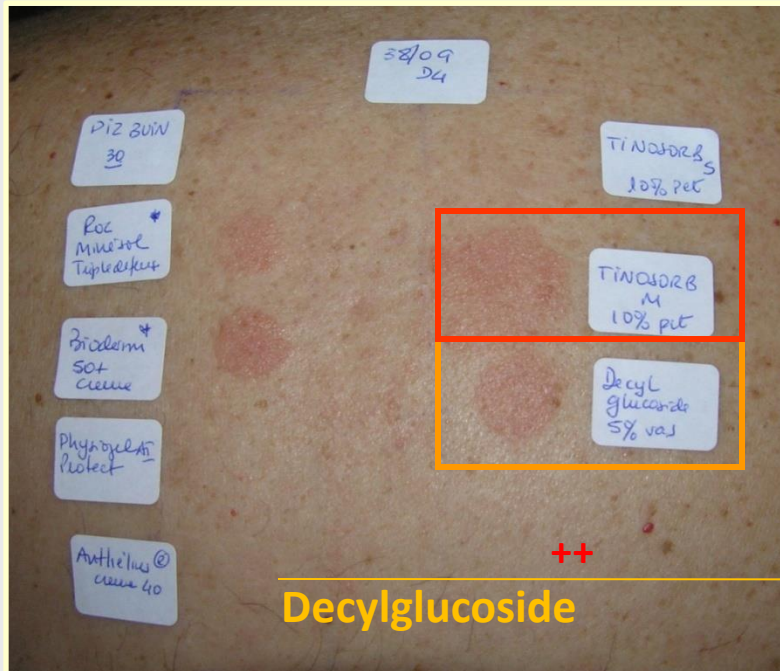
**Tinosorb M ++**  
Bis-benzotriazolil-  
tetrametilbutilfenol  
de metileno

**UVA – 5J/cm<sup>2</sup>**  
**Tinosorb M ++**

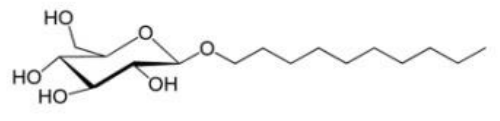
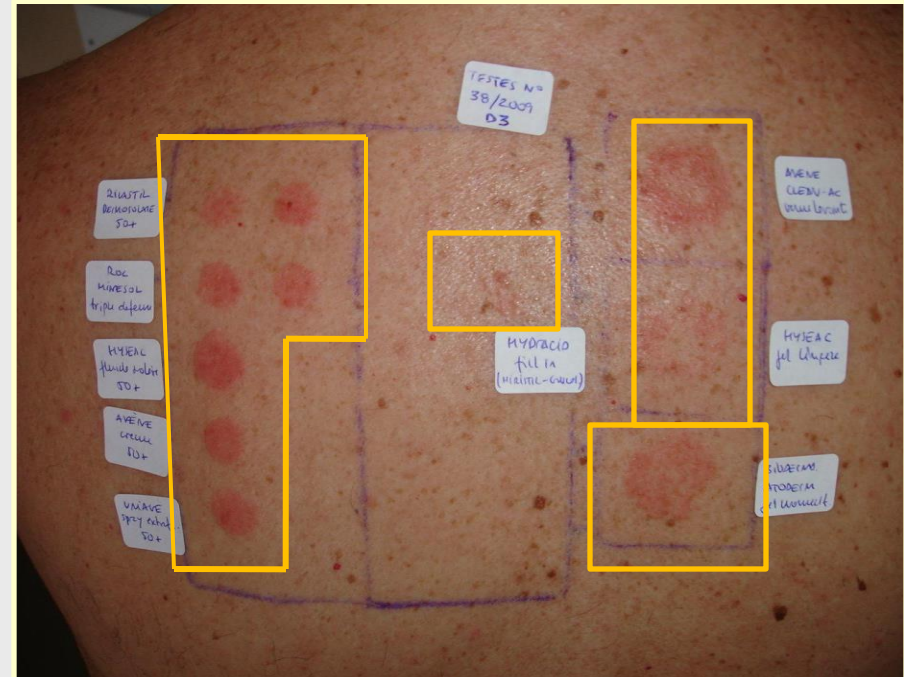


# Allergen "impurities"

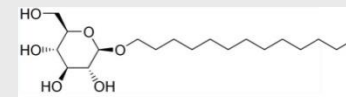
## Tinosorb M<sup>®</sup> and Decylglucoside



**Decylglucoside**



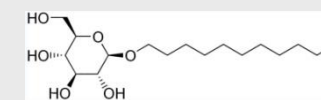
Lauryl glucoside 3% pet  
Lauryl polyglucose



**+? Miristilglucósido**



**++ Cocoilglucósido**



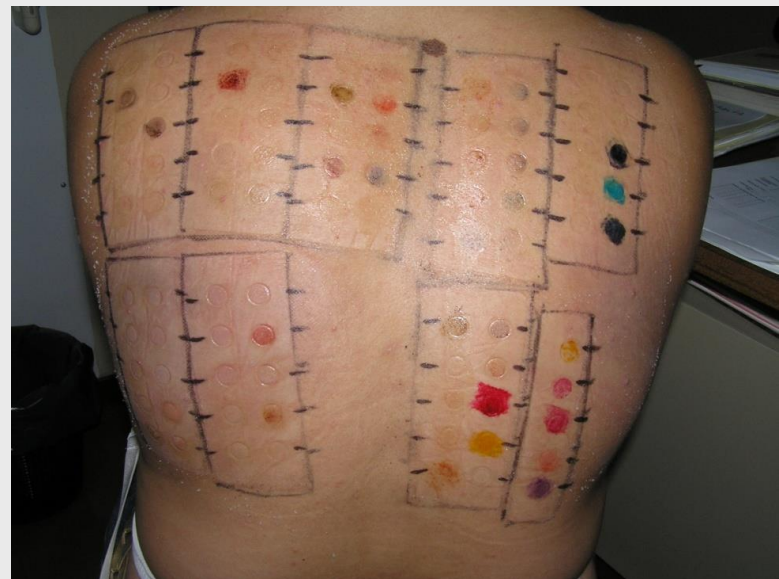
**++ Laurilglucósido**

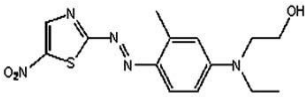
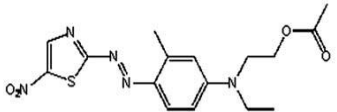


# Allergen “impurities”

## ■ Textile Dyes

- Allergens are real mixes as industrial dyes
- Azoic dyes  
DBlue 124/106  
DO1/DO3
- Reactivity to impurities in chromatograms



|   |  |
|---|--|
| <p>DB 106</p>  | <p>CAS: 68516-81-4<br/>CI 111935<br/>MW: 335</p> |
| <p>DB 124</p>  | <p>CAS: 15141-18-1<br/>CI 111938<br/>MW: 377</p> |



# Allergen "impurities"

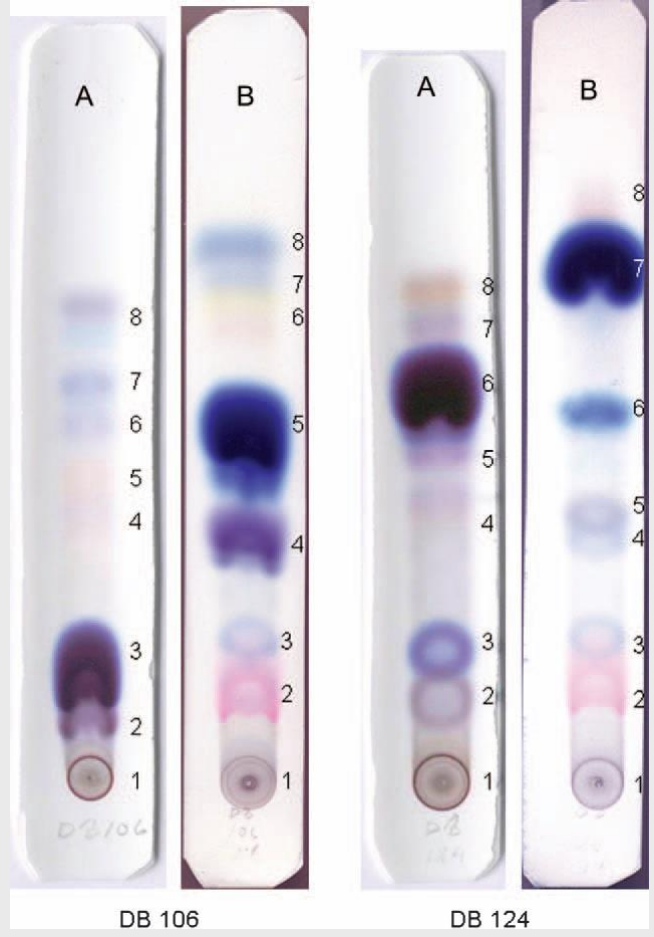
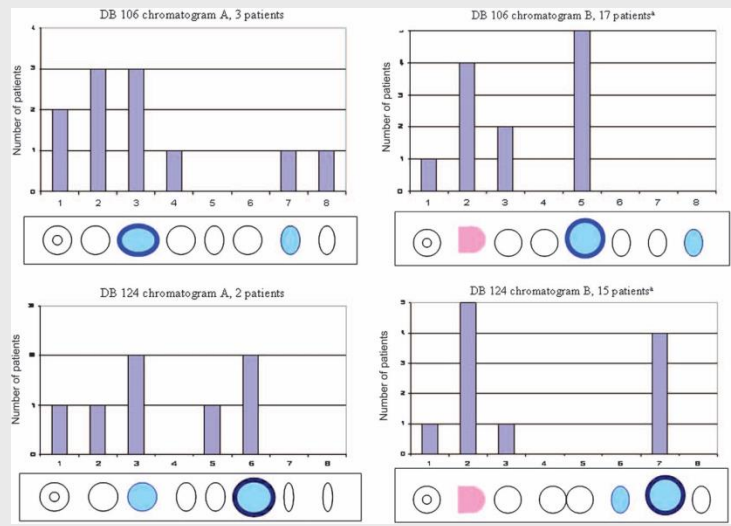
Contact Dermatitis 2009; 60: 270-278  
Printed in Singapore. All rights reserved

Copyright © 2009 John Wiley & Sons A/S  
CONTACT DERMATITIS

## Patch testing of patients allergic to Disperse Blue 106 and Disperse Blue 124 with thin-layer chromatograms and purified dyes

KRISTINA RYBERG<sup>1,2</sup>, AN GOOSSENS<sup>3</sup>, MARLINE INAKSSON<sup>1</sup>, BERGITTA GRUVBERGER<sup>1</sup>, ERIC ZIMMERSON<sup>1</sup>, LENA PERSSON<sup>1</sup> AND MAGNUS BRUZE<sup>1</sup>

<sup>1</sup>Department of Occupational and Environmental Dermatology, Lund University, Malmö University Hospital, Malmö, <sup>2</sup>Department of Dermatology, Norra Älvsborgs Länsjukhus (NÄL), Trollhättan, Sweden, and <sup>3</sup>Department of Dermatology, Contact Allergy Unit, University Hospital, K.U. Leuven, Leuven, Belgium



**Reactions to "impurities"**

# Patch testing ... in 2013



**Material, methods**  
**Human interpretation**  
**“In vivo” technique**

# Patch testing ... in 2013



## **“In vivo” technique**

made and interpreted by humans

→ Intra- and inter-individual variability  
tested patient / doctor



# Intra-individual variability

- Skin location

Comparison Left  $\leftrightarrow$  Right  
(concordance  $\pm$  95%)



Less  
intense  
reactions

- Variability in PT reactivity with time

- Chromium, Ni ...

- ? *Drugs, immune status, dermatitis elsewhere ...*



# Patch testing – Reading

## Late readings D6/D7

(corticosteroids, neomycin, pts on immunosuppressors)

ICDRG guidelines (-, **+**?, **+**, **++**, **+++**, **IR**)

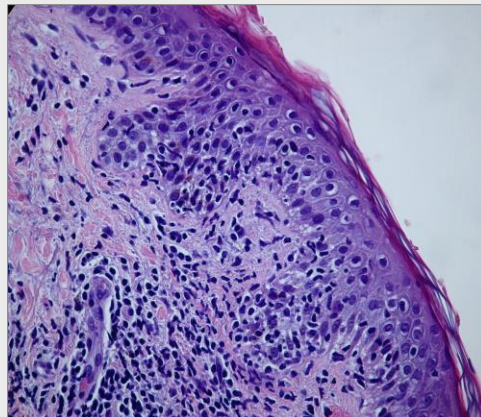
Doubtful reactions - +?

Irritant reactions (shampoo effect)



# Patch testing – Reading / interpretation

- Irritant v. allergic reactions
- Pharmacologic effect
- Reappraisal of some concepts  
“edge effect”; pustular reactions



# Patch testing – Reading / interpretation

- +? – meaning
  - May be significant
- 
- Repeat patch testing
  - ROAT, use test
- 
- In vitro test ?





# Patch testing Interpretation / **relevance**

- Back to patient history
- analysis of contacts  
(labeling; chemical analysis)



Get the most out of life in the sun safely

IPR-002897H-INGREDIENTS: Aqua, Homosalate, Glycerin, Benzophenone-3, Dimethicone, Butylene Glycol, Lipoic Acid/Diethylene Glycol/Glycerin Crosspolymer, Octocrylene, VP/Hexadecene Copolymer, Butyl Methoxybenzoyl Methane, Diethylhexyl 2,6-Naphthalate, Silica, Ethylhexyl Salicylate, C30-38 Olefin Copolymer, Stearate, PEG-100 Stearate, Squelane, Potassium Cetyl Phosphate, Trimethylsilyloxyethylacetate, Caprylyl Glycol, Glyceryl Stearate, PEG-100 Stearate, Squelane, Potassium Cetyl Phosphate, Trimethylsilyloxyethylacetate, Trisiloxane, Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Sclerotium Gum, Behenyl Alcohol, Polysorbate 60, Disodium EDTA, Sodium Hydroxide, Phenoxyethanol, Methylisothiazolinone, Parfum, Containe oxibenzona, Contien oxibenzona, Παράγει 2-οξροτο-4-μεθοξυβενζοφρονονη.

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S27 122214

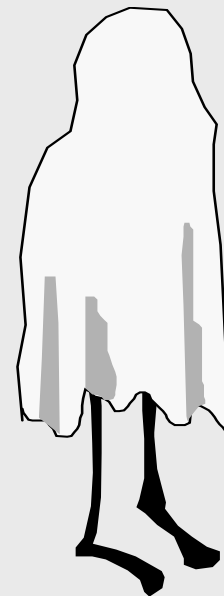
# Patch testing

## Interpretation / **relevance**

- Depends much on knowledge
  - Patient; doctor ..... available scientific data



ACD from  
corticosteroids  
“by proxy”





# Patch testing

## Interpretation / **relevance**

- Thiomersal → thiosalicylic acid → piroxicam  
thiosalicylic acid ← UVA --- piroxicam



Contact allergy



Photo-allergy from piroxicam

## ■ PhACD from ketoprofen

- Cross reactions  
benzofenones, fenofibrate
- Octocrylene
- Perfume mix 1 - ?  
Cinamic alcohol (ald)



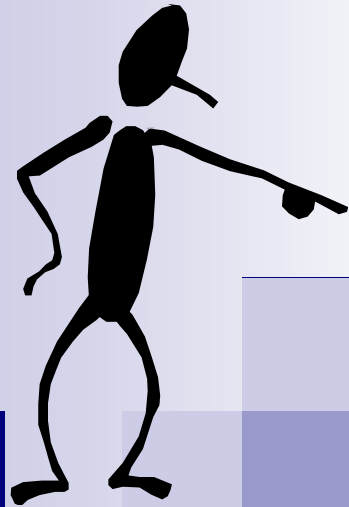
Courtesy An Goossens





# Patch testing – safety

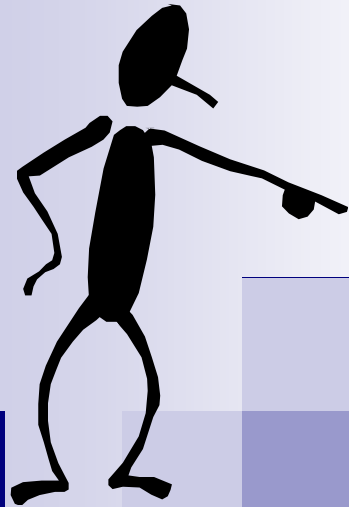
- generally SAFE
- Exuberant reactions (angry back)
- Aggravation of dermatitis
- Active sensitization (?)  
v. late reactions



# Patch testing ... in 2013

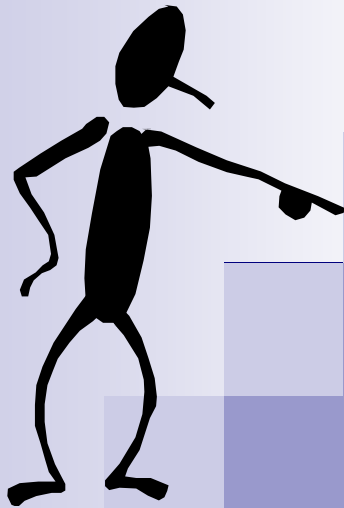
Widely available  
Safe technique  
Not expensive  
Time consuming  
“easy” to perform

**Everyone can apply a patch test**  
**The problems are**  
**the choice of allergens** (other tests)  
**reading ... and**  
**interpretation of the results**



# Patch testing ... in 2013

**GOLD STANDARD**  
**FOR THE DIAGNOSIS OF ACD**  
for detecting contact allergy



# Patch testing ...in 2013

It is not a final answer ...  
Sometimes just the beginning





# ROAT (repeated open application test) USE TEST

- **Defined** – Hannuksella et al. CD 1986
  - Developed Klaus Andersen / JD Johanssen
  - Developed M Bruze, M Isaksson
- 2 daily for 7 days → 4 weeks  
(stop if positive before)
- 5x5cm (3x3 – 10x10cm) antecubital fossa
- Positive = erythema and papules covering  
> 25% area

# ROAT (repeated open application test) USE TEST

## ■ Grading system

Negative

Weak pos: 25-50% erythem+infiltration, possibly papules

Moderately pos: >50% E+I, papules and few vesicles

Strongly pos: >50% E+I, papules >10-25



# ROAT (repeated open application test) USE TEST

- ... doubtful reaction at week 4 (?)
- ... time to become positive – significance ?
  
- **Widely available**; seldom used according to guidelines
- Time consuming
- Compliance



# Diagnosis of allergic contact dermatitis in 2013

- Clinical diagnosis
- Human skin testing
- **“In vitro” testing**



# “In vitro” testing

## ■ Allergen specific T cells (DCs)

- Proliferation and  $^3\text{H}$  incorporation
- Flow cytometry – activation markers

## □ Cytokine production from PBMC

- ELISA

- ELISpot ... MELISA

IFN- $\gamma$ , IL-2

IL-4, IL-13, IL-5



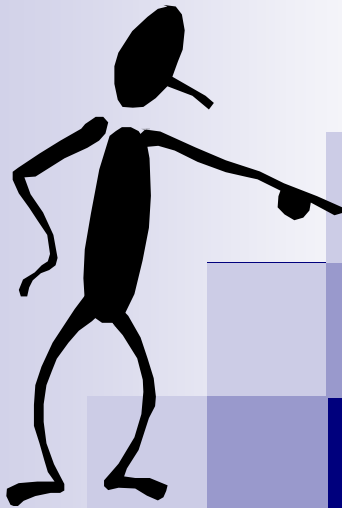
# “In vitro” testing

- Not feasible in every LAB
- Not feasible for many allergens (expensive)
- Standardization for limited nr. allergens  
Would need controls for new allergens
- Value of **individual** results?
  
- T cells in blood  $\neq$  effector T cells in skin
- Cytokine production – sub-phenotypes



# “In vitro” testing

- Good (?) correlation with PT results
- Distinction ACD from CA
- Is feasible also in pts persistent ACD  
*(no skin “free of dermatitis” for PT)*
- Less variability than patch testing
- ... intolerance to metal implants ??



# Diagnosis of allergic contact dermatitis in 2013

**CLINIC** ↔ **PATCH TESTING**

...

“in vitro” testing



# Diagnosis of ACD in 2014



## 12<sup>th</sup> Congress of the **ESCD**

European Society of Cutaneous Allergy  
and Contact Dermatitis

**BARCELONA** • 25<sup>th</sup> - 28<sup>th</sup> June 2014

[www.escd2014.com](http://www.escd2014.com)



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