In vivo tests:
Prick test, scratch test, intradermal test

Erlangen, 26.7.2013

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Diagnostic algorithm

1.) hx taking
2.) diagnostic measures
   - skin testing
   - laboratory tests
   - provocation tests
3.) result assessment (relevance ?)

*In vivo* test methods

- skin prick test (commercial test solutions, fresh material)
- scratch test
- intradermal (=%intracutaneous) test
- open patch test/rub test
- provocation tests

→ negative (0.9% sodium chloride) control
→ positive control (1.0% or 0.1% histamine hydrochloride for SPT and 0.01% for the intracutaneous test)
→ emergency plan (rescue medication at hand)
Skin prick test (SPT)

Technique:
- Disposable single-use metal needle or lancet
- Crossing the drop, bevel up, before making a slight infringement of the skin without causing bleeding
- Constant technique (pressure and time of applied pressure)
- 10-fold increase of allergen concentration: 2-2.5 fold increase of wheal area
- Duplicate SPT for routine, quadruplicate for scientific purposes (variation of MWD < 20%)

→ SPT is recommended as the best skin test (safety, technical performance, reproducibility)

Masse MS et al.. Allergy 2011; 66: 1415-19.
Skin scratch test and scratch chamber test

technique:

- 1 mm lancet
- 5 mm skin scratch-without bleeding
- with or without 12 mm Finn chamber

→ scratch test: low specificity
→ scratch chamber: low sensitivity for fresh apple (30%) and birch (60%) versus SPT (80%/100%)
→ not recommended

Niinimäki A. Contact dermatitis 1987; 16:11-20.
Dreborg S, Frew A & EAACI Subcommittee on skin tests. Allergy 1993 (suppl) 48-82.
Skin scratch test

- 1 mm lancet
- 5 mm skin scratch-without bleeding
- with or without 12 mm Finn chamber

- Scratch chamber: low sensitivity for fresh apple (30%) and birch (60%) versus SPT (80%/100%)

- Not recommended

Scratch test vs. skin prick test
- 0.1% histamine
- 0.9% sodium chloride
- birch
- carrot
- cat
Intradermal test (higher sensitivity than SPT)

technique:
- 26 gauge needle
- 1ml syringe
- 0.02-0.05 ml test solution
- test solutions: preferentially stabilized by addition of 0.03% human serum albumin; glycerol concentration ≤ 2%;
- 0.1-1% of the SPT-solution

→ more reproducible and higher sensitivity than SPT
→ for testing with low potency extracts
→ when SPT was negative (despite indicative hx)
→ for aeroallergen extracts: no advantage over SPT.

Dreborg S, Frew A & EAACI Subcommittee on skin tests. Allergy 1993 (suppl) 48-82.
Intradermal test (higher sensitivity than SPT)

<table>
<thead>
<tr>
<th>Honey bee venom concentration [μg/ml]</th>
<th>reactivity</th>
<th>Yellow jacket venom [μg/ml]</th>
<th>reactivity</th>
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<td>1. 0.00001</td>
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<td>2. 0.0001</td>
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<td>2. 0.0001</td>
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<td>5. 0.1</td>
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<td>6. 1.0</td>
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### Reading (semiquantitative)

<table>
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<tr>
<th></th>
<th>SPT wheal (mm Ø)</th>
<th>SPT erythema (mm Ø)</th>
<th>intradermal wheal (mm Ø)</th>
<th>intradermal erythema (mm Ø)</th>
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<td>&lt;3</td>
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<td>+</td>
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<tr>
<td>++</td>
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<td>&gt;6 pseudopods</td>
<td>&gt;20</td>
<td>&gt;15</td>
<td>&gt;40</td>
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</table>

Ring J. Angewandte Allergologie. 1988
Reading (semiquantitative)

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<tr>
<th></th>
<th>SPT wheal (mm Ø)</th>
<th>intradermal wheal (mm Ø)</th>
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<td>0</td>
</tr>
<tr>
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<td>&lt; 5</td>
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<td>+</td>
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<td>≥ 5 - &lt; 8</td>
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<tr>
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<td>≥ 4 - &lt; 5</td>
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<td>+++</td>
<td>≥ 5 - &lt; 6</td>
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<tr>
<td>++++</td>
<td>≥ 6 pseudopods</td>
<td>≥ 15</td>
</tr>
</tbody>
</table>

AWMF-Guideline No. 061/026; 2009
Reading (quantitative)

Mean wheal diameter (MWD): \((D + d)/2\),
\(D = \) the longest diameter of the wheal,
\(d = \) the longest diameter orthogonal to \(D\)

Positive:
\(\geq 3\) mm MWD (SPT), \(\geq 5\) mm MWD (intradermal test),
for occupational allergens:
\(\geq 1.5\) mm MWD (SPT)

Longest wheal diameter (LWD): \(D\)

\(\rightarrow \) „The longest wheal diameter alone is a better surrogate marker
of the wheal surface, in comparison with the mean diameter, but also easier
and faster to measure.
Therefore, it seems to be preferable for SPT evaluation.“

Dreborg S, Frew A & EAACI Subcommittee on skin tests. Allergy 1993 (suppl) 48-82.
van Kampen V et al.. EAACI position paper:
### Table 1 Performance of skin prick tests

1. Use standardized extracts when available.
2. Include a positive and a negative control solution.
3. Perform tests on normal skin.
4. Evaluate the patient for dermographism.
5. Determine and record medications taken by the patient and time of last dose.
6. Record the reactions after 15 min.
7. Measure the longest wheal diameter.

In vivo testing with non-standardized agents

- sterile, non-infectious material
- no intradermal tests with colored agent (iatrogenic tattoo)
- dilution series
- controls (allergic reaction, if ≤ 2/10 individuals show positive reaction)
- be aware of national legal requirements (e.g., Germany: § 67: duty of disclosure to the inspecting authority)
not all individuals with detectable allergen-specific IgE show clinical allergy at contact
→ some develop clinical allergy later and
→ in some the test may remain positive after clinical symptoms have disappeared.

Assessment

sensitization $\neq$ clinical allergy

(positive test) (clinical symptoms)

treatment

Kuehn HS et Gilfillan AM. Immunol Lett 2007; 113: 59-69
What if.....

medical history **positive**

↓ ?

test results **negative**
Causes for false-negative test results

<table>
<thead>
<tr>
<th>(False-)negative test result due to</th>
<th>in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>incomplete test material (missing components)</td>
<td>+</td>
</tr>
<tr>
<td>incorrect technique</td>
<td>+</td>
</tr>
<tr>
<td>cut-off set to high</td>
<td>-</td>
</tr>
<tr>
<td>allergen-specific blocking IgG-antibodies</td>
<td>-</td>
</tr>
<tr>
<td>refractory phase (following antibody depletion in severe anaphylactic reactions)</td>
<td>+</td>
</tr>
<tr>
<td>local IgE-production</td>
<td>+</td>
</tr>
<tr>
<td>different immunological mechanism (e.g. late-phase reaction) or non-immunological mechanism induces symptoms</td>
<td>+</td>
</tr>
<tr>
<td>concomitant medication (systemic H1-blocker, certain H2-blockers (ranitidine), ketotifen, phenothiazine-derivatives, tricyclic antidepressants, βadrenergic agonists, high dose corticosteroids, topical steroids in test site)</td>
<td>+</td>
</tr>
<tr>
<td>skin alterations due to underlying disease (diabetic neuropathy, chronic hemodialysis)</td>
<td>+</td>
</tr>
<tr>
<td>incomplete history concerning the culprit allergen exposure</td>
<td>+</td>
</tr>
</tbody>
</table>
Incomplete SPT material (missing components): occupational allergen sources

- n=115 individuals with baker’s asthma
- 4 commercial SPT-solutions (wheat/rye flour)
- specificity: 86-100%
- sensitivity (SPT compared to allergen-specific bronchial provocation): rye flour (SPT-solution): 40-50%. wheat flour (SPT-solution): 45-67%
- 30-60% false-negative

→ „Improvement and standardization of SPT extracts for wheat and rye flour is highly recommended.“

van Kampen V et al.. Allergy 2013; 68: 560-584.
van Kampen V et al.. Allergy 2013; 68: 651-658.
Sander I et al.. Allergy 2004; 59: 95-98.
Incomplete test material (missing components): drug allergies

- Immunological mechanisms: type I-type IV (Coombs & Gell)
- *in vivo* and *in vitro* tests: frequently false-negative (despite existing allergy)
- Missing components (metabolites)/inadequate allergen presentation
Nonirritating test concentrations for SPT an IDT

- for antibiotics
- for perioperative drugs
- for selected drugs

Non-immunological mechanism:

- NSAIDS
- local anaesthetics
- food additives

→ SPT and intradermal test negative
→ dose-dependant
→ provocation tests (route similar to regular application)
→ objective: find tolerated agent (therapeutic dose)
What if.....

medical history negative

? 

test results positive
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<tbody>
<tr>
<td>(False-)positive test result due to</td>
<td>in vivo</td>
</tr>
<tr>
<td>contaminated test material</td>
<td>+</td>
</tr>
<tr>
<td>test substance naturally contains histamine</td>
<td>+</td>
</tr>
<tr>
<td>test substance is a non-specific mast cell secretagogue</td>
<td>+</td>
</tr>
<tr>
<td>incorrect technique (distance of test sites is too low; reuse of test lancet)</td>
<td>+</td>
</tr>
<tr>
<td>skin condition of individual is unfit for testing (eczematous skin, urticaria factitia)</td>
<td>+</td>
</tr>
<tr>
<td>non-specific IgE-binding in individuals with total IgE &gt; 1000 IU/ml</td>
<td>-</td>
</tr>
<tr>
<td>carbohydrate specific IgE-binding</td>
<td>-</td>
</tr>
<tr>
<td>clinically silent cross-reactivity to homologous allergens (different primary sensitizing allergen source)</td>
<td>+</td>
</tr>
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Mahler V. Prick and Intracutaneous Testing and IgE Testing
Allergic to sodium chloride?

- 62yo cleaning woman
- previous SPT positive
- presentation for medical report on „allergy against sodium chloride“
- objective: early retirement

→ no relevance
  (false-positive *in vivo* test)
Summary

- skin prick test is the test of choice
- scratch test is obsolete
- intradermal test for low potency allergens
- use disposable metal test device (needle, lancet)
- the longest wheal diameter is feasible for recording of test reactions
- standardized extracts are available for most aeroallergens
- *in vivo* testing of occupational allergens and drugs follows its own rules
- false-positive and false-negative test results may occur