Allergy Testing: Skin Testing vs RAST testing

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Disclosures

• I have no relevant financial relationships with the manufacturers(s) of any commercial products(s) and/or provider of commercial services discussed in this CME activity.

• I do not intend to discuss an unapproved/investigative use of a commercial product/device in my presentation.
Learning Objectives

• Describe the role of skin testing and RAST testing in the diagnosis of allergic diseases
• Review when to order ST's and RAST testing
Common Allergic Diseases Seen in the Primary Care Office

- Atopic Dermatitis/Eczema
- Food Allergy
- Allergic Rhinitis
- Allergic Asthma
- Venom Hypersensitivity
Allergic Disease: Why is it important?

Prevalence

- According to most recent data from Asthma and Allergy Foundation of America, allergic disease affects 50 million Americans. ¹
  - Adults: allergic disease is the 5th-leading chronic disease: AR in 10-30% of adults²
  - Children: allergic disease is the 3rd-leading chronic disease: AR in up to 40% of children²
- Prevalence peaks in early ages³
  - In 80% of cases, symptoms develop before age 20³
  - 40% of cases prior to age 6³
  - 20% of cases prior to age 3³

Evaluation of the Patient with Allergy Symptoms: Why is Specific Allergy Diagnosis Important?

- Atopic dermatitis: may identify food or inhalants triggers
- Food allergy: may help determine likelihood of clinical allergy and possibly persistence & severity
- Asthma: identify specific triggers
- Allergic rhinitis: identify specific triggers
- Asthma and allergy medications only control symptoms: they do not prevent or provide lasting benefits after discontinuation
- If considering specific allergen immunotherapy, which is currently the only disease modifying treatment
Why Test For Specific IgE? Isn’t the Clinical History Good Enough?

- History alone is insufficient to diagnose specific allergen sensitivity
- Allergy tests help direct and optimize management
  - If non-atopic: results will allow you to focus on other etiologies
  - If atopic: will provide guidance for appropriate treatment
- Inappropriate treatment recommendations may result if allergen sensitivity is based on history alone
- This may cause unnecessary environmental controls and patient costs or failure to implement appropriate environmental controls
Diagnostic Algorithm for the Assessment of Human Allergic Disease

Clinical History & Physical Examination

Are there symptoms with exposure?

- **Yes**: Perform Diagnostic test for Specific IgE (skin test/In-vitro test)
  - **Positive Test?**
    - **Yes**: IgE-mediated Reaction Confirmed
    - **No**: Non IgE-mediated Reaction
  - **No**: Uncertain
  - **No** Consider provocation challenge

- **No**: Not allergic to that exposure
Diagnostic Allergy Testing
Serological Confirmation of Sensitization
Tests Performed in the Diagnostic Allergy Laboratory

- Allergen-specific IgE (over 200 allergen extracts)
  - Pollen (weeds, grasses, trees),
  - Epidermals, dust mites, molds,
  - Foods,
  - Venoms,
  - Drugs,
  - Occupational allergens (e.g., natural rubber latex)
- Total Serum IgE (anti-IgE; ABPA)
- Multi-allergen screen for IgE antibody
Evolution of Allergen-Specific IgE Assay Technology

**Historical Manual Chemistries**

- **RAST** = disc allergosorbent 1^o (transitioned) 1968
- **Hycor Hy-Tec** (paper disc based)
- **FAST** = Allergenics/Biowhittaker, fluorescent allergosorbent test
- **MAST** = Hitachi: thread pipette
- **EAST** = Sanofi Dignostics Pasteur
- **Magic Lite** = ALK/Corning/Bayer
- **Matrix** = Abbott

**Historical Semi-automated Chemistries**

- **Alastat**, Diagnostic Products Corp. (biotinylated-allergen)
- **AutoCAP**, Pharmacia (Allergen insolubilized on sponge)
Current Clinically-Used IgE Antibody Autoanalyzers

- **ImmunoCAP (250, 1000):** Phadia (changed from Pharmacia, Jan 06)

- **HyTec-288:** Hycor Biomedical-Agilent (June 07)

- **Immulite 2000/2500:** Siemens Medical Solutions Diagnostics (Jan 07)
RAST: First Generation

RAST 1\textsuperscript{st} on the market in 1974, considerable variability & questionable quantification-no longer in use and term is no longer appropriate

Allergen bound to paper disc

All antibody isotypes bind: Ig of A,M,G,E class

Bound IgE detected with polyclonal I\textsuperscript{125} Anti-IgE

Results reported as log-related classes or arbitrary units by interpolation of heterologous IgE anti-birch pollen curve

Serological Evaluation for Sensitization to Food

**Limitations**

- Cost-patient time & money
- Requires venipuncture/or other blood draw
  - Modest sensitivity/specificity lead to false positive and false negative
  - Although anyone can order still requires experienced clinician to optimally interpret data
- Reactions could occur despite a “negative” test
  - Several studies show reaction rates over 20% in patients with “undetectable” food specific serum IgE (with suspected allergy by history)
  - Different Lab assay systems are not interchangeable
## Cap Rast Levels

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Skin Test
Confirmation of Sensitization
Skin Testing

Photographs courtesy of Dr. Ed Philpot.
Common Asthma Skin Testing Antigens

- Mold
- Dust mites
- Pollens
- Cockroaches
- Animals
- Feathers
Allergy Skin Testing

• Skin testing remains the central test to confirm allergic sensitivity when it can be performed \(^1\)

• Skin testing is fast (15-30 minutes), safe, sensitive and involves minimally invasive procedures which can be cost effective

• When performed correctly, skin testing is reproducible

• Skin testing has demonstrated good correlation with results of nasal challenge\(^2\) and bronchial challenges \(^3\)

• Results of skin test should always be used as an adjunct to the clinical history and physical examination when making the diagnosis of allergic disease

\(^1\) Oppenheimer et al, Ann Allergy 2006;S1:6-12
\(^2\) Bousquet et al, Clin Allergy 17:529-36, 1987
\(^3\) Cockcroft et al, Am Rev Respir Dis 135:264-7., 1987
Allergy Skin Tests: General Rules

• The technician performing the skin tests and the clinician ordering and interpreting skin tests must understand the characteristics of the tests they are administering

• These include:
  • type of skin testing (intradermal vs percutaneous)
  • device used (single vs multiple puncture)
  • placement of tests (location and adjacent testing)
  • the quality and potency of the extracts being used
  • potential confounder of medications that may suppress skin test response.
Skin Test Devices

• Numerous studies have directly compared the performance of the multiple percutaneous devices.

• Percutaneous skin test devices vary in the degree of trauma they impart to the skin. Therefore they differ in the size of positive reactions and in the likelihood of inducing a false positive reaction at the site of the negative control.

• **Different devices require different criteria for what constitutes a positive reaction.**
Recording Skin Test Response

- A record of skin testing should indicate a minimum amount of information that will allow another physician to interpret the results, avoiding the need to repeat skin testing.

- This should include:
  - the concentration of extract employed - consider including manufacturer
  - method of testing - ID or PST including device
  - location of where testing was performed
  - size of the positive and negative control reactions - preferably actual measurement or tracings, but if a score is used include the grading system
“Qualitative scoring (0-4+) is no longer used by many clinicians because of interphysician variability in this method of scoring and interpretation”
Reporting Results

- Negative control (saline or 50% glycerinated HSA-saline)
- Positive control (histamine)
- Test allergen, medication, latex, venom, chemicals
- Timing of reading should be at the peak
- Histamine 8-10 minutes
- Mast cell secretagogues (9% codeine) 10-15 minutes
- Allergens (15-20 minutes)
Variables That Affect Skin Test Results

**Controllable**
- Medications:
  - H1 Antihistamines
  - H2 Antihistamines
  - Antidepressants
  - Corticosteroids
- Immunotherapy
- Relation to adjacent positive reactions
- Extract quality
- Skin testing devices

**Uncontrollable**
- Chronobiology:
  - Diurnal
  - Seasonal
  - Menstrual Cycle
- Age:
  - Specific IgE
  - Histamine Reactivity
- Location on Body:
  - Variations on Back
  - Back vs. Forearm
COMPARISON OF SKIN TESTING AND SEROLOGY?
### Allergen-Specific IgE

*In vitro (lab) and In-Vivo (skin tests)*

<table>
<thead>
<tr>
<th></th>
<th><em>In-vitro</em> IgE Antibody Serology</th>
<th><em>In-vivo</em> SPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>High sensitivity*</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>High specificity*</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>High reproducibility</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Quantitative results in kIU/L^</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>WHO Standard calibrated</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Quality assurance test program</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Can be used independently of pharmaceutical treatment</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Can be used independently of patient skin status</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Time factor</td>
<td>1-7 days</td>
<td>15-30 minutes</td>
</tr>
<tr>
<td>Cost factor</td>
<td>more expensive</td>
<td>inexpensive</td>
</tr>
<tr>
<td>Usefulness in motivating patients</td>
<td>obscure</td>
<td>dramatic</td>
</tr>
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</table>

Results may vary between specific bioassays

^Although all are expressed with same units, cannot compare results between different bioassays
Serological Evaluation for Sensitization to Food

Limitations

- Cost
- Does not assist in diagnosis on non-IgE mediated food hypersensitivity reactions
  - Enterocolitis, enteropathy, proctocolitis
- Identifies sensitization but not intrinsically diagnostic of clinical reactivity (in most cases)
  - Modest sensitivity/specificity lead to false positive and false negative
  - Requires experienced clinician to optimally interpret data
Why Skin Testing is Superior to In Vivo Testing Because:

• More cost and time efficient for patient
  – Results available on initial consultation allowed for development of specific treatment plan
• Predictive value in terms of presence of clinical allergy and possible severity
  – In some cases greater predictive value than in vivo test
• Ability to test to allergens that may be altered in extract preparation process e.g., natural foods
• Can also be used in component-resolved diagnosis
Food Allergy Evaluation
Office Based Evaluation of Food Allergy

• Primary Care Professional
  – Clinical history (symptoms, food, reaction consistency, alternative explanations, determination if likely IgE mediated)
  – Physical examination
  – Serological tests for food-specific IgE

• Allergist
  – Clinical history and physical examination
  – Serum and/or skin prick tests for food-specific IgE antibodies
  – Diagnostic elimination diets
  – Physician-supervised oral food challenges
Food Allergy Prevalence

- Adults
  - Adverse food reactions: no firm data
  - Food allergy: estimated 1%

- Children
  - Adverse food reactions: age < 6 yrs. - 8%
  - Food allergy: 1-3%
Pathogenesis: Allergens

- Glycoproteins
  - 14,000-40,000 daltons
  - Largely heat-resistant and acid stable
- GI tract has barriers to prevent entry of foreign proteins from entering the body.
Pathogenesis: Allergens

- **Adults**
  - Nuts, peanuts, fish, shellfish, eggs
- **Children**
  - Eggs, peanuts, milk, soy, fish, wheat
- **Societal eating patterns influence development of specific food hypersensitivities**
  - Boiled peanuts in Asian cultures,
  - Lack of Peanut Consumption in Sweden
Why can’t you test my child to everything?????
When to Test/What to Test

IgE associated clinical disorder? (Is testing for food allergy appropriate?)

Yes → Determination of potential triggers
• Requires careful history, consideration of epidemiology, pathophysiology
• Foods tolerated (should not be tested)
• Foods not often ingested, more likely triggers
• Foods commonly associated with severe reactions:
  • Peanut, nuts from trees, fish, shellfish, seeds
  • Common allergens for children with moderate-severe atopic dermatitis:
    • Egg, milk, wheat, soy

No → Alternative tests/advice

Selection of serological or skin tests
• select tests to confirm/exclude suspicions
• avoid “panels” of food allergens
• avoid testing tolerated foods
Diagnostic Laboratory Techniques
IgE-Mediated Food Hypersensitivity

- Prick skin tests: Positive tests are “suggestive”
  - Wheal diameter 3 mm > negative control
  - Positive predictive accuracy: < 50%
  - Negative predictive accuracy: > 90%

- Intradermal skin tests: Too non-specific

- IgE RAST: In good lab is similar to skin test
  - Positive: 3+ to 6+ in 6+ scoring system
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Value of the Immuno Cap Assay for Peanut Protein

Diagnostic Decision Point

Percentage of positive reactions

kU/L

5  10  15
5  10  15
2.5  5  10

Sample Case

Devin age 18 months with AD. No prior hx of anaphylactic reactions to foods

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<thead>
<tr>
<th>Food</th>
<th>Class</th>
<th>Level</th>
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<tr>
<td>Milk</td>
<td>III</td>
<td>3.2 kU/L</td>
</tr>
<tr>
<td>Egg</td>
<td>IV</td>
<td>17.6 kU/L</td>
</tr>
<tr>
<td>Soy</td>
<td>I</td>
<td>0.40 kU/L</td>
</tr>
<tr>
<td>Peanut</td>
<td>III</td>
<td>4.2 kU/L</td>
</tr>
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Sample Case # 2

• Connor age 3 with a history of multiple food allergies and recent anaphylaxis to unknown food at a party. Drinks milk with no problem, No exposure to Peanuts or tree nuts, shrimp etc.

• Cap Rast
  – Milk Class II 2.5 kU/L
  – Peanut Class III 7.5 kU/L
  – Cashew Class IV 22.7 kU/L
  – Almonds Class III 13.2 kU/L
  – Shrimp Class I 0.70 kU/L
Food-specific IgE concentrations predictive of clinical reactivity (adapted from Sampson HA)

- **Diagnostic Decision Point**

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<th>Allergen</th>
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<th>PPV</th>
<th>NPV</th>
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<td>Egg</td>
<td>7</td>
<td>61</td>
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<tr>
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<td>44</td>
<td>94</td>
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<td>82</td>
</tr>
<tr>
<td>Wheat</td>
<td>26</td>
<td>61</td>
<td>92</td>
<td>74</td>
<td>87</td>
</tr>
<tr>
<td>Tree nuts*</td>
<td>~15</td>
<td></td>
<td></td>
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When to Re-challenge?

- Are skin tests predictive of positive food challenges?
  - Study performed in 467 children suspected of food allergy
    - 555 food challenges
    - Positive in 55% of the patients, negative in 37% and inconclusive in 8%
    - Challenges were ALWAYS positive when the skin test diameter was >8mm for milk, >7mm for egg, and >8mm for peanut
Food-specific IgE concentrations predictive of clinical reactivity (adapted from Sampson HA)

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When to Re-challenge?

- Milk and soy allergy in infants
  - Every 6-12 months depending on initial reactions and intervening period
- Peanuts and tree nuts
  - Depending on exposure and history at 3-5 years of age. ONLY IN A CONTROLLED SETTING
- Eggs
  - Challenge eggs in baked goods at 2-3 years of age
  - Eggs as such at 4-5 years of age
Allergy to soy formula and to extensively hydrolyzed whey formula in infants with cow’s milk allergy: A prospective, randomized study with a follow-up to the age of 2 years. (J Peds 2003)

• Purpose: To study cumulative incidence of adverse reactions to soy formula in infants with confirmed cow’s milk allergy
• Study Population: 170 children with cow’s milk allergy (DBPCFC)
• Methods: Randomly assigned to receive either soy formula or eHF.
• Results: 10% with soy allergic reactions, 2.2% with eHF. Adverse reactions to soy were more common in infants under 6 months of age.
• Conclusions: Soy formula can be recommended safely in children over 6 months of age in cow’s milk allergy.
Natural History of Food Allergy

Egg

• Initial data shorter follow up
  – 9/24 resolved in one challenge study after 2.9 y
  – 11/25 resolved in another group, mean F/U 2.5 y

• subsequent reports from AD cohort of challenge proven patients - 80% by 5 y of age
Natural History of Food Allergy

Peanut

- Prevalence in population at 1.1%
- Previously regarded as a lifetime allergy
- Initial studies reported 3/15 and later studies with more rigorous criteria 4/83 (though only 17 challenged)
- Reports of resolution initially in the UK suggested that younger male infants may outgrow sensitivity
  - Problems with case definition
  - 10/15 may never have had allergy
- Two US studies both with problems
  - Cutaneous symptoms more likely to outgrow vs systemic, lower RAST levels, other food allergy
Over 13 allergenic components have been identified in peanuts. Of these, Ara h 1, 2, 3, 6, 8, and 9 are considered the most important markers of peanut sensitization and are predictive of an allergic response.

- Ara h 1, 2, and 3 are seed storage proteins.
- Ara h 2 is a more important predictor of clinical peanut allergy than Ara h 1 and 3, and is the one most often associated with severe reactions.
- Ara h 8 is a pathogenesis-related (PR)-10 protein, and sensitization to it is associated with a low risk of systemic reaction and a moderate risk of mild, localized symptoms (ie, oral allergy syndrome).
Peanut ImmunoCap

• Reactivity to Ara h 1, 2, or 3 is associated with a high risk for systemic reaction, including anaphylaxis.
• Reactivity to Ara h 9 is associated with a variable risk for systemic reaction, including anaphylaxis.
• Patients who exhibit reactivity to Ara h 1, 2, 3, and/or 9 should be counseled to avoid peanuts, foods that contain peanut products, and foods that have been processed in plants that also process peanuts.
• Reactivity to Ara h 8 and nonreactivity to Ara h 1, 2, 3, and 9 indicates a low risk of a systemic allergic reaction.
• Patients with only Ara h 8 sensitization may consider taking an oral food challenge test, and, if negative, they may not have to avoid peanuts or peanut-containing foods.
Cross reactivity data vs. Co sensitivity

• Peanut and tree nut are not cross-reactive Ag’s
  – co-sensitivity due to highly allergic Antigens in an allergic population
• Peanut and other legumes: soy have a 6% cross reactivity
• Fish
  – previous studies were based on RAST inhibition 45 - 83% based on the family
  – challenge study of 11 patients challenged to 10 different fish, 7 were sensitive to only 1 fish, 3 were sensitive to more than 2 fish, one was negative
Recommendations On Epi-Pen

• Dose is 0.01 ml/Kg of body Weight!!
• 2 Doses are available
  – 0.15 ml (Children 5-60 lbs)
  – 0.30 ml (Children and Adults over 60 lbs)
• Exceptions
  – May give higher dose Epi-Pen if high Risk for anaphylaxis and asthma as a Risk Factor.
• ALWAYS prescribe a 2 Pak and give as many as the patient or parent wants
Food Atopy Patch Tests (APT’s)

• Small amount of food placed in a Finn Chamber
  – Removed at 48 hours and read at 72 hours.

• Positive patch test rates range from 30% to 95% in children and adults

• Negative predictive values of greater than 90% (except milk, with a negative predictive value of 50%)
What’s Not Clear: APT’s

• Food patch testing NOT standardized in children and adults.

• NO evidence that APTs induce a local immune response reflecting the immunopathology seen in patients with EoE

• Despite this recommend in more challenging cases
• Peanut Introduction: LEAP study recommendations

• The guideline recommends home or physician-supervised feeding or exclusion of peanut based on the test results.

• If a blood test is used to screen and is positive to peanut (sIgE ≥ 0.35 kU_A/L), referral to a specialist with training and experience to perform and interpret the peanut SPT.
  – Safely perform medically supervised feeding tests is advised.

• The guideline discusses the manner of peanut introduction according to the test results, whether at home or under physician supervision.
• Peanut Introduction: LEAP study recommendations

• 1. Infants with severe eczema, egg allergy or both should have introduction of age-appropriate peanut-containing foods as early as 4 to 6 months to reduce the risk of peanut allergy.

• 2. Infants with mild to moderate eczema should have introduction of age-appropriate peanut-containing foods around 6 months, in accordance with family preferences and cultural practices, to reduce the risk of peanut allergy.

• 3. Infants without eczema or any food allergy may have age-appropriate peanut-containing foods freely introduced in the diet, together with other solid foods in accordance with family preferences and cultural practices.
Questions ???