Skin Testing Methods for Aeroallergen Diagnosis

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Disclosures

• None
Objectives

At the end of this lecture you should be able to:

1. Describe factors that confound skin test results

2. Explain how control tests are used in the interpretation of skin test results

3. Describe the advantages and disadvantages of different forms of allergy skin testing
Topics:

- Pathophysiology of skin whealing responses
- Medications that affect the skin whealing response
- Skin tests:
  - Prick
  - Intradermal
  - IDT
- Diluents
- Antigen Potency Preservation
- Positive control test (histamine)
- Negative control test (PNS)
- Glycerine control test
- Allergy Screens
- Test/treatment board preparation
- Modified quantitative testing
Goals of Allergy Testing

(a) confirm the suspicion of allergy
(b) identify the offending allergens
(c) determine degree of sensitivity

In vivo allergy skin tests can accomplish these three objectives.
Why Test the Skin?

- Antigen + Sensitized mast cell = Allergic reaction which leads to release of chemical mediators
- Classic wheal (edema) and flare (erythema) is easily seen on the skin
- Skin testing is an indirect measure of cutaneous mast cell reactivity due to presence of IgE
Why Test the Skin?

- Mast cells reside in the subepithelial layer of the respiratory tract including the nasolacrimal tract, GI tract, and skin.
- Of all of these areas, the skin is the most accessible organ to test.
- Skin testing is the best established test and most sensitive indicator of inhalant sensitivity.
- Skin Testing is an in-vivo challenge test.
What happens in an allergy skin test?
Allergy in a Nutshell
The “Abnormal” Skin Whealing Response to an allergenic liquid:

- After a delay of up to 5 minutes, the skin begins to swell due to increased vascular permeability and transudation of plasma proteins into the tissues, with a corresponding influx of water.

- This swelling produces the "wheal" response which is proportionate to the amount of mast cell activation, and is therefore a specific indicator of IgE mediated release of histamine and other mediators.

- Mediators also trigger sensory nerves in the skin, causing itching and erythema.

- This immediate response is maximal at about 10-20 minutes, and lasts for up to an hour.
Effect of Histamine

- Patient response
  - Mediated by cell receptors
    - Increased vascular permeability
      - Wheal
    - Vasodilation
      - Flare
    - Smooth muscle contraction
      - Wheeze
  - Irritation
    - Itch, sneeze

Iriyoshi, Clin Exp Allergy 1996;26:215
Biphasic histamine release after a single cutaneous antigen exposure

Charlesworth EN, J Clin Invest 1989;83:1519-1526
Early and Late Phase Responses

Immediate

Late-phase
Late Phase Response (LPR)

- A delayed inflammatory process consisting of a polymorphous inflammatory infiltrate occurring in response to mast cell activation by antigen.

- Neutrophils, eosinophils, lymphocytes, basophils orchestrate the late phase response.

- The LPR may be biphasic or may be a continuum of the immediate response.
Basic Immunology: Early and Late Phase Response

Mast

Preformed & Newly Formed Mediators

TNF-α → Endothelial Cell Activation

VCAM → Leukocyte Adhesion & Diapedesis

GM-CSF → Eosinophil Basophil Infiltration & Mediator Release

Eosinophil
Basophil
Infiltration
& Mediator Release

Early-Phase Response

Recruitment Phase

Late-Phase Response

Preformed & Newly Formed Mediators

Leukocyte Adhesion & Diapedesis

Eosinophil Basophil Infiltration & Mediator Release
Skin Testing for Allergy
Principles of Screening

- **Testing for presence of inhalant allergy should begin with an allergy screen.**
- Screening provides a rapid, efficient, and cost-effective method to assess the presence or absence of allergy.
- **Prick testing can be a sensitive and specific screening tool** for evaluating inhalant allergy
- **The simplest and most cost-effective method of screening would involve the use of a prick test battery of 12 to 14 antigens with appropriate positive and negative control tests.**

Krouse & Mabry OTO-HNS 2003;129:s33-49
The screening battery should represent classes of antigens to which the patient is exposed and which are common geographically:

- **Pollens**
  - one or two trees
  - one or two grasses
  - one or two weeds

- **Molds**
  - include at least two families of mold

- **Dust mites**
  - use at least one, if not both species

- **Epidermals**
  - should include cat
What do you do with negative or positive screens?

• *If the patient does not test positive to a screening panel of several common antigens, then the likelihood that the patient will demonstrate sensitivity to a broader panel of antigens is low.*

• Therefore no further testing is necessary with a negative allergy screen

• If inhalant screens demonstrate positive reactions, a broader panel of allergens may be tested prior to the provision of immunotherapy
Types of Skin Testing

• EPICUTANEOUS TESTS
  – Patch test
  – Scratch test
  – Prick test
    • single prick test
    • multi-test devices

• INTRACUTANEOUS TESTS
  – Intradermal Test
    • single intradermal
    • Intradermal dilutional testing (skin endpoint titration)
Epicutaneous testing
Epicutaneous test background

- Epicutaneous tests were among the earliest tests used for the diagnosis of inhalant allergy.
- Scratch tests—a superficial cut is made into the epidermis and antigen is applied to the denuded skin.
- Patch tests—Allergen is applied to skin under occlusion.
- Prick/puncture tests—a drop of antigen is introduced into the epidermis through a superficial needle prick or puncture.
Prick/puncture testing

- Commonly used by general allergists *without* other supplemental testing for the diagnosis of allergy and the provision of immunotherapy

- Immunotherapy based on prick/puncture results alone is less accurate and potentially more risky than with quantitative testing methods

- However, with care to detail and with the added safety of the vial test, immunotherapy based on prick testing alone can be safe and effective in the treatment of inhalant allergy
Technique of Prick Testing

• Strength of antigen pre-determined
  – usually concentrate 1:20 w/v

• Antigen is either placed on skin or “dipped”

• Skin is then “pricked” with sharp device

• Read wheal size after 20 mins

• Measure wheal in mm or use 1+ to 4+ grading system

• Positive and negative controls should also be used
Prick Testing Device

- **Dip the puncture device into a well containing antigen extract first**

- The skin is then punctured, which delivers the antigen into the epidermis

- Results compared with positive and negative controls
Multi-prick devices allow for more rapid testing

- Enables testing for multiple antigens and controls at one time
- The device pictured here has multiple fine tines that hold antigen concentrate in place by capillary action
- Designed to deliver a more consistent amount of antigen to a more consistent depth

Grading of skin prick responses

• Criteria somewhat vague

• A wheal that grows to a diameter of ≥ 3 mm more than a negative control can be considered positive

• Some consider the flare and itching as part of the positive response

• The 1+ to 4+ grading system is poorly reproducible

McCann & Ownby Annals of AAI
2002;89:368-71
Prick Testing Advantages

• Rapid
• Relatively safe
• Useful for screening
• Good correlation with intradermal tests
• Considered more specific than intradermal tests
• More specificity leads to less false positives
Disadvantages of Prick Testing

May not detect “low sensitivity” patients

– A positive prick test roughly correlates with an IDT #3 (1:2,500 w/v) or #4 (1:12,500 w/v) endpoint

Less sensitivity can lead to more false negatives

Lack of quantitative information requires arbitrary starting concentration for immunotherapy
Intradermal Testing
The Intradermal Test

• An intradermal test is performed by injecting antigen into the dermal layer of skin

4 mm wheal
Intradermal Testing Applications:

- A single intradermal test to confirm a previously placed prick test
- A single intradermal test as the ONLY skin test
- A single **screening** test **prior to** endpoint titration testing
- A series of multiple tests of consecutive or serial dilutions of antigens (IDT)
- A single intradermal test to confirm the safety of a new immunotherapy treatment vial (vial safety test)
Pathophysiology of Skin Whealing Responses

4 mm wheal → ?
The normal skin whealing response to an *inert* liquid:

- The intradermal injection of 0.01-0.05 mL of non-reactive liquid:
  - will produce a skin wheal of approximately 4 mm diameter, which enlarges to a *5 mm diameter* through physical spreading.\(^1\)
  
  - The same should occur with saline, PNS, or any allergenic extract to which the patient is NOT allergic

- After 10-20 minutes, no further growth should occur

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The normal skin whealing response to an allergenic extract in an allergic patient:

- When 0.01-0.05 mL of an allergen to which a patient is allergic is placed intradermally, the reaction produced causes the 4 mm wheal to enlarge, not just to 5 mm, but to a diameter of at least 7 mm
- This enlargement occurs within 10-20 minutes
Antigen dilutions used in intradermal testing:

<table>
<thead>
<tr>
<th>Concentrate</th>
<th>1:20 w/v*</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 Dilution</td>
<td>1:100 w/v</td>
</tr>
<tr>
<td>#2 Dilution</td>
<td>1:500 w/v</td>
</tr>
<tr>
<td>#3 Dilution</td>
<td>1:2,500 w/v</td>
</tr>
<tr>
<td>#4 Dilution</td>
<td>1:12,500 w/v</td>
</tr>
<tr>
<td>#5 Dilution</td>
<td>1:62,500 w/v</td>
</tr>
<tr>
<td>#6 Dilution</td>
<td>1:312,500 w/v</td>
</tr>
</tbody>
</table>

*sometimes the concentrate is 1:10 or 1:100 w/v
Dilutions for Intradermal Testing

Serial Dilution Titration Dilutions

Prior to dilution each vial contains 4ml normal saline and 0.4% phenol
Intradermal *Single*-dilution Tests (ID)

A single intradermal test
Single dilution Intradermal Testing Pros and Cons

- **Advantages:**
  - Moderately sensitive
  - Reproducible
- **Disadvantages:**
  - More subjective interpretation than with multiple dilution technique
  - Significant variation in wheal size and erythema
  - Poor quantitative information
  - Results vary with antigen dilution
    - Concentrated dose - false positives
    - Dilute dose - false negatives
- Interpretation can be improved with intradermal testing using multiple dilutions (IDT)
Intradermal Dilutional Testing (IDT)
Principles of Intradermal Dilutional Testing

- Consecutive dilutions of antigenic extracts are applied in a sequential manner from weakest to strongest.
- Positive whealing demonstrates the presence of allergic sensitization (qualitative test).
- Comparison of whealing responses at different dilutions shows to what degree the patient is sensitive (quantitative test).
- Quantitation of sensitivity allows determination of the safe initial starting dose for desensitization immunotherapy.
Create skin wheals using multiple dilutions for titration testing:

- Begin with a test dilution that is anticipated to be weak enough (#6 usually) to produce a negative response.
- Apply progressively stronger concentrations until either a reacting strength is confirmed or all wheals are negative down to a #2 dilution.
- If the patient is allergic to the test antigen, then you will produce a series of wheals that (a) progress from negative to positive and (b) show increasingly larger wheals with stronger dilutions.
- **All wheals produced should be 4mm in diameter and read at 10 minutes.**
Intradermal Dilutional Testing (IDT)

Progressive increase of strength of dilution produces whealing response that indicates reactivity with progressive increase in size of wheal of 2 mm or more.
Possible Wheal Responses

• The “negative” wheal
• The “positive” wheal
• The "endpoint of titration”
• The "confirming wheal”
• The "plateau reaction”
• The "flash response"
The “negative” wheal

- A negative wheal is one which grows less than 2 mm larger than the 4-5 mm wheal initially created; i.e., a wheal of 6 mm diameter or less.
The “positive” wheal

- A positive wheal is one which grows at least 2 mm larger than the 5 mm wheal initially created; i.e., a wheal of 7 mm diameter or greater

- If the patient is allergic to the applied antigen, then the application of progressively stronger concentrations will at some point produce a positive wheal, i.e., one which enlarges to a diameter of 7 mm or greater
The "endpoint of titration"

- The *first or weakest* antigen dilution which produces a positive wheal is termed the "endpoint of titration"

- In a classical titration test, it is the intradermal dilution where the patient’s response turns from negative to positive

- The endpoint represents the *level of sensitivity of the patient being tested* for that particular antigen

- The endpoint represents an antigen dilution which may safely be used to begin immunotherapy

The "confirming wheal"

- To confirm that the endpoint is authentic, place a "confirming" skin wheal using antigen one dilution stronger than the one which produced the endpoint wheal.

- This should produce a wheal which is at least 2 mm greater in diameter than the ‘endpoint’ wheal.

- Ideally this so-called “confirming wheal” is used to validate the endpoint.
Intradermal Dilutional Testing (IDT)

Progressive increase of strength of dilution produces whealing response that indicates reactivity with progressive increase in size of wheal of 2 mm or more.

endpoint

Confirming wheal
## Plateau Response

| 5 | 5 | 7 | 7 | 7 |

Instead of a typical confirming wheal, the next wheal grows to the same size as the first positive wheal.

This represents a "plateau reaction".

In such a case, it is safest to designate the first of the two positive wheals as the endpoint.

(However, according to strictest of definitions, there is no endpoint here, since there is no confirming wheal and thus there is no “progression” of whealing with stronger dilutions.)
Plateau Response

A true endpoint is shown in this plateau reaction, since there is a negative response, followed by a positive response, followed by a confirming wheal.

The positive wheal that immediately precedes the confirming wheal is the endpoint because it is the dilution that initiates progressive whealing.
Flash Response

An abnormally large wheal preceded by negative wheals

The etiology of the "flash response" is unknown

Postulated to be due to ingestion of a cross-reacting food (e.g., melons in patients allergic to ragweed) or age/potency of antigen

Repeating the test in a few days generally will yield a more conventional response
Flash Response

<table>
<thead>
<tr>
<th></th>
<th>#6</th>
<th>#5</th>
<th>#4</th>
<th>#3</th>
<th>#2</th>
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<tbody>
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<td>Day 1</td>
<td>5</td>
<td>5</td>
<td>13</td>
<td></td>
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</tr>
<tr>
<td>days later</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

Stop (do not test the next dilution)
How far does one titrate?

• As long as titrations are negative, antigens are tested down to the #2 dilution

• If no positive wheal occurs by that point, most clinicians will end the test, and consider the patient to be negative to that antigen

• Most clinicians will not test with a #1 dilution if a #2 was already negative

• The only time to place a #1 dilution for inhalant testing is to confirm a #2 endpoint
### Examples of Intradermal Dilution Testing (IDT)

<table>
<thead>
<tr>
<th>Test material</th>
<th>#6</th>
<th>#5</th>
<th>#4</th>
<th>#3</th>
<th>#2</th>
<th>#1</th>
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<td>5</td>
<td>7</td>
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<td></td>
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<tr>
<td>Antigen b</td>
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<td>5</td>
<td>6</td>
<td>6</td>
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<tr>
<td>Antigen c</td>
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<td>8</td>
<td>6</td>
<td>6</td>
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<td>Antigen d</td>
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<td>Antigen e</td>
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<td>5</td>
<td>8</td>
<td>11</td>
<td></td>
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</tr>
</tbody>
</table>
IDT tips to save time:

- Use simultaneous dual consecutive dilutions (e.g. #6 and #4)
- Titrate by extrapolation (e.g. only test #6, #4, #2. then fill in the blanks)
- For “non-brittle” patients, start with #4 dilution, titrate either direction
Warning:

- Performing intradermal tests with full strength extract concentrates, due to the high concentration of antigen, poses unacceptable risk of anaphylaxis

- Therefore, do not perform intradermal testing with full strength (undiluted) extract concentrates
Diluents and Preservatives
Test Board
Preparation Steps

• Prepare five-fold dilutions, beginning with a commercially available "concentrate" strength.

• The most popular diluent is PNS (phenolated normal saline).

• Label the dilutions according to the number of times they have been diluted.

• The first five-fold dilution is the "#1" dilution.

• The second five-fold dilution, is the "#2" dilution, etc.

• In clinical practice, it is almost never necessary to make or test with dilutions weaker than the #6 dilution.
Dilutions for Intradermal Testing

Serial Dilution Titration Dilutions

Prior to dilution each vial contains 4ml normal saline and 0.4% phenol

$5^{-9} \quad 5^{-8} \quad 5^{-7} \quad 5^{-6} \quad 5^{-5} \quad 5^{-4} \quad 5^{-3} \quad 5^{-2} \quad 5^{-1}$
Antigen dilution using Phenolated Normal Saline (PNS)

- Inexpensive, non-irritating, and thus is reliable for testing
- 0.4% phenol added for sterility
- Most commonly used diluent for testing solutions
Antigen Dilution using Human Serum Albumin (HSA)

- 0.4% phenol added for sterility
- More expensive than saline
- Albumin minimizes antigen adsorption to glass vial
- “Potential” risk of transmission of hepatitis virus and HIV
Re-make your intradermal test/treatment dilutions

• As one prepares dilutions 1-6 on a test/treatment board, the glycerine concentrations weaken, and so do the glycerine preservation properties

• The potency of most dilutions prepared on a test board for intradermal testing is unpredictable after 6-8 weeks, even if properly refrigerated

• Intradermal test/treatment dilutions should be kept refrigerated as much as possible, and replaced with newly prepared dilutions every 6-8 weeks
Antigen preservation using 50% glycerine

- Extract concentrates from the manufacturer contain 50% glycerine because:
  - 50% Glycerine preserves potency for long periods
  - (3 years?**)
  - 50% Glycerine maintains sterility for long periods
  - (3 years?**)
  - Glycerine prevents “walling” in glass vials

- However, *glycerine is NOT used as a diluent in skin testing dilutions* because it is irritating, and is associated with an unacceptably high likelihood of false positive reactions
Antigen preservation using 10 or 25% glycerine

- 10% glycerine preserves antigen potency for 3 months

- In fact, adding enough glycerine to achieve a 10% concentration in immunotherapy treatment vials for purposes of preserving potency for 3 months is a good idea

- 25% glycerine preserves antigen potency for 6-12 months
Stop!

Before you start your skin testing, there’s something else we have to discuss
There are factors other than allergy that can affect skin whealing responses!!

- *Before proceeding with skin testing* using allergenic extracts, it is necessary to determine that the skin:

  1. *responds normally to mast cell provocation* by exhibiting the usual effects of mast cell degranulation and mediator release, leading to normal wheal growth

  2. *does not respond abnormally* by exhibiting unsuspected degranulation and thus abnormal or unusual wheal growth to stimuli that ordinarily should not cause degranulation\(^1\)

- We accomplish this task with the help of taking a good history and utilizing positive and negative control tests

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Factors affecting the skin whealing response:

- Factors that inhibit the whealing response
  - Medications
    - Antihistamines
    - Tricyclic antidepressants
    - Systemic beta agonists
  - Age of patient
    - Both pediatric and geriatric patients are less sensitive
- Factors that enhance the whealing response
  - Positive skin tests that are too close together
  - Dermatopathology
    - Dermatographism
    - Eczema
    - Urticaria
  - Food cross-reactivity
  - Beta antagonists (beta blockers)
  - Glycerine sensitivity

Antihistamines (H1 histamine antagonists) Pharmacology

- Competitively antagonizes histamine by stabilizing H1 receptors, thereby making these receptors unsuitable for histamine binding

- Antihistamines antagonize in varying degrees most of the pharmacologic effects of histamine

- Therefore, all H1 antihistamines suppress the wheal and flare reaction
<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Generic Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banophren--Diphenhydramine</td>
<td>Scot-Tussin</td>
</tr>
<tr>
<td>Benadryl--Diphenhydramine</td>
<td>Allergy Relief</td>
</tr>
<tr>
<td>Calm-Aid--Diphenhydramine</td>
<td>Formula</td>
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<tr>
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<td>--Diphenhydramine</td>
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<td>Diphenhydramine</td>
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<td>Diphedryl--Diphenhydramine</td>
<td>Sominex--Diphenhydramine</td>
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<td>Diphen--Diphenhydramine</td>
<td>Twilite--Diphenhydramine</td>
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<tr>
<td>Genahist--Diphenhydramine</td>
<td>Tylenol PM--Diphenhydramine</td>
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<td>Antihistamine Name</td>
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<td>Actifed Sinus Day</td>
<td>Diphenhydramine</td>
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<td>Chlorpheniramine</td>
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**Some Antihistamine Brand Names & Generic Names**
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<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Generic Name</th>
</tr>
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<tbody>
<tr>
<td>Livostin</td>
<td>Levocabastine</td>
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<td>Claritin</td>
<td>Loratadine</td>
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<td>Quintadrill</td>
<td>Mequitazine</td>
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<td>Mizolastine</td>
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<td>Phenergan</td>
<td>Promethazine</td>
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<tr>
<td>Prorex 25 &amp; 50</td>
<td>Promethazine</td>
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<tr>
<td>PBZ &amp; PBZ-SR</td>
<td>Tripelennamine</td>
</tr>
</tbody>
</table>
Factors that can **enhance** the wheal and flare reaction

- Positive skin tests that are too close together
- Dermatopathology
  - Dermatographism
  - Eczema
  - Urticaria
- Food cross-reactivity
- Beta antagonists (beta blockers)
- Glycerine sensitivity
Too short a distance between positive adjacent tests can **enhance** the whealing response.

- Histamine controls or *positive* skin tests placed too near other tests can initiate axonal reflexes which drive the wheal and flare response.

- Thus, it is recommended that each skin test be separated by at least 2 cm.
Dermatographism-
An “Abnormal” Skin Whealing Response:

• In some patients the simple act of placing a needle beneath the skin, or even just scratching it can produce a wheal and flare response
Dermatographism
Dermatographism

• Most common physical urticaria
  – 5% of the atopic population
  – 1.5% of the overall population
• “Write on skin”
• Pruritic
Food/inhalant cross-reactivity can enhance the whealing response

- Some foods and inhalant allergens share similar surface epitopes
- Therefore if exposure to both are simultaneous, then the allergic reaction can be enhanced
- Example--melon ingestion in patients who are allergic to ragweed, and undergo ragweed skin testing, especially while in season
- May explain “flash” reactions during skin testing
Antagonism of Beta Receptors on the Mast Cell (β - Blockade) can enhance the whealing response

- Decreases intracellular cyclic AMP
- Lowers the threshold for mast cell and basophil degranulation
- Increases the size of the skin whealing response
- Increases total IgE production
- Increases circulating eosinophils 30%
- Increases mast cell sensitivity
- Increases mast cell mediator release
- Decreases response to beta agonists
- Increases severity of allergic symptoms
- Increases risk of anaphylaxis
- Increases difficulty treating anaphylaxis
Glycerine sensitivity can **enhance** the whealing response

- Glycerine is inherently irritating to the skin in some people
- Therefore an intradermal skin test from an antigen preserved in glycerine, especially a stronger antigen dilution (#1, #2, possibly #3), may be associated with wheal growth that is due to the glycerine preservative and not the antigen itself
Other factors which affect skin whealing responses, but which are not so critical to the **accuracy** of test results.

- Area of body tested
  - upper back > lower back > arm

- Potency of extract
Drugs that do **NOT** “appreciably” affect skin whealing responses

- H2 antagonists (e.g., ranitidine, cimetidine)\(^1\)
- Systemic corticosteroids\(^2\)
- Inhaled corticosteroids
- Leukotriene modifiers\(^3\)
- Cromolyn
- *Inhaled* Beta 2 Agonists

Topical, but not systemic steroids, alter the skin testing response

• Skin prick tests are not altered by short or long term treatment with systemic corticosteroids


• Prolonged topical steroids do reduce skin test responses

  Andersson and Popcorn JACI 1987;79:345-9
When Falling Asleep Feels the Easiest

<table>
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<tr>
<th>Circumstance</th>
<th>How Easy it is to Fall Asleep</th>
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<td>In Class</td>
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<td>At Work</td>
<td>80</td>
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<tr>
<td>In bed actually trying to sleep</td>
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Controls
Step 1 of all allergy skin testing is to apply positive, negative, and glycerine controls

• Positive control
• Negative (Diluent) control
• Glycerine control
• If the positive control test does not yield appropriate results, testing should be suspended, as the results of further tests will most likely not be reliable
Positive Control Test

• In order to assure that the wheal and flare capability of the patient's skin is intact, testing must include application of a positive control

• Histamine is the most popular positive control

• Codeine, which causes mast cell degranulation, can be used
How to apply the intradermal histamine control test:

- A 4 mm wheal applied using histamine at a strength of approximately 0.004 mg/mL should yield a 7 mm or larger wheal at 10 minutes.
- If this fails to produce a positive response, testing should be suspended for that day, and an investigation into the cause carried out.
- In vitro tests may be indicated in this situation.

How to prepare the intradermal histamine control

• The positive intradermal control test is administered using histamine at a strength of approximately 0.004 mg/mL, and can be made in one of 2 ways:

• **Method 1:**
  – Obtain aqueous (not glycerinated\(^1\)) histamine phosphate .275 mg/mL
  – Then mix 2 mL of this with 3 mL of PNS
  – Then make a # 2 dilution of this by titrating 2 times

• A positive wheal growth from glycerinated histamine may be due to the glycerine rather than the histamine
How to Prepare the Intradermal Histamine Control

• **Method 2:**
  
  – Obtain aqueous (not glycerinated) histamine 2.75 mg/5 mL
  
  – Then make a #3 dilution of this by titrating 3 times

1. A positive wheal growth from glycerinated histamine may be due to the glycerine rather than the histamine
Negative Intradermal Control Test

- To rule out dermatographism or other skin hypersensitivity, place an intradermal wheal using diluent or some other inert substance (phenolated saline or HSA).
- The 4 mm negative control wheal should not enlarge beyond 5 mm.
- “positive” wheal growth predicts false positive test results.
- If a negative control test yields significant wheal growth, in vitro testing may be appropriate for further testing.
Glycerine Control Test Rationale (for intradermal testing)

• Extract concentrates that are glycerinated contain 50% glycerine
• #1 dilutions created from these extracts contain 10% glycerine
• #2 dilutions created from these extracts contain 2% glycerine
Glycerine Control Test

Rationale (cont’d)

• Intradermal injection with solutions containing 10% and 2% glycerine can irritate

• Intradermal testing with #1 and #2 antigen dilutions may cause wheal growth from the glycerine and not the antigen

• Therefore if one tests with #2 and #1 antigen dilutions, the wheal sizes must be compared to those created from placement of #2 and #1 glycerine control wheals
Glycerine Control Test Technique

- Create glycerine control test dilutions by creating five fold dilution #s 1, 2, and 3 from a vial containing 50% glycerine
- Create 4mm wheals from dilutions #1 &/or #2 as needed** and read after 10 minutes
- If the #2 glycerine control test grows to 7mm or more, then the #3 glycerine control test should also be performed

** If you anticipate ID testing with #1 or #2 antigen dilutions
Glycerine control test technique and interpretation

• A “positive” wheal growth using glycerine controls does NOT necessarily lead to the need to abort further skin testing:
  • Instead one can compare these wheal growths to those created by comparable strengths of antigen test dilutions used during antigen testing
  • Consider antigen reaction “positive” if size is 2mm greater than glycerine control at same dilution
## Interpretation of IDT tests with glycerine controls

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*remember to apply a #3 glycerine control if the #2 is positive*
Review

- Pathophysiology of skin whealing
- Prick testing
- Intradermal testing
- Factors that influence skin test response
- Controls
Comparison of Testing methods and a blend of IDT and prick testing called MQT
Modified Quantitative Testing (MQT)

• Blends SPT with single dilution ID tests
• Uses SPT (multi-prick) as the initial test to estimate level of sensitivity
  – assumes that a 3 - 8 mm wheal suggests a level of reactivity ranging from a #3 to a #4 dilution
• Uses either a single weaker or stronger ID test to further estimate the “endpoint”

Modified Quantitative Testing (MQT) - Technique

- If SPT wheal is < 3 mm in diameter
  - place single ID dilution #2
- If SPT wheal is 3 mm - 8 mm in diameter
  - place single ID dilution #5
- If SPT wheal is > 8 mm in diameter
  - antigen is graded as a #6 endpoint
MQT Algorithm

SCREEN WITH MULTI-TEST

- < 3 mm
  - PUT ON #2
    - ≤ 6 mm
      - TEST NEGATIVE
    - ≥ 7 mm
      - + E.P. #3
  - ≥ 9 mm
    - + E.P. #6

- 3 – 8 mm
  - PUT ON #5
    - ≤ 5 mm
      - + E.P. #4
    - 7 – 8 mm
      - + E.P. #5
    - ≥ 9 mm
      - + E.P. #6
How well does MQT correlate with IDT?

- For non-fungal antigens
  - 83% concordance

- For fungal antigens
  - 84% concordance

- MQT and IDT yield 10% more positive tests than skin prick testing alone

- When MQT and IDT differ, MQT predicts safer starting dose of immunotherapy

Peltier & Ryan Otolaryngol Head Neck Surg 2007;137:246-249
Peltier & Ryan Otolaryngol Head Neck Surg 2006;134:240-244
Modified Quantitative Testing (MQT) Summary

• The assignment of endpoints to intradermal reactions is conservative
  – enhances safety of method

• Vial preparation is conducted using MQT endpoints in a similar fashion to IDT endpoints
Costs of Intradermal Dilutional Testing (IDT) versus Prick Testing

- IDT is more time consuming and labor-intensive than other forms of skin testing\(^1\)
- It requires more supplies
  - vials
  - syringes
  - expended antigen
  - more time consuming
- More technical expertise of tester
- More needle sticks

Costs of Intradermal Dilutional Testing (IDT) versus Prick Testing

• One study showed that the cost of performing a complete IDT battery is approximately three times greater than the cost of prick testing for the same number of antigens.

Reimbursement Disadvantages of IDT

- Insurers often fail to cover testing with IDT methods as an “unproven” or “experimental” technique.

- Even when IDT is covered, total reimbursement can be limited or reduced.

- Some insurers are beginning to place limits on the number of antigens and/or tests that can be conducted on a specific patient (often limited to 60 – 65).

- Thus the variable and fixed costs for IDT continue to remain high relative to other forms of testing.
IDT testing with strong dilutions?

- Intradermal testing with strong dilutions of antigen (1:500 to 1:1000 w:v) may lead to a significant number of false positive tests.

- A positive intradermal test with strong concentrations of antigens in the face of a negative prick test did not correlate with either antigen challenge nor with symptoms during relevant pollen seasons and thus may be falsely positive²-⁴.

- ID testing adds little to the sensitivity of skin prick testing, and may decrease its specificity²,³.


SPT versus IDT versus nasal antigen challenge

- One recent paper compared IDT to skin prick testing using nasal antigen challenge as a standard

- **IDT was less sensitive and less specific** than skin prick testing

- **Skin prick testing better** correlated with direct end organ challenge than did IDT

Gungor et al., ENT Journal, 2004;83:54-60.
Summary

- We have a variety of skin testing methods available for the diagnosis of inhalant allergy.
- The precise techniques used will depend upon practice characteristics and familiarity/confidence in the available methods.
- There is no one method that will meet the needs of all patients or physicians.
Summary

• A variety of factors can alter skin test results.

• Skin testing should begin with controls.

• Antigens must be stored appropriately to preserve potency.

• Skin test results must be correlated to the patient’s history.
Thank You