CLS 468 - Immunology Clinical Practice

جامعة الملك سعود
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Allergy Testing

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Outline

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I. Overview of Allergy

- **Allergies** arise if the body's immune system overreacts to foreign substances (allergens) that are usually harmless for most people, such as pollen or certain foods.
- Allergic reactions most often occur on the skin and in the airways and mucous membranes.
- **Immunoglobulin E (IgE)** is produced in response to allergens.
- **Basophils and mast cells** are recognized as important effector cells in immediate hypersensitivity responses.

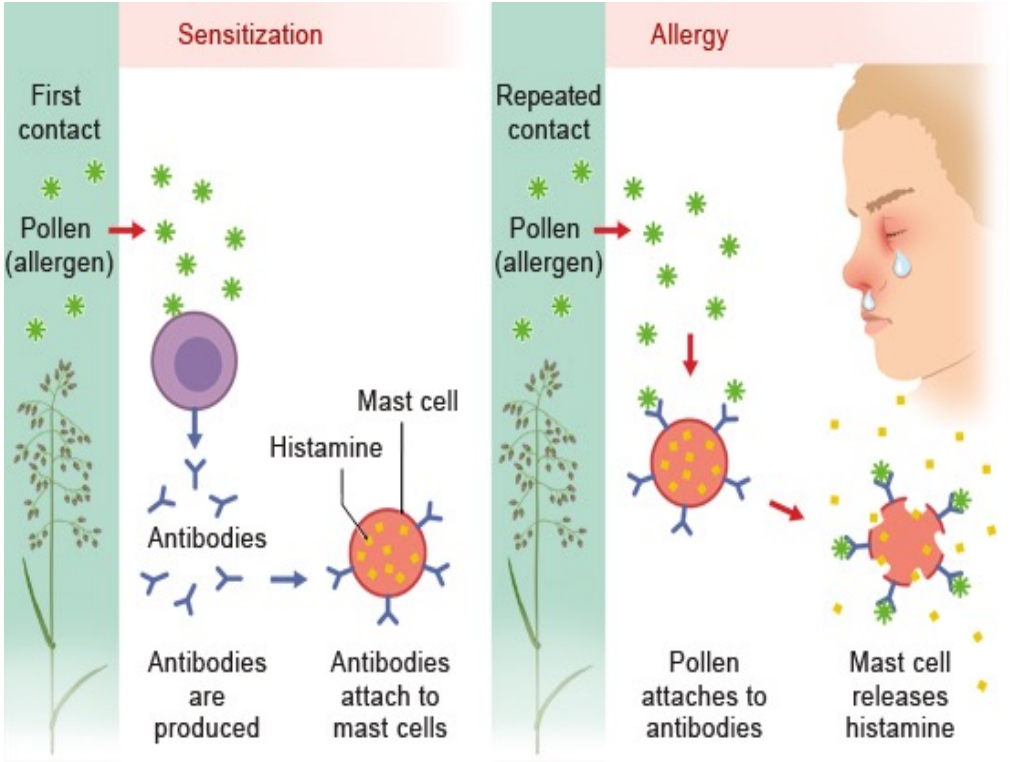

Figure 1. Development of allergy

II. Types of Allergy Testing

Allergy testing can be categorized into:

1. In vivo testing:
    a. **Skin Prick Test (SPT):** This represents the first level of approach for the diagnosis of type I, immediate, IgE-mediated allergy.
    b. **Intradermal Test (IDT):** This can be used to evaluate both immediate IgE-mediated allergy and delayed-type hypersensitivity, according to the time of read-out. It has increased sensitivity and decreased specificity compared to SPT.
    c. **Patch test:** This is used for delayed type, cell-mediated, hypersensitivity reactions. It has no relevance for IgE-mediated allergy.

II. Types of Allergy Testing

2. In vitro diagnosis of IgE-mediated allergic diseases
    a. **The total IgE assay** is nonspecific and provides only gross information.
    b. **Serum-specific IgE assays** against allergen sources/molecules are the most commonly used in vitro diagnostic approach. It can be performed by a single-plexed or multiplexed strategy.
    c. **The Basophil Activation Test (BAT)** is quite specific, but complex to perform, and therefore limited to selected situations.

1. In Vivo Allergy Testing

1.a. Skin Prick Test

- **Skin prick testing (SPT)** is the most frequently used method for the detection of IgE antibodies, due to its rapidity, simplicity, and low cost.
- Skin tests should include the relevant allergens in the given geographical area and ideally be carried out only using standardized allergenic extracts.
- approximately after 25 min the wheals are interpreted, if >3 mm it indicates a positive reaction.

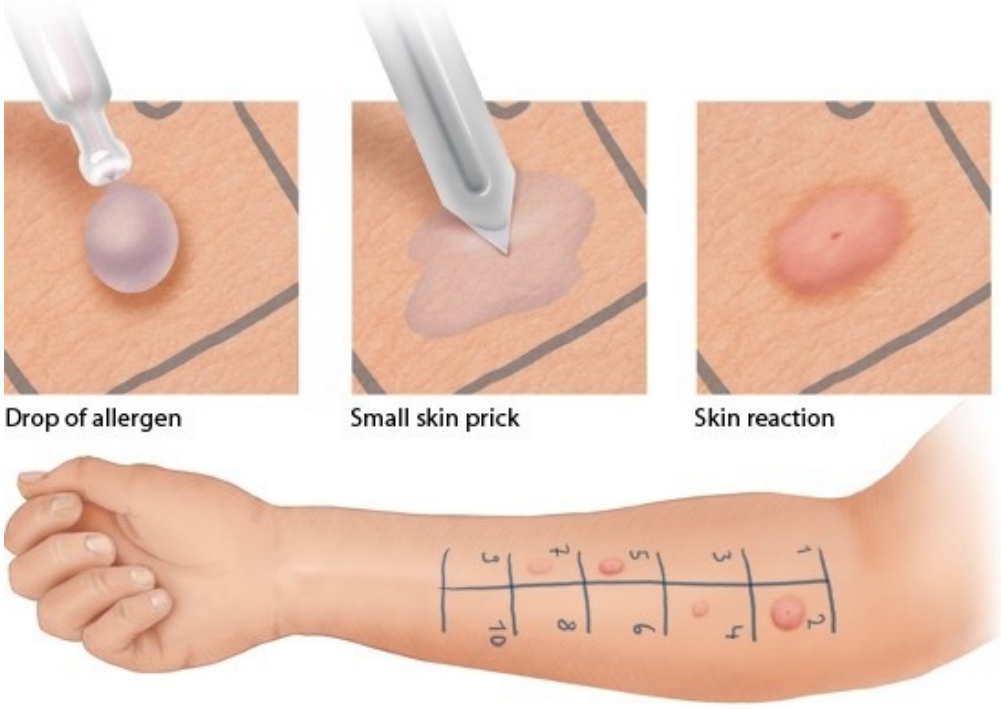

Figure 2. Skin prick testing procedure

1.b. Intradermal Test

- In a patient with a strong clinical suspicion of an IgE-mediated disease with negative skin prick tests, the intradermal test (IDT) can be considered.
- Intradermal testing (IDT) is important to reveal both immediate IgE-mediated allergy and delayed-type hypersensitivity.
- Uses in assessing hypersensitivity to drugs or Hymenoptera venoms.

1.b. Intradermal Test

Technique:

- 0.02 mL of allergens are injected intradermally with small needles to produce a small bleb, and the outcome measure is an increase in the size of the wheal with a flare reaction at 20 minutes.

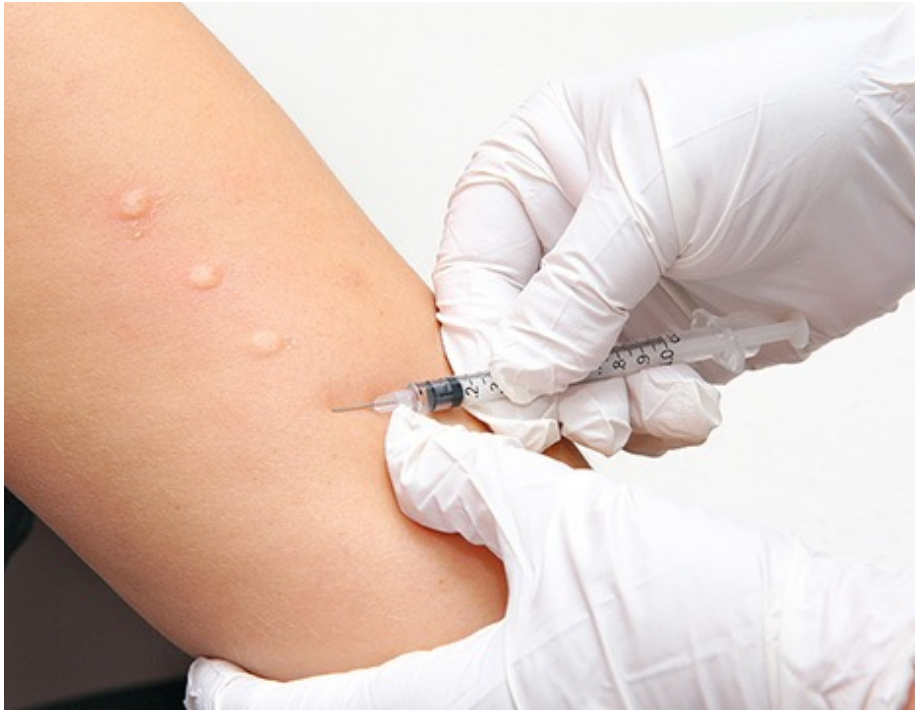

Figure 3. Intradermal test procedure

Skin Prick Test V.S Intradermal Test

Table 1. Relative advantages/disadvantages of prick and intradermal allergy skin testing.

	Prick test	Intradermal test
Simplicity	++++	++
Speed	++++	++
Interpretation of positive and negative reactions	++++	++
Discomfort	+	+++
False-positive reactions	Possible	Likely
False-negative reactions	Possible	Rare
Reproducibility	+++	++++
Sensitivity	+++	++++
Specificity	++++	+++
Indicative of IgE antibodies	Yes	Yes
Safety	++++	++
Testing of infants	Yes	Difficult

2. In Vitro Allergy Testing

2.a. The total IgE assay

- The total IgE assay is nonspecific and provides only gross information.
- Serum IgE concentration is largely age-dependent.
- Very high IgE levels are observed in parasitic infections.
- Total IgE values are reported in equivalence of kU/L.

2.b. Serum-specific IgE assays

Two ways of measuring specific IgE recognizing allergenic epitopes:

- **Singleplex**: single reagents.
- **Multiplex**: a pre-defined panel of a number of molecules to be tested simultaneously.

Example of Specific IgE allergens assays:

- **Food:** Egg white, milk, fish, wheat, peanut.
- **Molds and yeast:** aspergillus.
- **Weed pollens:** insects, and mites.
- **Epidermal and animal protein:** grass pollens.

Testing methodologies for specific IgE

Table 2. The most commonly used systems for specific IgE detection include the following distinct components.

Producer	Solid phase	Allergens	Patient's serum	Anti-IgE	Anti-IgE Labelling	Enzyme substrate	Stop solution	Reading system
RAST	Sephadex or paper	Extract	0.05 mL/ sample	Polyclonal	^{125} I	none	NN	Gamma-counter
Phadia	Polymer of hydrophilic, highly branched cellulose derivative enclosed in a capsule.	Extract or recombinant bound covalently to the solid phase	0.04 mL	Mouse monoclonal anti-human IgE	ß-Galattosidase	4-metilumbelliferil-ß-D-galattoside	Na Carbonate	Photometer
Siemens	Streptavidin-covered polystyrene ball conjugated with streptavidin-	Extract or recombinant allergens covalently to soluble biotinylated polylysine polymers.		anti-IgE antibody (mAb ? pAb?)	Alkaline phosphatase	4-methoxy-4-(3-phosphatephenyl)-spiro-(1,2-dioxetane-3,2′-adamantane)	N.S.	Light emission detector (chemiluminescence)
Hycor	Magnetic, streptavidin-coated microparticles incubated with a biotinylated allergen	Extract or recombinant	0.04 mL	A mixture of two mouse monoclonal Anti-IgE	Horseradish Peroxidase	acridin based chemiluminescent substrate	N.S.	Light emission detector (chemiluminescence)
Euroimmun	Paper	Extract or recombinant	1000 mL		Alkaline phospatase		Water	Scanner

The main reagents used in the assay

- **The reaction site is a** surface carrying the allergen-encapsulated hydrophilic carrier polymer to which the allergen is covalently coupled.
- **The allergen-containing reagent** can be represented by a solid-phase allegro-sorbent or liquid-phase conjugated allergen.
- **The nature of allergens** used for specific IgE: allergens can be both raw extract allergens or single molecules (obtained by recombinant DNA technology or by biochemical purification from natural extracts).
- **Sample:** both serum and plasma can be used.
- **The anti-human IgE Fc detection reagents (ε heavy-chain specific):** combinations of polyclonal and monoclonal (mAb) anti-human IgE and labeled human a-FcεR170 have also been used to detect human IgE.

The main reagents used in the assay

- Antibody labeling and detection methods:
  - Anti-human anti-Ab were originally labeled with ^{125}I (the original Radio Allergo-Sorbent Test – RAST).
  - Other labeling techniques using enzymes have been adopted such as:
    - b-Galactosidase (b-Gal) used in Phadia system.
    - Alkaline phosphatase (AP).
    - Horseradish Peroxidase (HPO).
  - enzyme substrates reagent: pNPP for AP, “perox” for HPO.

Automated testing for Allergy

- The **Phadia Laboratory Systems** (formerly known as ImmunoCAP®) include the Phadia 100, Phadia 250, Phadia 1000, Phadia 2500, and Phadia 5000.
- They are all **fully automated** laboratory systems <u>designed for the testing of allergy and autoimmune diseases.</u>

Phadia 200 instrument

Phadia 250 instrument

Phadia 2500 instrument

Figure 4. Phadia instruments models.

Phadia 250

1. Stop solution and development solution
2. Loading tray
3. Cooled storage chamber for 180 barcode-controlled EliA and ImmunoCAP carriers
4. Sample diluent and dilution plates for onboard dilution of samples.
5. Reagent barcode reader
6. Cooled conjugate compartment
7. Strip tray for calibrator strips and/or curve control strips.
8. Patient and control sample racks

Figure 5. Phadia 250 instrument.

Phadia 250 ImmunoCAP Reagent

- The reagents for the allergy assays are based on the well-established **ImmunoCAP** format.
- Over 500 different allergens and allergen components are available for allergy testing, with new analytes continually being developed and released.

Figure 6. ImmunoCAP reagents.

ImmunoCAP Assay Principle

Assay Principle

1. The **antigen** of interest, covalently bound to the ImmunoCAP well, reacts with the analyte in the patient sample.
2. After **washing** away unbound analyte, **enzyme-labeled antibodies** are added to form a complex.
3. After **incubation**, unbound enzyme label is **washed** away, and the bound complex is then incubated with a **developing agent**.
4. After stopping the reaction, the **fluorescence** of the eluate is measured. The higher the fluorescence, the more analyte that is present in the sample.

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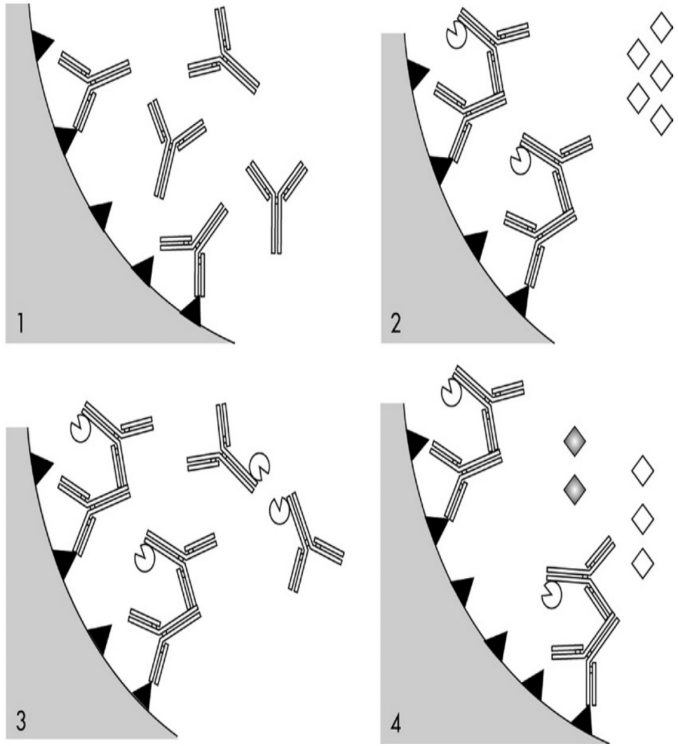

Figure 7. ImmunoCAP principle

ImmunoCAP Result

Result:

- Results for ImmunoCAP Allergen mixes are qualitative values and 0.35 kU/l is recommended as a **cut-off value**.

- Values ≥0.35 kU/l indicate **specific IgE antibodies** to one or more of the allergens coupled to ImmunoCAP Allergen mixes.

2.c. The Basophil Activation Test (BAT)

Cellular assays - basophil activation test:

- This test assesses selected and defined functions of effector cells within the allergic cascade.
- It is particularly helpful in cases of equivocal and/or negative results obtained with other in vitro and in vivo tests and in cases of discrepant results.
- **Basophils** express the high-affinity IgE receptor (FcεRI), and thus they carry specific IgE (sIgE) antibodies on their surface and degranulate when the allergen cross-links these sIgE/ FcεRI complexes.
- This degranulation of basophils can be detected and quantified by flow cytometry.

BAT Analysis

1. Surface-marker combinations for the identification of basophils include:
   - CCR3þ/ CD3-, or CD123þ/HLA-DR-, or IgEþ/CD203cþ.
   - The **only lineage-specific basophil marker** is CD203c.

2. Appearance and/or up-regulation of the desired activation/degranulation marker is investigated:
   - **CD63** is a degranulation marker that appears during compounded degranulation of the cell.
   - **CD203c** serves as an activation marker.
   - In the resting basophil, the expression of CD203c is low.
   - In the activated basophil a rapid and marked increase in CD203c is observed.

BAT Principle

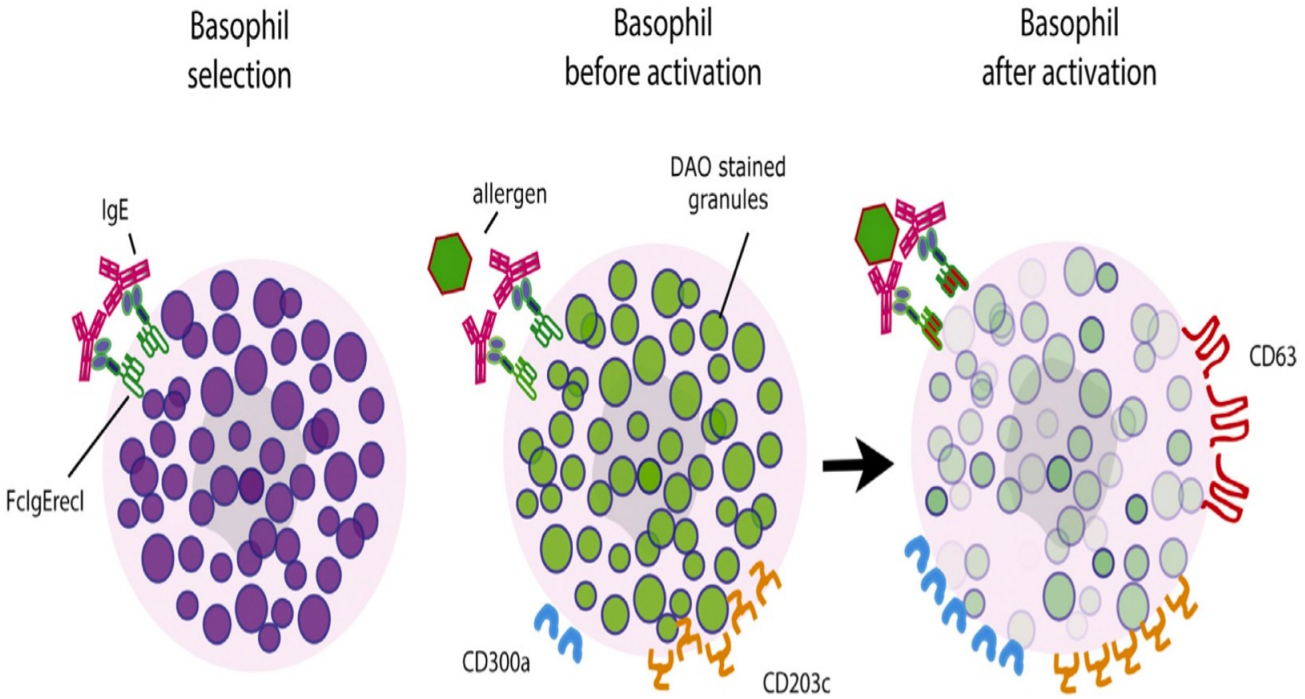

Figure 8. BAT Principle

BAT Analysis

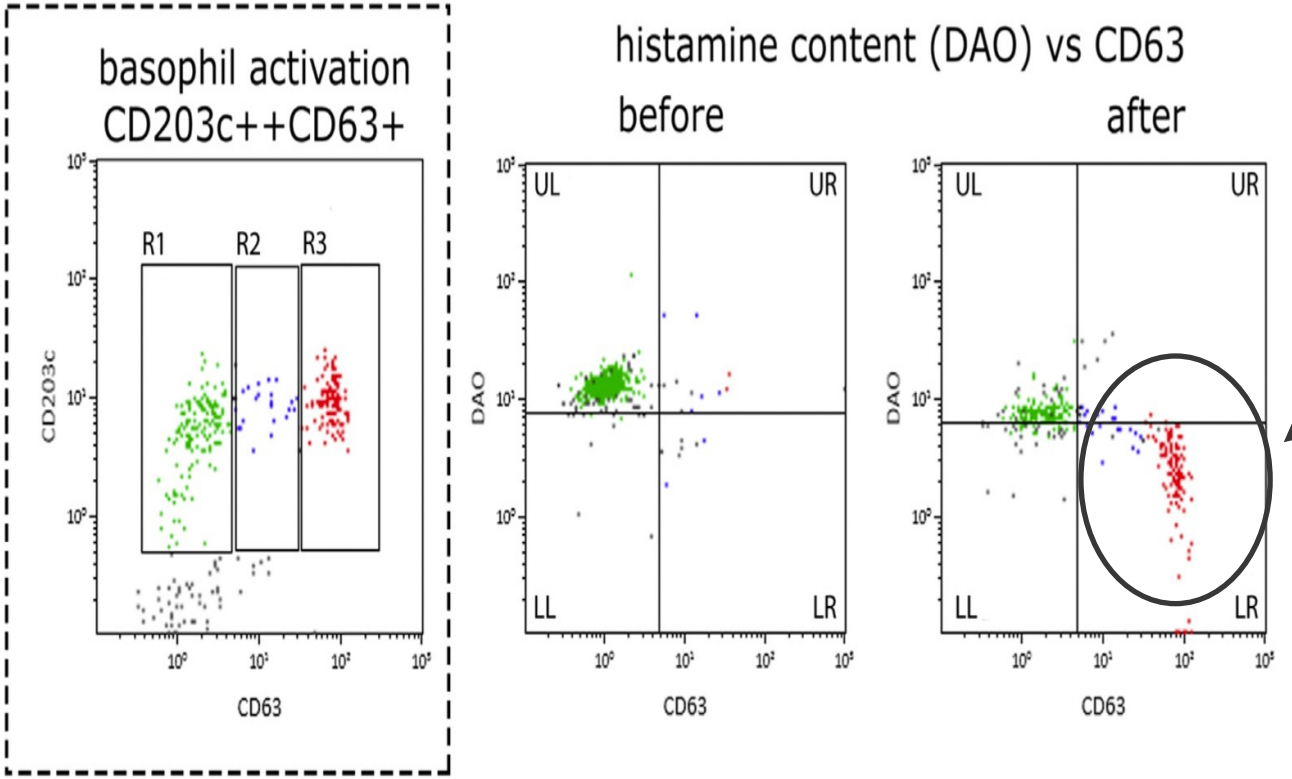

Figure 9. BAT result analysis.

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