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Allergy testing

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What is Allergy?

- Allergy is a hypersensitivity disorder of the immune system (hypersensitivity refers to undesirable reactions produced by the normal immune system)
- Allergic reactions occur when a person's immune system reacts to normally harmless substances
- Allergic reactions lead to an excessive activation of certain white blood cells called mast cells and basophils by a type of antibody called **Immunoglobulin E (IgE)**

What is atopy?

- Atopy is a personal and/or familial tendency, usually in childhood or adolescence, to become **sensitized** and produce IgE antibodies in response to ordinary exposure to allergens

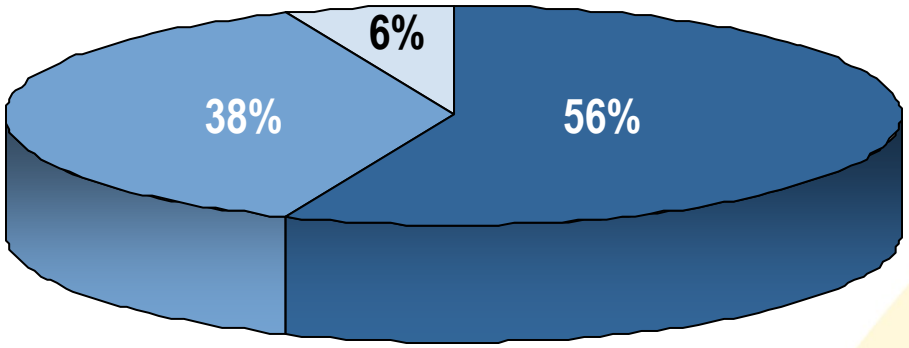
- Atopy may have a hereditary component

- Atopy is a clinical definition of an IgE antibody high-responder

- The term atopy can not be used until an **IgE sensitization** has been documented by IgE antibodies in serum or by a positive skin prick test

- A person with atopy typically presents with one or more of the following – eczema, allergic rhinitis, allergic conjunctivitis or allergic asthma

Most allergic children have non-allergic parents



German asthmatic children with:

- Non-atopic parents 56%
- One atopic parent 38%
- Two atopic parents 6%

The majority of prospectively affected children will <u>not</u> be identified at birth by family history *(Wahn, Allergy 2000)*

Current practice for diagnosis Of Allergy

History and clinical examination

↓

Suspicion of allergy

↓

Total IGE testing

↓

Skin Prick Test/Specific IGE testing*

* These tests detect allergic sensitization or the presence of allergen-specific IGE

Total IGE testing- Drawbacks

- Total IGE levels naturally increase from infancy to adolescence when they plateau and slowly decline with age
- There is a seasonal variation in Total IGE
- Low sensitivity and specificity
- Elevated in many non allergic conditions such as parasitic infections, vasculitis and rare hyper IGE syndrome

Hence a more specific test is needed that can definitely say whether the patient has allergy or not

Skin-Prick Test

- Immediate hypersensitivity skin tests are used to identify **specific IgE sensitization**
- The skin is marked for testing with a panel of appropriate allergens for the patient, selected on the basis of the clinical history and knowledge of the allergens commonly found in the locality
- A drop of allergen solution is placed onto the skin at each mark, and a fresh fine sterile needle is used to gently prick the skin through each drop, introducing a minute volume of allergen solution into the dermis
- The results are interpreted after 10-15 mins
- The presence of a raised wheal at the site of the allergen skin prick test of 3 mm or greater in diameter indicates the presence of IgE antibodies specific to that allergen

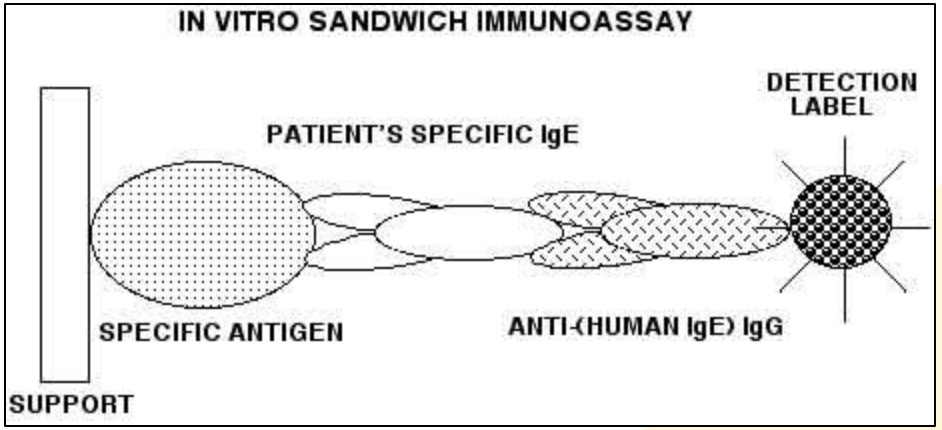
Skin-Prick Test - Disadvantages

- Safety - reactions occur!
- No standardization of procedure and reagents
- Poor reproducibility
- Labour intensive
- Drug interactions (cannot be done in patients on antihistaminics)
- Dependant on skin condition
- **No quantitative results** -Decision points not possible
- Patient’s discomfort

Skin-Prick Test or In-vitro test

- Both SPT and *in vitro* tests differ in their ability to detect sensitization depending on:
  - the quality of the extract used
  - and the technical detection limit of the test
- SPT is a tool mainly for specialists (experienced clinicians/nurses)
- *In vitro* tests can be used by "anyone"
- Interpretation of results is crucial for both tests!

Basic Principle of In-vitro allergy testing



- Basic principle is the formation of a molecular sandwich: allergen specific IgE in serum can be measured by sequential reaction of an insoluble support, a known allergen, the patient's serum containing an unknown amount of allergen-specific IgE, and anti-(human IgE), labeled in such a way that the whole sandwich can be detected and quantitated

- The sandwich technique depends on the immobilization of one of the two test reagents (usually the allergen) on a solid support. Soluble allergens must be bound to supports to create stable, insoluble immunosorbents that acquire the antigenicity of the allergen

- Many types of supports can be used
- These immunosorbents with attached allergens, when incubated with a test serum, will react with specific IgE antibody to form antigen-specific immune complexes
- Because the antigen is firmly attached to the support, the entire complex also stays attached to the support. The unreacted antibodies in the serum are then washed away, leaving only specific IgE bound to it's specific allergen, which is, in turn, attached to the support
- Labelled anti-(human IgE) IgG raised in another species (such as rabbit will react with antigenic determinants on the Fc portion of the patient's IgE antibody that is bound to the allergen-coated immunosorbent
- This forms a three-layered molecular sandwich: allergen+patient's allergen-specific IgE+anti-IgE, and the entire sandwich is bound to the support
- The amount of bound label then gives a relative measurement of the amount of specific IgE that was in the patient's serum
- Different assays are available using different support media and different detection labels (eg RAST, ELISA, CLIA and ImmunoCap)

History of RAST (radioallergosorbent test) and ImmunoCAP

- The market-leading RAST methodology was invented and marketed in 1974 by Pharmacia Diagnostics
- RAST used radiolabelled anti-human IGE antibody
- In 1989, Pharmacia Diagnostics AB replaced it with a superior test named the ImmunoCAP Specific IgE blood test
- ImmunoCAP is a fluorescent enzyme labeled assay
- ImmunoCAP is the only specific IgE assay to receive FDA approval to quantitatively report to its detection limit of 0.1kU/l
- In 2010 the United States National Institute of Allergy and Infectious Diseases recommended that the RAST test measurements of specific immunoglobulin E for the diagnosis of allergy be abandoned in favor of testing with mores more sensitive fluorescence enzyme-labeled assays

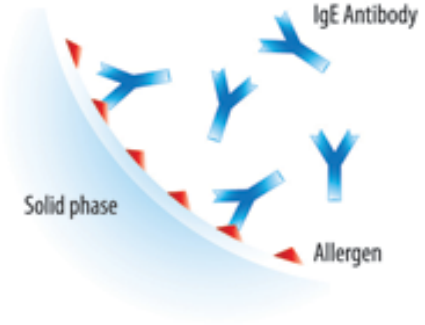
ImmunoCAP technology

Test Principle ImmunoCAP Specific IgE

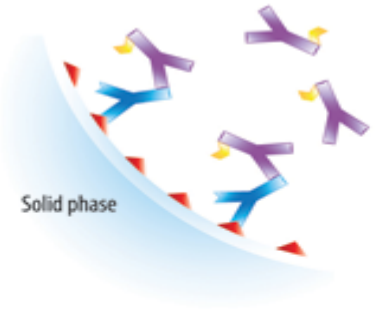
The technology is based on an extremely high total binding capacity, achieved through a high binding capacity per mg cellulose in combination with an optimal amount of cellulose in each solid phase. This ensures binding of all relevant antibodies, regardless of antibody affinity, still giving low non-specific binding.

The ImmunoCAP solid phase consists of a cellulose derivative enclosed in a capsule. The hydrophilic, highly branched polymer provides an ideal microenvironment for allergens, binding them irreversibly while maintaining their native structure.

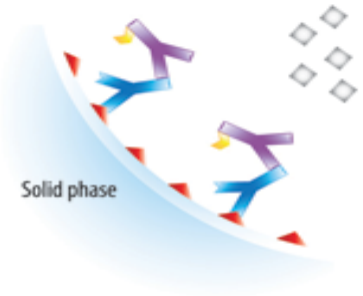
The test is designed as a sandwich immunoassay.



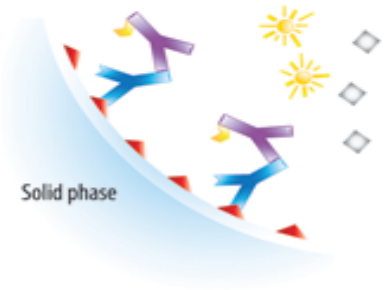
The allergen of interest, covalently coupled to the solid phase, reacts with the specific IgE in the patient sample.



After washing away non-specific IgE, enzyme-labelled antibodies against IgE are added to form a complex.



After incubation, unbound enzyme-labelled anti-IgE is washed away and the bound complex is then incubated with a developing agent.



After stopping the reaction, the fluorescence of the eluate is measured. The higher the fluorescence, the more specific IgE is present in the sample.

Allergy testing @ Metropolis

- Phadiatop Adult
- Phadiatop Infant
- Allergy Panels (by ImmunoCAP)
- Some Allergens by CLIA (outsourced)

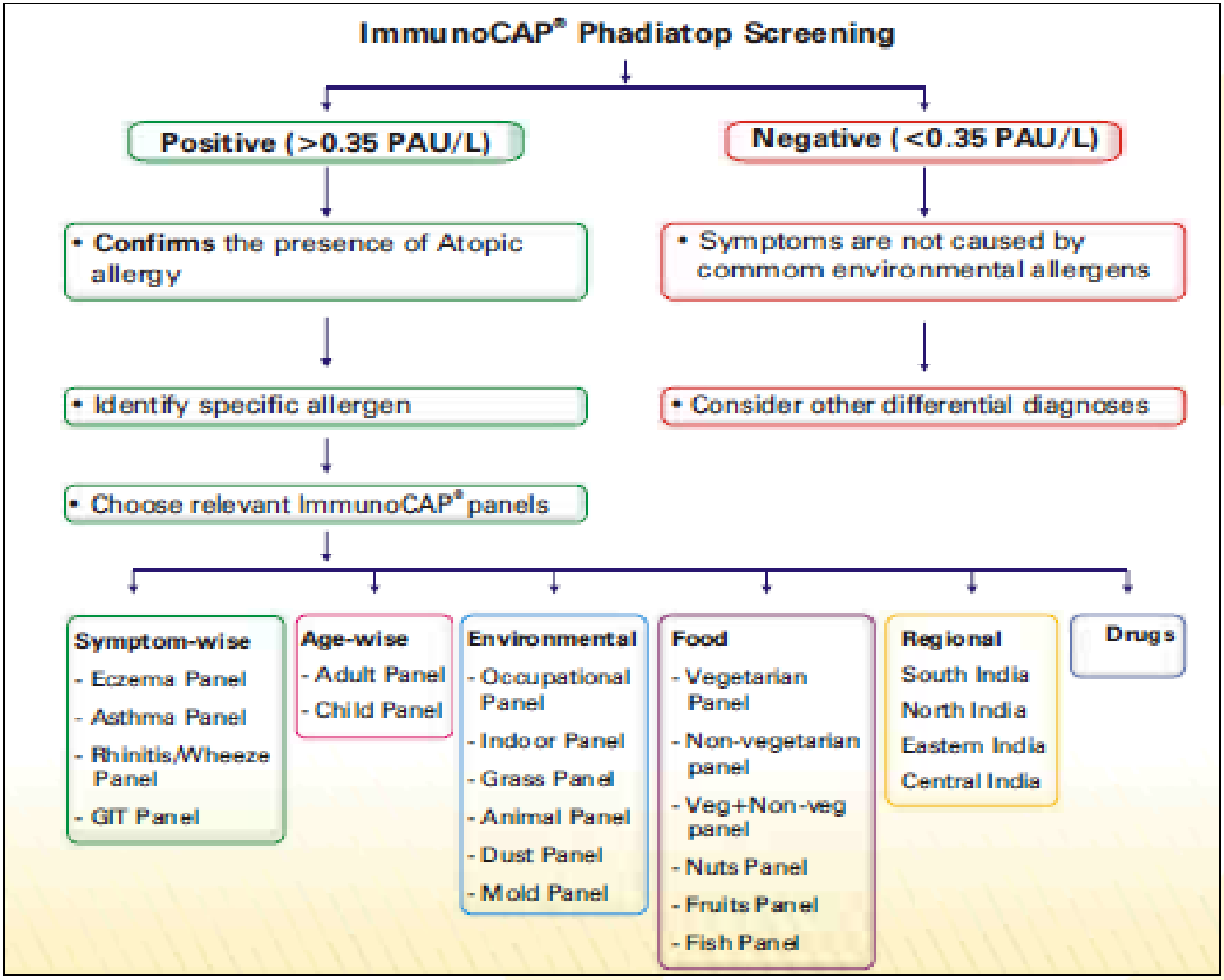
Phadiatop

- A screening test designed to differentiate between atopic and non-atopic patients
- Uses the ImmonoCAP technology
- Demonstrates the presence of IgE antibodies to **common** inhalant allergens
- Acts as an objective and reliable first step when testing for allergy
- Two age-dependent versions

  1. Phadiatop (>5 years of age) -Measures IgE abs to **common inhalant** allergen for the age

  2. Phadiatop Infant (0-4 years) - Measures IgE abs to common **food & inhalant** allergens for the age

Interpretation of Phadiatop results



Reporting of Phadiatop results

Semi-quantitative results (PAU/l)

- PAU/l – Phadia Arbitrary Units/l
- **PAU/l** is <u>not</u> equivalent to **kU_A/l**
- Limit of quantitation is 0.1 PAU/l*

*A use of 0.1 kU_A/l will lead to many more positive patients without any symptoms, which could be confusing (but in agreement with the intended use of Phadiatop – atopy test). Many labs have kept 0.35 kU/l as the cut-off.

Cost reduction:
– Phadiatop eliminates non-atopics and unnecessary testing

Sera from 35 patients aged 5 years or younger and sera from 95 patients 6 years and older were assayed for both total IgE level, allergen-specific IgE by RAST and either the Phadiatop Paediatric or Phadiatop procedures, depending on age. Good agreement was found allowing exclusion of specimens anticipated to be RAST negative with an expected reduction in RAST testing of up to 80% of the present workload, without significant exclusion of RAST positive specimens.

Reference: Salkie ML. J Clin Lab Analysis 1991;5:226-7

Can Phadiatop be used as an atopy test in children as well as in adults?

- Allergy to inhalant allergens is not common in the first year. Thus, small children rarely have IgE antibodies against inhalants. Phadiatop, designed as a test for the identification of atopic sensitivity to common inhalants, should therefore be expected to show increasing sensitivity with increasing age over the first year of life. The specificity is, however, consistently high for all ages.
- The multi-allergen fx5, covering the six most common foods, is a better choice for measuring atopy in very early childhood or Phadiatop Infant (<5 yrs)

- Reference: Zimmerman B et al. Alergologia e Inmunologia Clinica 1987;2:94.

How is Phadiatop designed?

- Phadiatop (Phadia differentiate atopy) is built around the atopy concept, which is the constitutional disposition to produce IgE antibodies to common environmental allergens, whether the patient has clinical symptoms or not

- The combination of allergens used in Phadiatop, and the way they are combined, is chosen with the aim of achieving an approximately 90% probability of correct classification of atopics and non-atopics

- A number of studies showing the excellent performance of Phadiatop have been reviewed and the average sensitivity and specificity was found to be above 90%

How can the result of Phadiatop in a patient be negative despite a positive single IgE test?

- This is not commonly seen, but can happen if the patient is monospecificically allergic to one or a few allergenic components weakly represented on the solid phase of Phadiatop.
- A number of studies showing the excellent performance of Phadiatop have been reviewed and commented on by Dr N Eriksson, who found the average sensitivity and specificity to be above 90%
- Reference: Eriksson N. Allergy 1990;45:285-292.

Why is the allergen composition of Phadiatop secret?

- The exact composition of Phadiatop is not essential to know for using the test.
- Phadiatop (Phadia differentiate atopy) is built around the atopy concept of Dr Pepys, which is the constitutional disposition to produce IgE antibodies to common environmental allergens, whether the patient has clinical symptoms or not. Therefore, Phadiatop should only be used in patients with allergy or allergy-like symptoms as an initial step aimed at confirming or excluding IgE sensitization, and not to identify the specific allergen. When atopy has been confirmed with Phadiatop, the specific allergen follow-up can begin. This follow-up can consist of not only common inhalant allergens, but also other suspected allergens in the environment of the patient.

- Reference: Pepys J. Atopy. In: Gell PGH, Coombes RRA, Lachmann PJ, editors. Clinical Aspects of Immunology. Blackwell Scientific Publications, Oxford. 1975:877-902.

Allergy panels @Metropolis

- Categories available at Metropolis-
  - Symptom-wise
  - Age-wise
  - Environmental
  - Food
  - Regional
  - Drugs
- Allergy panels also done by ImmunoCAP
- Doctors choose relevant panels based on the patients' symptoms and clinical history. For eg, Regional panel may be selected when a patient suddenly develops symptoms of allergy after moving to a new region in India

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Allergy panels @Metropolis

- Metropolis provides a group screening panel - This panel is specially created for those cases where choice of a specific category of panels is difficult either because of non-specific or incomplete history
- This panel screens for 9 categories of allergens along with Phadiatop and Total IGE
- This is more cost effective as the patient need not undergo testing of multiple panels to identify the allergen

All panels at Metropolis are customized for the Indian population and have been created through in-depth research and comparison with allergens used in Skin-Prick Tests by Allergologists

THANK YOU