

## SECOND EXERCISE

### ALLERGY DIAGNOSTIC TESTING. SPECIFIC IGE TESTING

Specific IgE testing can be done through skin testing or blood testing

#### **In Vivo (Skin) Testing**

First described in 1867 by Dr Charles Blackley, skin tests (prick/puncture and intracutaneous) have evolved as reliable, cost effective techniques for the diagnosis of IgE-mediated diseases.

Skin testing is minimally invasive, and when it is performed correctly it has good reproducibility, is easily quantified, and allows the evaluation of multiple allergens at one session. Skin testing alone or in combination with in vitro testing is relied upon for the evaluation of allergic rhinitis, asthma, eczema, food allergy, insect sting allergy, drug allergy (especially beta-lactam and local anesthetic allergy), occupational disease and anaphylaxis. Allergen extracts used for percutaneous and intracutaneous testing ideally should be of known composition and potency. Although a limited number of standardized extracts are commercially available, most inhalant and food extracts are not standardized. Before the recent availability of standardized extracts, the composition of nonstandardized, commercially available extracts varied greatly between the manufacturers. This situation is slowly improving with the introduction of bioequivalent extracts. Bioequivalency is defined as histamine equivalent prick (HEP) units. All extracts should be stored under cold (4°C) to ensure stability. Positive and negative controls should be performed with all tests. A 10-mg/mL histamine dehydrochloride control is available, and this is the preferred positive control for prick/puncture skin tests. Skin testing is usually done on the forearm or back.

#### Physiology of Skin Tests

Skin tests are performed by introducing a small quantity of allergen into the epidermis by pricking, puncturing, or scratching the skin or by intradermal injection.

Suitable concentration of an allergen extract.

Some materials may be used directly for testing epicutaneous testing, e.g., the fresh juice of fruit.

After the allergen has been introduced into the skin, it diffuses through the skin, where it binds to IgE antibodies (with specificity for the allergy), which are affixed to mast cells.

When an allergen can crosslink two or more mast cell-bound IgE antibodies, a signal is generated for mediator release.

Released mediators include preformed (histamine, tryptase, chymase, heparin) and newly synthesized (prostaglandins, leukotrienes, cytokines) cell products.

**The prick/puncture** method involves a skin testing device pricked through a droplet of allergenic extract. Histamine release is the major mediator that results in a hive at the prick site and surrounding erythema, called a wheal and flare. The wheal and flare is read in 15-20 minutes. It is measured in millimeters and compared with a positive control (histamine) wheal and flare and a negative control (usually glycerinated saline). A positive test is considered as a wheal equal to or larger to the histamine control (or greater than 3 mm). Skin testing can be performed at any age. Infants may have smaller positive tests, but the histamine is correlatively smaller. As there is a small risk of anaphylaxis, skin testing should not be performed on patients at risk for complications if they experience anaphylaxis. This includes pregnancy and unstable medical conditions, such as unstable asthma or reduced lung function, recent stroke, or recent cardiac event. Oral and nasal antihistamines should be stopped 3-7 days before skin testing. Other medications with an antihistaminic effect may alter skin tests as well, including H2 receptor antagonists (cimetidine, ranitidine), tricyclic antidepressants, and antiemetics. These medications should be discontinued prior to skin testing.

The peak reactivity of prick tests is 15 to 20 minutes at which time both wheal and erythema diameters should be recorded in millimeters and compared with positive and negative controls. Histamine control tests should be read 15 minutes after application at the peak of reactivity. The peak of allergen prick tests is usually 15 to 20 minutes after application. A prick test with a response of at least 3-mm diameter (with equivalent erythema) more than diluent control done at the same time is required as proof of the presence of cutaneous allergen specific IgE.

**Intradermal testing** is performed only after a negative prick skin test. There is a higher risk of anaphylaxis. Intradermal testing is more sensitive than prick skin testing, but false-positive results are common due to irritant reactions or intracutaneous bleeding. Intradermal testing typically involves injecting 0.01-0.02 mL of antigen into the dermis via a 27-gauge syringe to create a 2- to 3-mm intracutaneous bleb, similar to an intracutaneous tuberculosis test. The extracts are diluted to 100-1000 times less than the dilution used for skin tests. The wheal is measured after 10-20 minutes. A response is considered positive if the wheal is 7 mm or greater.

### **Patch Testing**

Patch testing is used to determine the causative agent for chronic eczematous conditions contributing to a delayed-type hypersensitivity reaction. An example is allergic contact dermatitis to jewelry containing nickel. In patch testing, the allergen is placed on the upper back under an occlusive bandage and removed in 48 hours. The skin is reassessed at 72-96 hours for erythema, papules, and vesicles under the area of contact

### **In Vitro Testing**

In vitro tests assess antigen-specific IgE by testing the patient's serum. Advantages to this method include the use of a single venipuncture that is not affected by medications. In vitro testing can be performed on patients with affected skin, such as dermatographism or atopic dermatitis. It is also a safer option if the patient is at risk for anaphylaxis. However, these tests are expensive compared with skin testing.

Current methods include enzyme-linked immunosorbent assay (ELISA), which uses antibodies linked to enzymes, as well as fluorescent enzyme immunoassays (FEIA) and chemiluminescent immunoassays, which use fluorescent generation with an enzyme. The panel chosen should be based on the patient's clinical history, as with skin testing.

#### Other Diagnostic Tests

1. Total IgE levels
2. Mast cells in the tissue and basophils in the blood (in cases of anaphylaxis)
3. Tryptase and histamine (useful in cases of an) Tryptase should be measured 60 minutes to 6 hours after the event; histamine 5-10 min. respectively
4. Basophil activation tests measure the release of histamine from blood basophils incubated with the allergen.