ELISA

Enzyme-Linked ImmunoSorbent Assay, or ELISA, is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries. In simple terms, in ELISA an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal. Thus in the case of fluorescence ELISA, when light is shone upon the sample, any antigen/antibody complexes will fluoresce so that the amount of antigen in the sample can be measured.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody which is linked to an enzyme through bioconjugation. Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample. Older ELISAs utilize chromogenic substrates, though newer assays employ fluorogenic substrates with much higher sensitivity.
Applications
Because the ELISA can be performed to evaluate either the presence of antigen or the presence of antibody in a sample, it is a useful tool both for determining serum antibody concentrations (such as with the HIV test or West Nile Virus) and also for detecting the presence of antigen. It has also found applications in the food industry in detecting potential food allergens such as milk, peanuts, walnuts, almonds, and eggs. The ELISA test, or the enzyme immunoassay (EIA), was the first screening test commonly employed for HIV. It has a high sensitivity. In an ELISA test, a person's serum is diluted 400-fold and applied to a plate to which HIV antigens have been attached. If antibodies to HIV are present in the serum, they may bind to these HIV antigens. The plate is then washed to remove all other components of the serum. A specially prepared "secondary antibody" — an antibody that binds to human antibodies — is then applied to the plate, followed by another wash. This secondary antibody is chemically linked in advance to an enzyme. Thus the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or fluorescence. ELISA results are reported as a number; the most controversial aspect of this test is determining the "cut-off" point between a positive and negative result.

History
Prior to the development of the EIA/ELISA, the only option for conducting an immunoassay was radioimmunoassay, a technique using radioactively-labeled antigens or antibodies. In radioimmunoassay, the radioactivity provides the signal which indicates whether a specific antigen or antibody is present in the sample. Radioimmunoassay was first described in a paper by Rosalyn Yalow and Solomon Berson published in 1960.

Because radioactivity poses a health threat, a safer alternative was sought. A suitable alternative to radioimmunoassay would substitute a non-radioactive signal in place of the radioactive signal. When certain enzymes (such as peroxidase) react with appropriate substrates (such as ABTS or 3,3',5,5'-Tetramethylbenzidine), they can result in changes in color, which can be used as a signal. However, the signal has to be associated with the presence of antibody or antigen, which is why the enzyme has to be linked to an appropriate antibody. This linking process was independently developed by Stratis Avrameas and G.B. Pierce. Since it is necessary to remove any unbound antibody or antigen by washing, the antibody or antigen has to be fixed to the surface of the container, i.e. the immunosorbent has to be prepared. A technique to accomplish this was published by Wide and Porath in 1966.

In 1971, Peter Perlmann and Eva Engvall at Stockholm University in Sweden, as well as Anton Schuurs and Bauke van Weemen in The Netherlands, independently published papers which synthesized this knowledge into methods to perform EIA/ELISA.
Allergy

Allergy is a disorder of the immune system that is often called atopy. Allergic reactions occur to environmental substances known as allergens; these reactions are acquired, predictable and rapid. Strictly, allergy is one of four forms of hypersensitivity and is called type I (or immediate) hypersensitivity. It is characterized by excessive activation of certain white blood cells called mast cells and basophils by a type of antibody, known as IgE, resulting in an extreme inflammatory response. Common allergic reactions include eczema, hives, hay fever, asthma, food allergies, and reactions to the venom of stinging insects such as wasps and bees.

Mild allergies like hay fever, are highly prevalent in the human population and cause symptoms such as allergic conjunctivitis, itchiness and runny nose. Similarly, conditions such as asthma are common, in which allergy plays a major role. In some people, severe allergies to environmental or dietary allergens, or to medication, occur that may result in life-threatening anaphylactic reactions and potentially death.

A variety of tests now exist to diagnose allergic conditions; these include testing the skin for responses to known allergens or analyzing the blood for the presence and levels of allergen-specific IgE. Treatments for allergies include allergen avoidance, use of antihistamines, steroids or other oral medications, immunotherapy to desensitize the response to allergen, and targeted therapy.

Classification and history
The concept "allergy" was originally introduced in 1906 by the Viennese pediatrician Clemens von Pirquet, after noting that some of his patients were hypersensitive to normally innocuous entities such as dust, pollen, or certain foods. Pirquet called this phenomenon "allergy" from the Greek words allos meaning "other" and ergon meaning "work". Historically, all forms of hypersensitivity were classified as allergies, and all were thought to be caused by an improper activation of the immune system. Later, it became clear that several different disease mechanisms were implicated, with the common link to a disordered activation of the immune system. In 1963, a new classification scheme was designed by Philip Gell and Robin Coombs that described four types of hypersensitivity reactions, known as Type I to Type IV hypersensitivity. With this new classification, the word "allergy" was restricted to only type I hypersensitivities (also called immediate hypersensitivity), which are characterized as rapidly developing reactions.

A major breakthrough in understanding the mechanisms of allergy was the discovery of the antibody class labeled immunoglobulin E (IgE) - Kimishige Ishizaka and co-workers were the first to isolate and describe IgE in the 1960s.

Signs and symptoms
Many allergens are airborne particles, such as dust or pollen. In these cases, symptoms arise in areas in contact with air, such as eyes, nose and lungs. For instance, allergic
rhinitis, also known as hay fever, causes irritation of the nose, sneezing, and itching and redness of the eyes. Inhaled allergens can also lead to asthmatic symptoms, caused by narrowing of the airways (bronchoconstriction) and increased production of mucus in the lungs, shortness of breath (dyspnea), coughing and wheezing.

Aside from these ambient allergens, allergic reactions can result from foods, insect stings, and reactions to medications like aspirin, and antibiotics such as penicillin. Symptoms of food allergy include abdominal pain, bloating, vomiting, diarrhoea, itchy skin, and swelling of the skin during hives or angiooedema. Food allergies rarely cause respiratory (asthmatic) reactions, or rhinitis. Insect stings, antibiotics and certain medicines produce a systemic allergic response that is also called anaphylaxis; multiple systems can be affected including the digestive system, the respiratory system, and the circulatory system. Depending of the rate of severity, it can cause cutaneous reactions, bronchoconstriction, edema, hypotension, coma and even death. This type of reaction can be triggered suddenly or the onset can be delayed. The severity of this type of allergic response often requires injections of epinephrine, sometimes through a device known as the Epi-Pen auto-injector. The nature of anaphylaxis is such that the reaction can seemingly be subsiding, but may recur throughout a prolonged period of time.

Substances that come into contact with the skin, such as latex are also common causes of allergic reactions, known as contact dermatitis or eczema. Skin allergies frequently cause rashes, or swelling and inflammation within the skin, in what is known as a "wheal and flare" reaction characteristic of hives and angiooedema.

**Cause**
Risk factors for allergy can be placed in two general categories, namely host and environmental factors. Host factors include heredity, sex, race and age, with heredity being by far the most important. There are recent increases in the incidence of allergic disorders, however, that cannot be explained by genetic factors alone. The four main candidate environmental factors are alterations in exposure to infectious diseases during early childhood, environmental pollution, allergen levels, and dietary changes.

**Genetic basis**
Allergic diseases are strongly familial: identical twins are likely to have the same allergic diseases about 70% of the time; the same allergy occurs about 40% of the time in non-identical twins. Allergic parents are more likely to have allergic children, and their allergies are likely to be stronger than those from non-allergic parents. However some allergies are not consistent along genealogies; parents who are allergic to peanuts, may have children who are allergic to ragweed, or siblings that are allergic to different things. It seems that the likelihood of developing allergies is inherited and due to some irregularity in the way the immune system works, but the specific allergen, which causes the development of an allergy, is not.

The risk of allergic sensitization and the development of allergies varies with age, with young children most at risk. Several studies have shown that IgE levels are highest in
childhood and fall rapidly between the ages of 10 and 30 years. The peak prevalence of hay fever is highest in children and young adults and the incidence of asthma is highest in children under 10. Overall, boys have a higher risk of developing allergy than girls, although for some diseases, namely asthma in young adults, females are more likely to be affected. Sex differences tend to decrease in adulthood. Ethnicity may play a role in some allergies, however racial factors have been difficult to separate from environmental influences and changes due to migration. Interestingly, it has been suggested that different genetic loci are responsible for asthma, specifically, in people of Caucasian, Hispanic, Asian, and African origins.

**Environmental factors**

International differences have been associated with the number of individuals within a population that suffer from allergy. Allergic diseases are more common in industrialized countries than in countries that are more traditional or agricultural, and there is a higher rate of allergic disease in urban populations versus rural populations, although these differences are becoming less defined.

Exposure to allergens, especially in early life, is an important risk factor for allergy. Alterations in exposure to microorganisms is the most plausible explanation, at present, for the increase in atopic allergy. Since children that live in large families or overcrowded households, or attend day care, have a reduced incidence of allergic disease, a relationship has been proposed between exposures to bacteria and viruses during childhood, and protection against the development of allergy, which has been called – the "hygiene hypothesis". Exposure to endotoxin and other components of bacteria may reduce atopic diseases. Endotoxin exposure reduces release of inflammatory cytokines such as TNF-α, IFNγ, interleukin-10, and interleukin-12 from white blood cells (leukocytes) that circulate in the blood. Certain microbe-sensing proteins, known as Toll-like receptors, found on the surface of cells in the body are also thought to be involved in these processes.

Gutworms and similar parasites are present in untreated drinking water in developing countries, and were present in the water of developed countries until the routine chlorination and purification of drinking water supplies. Recent research has shown that some common parasites, such as intestinal worms (e.g. hookworms), secrete chemicals into the gut wall (and hence the bloodstream) that suppress the immune system and prevent the body from attacking the parasite. This gives rise to a new slant on the hygiene hypothesis theory — that co-evolution of man and parasites has led to an immune system that only functions correctly in the presence of the parasites. Without them, the immune system becomes unbalanced and oversensitive. In particular, research suggests that allergies may coincide with the delayed establishment of gut flora in infants. However, the research to support this theory is conflicting, with some studies performed in China and Ethiopia showing an increase in allergy in people infected with intestinal worms. Clinical trials have been initiated to test the effectiveness of certain worms in treating some allergies. It may be that the term 'parasite' could turn out to be inappropriate, and in fact a hitherto unsuspected symbiosis is at work.
Pathophysiology
The pathophysiology of allergic responses can be divided into two phases. The first is an acute response that occurs immediately after exposure to an allergen. This phase can either subside or progress into a "late phase reaction" which can substantially prolong the symptoms of a response, and result in tissue damage.

Acute response
In the early stages of allergy, a type I hypersensitivity reaction against an allergen, encountered for the first time, causes a response in a type of immune cell called a TH2 lymphocyte, which belongs to a subset of T cells that produce a cytokine called interleukin-4 (IL-4). These TH2 cells interact with other lymphocytes called B cells, whose role is production of antibodies. Coupled with signals provided by IL-4, this interaction stimulates the B cell to begin production of a large amount of a particular type of antibody known as IgE. Secreted IgE circulates in the blood and binds to an IgE-specific receptor (a kind of Fc receptor called FceRI) on the surface of other kinds of immune cells called mast cells and basophils, which are both involved in the acute inflammatory response. The IgE-coated cells, at this stage are sensitized to the allergen.

If later exposure to the same allergen occurs, the allergen can bind to the IgE molecules held on the surface of the mast cells or basophils. Cross-linking of the IgE and Fc receptors occurs when more than one IgE-receptor complex interacts with the same allergenic molecule, and activates the sensitized cell. Activated mast cells and basophils undergo a process called degranulation, during which they release histamine and other inflammatory chemical mediators (cytokines, interleukins, leukotrienes, and prostaglandins) from their granules into the surrounding tissue causing several systemic effects, such as vasodilation, mucous secretion, nerve stimulation and smooth muscle contraction. This results in rhinorrhea, itchiness, dyspnea, and anaphylaxis. Depending on the individual, allergen, and mode of introduction, the symptoms can be system-wide (classical anaphylaxis), or localized to particular body systems; asthma is localized to the respiratory system and eczema is localized to the dermis.

Late-phase response
After the chemical mediators of the acute response subside, late phase responses can often occur. This is due to the migration of other leukocytes such as neutrophils, lymphocytes, eosinophils and macrophages to the initial site. The reaction is usually seen 2-24 hours after the original reaction. Cytokines from mast cells may also play a role in the persistence of long-term effects. Late phase responses seen in asthma are slightly different from those seen in other allergic responses, although they are still caused by release of mediators from eosinophils, and are still dependent on activity of TH2 cells.

Diagnosis
Before a diagnosis of allergic disease can be confirmed, the other possible causes of the presenting symptoms should be carefully considered. Vasomotor rhinitis, for
example, is one of many maladies that shares symptoms with allergic rhinitis, underscoring the need for professional differential diagnosis. Once a diagnosis of asthma, rhinitis, anaphylaxis, or other allergic disease has been made, there are several methods for discovering the causative agent of that allergy.

**Skin testing**
For assessing the presence of allergen-specific IgE antibodies, allergy skin testing is preferred over blood allergy tests because it is more sensitive and specific, simpler to use, and less expensive. Skin testing is also known as "puncture testing" and "prick testing" due to the series of tiny puncture or pricks made into the patient’s skin. Small amounts of suspected allergens and/or their extracts (pollen, grass, mite proteins, peanut extract, etc.) are introduced to sites on the skin marked with pen or dye (the ink/dye should be carefully selected, lest it cause an allergic response itself). A small plastic or metal device is used to puncture or prick the skin. Sometimes, the allergens are injected "intradermally" into the patient's skin, with a needle and syringe. Common areas for testing include the inside forearm and the back. If the patient is allergic to the substance, then a visible inflammatory reaction will usually occur within 30 minutes. This response will range from slight reddening of the skin to a full-blown hive (called "wheal and flare") in more sensitive patients. Interpretation of the results of the skin prick test is normally done by allergists on a scale of severity, with +/- meaning borderline reactivity, and 4+ being a large reaction. Increasingly, allergists are measuring and recording the diameter of the wheal and flare reaction. Interpretation by well-trained allergists is often guided by relevant literature. Some patients may believe they have determined their own allergic sensitivity from observation, but a skin test has been shown to be much better than patient observation to detect allergy.

If a serious life threatening anaphylactic reaction has brought a patient in for evaluation, some allergists will prefer an initial blood test prior to performing the skin prick test. Skin tests may not be an option if the patient has widespread skin disease or has not avoided antihistamines for several days.

**Blood testing**
Various blood allergy testing methods are also available for detecting allergy to specific substances. This kind of testing measures a "total IgE level" - an estimate of IgE contained within the patient's serum. This can be determined through the use of radiometric and colormetric immunoassays. Radiometric assays include the radioallergosorbent test (RAST) test method, which uses IgE-binding (anti-IgE) antibodies labeled with radioactive isotopes for quantifying the levels of IgE antibody in the blood. Other newer methods use colorimetric or fluorometric technology in the place of radioactive isotopes. Some "screening" test methods are intended to provide qualitative test results, giving a "yes" or "no" answer in patients with suspected allergic sensitization. One such method has a sensitivity of about 70.8% and a positive predictive value of 72.6% according to a large study.

A low total IgE level is not adequate to rule out sensitization to commonly inhaled allergens. Statistical methods, such as ROC curves, predictive value calculations, and
likelihood ratios have been used to examine the relationship of various testing methods to each other. These methods have shown that patients with a high total IgE have a high probability of allergic sensitization, but further investigation with specific allergy tests for a carefully chosen allergens is often warranted.

**Allergens**
An allergen is a nonparasitic antigen capable of stimulating a type-I hypersensitivity reaction in atopic individuals.

Most humans mount significant IgE responses only as a defense against parasitic infections. However, some individuals mount an IgE response against common environmental antigens. This hereditary predisposition is called atopy. In atopic individuals, non-parasitic antigens stimulate inappropriate IgE production, leading to type I hypersensitivity. Sensitivities vary from one person to another and it is possible to be allergic to an extraordinary range of substances.

**Types of allergies**
Dust, pollen and pet dander are all common allergens, but it is possible to be allergic to anything from chlorine to perfume. Food allergies are not as common as food sensitivity, but some foods such as peanuts (really a legume), nuts, seafood and shellfish are the cause of serious allergies in many people.

Officially, the Food and Drug Administration does recognize 8 foods as being common for allergic reactions in a large segment of the sensitive population, which includes, peanuts, tree nuts, eggs, milk, shellfish, fish, wheat and their derivatives, soy and their derivatives, and sulphites (chemical based, often found in flavors and colors in foods) at 10ppm and over. See the FDA website for complete details. It should be noted that other countries, due to differences in genetic profiles of its citizens and different levels of exposure to different foods, the "official" allergen list will change. Canada recognizes all eight of the allergens recognized by the US, and also recognizes sesame seeds.

A few people have been recorded to be allergic to certain chemicals found in almost all water[citation needed, and even water itself (see Aquagenic pruritus).

Poison ivy is a plant that will cause an allergic reaction in 70-85% of humans. But, given enough repeated contact—like any allergy, most human bodies will learn to fight the allergen.

An allergic reaction can be caused by any form of direct contact with the allergen—eating or drinking a food you are sensitive to (ingestion), breathing in pollen, perfume or pet dander (inhalation), or brushing your body against an allergy-causing plant (direct contact, generally resulting in hives). Other common causes of serious allergy are wasp, fire ant and bee stings, penicillin, and latex. An extremely serious form of an allergic reaction, which can kill in mere minutes, is called anaphylaxis. One form of treatment is the administration of sterile epinephrine (via "Epi-Pen") to the person experiencing anaphylaxis, which suppresses the body's overreaction to the food
ingested, and allows for time to be transported to a medical facility (it does not "cure" the allergic reaction).

**Common allergens**
In addition to foreign proteins found in foreign serum (from blood transfusions) and vaccines, common allergens include:

**Animal products**
cat allergy
fur and dander
cockroach calyx
dust mite excretion

**Drugs**
penicillin
sulfonamides
salicylates (also found naturally in numerous fruits)
local anaesthetics

**Foods**
celery and celeriac
corn or maize
eggs (typically albumen, the white)
fruit
pumpkin
legumes
beans
peas
peanuts
soybeans
milk

**Insect stings**
bee sting venom
wasp sting venom
mosquito stings

**Mold spores**

**Other**
latex
metal

**Plant pollens (hay fever)**
grass — ryegrass, timothy-grass
weeds — ragweed, plantago, nettle, artemisia vulgaris, chenopodium album, sorrel
trees — birch, alder, hazel, hornbeam, aesculus, willow, poplar, platanus, tilia, olea
The Most Common Allergens that Cause Allergies

**Pollen:** Plant pollens from trees, plants, grass, and weeds are disseminated by wind currents. These are the most difficult allergens to avoid entirely and include pollen from many different tree varieties such as birch, olive, mulberry, oak, western red cedar, elm, ash, hickory, poplar, sycamore, maple, cypress and walnut. Pollinating grasses include timothy, bermuda, orchard, sweet vernal, red top and blue grass. Pollinating weeds include ragweed, sagebrush, pigweed, tumbleweed, Russian thistle and cockleweed.

**Mold spores:** There are literally thousands of molds that can cause human allergic reactions. Microscopic mold spores can easily bypass the protective mucous of the ears, nose, throat, and lungs, leading to major allergies. Mold allergy can also be triggered by eating foods made with fungi, such as cheeses and pickled foods. Mushrooms, dried fruits, soy sauce, vinegar, or foods containing yeast can produce allergic symptoms from mold. Additionally, disruptions in the environment can cause a dramatic increase in mold production, and allergies. For example, mold overgrowth after Hurricane Katrina triggered “Katrina Cough” in humans.

**Medications:** There are thousands of drugs on the market. An allergic reaction to medication is individual to the person taking a specific medication. Discontinuing use of the medication that causes the allergic reaction usually suffices. In some cases, medications such as penicillin can cause anaphylaxis, a life-threatening allergic reaction. Other common allergy-causing medications include sulfonamides (any medication containing sulfa), salicylates (the main ingredient found in aspirin), and anesthetics.
Foods: Severe allergic reactions to foods have increased over the last several decades. Allergy-producing foods include (but are certainly not limited to) nuts; peanuts (which are actually legumes); sesame; seafood; egg whites; soybeans and soy products; milk and milk products; gluten in rye, wheat, and barley; and corn. It’s important to keep in mind that food allergies are very unique, and some people may be allergic to a wide variety of foods.

Insects and animals: Insect bites from bees, wasps, and ants can cause life-threatening allergic reactions in some people. If you’re allergic to bees, avoid all products that contain bee pollen. Allergies can also be triggered by cockroach waste and dust mite excretion. Pet hair and dander can also be major allergy culprits.

Latex: Certain people are highly allergic to latex. If so, make sure your healthcare professional knows that you’re allergic to latex to avoid exposure from medical equipment. Medical supplies and equipment may contain latex, such as rubber gloves, and tubing, for example.
Chemicals: The exponential use of chemicals in our modern-day society has contributed to the increased allergy potential. Thousands of chemicals are introduced into our environment daily with little known information about their toxicity to humans.

This experiment will test for the following allergens:

**Pollens**
--- Oak Pollen --- Timothy Grass --- Ragweed

**Mold**
--- Mold Spores

**Foods**
--- Nuts --- Seafood

**Other**
--- House Dust Mite --- Cat Dander
ELISA Procedure

1) Place the ELISA 96 well plate in the table. Note that rows 10, 11 & 12 will not be used.

2) Place one paper towel unfolded & flat on the table and mark it as “USED PIPETTES”.

3) Find the MicroCentrifuge tube labeled “Oak Pollen” and using one disposable pipette, place one drop of the Oak Pollen Antigens in the tube into wells 1 thru 9 in row “A”.

![DIagram of ELISA Procedure](image)
4) Place the disposable pipette on the towel marked “USED PIPETTES”.

5) Find the MicroCentrifuge tube labeled “Timothy Grass” and using one NEW disposable pipette, place one drop of the Timothy Grass Antigens in the tube into wells 1 thru 8 in row “B”.

6) Again, place the disposable pipette on the towel marked “USED PIPETTES”.

7) Repeat this procedure (using a new disposable pipette each time) for the tubes marked ragweed, mold spores, nuts, seafood, house dust mites and cat dander.
8) Carefully take the ELISA tray in the palm of your hand and quickly turn it upside
down into a small stack of paper towels to empty the wells. Tap the tray several times,
then place it back on the table right side up.

**What Did We Just Do??**

You just placed allergy antigens into the wells where they some of
them attached themselves to the wall of the well.

9) Find the large tube labeled “Wash” and using one NEW disposable pipette, place one
drop of the Wash solution into wells 1 thru 9 in rows A thru H. **Set this pipette aside,**
you will use it again for the Wash Solution.

10) Carefully take the ELISA tray in the palm of your hand and quickly turn it upside
down into a small stack of paper towels to empty the wells. Tap the tray several times,
then place it back on the table right side up.
11) Find the large tube labeled “Blocker” and using one NEW disposable pipette, place one drop of the Blocker Protein solution into wells 1 thru 9 in rows A thru H.

What Did We Just Do??

You washed away any allergy antigens that were not attached to the wall of the wells.

What Did We Just Do??

You blocked all the sites that allergy antigens were not attached to.
12) Carefully take the ELISA tray in the palm of your hand and quickly turn it upside down into a small stack of paper towels to empty the wells. Tap the tray several times, then place it back on the table right side up.

13) Find the large tube labeled “Wash” and using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1 thru 9 in rows A thru H. Set this pipette aside, you will use it again for the Wash Solution.

14) Carefully take the ELISA tray in the palm of your hand and quickly turn it upside down into a small stack of paper towels to empty the wells. Tap the tray several times, then place it back on the table right side up.

What Did We Just Do??

You washed away any blocker proteins that were not attached to the wall of the wells.
15) Find the large tube labeled “+Control” and using one NEW disposable pipette, place one drop of the Positive Control solution into wells 1 thru 3 in rows A thru H.

What Did We Just Do??

You added a solution containing all eight of the Antibodies used in this allergy test. The antibodies attached to the allergens in the wells.
16) Find the clear plastic tube labeled with a blue stopper labeled “Tube #”. This contains a sample of the patient’s blood plasma. The # indicates the number of the patient you are testing (names are not used for privacy). Using one NEW disposable pipette, place one drop of the Patient’s Plasma into wells 7 thru 9 in rows A thru H.

What Did We Just Do??

You added antibodies from the patient’s blood plasma. If the patient has formed antibodies to the antigen in the well, it means he has encountered it before and has formed an allergy response to it. If present, the antibodies will attach to the specific allergen in the well.
17) Carefully take the ELISA tray in the palm of your hand and quickly turn it upside down into a small stack of paper towels to empty the wells. Tap the tray several times, then place it back on the table right side up.

18) Find the large tube labeled “Wash” and using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1 thru 9 in rows A thru H. Set this pipette aside, you will use it again for the Wash Solution.

19) Carefully take the ELISA tray in the palm of your hand and quickly turn it upside down into a small stack of paper towels to empty the wells. Tap the tray several times, then place it back on the table right side up.

What Did We Just Do??

You washed away any antibodies that had not attached to antigens on the wall of the wells.
20) Find the large tube labeled “F Tag” and using one NEW disposable pipette, place one drop of the Fluorescent Tagged Antibodies into wells 1 thru 9 in rows A thru H.

21) Carefully take the ELISA tray in the palm of your hand and quickly turn it upside down into a small stack of paper towels to empty the wells. Tap the tray several times, then place it back on the table right side up.

What Did We Just Do??

You placed antibodies with fluorescent molecules attached into the wells. These antibodies will attach to any antibodies in the well. Antibodies will only be found in a well if they were placed there (as in the Positive Control) or if they were in the patients blood meaning that he has an allergy to the antigen placed in the well.
22) Find the large tube labeled “Wash” and using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1 thru 9 in rows A thru H.

![Diagram showing wash steps]

23) Carefully take the ELISA tray in the palm of your hand and quickly turn it upside down into a small stack of paper towels to empty the wells. Tap the tray several times, then place it back on the table right side up.

What Did We Just Do??

You washed any antibodies with fluorescent molecules attached that have NOT attached to antibodies in the well.
23) Obtain a Ultraviolet light and shine it over the ELISA plate. On your ELISA Test Data Sheet, mark which wells fluoresce. Use the ELISA Test Key below to determine which of the items tested showed positive for an allergy. Record your answers on the ELISA Test Data Sheet.

What Did We Just Do??

The ultraviolet light stimulated the fluorescent molecules attached to the antibodies attached to the antibodies that are attached to the antigens in the well. These fluorescent molecules then gave off a visible light.
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**ELISA Test Key**

Eight Allergens

- Negative Control
- Positive Control
- Not Used

- Oak Pollen
- Timothy Grass
- Ragweed
- Mold Spores
- Nuts
- Seafood
- House Dust Mite
- Cat Dander