Particle agglutination test

The presence of an antigen or anti-bodies in a blood sample can be found out using either the Particle Agglutination Test (PAT) or by the Enzyme-Linked Immunosorbent Assay (ELISA). The PAT is performed using substances that will facilitate the process of agglutination. Agglutination is the clumping together of particles which forms non-dissolvable or what is called as insoluble aggregates. The common substances used in the PAT are latex particles, preserved blood cells from mammals or birds, colloidal particles, and gelatine beads. The different components used in the PAT are microscope slides or test cards and a solution that facilitates agglutination which is made up of molecules that are analyte-specific which is attached to a constituent part or particle.

In a test using ELISA detects the presence of an antibody or antigen by affixing an undetermined amount of antigen to a test surface which in turn is washed by a specific antibody in order to attach it to the antigen. Because of this process the antibody becomes linked to an enzyme that will show a certain detectable signal after a substance is added to it during the final steps of the process. The signal emitted differs from what type of ELISA is used. For example a fluorescent ELISA a light of a correct wavelength that is shone on a sample will elicit a response from the antigen/antibody which will show incandescence. The antigen’s amount contained in the sample is then determined based on the intensity or magnitude of the sample’s incandescence.

While the PAT relies on the clumping together of particles to show the presence of an antibody, the ELISA is a more complex test which can only be performed following very specific protocols or steps to perform. In ELISA an

antigen with an undetermined amount is rendered immobile while place on a polystyrene microtiter plate or other substance that could provide a solid support and which does not interfere with the purity of the sample. The antigen is immobilized either by adsorption; the adhesion of molecules to a surface, or by capturing the particles of the antigen by another antibody that is particular to the same antigen. The process of adsorption is what is termed as non-specific immobilization while the capture of the antigen is what is termed as specific immobilization.

After the immobilization of an antigen the next step is to add a detection antibody. The addition of this antibody results in the formation of a complex with the existing antigen. The detection antibody can either be linked directly to an enzyme, or it can be noticed by a secondary antibody that is connected to an enzyme by a process called bioconjugation – this occurs when two biomolecules couple with each other in a chemical bonding wherein the two substances share pairs of electrons, or what is known as covalent bonding. In between each step of the process the plate used is washed in a solution of mild detergent so as to remove any antibodies or proteins that have not bonded specifically.

After the plate undergoes its final wash it is then developed using an enzymatic substrate, which means the enzyme has a molecule which it acts on. This enzyme substrate when added will produce a noticeable signal indicating how much the quantity of an antigen is in a given sample.

The PAT and ELISA are but two of the techniques used in laboratories in serology. Other techniques include gel diffusion tests, precipitation tests,

agglutination tests to which PAT belongs, immunological tests with markers which ELISA is one of the tests, and lastly there is the immunosorbent electron microscopy.

To further discuss the comparisons and contrasts of the ELISA and PAT let us discuss the general aspects of the two tests. As was mentioned above both ELISA and PAT are techniques used in serology to detect the presence of antibodies and antigens in a sample. However the two tests differ in the process or steps taken to obtain the same results.

PAT is an agglutination test. This means that its primary method to determine the presence of antibodies is by inducing the clumping or joining together of molecules in the test sample by either by using substances that are inert which carry the antigens or antibodies, or by using polystyrene spheres infused with immunoglobulin molecules, or by observing the loss of activity of antigens or what is called as neutralization.

What is observed by serologist when using PAT is the reaction called agglutination, which is a reaction that leads to the joining together or clumping of a specific antibody in a cell suspension as a reaction to a specific antigen.

While in ELISA what is observed is not the reaction of an antibody to the antigen but the level of activity made by the substance or markers chemically bound together to the antigen and antibody which in turn indicates the antibody – antigen reaction.

The PAT test was one of the first serological methods that was developed and which helped pave the way for developing diagnostic techniques and countermeasures to diseases. One early agglutination test is the Widal agglutination. The Widal agglutination, which was named after Fernand Widal, aided in the diagnosis of typhoid fever by showing the presence of an antibody in a sample obtained from an infected patient, in reaction against the antigens of the Salmonella typhi. What the Widal test did was to increase the suspicion of infection because of the presence of agglutination caused by a rise in of antibodies during the period of infection.

In the classic test for agglutination the process starts by placing a specimen in a microscope slide or a microtest plate well where the particles which act as carriers are added. This mixture is then stirred or disturbed and then it is given a few minutes to settle or is incubated. The results can be gleaned by observing agglutination as the carrier particles fuses or binds with the particular analytes contained in the sample. The results are seen visually by the naked eye or by using a microscope depending on the format of the test. Interpreting test results are relatively easy if the reactions are strong.

But in cases where there is a weak or minor reactions interpretation is quite difficult and in most cases may depend upon the wealth of experience and practice one has. In microwell plate assays, the resulting patterns produced by the particles after it has settled need an educated interpretation before the result can be determined.

The advantages of PAT are it is low cost, semi-quantitative tests results can be obtained, and test results are obtained in a relatively short span of time.

The disadvantages are there is a need to be very careful in the interpretation of marginal results. There is also the disadvantage related to problems that may arise in relation to specificity because substances that may interfere in many assays.

Unlike PAT, ELISA is a more reliable method which uses biochemicals to measure how much of a given substance in a biological liquid such as serum or urine mixes with another substance. This is done by means of the reaction that an antibody or antibodies has to its antigen which is a molecule that is identified by the immune system. The antigen always specifically binds itself to an antibody at the molecular level. Since ELISA can be used to test and identify the presence of an antigen or of an antibody in a sample, this method is a very useful tool to determine concentrations of serum antibodies such as when testing HIV and other viral diseases. ELISA is also helpful in determining potential allergens in food such as milk, eggs and nuts.

ELISA is also used in toxicology as a presumptive screen that is used for particular classes of drugs.

Since lab technicians, serologists, and other medical personnel conducting the PAT and ELISA tests are exposed to samples that might contain viral or bacterial strains, the need to ensure safety and also the purity of the samples through a high standard of quality necessitates developing certain protocols for laboratory work. Different hospitals and government departments dealing with lab samples have already developed their own manual or standards that must be observed while working with lab samples to ensure safety. The manual issued by the Industrial Accident Prevention

Association identifies potential hazards inside the lab brought about by chemical and biological agents, physical agents, the equipment and apparatus, and doing work that results in non-ergonomic movement and postures. The manual also discusses the ways by which to control or lessen the danger posed by these hazards and what are the steps that should be taken to have a safe lab environment.

The Mount Sinai Hospital’s Microbiology Department also has its own guidelines and policies which specifically deal with laboratory safety. The manual discusses the safety policy of the hospital and what are the protocols to be observed. These protocols include dealing with visitors to the lab, what constitutes good lab practice, how to ensure biological safety, chemical safety, physical hazards, specimen hazards, and what control, emergency, and preventive measures should be followed to realize laboratory safety. These and other similar measures contained in other manuals may vary from lab to lab, but the essence remains the same; that is to promote safety and institute standards of work that ensures an accident and hazard free lab environment.

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