SEROLOGICAL TESTS

Aim: In this laboratory hours, informations about the definition and properties of serological tests, the differences and principles of classical and rapid serological tests will be given. At the same time examples of both classical and rapid serological test results will be demonstrated and slide agglutination test will be performed.

Serological tests are the immunologic methods that are based on the specific in-vitro antigen-antibody reactions. The basic mechanism of these tests is the detection of an unknown reactant (eg. antibody) by using a known reactant (eg. antigen). Serological tests are the widely used methods for the laboratory diagnosis of infectious diseases by 2 major purpose:

- Detection and quantification of the agent-specific antibodies in the patient’s serum
- Detection of microbial antigens in the clinical specimens (e.g. blood, urine, stool, swab samples, etc).

Serological diagnosis of an acute infection can be done by the demonstration of seroconversion and/or agent-specific IgM antibodies in the serum samples. Seroconversion describes 4 fold or more increase in antibody levels of the serum samples which is collected at the acute and convalescent phase (10-15 days intervals) of an infection. As the detection of seroconversion is time-consuming, the agent-specific IgM antibodies –an indicative marker for the acute infection- can easily be detected in a single serum sample, by rapid immunologic methods. However, as the IgM antibodies may be persistant even after the acute infection in some cases, or may reappear in re-infections and/or re-activations, it should be kept in mind that the presence of agent-specific IgM is not a strict marker of the primary acute infection. The detection of IgA antibodies is also useful for the serologic diagnosis of mucosal infections.

PRECIPITATION TESTS

Precipitation is the formation of stable antigen-antibody complexes, known as precipitins, when a soluble antigen is mixed with its specific antibody. There are many types of qualitative and quantitative immunologic methods which based on precipitation mechanism.

1. Immunodiffusion techniques: These methods involve movement of antigen and/or antibody molecules within an agarose gel medium by the process of diffusion. When the reacting molecules diffuse and meet in optimal concentrations, a visible precipitin band or line is formed.

2. Immunelectrophoretic techniques: The principle of these methods is to facilitate and speed the movement of antigen and/or antibody molecules by applying voltage through the agarose gel medium. The reactants migrate in an electric field according to their surface charges and molecular weights, and form precipitin bands or “arc”s when meet in optimal concentrations. These techniques are named according to their mechanisms and modifications (e.g. counter current immunoelectrophoresis, rocket electrophoresis, isoelectric focusing, etc).

3. Turbidimetric and nephelometric techniques: The formed immunoprecipitins (antigen-antibody complexes) have the ability to scatter a beam of light as it passes through a sample. Thus the concentration of immunoprecipitins in a
solution (liquid medium) can be determined by measuring light scatter with an automated instrument. The analyzer is able to detect the amount of light intensity with a sensor by translating the absorbance units to electrical data and to give quantitative results.

AGGLUTINATION TESTS

Agglutination reactions occur when particulate antigens with multiple epitopes on their surface bind with bivalent specific antibody molecules to form aggregates or clumps. Particulate antigens, may occur naturally on red blood cells (RBCs) or bacterial cell surfaces or they may consist of commercially prepared antigen-coated inert carrier particles (latex). When the carrier particle is an RBC, the reaction is known as "hemagglutination".

Agglutination methods may be performed on the slides, in small tubes or in microtiter plates, and the specific antigen-antibody reaction is observed visually as small aggregates or clumping. These tests are used to detect unknown antigens found in a clinical sample, or identification of the bacteria by using known antiserum, and to detect or quantitate the antibodies found in the patient's serum by using known particulate antigens.

Antigen detection by slide agglutination: Clinical swab sample or a small piece of bacterial colony which had grown on the culture media, is put on a slide. Then antiserum (antibody) which is specific to the suspected antigen is dropped onto this sample. After mixing and homogenizing, the slide is shaken gently for 5-10 minutes. Positive reaction is evaluated with the presence of clumping.

Antibody titration by tube agglutination: Serial dilutions (1/40, 1/80, 1/160, 1/320, ...) of the patient's serum is prepared in small tubes, and the standard amount of suspension of interested microbe is added onto these tubes. After incubation at 37°C overnight, the agglutination reaction end-point is graded according to the presence or absence of clumping.

SOLID PHASE LABELED IMMUNOASSAYS

The basic and common mechanism of these methods is based on the immobilization of one of the reactants (antigen or antibody) on a solid phase and the demonstration of specific binding by using a labeled reactive (conjugate). Labeled immunoassays have become increasingly popular not only for the detection and/or quantification of microorganism antigens in clinical samples and specific antibodies in various infectious diseases, they are also used for the detection and/or quantification of various biologic substances in body fluids (e.g. hormones, enzymes, drugs, tumor markers, etc.). The conjugate molecules which are used in these tests, are the labeled antibody molecules obtained from laboratory animals against Fc region of human immunoglobulins (e.g. enzyme labelled anti-human IgG, IgM or IgA), or antigen-specific labeled antibodies. The labeled immunoassays are named after their selective labeling molecule.
<table>
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<th>Evaluation</th>
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<td>Enzyme immunoassay (ELISA, EIA)</td>
<td>Enzymes such as alkaline phosphatase or peroxidase</td>
<td>Polystiren beads or microplate wells</td>
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<td>Immunofluorescence (IF)</td>
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In routine laboratories, the most preferred methods are ELISA and IF, by which ready made commercial kits are used that include antigen or antibody coated plates, strips or slides with the other needed reactives. Chemiluminescence technology, the newest of the label detection, is generally applied by automated instruments.

**Antibody detection in sera by ELISA:** For this purpose, the solid phase (microplate wells) are coated with the antigens of interest. Negative and positive control sera and patients’ sera are dispersed into the correspondent wells and incubated at room temperature for 30 minutes. Then the wells are washed with phosphate buffer solution, in order to eliminate the non-specific bindings. Then the conjugate solution (enzyme labeled anti-human IgG) is dropped into all of the wells. The incubation and washing procedures are repeated. Next, colorogenic substrate solution (produce colour after reacting with enzyme) are dropped into the wells, and incubated for 15 minutes at room temperature. After stopping the reaction the intensity of color changes due to the specific bindings are evaluated in an automatic optic reader (spectrophotometer). The absorbance values obtained from patient samples are evaluated by comparing with the cut-off value calculated from control reference sera. The quantitative results are also available if standard sera with known concentrations are included into the study.

**Antigen detection in clinical samples by ELISA:** In this method, the solid phase is coated with specific antibodies against the searching antigens. The antigens which is found in the sample are captured by these antibodies. After non-specific bindings are eliminated by washing process, conjugate (antigen-specific enzyme-labeled antibodies) solution is added. This conjugate binds specifically to the antigen and the enzyme gives colorimetric reaction after the addition of the substrate. The results are evaluated spectrophotometrically.

**Direct immunofluorescence method (DFA):** This method is used in order to detect the antigens which may be found in the clinical samples. The sample is spread on a
slide, fixed by acetone or methyl alcohol, and stained for microbial antigen(s) using specific fluorescein conjugated antibodies. After incubation at 37°C for 30 minutes, the slide is examined under the fluorescence microscope. The detection of bright yellow-green color indicates antigen positivity in the sample.

**Indirect immunofluorescence method (IIFA):** This method is used for the detection of antibodies (IgG, IgM or IgA) specific for the microbial antigens in the patients’ sera. The serum sample is dropped on the slide which had already fixed with microbial antigen(s) of interest. After incubation, the bounded antibodies are detected by the use of fluorescein conjugated anti-human antibodies (anti-human IgG, anti-human IgM or anti-human IgA). The slides are examined under the fluorescence microscope, and the presence of bright yellow-green color indicates antibody positivity against the tested antigen.