SEROLOGICAL TESTS

Definition: the study of antigen-antibody reactions in vitro

Characters
- Specific: the antibody (Ab) reacts with the antigen (Ag) that induces its production
- Optimum temperature: 37°C up to 56 °C
- Type: When the antigen is:
  1. Particulate: the reaction is called agglutination (clumping of the antigen).
  2. Soluble: the reaction is called precipitation.
  4. Virus: virus neutralization reaction develops.
  5. When a third substance complement may enter the reaction, it is called complement fixation reaction.
- Optimal amounts of antigen and antibody are required, because increase any of them inhibit the reaction to be visible, this is called zone phenomenon i.e. the reaction is deviated toward one zone either antigen or antibody.

OBSERVABLE ANTIGEN-ANTIBODY REACTIONS: Some types of agglutination reactions:

<table>
<thead>
<tr>
<th>DIRECT AGGLUTINATION</th>
<th>HAEMAGGLUTINATION TEST</th>
<th>PASSIVE AGGLUTINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slide agglutination</td>
<td>Tubulation test</td>
<td>Soluble antigen is fixed on inert particles as latex (polyethylene) which act as a particulate antigen.</td>
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<tr>
<td>Rapid, simple and qualitative test, used in:</td>
<td>• When the antigen is an erythrocyte, the process is called haemagglutination. e.g. Influenza virus can agglutinate sheep, horse and chicken RBCs,</td>
<td>e.g. Rheumatoid factor (Latex fixation test), pregnancy test.</td>
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<tr>
<td>• Identification of unknown organism as Salmonella typhi.</td>
<td>• It is not true serological test (no Ag - Ab reaction).</td>
<td></td>
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<tr>
<td>• Blood grouping.</td>
<td>• Inhibition of this process by virus antibody is called haemagglutination inhibition test.</td>
<td></td>
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</tbody>
</table>

| Slide agglutination | Tube agglutination | |
|---------------------|-------------------|
| Rapid, simple and qualitative test, used in: | accurate & quantitative test, used in: |
| • Diagnosis of infectious diseases as brucellosis & enteric fever (Widal test). | • | |
| ➢ Titre: It is the highest dilution of the serum that gives positive reaction. Rising titre is diagnostic especially in results with low titre. | • | |

PRECIPITATION
- Definition: This is an Antigen-antibody reaction in which the antigen is in soluble form.
- In this case antigen is called precipitinogen and antibody is called precipitin.
- Example for precipitation is agar diffusion tests:
  ➢ Ag and Ab are allowed to diffuse against each other in agar gel:
  1- Single diffusion
  2-Double diffusion
RADIOIMMUNO ASSAY (RIA)

- Is based upon the competition between radio-labeled and unlabeled antigen for specific antibody sites, forming antigen-antibody complexes.
- Amount of bound against free-labeled antigen is made and the amount of unknown antigen is known from the standard curve.

Application of RIA:
- Estimation of hormones as T3, T4, F.S.H., testosterone and oestrogen.
- Serum proteins as AFP and CEA.
- Enzymes.
- Drugs as digoxin and morphine.
- Infectious diseases as HBs Ag and HIV.

IMMUNOFLUORESCENCE

Immunofluorescence is an antigen-antibody reaction where the antibodies are labeled with a fluorescent dye and the antigen-antibody complex is visualized using ultra-violet (fluorescent) microscope.

<table>
<thead>
<tr>
<th>DIRECT IMMUNOFLUORESCENCE</th>
<th>INDIRECT IMMUNOFLUORESCENCE</th>
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<tbody>
<tr>
<td>Used to detect antigen in clinical specimens using specific fluorochrome labeled antibody.</td>
<td>This is used to detect antibodies in patient serum. A second labeled antibody (anti-gamma globulin) is added, which binds the first unlabeled antibody.</td>
</tr>
</tbody>
</table>
| • Detect viral, parasitic, tumor antigens from patient specimens  
• Identification of anatomic distribution of an antigen within a tissue. | • Used to detect autoantibodies  
• eg anti-nuclear antibodies (ANA) found in the serum of patients with SLE |

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

- The assay is considered enzyme-linked because an enzyme is chemically linked to an antibody & immunosorbent refers to the fact that either antigens or antibodies are being adsorbed to plastic. It is used either to demonstrate unknown antigens or unknown antibody.

<table>
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<tr>
<th>Direct ELISA (Sandwich ELISA)</th>
<th>Indirect ELISA</th>
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<tr>
<td>Designed to detect an antigen</td>
<td>Designed to detect antibodies</td>
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</table>
| • Antibody specific for the tested antigen is coated on a plastic surface (e.g. microtiter plate)  
• The patient serum sample is added to the plate to test for the presence of the antigen that binds to the antibody.  
• Then a secondary antibody is added. If the antigen is present, a “sandwich” of antibody, antigen, and secondary antibody will form.  
• The secondary antibody is chemically linked to an enzyme. When the substrate is added, the enzyme converts the substrate from a colorless compound to a colored compound.  
• The amount of color produced will be proportional to the amount of antigen present and thus determines if the patient is positive for the antigen. | • The configuration that forms in the indirect ELISA is antigen, antibody, and secondary antibody.  
• Application of ELISA: as in RIA |