

7. Serological methods of disease diagnosis

Competencies

MI1.8: Describe the mechanisms of immunity and response of the host immune system to infections

MI8.15: Choose and interpret the results of the laboratory tests used in diagnosis of the infectious diseases

Specific Learning Objectives:

At the end the session, the students shall be able to,

- Enumerate different immunological tests available for diagnosis of infectious diseases
- Describe the principle, types and application of Agglutination test
- Describe the principle, and application of Precipitation test
- Describe the principle, application of ELISA test
- Describe the principle, types and application of Immunofluorescence test
- Discuss the principle, rapid point – of – care (POC) test with example

Exercise 7:

1) List various types of immunological techniques available for the diagnosis of infectious diseases.

| Disease | Immunological/ serological test |
|--------------------------|---------------------------------|
| Syphilis | Slide flocculation test |
| Diphtheria | Elek's gel precipitation test |
| Typhoid fever | Widal test |
| Acute Brucellosis | Standard agglutination test |
| Infectious mononucleosis | Paul Bunnell test |
| Atypical pneumonia | Cold agglutination test |
| Meningitis | Latex agglutination test |

| | |
|-----------|----------------------------------|
| Arthritis | Rheumatoid Arthritis factor (RA) |
|-----------|----------------------------------|

2) Describe the principle, types and applications of agglutination tests.

| Agglutination test principle: When a particulate or insoluble antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH the particles are clumped or agglutinated. | | |
|--|---|--|
| Types | Examples | Application |
| Direct Agglutination Test | Agglutination of Salmonella by its specific antibody | used for the serological test for visceral leishmaniasis |
| Indirect or Passive Agglutination Test | Latex beads or tanned erythrocytes | This is usually done to convert precipitation reaction into agglutination reaction, since the later are easier to perform an interpret and are more sensitive than precipitation reaction for detection of antibodies. |
| Reverse Passive Agglutination Test | Influenza, mumps, and measles have the ability to agglutinate RBCs without antigen–antibody reactions | To detect infectious bursal disease (IBD) virus antigen in various organs of experimentally infected chickens and field cases |

3) Describe the principle, types and applications of precipitation tests.

| Precipitation test principle: Antigen-antibody reaction, which occurs at the equivalence zone. At the equivalence region, the ratio of both antigen and antibody is equal, which brings out the formation of lattice or cross-linked structure. | | |
|---|-----------------|--|
| Types | Examples | Application |
| Slide flocculation test | VDRL | It is used for serodiagnosis of syphilis . VDRL test is most widely used simple and rapid test for syphilis. It is a non-treponemal test (non-specific test) used to investigate syphilis, other treponematoses, Yaws and Pinta. |
| Immunodiffusion test | Elek's gel | The Elek culture plate precipitin test is routinely used for the detection of exotoxin from toxigenic strains of <i>Corynebacterium diphtheriae</i> . |

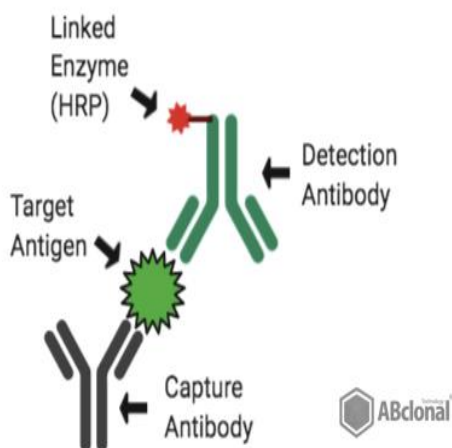
4) Describe the principle, types and applications of ELISA.

ELISA principle:

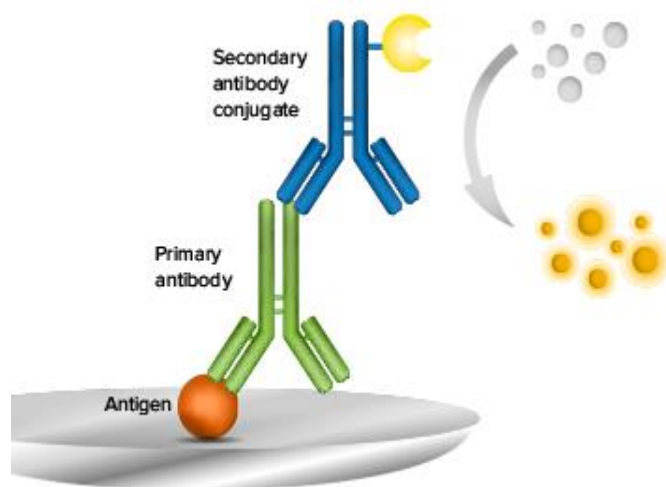
The specific antibodies bind the target antigen and detect the presence and quantity of antigens binding. In order to increase the sensitivity and precision of the assay, the plate must be coated with antibodies with high affinity. ELISA can provide a useful measurement of antigen-antibody concentration.

| Types | Examples | Application |
|-------------------|---|---|
| Direct ELISA | HIV | Detection of antigen in test serum |
| Indirect ELISA | HIV, Dengue etc. | Detects the presence of an antibody in a sample |
| Sandwich ELISA | Rotavirus, Enterotoxins of <i>E.coli</i> in feces | To detect the presence of antigen in a sample |
| Competitive ELISA | HIV antibodies | To detect antigen concentration in a sample |

5) Draw colored labeled diagrams depicting the principle of ELISA reactions in the space provided below.



Sandwich ELISA

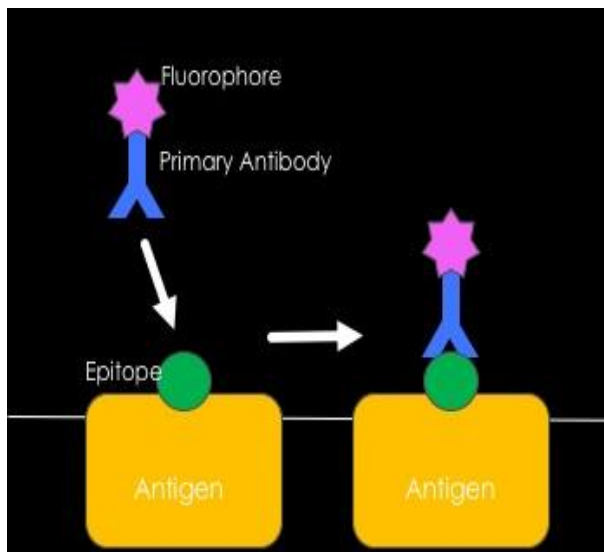


Indirect ELISA

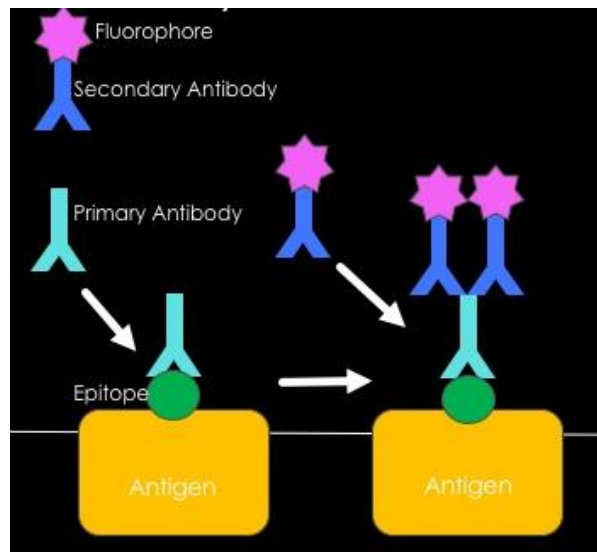
6) Describe the principle, types and applications of Immunofluorescence test.

| Immunofluorescence test principle: To absorbing high energy- shorter wavelength ultraviolet light rays by the fluorescent compounds and in turn emitting visible light rays with a low energy- longer wavelength. | | |
|---|--|---|
| Types | Examples | Application |
| Direct Immunofluorescence Assay | N. gonorrhoeae, T. palladium etc. | To diagnose diseases of the skin, kidney, and other organ systems |
| Indirect Immunofluorescence Assay | Leptospirosis, Amoebiasis, Taxoplasmosis | To detect circulating autoantibodies in patient serum |

7) Draw colored labeled diagrams of Direct and Indirect Immunofluorescence (IF) reactions below.



Direct IF test

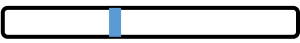




Indirect IF test

8) Discuss the principle of rapid point-of-care (POC) test with example

| Principle: POC (Bedside test) is mainly characterized by proximity to the patient, quantitative or semi-quantitative single measurements, short turnaround time, no sample preparation, no pipetting, use of pre-made reagents, user-friendly dedicated analytical instruments and instant, result-deduced therapeutic action. | | |
|---|--------------------|--------------------------------|
| Types | Examples | Application |
| Immuno-chromatographic test | Lateral flow assay | Used for detection of HBsAg |
| Flow Through test | Flow through assay | Used for detection HIV TRI-DOT |

9) Following are the results of immuno-chromatographic test for Hepatitis B infection done on three cases. Interpret your results and report (C=Control band, T=Test)

| Case | Result | Report |
|------|--|------------------|
| A | <div style="text-align: center;"> C T  </div> | Test is negative |
| B | <div style="text-align: center;"> C T  </div> | Test is positive |
| C | <div style="text-align: center;"> C T  </div> | Test is invalid |

Date:

Faculty Name & Signature