Application of immunological methods in the diagnosis of parasite in general.

- Serological diagnostic examinations include precipitation reaction, agglutination reaction, complement fixation test, immunofluorescence antibody assay, immunoenzymatic assay, radioimmunoassay, and immunoblotting.

- The serological diagnosis has evolved from serum sedimentation test and agglutination test to immunolabelling technique with efficiency requiring a trace amount of specimen and enzyme linked immunoblotting at the molecular level.

- These immunoassays can be applied to detect circulating antibody or antigen in infected human body, and are expected to be applicable for staging of an infection, identifying active stage of a new infection and assessing the therapeutic efficacy.

- The serological diagnosis is playing an increasingly important role in the clinical diagnosis that the etiological diagnosis fails to. Almost all the immunoassays are applicable for the diagnosis of parasitic diseases but not always effective.

Specimen Requirements

❖ **Specimens for antibody detection**

- Serum or plasma for most of the parasitic infections.
- Aqueous and vitreous eye fluids as well serum for toxocariasis and toxoplasmosis.
- CSF for cysticercosis and toxoplasmosis.

❖ **Specimen for antigen detection**

- Fresh or preserved stool samples for most of enteric protozoa.
- Venous blood with EDTA and capillary blood

(Note:- Polyvinyl alcohol fixed stool may be acceptable but not formaldehyde/formalin-fixed specimen)

African trypanosomiasis

- Caused by *Trypanosoma brucei gambiense*.
- Diagnosed by 3-step laboratory algorithm: Screening, Conformation and Staging.
- Detection of specific antibodies is the best method for screening, while diagnostic confirmation and staging require examination of csf.
- Card agglutination test for trypanosoma (CATT) is the most reliable test available for screening.
- CATT utilizes whole ,fixed, Coomassie blue-stained trypanosomes that react with serum IgG or IgM Abs to form visible precipitate/agglutination.

Amebiasis caused by Entamoeba histolytica

- *Entamoeba histolytica* is associated with both intestinal and extraintestinal infections.
- Antibody detection is the most useful in patients with extraintestinal disease, i.e. amebic liver abscess, when organisms are not generally found upon stool examination.
- Antigen used is crude soluble extracts of axenically cultured organisms.
- Enzyme immunoassay and indirect hemagglutination assay are mostly used.
- Immunodetection of *E. histolytica* antigens in fecal specimens may be used to distinguish the morphologically identical pathogenic *E. histolytica* and non-pathogenic *E. dispar.*
- The assay detect the galactose inhibitable adherence proteins, which is necessary for pathogenesis.

Chagas' disease

- Caused by *Tryanosoma cruzi*
- Immunodiagnosis is mehod of choice in chronic stage of infection , diagnosis.
- Diagnosis usually requires positive results from at least two different serological methods.
- Serological methods include IHA, IFA assay, complement fixation, EIA , immunoblotting, and radioimmunoprecipitation.
- Detect antibody that react with parasite antigens found in crude trypanosome extracts or in the excretory-secretory (ES) products of cultured parasites or recombinant proteins.

Cryptosporidiosis

- Caused by *cryptosporidium spp.*
- Microscopic identification of *cryptosporidium* parasites and detection of antigen in stool specimens are the current diagnostic test of choice.
- DFA test, identifies oocysts in concentrated or unconcentrated fecal samples by using a fluorescein isothiocyanate labeled monoclonal antibody.
- EIAs detects cryptosporidial antigen in the fresh or frozen stool samples.

Giardiasis

- Caused mainly by *Giardia lamblia.*
- DFA assay that employ fluorescein isothiocyanate-labelled monoclonal antibody for the detection of *Giardia* cyst.
- EIA are available in the microplate format for the detection of Giardia antigen in stool.

Leishmaniasis

- Caused by *Leishmania donovanii.*
- Immunodiagnosis technique detect antibody against cultured epimastigotes of various *Leishmania spp.*, or crude solubilized epimastigotes.
- Rapid immunochromatographic card test use recombinant proteins k39 or rK28.

Malaria

- Caused by *Plasmodium spp.*
- Rapid diagnostic technique uses histidine rich protein-2 of *Plasmodium falciparium* and lactate dehydrogenase, a pan malarial parasite antigens.
- Antibody against these antigen is detected in serum sample.

Toxoplasmasis

- Caused by *Toxoplasma gondii.*
- Sabin-Feldman dye test, Acetone (AC)- fixed versus formalin (HS)- fixed tachyzoites (AC/HS) agglutination test, and an IgG avidity test, as well as test for Toxoplasma-specific IgA and IgE are used as immunological techniques.