Diagnosis of Parasites

3rd stage/2nd Semester/2024-2025

Lec.4 **Indirect methods for diagnosing parasitic diseases:**

All types of parasites diagnostic methods are subject to varying degrees of **sensitivity** ,**specificity**, **reactivity** and are prone to obtaining **false-positive** or **false-negative** results; as such, all diagnostic procedures should employ various methods to ensure the most accurate possible diagnosis.

Detecting parasitic infections may perform by one or more of the following methods:

1-Microscopic method: Direct identification of the authentic parasite under microscope. Which will illustrated in the next lectures.

2-Moleculer methods: In which parasite DNA is detected in host tissues or body fluids.

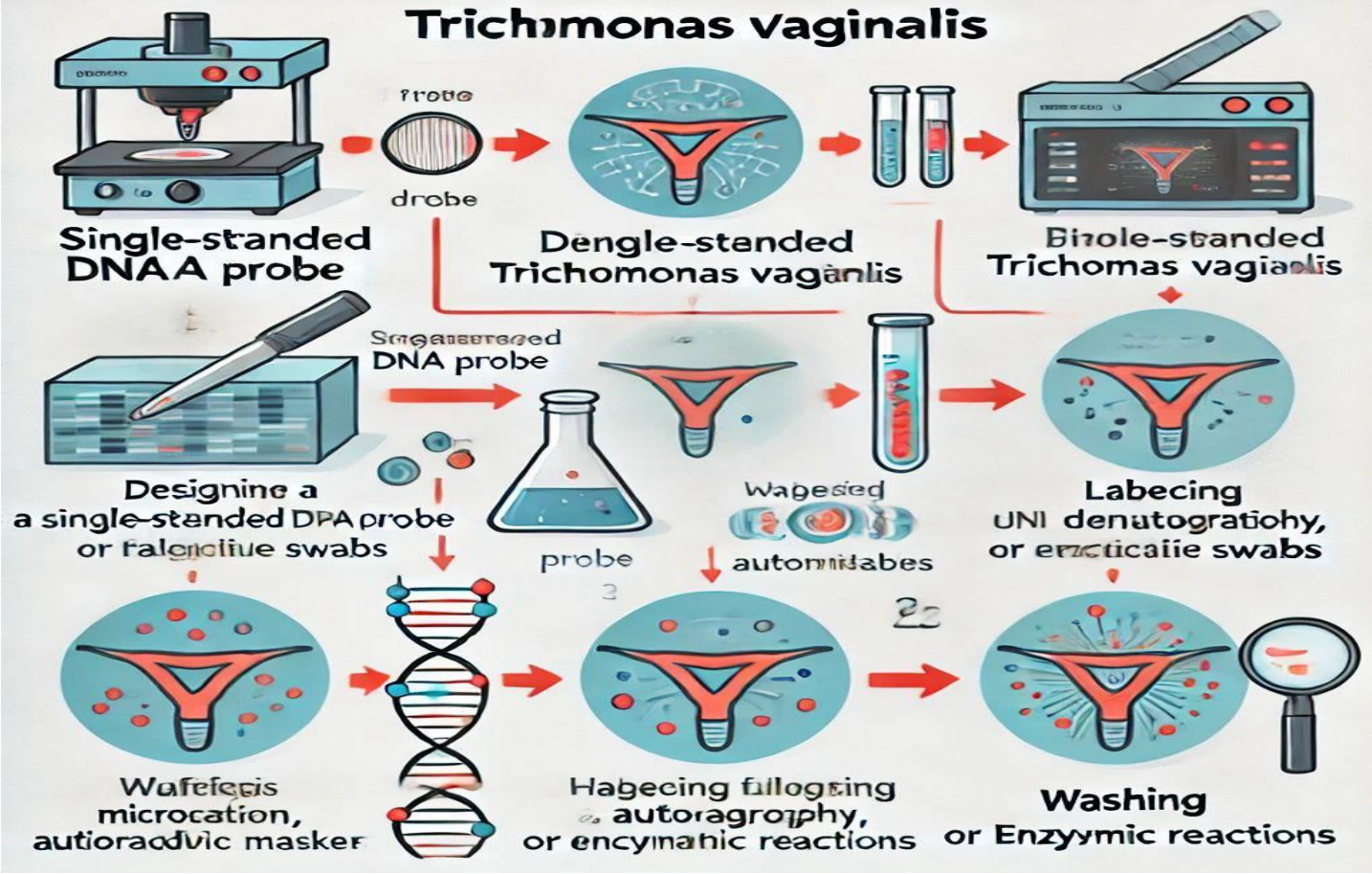
3-Serodiagnosis: which include indirect tracing of the parasitic invasion by tracking immune reaction in patient body in response to a parasitic infection (tracing either antigens of the invader parasite or antibody formed in the host body upon the parasitic infection).

Molecule methods:

a. DNA probe:

Performance of this test involves transfer of DNA-containing material of any sort (insect salivary glands or gut , human serum or feces , etc.) to a nitrocellulose membrane , where it is fixed . A DNA segment of known sequence, characteristic of the parasite DNA (produced by a cloning technique by means of hybridomans and then radioactively labeled), is hybridized to the DNA on the membrane. Radioactivity remaining after the membrane is washed signals the presence of the parasite.

This procedure is used primarily for the detection of ***Trichomonas vaginalis*.**



b- Polymerase chain reaction (PCR):

A method whereby low levels of specific DNA sequences may be amplified to reach the threshold of detection through action of the enzyme DNA polymerase.

This complicated procedure is used for the diagnosis of **babesiosis** and **toxoplasmosis.**

The general steps involved in performing PCR for *T. gondii:*

1. Sample Collection

Obtain biological samples such as blood, cerebrospinal fluid (CSF), tissue biopsies, or amniotic fluid, depending on the type of infection or diagnostic requirement.

2. DNA Extraction:

Extract genomic DNA from the sample using a DNA extraction kit or chemical lysis method.

The quality and quantity of extracted DNA should be assessed before proceeding with PCR.

3. Primer Selection:

Use specific primers targeting T. gondii DNA sequences. Common targets include:

4. PCR Setup:Prepare the PCR reaction mixture:

1.DNA template, 2.Primers, 3. DNA polymerased,

4.NTPs: The building blocks for DNA synthesis.

5.Buffer solution: To maintain the optimal pH and salt concentration.

6.Magnesium chloride ($MgCl_2$): A cofactor necessary for the polymerase activity

5. PCR Amplification ; Perform the PCR amplification in a thermal cycler, which typically involves the following steps:

Denaturation (94–98°C): The DNA template is heated to separate the double-stranded DNA into single strands.

Annealing (50–65°C): The primers bind to the complementary regions on the single-stranded DNA.

Extension (68–72°C): The polymerase extends the primers to synthesize new DNA strands.

This cycle is usually repeated 25–35 times to achieve amplification of the target region.

6. Detection of PCR Products: After amplification, analyze the PCR product by:

Agarose Gel Electrophoresis AND Real-Time PCR (qPCR):

7. Interpretation of Results

Positive Result: The presence of a specific DNA fragment corresponding to the *T. gondii* gene (e.g., B1 or SAG1) indicates an infection.

Negative Result: No amplification of the target DNA, suggesting the absence of *T. gondii.*

8. Confirmation

In some cases, the PCR product may be sequenced for further confirmation of the species or strain of *T. gondii*.

C. Immunoblot (IB):

is a laboratory technique used to detect specific proteins in a sample using antibodies. In parasitology, it helps identify parasite antigens, diagnose infections, and study immune responses.

A more sensitive version of the DNA probe in which a sample of DNA –containing material from serum cerebrospinal fluid, or other tissue or excreta is electrophoresed onto a polyacrylamide gel.

The electrophoretic ally separated proteins on the gel are then transferred (by blotting) onto a sheet where they are recognized by radioactively labeled specific antibodies, whose presence can be assayed after the sheet is washed.

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d. Westen blot (WB):

This type of immunoblot test is used in diagnosis of cysticercosis , echinococcosis , and schistosomiasis .

Here is a scientific diagram illustrating the steps of the Immunoblot (Western Blot) technique for parasite detection.

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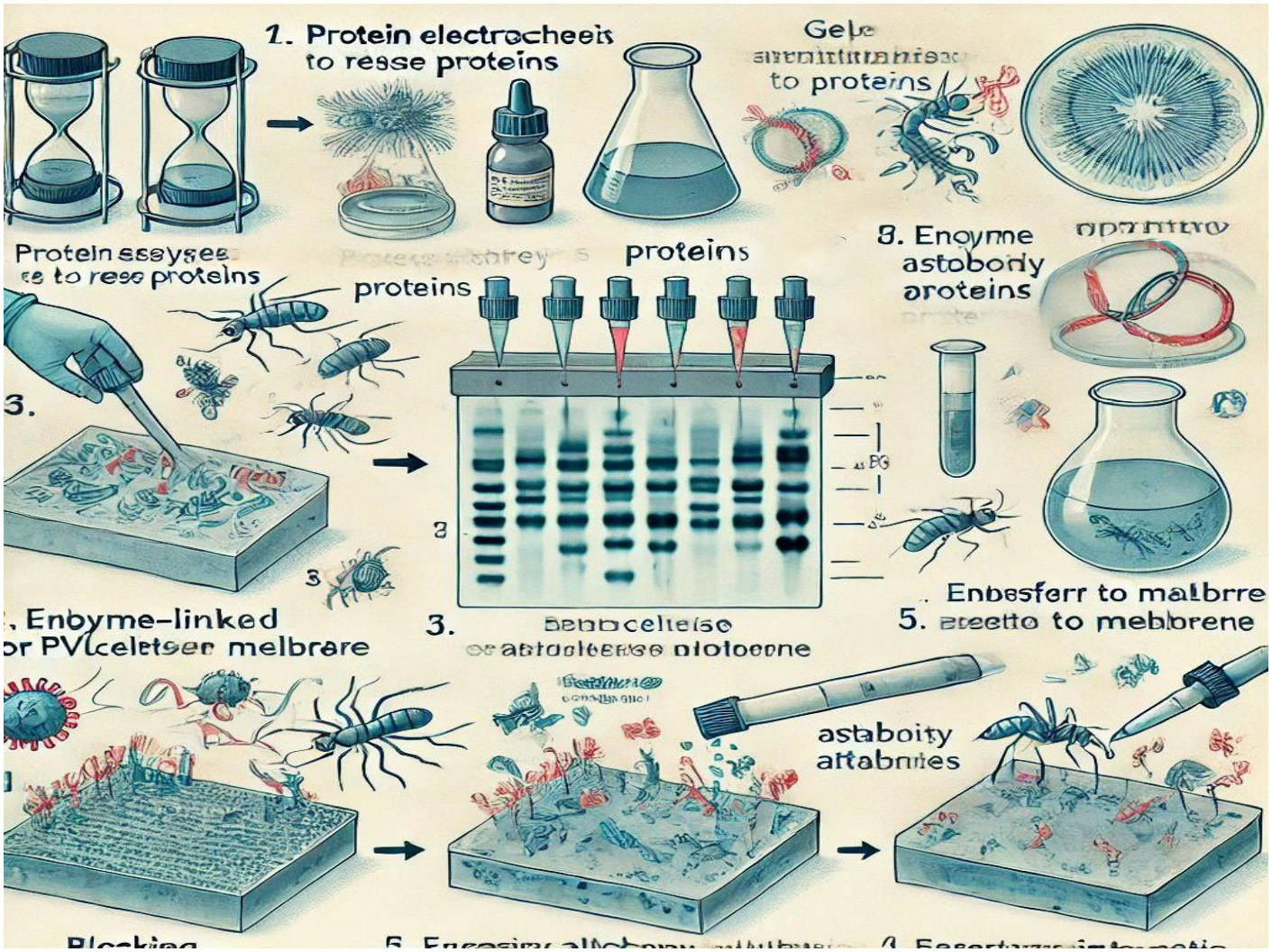


Serodiagnosis of the parasitic diseases:

1-Antibody depending methods:

a-Indirect hemagglutination(IHA); b-Indirect Fluorescent antibody (IFA);

c-Radioallergosorbent test (RAST).

2-Antigen depending methods:

a-Bentonite Flocculation (FB); b-Direct Immunoflurescence (DFA); c-Latex agglutination (LA)

3-Antibody and antigen depending methods:

a-Enzyme immunoassay (EIA); b-Enzyme-Linked immunobsorbant assay (ELIZA)

1-Antibody depending methods:

a-Indirect hemagglutination (IHA):

One of the older test modalities indirect hemagglutination depends on the agglutination (clumping) of antigen – coated sheep erythrocytes by antibodies in the test serum. It may be roughly quantitated by dilution of the test serum.

and is used for diagnosis of **amebiasis (E. histolytica / dispar complex). Chagas ,disease, cysticercosis , echinococcosis , filariasis and toxoplasmosis.**

B. Radioallergosorbent test (RAST):

Developed as a test for antibodies to certain allergens, RAST test specifically for IgG and IgE . Known parasite antigen is bound to complex carbohydrate matrix known as a sorbent .test serum is then added to the sorbent, which is washed and then allowed to react with a radioactively labeled antibody to human IgG or IgE . After removal of the excess labeled antibody , the presence and amount of radioactivity measure antibody present in the serum.

RAST is used for diagnosis of **anisakiasis and ascariasis .**

c. Indirect Fluorescent antibody (IFA):

A known parasite antigen (for example, blood smears containing plasmodium falciparum obtained from antibodies to that parasite .

Antibodies in the serum bind to antigen of the parasites, the slide is then washed to remove the serum, and then the slide is covered with a solution containing a fluorescent dye coupled with antihuman globulin . when this is in turn washed , any fluorescent dye remaining indicates the presence of the antibody in the serum specimen . by use of the appropriate antihuman globulin , one may test for IgG or IgM .

This test is used for diagnosis of **babesiosis**, **Chagas disease**, **cryptosporidiosis** , **giardiasis** , **leishmaniasis , malaria and toxoplasmosis**.

2-Antigen depending methods

a-Bentonite Flocculation (FB):

This test, of relatively low reactivity, is performed by coating particles of bentonite with the test antigen and observing flocculation on addition of the serum.

Titration is achieved by serial dilution of the serum.

This test is used primarily for the diagnosis of **trichinosis** , for which other tests are also available.

b-Direct Immunoflurescence (DFA):

Fluorescence of parasite antigen is induced by the introduction of monoclonal antibodies (produced in vitro against the parasite in question and fluorescently tagged)into a fluid or applied to a slide containing the parasite .the organism fluoresces when viewed by fluorescence microscopy .

This test is used for detection of **cryptosporidiosis** , **giardiasis**, and **trichomoniasis.**

c-Latex agglutination (LA):

See bentonite Flocculation . this procedure is the same , with substitution of latex particles for bentonite .Latex Agglutination is an older test ,

but it is still used for diagnosis of *Trichomonas vaginalis* infection.

3-Antibody and antigen depending methods:

a-Enzyme immunoassay (EIA):

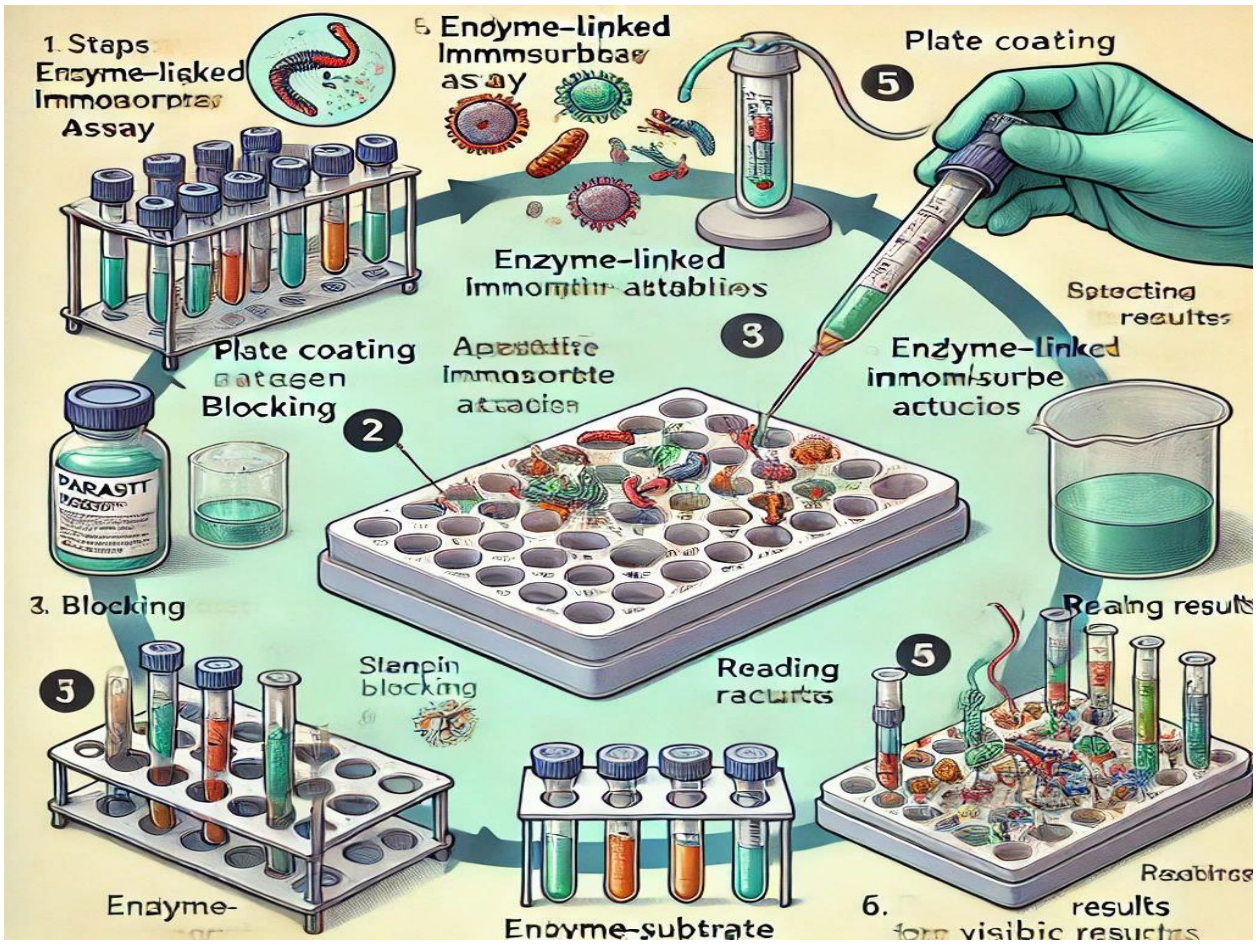
A general term for several different procedures that determine the presence or absence of a parasite by the responding antibody or antigen , and the enzyme substrate , with production of a color that may be assayed either qualitatively .

Enzyme immunoassay is the most widely used of all the types of tests for detection of parasitic infections.

b-Enzyme-Linked immunobsorbant assay (ELIZA):

A specific variation of enzyme immunoassay , the ELISA has become increasingly popular because of sensitivity and ease of interpretation . the appropriate antigen or antibody is bound to a solid support (microtiter wells , beads , test tube walls). The specimen to be tested (serum , cerebrospinal fluid ,feces) is added and reacts with the already present antigen or antibody . washing then removes unbound test material , after which enzyme-linked antibody or antigen is added , which reacts with the antigen or antibody of the test material . an additional washing removes all unbound material , and finally a solution which reacts with the remaining enzyme to produce a color change is added this change may be measured visually or colorimetrically .

ELISA (often listed simply as EIA) IS USED to diagnosis a wide variety of parasitic diseases.(cryptosporidiosis , giardiasis …etc).



Here is a scientific diagram illustrating the steps of the ELISA (Enzyme-Linked Immunosorbent Assay) technique for parasite detection.