DETECTION OF BACTERIAL, VIRAL AND PARASITIC DISEASES BY ELISA

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Fundamental Principle of ELISA

- **Antigen-antibody binding**: The core principle of all ELISAs is the highly specific binding reaction between an antigen and its corresponding antibody.
- **Immobilization**: Either a known antigen or an antibody is coated and immobilized onto a solid surface, most commonly the wells of a 96-well microtiter plate.
- **Enzyme conjugation**: An enzyme is linked to an antibody or antigen, acting as a label. This enzyme catalyzes a reaction with a substrate to produce a detectable signal, such as a colour change.
- **Signal measurement**: The intensity of the colour produced is measured with a spectrophotometer, which can provide both qualitative (positive/negative) and quantitative (concentration) results.

Types of ELISA for bacterial diagnosis

Indirect ELISA (for antibody detection)

This method is used to determine if a patient has been exposed to a particular bacterium by detecting the antibodies the patient's immune system has produced in response.

Procedure:

1. **Coat wells with antigen**: A known, purified antigen of the bacterium is coated onto the microplate wells.
2. **Add patient sample**: The patient's serum, containing unknown antibodies, is added. If specific antibodies are present, they will bind to the coated antigen.
3. **Add labeled secondary antibody**: An enzyme-linked secondary antibody, which is specific to the human antibodies, is added.
4. **Add substrate**: The substrate is added, and the enzyme catalyzes a reaction that produces a colour change.
5. **Measure and interpret**: The colour intensity is proportional to the amount of patient antibody present. A colour change indicates a positive result.

- **Key application**: Detecting antibodies to *Borrelia burgdorferi* for Lyme disease. The CDC-recommended two-tiered approach uses an initial ELISA screen followed by a Western blot to confirm positive or inconclusive results.

Sandwich ELISA (for antigen detection)

This highly sensitive and specific method is ideal for detecting bacterial antigens (like toxins or surface proteins) in a patient sample during an active infection.

Procedure:

1. **Coat wells with capture antibody**: A specific antibody (capture antibody) is coated onto the plate wells.
2. **Add patient sample**: The patient's sample (e.g., stool, serum) is added. If the bacterial antigen is present, it will bind to the capture antibody.
3. **Add labeled detection antibody**: An enzyme-linked detection antibody, which recognizes a different binding site (epitope) on the bacterial antigen, is added, creating a "sandwich."
4. **Add substrate**: The enzyme-linked antibody reacts with the substrate, producing a colour change.

- **Key application**: Diagnosing *E. coli* infections by detecting bacterial enterotoxins in fecal samples.

Competitive ELISA

This technique is often used to detect small bacterial antigens or antibodies. The signal is inversely proportional to the amount of the substance in the sample.

Procedure:

1. **Incubate sample and labeled antigen**: The patient's sample is mixed with a known, labeled bacterial antigen.
2. **Add to antibody-coated wells**: The mixture is added to wells that have been coated with specific antibodies. The unlabeled antigen from the patient sample and the labeled antigen compete for binding sites.
3. **Measure and interpret**: A higher concentration of antigen in the patient sample leads to less labeled antigen binding to the wells, resulting in a lower signal. A weaker colour change indicates a positive result.

Interpretation considerations

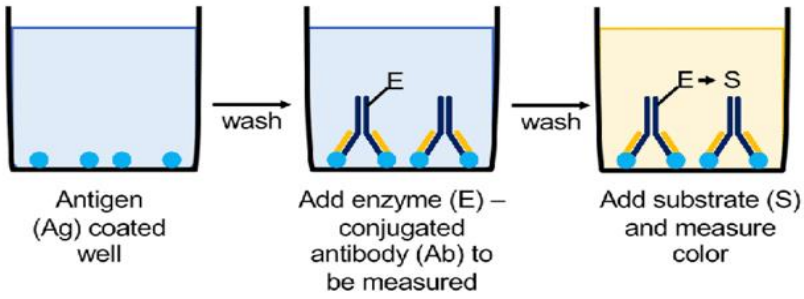
- **Sensitivity vs. specificity**: ELISA can be optimized for either high sensitivity (detecting most cases, but with more false positives) or high specificity (fewer false positives). The use of supplemental tests, like Western blot, helps confirm a diagnosis.

- **Timing of infection**: Antibody-based ELISAs are not effective for very recent infections, as it takes time for the body to produce a detectable immune response. Antigen-based ELISAs are better for detecting early or active infections.

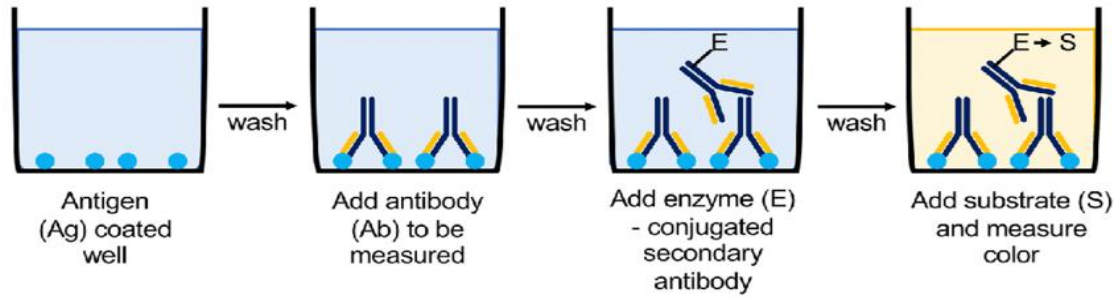
- **False positives/negatives**: Factors like cross-reactivity with other bacteria, non-specific binding, or low bacterial load can lead to inaccurate results. False negatives can also occur if the antibody level is below the detection limit of the test.

- **Standardization**: For some bacterial diseases, especially in children, the accuracy of commercial ELISA kits can vary significantly, so relying on validated tests or in-house assays is important.

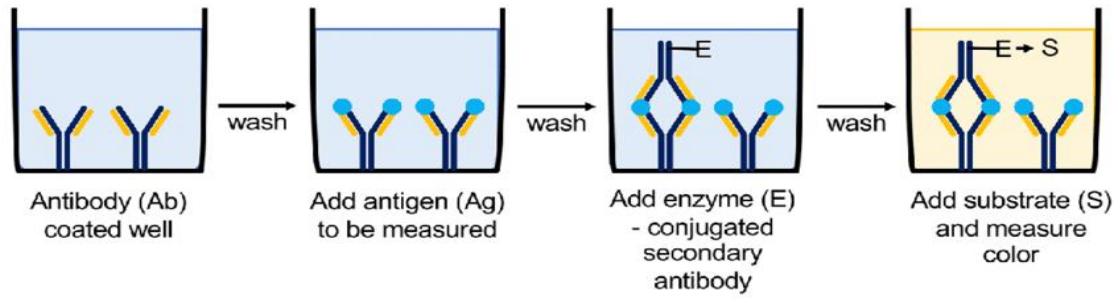
(a) Direct ELISA



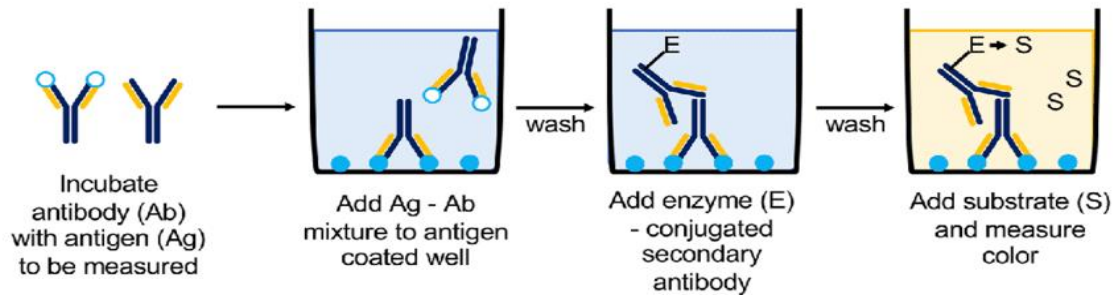
(b) Indirect ELISA



(c) Sandwich ELISA



(d) Competitive ELISA



ELISA (enzyme-linked immunosorbent assay) diagnoses bacterial, viral, and parasitic diseases by detecting specific antibodies or antigens in a patient's sample. It uses a micro-titre-plate coated with either antigens to find antibodies or antibodies to find antigens. The presence of the target molecule is indicated by a colour change after a series of enzyme-linked reactions.

How ELISA is used for different infections

- **Antibody detection:** An indirect ELISA is used to look for the body's immune response.
  - The microplate is coated with a specific antigen from a bacterium, virus, or parasite.
  - If the patient has antibodies against that pathogen, they will bind to the antigen.
  - An enzyme-linked antibody that binds to human antibodies is then added. If the patient's antibodies are present, this second antibody will attach.
  - A final enzyme-linked component causes a colour change, indicating the presence of a past or current infection.
- **Antigen detection:** A direct or sandwich ELISA is used to directly detect the pathogen itself.
  - The microplate is coated with antibodies that are specific to the pathogen's antigens.
  - A patient's sample is added. If the pathogen is present, its antigens will bind to the antibodies on the plate.
  - A final enzyme-linked antibody that binds to the pathogen's antigens is added, causing a colour change to show the presence of the pathogen.

Examples of diseases diagnosed by ELISA

- **Bacterial:** Lyme disease, brucellosis, and syphilis
- **Viral:** HIV, Hepatitis A, B, and C
- **Parasitic:** Amoebiasis, cysticercosis, malaria, schistosomiasis, and toxoplasmosis

Enzyme-linked immunosorbent assay (ELISA) is a versatile serological technique used to diagnose bacterial, viral, and parasitic diseases by detecting either specific antigens or the antibodies produced in response to them. The specific type of ELISA employed depends on the pathogen and the stage of infection.

DIAGNOSIS OF BACTERIAL DISEASES BY ELISA

ELISA can detect both bacterial antigens and the host's antibody response to them. It is a rapid and effective method for identifying many common bacterial pathogens, including:

- ***Bordetella pertussis:*** Causes whooping cough and is detected through ELISA.

- ***Chlamydia:*** Species like *Chlamydia pneumoniae* and *C. trachomatis*, which cause pneumonia and the sexually transmitted disease chlamydia, respectively, can be diagnosed with ELISA. It is employed to detect IgG and IgM antibodies in serum or plasma to diagnose chlamydial infections. This method can also be used to detect antibodies to genus-specific chlamydial antigens.

- ***Clostridium:*** This genus includes the bacteria that cause botulism (*C. botulinum*), tetanus (*C. tetani*), and *C. difficile* infection.

- ***Escherichia coli (E. coli):*** Pathogenic strains can cause gastroenteritis and are detectable via ELISA.

- ***Helicobacter pylori (gastritis/ulcers)****:* ELISA is used to detect *H. pylori* antibodies in serum, but this can't distinguish a current from a past infection. A more specific approach uses a sandwich ELISA to detect *H. pylori* antigens directly in stool samples, confirming an active infection.

- ***Borrelia burgdorferi (Lyme disease):*** ELISA is used to detect antibodies produced in response to the bacterium *Borrelia burgdorferi*, which causes Lyme disease. Indirect ELISA detects antibodies produced against the bacterium. Because false positives can occur, especially in low-prevalence areas, positive or equivocal ELISA results must be confirmed with a Western blot test.

- ***Salmonella:*** Including the serovar that causes typhoid fever, *Salmonella typhi*, is detectable using ELISA.

- ***Syphilis:*** ELISA is used to detect antibodies against *Treponema pallidum*, the bacteria that cause syphilis. ELISA tests detect antibodies (IgG, IgM, IgA) against this spirochete. These are often used as a first-line screening tool for syphilis.

DIAGNOSIS OF VIRAL DISEASES BY ELISA

An Enzyme-Linked Immunosorbent Assay (ELISA) is a versatile immunoassay widely used for diagnosing viral infections by detecting specific viral antigens or the antibodies produced in response to them. The choice of ELISA format depends on the specific diagnostic goal and the timing of the infection.

Fundamental principles of viral ELISA

- **Antigen-antibody specificity**: The test is based on the highly specific binding between viral antigens and the host's antibodies.

- **Enzyme conjugation**: An enzyme is chemically linked to an antibody or antigen, acting as a label.

- **Signal amplification**: The enzyme catalyzes a reaction with a substrate, producing a measurable signal, typically a colour change. This enzymatic amplification significantly increases the assay's sensitivity.
- **Immobilization**: Either a known viral antigen or a specific antibody is coated onto a solid surface, usually the wells of a polystyrene microtiter plate.
- **Measurement**: A spectrophotometer (ELISA plate reader) measures the intensity of the coloured product, which is proportional to the concentration of the target molecule.

Types of ELISA used for viral diagnosis

Indirect ELISA (for antibody detection)

This method is used to detect and quantify the antibodies produced by the patient's immune system in response to a viral infection. It is particularly useful for determining a patient's immune status or for tracking the course of an infection.

Procedure:

1. **Coat wells with antigen**: A purified viral antigen is coated onto the microplate wells.
2. **Add patient serum**: The patient's serum is added. If specific antibodies are present, they will bind to the coated viral antigen.
3. **Add enzyme-linked secondary antibody**: An enzyme-conjugated secondary antibody (anti-human immunoglobulin) is added, which binds to the patient's specific antibodies.
4. **Add substrate**: A chromogenic substrate is added, and the enzyme catalyzes a colour-producing reaction.
5. **Interpret results**: The colour intensity is measured. A darker colour indicates a higher concentration of virus-specific antibodies in the patient's serum.

Applications:

- **Screening for HIV**: Indirect ELISA is a common method for screening blood for HIV antibodies. Positive results are typically confirmed with more specific tests, like Western blot.
- **Hepatitis diagnosis**: Detecting antibodies for viruses like Hepatitis B and C.
- **Tracking infection**: Can be used to track the body's immune response to viruses like Rubella or CMV to distinguish between primary and reactivated infections.
- **Zika virus:** ELISA is used to test for antibodies against the Zika virus.
- **Herpes Simplex Virus (HSV):** ELISA can detect HSV antigens or antibodies in a sample.

Sandwich ELISA (for antigen detection)

This method is highly sensitive and specific for detecting viral antigens directly in a sample, making it ideal for the early stages of an infection before a strong antibody response has developed.

Procedure:

1. **Coat wells with capture antibody**: The microplate wells are coated with an unlabeled "capture" antibody specific to the viral antigen.
2. **Add patient sample**: The patient's sample (e.g., serum, respiratory swabs) is added. If the viral antigen is present, it binds to the capture antibody.
3. **Add enzyme-linked detection antibody**: An enzyme-conjugated detection antibody, which recognizes a different epitope on the viral antigen, is added. This creates a "sandwich" of the viral antigen between the two antibodies.
4. **Add substrate**: The substrate is added, and the enzyme catalyzes a colour-producing reaction.
5. **Interpret results**: The colour intensity is directly proportional to the amount of viral antigen present.

Applications:

- **COVID-19 antigen tests**: Rapid antigen tests for SARS-CoV-2 often use the sandwich ELISA principle.
- **Influenza virus (Flu):** ELISA is used to detect antibodies against the virus, such as H1N1.
- **Rotavirus detection**: Used to detect rotavirus antigens in stool samples to diagnose gastroenteritis.
- **Hepatitis B Surface Antigen (HBsAg)**: A common method for detecting the HBsAg marker, indicating an active Hepatitis B infection.

Competitive ELISA (for antibody or antigen detection)

In a competitive ELISA, the patient's sample (containing unknown antigens or antibodies) competes with a labeled reagent for a limited number of binding sites on an antibody or antigen. This format is particularly useful for detecting small viral antigens or when only a limited quantity of antibody is available.

Procedure (for antigen detection):

1. **Add sample and labeled antigen**: The patient's sample and a known amount of enzyme-linked viral antigen are added to wells coated with a specific antibody.

2. **Competition for binding**: The unlabeled antigen from the patient sample and the enzyme-linked antigen compete for binding sites on the coated antibody.

3. **Add substrate and interpret results**: A weaker colour signal indicates a higher concentration of antigen in the sample, as more unlabeled antigen outcompeted the labeled antigen for binding.

Applications:

- **HIV antibody detection**: In some competitive ELISA kits for HIV antibodies and p24 antigens in blood. The labelled antibody competes with the patient's antibodies for the antigen coated on the well. The absence of colour indicates a positive result.

- **Dengue virus:** ELISA is used to detect both dengue virus antigens (e.g., NS1) and antibodies.

DIAGNOSIS OF PARASITIC DISEASES BY ELISA

For many tissue-invading parasites, ELISA is a valuable diagnostic tool, as detecting the parasite itself can be challenging. ELISA is typically used to find antibodies produced in response to a parasitic infection. Examples include:

- ***African trypanosomiasis (Sleeping Sickness):*** Caused by *Trypanosoma brucei*, can be detected by indirect ELISA method.

- ***Chagas disease (American trypanosomiasis):*** **Pathogen is** *Trypanosoma cruzi*. Indirect ELISA is used to detect IgG antibodies against the parasite in the patient's serum. Serology is essential for confirming a diagnosis, as the parasite can be difficult to find in the blood during the chronic phase.

- ***Amebiasis:*** Caused by *Entamoeba histolytica*.

- ***Cysticercosis:*** Caused by the larval stage of the pork tapeworm *Taenia solium* can be detected by indirect ELISA technique.

- ***Echinococcosis*** **(Hydatid disease)*:*** Caused by *Echinococcus* tapeworms. Indirect ELISA is used to detect IgG antibodies *E. granulosus* and *E. multilocularis*. The sensitivity of the test depends on the integrity of the hydatid cyst.

- ***Fascioliasis:*** A liver fluke infection.

- ***Filariasis:*** Caused by parasitic worms *Wuchereria bancrofti* and *Brugia malayi*. ELISA method includes both antigen and antibody detection. An IgG4 ELISA for specific filarial antigens can offer improved specificity over older methods.

- ***Giardiasis:*** Sandwich ELISA can detect *Giardia lamblia* antigens in stool samples of human, which is often more sensitive than traditional microscopy, particularly in low-grade infections.

- ***Cryptosporidiosis****:* Similar to Giardia, Sandwich ELISA can detect *Cryptosporidium* antigens in stool. Species includes *Cryptosporidium parvum* and *Cryptosporidium hominis*. Antigen detection ELISA is used to identify *Cryptosporidium* antigens in stool samples.The method provides a sensitive and rapid alternative to microscopy for diagnosing active infection, especially in cases of low-grade infection.

- ***Malaria:*** Rapid diagnostic tests (RDTs) for malaria often use a modified sandwich ELISA format to detect parasitic antigens, though these are typically not plate-based.

- ***Schistosomiasis (Bilharzia):*** Caused by blood flukes of the genus *Schistosoma*. ELISA method used is Indirect ELISA to detect antibodies, especially against soluble egg antigens (SEA). Urine-based ELISA is also used. Method is a valuable tool for diagnosing chronic infections or low-level infestations where eggs may be difficult to detect in stool or urine.

- ***Toxocariasis:*** Caused by the larvae of *Toxocara* roundworms. *Toxocara canis* or *Toxocara cati*. ELISA method includes Indirect ELISA that detects antibodies against *Toxocara* excretory-secretory (TES) antigens. Method is essential for diagnosing this infection, which can cause symptoms in various organs. A positive ELISA with TES antigen is often combined with clinical signs and eosinophilia for diagnosis.

- ***Toxoplasmosis:*** Caused by *Toxoplasma gondii* can be detected by applying indirect ELISA.

- ***Trichinellosis:*** Serological indirect ELISA is used to detect antibodies to a roundworm infection caused by *Trichinella spiralis*, especially in swine and humans.

- ***Visceral leishmaniasis****:* Dot-ELISA offers a sensitive and economical alternative to splenic aspiration for detecting antibodies against *Leishmania* parasites.

- ***Fascioliasis****:* Dot-ELISA can detect antibodies against liver flukes.

- ***Other field-based screenings****:* Dot-ELISA can be used for various protozoan and metazoan diseases where a rapid, qualitative result is needed.

- ***Neurocysticercosis***: Pathogen is the larval stage of the tapeworm *Taenia solium*. ELISA method includes Indirect ELISA to detect antibodies in the blood or cerebrospinal fluid (CSF). It is especially useful for diagnosing central nervous system infections, which are difficult to confirm through other methods.