WESTERN BLOTTING TECHNIQE FOR DISEASE DIAGNOSIS

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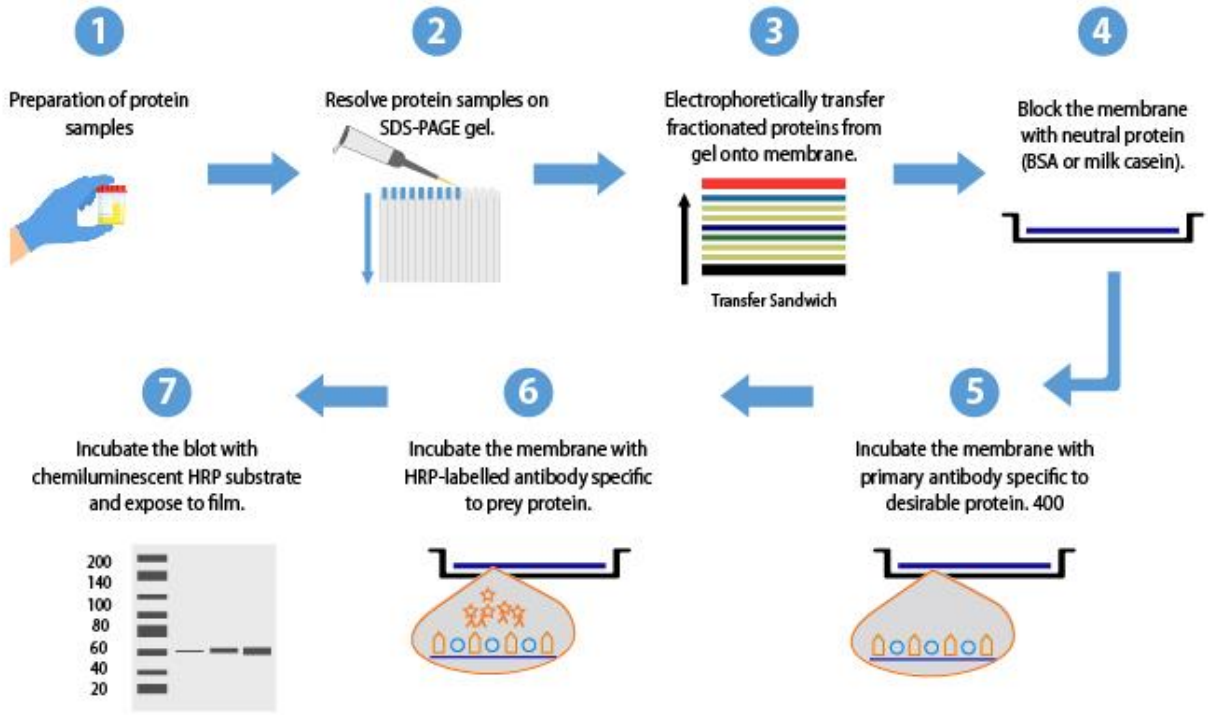
The Western blot technique is used to diagnose bacterial, viral, and parasitic diseases primarily as a **highly specific confirmatory test** following an initial screening with a more sensitive assay, such as ELISA. The technique detects specific antibodies produced by the patient's immune system in response to the infection, or sometimes the pathogen's antigens themselves, by identifying a characteristic pattern of protein bands.



General Diagnostic Principle

The Western blot procedure for disease diagnosis involves the following key steps:

1. **Protein Separation:** A complex mixture of proteins (antigens) from the suspected pathogen is separated by size using gel electrophoresis (SDS-PAGE).

2. **Transfer:** The separated proteins are transferred and immobilized onto a membrane (typically nitrocellulose or PVDF).

3. **Incubation with Patient Serum:** The membrane is incubated with the patient's serum. If the patient is infected, their specific antibodies will bind to the corresponding pathogen proteins on the membrane.

4. **Detection:** A secondary antibody, conjugated to an enzyme or fluorophore, is added. This secondary antibody binds to the patient's primary antibodies. The addition of a substrate then produces a visible signal (e.g., color or light) at the location of the target protein band(s).

5. **Analysis:** The resulting band pattern is compared to known positive and negative controls. The presence of specific bands at particular molecular weights confirms the presence of antibodies against the pathogen, thus confirming the diagnosis.

Applications in Specific Diseases

The Western blot is used in the diagnosis of various infectious diseases, including:

Disease Category	Example Disease(s)	Role of Western Blot	Specific Antigens Detected (Examples)
Viral	Human Immunodeficiency Virus (HIV)	Confirmatory test for HIV-1 and HIV-2 antibodies after a positive ELISA result. The CDC has recommended other rapid tests since 2014, but it is still used in some places.	Antibodies to viral proteins like gp41, gp120/160 (envelope), and p24 (core).
Bacterial	Lyme disease (Borreliosis)	Used as a more specific confirmatory test when initial screening (ELISA or IFA) is positive or equivocal.	Antibodies to *Borrelia burgdorferi* proteins (e.g., 43-44 kDa and 64 kDa bands for early diagnosis).
Parasitic	Visceral Leishmaniasis (VL)	Used to detect specific antibodies against *Leishmania infantum* antigens, helping to confirm the diagnosis.	Antibodies to immunodominant polypeptide fractions (e.g., 29 kDa, 51 kDa, and 62 kDa).
Parasitic	Trichinellosis	Useful for differential and early diagnosis to investigate cross-reactions from other parasitic infections.	Antibodies to specific *Trichinella* antigens (e.g., 43- to 44-kDa and 64-kDa bands).
Other	Bovine Spongiform Encephalopathy (BSE)	Recognized as a confirmatory test for this prion disease.	Anti-prion protein antibodies reacting with three distinctive bands.

Advantages and Limitations

- **Advantages:** High specificity due to the separation of individual proteins, which helps to reduce false positive results sometimes seen with less specific tests like ELISA. It also provides information on the molecular weight of the target protein(s) and can distinguish between different stages of infection or different but related pathogens based on banding patterns.

- **Limitations:** The procedure is time-consuming, labor-intensive, requires well-trained technical personnel, and is considered semi-quantitative at best. It can also produce indeterminate results, requiring further follow-up tests. The test may yield false negatives if

performed too early in the infection before the body has produced a sufficient amount of antibodies.

WESTERN BLOT AS A CONFIRMATORY TEST FOR DIAGNOSIS OF BACTERIAL DISEASE

Western blotting is used in the diagnosis of bacterial diseases primarily as a **confirmatory test** to verify the presence of specific antibodies produced by the patient's immune system in response to the infection. It is particularly valuable for its high specificity, which helps to minimize false positive results from initial, more sensitive screening tests like ELISA.

Mechanism of Use in Bacterial Diagnostics

The diagnostic process leverages the body's immune response and the core principles of the Western blot technique (separation by size and specific antibody binding).

1. **Antigen Preparation:** Specific proteins (antigens) from the target bacterium are prepared and separated by size using gel electrophoresis (SDS-PAGE).

2. **Transfer (Blotting):** The separated proteins are transferred from the gel onto a solid membrane (e.g., nitrocellulose or PVDF), where they become immobilized while maintaining their separation pattern.

3. **Patient Sample Incubation:** The membrane is incubated with the patient's serum, which is being tested for the presence of anti-bacterial antibodies. If the patient is infected, their antibodies will bind to the specific bacterial proteins on the membrane.

4. **Detection:** A secondary antibody, which is labeled with an enzyme or fluorophore and recognizes human antibodies, is added. This secondary antibody binds to any patient antibodies that are attached to the bacterial proteins.

5. **Visualization and Interpretation:** A substrate is added, which reacts with the label on the secondary antibody to produce a detectable signal (e.g., light or color). The pattern of bands is then analyzed. The presence of specific protein bands, corresponding to known bacterial antigens of a particular molecular weight, confirms exposure to the bacterium.

Advantages and Limitations in Diagnosis

- **Specificity:** The primary advantage is its high specificity, as it separates proteins by size before antibody detection, reducing false positives caused by cross-reactive antibodies that might occur in other tests.

- **Confirmatory Role:** Due to its specificity, it is considered the "gold standard" or a definitive confirmatory test in many diagnostic protocols.

- **Limitations:** The technique is time-consuming, labor-intensive, and requires experienced personnel to perform and interpret correctly. It may also produce false negatives in the very early stages of infection before the immune system has produced a sufficient amount of antibodies.

Key Applications in Diagnosis

Western blotting is widely used for:

- **Lyme Disease:** It is used as a confirmatory test after a positive or equivocal ELISA result to detect antibodies specific to *Borrelia burgdorferi* proteins (e.g., the 41 kDa flagellin, or 94 kDa, 31 kDa, and 21 kDa proteins for late-stage infection). Lyme disease is a multi-organ animal-borne disease, caused by spirochetes of Borrelia burgdorferi (Bb), which typically affect the skin, nervous system, musculoskeletal system and heart. A history of confirmed exposure to tick bites, typical signs and symptoms of Lyme borreliosis and positive tests for anti-Bb antibodies, are the basis of a diagnosis.
- **Syphilis:** Western blot is a useful adjunct or confirmatory test for *Treponema pallidum* infection, particularly for resolving questionable results from other serological tests. It provides a molecular characterization of the antibody response by visualizing characteristic banding patterns that are highly specific for syphilis.

- ***Helicobacter pylori* Infection:** While not a general practice, commercial Western blot assays, such as the Helico Blot 2.1, are used in some cases to detect specific IgG or IgA antibodies to *H. pylori* antigens (e.g., CagA, VacA, UreA, UreB proteins). This is especially useful for determining the presence of virulent *H. pylori* strains (like CagA-positive strains associated with gastric cancer) and for detecting past infections over several years.

- **Bartonellosis (e.g., Cat Scratch Disease and Endocarditis):** Western blot helps diagnose *Bartonella henselae* (cat scratch disease) and *Bartonella quintana* infections, particularly in cases of blood culture-negative endocarditis. It is useful for distinguishing between different *Bartonella* species infections and can be more sensitive than IFA for detecting specific IgM antibodies in the early stages of cat scratch disease.

- **Bovine Spongiform Encephalopathy (BSE - "Mad Cow Disease"):** In veterinary medicine, Western blot is recognized as a confirmatory test for BSE (a prion disease) by identifying characteristic bands produced by anti-prion protein antibodies.

- **Tuberculosis (Research/Specific Cases):** The technique has been used in research settings to study immune responses to specific *Mycobacterium tuberculosis* antigens, such as in the diagnosis of tuberculosis meningitis.

- **Other Bacterial Infections:** The technique has been used to detect specific antibodies for diseases such as tuberculosis meningitis (caused by *Mycobacterium tuberculosis*) and to assess the presence of virulence factors like cytolysin proteins from pathogenic bacterial strains such as *Enterococcus faecalis*.

- In all these cases, Western blotting provides a higher level of specificity than general antibody tests because it separates the bacterial proteins first, ensuring that the detected antibodies are binding to specific, identifying antigens rather than just general bacterial components that might cause cross-reactivity.

WESTERN BLOT APPLICATION FOR DIAGNOSIS OF VIRAL DISEASES:

Western blotting is used in the diagnosis of viral diseases primarily as a **highly specific confirmatory test** for antibodies produced by the patient's immune system in response to the infection, or sometimes to detect viral antigens directly. Its main advantage is its ability to differentiate between antibodies to various viral proteins, minimizing false positives from less specific screening tests.

Mechanism of Use in Viral Diagnostics

The method for viral diagnostics mirrors that of bacterial diagnostics, but uses viral components:

1. **Antigen Preparation:** Specific proteins from the target virus are separated by size using gel electrophoresis.
2. **Transfer (Blotting):** The separated viral proteins are transferred onto a membrane.
3. **Patient Sample Incubation:** The patient's serum is added. If the patient has been infected, their antibodies will bind to specific viral proteins on the membrane.
4. **Detection and Visualization:** A labeled secondary antibody is used to detect the patient's bound antibodies, producing a visual signal that confirms the presence of an antibody response to specific viral components.
5. **Interpretation:** The pattern of bands is compared to known positive controls. The presence of specific bands confirms exposure to the virus.

EXAMPLES:

a. As a confirmatory test for HIV infection

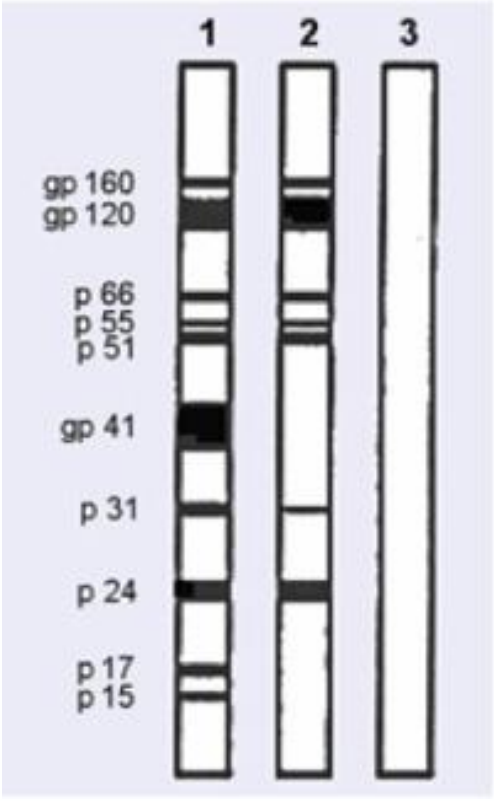
Human Immunodeficiency Virus (HIV) can be divided into two major types, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV-1 is related to viruses found in chimpanzees and gorillas living in western Africa. HIV-2 is related to viruses found in sooty mangabeys. HIV-1 viruses may be further divided into groups. The HIV-1 group M viruses predominate and are responsible for the AIDS pandemic.

Western Blot is the benchmark test for confirming a diagnosed HIV infection followed by the ELISA test. However, since 2014, CDC have recommended discontinuing the Western blot test for HIV, as other tests are now more reliable and enable a faster diagnosis.

In the Western blot test, the sample is separated with an electrical current and transferred onto a piece of blotting paper. And an enzyme is added to cause color changes that signal the presence of HIV antibodies.

Western blot HIV tests usually look for antibodies against the following HIV proteins:

- Proteins from the HIV envelope: gp41, and gp120/gp160.
- Proteins from the core of the virus: p17, p24, p55
- Enzymes that HIV uses in the process of infection: p31, p51, p66



A drawing of results of a western blot test #1: a control; #2: positive; #3: negative.

b. Western Blot application: as a confirmatory test for FIV infection

Feline immunodeficiency virus (FIV) is a lentivirus that causes an immunodeficiency syndrome in cats and is structurally similar to the human immunodeficiency virus (HIV).

Diagnosis of FIV requires an FIV antibody test. ELISA (enzyme-linked immunosorbent assay) is a simple test that can be done in most veterinary laboratories and clinics. Test results are available within 10 to 20 minutes. False positives can occur, so all cats that test positive should be retested with a Western blot assay. The Western blot detects the presence of FIV-antibodies in the cat's blood using slightly different technology. The Western Blot is considered the confirmatory test for FIV. If the western blot result shows positive, consider FIV-infected and continue appropriate management program and/or treatments. If the western blot shows a discrepant negative result, then the FIV infection may be in early stage or initial ELISA results may be incorrect.

c. **Hepatitis C Virus (HCV):** It is used as a supplementary or confirmatory test for individuals testing positive by antibody screening tests. Commercial "recombinant immunoblot assays" (RIBAs) use the Western blot principle to identify antibodies against multiple specific HCV antigens, increasing diagnostic specificity.

d. **Human T-lymphotropic Virus (HTLV) Types I and II:** Western blotting is the standard confirmatory test to distinguish between HTLV-I and HTLV-II infections after a positive screening result, detecting antibodies against specific viral core (gag, e.g., p19, p24) and envelope (env, e.g., gp21, gp46) proteins.

e. **Epstein-Barr Virus (EBV):** Western blot is sometimes used in specific diagnostic contexts to confirm or clarify the stage of EBV infection by detecting antibodies to specific viral capsid antigen (VCA), early antigen (EA), and nuclear antigen (EBNA) proteins.

f. **Human T-lymphotropic virus (HTLV):** The test is used to confirm infection with HTLV-I and HTLV-II by identifying antibodies against specific viral proteins.

WESTERN BLOTTING FOR DIAGNOSIS OF PARASITIC DISEASES

Western blotting is used in the diagnosis of parasitic diseases primarily to **confirm the presence of specific antibodies** against the parasite's antigens, providing a highly specific and reliable diagnostic method, especially in cases where clinical symptoms are non-specific or direct parasite detection is difficult. It is typically used as a confirmatory test after a positive result from an initial screening test.

Mechanism of Use in Parasitic Diagnostics

The application of Western blot for parasitic diseases involves the following general steps:

1. **Parasite Antigen Preparation:** Specific protein antigens are extracted from the target parasite.
2. **Electrophoresis and Blotting:** These antigens are separated by size via gel electrophoresis and transferred onto a membrane.
3. **Patient Antibody Detection:** The membrane is incubated with the patient's serum. Specific anti-parasite antibodies in the serum will bind to the corresponding antigens on the membrane.
4. **Confirmation and Interpretation:** A labeled secondary antibody is used to detect the bound antibodies, producing a characteristic band pattern that confirms exposure to the parasite.

Western blotting is used in the diagnosis of parasitic diseases primarily to **confirm the presence of specific antibodies** against a parasite's unique protein components. This method is crucial for its high specificity, which helps to minimize cross-reactivity issues common in other serological tests (like ELISA or IFA) that use crude parasite antigens.

Specific Examples of Diagnosis by Western Blotting

Western blotting is particularly valuable for parasitic infections where the immune response can be complex or where cross-reactions with other parasites are an issue:

- **Chagas Disease (caused by *Trypanosoma cruzi*):** Western blot is used as a confirmatory test, particularly in regions where it coexists with Leishmaniasis. It helps identify a specific, homogeneous band pattern (e.g., 28, 32, 38, 39, 40, and 48 kDa antigens) that is distinct from the patterns seen in other infections, thus improving specificity.
- **Toxocariasis (caused by *Toxocara canis* larvae):** Western blot with excretory-secretory (TES) antigens is more specific than ELISA and is recommended by health authorities like the CDC for confirmation. It helps identify specific low-molecular-weight fractions (e.g., 92 kDa and 35 kDa antigens) which are less likely to cross-react with antibodies from other helminthic infections.
- **Cysticercosis and Echinococcosis (Hydatid Disease):** Western blot is used in the diagnosis of these helminthic infections. For human hydatid cysts, high sensitivity and specificity have

been reported using antigens from hydatid fluid, with a specific 20 kDa band being a good candidate for diagnosis.

- **Trichinellosis (caused by *Trichinella* species):** Western blot helps to confirm diagnosis, especially in cases with ambiguous ELISA or immunofluorescence results. It can detect specific antibody bands (e.g., 43-44 kDa and 64 kDa) earlier in the course of infection than some other methods.

- **Visceral Leishmaniasis:** Western blot analysis of whole parasite antigens has been considered sensitive, especially when low serum antibody titers are present.

- **Amoebiasis:** Western blot has been utilized to detect antibodies to the parasite *Entamoeba histolytica*, especially in patients with extraintestinal manifestations like amoebic liver abscesses, where direct parasite detection in stool is difficult.

- **Onchocerciasis (River Blindness):** This is another key application. Western blot is used to confirm the diagnosis by detecting antibodies against specific larval antigens of the parasite *Onchocerca volvulus*, particularly the 14kDa, 16kDa, and 22kDa proteins. This is especially useful for early or light infections where microfilariae might not be easily detected in skin snips.

- **Paragonimiasis:** Western blot is highly sensitive and specific for detecting antibodies to *Paragonimus* (lung fluke) antigens in patient serum or pleural fluid, helping in the diagnosis of pulmonary paragonimiasis. The use of specific antigens like the 8 kDa protein improves diagnostic accuracy.

- **Fascioliasis:** Western blotting can be used to diagnose infections caused by *Fasciola hepatica* (liver fluke). It helps in the detection of antibodies to specific antigens, such as the 8 kDa, 12 kDa, 17 kDa, and 27 kDa components, which can differentiate *Fasciola* infection from other trematode infections.

- **Schistosomiasis (*mansoni* and *haematobium*):** While other methods are more common, Western blot can be used to detect specific antibodies to *Schistosoma* antigens (e.g., the 31/32 kDa protein band for *S. mansoni*), especially in low-endemic areas or for confirming chronic infections.

- **Toxoplasmosis:** While the primary diagnostic tools for *Toxoplasma gondii* infection are other serological tests (like ELISA, IFA, or dye test), Western blot can be employed in complex cases to confirm the presence of specific IgG antibodies and differentiate between acute and chronic infections by assessing the antibody profiles against different antigens.

Protozoan Infections

- **Chagas Disease (American Trypanosomiasis):** Caused by *Trypanosoma cruzi*, Western blot is an efficient method to confirm diagnosis and distinguish it from other Leishmania

infections by identifying a specific, homogeneous pattern of antigenic bands (e.g., 28, 32, 38, 39, 40, and 48 kDa).

- **Visceral Leishmaniasis:** Western blot is used in complex cases to detect antibodies against specific *Leishmania infantum* antigens (e.g., 29, 51, and 62 kDa fractions were found to have high frequency in positive sera). It is particularly useful for detecting specific immunodominant antigens when low antibody titers are present or for confirming diagnosis in immunocompromised patients (e.g., HIV/VL coinfection).

- **Toxoplasmosis (Congenital):** In cases of suspected congenital toxoplasmosis, Western blot is considered a valuable tool to differentiate between a mother's passively transferred antibodies and those actively produced by the newborn infant, which is crucial for early diagnosis and treatment.

- **Amoebiasis:** Western blot is used to detect antibodies to the invasive parasite *Entamoeba histolytica*, especially in cases of extraintestinal amoebiasis like liver abscesses where the parasite may not be present in stool samples.

- **Malaria (Research/Specific Cases):** While blood smears are the primary diagnostic tool, Western blot has been explored in research to diagnose *Plasmodium vivax* and *P. falciparum* infections, particularly in cases with very low parasitemia or long incubation periods, by using multiple stage-specific antigens (e.g., CSP-1, MSP-1, AMA-1).

Helminthic Infections (Worms)

- **Cysticercosis (Neurocysticercosis):** A highly specific Western blot test using specific larval glycoprotein antigens is the diagnostic test of choice recommended by the CDC for confirming infection of the brain tissue by the pork tapeworm larvae (*Taenia solium*).

- **Echinococcosis (Hydatid Disease):** Western blot helps confirm this tapeworm infection (*Echinococcus granulosus* or *E. multilocularis*) by detecting specific antibodies to antigens like Antigen 5 and Antigen B, aiding in the differentiation of hydatid cysts from other lesions.

- **Fascioliasis (Liver Flukes):** The American CDC recommends immunoblot for the confirmation of serodiagnosis of fascioliasis (*Fasciola hepatica* or *F. gigantica*). The test often targets specific immunodominant antigens around 17 kDa, 23 kDa, and 27 kDa to provide high sensitivity and 100% specificity.

- **Paragonimiasis (Lung Flukes):** Western blot with specific antigens from *Paragonimus westermani* or *P. kellicotti* is used to diagnose lung fluke infections. A specific 8 kDa band has high sensitivity (96%) and specificity (99%) for diagnosis of *P. westermani*, and a 21/23 kDa doublet is key for *P. kellicotti*.

- **Strongyloidiasis:** Western blot is a highly sensitive and specific serological method used for the diagnosis of chronic *Strongyloides stercoralis* infection, which can be difficult to diagnose via microscopy alone.
- **Onchocerciasis (River Blindness):** Western blot for IgG4 antibodies using the specific Ov20/OvS1 protein as an antigen is a sensitive and highly specific method for the diagnosis of this filarial infection, and can help differentiate it from other filarial infections.