Schick's Test

Overview:

- Schick's Test is a **diagnostic skin test** used to determine an individual's **susceptibility or immunity** to **diphtheria**, a serious bacterial infection caused by *Corynebacterium diphtheriae*.

Historical Context:

- Developed by **Dr. Béla Schick** in 1913, it was widely used before the advent of widespread diphtheria vaccination to assess the need for immunization.

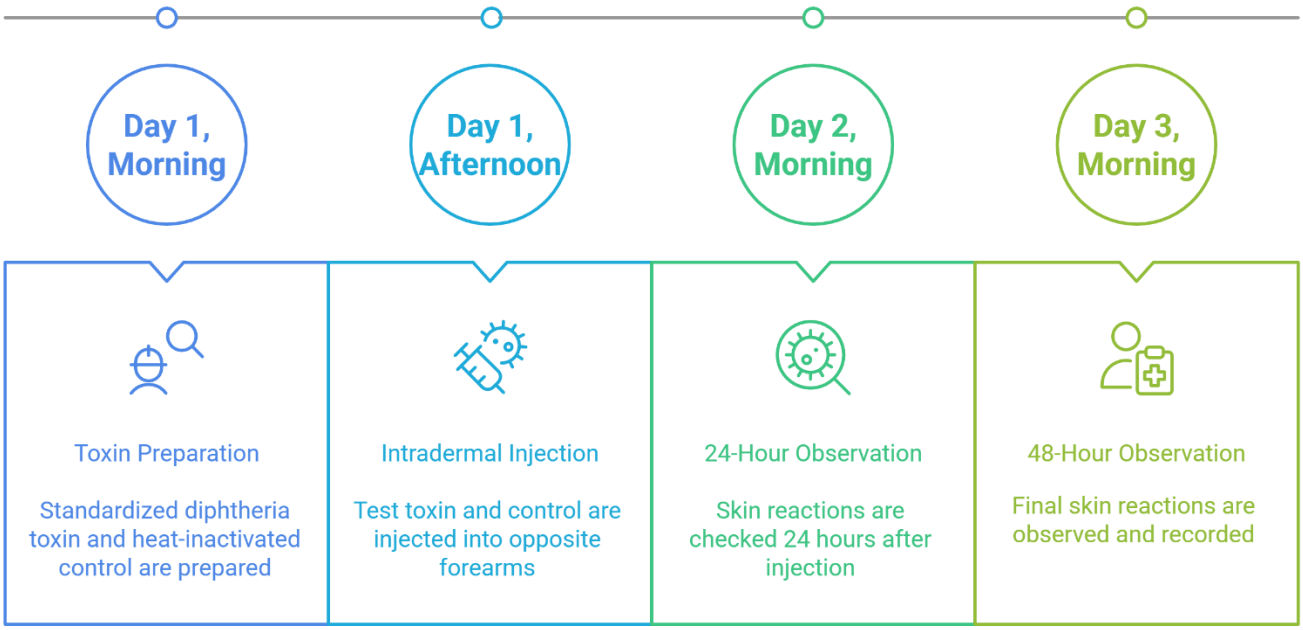
Purpose:

- To assess if a person has **protective immunity** (antibodies) against diphtheria toxin.

Principle:

- The test is based on an intradermal injection of a small amount of diphtheria toxin.
- If the person lacks antibodies to neutralize the toxin, a localized inflammatory reaction occurs.
- If the person has antibodies, they will neutralize the toxin and no reaction occurs.

Procedure:



1. Preparation of Test Toxin:

- A small amount (0.1 mL) of **diphtheria toxin** (standardized dose) is prepared.
- In the **opposite forearm**, a **heat-inactivated toxin (control)** is prepared in an identical volume.

2. Intradermal Injection:

- The **test toxin** is injected intradermally into the skin of one forearm.
- The **control (inactivated) toxin** is injected into the other forearm.

3. Observation Period:

- Skin reactions are observed for **48 hours**.

Interpretation:

1. Positive Schick Test (Susceptible):

- A **red, raised area** appears at the test site (not the control).
- Indicates the absence of protective antibodies (individual is susceptible to diphtheria).

2. Negative Schick Test (Immune):

- **No reaction** at the test site.
- Indicates the presence of circulating antibodies (immunity to diphtheria).

3. Pseudo-positive Reaction:

- Similar reaction occurs at **both sites** due to **hypersensitivity** to the protein or preservatives.
- Not due to toxin activity.

4. Pseudo-negative Reaction:

- Occurs when the person is immune but the toxin used is **not active** (faulty reagent).

Clinical Relevance:

- Rarely used now due to widespread vaccination (DPT vaccine) and serologic antibody testing (ELISA).
- Historically important in public health to assess immunity in populations.

ELISA Test (Enzyme-Linked Immunosorbent Assay)

Overview:

- ELISA is a plate-based immunoassay used to detect and quantify antibodies, antigens, proteins, and glycoproteins in biological samples such as blood, urine, or saliva.

Applications:

- Diagnosis of **infectious diseases**: HIV, Hepatitis B & C, COVID-19.
- Detection of hormones, tumor markers, allergens, and cytokines.
- Used in vaccine development and serosurveillance.

Principle:

- Relies on antigen-antibody interaction.

- The enzyme linked to an antibody or antigen acts on a **substrate** to produce a **color change**, which is measured spectrophotometrically.
- The color intensity is **proportional** to the amount of antigen or antibody present.

Types of ELISA:



1. Direct ELISA:

- Antigen is immobilized on a plate.
- A labeled antibody directly binds to the antigen.

2. Indirect ELISA:

- Antigen is immobilized.
- First, a **primary antibody** binds to the antigen.
- Then, a **secondary enzyme-linked antibody** binds to the primary antibody.

3. Sandwich ELISA:

- A **capture antibody** is immobilized on the plate.
- The **antigen** from the sample binds to it.
- A **detection antibody** (linked to enzyme) binds to the antigen.
- Used when antigen concentration is very low.

4. Competitive ELISA:

- Sample antigen competes with labeled antigen for antibody binding.
- Inverse relationship between color intensity and antigen concentration.

Procedure (Indirect ELISA Example):

1. **Coating**: Wells of microtiter plate are coated with known antigen.
2. **Blocking**: Non-specific binding sites are blocked (e.g., with BSA).
3. **Incubation with Sample**: Add serum from patient. If antibodies are present, they bind to the antigen.
4. **Addition of Secondary Antibody**: Enzyme-labeled anti-human antibody is added.
5. **Substrate Addition**: Enzyme substrate is added. Enzyme converts substrate to colored product.

6. **Measurement**: Color intensity is measured using a **spectrophotometer (ELISA reader)**. Intensity ∝ amount of antibody in sample.

Enzymes Commonly Used:

- **Horseradish Peroxidase (HRP)**
- **Alkaline Phosphatase (AP)**

Substrates:

- **TMB (3,3',5,5'-Tetramethylbenzidine)** – for HRP.
- **PNPP (p-Nitrophenyl phosphate)** – for AP.

Common Uses:

- Detection of HIV antibodies (HIV ELISA test).
- Detection of Hepatitis B surface antigen (HBsAg).
- Measurement of hormones **(e.g., hCG in pregnancy tests)**.
- Identification of allergens, cytokines, tumor markers, etc.

Advantages:

- High specificity and sensitivity.
- Suitable for large-scale screening.
- Can be automated.
- Quantitative or qualitative analysis.

Limitations:

- Requires multiple steps, increasing time.
- Possibility of false positives/negatives if cross-reactivity or poor washing.
- Requires careful standardization.

Western Blot Test

Overview:

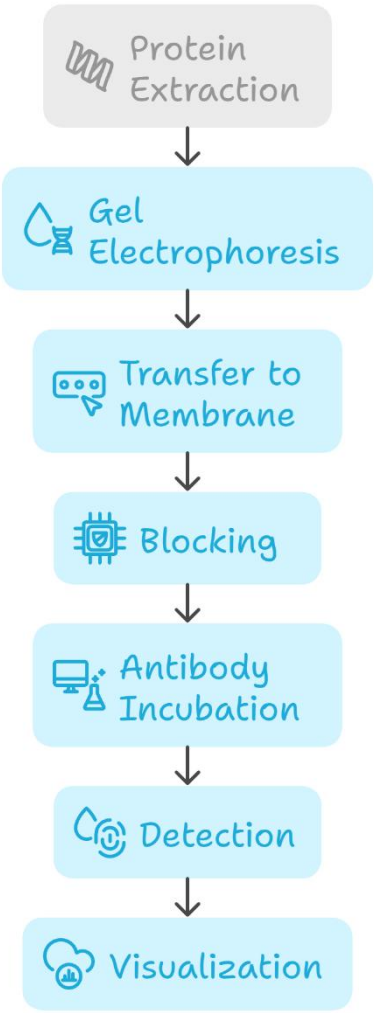
- The **Western Blot** (also called **immunoblotting**) is a laboratory method used to detect specific proteins in a sample.
- It combines gel electrophoresis and antibody-based detection, making it highly specific and confirmatory.
- It is commonly used as a confirmatory test for HIV, Lyme disease, and other infections or protein abnormalities.

Principle:

- Western blot works on two key steps:
    1. **Separation of proteins** by size using **SDS-PAGE** (sodium dodecyl sulfate–polyacrylamide gel electrophoresis).
    2. Detection of a specific protein using antibodies that bind specifically to the protein of interest, visualized using an enzyme-substrate reaction or chemiluminescence.

Step-by-Step Procedure:





1. Protein Extraction:

- Proteins are extracted from cells or tissue (e.g., viral proteins, cellular lysates).
- A detergent (like SDS) is used to denature the proteins and impart a uniform negative charge.

2. Gel Electrophoresis (SDS-PAGE):

- Proteins are loaded onto a polyacrylamide gel.
- An electric field causes proteins to migrate:
    - **Smaller proteins move faster**, separating proteins based on **molecular weight**.

3. Transfer (Blotting) to Membrane:

- The gel is placed against a nitrocellulose or PVDF membrane.
- Electric current is applied in a perpendicular direction to transfer proteins from the gel to the membrane.

4. Blocking:

- The membrane is incubated with a blocking solution (e.g., BSA or milk proteins) to prevent non-specific antibody binding.

5. Antibody Incubation:

- **Primary antibody**: Specifically binds to the target protein.
- **Secondary antibody**: Binds to the primary antibody and is **linked to an enzyme** (e.g., horseradish peroxidase).

6. Detection:

- A **substrate** (e.g., chemiluminescent or colorimetric) is added.

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