CHAPTER-VIII
DIAGNOSTIC TESTS

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SCHICK’S TEST:-

- The Schick test, invented between 1910 and 1911 is a test used to determine whether or not a person is susceptible to diphtheria. It was named after its inventor, Béla Schick (1877–1967), a Hungarian-born American paediatrician.

- The test is a simple procedure. A small amount (0.1 ml) of diluted (1/50 MLD) diphtheria toxin is injected intra dermally into the arm of the person. If a person does not have enough antibodies to fight it off, the skin around the injection will become red and swollen, indicating a positive result. This swelling disappears after a few days. If the person has an immunity, then little or no swelling and redness will occur, indicating a negative result.
Results can be interpreted as
Positive: when the test results in a wheal of 5–10 mm diameter
Pseudo-positive: when there is only a red colour inflammation and it disappears rapidly

Negative reaction:
pseudo negative reaction:
The test was created when immunizing agents were scarce and not very safe, however as newer and safer toxoids were made available there was no more requirement for susceptibility tests
ELISA TEST:

- Enzyme-linked immunosorbent assay (ELISA), is a popular format of a "wet-lab" type analytic biochemistry assay that uses one sub-type of heterogeneous, solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample.

- The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. Attempting to detect (and quantify) the presence of the antigen in the sample proceeds as follows: Antigens from the sample are attached to a surface. Then, a further specific antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate.
Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bio conjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.
TYPES

Indirect ELISA

Competitive ELISA

Sandwich ELISA
**Virus Sample on Surface**

- Antibody with enzyme conjugate attached to viral antigen

- Substrate and enzyme interaction create color change for detection
A sandwich ELISA. (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form
A descriptive animation of the application of sandwich ELISA to HIV can be found.
Human anti-IgG, double antibody sandwich ELISA
Applications

Human anti-IgG, double antibody sandwich ELISA

Because the ELISA can be performed to evaluate either the presence of antigen or the presence of antibody in a sample, it is a useful tool for determining serum antibody concentrations (such as with the HIV test or West Nile virus). It has also found applications in the food industry in detecting potential food allergens, such as milk, peanuts, walnuts, almonds, and eggs. ELISA can also be used in toxicology as a rapid presumptive screen for certain classes of drugs.

Enzyme-linked immunosorbent assay plate
Enzyme-linked immunosorbent assay plate
WESTERN BLOT TEST AND SOUTHERN BLOT TEST

The method originated in the laboratory of George Stark at Stanford. The name *Western blot* was given to the technique by W. Neal Brunette and is a play on the name Southern blot, a technique for DNA detection developed earlier by Edwin Southern. Detection of RNA is termed northern blot.
Steps in a western blot

Tissue preparation

- Samples can be taken from whole tissue or from cell culture. Solid tissues are first broken down mechanically using a blender (for larger sample volumes), using a homogenizer (smaller volumes), or by sonication. Cells may also be broken open by one of the above mechanical methods. However, virus or environmental samples can be the source of protein and thus western blotting is not restricted to cellular studies only.

- Assorted detergents, salts, and buffers may be employed to encourage lysis of cells and to solubilize proteins. Protease and phosphatase inhibitors are often added to prevent the digestion of the sample by its own enzymes. Tissue preparation is often done at cold temperatures to avoid protein denaturation and degradation.

- A combination of biochemical and mechanical techniques – comprising various types of filtration and centrifugation – can be used to separate different cell compartments and organelles.
GEL ELECTROPHORESIS

The proteins of the sample are separated using gel electrophoresis. Separation of proteins may be by isoelectric point (pI), molecular weight, electric charge, or a combination of these factors. The nature of the separation depends on the treatment of the sample and the nature of the gel. This is a very useful way to identify a protein.

By far the most common type of gel electrophoresis employs polyacrylamide gels and buffers loaded with sodium dodecyl sulfate (SDS). SDS-PAGE (SDS polyacrylamide gel electrophoresis) maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure (e.g. disulfide bonds [S-S] to sulfhydryl groups [SH and SH]) and thus allows separation of proteins by their molecular weight. Sampled proteins become covered in the negatively charged SDS and move to the positively charged electrode through the acrylamide mesh of the gel. Smaller proteins migrate faster through this mesh and the proteins are thus separated according to size (usually measured in kilodaltons, kDa). The concentration of acrylamide determines the resolution of the gel - the greater the acrylamide concentration the better the resolution of lower molecular weight proteins. The lower the acrylamide concentration the better the resolution of higher molecular weight proteins. Proteins travel only in one dimension along the gel for most blots.

Samples are loaded into wells in the gel. One lane is usually reserved for a marker or ladder, a commercially available mixture of proteins having defined molecular weights, typically stained so as to form visible, coloured bands. When voltage is applied along the gel, proteins migrate through it at different speeds dependent on their size. These different rates of advancement (different electrophoretic mobilities) separate into bands within each lane.
Transfer

In order to make the proteins accessible to antibody detection, they are moved from within the gel onto a membrane made of PVDF or another membrane ( ). The primary method for transferring the proteins is called _______ and

and a stack of filter papers on top of that. The entire stack is placed in a buffer solution which moves up the paper by _______
*If proteins are hydrophobic, use PVDF membrane instead.
Western blot using radioactive detection system

Analysis

After the unbound probes are washed away, the western blot is ready for detection of the probes that are labeled and bound to the protein of interest. In practical terms, not all westerns reveal protein only at one band in a membrane. Size approximations are taken by comparing the stained bands to that of the marker or ladder loaded during electrophoresis. The process is repeated for a structural protein, such as actin or tubulin, that should not change between samples. The amount of target protein is normalized to the structural protein to control between groups. This practice ensures correction for the amount of total protein on the membrane in case of errors or incomplete transfers.

Colorimetric detection :-

The colorimetric detection method depends on incubation of the western blot with a substrate that reacts with the reporter enzyme (such as peroxidase) that is bound to the secondary antibody. This converts the soluble dye into an insoluble form of a different color that precipitates next to the enzyme and thereby stains the membrane. Development of the blot is then stopped by washing away the soluble dye. Protein levels are evaluated through densitometry (how intense the stain is) or spectrophotometry.
**Chemiluminescent detection**:

Chemiluminescent detection methods depend on incubation of the western blot with a substrate that will luminance when exposed to the reporter on the secondary antibody. The light is then detected by photographic film, and more recently by CCD cameras which capture a digital image of the western blot. The image is analysed by densitometry, which evaluates the relative amount of protein staining and quantifies the results in terms of optical density. Newer software allows further data analysis such as molecular weight analysis if appropriate standards are used.
WIDAL TEST

Test whereby bacteria causing typhoid fever are mixed with serum containing specific antibodies obtained from an infected individual. It is a presumptive serological test for enteric fever or undulant fever. In case of *Salmonella* infections, it is a demonstration of the presence of O-soma false-positive result. Test results need to be interpreted carefully in the light of past history of enteric fever, typhoid vaccination, and the general level of antibodies in the populations in endemic areas of the world. Typhidot is the other test used to ascertain the diagnosis of typhoid fever. As with all serological tests, the rise in antibody levels needed to perform the diagnosis takes 7–14 days, which limits its applicability in early diagnosis. Other means of diagnosing *Salmonella typhi* (and *paratyphi*) include cultures of blood, urine and faeces. These organisms produce H$_2$S from thiosulfate and can be easily identified on differential media such as Bismuth sulfite agar.
Often 2-mercaptoethanol is added to the Widal test. This agent more easily denatures the IgM class of antibodies, so if a decrease in the titer is seen after using this agent, it means that the contribution of IgM has been removed leaving the IgG component. This differentiation of antibody classes is important; as it allows for the distinction of a recent (IgM) from an old infection (IgG).

The Widal test is positive if TO antigen titer is more than 1:160 in an active infection, or if TH antigen titer is more than 1:160 in past infection or in immunized persons. A single Widal test is of little clinical relevance due to the number of cross reacting infections, including malaria. If no other tests (either bacteriologic culture or more specific serology) are available, a fourfold increase in the titer (e.g., from 1:40 to 1:160) in the course of the infection, or a conversion from an IgM reaction to an IgG reaction of at least the same titer, would be consistent with a typhoid infection.
The **buffy coat** is the fraction of an anticoagulated blood sample that contains most of the white blood cells and platelets following density gradient centrifugation of the blood.

**Description**

After centrifugation, one can distinguish a layer of clear fluid (the plasma), a layer of red fluid containing most of the red blood cells, and a thin layer in between. Making up less than 1% of the total volume of the blood sample, the buffy coat (so-called because it is usually buff in hue), contains most of the white blood cells and platelets. The buffy coat is used, for example, to extract DNA from the blood of mammals (since mammalian red blood cells are anucleate and do not contain DNA).

The buffy coat is usually whitish in color, but is sometimes green if the blood sample contains large amounts of neutrophils (which are high in green myeloperoxidase). The layer next to buffy coat contains granulocytes and red blood cells.
The QBC Test, developed by Becton and Dickenson Inc., is a new method for identifying the malarial parasite in the peripheral blood. It involves staining of the centrifuged and compressed red cell layer with acridine orange and its examination under UV light source. It is fast, easy and claimed to be more sensitive than the traditional thick smear examination.

**Method:** The QBC tube is a high-precision glass hematocrit tube, pre-coated internally with acridine orange stain and potassium oxalate. It is filled with 55-65 microliters of blood from a finger, ear or heel puncture. A clear plastic closure is then attached. A precisely made cylindrical float, designed to be suspended in the packed red blood cells, is inserted. The tube is centrifuged at 12,000 rpm for 5 minutes. The components of the buffy coat separate according to their densities, forming discrete bands. Because the float occupies 90% of the internal lumen of the tube, the leukocyte and the thrombocyte cell band widths and the top-most area of red cells are enlarged to 10 times normal. The QBC tube is placed on the tube holder and examined using a standard white light microscope equipped with the UV microscope adapter, an epi-illuminated microscope objective. Fluorescing parasites are then observed at the red blood cell/white blood cell interface. The key feature of the method is centrifugation and thereby concentration of the red blood cells in a predictable area of the QBC tube, making detection easy and fast. Red cells containing Plasmodia are less dense than normal ones and concentrate just below the leukocytes, at the top of the erythrocyte column. The float forces all the surrounding red cells into the 40 micron space between its outside circumference and the inside of
Precision-Bore Capillary

Plasma

Precision Plastic Float

Platelet Layer

Gametocytes of *P. falciparum*

Lymphocyte/Monocyte Layer

Mature Asexual Forms

Eosinophils (in some species)

Granulocyte Layer

Thin Layer of Red Blood Cells

Region in which parasitized cells are concentrated

Thick Layer of Red Blood Cells
MANTAOUX PERIPHERAL SMEAR TEST

- A positive Mantoux test (MT) or tuberculin skin test (TST) indicates infection with
- Mycobacterium tuberculosis and the test may thus be used to support the clinical diagnosis of active TB. However, MT maybe negative in patients with active TB and positive as a consequence of exposure to mycobacteria other than
- M. tuberculosis and BCG vaccination and the current indication for MT is solely for detection of latent TB. ESR is a non-specific marker of inflammation and is elevated in a number of infectious and non-infectious conditions. Before using MT (with or without ESR) as a diagnostic test, it is important to assess how the test performs in patients with confirmed diagnosis of tuberculosis and in healthy individuals. In this study, we sought to address this question and studied the utility of Mantoux test in the diagnosis of active tuberculosis in a BCG-vaccinated, TB
The diagnosis of TB is made on the basis of laboratory test results. The standard test for tuberculosis—which is the so-called tuberculin skin test—detects the presence of infection, not of active TB. Tuberculin is an extract prepared from cultures of *M. tuberculosis*. It contains substances belonging to the bacillus (antigens) to which an infected person has been sensitized. When tuberculin is injected into the skin of an infected person, the area around the injection becomes hard, swollen, and red within one to three days. Today skin tests utilize a substance called purified protein derivative (PPD) that has a standard chemical composition and is therefore is a good measure of the presence of tubercular infection. The PPD test is also called the Mantoux test. The Mantoux PPD skin test is not, however, 100% accurate; it can produce false positive as well as false negative results. What these terms mean is that some people who have a skin reaction are not infected (false positive) and that some who do not react are in fact infected (false negative). The PPD test is, however, useful as a screener. Anyone who has suspicious findings on a chest x ray, or any condition that makes TB more likely should have a PPD test. In addition, those in close contact with a TB patient and persons who come from a country where TB is common also should be tested, as should all healthcare personnel and those living in crowded conditions or institutions.
**MALARIAL PARASITE**

- *Plasmodium* is a genus of Apicomplexan parasites. Infection by these organisms is known as malaria. The genus *Plasmodium* was described in 1885 by Ettore Marchiafava and Angelo Celli. Currently over 200 species of this genus are recognized and new species continue to be described.

- Of the over 200 known species of *Plasmodium*, at least 11 species infect humans. Other species infect other animals, including monkeys, rodents, birds, and reptiles. The parasite always has two hosts in its life cycle: a vector—usually a mosquito—and a vertebrate host.

- Life cycle

- The life cycle of *Plasmodium* while complex is similar to several other species in the Haemosporidia.
All the *Plasmodium* species causing malaria in humans are transmitted by mosquito species of the genus *Anopheles*. Species of the mosquito genera *Aedes*, *Culex*, *Culiseta*, *Mansonía* and *Theobaldia* can also transmit malaria but not to humans. Bird malaria is commonly carried by species belonging to the genus *Culex*. The life cycle of *Plasmodium* was discovered by Ross who worked with species from the genus *Culex*.

Both sexes of mosquitoes live on nectar. Because nectar's protein content alone is insufficient for oogenesis (egg production) one or more blood meals is needed by the female. Only female mosquitoes bite. Sporozoites from the saliva of a biting female mosquito are transmitted to either the blood or the lymphatic system of the recipient. It has been known for some time now that the parasites block the salivary ducts of the mosquito and as a consequence the insect normally requires multiple attempts to obtain blood. The reason for this has not been clear. It is now known that the multiple attempts by the mosquito may contribute to immunological tolerance of the parasite. The majority of sporozoites appear to be injected into the subcutaneous tissue from which they migrate into the capillaries. A proportion are ingested by macrophages and still others are taken up by the lymphatic system where they are presumably destroyed. ~10% of the parasites inoculated by the mosquitoes may remain in the skin where they may develop into infective merozoites.