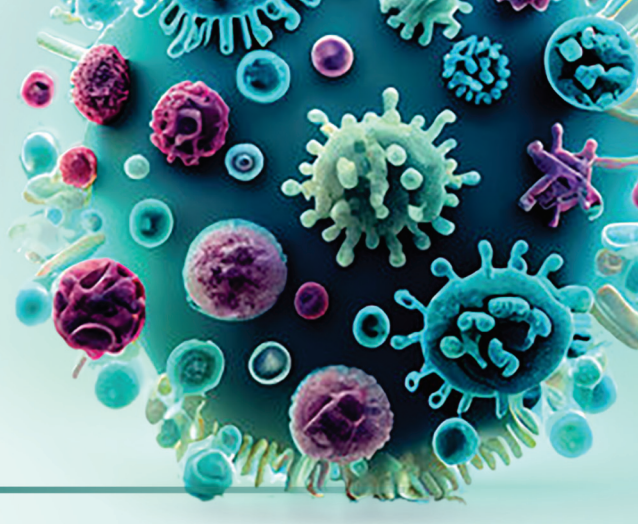


Section

1

General Microbiology Practicals

1. Rules and Safety Precautions to be Observed in the Microbiology Laboratory
2. GLP, Biohazard, Biosafety Levels and Biosafety Cabinets
3. Universal Presence of Microbes
4. Microscope: General Instructions about Use and Care
5. Microscopy and Micrometry
6. Methods of Sterilization and Disinfection
7. Biomedical Waste Disposal
8. Table of Commonly Used Culture Media in Microbiology and their Uses
9. Demonstration of Common Culture Media and Biochemical Reactions
10. Culture Methods
11. Description of the Appearance of Growth on Solid or in Liquid Media
12. Demonstration of Methods of Antimicrobial Susceptibility Testing
13. Miscellaneous Arthropods of Medical Importance (Including Tables)
14. Demonstration of Methods for Diagnosis of Viral Infections
15. Antigen–Antibody Reactions

CHAPTER 1

Rules and Safety Precautions to be Observed in the Microbiology Laboratory

1. Wash your hands with soap and water after handling the infectious material.
2. Wear laboratory coats in the laboratory.
3. Keep the nails clean and short.
4. Tie up long hair, while working in the laboratory.
5. No eating, drinking and smoking is permitted in the laboratory.
6. Decontaminate the working area with appropriate disinfectant after spillage of potentially infected material.
7. Carry out the laboratory procedure under sterile conditions.
8. Avoid mouth pipetting.
9. Perform all the laboratory procedures in a way that minimize the aerosol formation.
10. Bring practical manual in all the practical classes.
11. Draw properly labeled diagrams where applicable and get it corrected from your teachers.
12. Leave your microscope and work seat clean before going out of practical lab.
13. Keep your bag away from working tables.
14. Come exactly on time for practical and tutorials.

CHAPTER 2

GLP, Biohazard, Biosafety Levels and Biosafety Cabinets

Good Laboratory Practices

The purpose of good laboratory practice (GLP) is to promote a set of standards for ensuring the quality, reliability, reproducibility and integrity of studies and the reporting of verifiable conclusions and the traceability of data.

Biohazard

Biological hazards or biohazards refer to biological substances that pose a threat to the health of humans. This can include medical waste or samples of a bacteria, viruses, or toxins (from a biological source) that can affect human health or carry significant health risk.

Biohazard symbol is used as a warning symbol for laboratories and those dealing with potentially hazardous material.

Risk groups are classifications that describe the relative hazard posed by infectious agents or toxins in the laboratory. The World Health Organization (WHO) defines the risk groups as:

WHO Risk Group 1 (no/low individual and community risk): A microorganism that is unlikely to cause human disease or animal disease.

WHO Risk Group 2 (moderate individual risk, low community risk): A pathogen that can cause human or animal disease but is unlikely to spread to the community, and effective treatment and preventative measures are available and the risk of spread of infection is limited, e.g. *Staphylococcus aureus*, *Aeromonas hydrophila*, *Corynebacterium diphtheriae*, *Escherichia coli*, *Legionella*, *Sporothrix*, *Microsporum* spp., *Ascaris*, *C. parvum*, herpesviruses, hepatitis viruses.

WHO Risk Group 3 (high individual risk, low community risk): A pathogen that usually causes serious human or animal disease and spread from one infected individual to another through inhalation and effective treatment and preventive measures are available, e.g. *Bartonella*, *Burkholderia* spp., *M. tuberculosis, Histoplasma*, chikungunya virus, SARS-CoV, yellow fever, prions, HIV.

WHO Risk Group 4 (high individual and community risk): A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available, e.g. Ebola and Marburg viruses, KFD virus, Hendra virus. No bacterial, fungal or parasitic agents fall in this category.

Biological safety levels (BSL) or containment levels are a series of protections designed to safeguards and protect laboratory personnel, as well as the surrounding environment and community. These levels, which are ranked from one to four, are selected based on the agents or organisms that are being researched or worked on in any given laboratory setting.

BSL-1

As the lowest, BSL applies to work with agents on **WHO Risk Group 1**. Research with these agents is generally performed on standard open laboratory benches without the use of special containment equipment. BSL-1 labs are not usually isolated from the general building. There is prohibition of food, drink and smoking materials in lab setting. Personal protective equipment (PPE) is to be worn. Daily decontamination of all work surfaces when work is complete. Infection materials are also decontaminated prior to disposal. Hand washing sink must be available.

BSL-2

This BSL covers laboratories that work with agents **WHO Risk Group 2**. In addition to BSL-1 expectation, the following are to be adhered to appropriate PPE must be worn, including eye protection and face shields. [All procedures that can cause infection from aerosols or splashes are performed within a Class 1 biological safety cabinet (BSC).] A suitable decontamination method is available for proper disposals. The laboratory has self-closing, lockable doors. A sink and eyewash station should be readily available.

Access to a BSL-2 lab is far more restrictive than a BSL-1 laboratory. Outside personnel, or those with an increased risk of contamination, are often restricted from entering when work is being conducted.

BSL-3

Again building upon the two prior biosafety levels, a BSL-3 laboratory typically includes work on WHO Risk Group 2 microbes. The microbes are so serious that the work is often strictly controlled and registered with the appropriate government agencies. Laboratory personnel are also under medical surveillance and could receive immunizations for microbes they work with.

Common requirements in a BSL-3 laboratory include: Standard PPE along with respirators and gowns. All work with microbes must be performed within Class 2 BSC. Access hands-free sink and eyewash are available near the exit. Sustained directional airflow to draw air into the laboratory from clean areas towards potentially contaminated areas (exhaust air cannot be re-circulated). A self-closing set of locking doors with access away from general building corridors.

Access to a BSL-3 laboratory is restricted and controlled at all times.

BSL-4

BSL-4 labs are rare. However, some do exist in a small number of places in the US and around the world. As the highest level of biological safety, a BSL-4 laboratory consists of work with highly dangerous and exotic microbes, i.e. WHO Risk Group 4. Examples of such microbes include: Ebola and Marburg viruses.

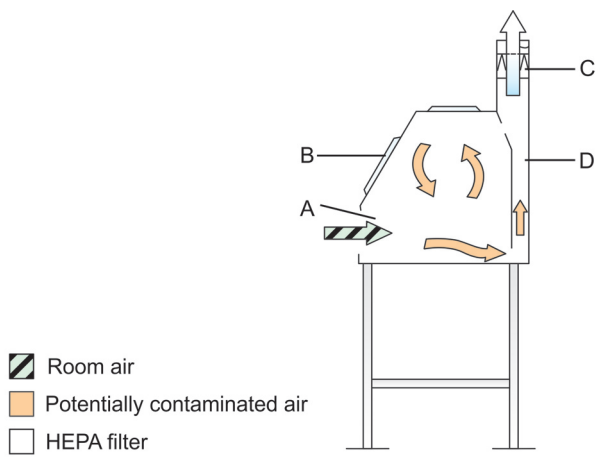
BSL-4 laboratories have the following containment requirements: Personnel are required to change clothing before entering, shower upon exiting. Decontamination of all materials before exiting. Personnel must wear appropriate PPE from prior BSL levels, as well as a full body, air-supplied, positive pressure suit. A Class III biological safety cabinet is required.

Biosafety level	*BSL-1*	*BSL-2*	*BSL-3*	*BSL-4*
Description	• No containment • Defined organisms • Unlikely to cause disease	• Containment • Moderate risk • Disease of varying severity	• High containment • Aerosol transmission • Serious/potentially lethal disease	• Max containment • "Exotic," high-risk agents • Life-threatening disease
Sample organisms	*E. coli*	Influenza, HIV, Lyme disease	Tuberculosis	Ebola virus
Pathogen type	Agents that present minimal potential hazard to personnel and the environment	Agents associated with human disease and pose moderate hazards to presonnel and the environment	Indigenous or exotic agents, agents that present a potential for aerosol transmission and agents causing serious or potentially lethal disease	Dangerous and exotic agents that pose a high risk of aerosol-transmitted laboratory infections and life-threatening disease

Biosafety Cabinets

Class I BSC

Room air is drawn in through the front opening and reaches the operator's arms to reach the work surface inside the cabinet while he/she observes the work surface through a glass window. The window can also be fully raised to provide access to the work surface for cleaning or other purposes. The directional flow of air whisks aerosol particles that may be generated on the work surface away from the laboratory worker and is then discharged from the BSC through a high efficiency particulate air (HEPA) filter.



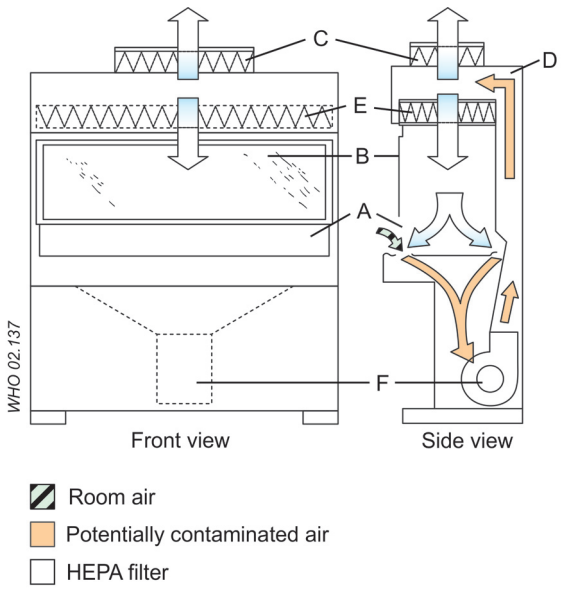
Schematic diagram of a Class I biological safety cabinet.
A. Front opening; B. Sash; C. Exhaust HEPA filter; D. Exhaust plenum.

Class II BSC

It is a ventilated cabinet, which provides personnel, product and environmental protection. It is commonly found in clinical and research laboratories working with infectious agents in Risk Groups 2, 3 and 4 (if positive-pressure suits are used) or with tissue culture.

There are four types (A1, A2, B1 and B2) of Class II BSCs. The main differences between the types are the ratio of air exhausted from the BSC to the air that is re-circulated within the BSC, and the type of exhaust system present.

About 90% of all biosafety cabinets installed are Type A2 cabinets. There is a limited need for Class II Type B BSCs. In addition, Class II Type B BSCs require very specific installation and operating conditions to function correctly.

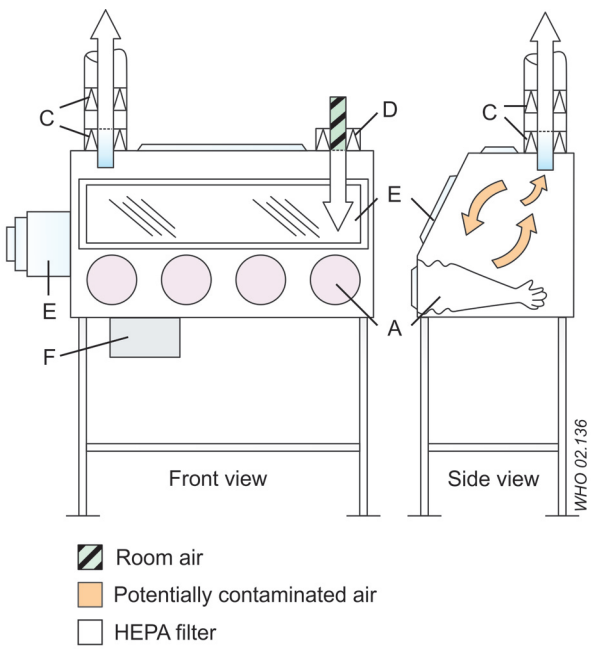


Schematic diagram of a Class I biological safety cabinet.
A. Front opening; B. Sash; C. Exhaust HEPA filter; D. Rear plenum; E. Supply HEPA filter; F. Blower.

Class III BSC

This type of cabinet is totally enclosed and is tested under pressure to ensure that no particles can leak from it into the room. Supply air is HEPA-filtered and exhaust air is discharged to atmosphere through two HEPA filters. The operator access the work surface by means of heavy-duty rubber gloves which form part of the cabinet. Airflow is maintained by a dedicated exhaust system exterior to the cabinet, which keeps the cabinet interior under negative pressure.

It may be connected to a double-door autoclave used to decontaminate all materials entering or exiting the cabinet.



Schematic diagram of a Class I biological safety cabinet.
A. Glove ports for arm-length gloves; B. Sash; C. Double-exhaust HEPA filters; D. Supply HEPA filter; E. Double-ended autoclave or pass-through box; F. Chemical dunk tank. Connection of the cabinet exhaust to an independent building exhaust air system is required

BIOSAFETY CABINETS

Biosafety cabinets

Features of Class I, II, and III Biological Safety Cabinets (BSCs)

Type	*Face velocity (m/s)*	*Airflow (re-circulated)*	*Airflow (exhausted)*	*Exhaust system*
Class I	0.36	0	100	Hard duct
Class IIA1	0.38–0.51	70	30	Exhaust to room or thimble connection
Class IIA2	0.51	70	30	Exhaust to room or thimble connection
Class IIB1	0.51	30	70	Hard duct
Class IIB2 (total exhaust BSC)	0.51	0	100	Hard duct
Class III	NA	0	100	Hard duct

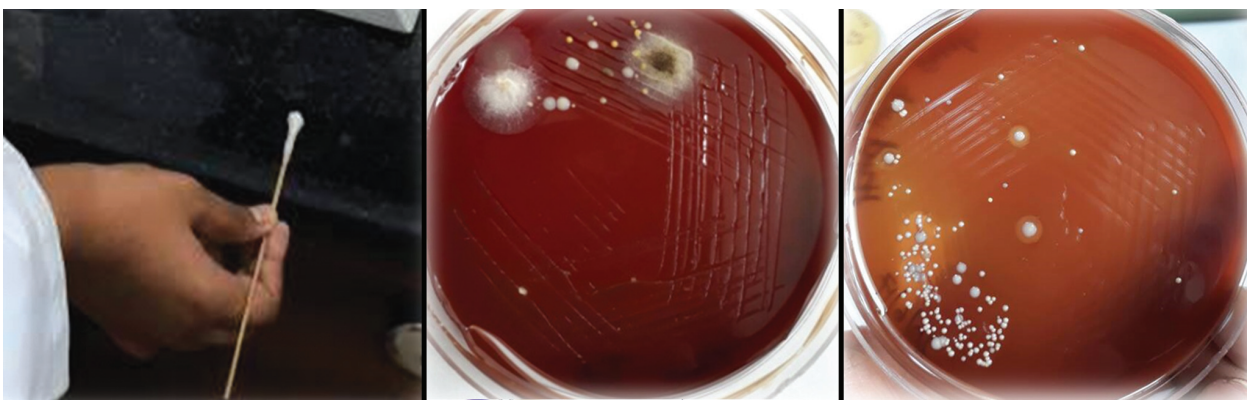
CHAPTER

3

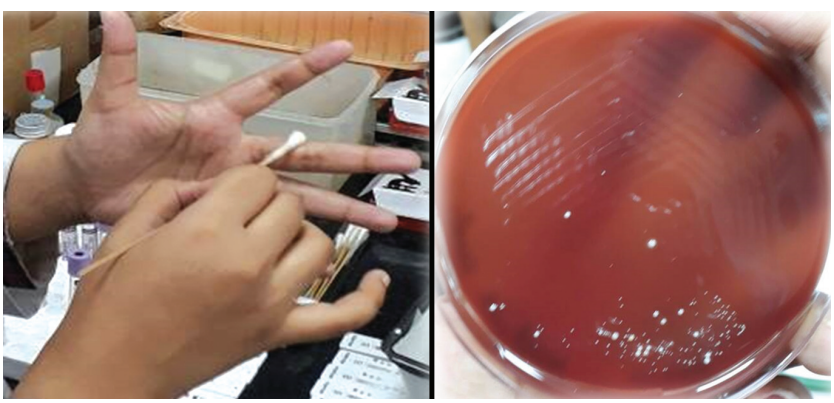
Universal Presence of Microbes

Demonstrate the universal presence of microorganisms on animate and inanimate surface.

Method: Using a sterile swab, swab an area on the table top/on hand between the webs of fingers and inoculate on blood agar culture plate. Leave a plate of blood agar exposed in air for 1 hour, cover and incubate the plates overnight by 37°C. Note the growth of microorganisms on the culture plates the next day.



1. On the table top



2. Between the webs of fingers

3. From the air after plate is exposed for 1 hour in air

Inference: Microorganisms are present universally.

CHAPTER 4

Competency: MI1.2 **Date:**

Microscope: General Instructions about Use and Care

1. Always observe an object with the body of the microscope in a perpendicular position. Do not tilt the microscope.
2. Train yourself to look with both eyes open, to reduce eye strain.
3. Do not attempt to take any part of the microscope apart.
4. Use artificial source of light provided on the work table. Use the plain side of mirror.
5. Never leave oil on the objectives. Wipe gently after the use and if oil has been left on for sometime, wipe with lens-paper or soft cloth moistened with xylene.
6. Do not touch the slide with objective lens. Always focus away from the slide, thus avoiding damage to the lens and slide.
7. Partly close the diaphragm for unstained preparation and keep the condenser down.
8. Always focus first with low power and then swing 'high power' in position and bring object into focus with fine adjustment. These two objectives are used for stool, urine, and hanging drop preparations.
9. Oil immersion lens is used for bacterial preparations, etc. Place a drop of oil on slide, lower the lens to touch the oil and focus with fine adjustment. The condenser should be right up when oil immersion is being used.
10. Objectives are as follows:
    - Low power: 16 mm
    - High power: 4 mm focal length
    - Oil immersion: 1.8 mm

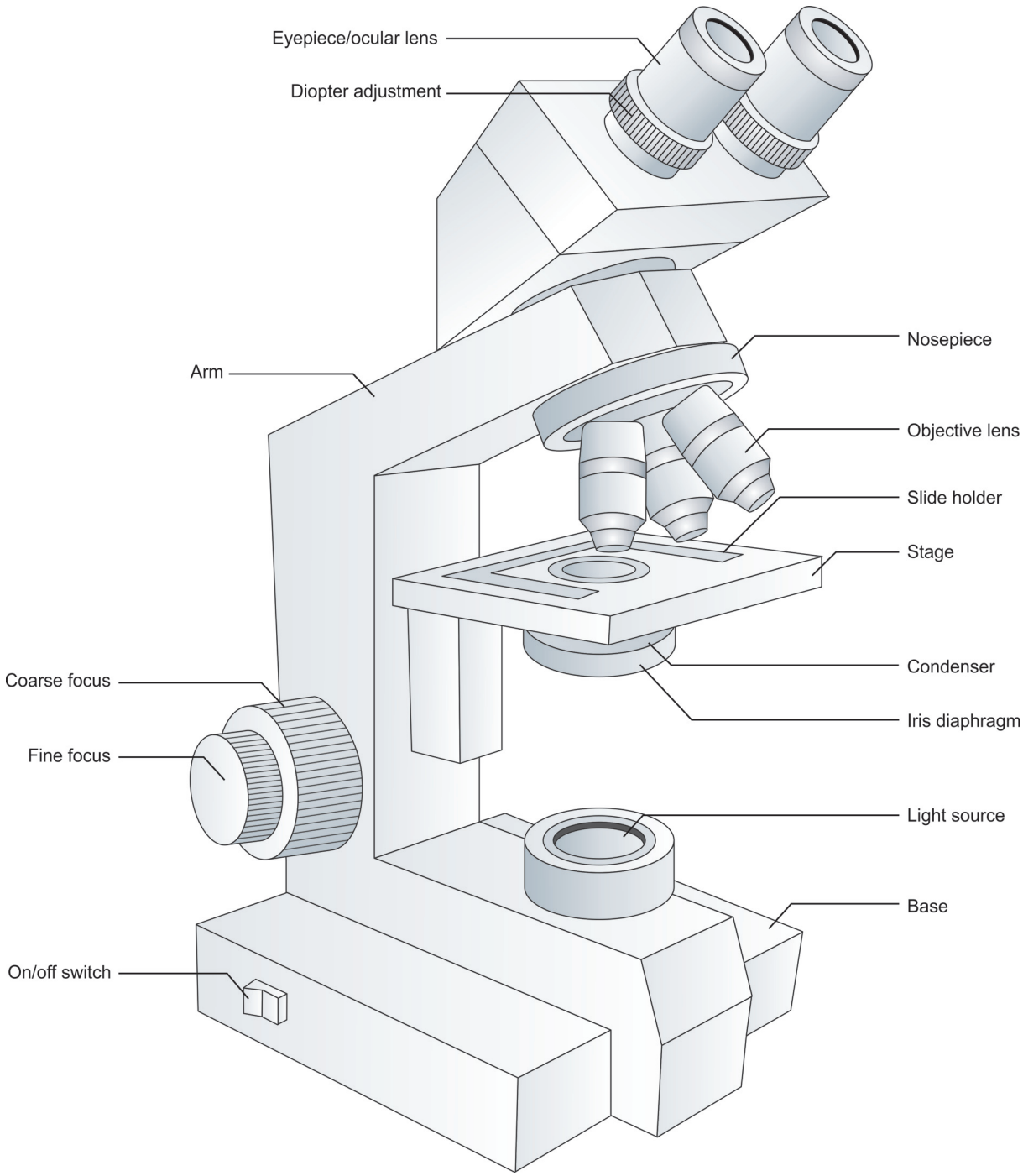
    These figures are working distances or focal lengths and very short in the last two, the fine adjustment must be used for focusing.
11. Magnification

    Usual corrected tube length = 16 cm = 160 mm
    Distance of image = 16 cm = 160 mm
    Distance of object = Focal length of objective
    Using low power and eyepiece (EL): 10X

$$\text{Magnification} = \frac{\text{Distance of image}}{\text{Distance of object}} \times \text{Magnification of eyepiece}$$

$$= \frac{160}{16} \times 10$$

12. Always leave your microscope lenses clean, oil and dust-free after you finish your practical.



Parts of a light microscope

CHAPTER 5

Competency: MI1.2 **Date:**

Microscopy and Micrometry

Microscope: Instrument to magnify and resolve microbes and very small objects.

Microscopy: It deals with magnification of object so as to show the finest details of the object.

Principle: The light rays pass through the object, the objective and series of lenses to form a magnified and resolved image of the object. Real, inverted and enlarged image is formed by the objective lens, while virtual erect and enlarged image is formed by eyepiece lens which is seen by the observer.

Parts of a Light Microscope

1. Base—system rests over it.
2. Foot—horseshoe shaped
3. Mirror—directs light in optical system
   Plane mirror to be used for point source of light
   Concave mirror for natural light
4. Condenser—regulates amount of light entering
5. Stage—to place the slide
6. Eyepiece—objects viewed with this
7. Coarse and fine adjustment screw—for focusing clearly and properly.

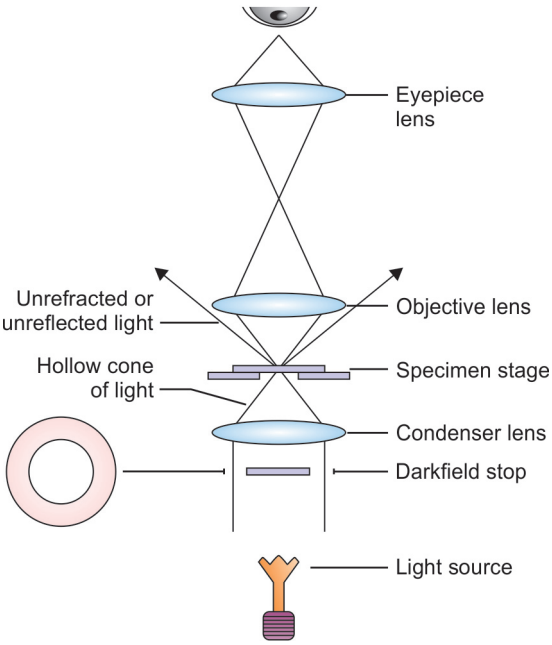
Position of Condenser

10X: Lowest
40X: Middle
100X: Highest

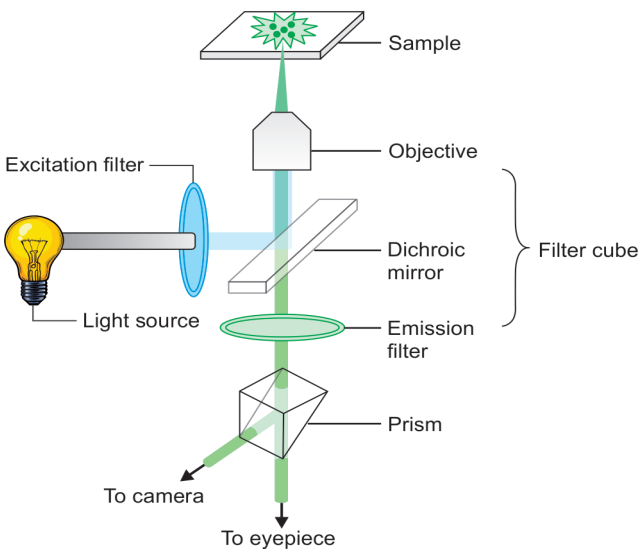
Types of Microscope

1. **Light/optical microscope:** Uses visible light and a system of lenses to magnify images of small samples.
2. **Phase contrast microscope:** The phase contrast microscope uses the fact that the light passing through a transparent part of the specimen travels slower and due to this is shifted compared to the uninfluenced light. This difference in phase is not visible to the human eye. However, the change in phase can be increased to half a wavelength by a transparent phase-plate in the microscope and thereby causing a difference in brightness. This makes the transparent object shine out in contrast to its surroundings.
   Uses:
   - Identification of cellular structures.
   - Motility of organisms, cell division can be observed in real time.
3. **Darkfield microscope:** The darkfield microscope creates a contrast between the background and the specimen by adding a special stop condenser. The background appears dark and the specimen bright as the stop condenser prevents all the transmitted light reaching the specimen and only the oblique scattered light reaches the specimen and the lens system.

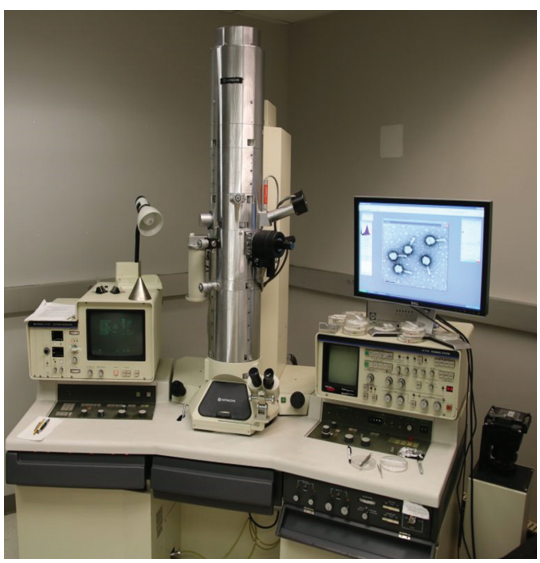
Phase-contrast microscope



Darkfield microscope



Fluorescent microscope



Electron microscope

Uses:
- Identification of treponemas.
- Motility of organisms.
- Observation of thin fragile organisms which cannot be stained.

4. **Fluorescent microscope:** The sample (cells/organism) is stained with a fluorescent dye and UV light source is used. The UV rays passes through the excitation filter and the objective lens on the sample. The reflected higher wavelength wave passes through the beam splitter and emission filter to reach the eyepiece in a fashion that the wavelength of transmitted ray matches the emission characteristics of the fluorescent dye. So, the sample appears colorful on a dark background.
   Uses:
   - Rapid identification of microorganisms, e.g. identification of TB bacilli in sputum, QBC for malaria.
   - Identification of specific protein and DNA in tissue sections.
5. **Electron microscope:** A beam of accelerated electrons is used as a source of light which is passed in a vacuum stack with array of electrostatic and electromagnetic lenses. This beam then passes through the specimen which in part scatters them. This reflected electronic beam carries the structural detail of the specimen and reaches the objective lens which is then further magnified by it. The real image formed is photographically captured and can be seen on a computer screen.
   Uses:
   - High velocity of electrons and low aperture of the EM allow higher magnification up to 0.3–0.5 nm.
   - Visualization of minute cellular detail is possible.
   - Visualization of virus

Micrometry: Measurement of size of microscopic objects is called micrometry.

Basic Principle of Micrometry

Micrometry deals with the measurement of microscopic objects like blood cells, microorganisms, etc. It has two components: The eyepiece micrometer and the stage micrometer.

1. The eyepiece micrometer is a graduated scale from 0 to 10 without standardized measurement.
2. The stage micrometer is a slide with a microscopic 1 mm scale on it. Each division of stage micrometer measures 1/100th mm, i.e. 10 µm (0.001 mm).

After focusing both the eyepiece and the stage micrometer, the scales are aligned and total number of stage micrometer division within 100 divisions of eyepiece micrometer are counted. After this, total length of eyepiece micrometer is calculated and divisional measurement is calculated by unitary method. After obtaining the measurement of single eyepiece division the stage micrometer is removed and the slide with the sample is focused. Number of eyepiece division within a single cell is counted and multiplied by the eyepiece division.

Remarks: The measurement of eyepiece division is not constant and changes with the magnification of the objective and the tube length.

Example: At 40X magnification, 100 eyepiece divisions are equivalent to 25 stage divisions, i.e.

100 eyepiece division × size of each eyepiece division (n) = 25 stage divisions × size of each stage division (10 µm)

$$n = \frac{25 \times 10\ \mu m}{100}$$

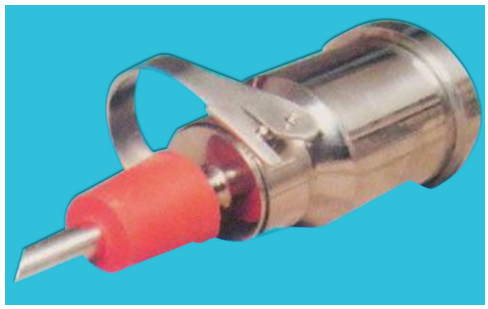
$$n = 2.5\ \mu m$$

Focus a Giemsa stained blood slide and calculate number of eyepiece divisions within a RBC.

Suppose, 1 RBC = 3 eyepiece divisions

Then diameter of 1 RBC = 3 × 2.5 µm = 7.5 µm

A pair of disposable surgical rubber gloves of size 6.5



Seitz filter

Sintered glass filter

Millipore membrane filter available in pore sizes 0.22 µm/0.45 µm

Sterile disposable plastic syringe of 2 ml capacity with 18G hypodermic needle

Glass syringe of 100 ml capacity

Universal container (McCartney bottle)

Bijou bottle

Glass Petri plate

Sterile swab

CHAPTER

6

Competency: MI1.5, 8.5, 8.6, 8.7, 8.8 **Date:**

Methods of Sterilization and Disinfection

METHODS OF STERILIZATION	
METHODS	**EXAMPLE**
PHYSICAL METHODS	
I. HEAT	
1. Moist heat	
a. Below 100°C	Vaccine bath; pasteurization; inspissation
b. At 100°C	Boiling steaming, tyndallization
c. Above 100°C	Autoclave: Dressings, surgical instruments, culture media
2. Dry heat	
a. Flaming, red heat	Bacteriological loupe
b. Hot air oven	Lab glasswares, powders, oils; liquid paraffin, glycerol, scalpels, scissors, and blades
II. IONISING RADIATION	
Gamma rays	Disposable syringe needles/gloves/catheters/swabs
III. FILTRATION	
1. Seitz filter	Serum, vaccine
2. Sintered glass filter	Serum, vaccine
3. Millipore filter	Serum, vaccine
CHEMICAL METHODS	
Chemicals	Glutaraldehyde
GASEOUS STERILANTS	
Gas: Ethylene oxide (EtO)	Disposable syringe needles/gloves/catheters
Plasma sterilization	Arthroscopes, laparoscopes, spine sets
METHODS OF DISINFECTION	
Alcohols	70% ethylalcohol, isopropyl alcohol
Phenols	Cresol; chlorhexidine, hexachlorophene
Halogens	Sodium hypochlorite, iodine
Aldehydes	Formaldehyde
Antiseptics	Mercuric chloride; acriflavin

LABORATORY AUTOCLAVE

Principle

Water boils when vapour pressure equals that of the surrounding atmosphere. At normal pressure water boils at 100°C. When the atmospheric pressure is raised as in a closed vessel like autoclave, the temperature at which water boils also increases. Steam at higher temperature has a greater penetration power.

Temperature = 121°C;
Time = 15 minutes
Pressure = 15 lbs/square inch

Vertical autoclave

Horizontal autoclave

HOT AIR OVEN

Principle

Hot air ovens are electrical devices which use dry heat to sterilize. Hot air ovens use extremely high temperatures over several hours to destroy microorganisms and bacterial spores. The ovens use conduction to sterilize items by heating the outside surfaces of the item, which then absorbs the heat and moves it towards the center of the item. Generally, they use a thermostat to control the temperature. Their double-walled insulation keeps the heat in and conserves energy, the inner layer being a poor conductor and outer layer being metallic.

The commonly used temperatures and time that hot air ovens need to sterilize materials are:
170°C for 30 minutes (1/2 hour)
160°C for 60 minutes (1 hour)
150°C for 150 minutes (2½ hours)

Hot air oven

Ethylene oxide (EO) sterilizer

Plasma sterilizer

CHAPTER 7

Competency: MI8.5, 8.6, 8.7, 8.8 **Date:**

Biomedical Waste Disposal

DISCARDING HAZARDOUS MATERIAL

Colour Codes for Discard of Biomedical Wastes

(BMW Rules 2016 with Central Pollution Control Board Revision 5)

Category	*Type of BMW*	*Type of bag*
Yellow	• Disposable masks • Disposable nonplastic coverall/gown • Shoe covers • Head covers • Wastes contaminated blood/body fluids • Blood bags • Expired/discarded cytotoxic drugs • Discarded/expired vaccines	Yellow colored nonchlorinated plastic bags
Red	• Goggles and face-shields • Gloves • Blood bags • Syringe, IV set, nasogastric tubes, central line catheter • Urine catheter with urobags • Disposable plastic coverall/gown • Used automated blood culture bottles	Red colored nonchlorinated plastic bags
White	• Waste sharps, e.g. needles, scalpels	Puncture-proof, leakproof, tamper-proof container/box
Blue	• Glassware, e.g. glass ampoules • Microscopy slides • Metallic implants	Puncture-proof, leakproof, tamper-proof container/box
General green	• Biodegradable general wastes, e.g. leftover food	
General blue	• Nonbiodegradable general wastes, e.g. disposable plates and cutlery	

- All bin should have foot operated lids.
- Bin bags should be double layered as to ensure adequate strength and no-leaks.
- Collection bin for Covid waste should be labelled as "COVID-19".

CHAPTER

8

Table of Commonly Used Culture Media in Microbiology and their Uses

COMMONLY USED CULTURE MEDIA IN MICROBIOLOGY AND THEIR USES

Name of media	***Type of media***	***Important constituents***	***Uses***
Peptone water	Basal media	1% Peptone NaCl, water	• Sugar fermentation test after addition of 1% respective sugar to peptone water • Sub-culture of bacteria
Nutrient agar	Basal media	Peptone water Meat extract agar	Growth of nonfastidious bacteria
Blood agar	Enriched media	Nutrient agar 5% sheep blood	Growth of aerobic and anaerobic bacteria
Chocolate agar	Enriched media	Heated blood agar	Growth of fastidious bacteria, e.g. Neisseria
MacConkey agar	Differential media or mildly selective media	Peptone Lactose Bile salt Neutral red (indicator) agar	Differentiating lactose fermenters (LF) from nonlactose fermenting (NLF) gram-negative bacteria
Löffler's serum slope	Enriched media	Glucose broth 1 part Serum 3 part	Selective growth of *Corynebacterium diphtheriae*
Tellurite blood agar	Selective media	Blood agar Potassium tellurite (0.4%)	Growth of *Corynebacterium diphtheriae* (black colonies)
Xylose lysine deoxycholate /(XLD) agar	Moderately selective media	Mainly ..... Xylose Lysine Na-deoxycholate Phenol red (indicator) Lactose, sucrose, agar	Growth of *Salmonella* spp. (red colonies with black centre) and *Shigella* spp. (red colonies without black centre)
Wilson and Blair bismuth sulphite agar	Highly selective media	Peptone Beef extract Dextrose (glucose) Disodium phosphate Ferrous sulphate Bismuth sulphite Brilliant green (indicator) agar	Black colonies of *Salmonella typhi*

Contd.

Contd.

Name of media	*Type of media*	*Important constituents*	*Uses*
Löwenstein-Jensen (LJ) medium	Selective media	Hen's egg (solidifying agent) Malachite green (selective agent) L-asperagine Glycerol Mineral salts	Growth of *Mycobacterium TB* (rough, tough, buff) and nontuberculous mycobacteria (yellow)
Thiosulphate citrate bile-salt sucrose (TCBS) agar	Highly selective media	Bile Sucrose Bromothymol blue (indicator) Salts Agar Water	Differentiating *Vibrio cholerae* (yellow sucrose ferming) from *V. parahaemolyticus* (nonsucrose fermenting green colonies)
Müeller Hinton agar	Nonselective Nondifferential medium	Beef infusion Casein Starch Agar Water (cation adjusted)	Antimicrobial sensitivity testing (AST)
Robertson's cooked meat broth	Enriched media	Nutrient broth Cooked meat particles Yeast extract Dextrose	Used for growth of anaerobic organisms
Thioglycolate broth	Enrichment broth	Casein Dextrose, NaCl Yeast extract Na thioglycollate Cystine, resazurin Agar	Used for growth of anaerobic organisms
Selenite F broth	Enrichment broth	Peptone water Na-selenite	Selective culture of feces to grow fecal pathogens for 4–6 hours followed by SC
Alkaline peptone water	Enrichment broth	Peptone water at pH 9.0	Selective culture of *Vibrio cholerae* from feces for 4–6 hours followed by SC
Brain heart infusion broth/agar	BHIB-liquid BHIA-solid Biphasic BHIB/A	Calf brain Salts Glucose Agar to solidify	Growth of variety of fastidious and nonfastidious bacteria from blood culture in BHIB
Sabouraud dextrose agar (SDA)	Selective media	Peptone Dextrose 4% Water Agar	Growth of fungi

CHAPTER 9

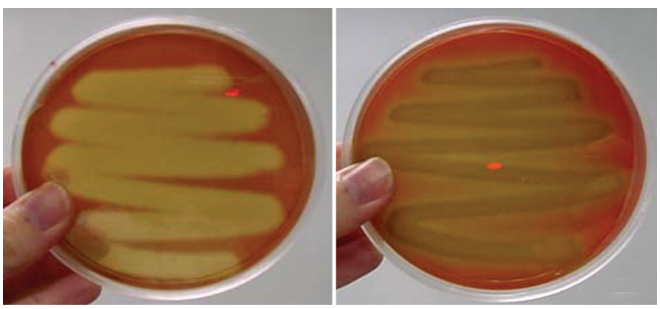
Competency: MI1.1 **Date:**

Demonstration of Common Culture Media and Biochemical Reactions

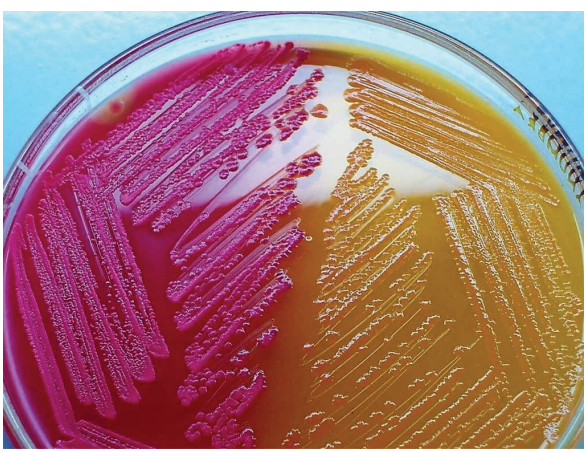
The following media are commonly used for gram-negative bacilli of the family Enterobacteriaceae.

Blood Agar

- It is an **enriched medium.**
- Contains **5% sheep blood** in nutrient agar.
- Helps to differentiate between **α and β hemolysis.**
- Helps to **study swarming** by *Proteus* spp.
- Preparation: Autoclaved nutrient agar is cooled to 50°C followed by addition of defibrinated sheep blood aseptically.



Beta hemolysis Alpha hemolysis



Lactose fermentor colonies Nonlactose fermentor colonies

MacConkey agar

MacConkey Agar

- It is a **mildly selective media** as it selects out lactose fermenting (**LF) pink colonies** from nonlactose fermenting (**NLF) pale colonies**.
- It is also an **indicator medium** and contains neutral red indicator.
- Contains bile salt (sodium taurocholate), hence **prevents swarming.**

Xylose Lysine Deoxycholate (XLD) Agar

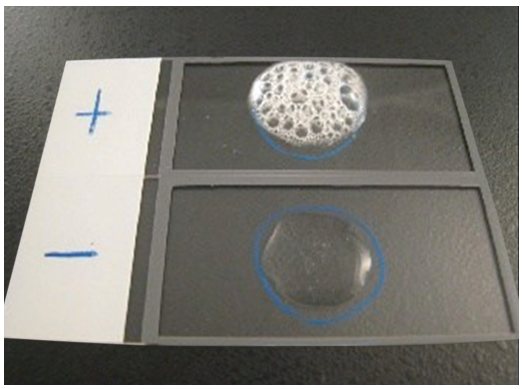
Moderately selective media for:

- *Salmonella* (pink colonies with black centre)
- *Shigella* (pink colonies)

XLD agar

BIOCHEMICAL TESTS

Catalase Test



Principle: Bacteria that produce enzyme catalase breaks down H_2O_2 into H_2O and O_2. The O_2 is released as bubbles.

Reagent used: 3% H_2O_2

A drop of 3% H_2O_2 is put on a glass slide. With a wooden applicator stick bacteria is transferred from fresh culture to H_2O_2 on slide and observed for immediate and sustained bubbles formation.

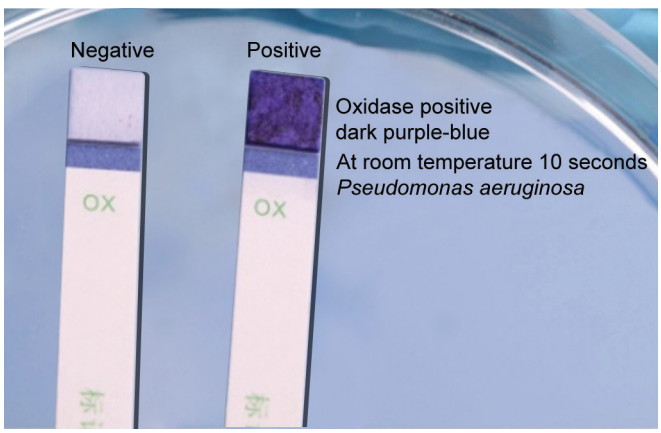
Catalase producing bacteria: Staphylococcus, all gram-negative organisms of family Enterobacteriaceae.

Oxidase Test

Reagent: Tetramethyl *para*-phenylenediamine dihydrochloride.

Test:

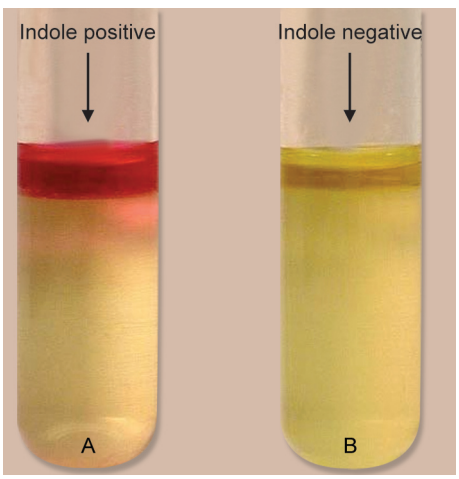
- A strip impregnated with the reagent is taken.
- A small portion of the colony is picked-up with the corner of a sterile coverslip and smeared onto the strip.
- Change of color to purple within 30 sec signifies positive reaction.



Oxidase test

Principle: The cytrochrome oxidase enzyme of certain bacteria is able to oxidize the reagent producing the coloured product.

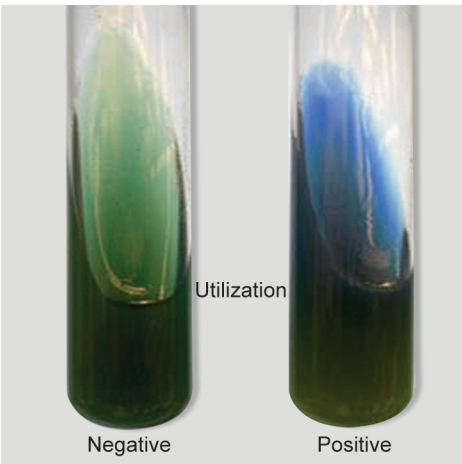
Example: Positive—*Pseudomomas* spp., *Vibrio* spp.
Negative—members of family Enterobacteriaceae



Indole production test

Indole Production Test

- **Principle of test:** Ability of an organisn to split indole from tryptophan.
- **Reagent used**: Kovac's reagent
- **Indole producing organisms:** *E. coli, Proteus vulgaris*.



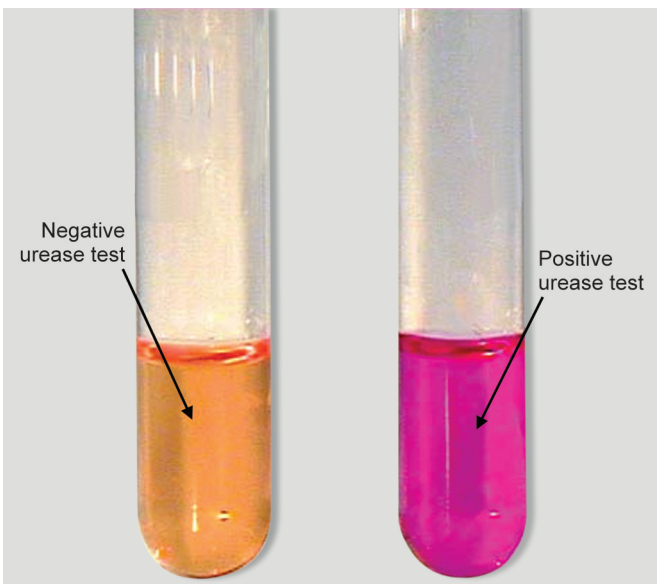
Citrate utilization test

Citrate Utilization Test

- **Principle:** Ability of an organism to utilize citrate as the sole source of carbon for metabolism resulting in alkalinity.
- **Medium:** Simmon's citrate medium
- **Indicator:** Bromothymol blue
- **Citrate utilizing organisms:** *Klebsiella, Salmonella, Enterobacter.*

Urease Production Test

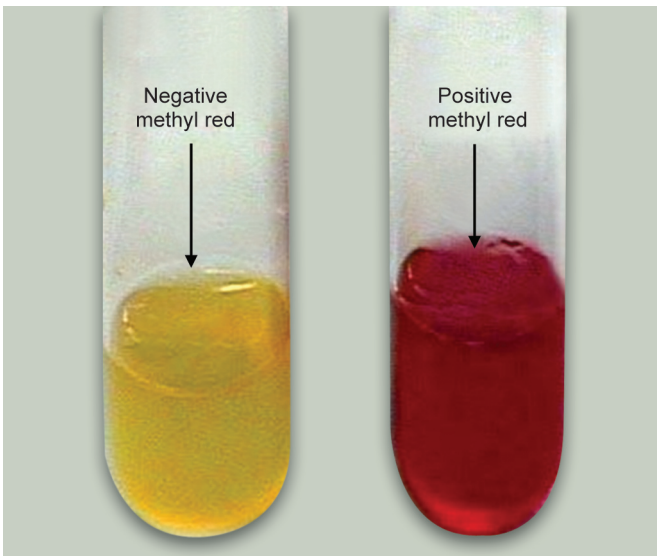
- **Principle:** Ability of an organism to produce the enzyme urease which hydrolyzes urea to ammonia resulting in pink color.
- **Medium:** Christensen's urea agar
- **Indicator:** Phenol red
- **Urease producing organisms:** *Proteus, Providencia, Helicobacter pylori, Klebsiella, Cryptococcus* (yeast fungus)



Urease production test

Methyl Red (MR) Test

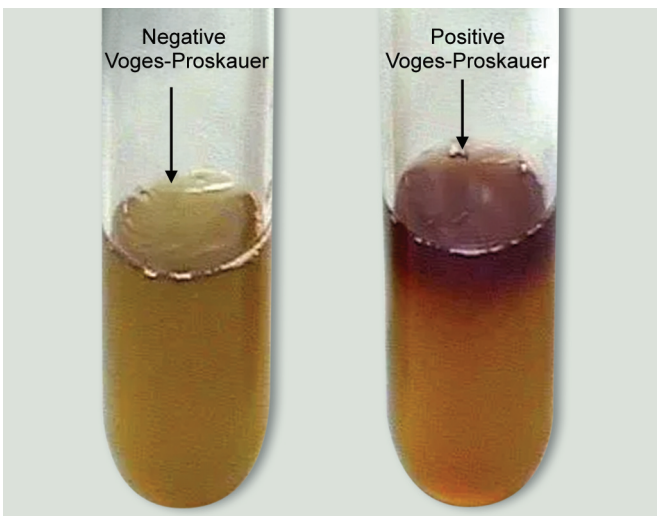
- **Principle:** Ability of an organism to produce and maintain stable acid end-products from glucose fermentation and overcome buffering capacity of the system.
- **Medium:** Glucose phosphate broth
- **MR positive organisms:** *E. coli*, *Yersinia* spp.



Methyl red test

Voges-Proskauer (VP) Test

- **Principle:** Ability of an organism to produce neutral end product acetylmethylcarbinol/acetoin from glucose fermentation
- **Medium:** Glucose phosphate broth
- **VP positive organisms:** Klebsiella, Enterobacter



Voges-Proskauer test

Triple Sugar Iron (TSI) Test

Principle: Tests the production of acid and gas from fermentation of three sugars—lactose, sucrose, glucose (present in ratio of 10:10:1 parts) and also the ability to produce H_2S.

Medium: Has a butt and slant. Contains lactose, sucrose, glucose (present in ratio of 10:10:1 parts). Indicator is phenol red.

Interpretation

Glucose fermentation: Acidic (yellow) butt
Sucrose/lactose fermentation: Acidic (yellow) slant
Both glucose and sucrose/lactose fermentation: Acidic (yellow) slant and butt
Gas production: Cracks in medium/medium lifted up
H_2S production: Blackening of the medium
No fermentation: Both slant and butt alkaline

Slant/butt	*Glucose*	*Lactose*	*Sucrose*
K/A	+	–	–
A/A	+	+	+/–
K/K	–	–	–

Organism	*Slant/butt*	*Gas*	*H_2S*
E. coli/Klebsiella	A/A	+	–
Proteus	A/A	–	+
S. typhi	K/A	–	+
Shigella	K/A	–	–
Pseudomonas	K/A	–	–

A/A + gas K/A K/A + H_2S K/A

CHAPTER 10

Competency: MI1.1 **Date:**

Culture Methods

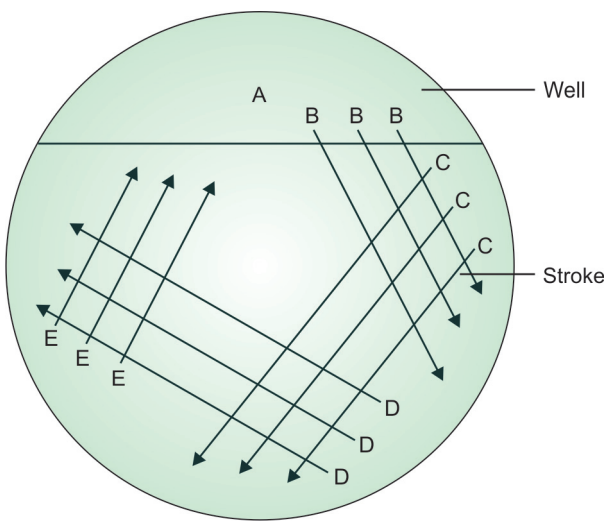
The purpose of culture is

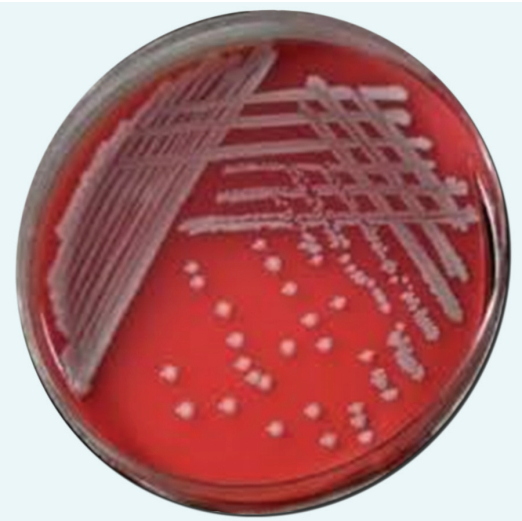
- To isolate bacteria in pure form
- To identify the bacteria
- To be able to do antibacterial sensitivity testing
- Demonstrate the properties of the bacteria
- For research purposes
- Maintain a stock of the bacteria

Different methods of culture of bacteria are as follows.

Culture on Solid Medium

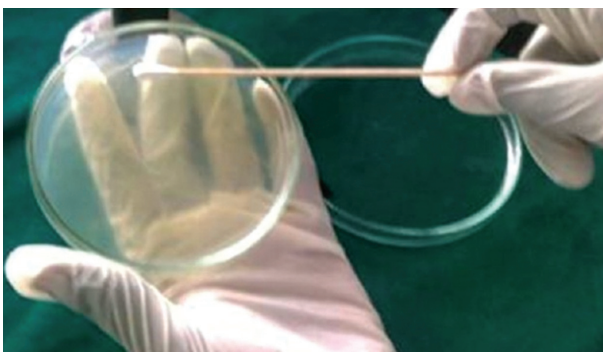
1. **Streak culture** which is most commonly used for inoculation of specimens for isolation or for obtaining isolated colonies of different bacteria from a mixed culture.
   A loopful of the specimen is smeared onto the solid media to form an oval shapes well of primary inoculum (A). This is then spread over the media by streaking parallel lines onto the media to form secondary (B) followed by tertiary (C) and subsequent inoculums (D & E) ending in a feathery tail. The loop may be heated in between different set of streaks to obtain isolated colonies which is the target of this type of culture.

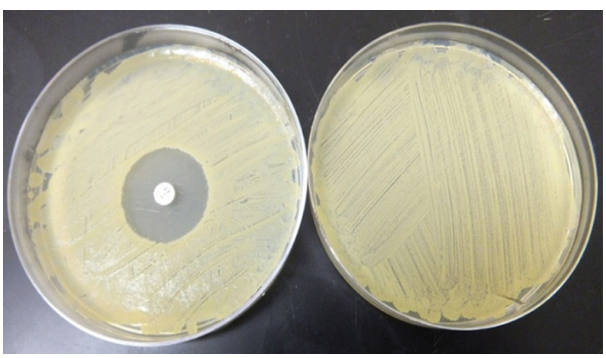




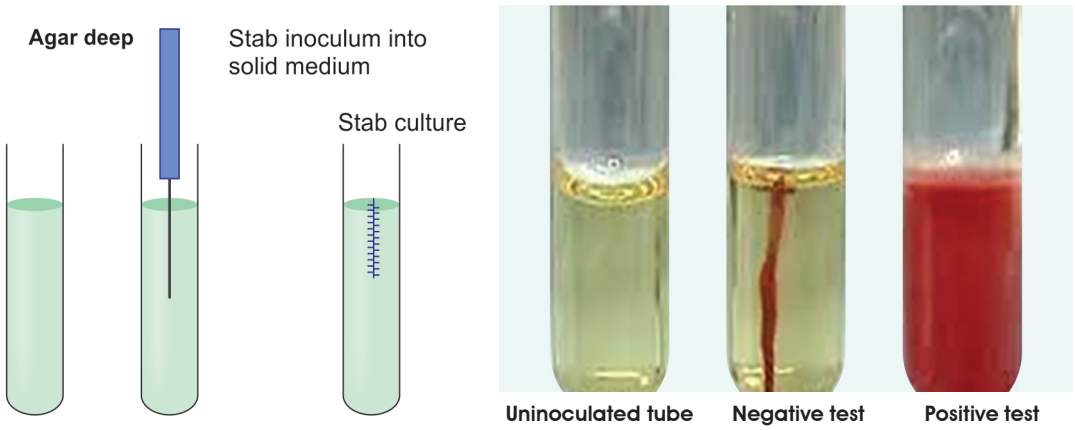
On incubation the growth may be confluent at the original inoculation but becomes progressively thinner and isolated colonies are obtained over the final series of streaks.

2. **Lawn culture or carpet culture** provides an uniform growth of the bacteria for antibiotic sensitivity testing or phage typing. It is prepared by either flooding the culture plate with a pure liquid culture or suspension of the bacterium and then pipetting out the excess inoculum or the surface of the plate may be inoculated by a swab soaked in liquid culture or suspension of the bacterium.



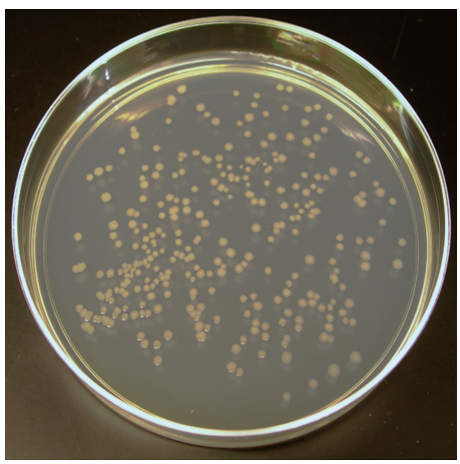


3. **Stab culture** is prepared by charging a long straight wire dipped in a liquid culture and then into a tube of suitable culture medium like nutrient agar or gelatin to demonstrate certain characteristics of the bacterium like motility or gelatin liquefaction.



4. **Stroke culture** is made on agar slope or slants in tubes for providing a pure growth of the bacterium for demonstrating certain properties.

5. **Pour plate culture** is prepared by first melting agar medium with subsequent cooling, then adding the bacterial inoculums and then pouring onto a sterile Petri dish and allowing it to set and then after incubation of the plate colonies will be seen well distributed in the depth of the medium. It is used for quantitative urine culture as it gives the viable counts.



Culture on Liquid Medium

These are inoculated by touching suitable liquid medium with charged loop or straight wire or by adding inoculums with pipettes or syringes. This method is used for blood culture or for preparing inocula containing antibiotics for micro or macro broth dilutional method for antibiotic sensitivity testing.

CHAPTER 11

Competency: MI1.1 **Date:**

Description of the Appearance of Growth on Solid or in Liquid Media

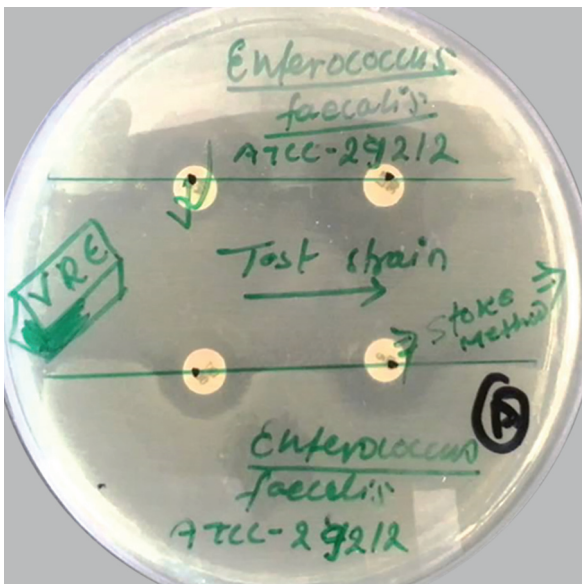
Aim: To describe colony characteristics of bacterial growth on solid and liquid media.

Description of Colonies on Solid Media

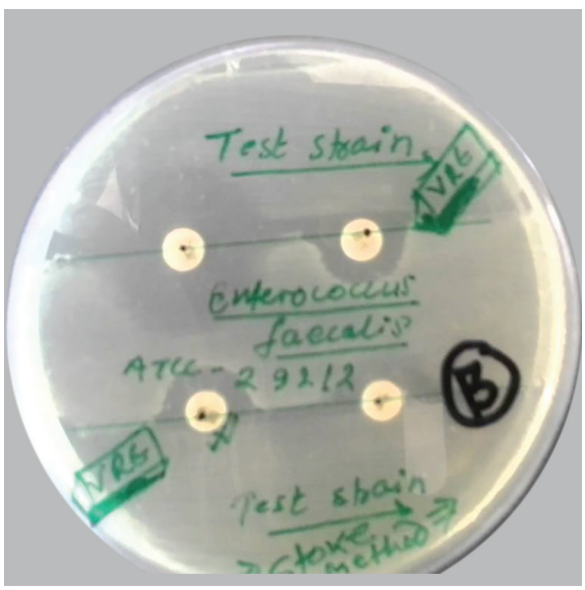
1. Name of the media	:	Blood agar, MacConkey agar, etc.
2. Shape	:	Circular, irregular, radial
3. Size	:	In millimeters (approx.), pinpoint, pinhead
4. Elevation	:	Raised, low convex, dome shaped, umbonate
5. Surface	:	Smooth, rough, granular, dull or glistening
6. Edge	:	Entire, crenate, spreading
7. Color	:	Colored by reflected and transmitted light, fluorescent
8. Opacity	:	Transparent, translucent or opaque
9. Consistency	:	Butyrous/mucoid/friable
10. Emulsifiability	:	Easy or difficult
11. Pigment	:	Pigmented—diffusible or nondiffusible/nonpigmented
12. Change in medium	:	Haemolysis on blood agar
13. Odor	:	Present seminal/fishy/earthy or absent

Growth in Liquid Medium

1. Degree	:	None/scanty/moderate/abundant
2. Turbidity	:	Present or absent
3. Deposit	:	Present or absent
4. Surface pellicle	:	Present or absent
5. Odor	:	Absent or fishy/earthy
6. Pigment	:	Pigmented/nonpigmented
7. Motility (after 2 hours of incubation)	:	Motile or nonmotile



Stokes method: Test strain in centre and control strains above and below



Modified Stokes method: Control strains in centre and test strains above and below. Two separate strains can be tested

CHAPTER 12

Competency: MI1.6 **Date:**

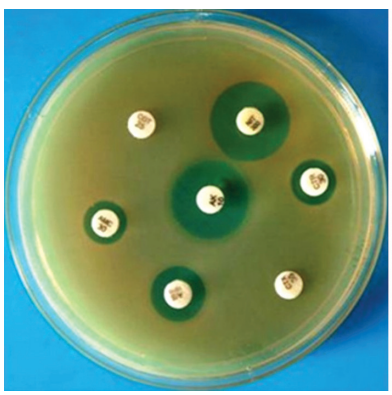
Demonstration of Methods of Antimicrobial Susceptibility Testing

Aim: Antimicrobial susceptibility testing (AST) measures the ability of an antimicrobial agent to inhibit growth of microorganism *in vitro*. It is an essential step for properly treating infectious diseases and monitoring antimicrobial resistance (AMR) in various pathogens.

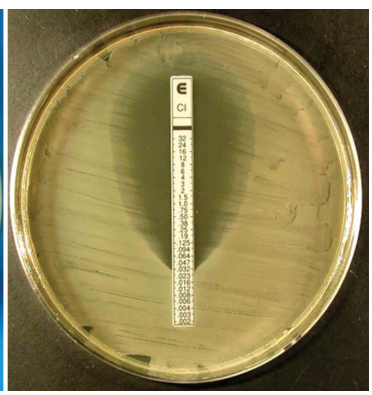
Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism following overnight incubation.

Diffusion Method

- Media used: **Müeller Hinton agar**
- Methods used:
  - **Kirby-Bauer method** (using controls ATCC *S. aureus* 25923, *E. coli* 25922, *Pseudomonas* 27853)
  - **Stokes method** (controls NCTC *S. aureus* 6571, *E. coli* 10418, *Pseudomonas* 10662)
  - **E-test** using E-strip



Kirby-Bauer method



E-test

Dilution Method

- Agar dilution
- Broth dilution: Macro broth dilution and micro broth dilution

Automated Systems Used

- MicroScan WalkAway
- Vitek 1 and 2
- Sensititre

ENTOMOLOGY

Phylum: Arthropoda
Class: Insecta, Arachnida, Crustaceae

Characteristics	Class Insecta	Class Arachnida	Class Crustaceae
Body divided into	Head Thorax Abdomen	Cephalothorax Abdomen	Cephalothorax Abdomen
Legs	3 pairs	**Ticks:** Adult—4 pairs Larvae—3 pairs **Mites:** Egg and larva—3 pairs Nymph and adult—4 pairs	5 pairs
Antenna	1 pair	Absent	2 pairs
Wings	**Absent**: Lice, flea, bug **Present:** **1 pair**—mosquito, sandfly, housefly, tsetse fly **2 pairs**—cockroach, reduviid bug	Absent	Absent
Examples	Mosquito, fly, lice, flea, bug	Ticks and mites	Cyclops, diaptomus crab, cray fish, fairy shrimps

ENTOMOLOGY

DISEASES TRANSMITTED BY CLASS INSECTA

	Mosquito-borne diseases
Anopheles	Malaria; Chittoor virus
Culex	Filariasis; Japanese encephalitis; West Nile fever; sindbis virus
Aedes	Dengue; chikungunya; yellow fever; viral hemorrhagic fever
Mansonoides	Filariasis
	Fly-borne diseases
Sandfly	Leishmaniasis; sandfly fever; bartonellosis; oriental sore
Tsetse fly (Glossina)	African trypanosomiasis (sleeping sickness)
Housefly (*Musca* spp.)	Mechanical carrier of diarrhea; dysentery; typhoid fever; trachoma; conjunctivitis; anthrax; yaws; myiasis
	Lice-borne diseases
Head louse (*P. capitis*)	Epidemic typhus; mechanical irritation, dermatitis
Body louse (*P. corporis*)	Epidemic typhus; trench fever; relapsing fever
Pubic louse (*Phthirus pubis*)	No disease in India
	Flea-borne diseases
Rat flea (*Xenopsylla cheopis*)	Plague; endemic typhus; murine typhus; cestode infection due to *H. diminuta*
	Bug-borne diseases
Cone nosed bugs (reduviid bugs)	Chagas disease (American trypanosomiasis)

ENTOMOLOGY

DISEASES TRANSMITTED BY CLASS ARACHNIDA

	Tick-borne diseases
Hard tick (ixodid tick) *Dermacentor* spp., *Rhipicephalus* spp. *Haemaphysalis, Ixodes, Amblyomma*	Relapsing fever; tularemia; babesiosis; Lyme disease; Rocky Mountain spotted fever; viral encephalitis (KFD) viral hemorrhagic fever; RSSE
Soft tick—Argasidae	Relapsing fever, KFD
	Mite-borne diseases
Scrub mite—trombiculid	Scrub typhus
Itch mite (*Sarcoptes scabiei*)	Scabies
Dust mite	Allergy
Rat mite	Rickettsialpox

DISEASES TRANSMITTED BY CLASS CRUSTACEAE (WATER FLEA)

	Crustaceae-borne diseases
Small crustaceans (cyclops, diaptomus)	Dracunculosis, *D. latum* infection
Big crustaceans (crab, cray fish, fairy shrimps)	Transmitting the metacercariae of *P. westermani* (lung fluke)

CHAPTER

13

Date:

Miscellaneous Arthropods of Medical Importance (Including Tables)

Aim: To study characteristic features of various insects/vectors and diseases transmitted to man.

1. *Anopheles* mosquito
2. *Aedes* mosquito
3. Mosquito larvae
4. Housefly
5. Tsetse fly
6. Sandfly
7. Cyclops
8. Hard tick
9. Soft tick
10. Rat flea
11. Itch mite
12. Louse

Characteristic Features of Adult *Anopheles* Mosquito

Adult *Anopheles* mosquito

- Adult sits at an angle.
- Wings are spotted at the periphery.
- Diseases transmitted—malaria, Chittoor virus.

Characteristic Features of Adult *Aedes* Mosquito

Adult *Aedes* mosquito

- White stripes on its black body and legs (Tiger mosquito)
- Bites during daytime
- Breeds in clean stagnant water
- Abundant during rainy season
- Diseases transmitted—chikungunya, dengue, yellow fever.

Mosquito Larvae

Anophelini (Anopheles) larva

- Rests parallel to water surface
- No siphon tube
- Palmate hairs present on abdominal segments.

Culicine (Culex, Aedes, Mansonia) larva

- Suspended with head downwards at an angle to water surface.
- Siphon tube present
- No palmate hairs

Characteristic Features of Housefly

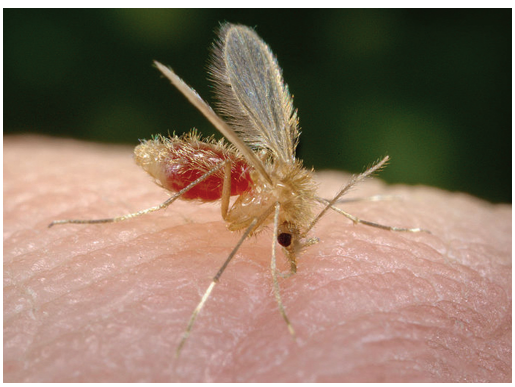
- A pair of antennae, short retractile proboscis, compound eye
- Thorax bears a pair of wings and three pairs of hairy legs
- Act as mechanical transmitters of diseases like enteric fever, gastroenteritis causing agents, trachoma, myiasis, etc.

Housefly

Tsetse fly

Characteristic Features of Tsetse Fly

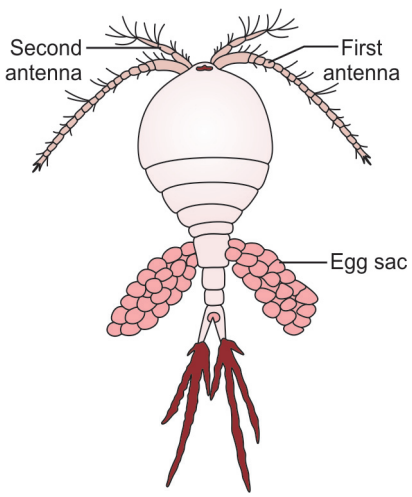
- Yellow or dark brown—resembles housefly
- Wings when folded overlap each other like blades of scissors
- Rigid, nonretractile proboscis adapted for skin piercing
- Disease transmitted trypanosomiasis—"sleeping sickness".



Sandfly

Characteristic Features of Sandfly

- Small insect 1.5–2 mm in length—body and wings densely haired
- Long slender and hairy antennae, palpi and proboscis
- Thorax bears a pair of wings and three pairs of legs
- Wings are upright and lanceolate, hairy—second longitudinal vein branches twice with first branching at the middle of the wing—characteristic of the genus *Phlebotomus*.
- Legs are long and slender and out of proportion to the body
- Sandflies hop and do not fly.
- Transmit leishmaniasis.



Cyclops

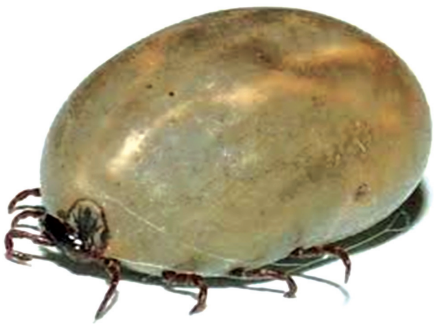
Characteristic Features of Cyclops

- Not more than 1 mm in length
- Pear-shaped semi-transparent body
- Forked tail
- 2 pairs of antennae
- 5 pairs of legs
- A small pigmented eye
- Transmits dracunculiasis and fish tapeworm (*Diphyllobothrium latum*) infestation

Hard tick

Characteristic Features of Hard Tick

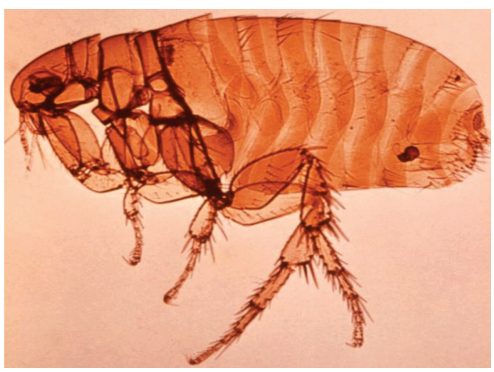
- Scutum present—entire in males, small portion in front in females
- Head anterior
- Several hundred or thousand eggs laid at one sitting
- Cannot starve—bites day and night
- Diseases transmitted: Babesious, tularemia, tick typhus Lyme disease



Soft tick

Characteristic Features of Soft Tick

- Scutum absent
- Head ventral—not seen from above
- Laid in batches 20–100 over a long period of time
- Can starve for a year or more
- Diseases transmitted—borreliasis, relapsing fever, Q-fever, KFD



Rat flea

Characteristic Features of Rat Flea

- The insect is laterally flattened
- Conical head without a neck—piercing mouth parts project downwards and are conspicuous
- Three segmented thorax—pro-, meso- and meta-thorax—no wings
- Three pairs of strong legs
- 10 segmented abdomen—males have a coiled structure—the penis; females have a short, stumpy structure—the spermatheca
- **Cannot fly—jumps** vertically 4 inches when starved or 3 inches when fed and can jump horizontally up to 6 inches
- Diseases transmitted: Endemic typhus and bubonic plague.

Itch mite

Characteristic Features of Itch Mite

- 0.4 mm size
- **Body:** No demarcation between cephalothorax and abdomen.
  - 2 pairs of legs in front—suckers
  - 2 pairs of legs behind—long bristle
- Male has sucker in all the legs except the 3rd pair which distinguishes it from female.
- Disease transmitted: Scabies

Characteristic Features of Louse

- Head is pointed in front, 5 jointed antennae, mouth parts adapted for sucking blood
- Thorax fused mass, square-shaped
- Strongly built three pairs of legs with claws
- 9-segmented elongated abdomen
- **Diseases transmitted:**
  - Epidemic typhus
  - Relapsing fever (epidemic)
  - Trench fever
  - Dermatitis
- **Species:**
  - Head louse—*Pediculus capitis*
  - Body louse—*Pediculus corporis*
  - Pubic or crab louse—*Phthirus pubis*

Head louse

Body louse

CHAPTER

14

Competency: MI1.1, 1.7 **Date:**

Demonstration of Methods for Diagnosis of Viral Infections

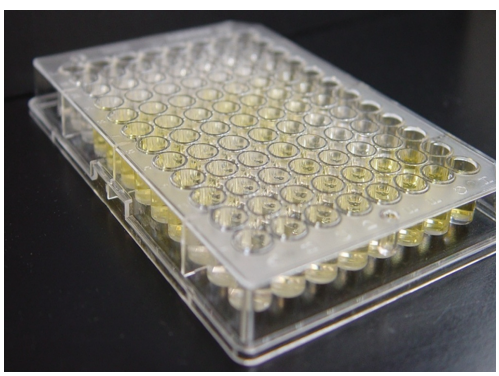
Aim: To study the different routes of inoculation for isolation of viruses from embryonated hen's egg and different methods for diagnosis of viruses.

Demonstration

1. Tissue culture bottle
2. Viral transport medium
3. Microtitre plate
4. Methods of inoculation of embryonated eggs
5. Hemagglutination test
6. Multinucleated giant cells of HSV
7. Negri body

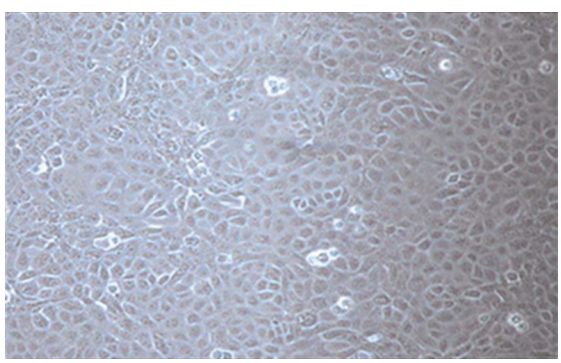
Microtitre Plate

- It is a flat plate with multiple "wells" (96 wells).
- Made of polystyrene, polypropylene or polycarbonate.
- Wells have either a conical/flat/round bottom.
- Uses:
  - ELISA for diagnosis of HIV, HBV, HCV.
  - Hemagglutination test
  - Hemagglutination inhibition test
  - Complement fixation.
  - TPHA test (*Treponema pallidum* hemagglutination)



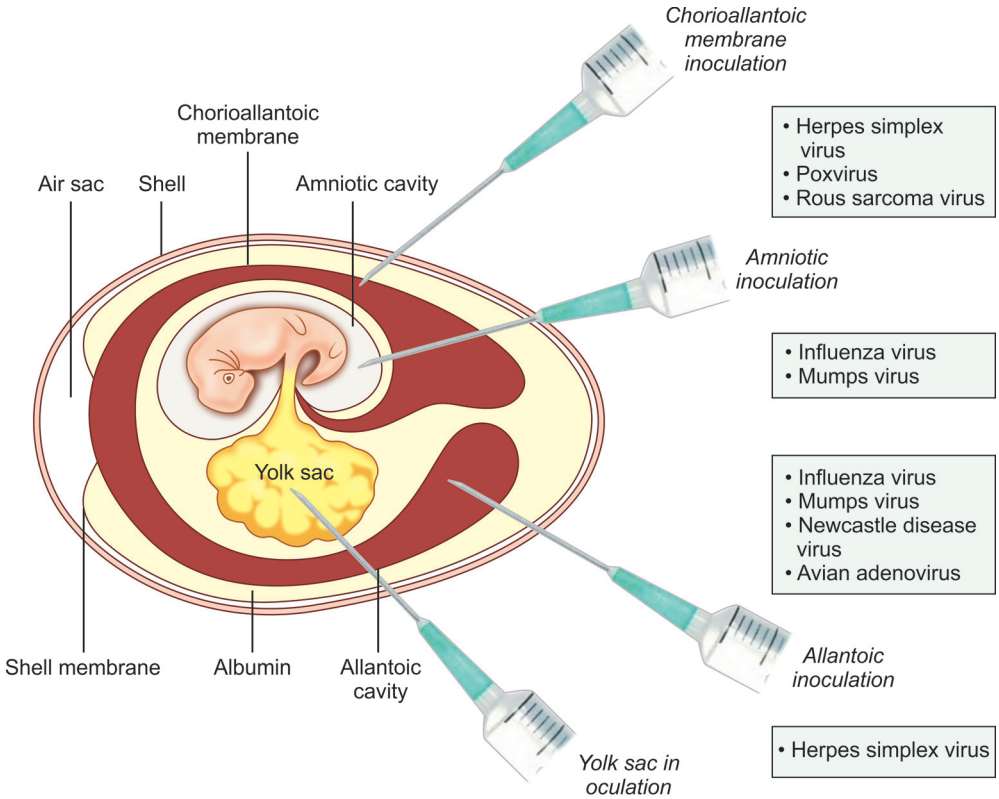
Microtitre plate

Tissue culture bottles

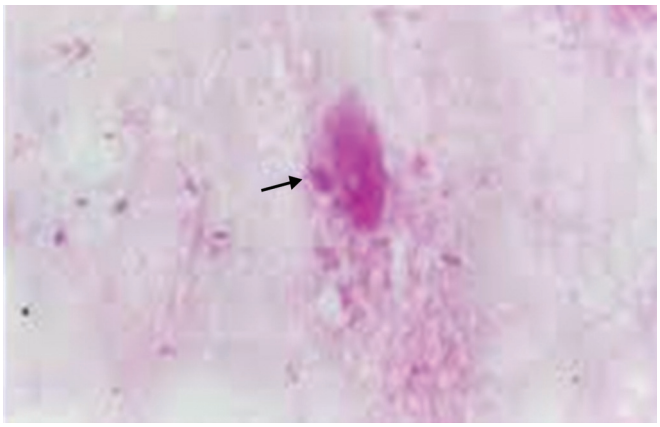


A monolayer of vero cell (vervet monkey kidney cell, continuous cell line)

Viral transport medium (pink to red)



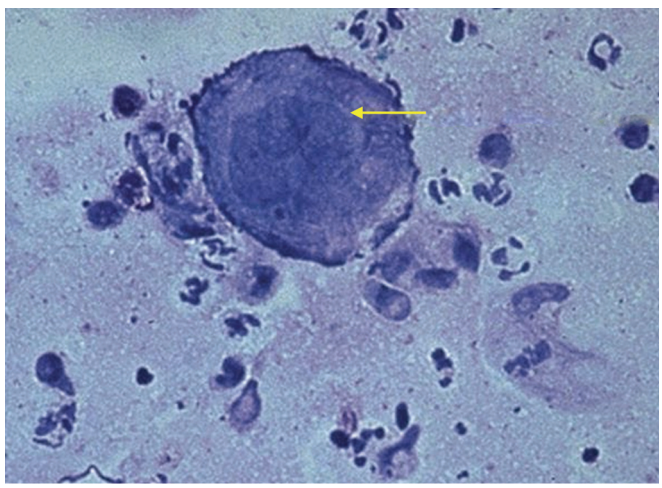
Growth of viruses in embryonated hen's egg
An embryonated chicken egg showing the different compartments in which viruses may grow. The different routes by which viruses are inoculated into eggs are indicated.



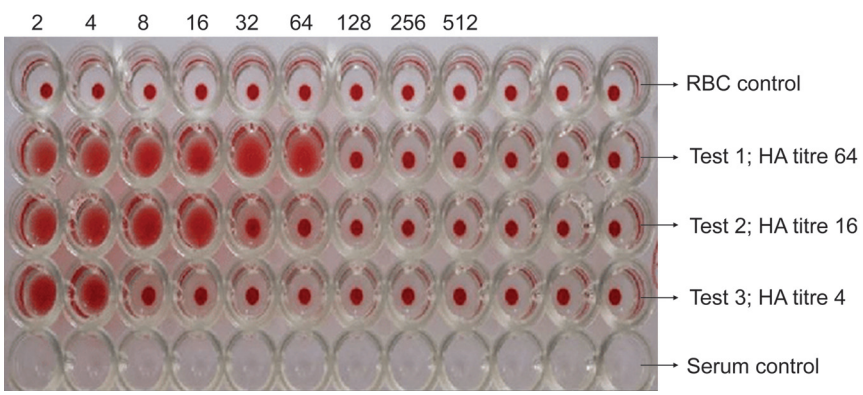
Impression smear from hippocampus of a rabid dog showing magenta colored intracellular Negri body (arrow) in a neuron. Seller's stain

Negri Body

- Specific intracytoplasmic eosinophilic inclusion body found in the cytoplasm of large neurons (1–10/cell) in rabies
- 2–20 µm, sharply defined, spherical/oval/elongated
- Cherry red to magenta colored, uniformly stained
- Larger Negri bodies contain blue staining granules/inner bodies, often arranged in concentric layers
- Part of the brain that best demonstrates Negri bodies is the Ammon horn of hippocampus
- Stain used: Seller stain
- Negri bodies are found in infection with street virus but not fixed virus
- Sensitivity of Negri bodies in rabies is 65–68%.



Giemsa stained Tzanck smear from genital ulcer showing multinucleated giant cells of HSV-2



Hemagglutination test

CHAPTER 15

Competency: MI1.8, 8.15 **Date:**

Antigen–Antibody Reactions

IMMUNOLOGY

Antigen (Ag)–antibody (Ab) reaction (conventional)—agglutination and precipitation
Antigen–antibody reaction (newer)—ELISA, ELFA, CLIA, IFA, Western blot, rapid methods.

ANTIGEN–ANTIBODY REACTION

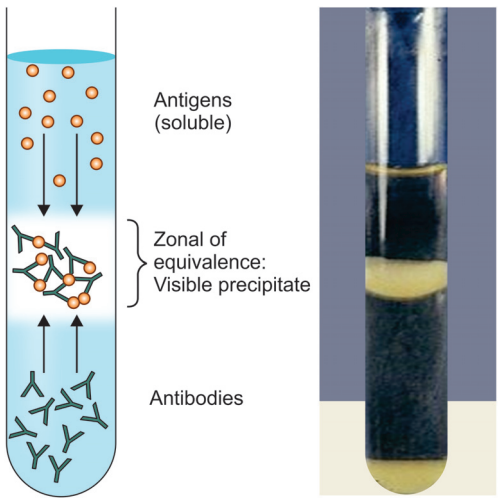
At the end of the session, the students shall be able to:

1. Enumerate different immunological tests available for diagnosis of infectious diseases.
2. Describe the principle, types and application of agglutination test.
3. Describe the principle, and application of precipitation test.
4. Describe the principle, application of ELISA test.
5. Describe the principle, types and application of immunofluorescence test.

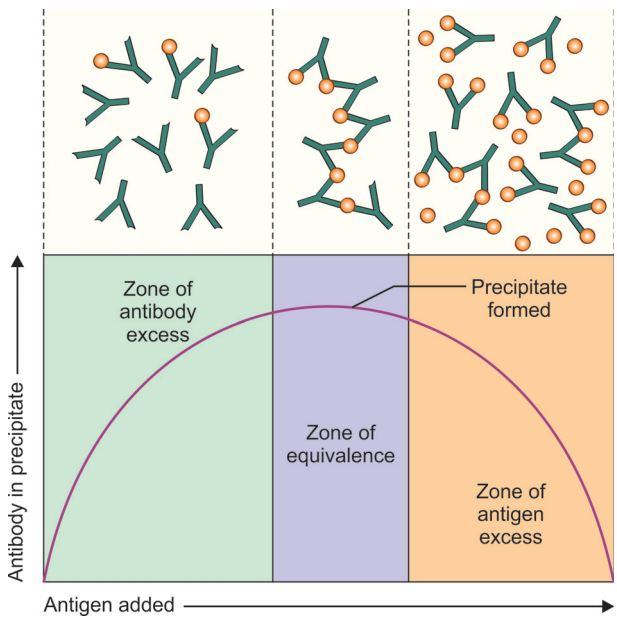
CONVENTIONAL METHODS

PRECIPITATION REACTIONS

When a **soluble antigen** reacts with its antibody in the presence of optimal temperature, pH, electrolytes, it leads to formation of antigen–antibody complex.



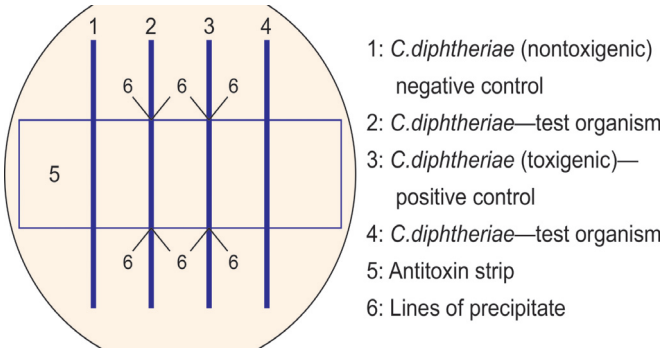
Precipitation test



Precipitation curve

TYPES

- Immunodiffusion: On gel or agar medium insoluble precipitation bands are seen. Or in presence of electric current is known as immune electrophoresis.
- Flocculation: Insoluble floccules form in liquid medium.



Elek's gel precipitation test

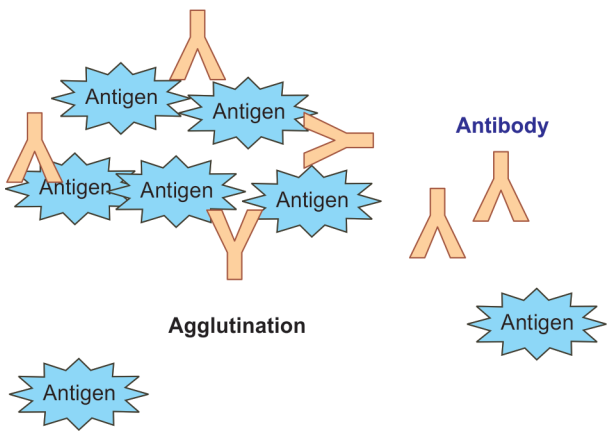
Slide Flocculation Test

Procedure: A drop of antigen is mixed with a drop of patients serum containing antibody on a slide, the precipitate formed remain suspended as floccules.

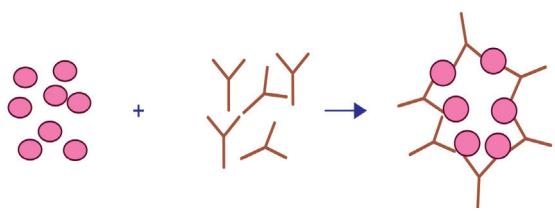
Venereal Disease Research Laboratory (VDRL) test.

AGGLUTINATION REACTION

When a **particulate or insoluble antigen** is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are agglutinated.



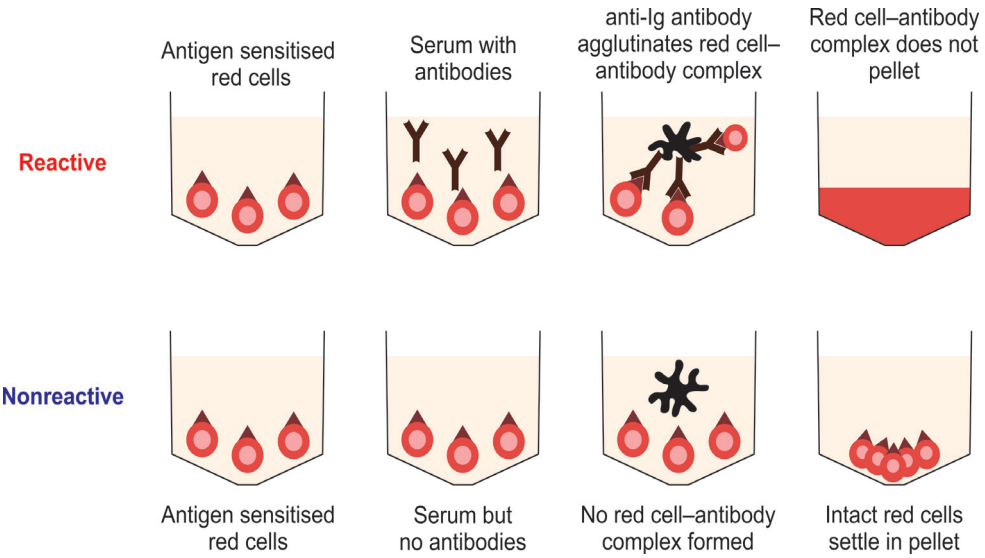
- **Direct agglutination reaction** (slide or tube agglutination reaction)



Direct agglutination reaction

- **Indirect or passive agglutination test:** For antibody detection

a. Indirect haemagglutination assay (IHA) reaction



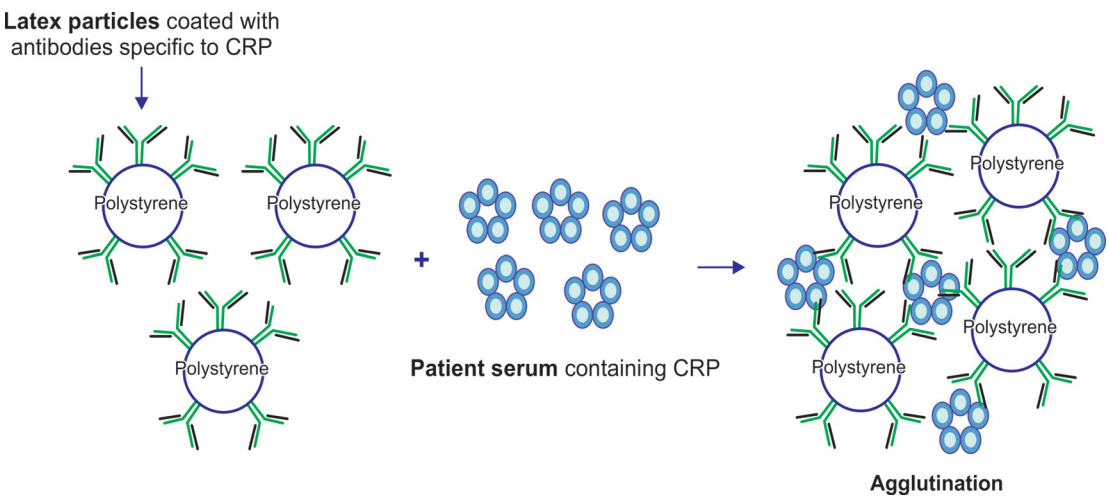
Indirect haemagglutination

b. Latex agglutination test (LAT)/reaction: For antibody

- **Reverse passive agglutination test:** For antigen detection

(a) Reverse passive haemagglutination assay (RPHA)/test: HBsAg detection

(b) Latex agglutination test, e.g. CRP, Cryptococcus antigen



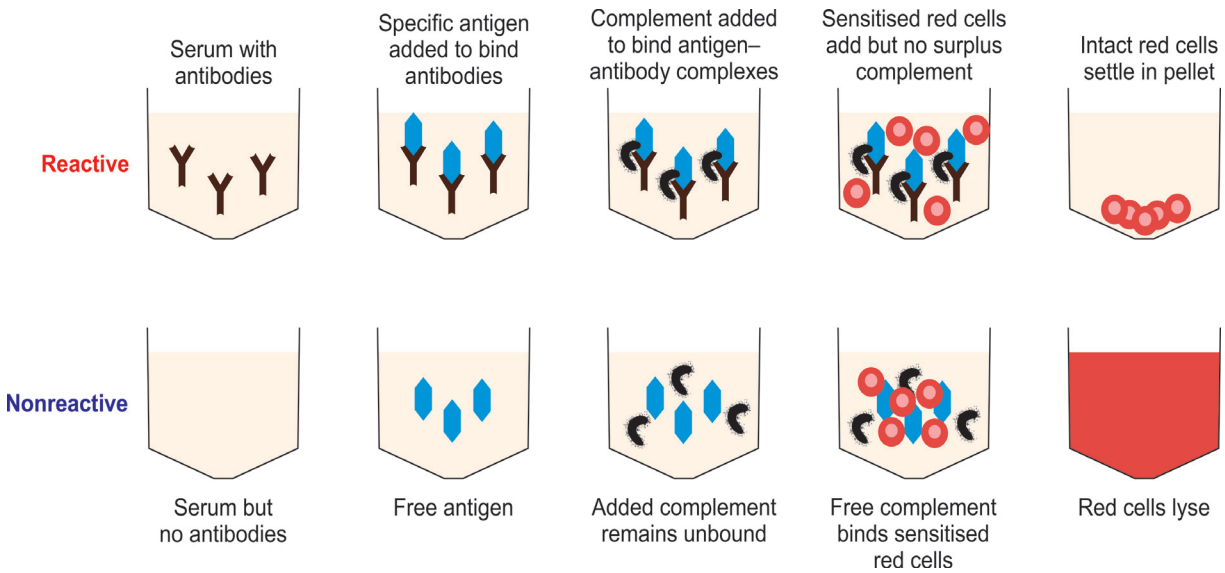
c. Conglutination test: *Staphylococcus aureus* detection

- **Haemagglutination Test**

(a) Direct haemagglutination test, e.g. Paul-Bunnell test, blood group

COMPLEMENT FIXATION TEST

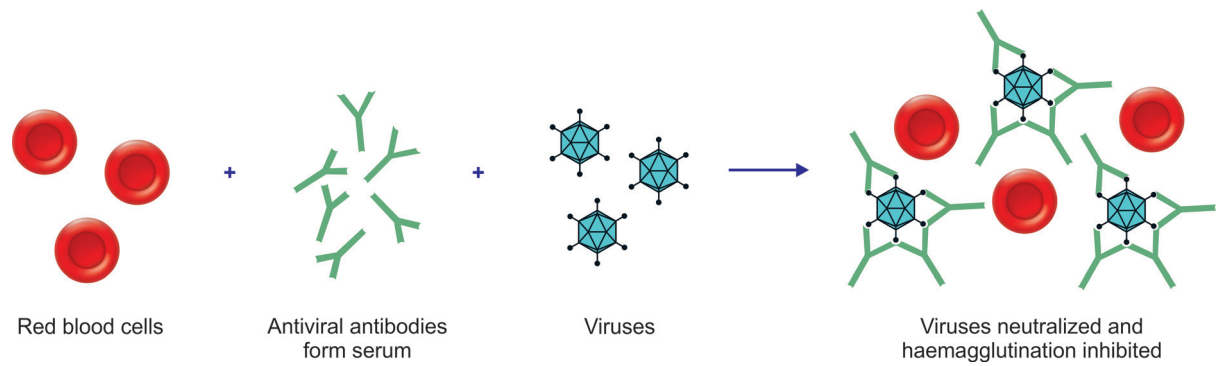
Detection of antibodies to Chlamydia, Mycoplasma, arboviral infections.



Complement fixation test

NEUTRALIZATION TEST

a. Viral neutralization test
b. Plaque inhibition test
c. Toxin-antitoxin neutralization test (ASO, Schick test, Nagler's reaction)
d. Haemagglutination inhibition (HAI) test: Influenza



NEWER TECHNIQUES

Enzyme-linked Immunosorbent Assay

It detects either the antigen or the antibody in the specimen by using enzyme–substrate–sorbent–chromogen system for detection.

Principle of ELISA: An adsorbing material is used (polystyrene plate) to adsorb antigen or antibody. Then enzyme is used to label these components. It reacts with the substrate which activates the chromogen to produce color (e.g. horseradish peroxidase).

Substrate chromogen system is added to complete the reaction. The color change is detected by spectrophotometry.

Ag + Ab complex–enzyme–substrate–activates chromogen–color–detected by ELISA reader.

TYPES OF ELISA

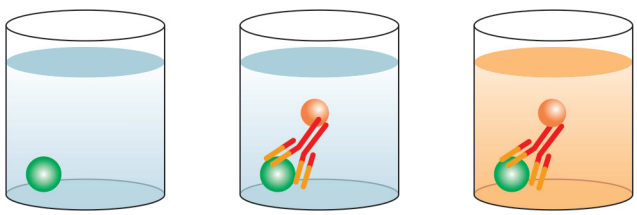
- **Direct ELISA:** In a direct ELISA, an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein. Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample. Since only one antibody is used in a direct ELISA, they are less specific than a sandwich ELISA.

  When to use: Assessing antibody affinity and specificity. Investigating blocking/inhibitory interactions.

  Advantages: Fast and simple protocol

  Disadvantages: Less specific since you are only using 1 antibody.

  Potential for high background if all proteins from a sample are immobilized in well.

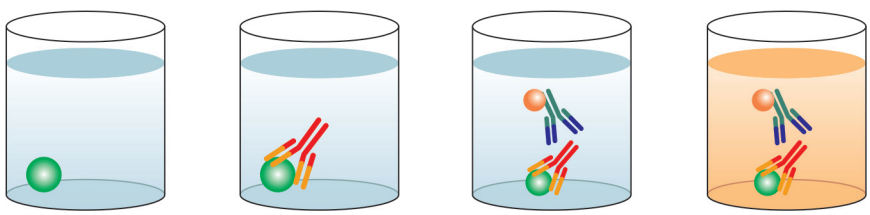


- **Indirect ELISA:** An indirect ELISA is similar to a direct ELISA in that an antigen is immobilized on a plate, but it includes an additional amplification detection step. First, an unconjugated primary detection antibody is added and binds to the specific antigen. A conjugated secondary antibody directed against the host species of the primary antibody is then added. Substrate then produces a signal proportional to the amount of antigen bound in the well.

  When to Use: Measuring endogenous antibodies.

  Advantages: Amplification using a secondary antibody.

  Disadvantages: Potential for cross-reactivity caused by secondary antibody.

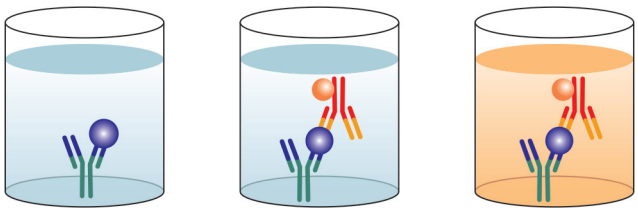


- **Sandwich ELISA:** Sandwich ELISAs are the most common type of ELISA. Two specific antibodies are used to sandwich the antigen, commonly referred to as matched antibody pairs. Capture antibody is coated on a microplate, sample is added, and the protein of interest binds and is immobilized on the plate. A conjugated-detection antibody is then added and binds to an additional epitope on the target protein. Substrate is added and produces a signal that is proportional to the amount of analyte present in the sample. Sandwich ELISAs are highly specific, since two antibodies are required to bind to the protein of interest.

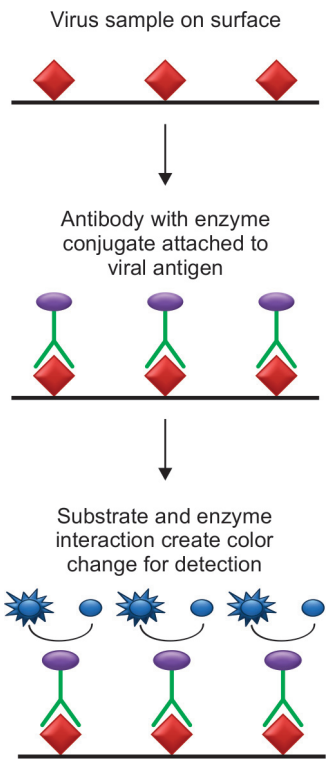
  When to Use: Determining analyte concentration in a biological sample.

  Advantages: Highest specificity and sensitivity. Compatible with complex sample matrices.

Disadvantages: Longer protocol. Challenging to develop.



- **MAC ELISA**



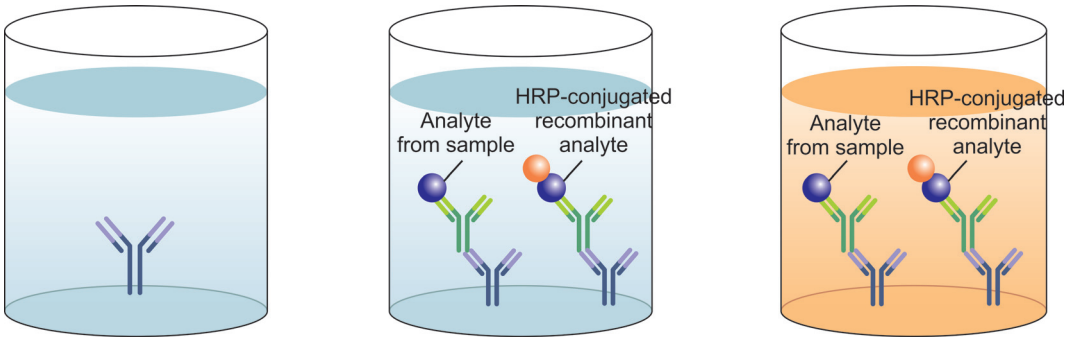
- **Competitive ELISA**

  Competitive ELISAs are commonly used for small molecules, when the protein of interest is too small to efficiently sandwich with two antibodies. Similar to a sandwich ELISA, a capture antibody is coated on a microplate. Instead of using a conjugated detection antibody, a conjugated antigen is used to complete for binding with the antigen present in the sample. The more antigen present in the sample, the less conjugated antigen will bind to the capture antibody. Substrate is added and the signal produced is inversely proportional to the amount of protein present in the sample.

  When to Use: Determining concentrations of a small molecules and hormones.

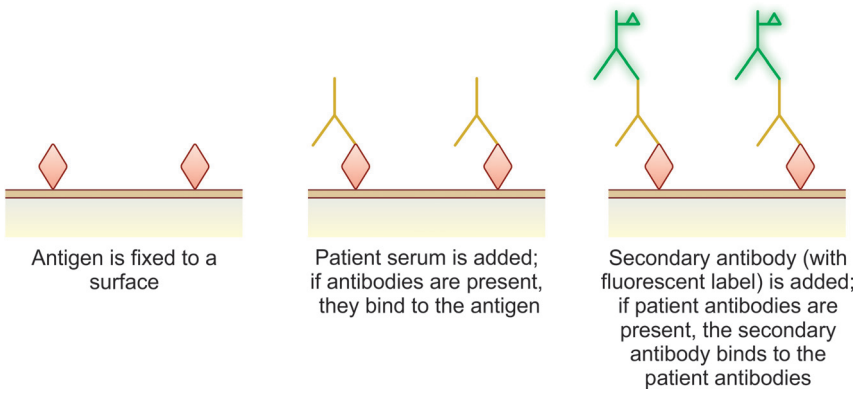
  Advantages: Ability to quantitate small molecules.

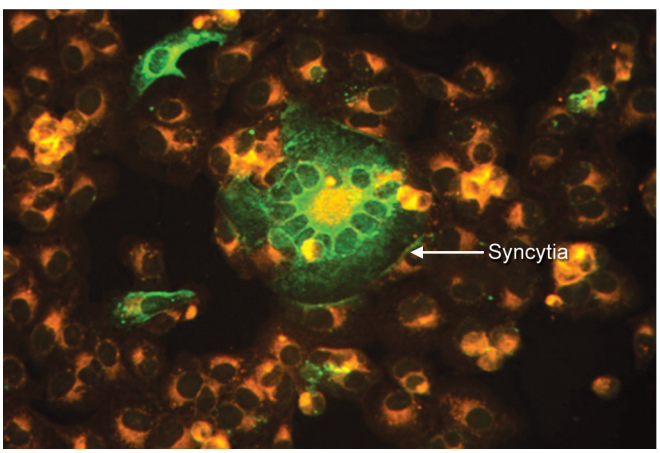
  Disadvantages: Less specific since you are only using 1 antibody. Requires a conjugated antigen.



IMMUNOFLUORESCENCE ASSAY (IFA)

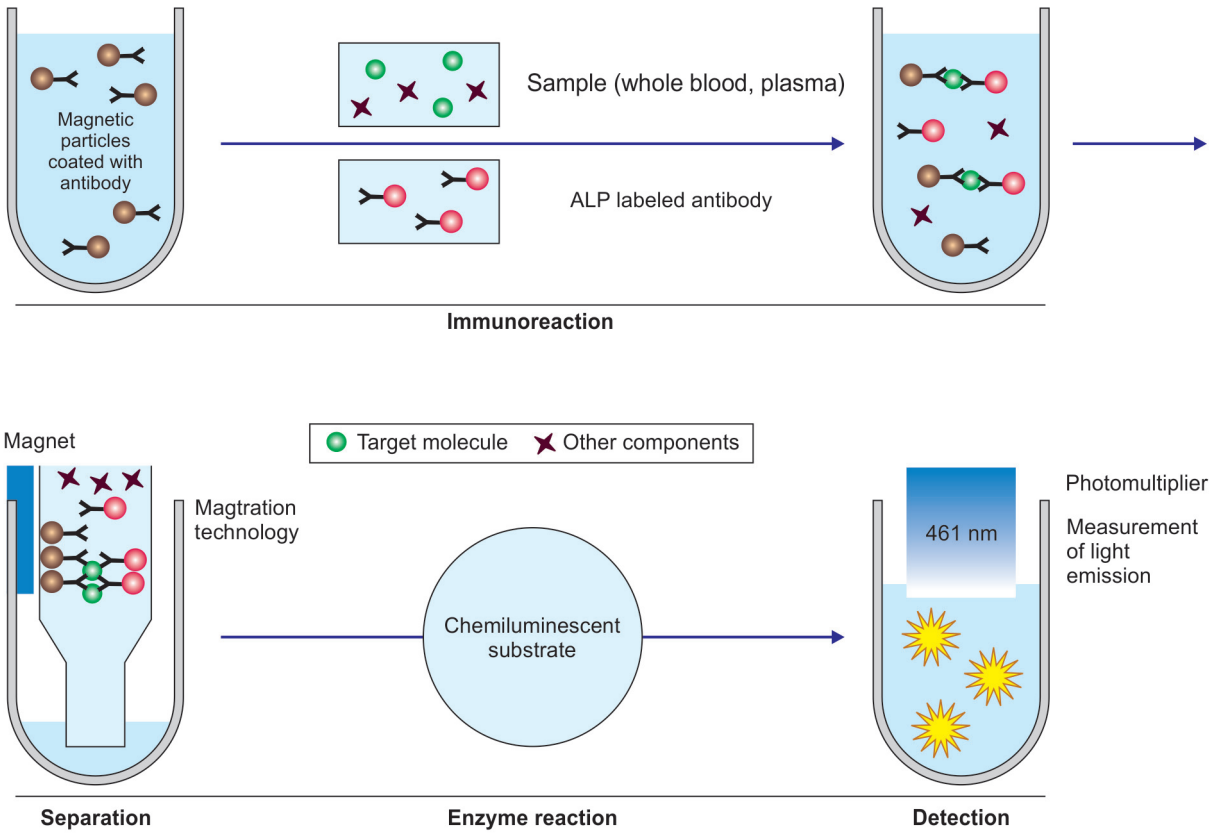
Principle: Specific Ab binds to the Ag of interest. Fluorescent dyes are coupled to these Ag–Ab complexes in order to visualize the Ag of interest using immunofluorescent microscope.





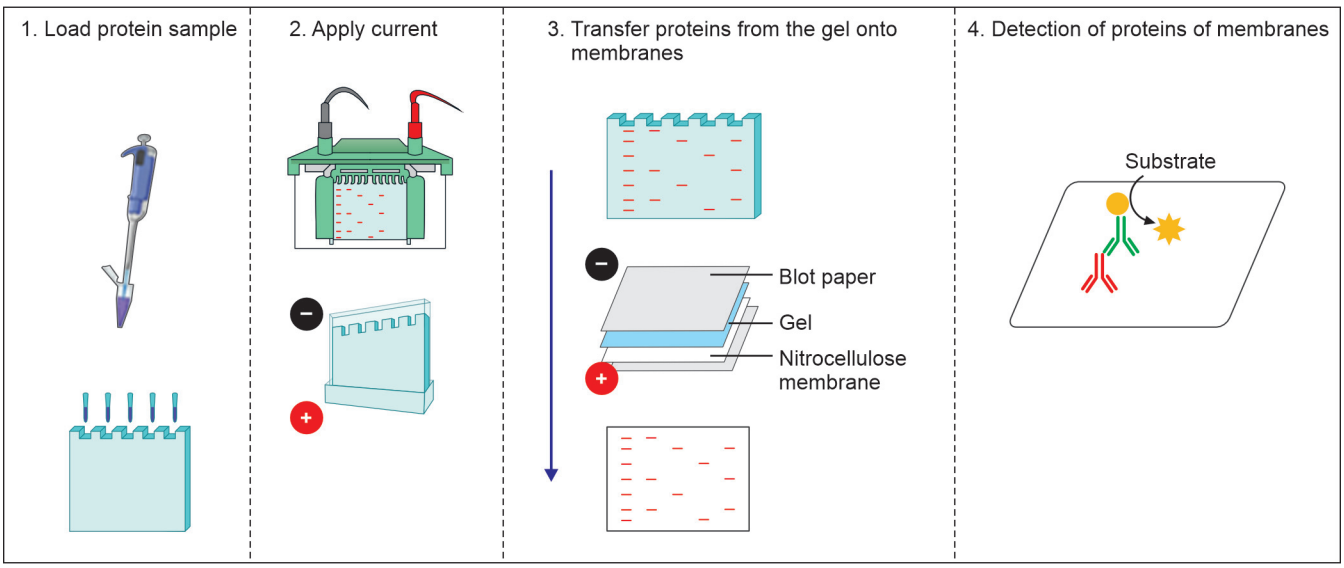
CHEMILUMINESCENCE-LINKED IMMUNOASSAY (CLIA)

Principle: In the presence of complimentary Ag and Ab, the paratope of the Ab binds to the epitope of the Ag to form Ag–Ab complex which is estimated using labeled Ab, chemiluminescent substrates and H_2O_2 as enhancer resulting in generation of light whose intensity is directly proportional to amount of labeled immune complex, e.g. detection of SARS-CoV-2 Ab.



WESTERN BLOT

Also called protein immunoblotting used to detect specific proteins in a given sample.



Principle: Uses SDS-PAGE to separate various proteins in a sample based on their molecular weight which are then blotted or transferred onto a matrix (generally NC paper) where they are stained with Ab specific to the target protein by immunoassay. WB can detect as low as 1 ng of target protein due to high resolution of gel electrophoresis and strong specificity and high sensitivity of the immunoassay, e.g. p-24 Ag of HIV in window period.