B.Sc. ZOOLOGY
ELECTIVE I
MEDICAL LABORATORY TECHNIQUES (MLT) I

UNIT I

General and personal care in the laboratory.

Laboratory instruments: Autoclave, hot air oven, incubators, water bath, Centrifuge, Refrigerator, Colorimeter, H meter, Heamoglobinometer.

UNIT II

Preparation and uses of reagents – normal saline - Turkey's fluid, Hayem's fluid, Leishamn's stain Wright stain, Carnoy's fluid and Bovin's fluid - Acetocalamine.

UNIT III

RBC, WBC, Total count and Erythrocyte Sedimentation rate (ESR), platelet count, clotting time,

bleeding time. Blood pressure apparatus, ECG,

UNIT IV

Examination of urine and faeces –microscopic examination of sediments. Methods of bacterial culture. Examination of cerebrospinal fluid, Semen analysis, sperm motility- sperm count and morphology.

UNIT V

Examination of parasites - Malarial parasites, Plasmodium, *Endameba histolytica*, *Ascaris lumbricoids, Taenia solium.*

REFERENCES:

1. Medical Laboratory Technology vol I, II, III –Kanai L. Mukherjee, Tata McGraw Hill Publishing Ltd.,New Delhi.

2. Medical Laboratory Technology – Ramanik Sood – Jaypee Brother's Medical Publishers (P) Ltd., NewDelhi.

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Basic Laboratory Safety

Objectives: Upon completion of the lecture, accompanying video, required readings and clinical rotations, the student will be able to:

1) List and describe the appropriate safety procedures practiced in the clinical laboratory that pertain to general laboratory safety and awareness:
   a) personal safety
   b) eye safety
   c) handling of biologically hazardous material
   d) handling of needles and sharps

2) Discuss the significance of OSHA to the regulation of safe practices within the clinical laboratory.

3) Define the term 'Universal Precautions' and state its importance in the handling of potential biohazardous materials.

4) Discuss appropriate safety procedures practiced in the clinical laboratory when handling all chemicals, flammables, ether and compressed gases.

5) Discuss the information provided by and the appropriate use of the Material Safety Data Sheet (MSDS).

6) Discuss appropriate safety precautions practiced in the clinical laboratory when handling radioactive materials.

7) Discuss appropriate procedures practiced in the clinical laboratory for fire, electrical, and severe weather safety.

READING:
Linne & Ringsrud, Clinical Laboratory Science – The Basics and Routine Techniques, 4th ed., 1999, C. V. Mosby, pp 23-42.

SAFETY IN THE LABORATORY

Purpose: Faulty technique is one of the chief causes of accidents and, because it involves the human element, is one of the most difficult to cope with. The purpose of this discussion is to help the student understand proper laboratory safety, to increase his awareness of the possible risks or hazards involved with laboratory work and to realize the laboratory is generally a safe place to work if safety guidelines are properly followed.

I. Standard Operating Procedures

A. General Personal Safety

1. Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in areas where specimens are handled.

2. Food and drink are not stored in refrigerators, freezers, cabinets, or on shelves, countertops, or bench tops where blood or other potentially infectious materials are stored or in other areas of possible contamination.

3. Long hair, ties, scarves and earrings should be secured.

4. Keep pens and pencils OUT OF YOUR MOUTH!!

5. Appropriate Personal Protective Equipment (PPE) will be used where indicated:

   Lab coats or disposable aprons should be worn in the lab to protect you and your clothing from contamination. Lab coats should not be worn outside the laboratory.

   Lab footwear should consist of normal closed shoes to protect all areas of the foot from possible puncture from sharp objects and/or broken glass and from contamination from corrosive reagents and/or infectious materials.

   Gloves should be worn for handling blood and body fluid specimens, touching the mucous membranes or non-intact skin of patients, touching items or surfaces soiled with blood or body fluid, and for performing venipunctures and other vascular access procedures. Cuts and abrasions should be kept bandaged in addition to wearing gloves when handling biohazardous materials.

   Protective eyewear and/or masks may need to be worn when contact with hazardous aerosols, caustic chemicals and/or reagents is anticipated.

6. **NEVER MOUTH PIPETTE!!** Mechanical pipetting devices must be used for pipetting all liquids.

7. Frequent hand washing is an important safety precaution, which should be practiced after contact with patients and laboratory specimens.

   Proper hand washing techniques include soap, running water and 10-15 seconds of friction or scrubbing action. Hands should be dried and the paper towel used to turn the faucets off.

   Hands are washed:

   a. After completion of work and before leaving the laboratory.
   b. After removing gloves.
   c. Before eating, drinking, smoking, applying cosmetics, changing contact lenses or using lavatory facilities.
   d. Before all other activities which entail hand contact with mucous membranes or breaks in the skin.
   e. Immediately after accidental skin contact with blood or other potentially infectious materials.
   f. Between patient contact and before invasive procedures.

8. Laboratory work surfaces must be disinfected daily and after a spill of blood or body fluid with a 1:10 dilution of Clorox in water.

B. Eye Safety

1. **KNOW WHERE THE NEAREST EYE WASH STATION IS LOCATED AND HOW TO OPERATE IT.**

2. **Eye goggles should be worn:**

   a. When working with certain caustic reagents and/or solvents, or concentrated acids and bases.
   b. When performing procedures that are likely to generate droplets/aerosols of blood or other body fluid.
   c. When working with reagents under pressure.
   d. When working in close proximity to ultra-violet radiation (light).

3. Wearing contact lenses in the laboratory is discouraged and requires extra precaution if worn. Gases and vapors can be concentrated under the lenses and cause permanent eye damage. Furthermore, in the event of a chemical splash into an eye, it is often nearly impossible to remove the contact lens to irrigate the eye because of involuntary spasm of the eyelid. Persons who must wear contact lenses should inform their supervisor to determine which procedures would require wearing no-vent goggles.

C. Safe Handling of Biologically Hazardous Material

1. **YOU SHOULD HANDLE ALL PATIENT SAMPLES AS POTENTIALLY BIOHAZARDOUS MATERIAL.** This means UNIVERSAL PRECAUTIONS should be followed at all times!!

2. **When working in the laboratory:**

   a. Wear protective closing (lab coat, gloves. If you have a cut/abrasion, also wear a band-aid.
   b. Avoid spillage and aerosol formation.
   c. Hands should be washed immediately and thoroughly if contaminated with blood or other body fluids.
   d. Gloves should be removed before handling a telephone, computer keyboard, etc., and must NOT be worn outside the immediate work area. Hands should always be washed immediately after gloves are removed.
   e. You should wash your hands after completing laboratory activities and before leaving the area. All protective clothing should be removed prior to leaving the lab.
   f. All biohazardous material should be discarded in a biohazard bag to be autoclaved.
   g. All counter and table tops should be disinfected with a proper disinfecting solution:
      1) At the beginning of the day.
      2) If you should spill a patient sample.
      3) At the end of the day.

3. **When performing venipuncture:**

   a. Wear clean gloves for each patient you draw.
   b. Wash your hands whenever you change gloves.
   c. Dispose of contaminated needle, syringe and test tubes in a proper biohazardous receptacle.
   d. When drawing blood from a patient in an isolation room.

      1) All material taken into this room must remain in the room.
      2) Label all tubes drawn from this patient with isolation stickers.

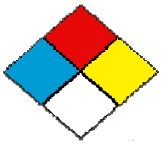
4. **Proper handling of SHARPS:**

   a. Contaminated needles and other sharps are never broken, bent, recapped or re-sheathed by hand.
   b. Used needles are not removed from disposable syringes.

c. Needles and sharps are disposed of in impervious containers located near the point of use.

II. Chemical and Gas Safety

To provide a safe working environment, all personnel should be aware of potentially hazardous materials and the proper way of handling this material. Avoid unnecessary exposure to chemicals. Occupational Safety and Health Administration (OSHA) requires any necessary information in the form of MATERIAL SAFETY DATA SHEETS (MSDS) concerning the handling of hazardous materials to be available to all laboratory personnel, so that they may achieve and maintain safe working conditions.



Flammable (Red); Instability (Yellow); Health (Blue) and Special Notice (White)

NFPA Chemical Hazard Sign

A. Toxic and Corrosive Materials (acids and alkali):

Toxic or Poison Hazard

Corrosive Hazard

1. To avoid dangerous splatter, **ALWAYS ADD ACID TO WATER!**
2. Toxic materials should be labeled with special tape when used in compounded reagents and stored in separate containers. These materials should be handled carefully and kept in the hood during preparation.
3. Acids and alkali should be carried by means of special protective carriers when transported.
4. Acid and alkali spills should be covered and neutralized by using the material from the 'spill bucket'. All material, spill and compound, should be swept up and placed in a plastic bucket for proper disposal.
5. In case of spillage, wash all exposed human tissue (including eyes) generously with water and notify your supervisor for proper reporting of the incident.

B. Carcinogens

1. All laboratory chemicals identified as carcinogens must be labeled **CARCINOGEN**.
2. When working with these substances, protective clothing and gloves should be worn.

C. **Flammable Compounds**

1. All flammable reagents should be kept in the flammable storage facilities (closet or refrigerator) at all times when not in use.
2. Any solutions compounded from these reagents should be labeled as flammable.
3. Flammable substances should be handled in areas free of ignition sources.
4. Flammable substances should never be heated using an open flame.
5. Ventilation is one of the most effective ways to prevent accumulation of explosive levels of flammable vapors. An exhaust hood should be used whenever appreciable quantities of flammables are handled.
6. Flammable compounds should be placed in proper receptacle for disposal.

D. **Ether Precautions (flammable compound)**

1. These compounds tend to react with oxygen to form explosive peroxides. When ether containers are opened they are to be dated and all material remaining after six (6) months must be disposed of immediately.
2. Disposal of ether compounds is through the Hazardous Materials Office.
3. Ether compounds will be stored in an explosion-proof refrigerator. (boiling point of ether is approximately room temperature)

E. **Compressed Gases**

1. The storage of all compressed gases shall be in containers designed, constructed, tested and maintained in accordance with the U.S. Department of Transportation Specifications and Regulations.
2. In the laboratory, gas containers are to be limited to the number of containers in use at any time. Low pressure (LP) gases shall also be limited to the smallest size container.
3. Containers shall be securely strapped, chained or secured in a cylinder stand so they cannot fall.
4. Oxidizing gases should be separated from flammable gasses.

III. Radiation Safety

A. No eating, drinking, smoking permitted!
B. Radioactive material should be labeled as radioactive and stored in a proper container so as to prevent spillage or leakage.
C. These materials must be handled carefully. Remember: **the amount of radiation exposure decreases with distance.**
D. Radioactive spills should be absorbed with absorbent toweling. The area should be cleaned with soap and water and then decontaminated with a product such as

'count-off'. The area of the spill is then monitored for any residual radioactivity. If the area is not decontaminated, the above regimen is repeated and re-monitored.

E. In the case of a radioactive spill in a high traffic area, the area will be 'roped off' until proper decontamination has been achieved.

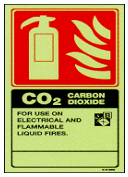
F. In the case of a major radioactive spill, all personnel in the area must be notified. The appropriate safety officer must be notified and all attempts to keep contamination at a minimum must be used.

IV. Fire Safety

A. KNOW WHERE ALL FIRE EXITS, FIRE EXTINGUISHERS AND FIRE ALARMS ARE LOCATED!

B. **KNOW HOW TO PROPERLY OPERATE APPROPRIATE FIRE ALARMS AND FIRE SAFETY EQUIPMENT!**

Portable fire extinguishers are classified by their ability to handle specific classes of fires:



For burning combustible materials (wood, paper, clothing, trash). **GREEN TRIANGLE WITH THE LETTER 'A'**, uses water or an all-purpose dry chemical.

For burning liquids: **RED SQUARE WITH THE LETTER 'B',** uses foam, a dry chemical or carbon dioxide.

For electrical fires: **BLUE CIRCLE WITH THE LETTER 'C'** uses non-conducting extinguishing agents (carbon dioxide or a dry chemical).

Multipurpose: Recommended for all types of fire. Most common extinguisher found in most clinical laboratories.

C. Know the proper procedure for notifying colleagues and proper personnel of a fire.

R A C E

1. **R**escue those in danger
2. **A**larm
   a. Activate the fire pull station
   b. Notify switchboard operator of the location, your name and the type of fire, if known
3. **C**ontain the fire by closing all doors and windows
4. **E**xtinguish the fire, if possible. Do not re-enter a room that has already been closed.

   Evacuate

V. Electrical Safety

A. The use of extension cords is prohibited.
B. All equipment must be properly grounded.
C. Never operate electrical equipment with fluid spillage in the immediate are or with wet hands.
D. Never use plugs with exposed or frayed wires.
E. If there are sparks or smoke or any unusual evens occur, shut down the instrument and notify the manager or safety officer. Electrical equipment that is not working properly should not be used.
F. If a person is shocked by electricity, shut off the current or break contact with the live wire immediately. Do not touch the victim while he is in contact with the source of current unless you are completely insulated against shock. If the victim is unconscious, call **911 (at UTMB)** to report the incident and request assistance.

VI. Severe Weather Safety

A. When the tornado-warning message is heard on the hospital public address system, all personnel should move to a safe area. Safe areas are considered to be:
    1. below ground level if possible
    2. inside, interior halls in an east/west corridor, away from windows
    3. inside, interior windowless rooms
B. Stair towers should be used for evacuation
C. Elevators should be used only in emergency
D. No one will leave the building until the 'all clear' is announced

VII. General Procedures and Equipment

A. Cracked or chipped glassware should not be used.
B. Centrifuges should not be used without the covers completely closed.
C. When removing tops from evacuated test tubes, care must be taken to prevent aerosol formation.

VIII. In Case of Accidents

A. Accidental Needle Stick
    1. Bleed wound.
    2. Wash wound thoroughly with soap.
    3. Notify the supervisor of the incident and report to Student Health with an incident report form.
    4. May need to get blood tested for hepatitis.

B. If you should wound yourself in the laboratory:
    1. Any type of accident should be brought to the attention of the Teaching Supervisor of the area.

IX. Summary…….USE COMMON SENSE!!!

Autoclave Definition

An autoclave is a machine that provides a **physical method of sterilization** by killing bacteria, viruses, and even spores present in the material put inside of the vessel using steam under pressure.

- Autoclave sterilizes the materials by heating them up to a particular temperature for a specific period of time.
- The autoclave is also called a steam sterilizer that is commonly used in healthcare facilities and industries for various purposes.
- The autoclave is considered a more effective method of sterilization as it is based on moist heat sterilization.

Autoclave Parts/ Components

The simplest form of the autoclave is the pressure cooker types or laboratory bench autoclaves. The following is the detailed description of different components/ parts of an autoclave:

a. Pressure Chamber

- The pressure chamber is the main component of a steam autoclave consisting of an inner chamber and an outer jacket.
- The inner chamber is made up of stainless steel or gunmetal, which is present inside the out chamber made up of an iron case.
- The autoclaves used in healthcare laboratories have an outer jacket that is filled with steam to reduce the time taken to reach the sterilization temperature.
- The inner chamber is the case where the materials to be sterilized are put.
- The size of the pressure chamber ranges from 100 L to 3000 L.

b. Lid/ Door

- The next important component of an autoclave is the lid or door of the autoclave.
- The purpose of the lid is to seal off the outside the atmosphere and create a sterilized condition on ht inside of the autoclave.
- The lid is made airtight via the screw clamps and asbestos washer.
- The lid consists of various other components like:

Pressure gauge

- A pressure gauge is present on the lid of the autoclave to indicate the pressure created in the autoclave during sterilization.
- The pressure gauge is essential as it assures the safety of the autoclave and the working condition of the operation.

Pressure releasing unit/ Whistle

- A whistle is present on the lid of the autoclave is the same as that of the pressure cooker.
- The whistle controls the pressure inside the chamber by releasing a certain amount of vapor by lifting itself.

Safety valve

- A safety valve is present on the lid of autoclave, which is crucial in cases where the autoclave fails to perform its action or the pressure inside increases uncontrollably.
- The valve has a thin layer of rubber that bursts itself to release the pressure and to avoid the danger of explosion.

c. Steam generator/ Electrical heater

- An electrical steam generator or boiler is present underneath the chamber that uses an electric heating system to heat the water and generate steam in the inner and the outer chamber.
- The level of water present in the inner chamber is vital as if the water is not sufficient; there are chances of the burning of the heating system.

- Similarly, if the water is more than necessary, it might interfere with the trays and other components present inside the chamber.

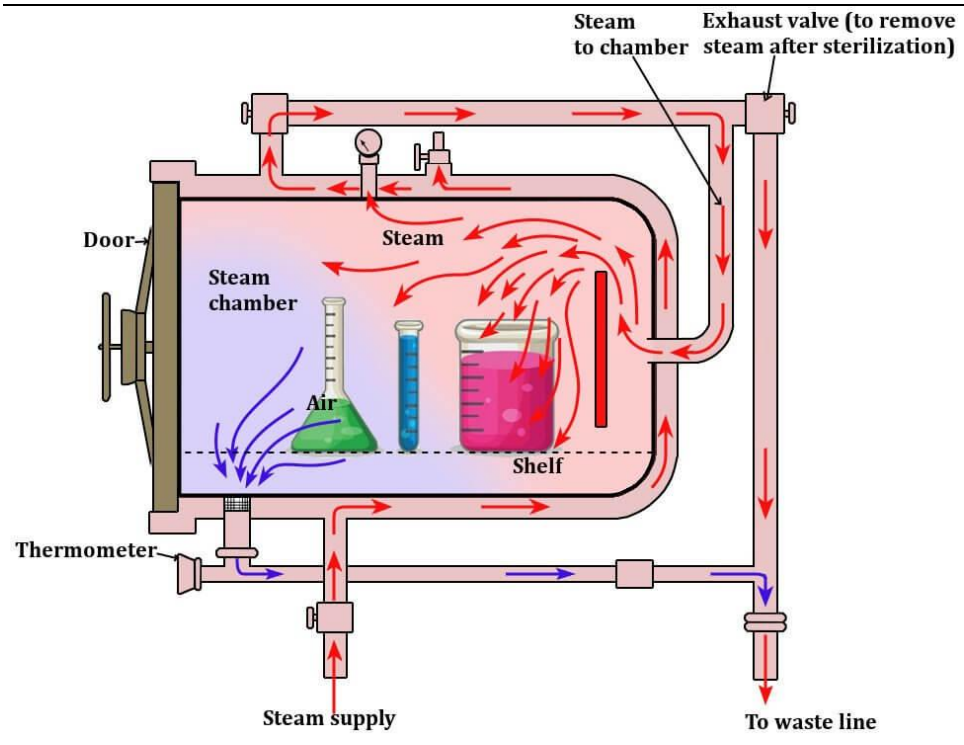
d. Vacuum generator (if applicable)

- In some types of autoclaves, a separate vacuum generator is present which pulls out the air from the inside of the chamber to create a vacuum inside the chamber.
- The presence of some air pockets inside the chamber might support the growth of different microorganisms. This is why the vacuum chamber is an important component of an autoclave.

e. Wastewater cooler

- Many autoclaves are provided with a system to cool the effluent before it enters the draining pipes.
- This system prevents any damage to the drainage pipe due to the boiling water being sent out of the autoclave.

Autoclave Principle/ Working



- The autoclave works on the principle of moist heat sterilization where steam under pressure is used to sterilize the material present inside the chamber.
- The high pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization.
- Water usually boils at 100°C under normal atmospheric pressure (760 mm of Hg); however, the boiling point of water increases if the pressure is to be increased.
- Similarly, the high pressure also facilitates the rapid penetration of heat into deeper parts of the material, and moisture present in the steam causes the coagulation of proteins causing an irreversible loss of function and activity of microbes.
- This principle is employed in an autoclave where the water boils at 121°C at the pressure of 15 psi or 775 mm of Hg.
- When this steam comes in contact on the surface, it kills the microbes by giving off latent heat.
- The condensed liquid ensures the moist killing of the microbes.
- Once the sterilization phase is completed (which depends on the level of contamination of material inside), the pressure is released from the inside of the chamber through the whistle.
- The pressure inside the chamber is then restored back tot eh ambient pressure while the components inside remain hot for some time.

Procedure for running an autoclave

In general, an autoclave is run at a temperature of 121° C for at least 30 minutes by using saturated steam under at least 15 psi of pressure. The following are the steps to be followed while running an autoclave:

1. Before beginning to use the autoclave, it should be checked for any items left from the previous cycle.
2. A sufficient amount of water is then put inside the chamber.

3. Now, the materials to be sterilized are placed inside the chamber.
4. The lid is then closed, and the screws are tightened to ensure an airtight condition, and the electric heater is switched on.
5. The safety valves are adjusted to maintain the required pressure in the chamber.
6. Once the water inside the chamber boils, the air-water mixture is allowed to escape through the discharge tube to let all the air inside to be displaced. The complete displacement can be ensured once the water bubbles cease to come out from the pipe.
7. The drainage pipe is then closed, and the steam inside is allowed to reach the desired levels (15 lbs in most cases).
8. Once the pressure is reached, the whistle blows to remove excess pressure from the chamber.
9. After the whistle, the autoclave is run for a holding period, which is 15 minutes in most cases.
10. Now, the electric heater is switched off, and the autoclave is allowed to cool until the pressure gauge indicates the pressure inside has lowered down to that of the atmospheric pressure.
11. The discharge pipe is then opened to allow the entry of air from the outside into the autoclave.
12. Finally, the lid is opened, and the sterilized materials are taken out of the chamber.

Video: How to use an autoclave (BioNetwork)

Types of autoclave

There are different types of autoclaves present in the market, some of which are:

Pressure cooker type/ Laboratory bench autoclaves (N-type)

- These, as domestic pressure cookers, are still in use in many parts of the world.

- The more modern type has a metal chamber with a secure metal lid that can be fastened and sealed with a rubber gasket.
- It has an air and steam discharge tap, pressure gauge, and safety valve. There is an electric immersion heater in the bottom of the chamber.

Gravity displacement type autoclave

- This is the common type of autoclave used in laboratories.
- In this type of autoclave, the steam is created inside the chamber via the heating unit, which then moves around the chamber for sterilization.
- This type of autoclave is comparatively cheaper than other types.

Positive pressure displacement type (B-type)

- In this type of autoclave, the steam is generated in a separate steam generator which is then passed into the autoclave.
- This autoclave is faster as the steam can be generated within seconds.
- This type of autoclave is an improvement over the gravity displacement type.

Negative pressure displacement type (S-type)

- This is another type of autoclave which contains both the steam generator as well as a vacuum generator.
- Here, the vacuum generator pulls out all the air from inside the autoclave while the steam generator creates steam.
- The steam is then passed into the autoclave.
- This is the most recommended type of autoclave as it is very accurate and achieves a high sterility assurance level.
- This is also the most expensive type of autoclave.

Figure: Types of Autoclave. Image Source: Microbe Online.

Uses of autoclave

Autoclaves are important devices to ensure the sterilization of materials containing water as they cannot be sterilized by dry heat sterilization. Besides, autoclaves are used for various other purposes.

1. They are used to decontaminate specific biological waste and sterilize media, instruments, and labware.
2. Regulated medical waste that might contain bacteria, viruses, and other biological materials are recommended to be inactivated by autoclaving before disposal.
3. In medical labs, autoclaves are used to sterilize medical equipment, glassware, surgical equipment, and medical wastes.
4. Similarly, autoclaves are used for the sterilization of culture media, autoclavable containers, plastic tubes, and pipette tips.

Advantages

- A dry heat cabinet is easy to install and has relatively low operating costs
- It penetrates materials
- It is nontoxic and does not harm the environment
- it is noncorrosive for metal and sharp instruments.

2. Biological controls: paper strips containing 10^6spores of Clostridium tetani

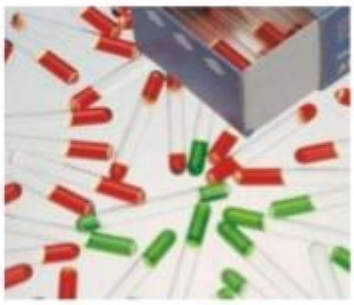
- Place strips in oven along with other material for the sterilization
- Later culture the strips in thioglycollate broth at 37 °C for 5 days
- Growth in medium indicates failure of sterilization

Quality control

to check whether the equipment is working properly

1. Chemical controls: Browne's tubes

Color change from red to green



- Used for glassware, forceps, swabs, water impermeable oils, waxes & powders

- Before placing in hot air oven

– Dry glassware completely
– Plug test tubes with cotton wool
– Wrap glassware in Kraft papers

- Don’t over load the oven

- Allow free circulation of air between the material

- The contents must not be removed from the oven immediately as a slow cooling period is necessary – ideally when the temperature has reduced down to 50°c, but no less.

- The reason for the gradual cooling period is to avoid the cracking of glassware as well as preventing air (that could potentially contain contaminating organisms) entering the oven.

Working

- The most common time-temperature relationships for sterilization with hot air sterilizers are

- 170°C (340°F) for 30 minutes,
- 160°C (320°F) for 60 minutes, and
- 150°C (300°F) for 150 minutes or longer depending up the volume.

Principle

- Sterilizing by dry heat is accomplished by conduction. The heat is absorbed by the outside surface of the item, then passes towards the Centre of the item, layer by layer. The entire item will eventually reach the temperature required for sterilization to take place.

- Dry heat does most of the damage by oxidizing molecules. The essential cell constituents are destroyed and the organism dies. The temperature is maintained for almost an hour to kill the most difficult of the resistant spores.

- Dry heat sterilization technique requires longer exposure time (**1.5 to 3 hours**) and higher temperatures than moist heat sterilization.

Fiber crops				
Cotton	*Gossypium spp.*	103	17	Fine
Vegetables				
Cucumber	*Cucumis sativus*	130	1	Fine
Pumpkin	*Cucurbita moschata*	130	1	Fine
Cabbage	*B olaracea var. capitata*	103	17	No
Cauliflower	*B. olaracea var. botrytis*	103	17	No
Tomato	*Lycopersicon esculentum*	130	1	No
Brinjal	*Solanum melongena*	103	17	No
Okra	*Abelmoscus esculentus*	1	130	Coarse
Carrot	*Daucus carota*	130	1	No
Raddish	*Raphanus sativus*	103	17	No
Beet	*Beta vulgaris*	130	1	Coarse

Specification of drying duration and temperature for seed moisture test (ISAT)

Common Name	Scientific Name	Drying temperature (°C)	Drying time (hr)	Grinding
CEREALS				
Paddy	*Oryza sativa*	130	2	Fine
Wheat	*Triticum spp.*	130	2	Fine
Barley	*Hardeum spp.*	130	2	Fine
Maize	*Zea mays*	130	4	Fine
Sorghum	*Sorghum bicolor*	130	2	Fine
Pearl millet	*Pennisaetum typhoides*	130	1	Fine
Pulses				
Black gram	*Vigna mungo*	130	1	Coarse
Cow pea	*V. sinensis*	130	1	Coarse
Green gram	*V. radiate*	130	1	Coarse
Pegeon pea	*Cajanus cajan*	130	1	Coarse
Soybean	*Glycine max*	103	17	Coarse
French bean	*Phaseolus vulgaris*	130	1	Coarse
Chick pea	*Cicer arietinum*	130	1	Coarse
Mustard	*Brassica spp.*	130	2	Fine
Oil seeds				
Safflower	*Carthomus tinctorius*	103	17	No
Castor	*Ricinus communis*	103	17	Coarse
Ground nut	*Arachis hypogea*	103	17	Coarse
Sunflower	*Helianthus annus*	103	17	No
Onion	*Allium cepa*	103	17	No

USE THE RESULTS OF THE WEIGHING TO CALCULATE MOISTURE CONTENT

- Moisture content is calculated on a wet weight basis and expressed to one decimal place.
- Replicates should not differ by more than 0.2%.
- For samples tested without pre-drying use the following formula:

$$\%\text{Moisture content} = \frac{\text{weight of fresh seeds} - \text{weight of dry seeds}}{\text{weight of fresh seeds}} \times 100$$

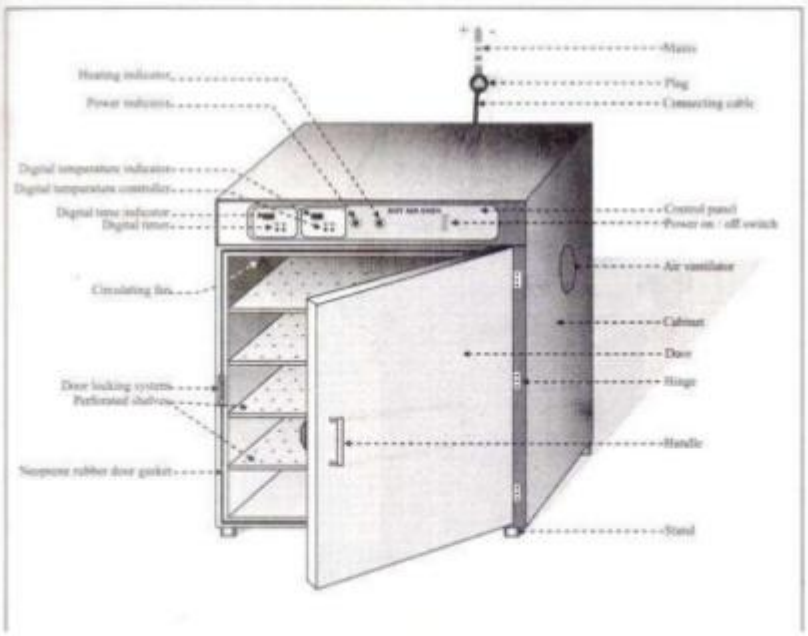
Removing the seed samples from oven

Placing the seed sample in oven for seed moisture determination

CONT….

- We offer double walled thermostatically controlled hot air oven.
- The inner chamber of this hot air oven are made of alluminium/stainless steel. Outer body is made of mild steel.
- Beaded heating Elements are placed under the ribs at the bottom and sides. these are also availble with special arrangement for digital temperature conteoller, alarm system, timer, blower etc.

HOT AIR OVEN


Mains
Plug
Connecting cable
Heating indicator
Power indicator
Digital temperature indicator
Digital temperature controller
Digital time indicator
Digital timer
Control panel
Power on / off switch
Air ventilator
Circulating fan
Cabinet
Door
Hinge
Door locking system
Perforated shelves
Handle
Neoprene rubber door gasket
Stand

CONT…

- The standard method for determining moisture in seed is the hot air oven method.

- the principle of this method is the elimination of water from the seed by heating under precisely controlled condition.

INTRODUCTION

- An oven provides a temperature higher than that of atmosphere.

- The temperature range covered by ovens is between **50-250°C.**

- These are used for rapid evaporation of materials, rapid drying and for sterilization of articles that can be sterilized by dry heat.

Disadvantages

- Time consuming method because of slow rate of heat penetration and microbial killing.
- High temperatures are not suitable for most materials.

Hot air oven

Hot air ovens are electrical devices which use dry heat to sterilize. They were originally developed by Pasteur.[1] 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. There is also an air filled space in between to aid insulation. An air circulating fan helps in uniform distribution of the heat. These are fitted with the adjustable wire mesh plated trays or aluminium trays and may have an on/off rocker switch, as well as indicators and controls for temperature and holding time. The capacities of these ovens vary. Power supply needs vary from country to country, depending on the voltage and frequency (hertz) used. Temperature sensitive tapes or biological indicators using bacterial spores can be used as controls, to test for the efficacy of the device during use.

Advantages and disadvantages

They do not require water and there is not much pressure build up within the oven, unlike an autoclave, making them safer to work with. This also makes them more suitable to be used in a laboratory environment. They are much smaller than autoclaves but can still be as effective. They can be more rapid than an autoclave and higher temperatures can be reached compared to other means. As they use dry heat instead of moist heat, some organisms like prions, may not be killed by them every time, based on the principle of thermal inactivation by oxidation.[*citation needed*]

Usage

A complete cycle involves heating the oven to the required temperature, maintaining that temperature for the proper time interval for that temperature, turning the machine off and cooling the articles in the closed oven till they reach room temperature. The standard settings for a hot air oven are:

- 1.5 to 2 hours at 160 °C (320 °F)
- 6 to 12 minutes at 190 °C (374 °F)

....plus the time required to preheat the chamber before beginning the sterilization cycle. If the door is opened before time, heat escapes and the process becomes incomplete. Thus the cycle must be properly repeated all over.

These are widely used to sterilize articles that can withstand high temperatures and not get burnt, like glassware and powders. Linen gets burnt and surgical sharps lose their sharpness.

Incubator (culture)

An **incubator** is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the CO_2 and oxygen content of the atmosphere inside. Incubators are essential for much experimental work in cell biology, microbiology and molecular biology and are used to culture both bacterial and eukaryotic cells.

Louis Pasteur used the small opening underneath his staircase as an incubator. Incubators are also used in the poultry industry to act as a substitute for hens. This often results in higher hatch rates due to the ability to control both temperature and humidity. Various brands of incubators are commercially available to breeders.

The simplest incubators are insulated boxes with an adjustable heater, typically going up to 60 to 65 °C (140 to 150 °F), though some can go slightly higher (generally to no more than 100 °C). The most commonly used temperature both for bacteria such as the frequently used E. coli as well as for mammalian cells is approximately 37 °C (99 °F), as these organisms grow well under such conditions. For other organisms used in biological experiments, such as the budding yeast Saccharomyces cerevisiae, a growth temperature of 30 °C (86 °F) is optimal.

More elaborate incubators can also include the ability to lower the temperature (via refrigeration), or the ability to control humidity or CO_2 levels. This is important in the cultivation of mammalian cells, where the relative humidity is typically >80% to prevent evaporation and a slightly acidic pH is achieved by maintaining a CO_2 level of 5%.

History of the laboratory incubator

From aiding in hatching chicken eggs to enabling scientists to understand and develop vaccines for deadly viruses, the laboratory incubator has seen numerous applications over the years it has been in use. The incubator has also provided a foundation for medical advances and experimental work in cellular and molecular biology.

An incubator is made up of a chamber with a regulated temperature. Some incubators also regulate humidity, gas composition, or ventilation within that

chamber. While many technological advances have occurred since the primitive incubators first used in ancient Egypt and China, the main purpose of the incubator has remained unchanged: to create a stable, controlled environment conducive to research, study, and cultivation.

The earliest incubators

The earliest incubators were found thousands of years ago in ancient Egypt and China, where they were used to keep chicken eggs warm.[1] Use of incubators revolutionized food production, as it allowed chicks to hatch from eggs without requiring that a hen sit on them, thus freeing the hens to lay more eggs in a shorter period of time. Both early Egyptian and Chinese incubators were essentially large rooms that were heated by fires, where attendants turned the eggs at regular intervals to ensure even heat distribution.

In the 16th and 17th century

The incubator received an update in the 16th century when Jean Baptiste Porta drew on ancient Egyptian design to create a more modern egg incubator. While he eventually had to discontinue his work due to the Spanish Inquisition, Rene-Antoine Ferchault de Reaumur took up the challenge in the middle of the 17th century.[2] Reaumur warmed his incubator with a wood stove and monitored its temperature using the Reaumur thermometer, another of his inventions.

In the 19th century

In the 19th century, researchers finally began to recognize that the use of incubators could contribute to medical advancements. They began to experiment to find the ideal environment for maintaining cell culture stocks. These early incubators were simply made up of bell jars that contained a single lit candle. Cultures were placed near the flame on the underside of the jar's lid, and the entire jar was placed in a dry, heated oven.

In the late 19th century, doctors realized another practical use for incubators: keeping premature or weak infants alive. The first infant incubator, used at a women's hospital in Paris, was heated by kerosene lamps. Fifty years later, Julius H. Hess, an American physician often considered to be the father of neonatology, designed an electric infant incubator that closely resembles the infant incubators in use today.[3]

In the 20th century

The next innovation in incubator technology came in the 1960s, when the CO_2 incubator was introduced to the market.[4] Demand came when doctors realized that they could use CO_2 incubators to identify and study pathogens found in patients' bodily fluids. To do this, a sample was harvested and placed onto a sterile dish and into the incubator. The air in the incubator was kept at 37 degrees Celsius, the same temperature as the human body, and the incubator maintained the atmospheric carbon dioxide and nitrogen levels necessary to promote cell growth.

At this time, incubators also began to be used in genetic engineering. Scientists could create biologically essential proteins, such as insulin, with the use of incubators. Genetic modification could now take place on a molecular level, helping to improve the nutritional content and resistance to pestilence and disease of fruits and vegetables.

Today

Incubators serve a variety of functions in a scientific lab. Incubators generally maintain a constant temperature, however additional features are often built in. Many incubators also control humidity. Shaking incubators incorporate movement to mix cultures. Gas incubators regulate the internal gas composition. Some incubators have a means of circulating the air inside of them to ensure even distribution of temperatures. Many incubators built for laboratory use have a redundant power source, to ensure that power outages do not disrupt experiments. Incubators are made in a variety of sizes, from tabletop models, to warm rooms, which serve as incubators for large numbers of samples.

Incubator Definition

Incubator, in microbiology, is an insulated and enclosed device that provides an optimal condition of temperature, humidity, and other environmental conditions required for the growth of organisms.

An incubator is a piece of vital laboratory equipment necessary for the cultivation of microorganisms under artificial conditions.

An incubator can be used for the cultivation of both unicellular and multicellular organisms.

Components/Parts of Incubator

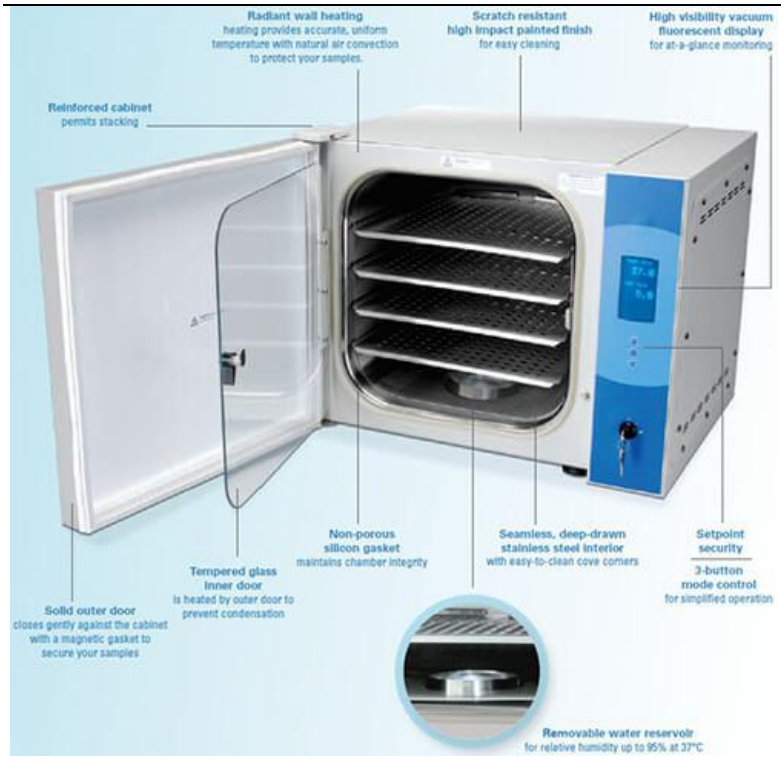


Image Source: McQueen Laboratory.

A microbial incubator is made up of various units, some of which are:

Cabinet

- The cabinet is the main body of the incubator consisting of the double-walled cuboidal enclosure with a capacity ranging from 20 to 800L.
- The outer wall is made up of stainless steel sheets while the inner wall is made up of aluminum.
- The space between the two walls is filled with glass wool to provide insulation to the incubator.
- The insulation prevents heat loss and in turn, reduces the electric consumption, thereby ensuring the smooth working of the device.
- The inner wall of the incubator is provided with inward projections that support the shelves present inside the incubator.

Door

- A door is present in all incubators to close the insulated cabinet.
- The door also has insulation of its own. It is also provided with a glass that enables the visualization of the interior of the incubator during incubation without disturbing the interior environment.
- A handle is present on the outside of the door to help with the maneuvering of the door.

Control Panel

- On the outer wall of the incubator is a control panel with all the switches and indicators that allows the parameters of the incubator to be controlled.

- The control panel also has a witch to control the thermostat of the device.

Thermostat

- A thermostat is used to set the desired temperature of the incubator.
- After the desired temperature is reached, the thermostat automatically maintains the incubator at that temperature until the temperature is changed again.

Perforated shelves

- Bound to the inner wall are some perforated shelves onto which the plates with the culture media are placed.
- The perforations on the shelves allow the movement of hot air throughout the inside of the incubator.
- In some incubators, the shelves are removable, which allows the shelves to be cleaned properly.

Asbestos door gasket

- The asbestos door gasket provides an almost airtight seal between the door and the cabinet.
- This seal prevents the outside air from entering the cabinet and thus, creating an isolated hot environment inside the cabinet without being interrupted by the external environment.

L-shaped thermometer

- A thermometer is placed on the top part of the outer wall of the incubator.
- One end of the thermometer provided with gradations remains outside of the incubator so that temperature can be read easily.
- The next end with the mercury bulb is protruded slightly into the chamber of the incubator.

HEPA filters

- Some advanced incubators are also provided with HEPA filters to lower the possible contamination created due to airflow.
- AN air-pump with filters creates a closed-loop system so that the air flowing inside the incubator generates less contamination.

Humidity and gas control

- The CO_2 incubators are provided with a reservoir underneath the chamber that contains water.
- The water is vapourised to maintain the relative humidity inside the chamber.
- Similarly, these incubators are also provided with gas chambers to give the desired concentration of CO_2 inside the incubator.

Principle/ Working of Incubator

- An incubator is based on the principle that microorganisms require a particular set of parameters for their growth and development.
- All incubators are based on the concept that when organisms are provided with the optimal condition of temperature, humidity,

oxygen, and carbon dioxide levels, they grow and divide to form more organisms.

- In an incubator, the thermostat maintains a constant temperature that can be read from the outside via the thermometer.
- The temperature is maintained by utilizing the heating and no-heating cycles.
- During the heating cycle, the thermostat heats the incubator, and during the no-heating period, the heating is stopped, and the incubator is cooled by radiating heat to the surrounding.
- Insulation from the outside creates an isolated condition inside the cabinet, which allows the microbes to grow effectively.
- Similarly, other parameters like humidity and airflow are also maintained through different mechanisms that create an environment similar to the natural environment of the organisms.
- Similarly, they are provided with adjustments for maintaining the concentration of CO_2 to balance the pH and humidity required for the growth of the organisms.
- Variation of the incubator like a shaking incubator is also available, which allows for the continuous movement of the culture required for cell aeration and solubility studies.

Procedure for running an incubator

Once the cultures of organisms are created, the culture plates are to be placed inside an incubator at the desired temperature and required period

of time. In most clinical laboratories, the usual temperature to be maintained is 35–37°C for bacteria.

The following are the steps to be followed while running an incubator:

1. Before using the incubator, it should be made sure that no remaining items are present in the incubator from the previous cycles. However, in some cases, if the same incubator is being used for multiple organisms, and they require the same set of parameters, they can be placed together in the same incubator.
2. The door of the incubator is then kept closed, and the incubator is switched on. The incubator has to be heated up to the desired temperature of the growth of the particular organism. The thermometer can be used to see if the temperature has reached.
3. In the meantime, if the organism requires a particular concentration of CO_2 or a specific humidity, those parameters should also be set in the incubator.
4. Once all the parameters are met, the petri dish cultures are placed on the perforated shelves upside down, i.e., media uppermost. This is necessary because if the plates are incubated normally, condensation collects on the surface of the medium and prevents the formation of isolated colonies.
5. If it is necessary to incubate Petri dish cultures for several days, the plates are sealed with adhesive tapes or are placed in plastic bags or plastic food containers.

6. Now, the door is locked, and the plates are kept inside for the required time before taking them out.

Types of incubators

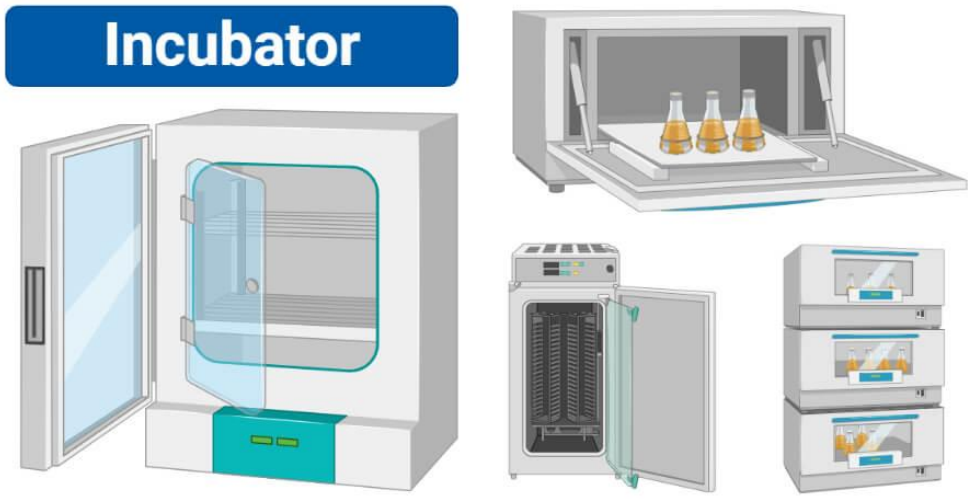


Figure: Some Incubators used in Microbiology Lab. Image created using bioredner.com

On the basis of the presence of a particular parameter or the purpose of the incubator, incubators are divided into the following types:

Benchtop incubators

- This is the most common type of incubator used in most of the laboratories.
- These incubators are the basic types of incubators with temperature control and insulation.

CO2 incubators

- CO2 incubators are the special kinds of incubators that are provided with automatic control of CO2 and humidity.

- This type of incubator is used for the growth of the cultivation of different bacteria requiring 5-10% of CO_2 concentration.
- For humidity control, water is kept underneath the cabinet of the incubator.

Cooled incubators

- For incubation at temperatures below the ambient, incubators are fitted with modified refrigeration systems with heating and cooling controls.
- This type of incubator is called the cooling incubator.
- In the cooling incubator, the heating and cooling controls should be appropriately balanced.

Shaker incubator

- A thermostatically controlled shaker incubator is another piece of apparatus used to cultivate microorganisms.
- Its advantage is that it provides a rapid and uniform transfer of heat to the culture vessel, and its agitation provides increased aeration, resulting in acceleration of growth.
- This incubator, however, can only be used for broth or liquid **culture media**.

Portable incubator

- Portable incubators are smaller in size and are used in fieldwork, e.g. environmental microbiology and water examination.

Uses of Incubator

Incubators have a wide range of applications in various areas including cell culture, pharmaceutical studies, hematological studies, and biochemical studies.

Some of the uses of incubators are given below:

1. Incubators are used to grow microbial culture or cell cultures.
2. Incubators can also be used to maintain the culture of organisms to be used later.
3. Some incubators are used to increase the growth rate of organisms, having a prolonged growth rate in the natural environment.
4. Specific incubators are used for the reproduction of microbial colonies and subsequent determination of biochemical oxygen demand.
5. These are also used for breeding of insects and hatching of eggs in zoology.
6. Incubators also provide a controlled condition for sample storage before they can be processed in the laboratories.

Precautions

The following precautions are to be followed while running an incubator:

1. As microorganisms are susceptible to temperature change, the fluctuations in temperature of the cabinet by repeatedly opening the door should be avoided.

2. The required parameters growth of the organism should be met before the culture plates are placed inside the cabinet.
3. The plates should be placed upside down with the lid at the bottom to prevent the condensation of water on to the media.
4. The inside of the incubators should be cleaned regularly to prevent the organisms from settling on the shelves or the corners of the incubator.
5. While running the incubator for an extended period of time, sterile water should be placed underneath the shelves to prevent the culture media from drying out.

WATER BATH

- A water bath is a device that maintains water at a constant temperature.
- It allows the heating of small amounts of fluids over a period of time without changing the concentration of constituents by evaporation.
- It is also used when several tubes are to be handled while maintaining the temperature of the contents,e.g., in coagulation tests.

- Water bath is an instrument used for maintaining a uniform temperature throughout the fluid contained in a glass container by keeping it in pre–heated water.

- It also prevents excessive evaporation of the fluid being heated.

Components

- It is made up of insulated metal, usually stainless steel or of heat resistant glass with or without an insulated lid.
- An electric element to heat the water contained in the trough.
- A propeller or stirrer to circulate the water in the trough in order to maintain a uniform temperature throughout the trough.

- A thermometer to check the temperature. This may be in-built or placed separately in the trough.
- A thermostat to maintain the temperature at a constant level.

Water Bath Controls:

Temperature Control:

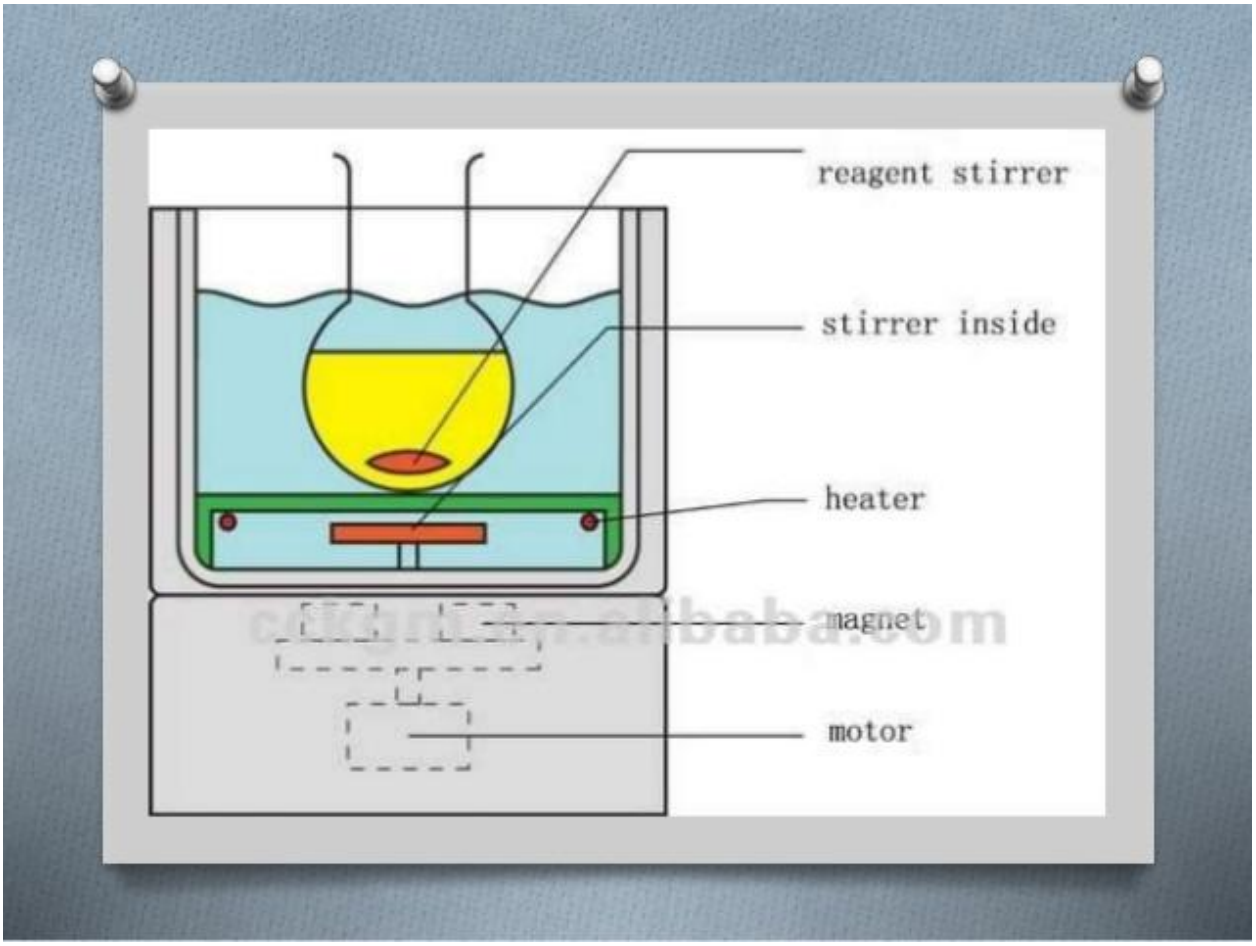
- All water baths have a control to set temperature. This control can be digital or a dial. Often there is an indicator light associated with this control. When the light is on the water bath is heating. When the water bath reaches the set temperature, it will cycle on and off to maintain constant temperature.

Safety control

- Most water baths have a second control called the safety. This control is set at the maximum temperature the water bath should attain.
- It is usually set just above the temperature control. Often an indicator light is associated with the safety control.
- If the water bath reaches the temperature that the safety control is set at, the light will go on. It will be impossible for the water bath to heat higher than the safety setting even when the temperature setting is higher.

- **Shaking Control:**

Shaking water baths have additional controls for shaking. The shaking mechanism can be turned on or off. The speed of shaking can also be set.


reagent stirrer
stirrer inside
heater
magnet
motor

Operating procedure

- Fill it with clean (preferably distilled) water to a desired level and then switch it on.
- Set the thermostat to desired temperature and allow the water to warm to that temperature.
- Check the temperature from the thermometer.

Precautions and maintenance

- Clean from inside and change the water daily.
- This will prevent encrustation of stirrer, heat probe and thermostat with salts contained in raw water.
- It will also prevent the growth of fungi and algae.

- Keep the lid closed when not in use to prevent evaporation of water.
- Periodically check and counter-check the water temperature with internal as well as with an external thermometer.
- The thermometer should be placed in such a way that it is away from the heating element and the walls.

Uses

- It provide Indirect heat.
- Used for warming blood bag blood.
- Used for incubation of test such as PT, APTT and Coombs test.

Refrigerator:

As already brought out, Refrigeration has two main uses- one is air conditioning and other is food preservation.

Here we are going to study the refrigeration in very details.

Definition:

The Refrigerator is the cooling household appliance system where removing the heat from the system to surrounding by the heat pump
so that the inside of the fridge is cooled to a temperature below the ambient temperature of the room.

Main Parts or Component of Refrigerator:

It consists of the 4 main components or Parts:

1. Evaporator:

In most of the common refrigerators, evaporator coils are wound around the freezer cabinet.

Sometimes they are placed between two seats to form a freezer cabinet.
The coil has no fins due to the frosting that collects on it over a period and due to low draft by natural convection.

In frost-free refrigerators, the evaporated is finned and the air is circulated over it by the fan.

The Frost on the evaporator is melted by a very low capacity heating element.

During the cyclical cut off period of the refrigerator, the electric heater switch on the defrost the evaporator. It is switched off before the restart of the compressor.

In this refrigerators, there is forced air circulation due to a finned evaporator and independent compartments.

2. Compressor:

It is hermetically sealed unit with the reciprocating compressor as the most common type of compressor.

In some large refrigerators, use of rotary or screw compressor is possible. But the use is limited due to the high cost.

The compressor unit is mounted on the rear side of the refrigerator.

The compressor motor capacity ranges from 1/8 to 1/4 HP depending upon the size of the refrigerator.

The starting relay also has overload protection to protect the compressor motor against higher current due to low voltage or tripling of the power supply.

3. Condenser:

In normal refrigerators, a condenser is installed on the rear side. The air circulation is by natural convection.

In the Frost free refrigerators, the condenser tube is on the inside of the outer cabinet of the refrigerator.

This arrangement prevents condensation on the outer cabinet in humid condition. It also allows placing the refrigerated close to the wall.

4. Expansion device:

In this system, the capillary tube is used as an expansion device.

The capillary is long due to the large difference between condenser pressure and evaporator pressure.

The capillary is sometimes soldered on the outside of the suction line and insulated to provide liquid-suction heat exchanger.

There is a dryer strainer before the capillary tube.

Working Principle:

This is the application of food preservation at the end user level. A refrigerator is an insulated box in which low temperature is maintained to facilitate salt or medium-term storage of food.

The refrigerator is usually specified as per volumetric capacity on the inside.

It is available in the capacity of 65 liters, 100 liters, 165 liters, 275 liters, to double door refrigerator of capacity as large as 1000 liters.

Large size refrigerator comes with independent compartments as per food categories.

So you get freezer cabinet, vegetable pulls out compartment and so on.

Most of the refrigerator comes with painting Stell sheet cabinet on the outside with strong industrial plastic inner cabinet for rust proofing shown in the figure.

In early refrigerators, cork expanded polystyrene (Thermocol) sheets or granules were used for insulations.

But nowadays polyurethane foam is the most common insulation used in in the refrigerator.

The door of the cabinet is provided with a neoprene rubber gasket to prevent leakage of air from the cabinet.

The inner cabinet and the inner panel of the door are suitably formed to provide trays for different categories of food.

Small refrigerator 165 liters the circulation of air for cooling is by natural convection.

This allows a range of temperature from -20 degree Celsius in the freezer cabinet at the top to about 5 degree Celsius in the vegetable tray located at the bottom.

The trays are not solid to allow air flow through the trays for circulation of air.

The temperature is maintained within a range by cyclic control.

The compressor cuts off at lower end i.e cut off temperature and restarts at Higher end i.e cut in temperature.

Advantages, Disadvantages, Application of Refrigerator:

Application:

Refrigerators are used mainly in keeping the refrigeration space cold.

By keeping the refrigeration space cold, the presence of micro-organisms such as Bacteria, Fungi is slowed down. They are not able to multiply fast enough to damage cells present in our foods and drinks.

Therefore, our food and drinks could be preserved over a long duration.

In the System, the bacteria and fungi become inactive under extremely lower temperatures.

Refrigerators are also used for medical and scientific purposes for the same reason.

For medical use, items such as blood and vaccines are stored in it for preservation.

For Scientific use, experimental samples are stores in it.

Used for making ice and ice-creams apart from storage of food.

Many drugs which are to be kept away from direct sunlight and heat like insulin are also stored in refrigerators for longevity.

In Brief:

- Cold storages
- Fish and meat processing
- Concrete cooling
- Paint cooling
- Chemical formulation
- Pharma industries

COLORIMETER – PRINCIPLE, COMPONENTS, WORKING & APPLICATIONS

February 25, 2018

SAHIL BATRA

1 Comment

⇒A Colorimeter involves the measurement of Color and is the widely used method for finding the concentration of biochemical compounds. It Measures absorbance and wavelength between 400 to 700 nm (nanometer) i.e. from the visible spectrum of light of the electromagnetic spectrum.

⇒**Absorption of light** – Light falling on a colored solution is either absorbed or transmitted. A colored solution absorbs all the colors of white light and selectively transmits only one color. This is its own color.

PRINCIPLE OF COLORIMETER

⇒**A colorimeter** is based on the photometric technique which states that When a beam of incident light of intensity I_0 passes through a solution, a part of the incident light is reflected (I_r), a part is absorbed (I_a) and rest of the light is transmitted (I_t)

Thus,

$$I_0 = I_r + I_a + I_t$$

⇒In colorimeter, (I_r) is eliminated because of the measurement of (I_0) and It is sufficient to determine the (I_a). For this purpose, the amount of light reflected (I_r) is kept constant by using cells that have identical properties. (I_0) & (I_t) is then measured.

⇒The mathematical relationship between the amount of light absorbed and the concentration of the substance can be shown by the two fundamental laws of photometry on which the colorimeter is based.

Beer's Law

⇒This law states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution.

$$\text{Log}_{10}\, I_0/I_t = a_s c$$

where,

a_s = Absorbency index

c = Concentration of Solution

Lambert's Law

⇒The Lambert's law states that the amount of light absorbed is directly proportional to the length and thickness of the solution under analysis.

$$A = \log_{10}\, I_0/I_t = a_s b$$

Where,

A = Absorbance of test

a_s = Absorbance of standard

b = length / thickness of the solution

The mathematical representation of the combined form of Beer-Lambert's law is as follows:

$$\text{Log}_{10}\, I_0 / I_t = a_s bc$$

If *b* is kept constant by applying Cuvette or standard cell then,

$$\text{Log}_{10}\, I_0/I_t = a_s c$$

The absorbency index as is defined as

$$a_s = A/cl$$

Where,

c = concentration of the absorbing material (in gm/liter).

l = distance traveled by the light in solution (in cm).

In simplified form,

The working principle of the colorimeter is based on Beer-Lambert's law which states that the amount of light absorbed by a color solution is directly proportional to the concentration of the solution and the length of a light path through the solution.

$$A \propto cl$$

Where,

A = Absorbance / Optical density of solution

c = Concentration of solution

l = Path length

$$A = \in cl$$

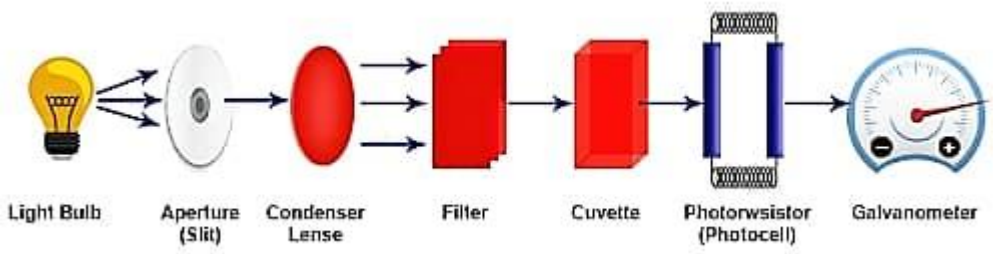
$\in$ = Absorption coefficient

PARTS OF COLORIMETER

There are 5 essential parts in a calorimeter……

⇒**Light Source** – The most common source of light used in colorimeter is a tungsten filament.

⇒ **Monochromator** – To select the particular wavelength filter or monochromators are used to split the light from the light source.



⇒**Sample holder** – Test tube or Cuvettes are used to hold the color solutions they are made up of Glass at the visible wavelength.

⇒**Photo Detector System** – when light falls on the detector system, an electric current is generated, this reflects the Galvanometer reading.

⇒ **Measuring device** – The current from the detector is fed to the measuring device, the Galvanometer, shows the meter reading that is directly proportional to the intensity of light.

WORKING OF THE COLORIMETER

⇒When using a colorimeter, it requires being calibrated first which is done by using the standard solutions of the known concentration of the solute that has to be determined in the test solution. For this, the standard solutions are filled in the cuvettes and placed in the cuvette holder in the colorimeter.

⇒There is a ray of light with a certain wavelength that is specific for the assay is directed towards the solution. Before reaching the solution the ray of light passes through a series of different filters and lenses. These lenses are used for navigation of the colored light in the colorimeter and the filter splits the beam of light into different wavelengths and allows the required wavelength to pass through it and reaches the cuvette containing the standard or test solutions. It analyzes the reflected light and compares it with a predetermined standard solution.

⇒When the monochromatic light (light of one wavelength) reaches the cuvette some of the light is reflected, some part of the light is absorbed by the solution and the remaining part is transmitted through the solution which falls on the photodetector system. The photodetector system measures the intensity of transmitted light and converts it into the electrical signals that are sent to the galvanometer.

⇒The galvanometer measures the electrical signals and displays them in the digital form. That digital representation of the electrical signals is the absorbance or optical density of the solution analyzed.

⇒If the absorption of the solution is higher than there will be more light absorbed by the solution and if the absorption of the solution is low then more lights will be transmitted through the solution which affects the galvanometer reading and corresponds to the concentration of the solute in the solution. By putting all the values in the formula given in the below section one can easily determine the concentration of the solution.

APPLICATIONS OF THE COLORIMETER

⇒The colorimeter is commonly used for the determination of the concentration of a colored compound by measuring the optical density or its absorbance.

⇒It can also be used for the determination of the course of the reaction by measuring the rate of formation and disappearance of the light-absorbing compound in the range of the visible spectrum of light.

⇒By colorimeter, a compound can be identified by determining the absorption spectrum in the visible region of the light spectrum.

⇒Here is the formula used for determining the concentration of a substance in the test solution.

$$A = \in cl$$

⇒For two solutions i.e. Test and standard,

$\in$ = Constant

l = Constant (using the same Cuvette or Standard cell)

$$A_T = C_T \quad \text{..... (i)}$$

$$A_S = C_S \quad \text{..... (ii)}$$

⇒From (i) & (ii),

$$A_T \times C_S = A_S \times C_T$$

$$C_T = (A_T/A_S) \times C_S$$

Where,

C_T = Concentration of the Test solution

A_T = Absorbance/ Optical density of the test solution

C_S = Concentration of the standard

A_S = Absorbance / Optical density of the standard solution

pH meter

A **pH meter** is a scientific instrument that measures the hydrogen-ion activity in water-based solutions, indicating its acidity or basicity expressed as pH.[2] The pH meter measures the difference in electrical potential between a pH electrode and a reference electrode, and so the pH meter is sometimes referred to as a "potentiometric pH meter". The difference in electrical potential relates to the acidity or pH of the solution.[3] The pH meter is used in many applications ranging from laboratory experimentation to quality control.[4]

Applications[edit]

The rate and outcome of chemical reactions taking place in water often depends on the acidity of the water, and it is therefore useful to know the acidity of the water, typically measured by means of a pH meter.[5] Knowledge of pH is useful or critical in many situations, including chemical laboratory analyses. pH meters are used for soil measurements in agriculture, water quality for municipal water supplies, swimming pools, environmental remediation; brewing of wine or beer; manufacturing, healthcare and clinical applications such as blood chemistry; and many other applications.[4]

Advances in the instrumentation and in detection have expanded the number of applications in which pH measurements can be conducted. The devices have been miniaturized, enabling direct measurement of pH inside of living cells.[6] In addition to measuring the pH of liquids, specially designed electrodes are available to measure the pH of semi-solid substances, such as foods. These have tips suitable for piercing semi-solids, have electrode materials compatible with ingredients in food, and are resistant to clogging.[7]

Principle of operation[edit]

Potentiometric pH meters measure the voltage between two electrodes and display the result converted into the corresponding pH value. They comprise a simple electronic amplifier and a pair of electrodes, or alternatively a combination electrode, and some form of display calibrated in pH units. It usually has a glass electrode and a reference electrode, or a combination electrode. The electrodes, or probes, are inserted into the solution to be tested.[8]

The design of the electrodes is the key part: These are rod-like structures usually made of glass, with a bulb containing the sensor at the bottom. The glass electrode for measuring the pH has a glass bulb specifically designed to be selective to hydrogen-ion concentration. On immersion in the solution to be tested, hydrogen ions in the test solution exchange for other positively charged ions on the glass bulb, creating an electrochemical potential across the bulb. The electronic amplifier detects the difference in electrical potential between the two electrodes generated in the measurement and converts the potential difference to pH units. The magnitude of the electrochemical potential across the glass bulb is linearly related to the pH according to the Nernst equation.

The reference electrode is insensitive to the pH of the solution, being composed of a metallic conductor, which connects to the display. This conductor is immersed in an electrolyte solution, typically potassium chloride, which comes into contact with the test solution through a porous ceramic membrane.[9] The display consists of a voltmeter, which displays voltage in units of pH.[9]

On immersion of the glass electrode and the reference electrode in the test solution, an electrical circuit is completed, in which there is a potential difference created and detected by the voltmeter. The circuit can be thought of as going from the conductive element of the reference electrode to the surrounding potassium-chloride solution, through the ceramic membrane to the test solution, the hydrogen-ion-selective glass of

the glass electrode, to the solution inside the glass electrode, to the silver of the glass electrode, and finally the voltmeter of the display device.[9] The voltage varies from test solution to test solution depending on the potential difference created by the difference in hydrogen-ion concentrations on each side of the glass membrane between the test solution and the solution inside the glass electrode. All other potential differences in the circuit do not vary with pH and are corrected for by means of the calibration.[9]

For simplicity, many pH meters use a combination probe, constructed with the glass electrode and the reference electrode contained within a single probe. A detailed description of combination electrodes is given in the article on glass electrodes.[10]

The pH meter is calibrated with solutions of known pH, typically before each use, to ensure accuracy of measurement.[11] To measure the pH of a solution, the electrodes are used as probes, which are dipped into the test solutions and held there sufficiently long for the hydrogen ions in the test solution to equilibrate with the ions on the surface of the bulb on the glass electrode. This equilibration provides a stable pH measurement.[12]

pH electrode and reference electrode design[edit]

Details of the fabrication and resulting microstructure of the glass membrane of the pH electrode are maintained as trade secrets by the manufacturers.[13]:125 However, certain aspects of design are published. Glass is a solid electrolyte, for which alkali-metal ions can carry current. The pH-sensitive glass membrane is generally spherical to simplify the manufacture of a uniform membrane. These membranes are up to 0.4 millimeters in thickness, thicker than original designs, so as to render the probes durable. The glass has silicate chemical functionality on its surface, which provides binding sites for alkali-metal ions and hydrogen ions from the solutions. This provides an ion-exchange capacity in the range of 10^{-6} to 10^{-8} mol/cm^2. Selectivity for hydrogen ions (H^+) arises from a balance of ionic charge, volume requirements versus other ions, and the coordination number of other ions. Electrode manufacturers have developed compositions that suitably balance these factors, most notably lithium glass.[13]:113–139

The silver chloride electrode is most commonly used as a reference electrode in pH meters, although some designs use the saturated calomel electrode. The silver chloride

electrode is simple to manufacture and provides high reproducibility. The reference electrode usually consists of a platinum wire that has contact with a silver/silver chloride mixture, which is immersed in a potassium chloride solution. There is a ceramic plug, which serves as a contact to the test solution, providing low resistance while preventing mixing of the two solutions.[13]:76–91

With these electrode designs, the voltmeter is detecting potential differences of ±1400 millivolts.[14] The electrodes are further designed to rapidly equilibrate with test solutions to facilitate ease of use. The equilibration times are typically less than one second, although equilibration times increase as the electrodes age.[13]:164

Maintenance[edit]

Because of the sensitivity of the electrodes to contaminants, cleanliness of the probes is essential for accuracy and precision. Probes are generally kept moist when not in use with a medium appropriate for the particular probe, which is typically an aqueous solution available from probe manufacturers.[11][15] Probe manufacturers provide instructions for cleaning and maintaining their probe designs.[11] For illustration, one maker of laboratory-grade pH gives cleaning instructions for specific contaminants: general cleaning (15-minute soak in a solution of bleach and detergent), salt (hydrochloric acid solution followed by sodium hydroxide and water), grease (detergent or methanol), clogged reference junction (KCl solution), protein deposits (pepsin and HCl, 1% solution), and air bubbles.[15][16]

Calibration and operation[edit]

German Institute for Standardization[17]Very precise measurements necessitate that the pH meter is calibrated before each measurement. More typically calibration is performed once per day of operation. Calibration is needed because the glass electrode does not give reproducible electrostatic potentials over longer periods of time.[13]:238–239

Consistent with principles of good laboratory practice, calibration is performed with at least two standard buffer solutions that span the range of pH values to be measured. For general purposes, buffers at pH 4.00 and pH 10.00 are suitable. The pH meter has

one calibration control to set the meter reading equal to the value of the first standard buffer and a second control to adjust the meter reading to the value of the second buffer. A third control allows the temperature to be set. Standard buffer sachets, available from a variety of suppliers, usually document the temperature dependence of the buffer control. More precise measurements sometimes require calibration at three different pH values. Some pH meters provide built-in temperature-coefficient correction, with temperature thermocouples in the electrode probes. The calibration process correlates the voltage produced by the probe (approximately 0.06 volts per pH unit) with the pH scale. Good laboratory practice dictates that, after each measurement, the probes are rinsed with distilled water or deionized water to remove any traces of the solution being measured, blotted with a scientific wipe to absorb any remaining water, which could dilute the sample and thus alter the reading, and then immersed in a storage solution suitable for the particular probe type.[18]

Types of pH meters[edit]

pH meters range from simple and inexpensive pen-like devices to complex and expensive laboratory instruments with computer interfaces and several inputs for indicator and temperature measurements to be entered to adjust for the variation in pH caused by temperature. The output can be digital or analog, and the devices can be battery-powered or rely on line power. Some versions use telemetry to connect the electrodes to the voltmeter display device.[13]:197–215

Specialty meters and probes are available for use in special applications, such as harsh environments[19] and biological microenvironments.[6] There are also holographic pH sensors, which allow pH measurement colorimetrically, making use of the variety of pH indicators that are available.[20] Additionally, there are commercially available pH meters based on solid state electrodes, rather than conventional glass electrodes.[21]

History

concept of pH was defined in 1909 by S. P. L. Sørensen, and electrodes were used for pH measurement in the 1920s.[22]

In October 1934, Arnold Orville Beckman registered the first patent for a complete chemical instrument for the measurement of pH, U.S. Patent No. 2,058,761, for his "acidimeter", later renamed the pH meter. Beckman developed the prototype as an assistant professor of chemistry at the California Institute of Technology, when asked to devise a quick and accurate method for measuring the acidity of lemon juice for the California Fruit Growers Exchange (Sunkist).[23]:131–135

Incubator

An **incubator** is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the CO_2 and oxygen content of the atmosphere inside.

Incubators are essential for much experimental work in cell biology, microbiology and molecular biology and are used to culture both bacterial and eukaryotic cells.

Louis Pasteur used the small opening underneath his staircase as an incubator. Incubators are also used in the poultry industry to act as a substitute for hens.
This often results in higher hatch rates due to the ability to control both temperature and humidity. Various brands of incubators are commercially available to breeders.

The simplest incubators are insulated boxes with an adjustable heater, typically going up to 60 to 65 °C (140 to 150 °F), though some can go slightly higher (generally to no more than 100 °C).

The most commonly used temperature both for bacteria such as the frequently used E. coli as well as for mammalian cells is approximately 37 °C (99 °F), as these organisms grow well under such conditions.

For other organisms used in biological experiments, such as the budding yeast Saccharomyces cerevisiae, a growth temperature of 30 °C (86 °F) is optimal. More elaborate incubators can also include the ability to lower the temperature (via refrigeration), or the ability to control humidity or CO_2 levels.

This is important in the cultivation of mammalian cells, where the relative humidity is typically >80% to prevent evaporation and a slightly acidic pH is achieved by maintaining a CO_2 level of 5%.

History of the laboratory incubator.

From aiding in hatching chicken eggs to enabling scientists to understand and develop vaccines for deadly viruses, the laboratory incubator has seen numerous applications over the years it has been in use.
The incubator has also provided a foundation for medical advances and experimental work in cellular and molecular biology.

An incubator is made up of a chamber with a regulated temperature. Some incubators also regulate humidity, gas composition, or ventilation within that chamber.

While many technological advances have occurred since the primitive incubators first used in ancient Egypt and China, the main purpose of the incubator has remained unchanged: to create a stable, controlled environment conducive to research, study, and cultivation.

The earliest incubators

The earliest incubators were found thousands of years ago in ancient Egypt and China, where they were used to keep chicken eggs warm.[1]

Use of incubators revolutionized food production, as it allowed chicks to hatch from eggs without requiring that a hen sit on them, thus freeing the hens to lay more eggs in a shorter period of time.

Both early Egyptian and Chinese incubators were essentially large rooms that were heated by fires, where attendants turned the eggs at regular intervals to ensure even heat distribution.

In the 16th and 17th century

Reaumur thermometer

The incubator received an update in the 16th century when Jean Baptiste Porta drew on ancient Egyptian design to create a more modern egg incubator.

While he eventually had to discontinue his work due to the Spanish Inquisition, Rene-Antoine Ferchault de Reaumur took up the challenge in the middle of the 17th century.

Reaumur warmed his incubator with a wood stove and monitored its temperature using the Reaumur thermometer, another of his inventions.

In the 19th century

In the 19th century, researchers finally began to recognize that the use of incubators could contribute to medical advancements. They began to experiment to find the ideal environment for maintaining cell culture stocks.

These early incubators were simply made up of bell jars that contained a single lit candle. Cultures were placed near the flame on the underside of the jar's lid, and the entire jar was placed in a dry, heated oven. In the late 19th century, doctors realized another practical use for incubators: keeping premature or weak infants alive.

The first infant incubator, used at a women's hospital in Paris, was heated by kerosene lamps. Fifty years later, Julius H. Hess, an American physician often considered to be the father of neonatology, designed an electric infant incubator that closely resembles the infant incubators in use today.

In the 20th century

The next innovation in incubator technology came in the 1960s, when the CO_2 incubator was introduced to the market.

Demand came when doctors realized that they could use CO_2 incubators to identify and study pathogens found in patients' bodily fluids.

To do this, a sample was harvested and placed onto a sterile dish and into the incubator.

The air in the incubator was kept at 37 degrees Celsius, the same temperature as the human body, and the incubator maintained the atmospheric carbon dioxide and nitrogen levels necessary to promote cell growth.

At this time, incubators also began to be used in genetic engineering. Scientists could create biologically essential proteins, such as insulin, with the use of incubators.

Genetic modification could now take place on a molecular level, helping to improve the nutritional content and resistance to pestilence and disease of fruits and vegetables.

Today

Incubators serve a variety of functions in a scientific lab. Incubators generally maintain a constant temperature, however additional features are often built in. Many incubators also control humidity. Shaking incubators incorporate movement to mix cultures.

Gas incubators regulate the internal gas composition. Some incubators have a means of circulating the air inside of them to ensure even distribution of temperatures. Many incubators built for laboratory use have a redundant power source, to ensure that power outages do not disrupt experiments.

Incubators are made in a variety of sizes, from tabletop models, to warm rooms, which serve as incubators for large numbers of samples

Laboratory Reagents Required in Preparation of Permanent Slides

Wright's Stain

Technique

This is a Romanowsky type of metachromatic stain that is prepared by mixing specially treated methylene blue dye with eosin.

The acidic portion of the stain unites with the basic components of the cells such as hemoglobin, and thus they are referred to as eosinophilic and are stained pink or red.

The acidic components of the cell, such as the nucleic acids on the other hand take the basic dye and stain blue or purple.

PH has to be controlled using a buffer of 6.4 to 6.7 to avoid poor staining.

Preparation

- Measure 1.0 grams of wright's stain powder and 400 ml of methanol (methyl alcohol),
- Add a few glass beads to assist in dissolving and add the ethanol to the stain,
- Mix well at intervals until the powder has completely dissolved (do this by warming in 37 C water bath to aid in the dissolving),
- Label the bottles and mark it as flammable and toxic,
- Tightly atop and store at room temperature in the dark

Procedure

1- Prepare a fill of the sample and allow drying on a slide,

2- Prepare three containers, and fill one with one step Wright's Stain and the other two with distilled water,

3- Keep the stain tightly covered when not in use to avoid evaporation (always replace the stain once it becomes insufficient)

4- Always replace distilled water once iridescent scum start forming on the surface, or when it starts turning blue,

5- Dip the slide in the stain for 15 to 20 seconds,

6- Dip the slide in distilled water in the second container for 15- 45 seconds,

7- Dip the slide in container 3 for 25 seconds using quick dips,

8- Wipe the back of the slide,

9- Dry the slide on a vertical position, on the absorbent surface and avoid blotting the smear,

10- Apply oil to examine microscopically,

These steps should be repeated two times for marrow smears.

Leishman's stain for blood cells and parasites

Leishman 1901.

Leishman stain

0.15% Leishman powder in 100% methanol. Use after 24hrs.

Phosphate buffer (Sorensen)

Stock A: 0.2M sodium di-hydrogen orthophosphate (mw 156).
To prepare dissolve 3.12g in 100ml distilled water.

Stock B: 0.2M di-sodium hydrogen orthophosphate (mw 142).
To prepare dissolve 2.83g in 100ml ditilled water.

For pH 6.8 add 25.5ml of A to 24.5ml of B and make up to 100ml with distilled water.

Prepare cytospin slide or smear of sample.

- **Either** spray the wet preparation with Smear Fix leave for 1-2 mins, wash off the fixative with distilled water and drain....
- **Or** lower the slide gently into a coplin jar of acetic alcohol (3% acetic acid in 95% methanol), fix for 1 minute, wash off the fixative with distilled water and drain.
- Put slides on a rack and cover with 1ml of Leishman stock - 20 seconds.
- Add 2ml of pH6.8 buffer and tip the rack up and down to mix the solutions, stain for 7 minutes.
- Rinse quickly in distilled water then treat with pH6.8 buffer - 2 minutes.
- Rinse quickly in distilled water, shake off the excess and dry on a warm (50^{o}C) hotplate, or carefully blot dry with fibre-free blotting paper.
- Clear and mount.

Red blood cells - red to yellowish red
Neutrophils - dark purple nuclei, pale pink cytoplasm, reddish-lilac small granules
Eosinophils - blue nuclei, pale pink cytoplasm, red to orange-red large granules
Basophils - purple to dark blue nucleus, dark purple, almost black large granules
Lymphocytes - dark purple to deep bluish purple nuclei, sky blue cytoplasm
Platelets - violet to purple granules
Parasites (Leishmania, malaria, etc.) - dark blue-black.

Histological Staining Techniques

Bouin's fluid fixation

Contributed by Martin Fitzpatrick, University of Birmingham, United Kingdom

Fixation of histological samples with Bouin's fluid

- Prepare 75ml saturated aqueous solution of picric acid
- Add 25ml formalin (40% aqueous solution of formaldehyde) to give 100ml total volume
- Add 5ml glacial acetic acid
- Fix tissue by submersion in Bouin's fluid for 6 hours
- Transfer fixed tissue to 70% alcohol

Carnoy's fluid fixation

Contributed by Martin Fitzpatrick, University of Birmingham, United Kingdom

Fixation of samples in Carnoy's fluid

- In a fume hood pour 60ml of ethanol into a suitable container
- Add 30ml choloroform
- Add 10ml glacial acetic acid to give a total volume of 100ml
- Place tissue into fixative for 1-3 hours
- Process fixed tissues immediately or transfer to 80% alcohol for storage

ACETOCARMINE STAINING

Acetocarmine preparation (1% solution)

Carmine is a basic dye that is prepared from the insect *Coccus cacti*. Dissolve 10 g carmine (Fisher C579-25) in 1 L of 45% glacial acetic acid, add boileezers, and reflux for 24 h. Filter into dark bottles and store at 4°C. This solution can be stored for a long time. Staining can be intensified by adding ferric chloride ($FeCl_2 \cdot 6H_2O$); add 5 mL of a 10 % ferric chloride solution per 100 mL of % acetocarmine.

Acetocarmine staining

To stain plant chromosomes, a 1% solution of carmine in 45% acetic acid is used. Freshly fixed material is transferred into 1% acetocarmine for at least 30 min and then analyzed by the squash method. If the material was fixed for a longer time, it requires a longer staining time (up to several days) to reach

good contrast. If the material is to be analyzed immediately, fix and stain the tissue in one step using the 1% acetocarmine solution.

Chromosome squash technique

Drain off the fixative and place the roots in 1% acetocarmine for 1 to 3 h. Heat until the acetocarmine begins to boil. Cut off the root cap with a razor blade and squeeze the meristematic tissue out with a lancet needle. Add a drop of acetocarmine or 45% acetic acid. Place a razor blade (double-edged) to one side and add a cover slip. Tap the cover slip gently with the needle end of a probe. Slide the razor blade out and heat to a point just below boiling (steam will form beneath the slide). Then, quickly squash with thumb or forefinger between two layers of filter paper. Be careful to not move the cover slip at this point.

Hayem's Solution

Ingredients:

- Mercuric chloride – 0.25 gm
- Sodium sulphate – 2.50 gm
- Sodium chloride – 0.50 gm
- Distilled water – 100.0ml
- Final pH (at 25°C) – 5.9±0.1

- **Red cell counting**
- The principle consists of diluting a sample of blood with a suitable fluid (Hayem's solution) and counting the cells in a small portion of this under the microscope. The most important items in the apparatus are the **counting chamber** and the **diluting system**.
- Red cells are lysed by glacial acetic acid. Gentian violet slightly stains the leukocyte nuclei. The blood specimen is diluted 1:20 (20:380 in some laboratories) in a White blood cell pipette with the diluting fluid, and the cells are counted under the low power of the microscope using a counting chamber.The

number of cells in undiluted blood is reported per cumm(µl) of the whole blood.

CompositionofW.B.C.DilutingFluid (Turkey's Fluid) Ingredients

- Glacial acetic acid.2.00ml
  GentianViolet.(1%w/v)1.00gm.
  Distilledwater.97.00ml
  FinalpH(at25°C)2.2±0.2

Principle of W.B.C. Diluting Fluid
WBC diluting fluid is used to perform the WBC(leucocyte) count.

How to Make a Saline Solution

Materials

non-iodized salt

distilled water

Solution should contain 9 grams of salt for every liter of water.

Steps

1. Heat water until it reaches a boil.
2. Dissolve salt into boiling water.
3. Keep solution sterile by placing lid on container.
4. Sterilze any containers before storing.
5. Discard solution after a few days.

ThoughtCo.

Normal Saline (0.9% NaCl)

- Contains sodium and chloride ions in water and it is isotonic with extracellular fluid
- Cell membrane is impermeable to Na^+ and Cl^- ions owing to the presence of the energy dependant Na^+ / K^+ - ATPase
- Intravenous infusion of an isotonic solution of sodium chloride will expand only the extracellular compartment

Crystalloids

0.9% Normal Saline

- Contains: Na^+ 154 mmol/l, Cl^- 154 mmol/l
- Osm : 308mosm/l, pH 6.0
- IsoOsmolar compared to normal plasma.

- Indication :
  - Intravascular resuscitation and replacement of salt loss e.g. diarrhoea and vomiting.
  - Also for diluting packed RBCs prior to transfusion
  - Used for diluting Drugs

USES OF NORMAL SALINE SOLUTION:

- Saline has many uses in medicine.

1. Applied to the effected area to clean the wound
2. Help remove contact lenses
3. Help with the dry eyes
4. By injection into vein it is used to treat dehydration
5. It is used to dilute other medications to be given by injection

Turk's fluid

Composition:

- Glacial acetic acid= 1.5ml→lyses RBC
- 1% gention violet= 1ml→ stain nuclei of WBC
- Distilled water= upto 100ml→ solvent
- Thymol= 1 pinch→prevent growth of fungus

The diluting fluid is not isotonic and the very dilute acid solution lyses RBC but not WBC

WBC (TURK'S) DILUTING FLUID:

It is prepared as follows:

- **a) Glacial acetic acid: 2.0 ml**
- **b) 1 % (w/v) gentian violet: 1.0 ml**
- **c) Distilled water: 97 ml**

Principle

- The sample of blood is diluted with a turk's fluid which destroy RBC and stain the nuclei of leucocyte. The cell are then counted in a calibrated chamber and their number in undiluted blood reported as leucocyte/cumm.

Composition of Hayem's fluid:

a. Sodium chloride -0.5 gm
b. Sodium sulfate -2.5 gm
c. Mercuric chloride -0.25 gm
d. Distilled water – 100 ml.

RBC DILUTING FLUID

- RBCs are around only 5 millions/cumm of blood.
- Counting this much number is highly imposible.
- Therefore the blood sample is diluted with the help of RBC diluting fluid.
- It fixes and preserves RBCs.
- It is isotonic to RBCs.

DILUTING FLUID

- Two types of diluting fluids are used for RBC counting.

1) Hayem's fluid
2) Dacie's fluid

Composition of Dacie's fluid:

i. Trisodium citrate – 3 gm.
ii. Formalin – 1 ml.
iii. Distill water – 99 ml.

This diluting fluid is cheap and commonly used.

Leishman Stain:

Preparation

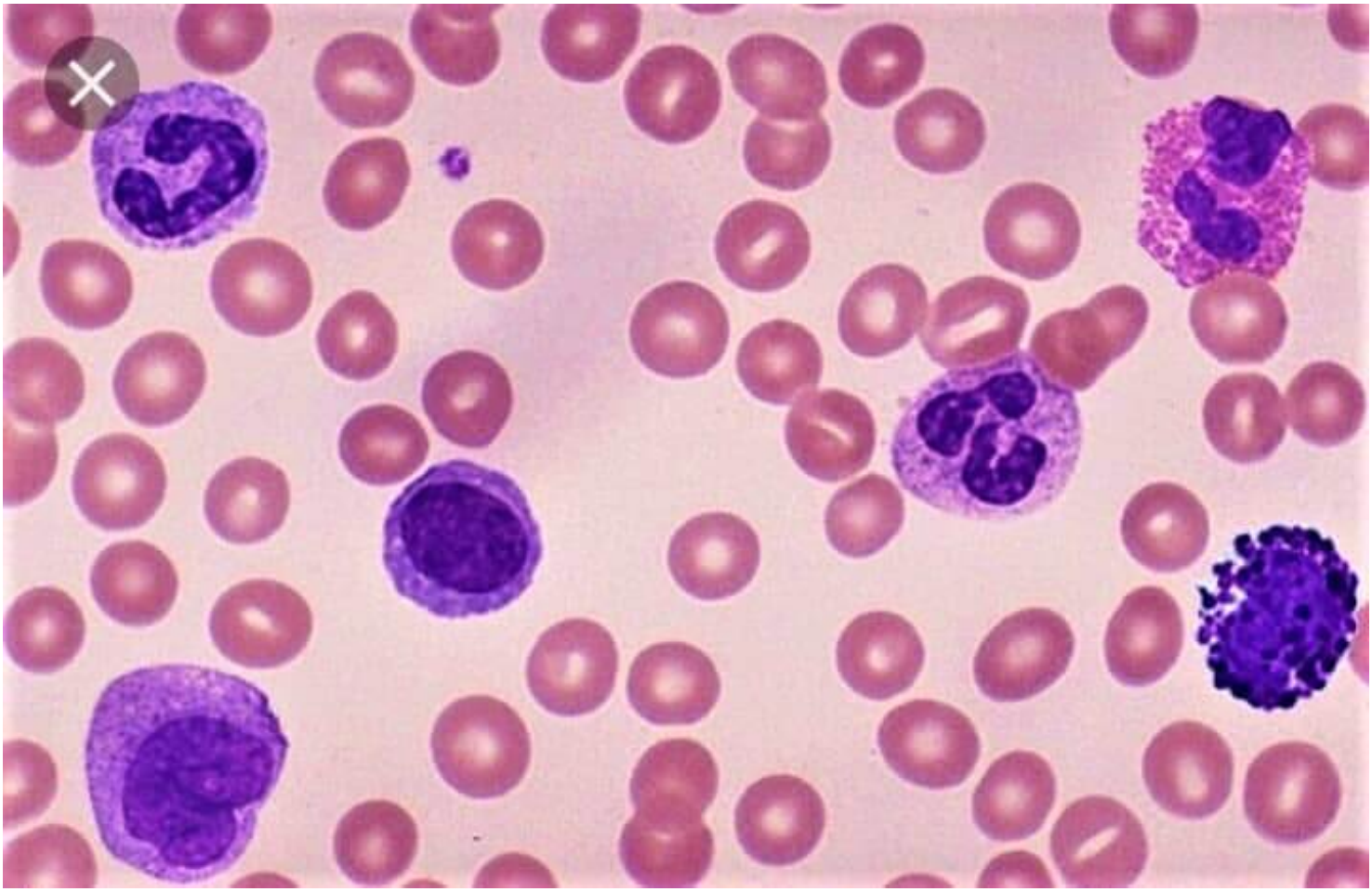
- Dissolve 0.2 g of powdered Leishman's dye in 100 ml of acetone-free methyl alcohol in a conical flask.
- Warm it to 50°C for half an hour with occasional shaking.
- Cool it and filter it.

Procedure for staining

- Pour Leishman's stain dropwise (counting the drops) on the slide and wait for 2 minutes. This allows fixation of the PBF in methyl alcohol.
- Add double the quantity of buffered water dropwise over the slide (i.e. double the number of drops).
- Mix by rocking for 8 minutes.
- Wash in water for 1 to 2 minutes.
- Dry in air and examine under oil immersion lens of the microscope.

Comparative chart of common Romanowsky stain

Wright stain	Leishman stain	Giemsa stain
-Wright's stain powder:- 0.25gm -Acetone free methanol: - 100 ml Fixation:- 1-2 mints Staining time:- 5-7 min Dilution:- by equal of buffer water	Leishman's powder:-0.15 gm Acetone free methanol : - 100 ml Fixation:- 2 mints. Staining time:- 7-10 min Dilution :- by double volume of buffer water.	Giemsa powder:-0.3gm Glycerin:- 25 ml Acetone free methanol:- 25 ml Fixation: separate prefixation in methanol 3-5 mints due to less volume of methanol contained in composition. Staining time:- 15-20 Dilution- 1:10 with buffer water before staining.



Wright Stain

- **“Romanovsky-type” stain**
  -
  - **A.K.A. Wright-Giemsa; utilized in peripheral blood smears.**
  - **Contains mixtures of methylene blue, azure, and eosin.**
  - **Metachromasia: property of methylene blue and toluidine blue dyes.**
    - **Cell components stain a different color than the dye itself.**
      - **Mast cell granules, cartilage, and mucin will stain purple.**

Uses of Romanowsky stain

- use to stain the blood smear for differential leucocyte count and also for lupus erythematous (LE cell)
- to stain the bone marrow
- to study the blood parasite e.g.: malaria parasite.(we do Giemsa stain)

Comparative chart of common Romanowsky stain

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7. Carnoy's fluid:

Absolute alcohol	60ml
Chloroform	30ml
Acetic acid	10ml

-Rapidly penetrating & acting fixative

-Due to quick fixation, it can be used for urgent diagnosis.

-Glycogen is preserved.

CYTOLOGICAL FIXATIVES

I. **NUCLEAR FIXATIVES:**

1. Carnoy's Fluid:

- Absolute alcohol – 60ml
- Chloroform – 30ml
- Glacial acetic acid – 10ml
- Penetrates very rapidly
- Excellent nuclear fixation
- Preserves **Nissl substance& glycogen**
- chromosomes
- Destroys cytoplasmic elements.
- Rapid fixative – urgent diagnosis.

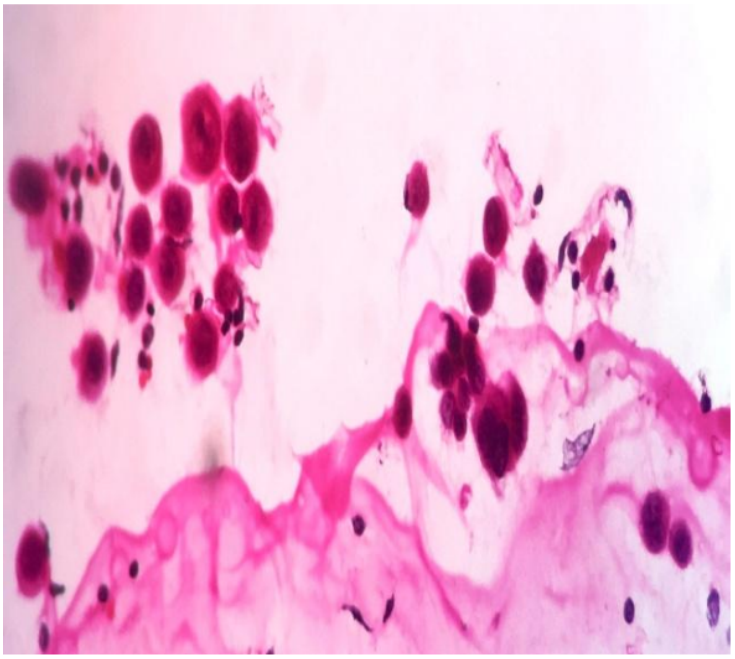


Figure 3: Pleural fluid –NSRT (H&E, 40x)

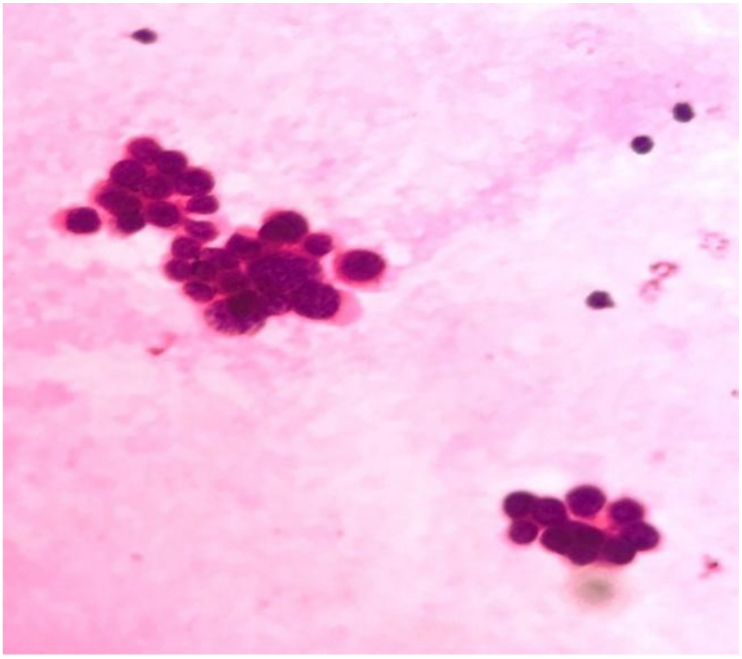


Figure 4: Pleural –Carnoy's fluid (H&E, 40x)

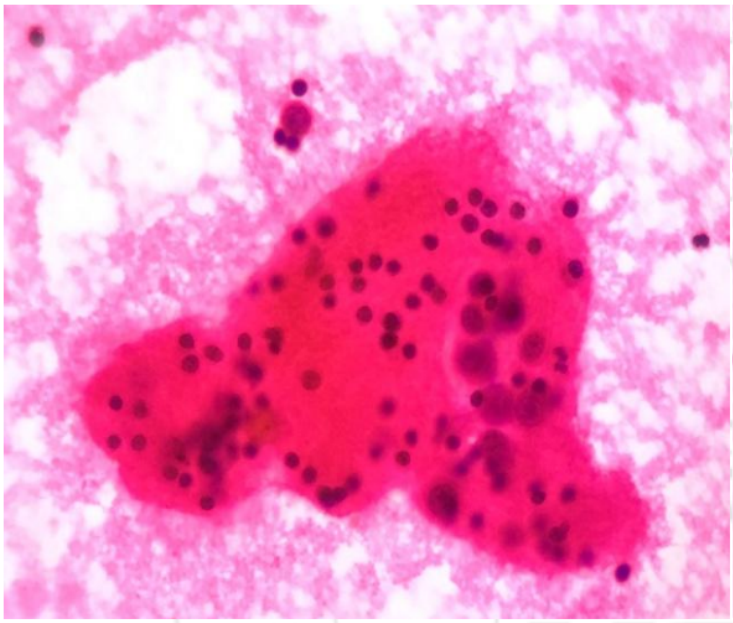


Figure 3: Asitic fluid –NSRT (H&E, 40x)

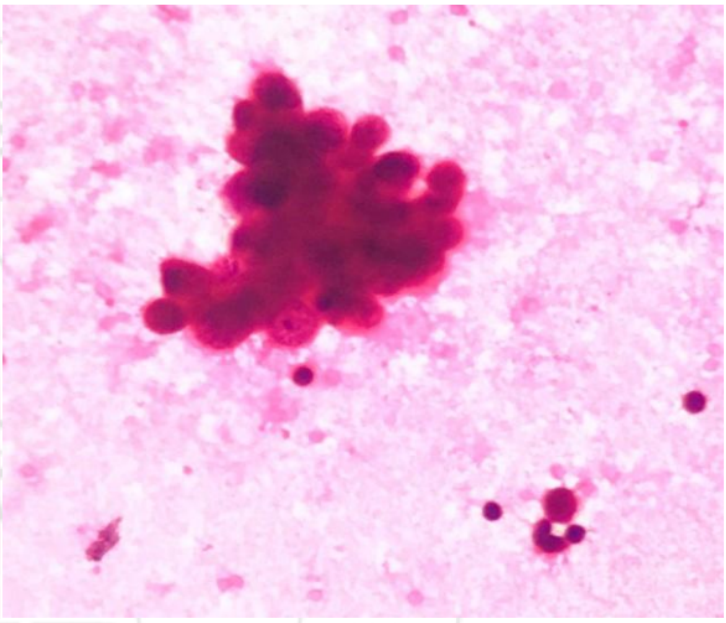


Figure 4: Asitic fluid–Carnoy's fluid (H&E, 40x)

BOUIN'S FLUID

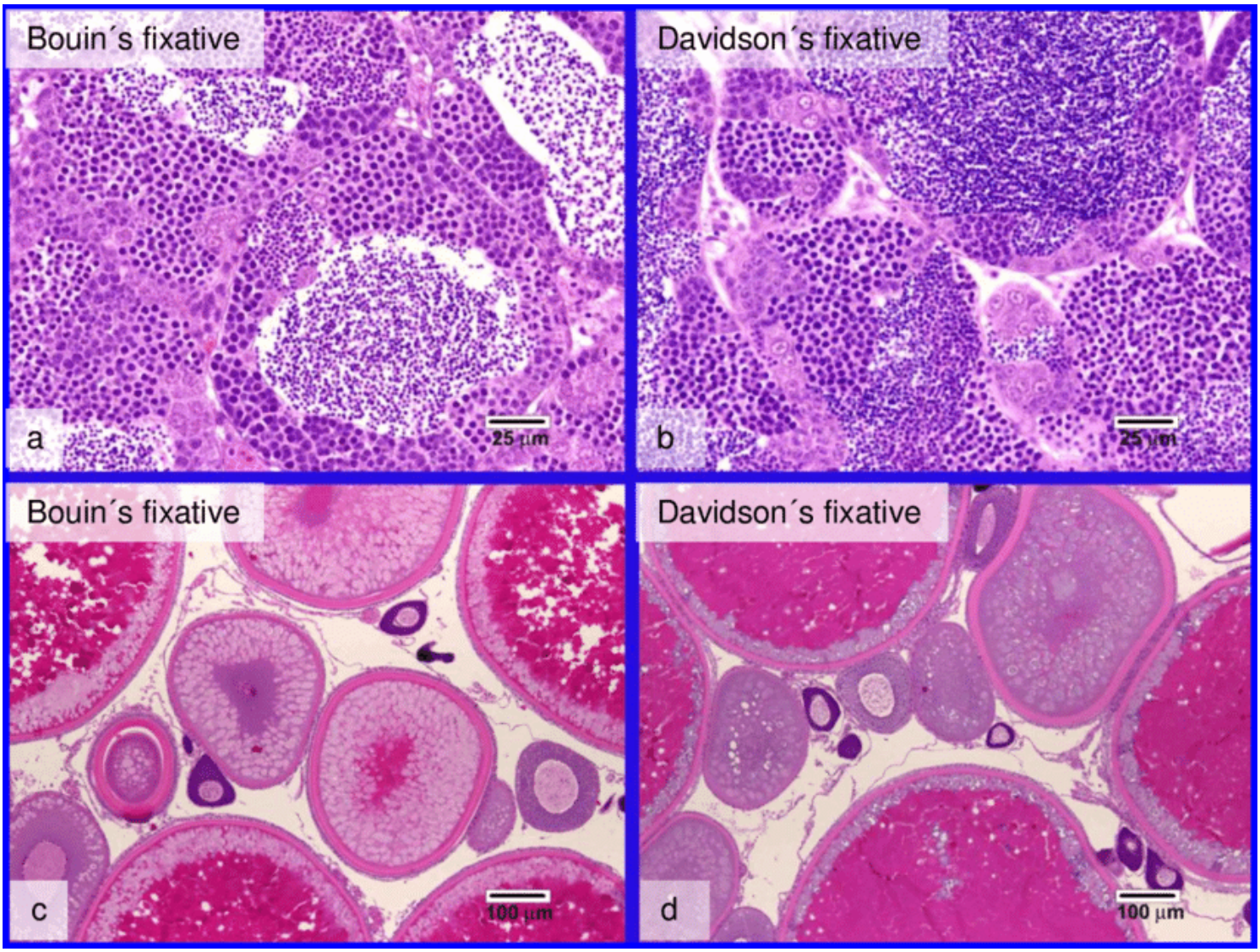
- Picric acid saturated aqueous solution - 75ml
- Formalin - 25ml
- Glacial acetic acid - 5ml

Fixation time

1 to 12 hours

7. Bouin's fluid:

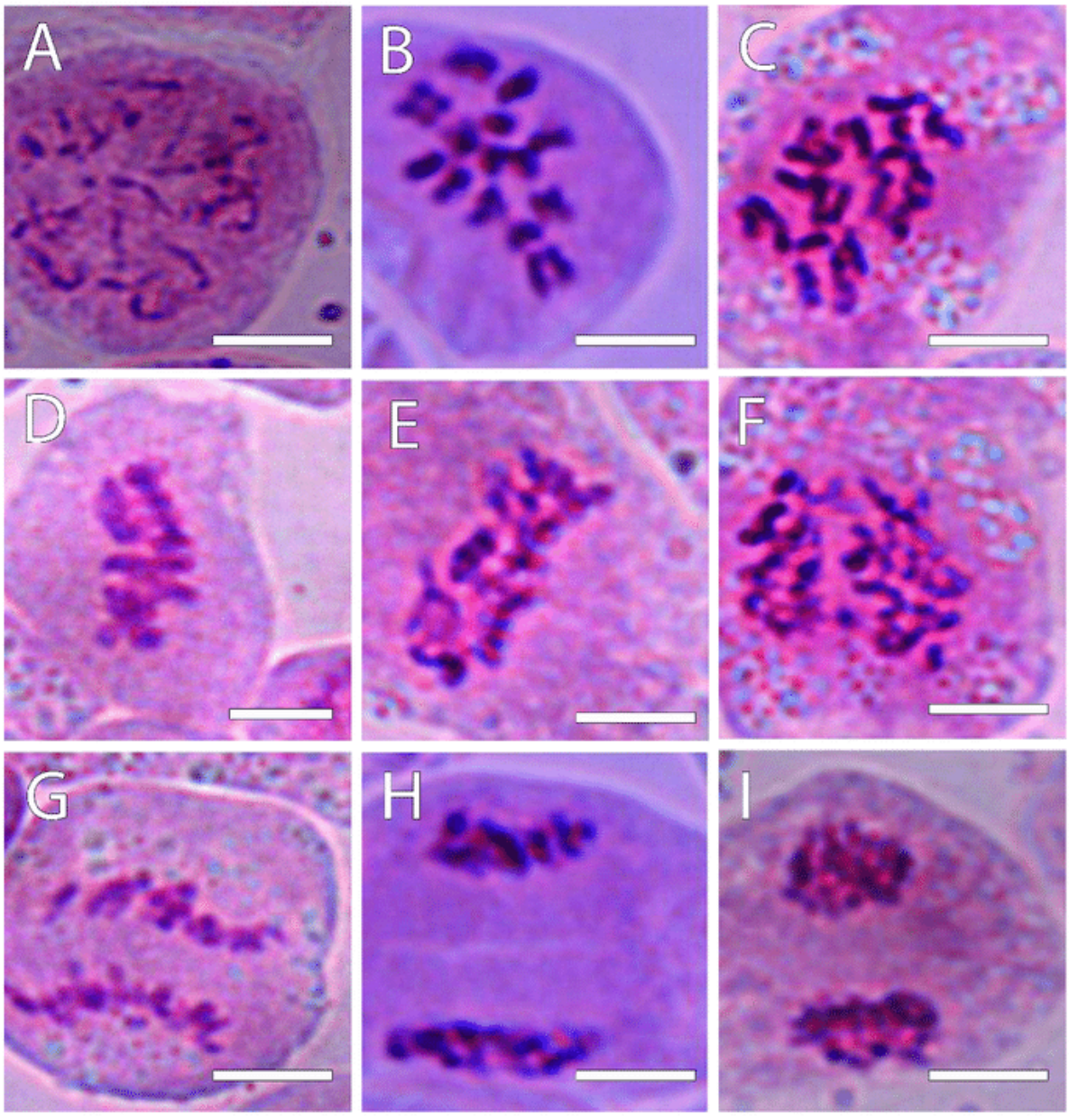
- Picric acid – 75ml
- Formalin – 25ml
- Glacial acetic acid – 5ml
- Rapid & even peneration
- Fixed tissue gives brilliant staining with trichome methods
- Used to demonstrate **glycogen.**
- Good for **GIT biopsies**


Bouin´s fixative
a
25 µm
Davidson´s fixative
b
25 µm
Bouin´s fixative
c
100 µm
Davidson´s fixative
d
100 µm

WHY ACETOCARMINE STAIN IS USED IN MITOSIS

Staining is a technique used in microscopic studies of biological samples to enhance the contrast of the biological sample under the microscope.

- Acetocarmine is a DNA-specific stain used for the visualization of super-coiled chromosomes during the different stages of mitosis
- Acetocarmine specifically stain chromosomes keep the cytoplasm colorless. Therefore, it can be used to visualize chromosomes in mitotic studies.
- Acetocarmine is lightly-toxic than the other nucleic acid stains such as aceto-orcein.
- Acetocarmine is cheaper than other types of stains.


A
B
C
D
E
F
G
H
I

சாதாரண உப்பு கரைசல்: எப்படி தயாரிப்பது

சாதாரண உப்பு என்பது உப்பு மற்றும் தண்ணீரின் கலவையாகும். இது உப்பு செறிவு கண்ணீர், இரத்தம் மற்றும் பிற உடல் திரவங்களுக்கு (0.9% உமிழ்நீர்) ஒத்திருப்பதால் இது சாதாரணமானது என்று அழைக்கப்படுகிறது. இது ஐசோடோனிக் தீர்வு என்றும் அழைக்கப்படுகிறது. சாதாரண உப்பு இனிமையானது மற்றும் பயன்படுத்தும்போது எரியாது அல்லது கொட்டாது. இது இதற்குப் பயன்படுத்தப்படுகிறது:

காண்டாக்ட் லென்ஸ்கள் கழுவுதல்

நாசி பத்திகளை கழுவுதல் (நாசி நீர்ப்பாசனம்)

சிறுநீர்ப்பை சுத்தப்படுத்துதல் (சிறுநீர்ப்பை பாசனம்)

சாதாரண உப்பு கரைசலை செய்வது எப்படி

ஒரு சுத்தமான கண்ணாடி பாட்டில் அல்லது மூடியுடன் கூடிய ஜாடி

அட்டவணை உப்பு

மூடியுடன் பானை

வழிமுறைகள்:

ஒரு கப் தண்ணீர் மற்றும் ½ டீஸ்பூன் உப்பு பானையில் வைக்கவும். மூடி வைக்கவும்.

மூடியுடன் 15 நிமிடங்கள் வேகவைக்கவும் (ஒரு டைமரை அமைக்கவும்).

ஒரு அறை வெப்பநிலையில் குளிரும் வரை பான் ஒதுக்கி வைக்கவும்.

கடாயில் இருந்து உப்பு மற்றும் தண்ணீரை (சாதாரண உப்பு) ஜாடி அல்லது பாட்டில் கவனமாக ஊற்றி மூடி வைக்கவும்.

சாதாரண உப்பு தீர்வு: சில முக்கியமான புள்ளிகள்

புதிய சாதாரண உமிழ்நீரை மட்டுமே பயன்படுத்துவது மிகவும் முக்கியம். பாக்டீரியாக்கள் உமிழ்நீரில் வளர்ந்து தொற்றுநோய்களை ஏற்படுத்தும்.

ஒரு சுத்தமான கண்ணாடி குடுவை அல்லது பாட்டிலைப் பயன்படுத்துங்கள், இது சமீபத்தில் ஒரு பாத்திரங்கழுவி மிகவும் சூடான சவக்காரம் கொண்ட தண்ணீரில் கழுவப்பட்டது.

கரைசலை குடிக்க வேண்டாம்.

தீர்வு மேகமூட்டமாக வளர்ந்தால் அல்லது அழுக்காகத் தெரிந்தால் அதைத் தூக்கி எறியுங்கள்.

உமிழ்நீரை ஒரு பாட்டில் அல்லது ஒரு கிளாஸில் அதிகபட்சம் 24 மணி நேரம் வைத்திருங்கள். பயன்படுத்தப்படாத எந்தவொரு தீர்வையும் தூக்கி எறிந்து, கொள்கலனைக் கழுவி, புதிய தீர்வை உருவாக்கவும்.

இயல்பான உமிழ்நீர் தீர்வு (என்.எஸ்.எஸ்) பொதுவாக பல்வேறு ஆய்வக நடைமுறைகளில் பயன்படுத்தப்படுகிறது, இது செல்கள் செல்களைத் தயாரிப்பது குறுக்குவெட்டுக்கு இடைநீக்கம், மறுஉருவாக்கங்களை நீர்த்துப்போகச் செய்வது, மல பரிசோதனைகள், செரோலாஜிக்கல்

சோதனைகள், நோயறிதல் சோதனைகள் போன்றவற்றில் நீர்த்தங்களை உருவாக்குவது.

வணிக ரீதியாக தயாரிக்கப்பட்ட இயல்பான உமிழ்நீர் தீர்வு சந்தையில் கிடைக்கிறது, ஆனால் தேவைப்படும் போதெல்லாம் அதை ஆய்வகத்தில் கைமுறையாக எளிதாக தயாரிக்கலாம். சாதாரண உப்புத் தீர்வு வெறுமனே 0.85% சோடியம் குளோரைடு (NaCl) கரைசலாகும், இது சோடியம் குளோரைடு படிகங்களின் கணக்கிடப்பட்ட அளவை தேவையான அளவு வடிகட்டிய நீரில் கரைத்து ஆய்வகத்தில் தயாரிக்கலாம்

சாதாரண சால்ன் தீர்வுக்கான கணக்கீடு.....

ஒரு சாதாரண உமிழ்நீர் தீர்வு 0.85% சோடியம் குளோரைடு கரைசலாகும்.

அதாவது 100 மில்லி வடிகட்டிய நீரில் 0.85 கிராம் சோடியம் குளோரைடு.

1 நமக்குத் தேவையான 1 எல் சாதாரண உப்புத் தீர்வைத் தயாரிப்பதற்கு,

W1 / V1 = W2 / V2

W1 = 100 மில்லி என்எஸ் = 0.85 கிராம் தேவைப்படும் சோடியம் குளோரைடு அளவு

வி 1 = என்எஸ் = 100 மில்லி தயாரிக்க 0.85 கிராம் சோடியம் குளோரைட்டுக்கு தேவையான அளவு

W2 = தேவையான அளவு சாதாரண உப்பு கரைசலை உருவாக்க சோடியம் குளோரைட்டின் அளவு

வி 2 = 1000 மில்லி (அல்லது சாதாரண அளவு உப்பு தயாரிக்க விரும்பும் அளவு)

0.85 கிராம் / 100 மில்லி = டபிள்யூ 2 கிராம் / 1000 மில்லி

0.85 கிராம் × 1000 மிலி = டபிள்யூ 2 × 100 மிலி

W2 = 0.85 × 1000 மிலி / 100 மிலி

W2 = 8.5 கிராம்

தேவை....

எடையுள்ள அளவு

வால்யூமெட்ரிக் பிளாஸ்க் / பீக்கர்

அசை

புனல்

சாதாரண சால்ன் தீர்வுக்கான செயல்முறை.....

8. எடையுள்ள அளவின் உதவியுடன் சோடியம் குளோரைடு (NaCl) 8.5 கிராம் எடையுள்ளதாக இருக்கும்.

இப்போது 500 மில்லி வடிகட்டிய நீரை வால்யூமெட்ரிக் பிளாஸ்கில் அல்லது ஒரு பீக்கரில் எடுத்து 8.5 கிராம் NaCl ஐ சேர்க்கவும்.

உள்ளடக்கங்களை கலக்க மெதுவாக குடுவை சுழற்றுங்கள் அல்லது அசைப்பவரின் உதவியுடன் நீங்கள் ஒரு பீக்கரில் தீர்வு செய்கிறீர்கள் என்றால் கிளறவும்.

NaCl முழுவதுமாக கரைந்ததும் வடிகட்டிய நீரைச் சேர்த்து இறுதி அளவை 1 லிட்டராக மாற்றவும்.

வால்யூமெட்ரிக் பிளாஸ்கின் வாயில் காற்று-இறுக்கமான ஸ்டாப்பரைச் செருகவும், தீர்வை ஒரே மாதிரியாக மாற்ற மெதுவாக அசைக்கவும் அல்லது நீங்கள் ஒரு பீக்கரைப் பயன்படுத்துகிறீர்கள் என்றால் ஒரு ஸ்ட்ரைரரைப் பயன்படுத்தி கரைசலை நன்றாகக் கிளறவும்

பயன்படுத்தும் நோக்கம்:

W.B.C க்கு திரவத்தை நீர்த்துப்போகச் செய்தல்.

அறிமுகம்:

பனிப்பாறை அசிட்டிக் அமிலம் சிவப்பு அணுக்களை லைஸ் செய்கிறது. ஜெண்டியன் வயலட் லுகோசைட்டுகளின் கருக்களை சிறிது கறைபடுத்துகிறது. இரத்த மாதிரி ஒரு WBC குழாயில் 1:20 நீர்த்த திரவத்துடன் நீர்த்தப்படுகிறது மற்றும் செல்கள் ஒரு எண்ணும் அறையைப் பயன்படுத்தி நுண்ணோக்கியின் குறைந்த சக்தியின் கீழ் கணக்கிடப்படுகின்றன. முழு இரத்தத்தின் மிமீ 3 () 1) க்கு நீர்த்த இரத்தத்தில் உள்ள உயிரணுக்களின் எண்ணிக்கை தெரிவிக்கப்படுகிறது

இந்த கட்டுரையில் நிரந்தர ஸ்லைடுகளைத் தயாரிப்பதற்குத் தேவையான ஆறு முக்கியமான ஆய்வக உலைகளைப் பற்றி விவாதிப்போம். ஆய்வக எதிர்வினைகள்: 1. முகவர்களை சரிசெய்தல் மற்றும் கொல்வது 90% ஆல்கஹால் 2. கறை 3. ஆல்கஹால் தரங்கள் 4. உடலியல் உமிழ்நீர் 5. மேயரின் அல்புமென்ஸ் 6. பாதுகாப்புகள்.

Bouin இன் திரவம் (அக்வஸ்):

பிக்ரிக் அமிலம்

(நிறைவுற்ற அக்வஸ் கரைசல்): 75 எம்.எல்.

அசிட்டிக் அமிலம் பனிப்பாறை 5 எம்.எல்.

ஃபார்மால்டிஹைட்: 25 எம்.எல்.

தயாரிப்பு:

வடிகட்டிய நீரில் பிக்ரிக் அமிலத்தின் நிறைவுற்ற கரைசலைத் தயாரிக்கவும். பிக்ரிக் அமிலத்தின் 75 மில்லி நிறைவுற்ற கரைசலில் 25 மில்லி ஃபார்மால்டிஹைட் மற்றும் 5 மில்லி பனிப்பாறை அசிட்டிக் அமிலம் சேர்க்கவும். நன்கு கலக்கவும்

கார்னாயின் சரிசெய்தல்

குளோரோஃபார்ம்: 30 மில்லி

அசிட்டிக் அமிலம் (பனிப்பாறை): 30 மில்லி

முழுமையான ஆல்கஹால்: 60 மில்லி

தயாரிப்பு:

உலைகளை கலக்கவும்; ஆவியாவதைத் தடுக்க நன்கு பொருத்தப்பட்ட திருகு மூடிய பாட்டில் வைக்கவும்.

கார்போஹைட்ரேட்டுகள், கிளைகோஜன், நிசெல் பொருளுக்கு மட்டுமே சிறந்தது; சலவை திரவம்: முழுமையான ஆல், கழுவும் நேரம்: 2 முதல் 3 மணி வரை; நிர்ணயிக்கும் நேரம்: 3 முதல் 6 மணி வரை.

அசிட்டோகார்மைன்:

பனிப்பாறை அசிட்டிக் அமிலம்: 45 எம்.எல்

காய்ச்சி வடிகட்டிய நீர்: 55 எம்.எல்

கார்மைன் தூள்: 0.5-1.0 கிராம்.

தயாரிப்பு:

45 மில்லி பனிப்பாறை அசிட்டிக் அமிலம் மற்றும் 55 மில்லி வடிகட்டிய நீர் மற்றும் பருத்தி சொருகலுடன் ஒரு கூம்பு பிளாஸ்கில் கொதிக்க வைக்கவும். 0.5 முதல் 1.0 கிராம் கார்மைன் தூள் சேர்க்கவும். நன்றாக குலுக்கி, குளிர்ந்து வடிகட்டி, பங்கு தீர்வாக சேமிக்கவும்

அசிட்டிக் அமிலம் மற்றும் தண்ணீரை கலக்கவும். கொதிக்க வெப்பம் மற்றும் கார்மைன் தூள் சேர்க்க. குலுக்கி, குளிர்ந்து வடிகட்டவும். FeCl3 இன் படிகத்தைச் சேர்க்கவும்.

லீஷ்மேனின் கறை:

லீஷ்மேனின் கறை: 0.15 கிராம்.

முழுமையான ஆல்கஹால்: 100 எம்.எல்

தயாரிப்பு:

0.1 கிராம் லீஷ்மேனின் தூள் (சாயத்தை) 100 மில்லி முழுமையான மீதில் ஆல்கஹால் அறை வெப்பநிலையில் அவ்வப்போது கிளறி கரைக்கவும்.

மலேரியா ஒட்டுண்ணி மற்றும் பிற புரோட்டோசோவான்களை இரத்த ஸ்மியர்ஸில் கறைப்படுத்த கறை பயன்படுத்தப்படுகிறது.

உதிரிபாகங்கள்

சாயத்தை ஒரு தூளாக வாங்கலாம், பின்னர் அது மெத்தனால் கலக்கப்படுகிறது அல்லது ஒரு ஆயத்த தீர்வு பெறப்படலாம்.

ரைட்டின் கறை என்பது ஒரு வகை ரோமானோவ்ஸ்கி கறை ஆகும், இது பொதுவாக இரத்த ரத்த ஸ்மியர்ஸின் வழக்கமான கறைகளுக்கு ஹெமாட்டாலஜி ஆய்வகத்தில் பயன்படுத்தப்படுகிறது. எலும்பு மஜ்ஜை ஆஸ்பைரேட்டுகள், சிறுநீர் மாதிரிகள் மற்றும் இரத்த ஸ்மியர்ஸில் மலேரியா ஒட்டுண்ணிகளை நிரூபிக்கவும் இது பயன்படுத்தப்படுகிறது.

1902 ஆம் ஆண்டில் ரோமானோவ்ஸ்கி கறையின் மாற்றத்தின் அடிப்படையில் கறையை உருவாக்கிய ஜேம்ஸ் ஹோமர் ரைட்டுக்கு ரைட்டின் கறை பெயரிடப்பட்டது. கறை இரத்த அணுக்களுக்கு இடையில் எளிதில் வேறுபடுகிறது, எனவே வேறுபட்ட WBC எண்ணிக்கையைச் செய்வதற்கும் இரத்த அணுக்களின் உருவ அமைப்பை மதிப்பீடு செய்வதற்கும் பரவலாகப் பயன்படுத்தப்பட்டது

கொள்கை

ரைட்டின் கறை என்பது ஈசின் மற்றும் மெத்திலீன் நீல கலவையை உள்ளடக்கிய ஒரு பாலிக்ரோமடிக் கறை ஆகும். ரைட் கறை மெத்தனால் அடிப்படையிலானது என்பதால், கறை படிவதற்கு முன்பு அதை சரிசெய்யும் படி தேவையில்லை. இருப்பினும், ஈரப்பதமான

நாட்களில் அல்லது வயதான கறையுடன் ஏற்படக்கூடிய நீர் கலைப்பொருட்களைக் குறைக்க சரிசெய்தல் உதவுகிறது.

மெத்தனால் செல்களை ஸ்லைடில் சரிசெய்கிறது. ஈசின் ஒய் ஒரு அமில அனானிக் சாயம் மற்றும் மெத்திலீன் நீலம் அடிப்படை கேஷனிக் சாயமாகும். இடையக நீரில் நீர்த்தும்போது, அயனியாக்கம் ஏற்படுகிறது. ஈசின் ஹீமோகுளோபின் மற்றும் ஈசினோபிலிக் துகள்கள் போன்ற அடிப்படை கூறுகளை ஒரு ஆரஞ்சு முதல் இளஞ்சிவப்பு நிறத்தில் கறைபடுத்துகிறது. மெத்திலீன் நீல நிறமானது நீல நிற நிழல்களில் நியூக்ளிக் அமிலம் மற்றும் பாசோபிலிக் துகள்கள் போன்ற அமில செல்லுலார் கூறுகள். உயிரணுக்களின் நடுநிலை கூறுகள் சாயத்தின் இரு கூறுகளால் கறைபட்டு, மாறுபட்ட வண்ணங்களை உருவாக்குகின்றன

ஹைமின் தீர்வு

தேவையான பொருட்கள்:

மெர்குரிக் குளோரைடு - 0.25 கிராம்
சோடியம் சல்பேட் - 2.50 கிராம்
சோடியம் குளோரைடு - 0.50 கிராம்
காய்ச்சி வடிகட்டிய நீர் - 100.0 மிலி
இறுதி pH (25 ° C இல்) - 5.9 ± 0.1

சிவப்பு செல் எண்ணிக்கை

இரத்தத்தின் மாதிரியை பொருத்தமான திரவத்துடன் (ஹயெமின் தீர்வு) நீர்த்துப்போகச் செய்வதும், நுண்ணோக்கின் கீழ் ஒரு சிறிய பகுதியிலுள்ள செல்களை எண்ணுவதும் இந்தக் கொள்கையில் அடங்கும். எந்திரத்தில் மிக முக்கியமான பொருட்கள் எண்ணும் அறை மற்றும் நீர்த்த அமைப்பு.

PREPARATION OF NORMAL SALINE SOLUTION

Normal Saline Solution (NSS) is commonly used in various laboratory procedures like in the preparation of Red cells Suspension for the crossmatch, for preparing dilutions of Reagents, for stool examinations, to make the dilutions in serological tests, diagnostic tests etc.

There are commercially prepared Normal saline solution available in the market but it can easily be prepared manually in the laboratory whenever required. The normal saline solution is simply the 0.85% Sodium chloride (NaCl) solution which can be prepared in the laboratory by dissolving the calculated amount of Sodium chloride crystals in the required quantity of Distilled water.

The Normal saline solution is prepared as follows……

CALCULATION FOR NORMAL SALINE SOLUTION…..

⇒ A normal saline solution is the 0.85% sodium chloride solution.

⇒ That means 0.85 gm of sodium chloride in 100 ml distilled water.

⇒ For preparing 1 L normal saline solution we require,

$$W_1 / V_1 = W_2 / V_2$$

W_1 = Required quantity of sodium chloride for 100 ml NS = 0.85 gm

V_1 = Required volume for 0.85 gm sodium chloride to make NS = 100 ml

W_2 = Required Quantity of Sodium chloride to make the desired quantity of Normal Saline solution

V2 = 1000 ml (or desired quantity of Normal saline to be prepared)

$$0.85 \text{ gm} / 100 \text{ ml} = W2 \text{ gm} / 1000 \text{ ml}$$

$$0.85 \text{ gm} \times 1000 \text{ ml} = W2 \times 100 \text{ ml}$$

$$W2 = 0.85 \times 1000 \text{ ml} / 100 \text{ ml}$$

$$W2 = 8.5 \text{ gm}$$

Thus, to prepare 1000 ml of Normal saline solution 8.5 gm sodium chloride crystals should be dissolved in 1000 ml of distilled water.

SODIUM CHLORIDE CRYSTALS
APPARATUS REQUIRED.....

- Weighing scale
- Volumetric flask/beaker
- Stirrer
- Funnel

CHEMICALS REQUIRED.....

- Distilled water
- Sodium chloride (NaCl)

PROCEDURE FOR NORMAL SALINE SOLUTION.....

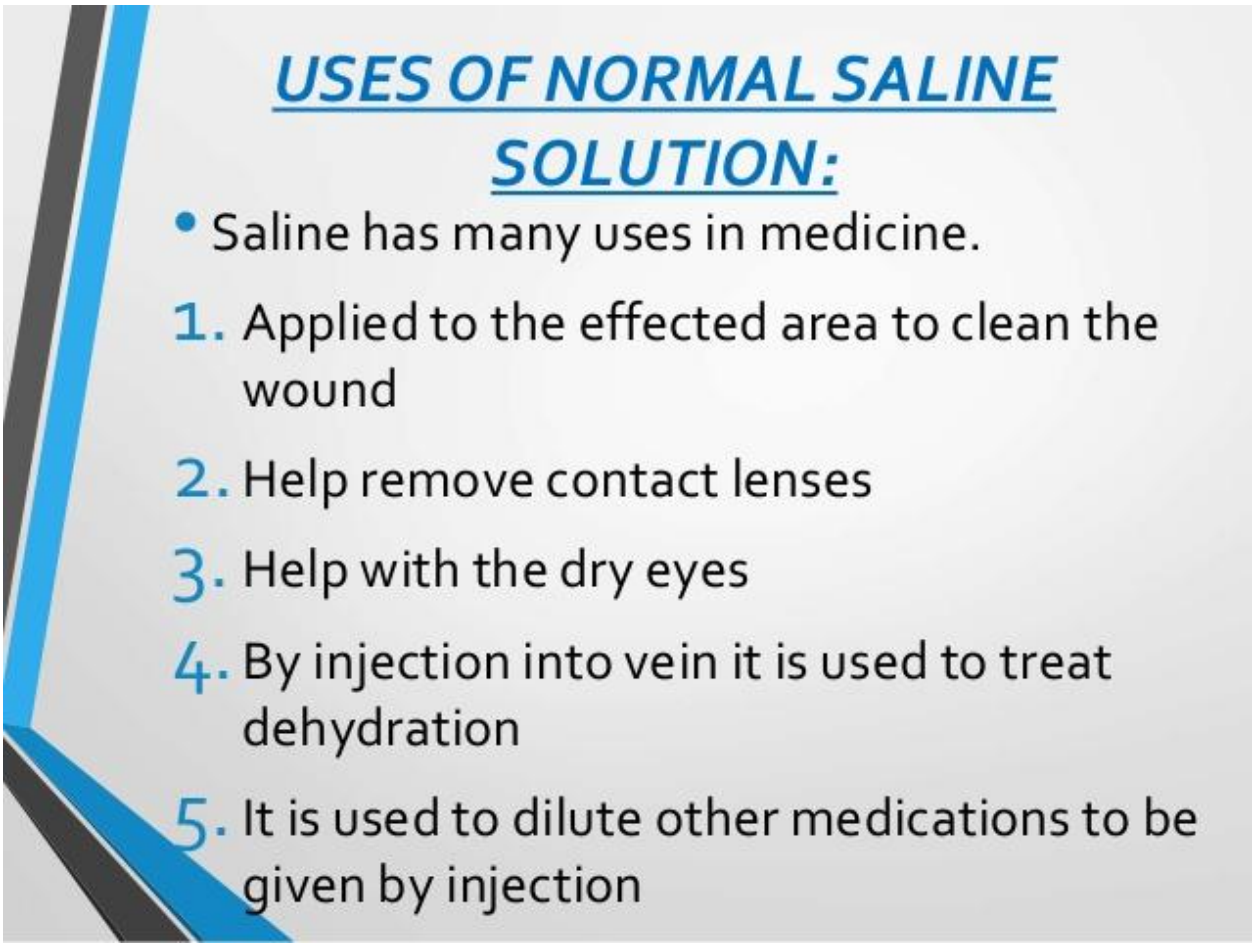
⇒ Weigh 8.5 gm of Sodium chloride (NaCl) with the help of weighing scale.

⇒ Now take 500 ml of distilled water in the volumetric flask or in a Beaker and to this add 8.5 gm NaCl.

⇒ Swirl the flask gently to mix the contents or stir in case you are making the solution in a beaker with the help of stirrer.

⇒ When NaCl dissolves completely then add distilled water and make the final volume 1 liter.

⇒ Insert an air-tight stopper into the mouth of the volumetric flask and shake it gently to make the solution homogeneous or if you are using a beaker stir the solution well using a stirrer.



W.B.C. Diluting Fluid

R016

Intended Use

WBC diluting fluid is used for perfoming the WBC (Leucocyte) count.

Composition**

Ingredients	
Glacial acetic acid	2.00 ml
Gentian Violet (1% w/v)	1.00 gm
Distilled water	97.00 ml
Final pH (at 25°C)	2.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

1) Draw EDTA anticoagulated blood to 0.5 mark in the capillary end of WBC pipette.
2) Carefully, wipe excess blood outside the pipette by using cotton.
3) Draw diluting fluid up to 11 mark.
4) Mix the contents in pipette and after 5minutes by discarding few drops, fill the counting chamber and allow the cells to settle for 2-3 minutes.
5) Focus on 1 of the "W" marked areas (each having 16 small squares) by turning objective to low powder.10X
6) Count cells in all 4 "W" marked corner squares.

Principle And Interpretation

WBC diluting fluid is used for perfoming the WBC (Leucocyte) count. Glacial acetic acid lyses the red cells. Gentian violet slightly stains the nuclei of the leucocytes. The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid and the cells are counted under low power of the microscope by using a counting chamber. The number of cells in undiluted blood is reported per cumm (µl) of whole blood.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Performance and Evaluation

Performace of the product is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Purple coloured, clear solution.

Clarity
Clear with no insoluble particles.

pH
2.00-2.40

Calculation

Number of WBCs/cumm(µl)of whole blood =No. of WBCs counted X Dilution /Area counted X Depth of fluid
where,Dilution=20; Area counted= 4x1sq.mm=4sq.mm ; Depth =0.1mm(constant)

No.of leucocytes / cu mm(µl) of whole blood = No.of cells counted X 20 / 4 X 0.1
= No.of cells counted X50

Storage and Shelf Life

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Text book of Medical Laboratory Technology; Praful B.Godkar
2. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
3. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.

Revision : 02 / 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, B /4-6 , MIDC, Dindori, Nashik MH www.himedialabs.com
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

UNIT – III

Complete blood count

A **complete blood count** (**CBC**), also known as a **full blood count** (**FBC**), is a set of medical laboratory tests that provide information about the cells in a person's blood. The CBC indicates the counts of white blood cells, red blood cells and platelets, the concentration of hemoglobin, and the hematocrit (the volume percentage of red blood cells). The red blood cell indices, which indicate the average size and hemoglobin content of red blood cells, are also reported, and a white blood cell differential, which counts the different types of white blood cells, may be included.

The CBC is often carried out as part of a medical assessment, and can be used to monitor health or diagnose diseases. The results are interpreted by comparing them to reference ranges, which vary with gender and age. Conditions like anemia and thrombocytopenia are defined by abnormal complete blood count results. The red blood cell indices can provide information about the cause of a person's anemia such as iron deficiency and vitamin B12 deficiency, and the results of the white blood cell differential can help to diagnose viral, bacterial and parasitic infections and blood disorders like leukemia. Not all results falling outside of the reference range require medical intervention.

The CBC is performed using basic laboratory equipment or an automated hematology analyzer, which counts cells and collects information on their size and structure. The concentration of hemoglobin is measured, and the red blood cell indices are calculated from measurements of red blood cells and hemoglobin. Manual tests can be used to independently confirm abnormal results. Approximately 10–25% of samples require a manual blood smear review, in which the blood is stained and viewed under a microscope to verify that the analyzer results are consistent with the appearance of the cells and to look for abnormalities. The hematocrit can be determined manually by centrifuging the sample and measuring the proportion of red blood cells, and in laboratories without access to automated instruments, blood cells are counted under the microscope using a hemocytometer.

In 1852, Karl Vierordt published the first procedure for performing a blood count, which involved spreading a known volume of blood on a microscope slide and counting every cell. The invention of the hemocytometer in 1874 by Louis-Charles Malassez simplified the microscopic analysis of blood cells, and in the late 19th century, Paul Ehrlich and Dmitri Leonidovich Romanowsky developed techniques for staining white and red blood cells that are still used to examine blood smears. Automated methods for measuring hemoglobin were developed in the 1920s, and Maxwell Wintrobe introduced the Wintrobe hematocrit method in 1929, which in turn allowed him to define the red blood cell indices.

A landmark in the automation of blood cell counts was the Coulter principle, which was patented by Wallace H. Coulter in 1953. The Coulter principle uses electrical impedance measurements to count blood cells and measure their sizes, a technology that remains in use in many automated analyzers. Further research in the 1970s involved the use of optical measurements to count and identify cells, which enabled the automation of the white blood cell differential.

Red blood cells, (RBC)

ample CBC in microcytic anemia

Analyte	Result	Normal range
Red cell count	5.5 x 10^{12}/L	4.5–5.7
White cell count	9.8 x 10^{9}/L	4.0–10.0
Hemoglobin	123 g/L	133–167
Hematocrit	0.42	0.35–0.53
MCV	76 fL	77–98
MCH	22.4 pg	26–33
MCHC	293 g/L	330–370
RDW	14.5%	10.3–15.3

An example of CBC results showing a low hemoglobin, mean red cell volume (MCV), mean red cell hemoglobin (MCH) and mean red blood cell hemoglobin content (MCHC). The person was anemic. The cause could be iron deficiency or a hemoglobinopathy.[108]

Red blood cells deliver oxygen from the lungs to the tissues and on their return carry carbon dioxide back to the lungs where it is exhaled. These functions are mediated by the cells' hemoglobin. The analyzer counts red blood cells, reporting the result in units of 106 cells per microlitre of blood (× 106/μL) or 1012 cells per litre (× 1012/L), and measures their average size, which is called the mean cell volume and expressed in femtolitres or cubic micrometres. By multiplying the mean cell volume by the red blood cell count, the hematocrit (HCT) or packed cell volume (PCV), a measurement of the percentage of blood that is made up of red blood cells, can be derived; and when the hematocrit is performed directly, the mean cell volume may be calculated from the hematocrit and red blood cell count. Hemoglobin, measured after the red blood cells are lysed, is usually reported in units of grams per litre (g/L) or grams per decilitre (g/dL). Assuming that the red blood cells are normal, there is a constant relationship between hemoglobin and hematocrit: the hematocrit percentage is approximately three times greater than the hemoglobin value in g/dL, plus or minus three. This relationship, called the rule of three, can be used to confirm that CBC results are correct.

Two other measurements are calculated from the red blood cell count, the hemoglobin concentration, and the hematocrit: the mean corpuscular hemoglobin and the mean corpuscular hemoglobin concentration. These parameters describe the hemoglobin content of each red blood cell. The MCH and MCHC can be confusing; in essence the MCH is a measure of the average amount of hemoglobin per red blood cell. The MCHC gives the average proportion of the cell that is hemoglobin. The MCH does not take into account the size of the red blood cells whereas the MCHC does. Collectively, the MCV, MCH, and MCHC are referred to as the red blood cell indices. Changes in these indices are visible on the blood smear: red blood cells that are abnormally large or small can be identified by comparison to the sizes of white blood cells, and cells with a low hemoglobin concentration appear pale. Another parameter is calculated from the initial measurements of red blood cells: the red blood cell distribution width or RDW, which reflects the degree of variation in the cells'

An abnormally low hemoglobin, hematocrit, or red blood cell count indicates anemia. Anemia is not a diagnosis on its own, but it points to an underlying condition affecting the person's red blood cells.[88] General causes of anemia include blood loss, production of

defective red blood cells (ineffective erythropoeisis), decreased production of red blood cells (insufficient erythropoeisis), and increased destruction of red blood cells (hemolytic anemia). Anemia reduces the blood's ability to carry oxygen, causing symptoms like tiredness and shortness of breath. If the hemoglobin level falls below thresholds based on the person's clinical condition, a blood transfusion may be necessary.

An increased number of red blood cells, which usually leads to an increase in the hemoglobin and hematocrit, is called polycythemia. Dehydration or use of diuretics can cause a "relative" polycythemia by decreasing the amount of plasma compared to red cells. A true increase in the number of red blood cells, called absolute polycythemia, can occur when the body produces more red blood cells to compensate for chronically low oxygen levels in conditions like lung or heart disease, or when a person has abnormally high levels of erythropoietin (EPO), a hormone that stimulates production of red blood cells. In polycythemia vera, the bone marrow produces red cells and other blood cells at an excessively high rate.

Evaluation of red blood cell indices is helpful in determining the cause of anemia. If the MCV is low, the anemia is termed microcytic, while anemia with a high MCV is called macrocytic anemia. Anemia with a low MCHC is called hypochromic anemia. If anemia is present but the red blood cell indices are normal, the anemia is considered normochromic and normocytic

The term hyperchromia, referring to a high MCHC, is generally not used. Elevation of the MCHC above the upper reference value is rare, mainly occurring in conditions such as spherocytosis, sickle cell disease and hemoglobin C disease.

An elevated MCHC can also be a false result from conditions like red blood cell agglutination (which causes a false decrease in the red blood cell count, elevating the MCHC) or highly elevated amounts of lipids in the blood (which causes a false increase in the hemoglobin result).

Microcytic anemia is typically associated with iron deficiency, thalassemia, and anemia of chronic disease, while macrocytic anemia is associated with alcoholism, folate and B12

deficiency, use of some drugs, and some bone marrow diseases. Acute blood loss, hemolytic anemia, bone marrow disorders, and various chronic diseases can result in anemia with a normocytic blood picture

The MCV serves an additional purpose in laboratory quality control. It is relatively stable over time compared to other CBC parameters, so a large change in MCV may indicate that the sample was drawn from the wrong patient

A low RDW has no clinical significance, but an elevated RDW represents increased variation in red blood cell size, a condition known as anisocytosis

Anisocytosis is common in nutritional anemias such as iron deficiency anemia and anemia due to vitamin B12 or folate deficiency, while people with thalassemia may have a normal RDW. Based on the CBC results, further steps can be taken to investigate anemia, such as a ferritin test to confirm the presence of iron deficiency, or hemoglobin electrophoresis to diagnose a hemoglobinopathy such as thalassemia or sickle cell disease.

White blood cells (WBC)

Sample CBC in chronic myeloid leukemia

Analyte	Result	Analyte	Result
White cell count	98.8 x 10^9/L	**Neutrophils**	48%
		Lymphocytes	3%
Hemoglobin	116 g/L	**Monocytes**	4%
Hematocrit	0.349 L/L	**Eosinophils**	3%
MCV	89.0 fL	**Basophils**	21%
Platelet count	1070 x 10^9/L	**Band neutrophils**	8%
		Metamyelocytes	3%
		Myelocytes	8%
		Blast cells	2%

The white blood cell and platelet counts are markedly increased, and anemia is present. The differential count shows basophilia and the presence of band neutrophils, immature granulocytes and blast cells.

White blood cells defend against infections and are involved in the inflammatory response. A high white blood cell count, which is called leukocytosis, often occurs in infections, inflammation, and states of physiologic stress. It can also be caused by diseases that involve abnormal production of blood cells, such as myeloproliferative and lymphoproliferative disorders. A decreased white blood cell count, termed leukopenia, can lead to an increased risk of acquiring infections, and occurs in treatments like chemotherapy and radiation therapy and many conditions that inhibit the production of blood cells. Sepsis is associated with both leukocytosis and leukopenia. The total white blood cell count is usually reported in cells per microlitre of blood (/μL) or 109 cells per litre (× 109/L)

In the white blood cell differential, the different types of white blood cells are identified and counted. The results are reported as a percentage and as an absolute number per unit volume. Five types of white blood cells—neutrophils, lymphocytes, monocytes, eosinophils, and basophils—are typically measured Some instruments report the number of immature granulocytes, which is a classification consisting of precursors of neutrophils; specifically, promyelocytes, myelocytes and metamyelocytes they are identified in the manual differential.

Differential results are useful in diagnosing and monitoring many medical conditions. For example, an elevated neutrophil count (neutrophilia) is associated with bacterial infection, inflammation, and myeloproliferative disorders, while a decreased count (neutropenia) may occur in individuals who are undergoing chemotherapy or taking certain drugs, or who have diseases affecting the bone marrow. Neutropenia can also be caused by some congenital disorders and may occur transiently after viral or bacterial infections in children. People with severe neutropenia and clinical signs of infection are treated with antibiotics to prevent potentially life-threatening disease.

An increased number of band neutrophils—young neutrophils that lack segmented nuclei—or immature granulocytes is termed left shift and occurs in sepsis and some blood disorders, but is normal in pregnancy. An elevated lymphocyte count (lymphocytosis) is associated with viral infection and lymphoproliferative disorders like chronic lymphocytic leukemia; elevated monocyte counts (monocytosis) are associated with chronic inflammatory states; and the eosinophil count is often increased (eosinophilia) in parasitic infections and allergic conditions. An increased number of basophils, termed basophilia, can occur in myeloproliferative disorders like chronic myeloid leukemia and polycythemia vera. The presence of some types of abnormal cells, such as blast cells or lymphocytes with neoplastic features, is suggestive of a hematologic malignancy.

Erythrocyte sedimentation rate (ESR)

The erythrocyte sedimentation rate (ESR or sed rate) is the rate at which red blood cells in anticoagulated whole blood descend in a standardized tube over a period of one hour. It is a common hematology test, and is a non-specific measure of inflammation. To perform the test, anticoagulated blood is traditionally placed in an upright tube, known as a Westergren tube, and the distance which the red blood cells fall is measured and reported in mm at the end of one hour.

Since the introduction of automated analyzers into the clinical laboratory, the ESR test has been automatically performed.

The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential). When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other. The red cells form stacks called rouleaux which settle faster, due to their increased density. Rouleaux formation can also occur in association with some lymphoproliferative disorders in which one or more paraproteins are secreted in high amounts. While abnormal in humans, rouleaux formation can be a normal physiological finding in horses, cats, and pigs.

The ESR is increased in inflammation, pregnancy, anemia, autoimmune disorders (such as rheumatoid arthritis and lupus), infections, some kidney diseases and some cancers (such as lymphoma and multiple myeloma). The ESR is decreased in polycythemia, hyperviscosity, sickle

cell anemia, leukemia, chronic fatigue syndrome, low plasma protein (due to liver or kidney disease) and congestive heart failure. Although increases in immunoglobulins usually increase the ESR, very high levels can reduce it again due to hyperviscosity of the plasma. This is especially likely with IgM-class paraproteins, and to a lesser extent, IgA-class. The basal ESR is slightly higher in females

Stages

Erythrocyte sedimentation rate (ESR) is the measure of ability of erythrocytes (red blood cell) to fall through the blood plasma and accumulate together at the base of container in one hour.

There are three stages in erythrocyte sedimentation:

1. Rouleaux formation
2. Sedimentation or settling stage
3. Packing stage - 10 minutes (sedimentation slows and cells start to pack at the bottom of the tube)

In normal conditions, the red blood cells are negatively charged and therefore repel each other rather than stacking. ESR is also reduced by high blood viscosity, which slows the rate of fall.

Causes of elevation

he rate of erythrocyte sedimentation is affected by both inflammatory and non-inflammatory conditions.

Inflammation

In inflammatory conditions, fibrinogen, other clotting proteins, and alpha globulin are positively charged, thus increasing the ESR.[9] ESR begins to rise at 24 to 48 hours after the onset of acute self-limited inflammation, decreases slowly as inflammation resolves, and can take weeks to months to return to normal levels. For ESR values more than 100 mm/hour, there is a 90% probability that an underlying cause would be found upon investigation.

Non-inflammatory conditions

In non-inflammatory conditions, plasma albumin concentration, size, shape, and number of red blood cells, and the concentration of immunoglobulin can affect the ESR. Non-inflammatory conditions that can cause raised ESR include anemia, kidney failure, obesity, ageing, and female sex. ESR is also higher in women during menstruation and pregnancy. The value of ESR does not change whether dialysis is performed or not. Therefore, ESR is not a reliable measure of inflammation in those with kidney injuries as the ESR value is already elevated.

Causes of reduction

An increased number of red blood cells (polycythemia) causes reduced ESR as blood viscosity increases. Hemoglobinopathy such as sickle-cell disease can have low ESR due to an improper shape of red blood cells that impairs stacking.

Medical uses

Diagnosis

ESR can sometimes be useful in diagnosing diseases, such as multiple myeloma, temporal arteritis, polymyalgia rheumatica, various auto-immune diseases, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease and chronic kidney diseases. In many of these cases, the ESR may exceed 100 mm/hour.

It is commonly used for a differential diagnosis for Kawasaki's disease (from Takayasu's arteritis; which would have a markedly elevated ESR) and it may be increased in some chronic infective conditions like tuberculosis and infective endocarditis. It is also elevated in subacute thyroiditis also known as DeQuervain's.

In markedly increased ESR of over 100 mm/h, infection is the most common cause (33% of cases in an American study), followed by cancer (17%), kidney disease (17%) and noninfectious inflammatory disorders (14%). Yet, in pneumonia the ESR stays under 100.

The usefulness of the ESR in current practice has been questioned by some, as it is a relatively imprecise and non-specific test compared to other available diagnostic tests.

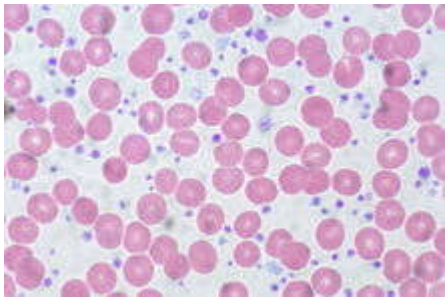
Disease severity

It is a component of the PCDAI (Pediatric Crohn's Disease Activity Index), an index for assessment of the severity of inflammatory bowel disease in children.

Monitoring response to therapy

The clinical usefulness of ESR is limited to monitoring the response to therapy in certain inflammatory diseases such as temporal arteritis, polymyalgia rheumatica and rheumatoid arthritis. It can also be used as a crude measure of response in Hodgkin's lymphoma. Additionally, ESR levels are used to define one of the several possible adverse prognostic factors in the staging of Hodgkin's lymphoma.[citation needed]

Platelets



Platelets play an essential role in clotting. When the wall of a blood vessel is damaged, platelets adhere to the exposed surface at the site of injury and plug the gap. Simultaneous activation of the coagulation cascade results in the formation of fibrin, which reinforces the platelet plug to create a stable clot. A low platelet count, known as thrombocytopenia, may cause bleeding if severe. It can occur in individuals who are undergoing treatments that suppress the bone marrow, such as chemotherapy or radiation therapy, or taking certain drugs, such as heparin, that can induce the immune system to destroy platelets. Thrombocytopenia is a feature of many blood disorders, like acute leukemia and aplastic anemia, as well as some autoimmune diseases. If the platelet count is extremely low, a platelet transfusion may be performed. Thrombocytosis, meaning a high platelet count, may occur in states of inflammation or trauma, as well as in iron deficiency, and the platelet count may reach exceptionally high levels in people with essential thrombocythemia, a rare blood disease.The platelet count can be reported in units

of cells per microlitre of blood (/μL), 103 cells per microlitre (× 103/μL), or 109 cells per litre (× 109/L).

The mean platelet volume (MPV) measures the average size of platelets in femtolitres. It can aid in determining the cause of thrombocytopenia; an elevated MPV may occur when young platelets are released into the bloodstream to compensate for increased destruction of platelets, while decreased production of platelets due to dysfunction of the bone marrow can result in a low MPV. The MPV is also useful for differentiating between congenital diseases that cause thrombocytopenia. The immature platelet fraction (IPF) or reticulated platelet count is reported by some analyzers and provides information about the rate of platelet production by measuring the number of immature platelets in the blood.

Bleeding time

Bleeding time is a medical test done on someone to assess their platelets function. It involves making a patient bleed then timing how long it takes for them to stop bleeding.

Indications

The term template bleeding time is used when the test is performed to standardized parameters.

The bleeding time test is a method indicated when other more reliable and less invasive tests for determining coagulation are not available. However, it remains the most reliable way of assessing clinical bleeding in patients with uremia. Historically it was indicated whenever the physician needed information about platelet activation

Process

It involves cutting the underside of the subject's forearm, in an area where there is no hair or visible veins. The cut is of a standardized width and depth, and is done quickly by an automatic device.

A blood pressure cuff is used above the wound, to maintain venous pressure at a specified value. The time it takes for the bleeding to stop (i.e. the time it takes for a platelet plug to form) is measured. Cessation of bleeding can be determined by blotting away the blood every several seconds until the site looks "glassy".

IVY method

The IVY method is the traditional format for this test. While both the IVY and the Duke method require the use of a sphygmomanometer, or blood pressure cuff, the IVY method is more invasive than the Duke method, utilizing an incision on the ventral side of the forearm, whereas the Duke method involves puncture with a lancet or special needle. In the IVY method, the blood pressure cuff is placed on the upper arm and inflated to 40 mmHg. A lancet or scalpel blade is used to make a shallow incision that is 1 millimeter deep on the underside of the forearm.

A standard-sized incision is made around 10 mm long and 1 mm deep. The time from when the incision is made until all bleeding has stopped is measured and is called the bleeding time. Every 30 seconds, filter paper or a paper towel is used to draw off the blood.

The test is finished when bleeding has stopped.

A prolonged bleeding time may be a result from decreased number of thrombocytes or impaired blood vessels. However, the depth of the puncture or incision may be the source of error.

Normal values fall between 3 – 10 minutes depending on the method used.

A disadvantage of Ivy's method is closure of puncture wound before stoppage of bleeding.

Duke method

With the Duke method, the patient is pricked with a special needle or lancet, preferably on the earlobe or fingertip, after having been swabbed with alcohol. The prick is about 3–4 mm deep. The patient then wipes the blood every 30 seconds with a filter paper. The test ceases when bleeding ceases. The usual time is about 2–5 minutes.

This method is not recommended and cannot be standardized because it can cause a large local hematoma

Interpretation

leeding time is affected by platelet function, certain vascular disorders and von Willebrand Disease—not by other coagulation factors such as haemophilia. Diseases that cause

prolonged bleeding time include thrombocytopenia, disseminated intravascular coagulation (DIC), Bernard-Soulier disease, and Glanzmann's thrombasthenia.

Aspirin and other cyclooxygenase inhibitors can significantly prolong bleeding time. While warfarin and heparin have their major effects on coagulation factors, an increased bleeding time is sometimes seen with use of these medications as well.

People with von Willebrand disease usually experience increased bleeding time, as von Willebrand factor is a platelet adhesion protein, but this is not considered an effective diagnostic test for this condition. It is also prolonged in hypofibrinogenemia

Clotting time

Clotting time is the time required for a sample of blood to coagulate in vitro under standard conditions.

There are various methods for determining the clotting time, the most common being the capillary tube method. It is affected by calcium ion levels and many diseases. Normal value of clotting time is 2-8 minutes.

For the measurement of clotting time by test tube method, blood is placed in a glass test tube and kept at 37° C. The required time is measured for the blood to clot.

There are several other methods, including testing for those on blood thinners, such as heparin or warfarin. Activated partial thromboplastin time (aPTT) is used for heparin studies and the normal range is 20–36 seconds, depending upon which type of activator is used in the study. Prothrombin time (PT) is used for warfarin studies and the normal values differ for men and women. PT time for adult males' normal range is 9.6–11.8 seconds, while adult females' normal range is 9.5–11.3 seconds.[2] Internationalized normalized ratio (INR) is also a warfarin study, with normal ranges of 2–3 for standard warfarin and 3–4.5 for high-dose warfarin.

Examination of Urine

Benedict's Test is used to test for simple carbohydrates. The **Benedict's test** identifies reducing sugars (monosaccharide's and some disaccharides), which have free ketone or aldehyde functional groups. Benedict's solution can be used to test for the presence of glucose in urine.

Composition and Preparation of Benedict's Solution

Benedict's solution is a deep-blue alkaline solution used to test for the presence of the aldehyde functional group, – CHO.

Anhydrous sodium carbonate = 100 gm
Sodium citrate – 173 gm
Copper(II) sulfate pentahydrate = 17.3 gm

One litre of Benedict's solution can be prepared from 100 g of anhydrous sodium carbonate, 173 g of sodium citrate and 17.3 g of copper(II) sulfate pentahydrate.

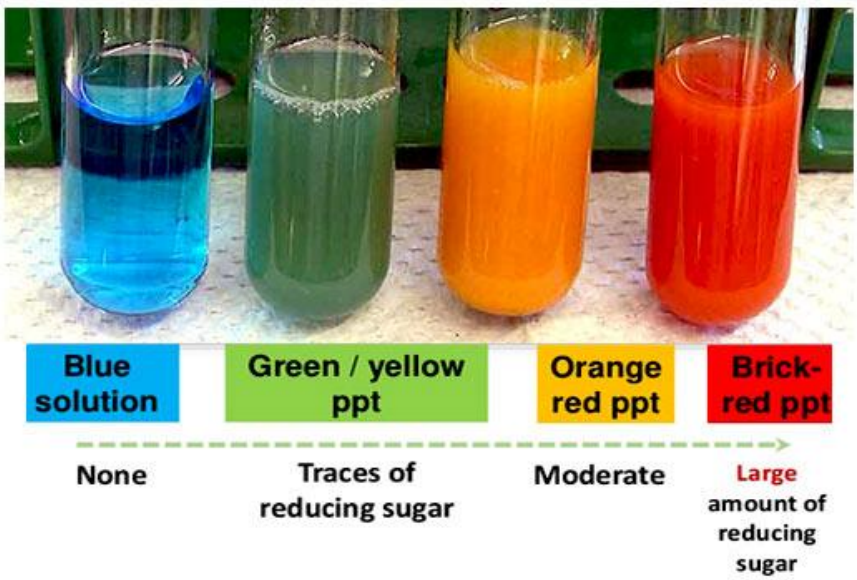
Procedure of Benedict's Test

1. Approximately 1 ml of sample is placed into a clean test tube.
2. 2 ml (10 drops) of Benedict's reagent (CuSO4) is placed in the test tube.
3. The solution is then heated in a boiling water bath for 3-5 minutes.
4. Observe for color change in the solution of test tubes or precipitate formation.

Result Interpretation of Benedict's Test

If the color upon boiling is changed into green, then there would be 0.1 to 0.5 percent sugar in solution.

If it changes color to yellow, then 0.5 to 1 percent sugar is present. If it changes to orange, then it means that 1 to 1.5 percent sugar is present. If color changes to red,then 1.5 to 2.0 percent sugar is present. And if color changes to brick red,it means that more than 2 percent sugar is present in solution.



Positive Benedict's Test: Formation of a reddish precipitate within three minutes. Reducing sugars present. Example: Glucose
Negative Benedict's Test: No color change (Remains Blue). Reducing sugars absent. Example: Sucrose.

Methodology

A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 rpm) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube. The supernate is decanted and a volume of 0.2 to 0.5 ml is left inside the tube. The sediment is resuspended in the remaining supernate by flicking the bottom of the tube several times. A drop of resuspended sediment is poured onto a glass slide and coverslipped.

Visual exam

A lab technician examines the urine's appearance. Urine is typically clear. Cloudiness or an unusual odor may indicate a problem, such as an infection.

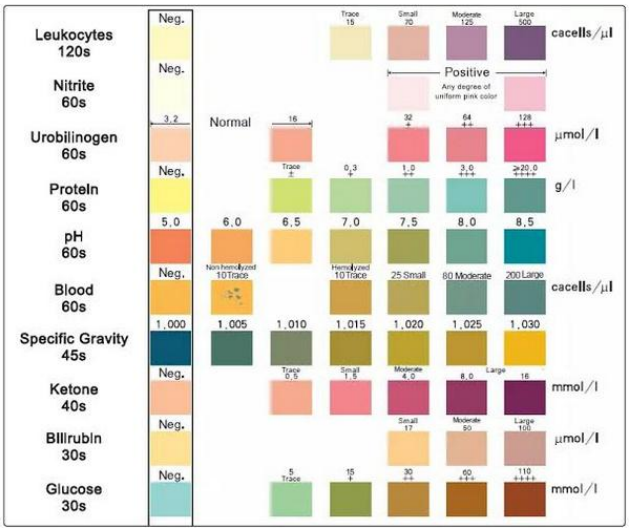
Blood in the urine may make it look red or brown. Urine color can be influenced by what you've just eaten. For example, beets or rhubarb may add a red tint to your urine.

Dipstick test

A dipstick — a thin, plastic stick with strips of chemicals on it — is placed in the urine to detect abnormalities. The chemical strips change color if certain substances are present or if their levels are above normal. A dipstick test checks for:

- **Acidity (pH).** The pH level indicates the amount of acid in urine. Abnormal pH levels may indicate a kidney or urinary tract disorder.
- **Concentration.** A measure of concentration, or specific gravity, shows how concentrated particles are in your urine. A higher than normal concentration often is a result of not drinking enough fluids.
- **Protein.** Low levels of protein in urine are normal. Small increases in protein in urine usually aren't a cause for concern, but larger amounts may indicate a kidney problem.
- **Sugar.** Normally the amount of sugar (glucose) in urine is too low to be detected. Any detection of sugar on this test usually calls for follow-up testing for diabetes.
- **Ketones.** As with sugar, any amount of ketones detected in your urine could be a sign of diabetes and requires follow-up testing.

- **Bilirubin.** Bilirubin is a product of red blood cell breakdown. Normally, bilirubin is carried in the blood and passes into your liver, where it's removed and becomes part of bile. Bilirubin in your urine may indicate liver damage or disease.
- **Evidence of infection.** If either nitrites or leukocyte esterase — a product of white blood cells — is detected in your urine, it may be a sign of a urinary tract infection.
- **Blood.** Blood in your urine requires additional testing — it may be a sign of kidney damage, infection, kidney or bladder stones, kidney or bladder cancer, or blood disorders.



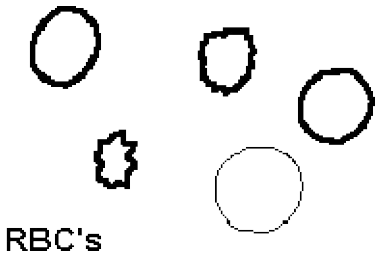
Microscopic Examination

In healthy people, the urine contains small numbers of cells and other formed elements from the entire urinary tract, and epithelial cells from the kidney, ureter , bladder, and urethra. In renal disease, the urine often contains increased numbers of substances discharged from an organ that is otherwise accessible only by biopsy or surgery. A microscopic examination of urine sediment detects the presence and amounts of:

- Red blood cells
- White blood cells
- Bacteria and yeast
- Casts
- Epithelial cells
- Crystals

Red Blood Cells

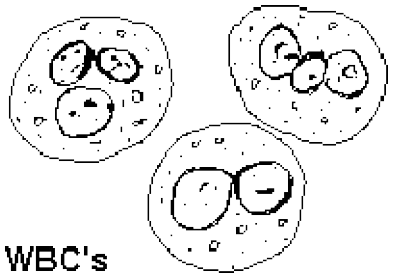
Hematuria is the presence of abnormal numbers of red cells in urine due to: glomerular damage, tumors which erode the urinary tract anywhere along its length, kidney trauma, urinary tract stones, renal infarcts, acute tubular necrosis, upper and lower uri urinary tract infections, nephrotoxins, and physical stress. Red cells may also contaminate the urine from the vagina in menstruating women or from trauma produced by bladder catherization. Theoretically, no red cells should be found, but some find their way into the urine even in very healthy individuals. However, if one or more red cells can be found in every high power field, and if contamination can be ruled out, the specimen is probably abnormal.



RBC's may appear normally shaped, swollen by dilute urine (in fact, only cell ghosts and free hemoglobin may remain), or crenated by concentrated urine. Both swollen, partly hemolyzed RBC's and crenated RBC's are sometimes difficult to distinguish from WBC's in the urine. In addition, red cell ghosts may simulate yeast. The presence of dysmorphic RBC's in urine suggests a glomerular disease such as a glomerulonephritis. Dysmorphic RBC's have odd shapes as a consequence of being distorted via passage through the abnormal glomerular structure.

White Blood Cells

Pyuria refers to the presence of abnormal numbers of leukocytes that may appear with infection in either the upper or lower urinary tract or with acute glomerulonephritis. Usually, the WBC's are granulocytes. White cells from the vagina, especially in the presence of vaginal and cervical infections, or the external urethral meatus in men and women may contaminate the urine.



If two or more leukocytes per each high power field appear in non-contaminated urine, the specimen is probably abnormal. Leukocytes have lobed nuclei and granular cytoplasm.

Bacteria

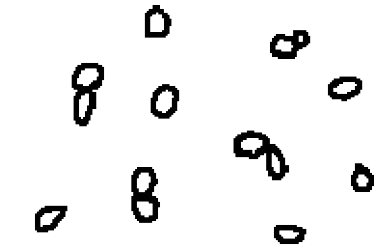
Bacteria are common in urine specimens because of the abundant normal microbial flora of the vagina or external urethral meatus and because of their ability to rapidly multiply in urine standing at room temperature.

Therefore, microbial organisms found in all but the most scrupulously collected urines should be interpreted in view of clinical symptoms.

Diagnosis of bacteriuria in a case of suspected urinary tract infection requires culture. A colony count may also be done to see if significant numbers of bacteria are present. Generally, more than 100,000/ml of one organism reflects significant bacteriuria. Multiple organisms reflect contamination. However, the presence of any organism in catheterized or suprapubic tap specimens should be considered significant.

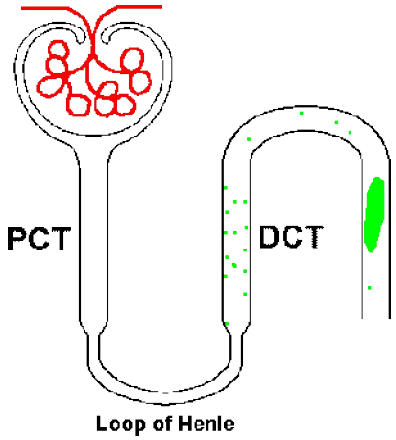
Yeast

Yeast cells may be contaminants or represent a true yeast infection. They are often difficult to distinguish from red cells and amorphous crystals but are distinguished by their tendency to bud. Most often they are Candida, which may colonize bladder, urethra, or vagina.

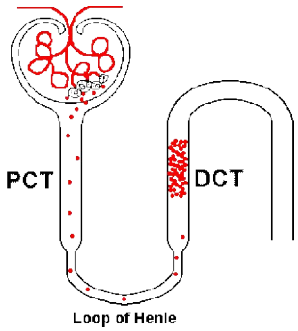


Casts

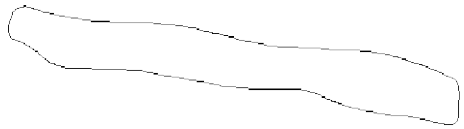
Urinary casts are formed only in the distal convoluted tubule (DCT) or the collecting duct (distal nephron). The proximal convoluted tubule (PCT) and loop of Henle are not locations for cast formation. Hyaline casts are composed primarily of a mucoprotein (Tamm-Horsfall protein) secreted by tubule cells. The Tamm-Horsfall protein secretion (green dots) is illustrated in the diagram below, forming a hyaline cast in the collecting duct:



Even with glomerular injury causing increased glomerular permeability to plasma proteins with resulting proteinuria, most matrix or "glue" that cements urinary casts together is Tamm-Horsfall mucoprotein, although albumin and some globulins are also incorporated. An example of glomerular inflammation with leakage of RBC's to produce a red blood cell cast is shown in the diagram below:



The factors which favor protein cast formation are low flow rate, high salt concentration, and low pH, all of which favor protein denaturation and precipitation, particularly that of the Tamm-Horsfall protein. Protein casts with long, thin tails formed at the junction of Henle's loop and the distal convoluted tubule are called cylindroids. Hyaline casts can be seen even in healthy patients.

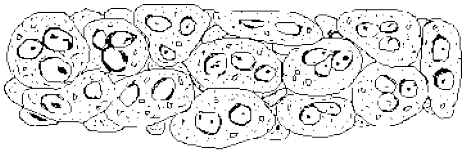


Hyaline Cast

Red blood cells may stick together and form red blood cell casts. Such casts are indicative of glomerulonephritis, with leakage of RBC's from glomeruli, or severe tubular damage.

Red Blood Cell Cast

White blood cell casts are most typical for acute pyelonephritis, but they may also be present with glomerulonephritis. Their presence indicates inflammation of the kidney, because such casts will not form except in the kidney.



White Blood Cell Cast

When cellular casts remain in the nephron for some time before they are flushed into the bladder urine, the cells may degenerate to become a coarsely granular cast, later a finely granular cast, and ultimately, a waxy cast. Granular and waxy casts are be believed to derive from renal tubular cell casts. Broad casts are believed to emanate from damaged and dilated tubules and are therefore seen in end-stage chronic renal disease.

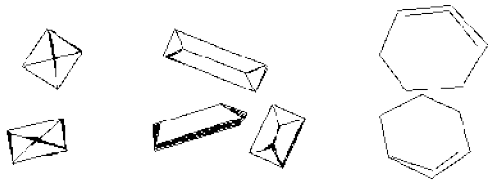
Granular Cast

The so-called telescoped urinary sediment is one in which red cells, white cells, oval fat bodies, and all types of casts are found in more or less equal profusion. The conditions which may lead to a telescoped sediment are: 1) lupus nephritis 2) hypertensive emergency 3) diabetic glomerulosclerosis, and 4) rapidly progressive glomerulonephritis.

In end-stage kidney disease of any cause, the urinary sediment often becomes very scant because few remaining nephrons produce dilute urine.

Crystals

Common crystals seen even in healthy patients include calcium oxalate, triple phosphate crystals and amorphous phosphates.



Very uncommon crystals include: cystine crystals in urine of neonates with congenital cystinuria or severe liver disease, tyrosine crystals with congenital tyrosinosis or marked liver impairment, or leucine crystals in patients with severe liver disease or with maple syrup urine disease.

Examination of faeces

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract. These conditions can include infection (such as from parasites, viruses, or bacteria), poor nutrient absorption, or cancer.

For a stool analysis, a stool sample is collected in a clean container and then sent to the laboratory. Laboratory analysis includes microscopic examination, chemical tests, and microbiologic tests. The stool will be checked for color, consistency, amount, shape, odor, and the presence of mucus. The stool may be examined for hidden (occult) blood, fat, meat fibers, bile, white blood cells, and sugars called reducing substances. The pH of the stool also may be measured. A stool culture is done to find out if bacteria may be causing an infection.

PhysicalExamination

A) Consistency: Normal consistency – well formed.

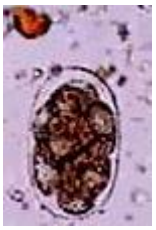
Abnormal Consistency	**Expected Reasons**
Pale, bulky, frothy	Poor fat digestion.
Hard	Constipation.
Flattened and ribbon like	Obstruction in the lumen of the bowel.

Semisolid	Digestive upset, mild diarrhea or after taking a laxative.
Watery	Bacterial infection or after taking a purgative.
Rice water stools	Cholera.

B) Color: Normal color – light to dark brown (due to the presence of bile pigments)

Abnormal Color	**Possible Reasons**
Black	Bleeding in the upper gastrointestinal tract, or iron administration in a deficiency like anemia.
Bright red	Bleeding piles, bleeding at the lower level of the gastrointestinal tract or contamination with menstrual blood.
Fresh blood, mucous clay colored	Amoebic dysentery, jaundice or obstruction to the flow of bile to the intestine.

White	After barium meal given for X–rays.

 **Hook worm egg**

C) Presence of adult worms or their parts like:

- Round worms.
- Pin worms.
- Whip worms.
- Hook worms.
- Tape worms.

ChemicalExamination

Normal stools are slightly acidic, slightly alkaline or neutral. The pH values range from 5.8 to 7.5.

1. Strongly acidic stool (pH below 5.5) indicates an excess of carbohydrates in the diet. It is non–pathologic. But, if fermentation is present, it may be due to lactose intolerance. Then, it is pathologic.
2. Strongly alkaline stool (pH above 7.5) indicates an excess of protein in the diet. It is non–pathologic.
3. Occult blood: Generally it is not present. If there, it indicates either infection or some disorder of the digestive system.
4. Reducing substances: They are generally found in stools of infants suffering from diarrhea.

MicroscopicExamination

The microscopic examination of faeces can be very helpful in the identification of helminths, protozoa and undigested foodstuffs.

There are 2 basic methods:

- Direct wet preparation.
- Faecal sedimenation.

As with all laboratory tests, ensure that protective clothing is worn, long hair tied back out of the way and any jewellery removed. Always check the microscope for safety prior to use, paying particular attention to the plug and electrical cable. Ensure that it is situated on a flat, clean, dry surface.

Direct Wet Preparation:

1. Place 1 drop of saline onto a microscope slide.
2. Add an equal amount of fresh faeces.
3. Add stain as necessary if looking for undigested food stuffs:
    - Lugol's iodine = stains starch granules blue-black.
    - New methylene blue = stains undigested meat fibres.
    - Sudan III = stains faecal fat orange.
4. Thoroughly mix the sample.
5. Transfer a small drop by pipette to a clean labelled slide.
6. Make a thin smear and place a cover slip over the slide.
7. Transfer the slide to the stage of the microscope and examine under low power for worm eggs and undigested food stuffs.
8. Use medium power to look for protozoa, or flame fix the slide for examination under oil immersion.

Faecal Sedimentation

1. Place 2g of fresh faeces into a screw-top glass jar.
2. Add a small amount of tap water, replace the cap, agitate and strain.
3. Half fill a centrifuge tube with the strained fluid.
4. Spin at 1500rpm for 5 minutes.
5. Pour off the supernatant.
6. Transfer a small amount of sediment to a labelled microscope slide with a Pasteur pipette.
7. Add a stain as for direct wet preparations if looking for undigested foodstuffs.
8. Place a cover slip over the slide.
9. Transfer the slide to the stage of the microscope and examine under low power for worm eggs and undigested foodstuffs.

This is the preliminary examination to find the cause of diarrhea.

1. **Presence of Leukocytes** Normally there are no WBCs.
    1. WBCs only appear in infection or inflammation.
    2. Their presence is important in case of diarrhea or dysentery.
    3. >3 WBCs /high field are seen in ulcerative colitis and bacterial infection.
    4. Greater numbers of WBCs indicate invasive pathogens.
2. Virus and parasites don't cause WBCs in the stool.
3. Increased number of WBCs in the stool.
    1. Bacillary dysentery.
    2. chronic ulcerative colitis.
    3. Shigellosis.
    4. salmonella infection.
    5. Yersinia infection.
    6. Invasive E.coli diarrhea.
    7. Fistula of anus or rectum.
    8. Localized abscess.

4. Few WBCs are seen in amoebiasis.
    1. Also, WBCs are seen in typhoid.
5. **The absence of WBCs seen in some of the diarrhoeal conditions alike:**
    1. Cholera.
    2. Viral diarrhea.
    3. Drug-induced diarrhea.
    4. Amoebic colitis.
    5. Non-invasive E.coli diarrhoea.
    6. Parasitic infestation.
    7. Toxigenic bacterial infection.
6. **Presence of Red Blood Cells in the stool.**Blood in the stool can be:
    1. Bright red from the bleeding in the lower GI tract.
    2. Maroon in color.
    3. Black and tarry from bleeding from the upper GI tract.
    4. Occult blood (not visible to the naked eye).
    5. **Causes of blood in stool:**
        1. Hemorrhoids.
        2. Cancer.
        3. Dysentery.
7. Make smear from the mucus area or from the watery stool.
    1. Please see more details in the occult blood.

Stool findings (Physical features)	**Possible Causes**
1. Diarrhea mixed with blood and mucous	Typhoid, Amoebiasis and large colon carcinoma
2. Diarrhea mixed with Pus	Ulcerative colitis, Salmonellosis, Intestinal tuberculosi

and mucous	Shigellosis, Regional enteritis, and acute diverticulitis
3. Patty stool with high-fat contents	Cystic fibrosis and CBD – obstruction
4. Formed stool with attached mucous	Constipation, Mucous colitis, and excessive straining
5. Small, hard dark balls like	Constipation
6. Clay-colored, pasty and little odor	Bile duct obstruction, and barium ingestion.
7. Black, tarry, sticky, watery, voluminous	Upper GI tract bleeding, Noninvasive infections li Cholera, Staphylo.coccal food poisoning, and Toxigenic Coli and Disaccharidase deficiency

1. **Ova and parasites**. Normally there are no parasites or eggs in the stool sample.
    1. Multiple stool sample is needed to rule out the parasitic infestation, at least three consecutive days.
    2. An abnormal result means parasites or eggs are present in the stool. Such infections include:
    3. Roundworms: Ascaris lumbricoides.
    4. Hookworms: Necator americanus.
    5. Pinworms: Enterobius vermicularis.
    6. Whipworm: Trichuris trichiura.
    7. Tapeworms: Diphyllobothrium latum, Taenia saginata, and Taenia solium

8. Protozoa: Entamoeba histolytica (an amoeba), and Giardia lamblia (a flagellate)
9. Strongyloidiasis.

2. **Presence of Fat**. The fat in the stool shows the possibility of :
    1. Malabsorption.
    2. Deficiency of pancreatic digestive enzyme.
    3. Deficiency of Bile.
3. **Meat fibers. and muscle fibers** are seen in the stool. Their presence show defect in the digestion.
    1. The increased amount of meat fibers are found in:
        1. Malabsorption syndrome.

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Chapter: Microbiology and Immunology: Culture Methods

| Study Material, Lecturing Notes, Assignment, Reference, Wiki description explanation, brief detail |

Methods of Culture

Various methods are used for culturing of bacteria. These include (a) streak culture, (b) lawn culture, (c) pour-plate culture, (d) stroke culture, (e) stab culture, and (f) liquid culture.

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Methods of Culture

Various methods are used for culturing of bacteria. These include (*a*) streak culture, (*b*) lawn culture, (*c*) pour-plate culture, (*d*) stroke culture, (*e*) stab culture, and (*f*) liquid culture.

Streak Culture

Streak culture is the most useful method for obtaining discrete colonies of the bacteria. It is carried out by streaking on the surface of a solid media plate using a platinum or nichrome loop of 2–4 mm diameter. In this method, a loopful of the inoculum is placed near the peripheral area of the plate. The inoculum is then spread with the loop to about one-fourth of the plate with close parallel strokes. From the primary inoculum, it is spread thinly over the plate by streaking with the loop in parallel lines. The loop is flamed and cooled in between the streaks to obtain isolated colonies. The inoculated culture plate is incubated at 37°C overnight for demonstration of colonies. Confluent growth occurs at the primary inoculum, but becomes progressively thinner, and well-separated colonies are demonstrated on the final streaks of the inoculum (Fig. 5-1). Single isolated colonies obtained by this method are very useful to study various properties of bacteria. Streak culture is the most useful method for obtaining discrete colonies of the bacteria.

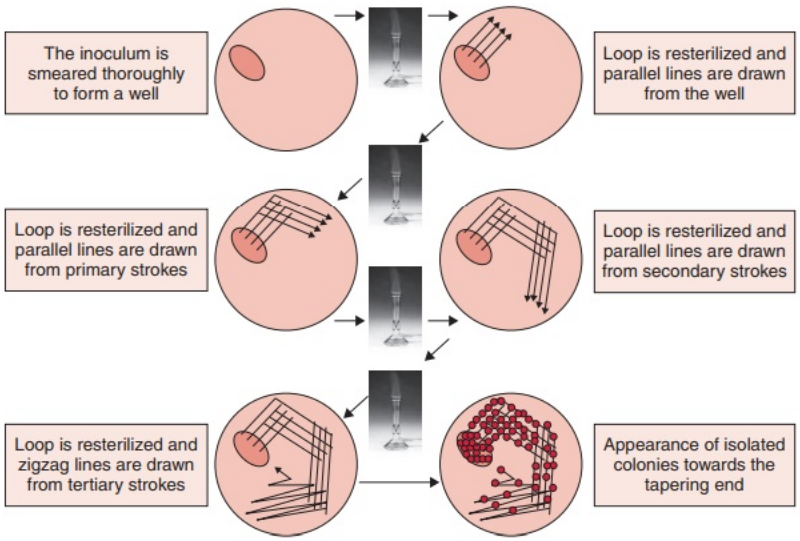

FIG. 5-1. Diagrammatic representation showing streak culture.

Lawn Culture

The lawn culture provides a uniform layer of bacterial growth on a solid medium. It is carried out by flooding the surface of the solid media plate with a liquid culture or suspension of bacteria, pipetting off the excess inoculum, and finally incubating the plate overnight at 37°C. Alternatively, the cul-ture plate may be inoculated by a sterile swab soaked in liquid bacterial culture or suspension and incubating overnight for demonstration of the bacterial colonies.

Pour-Plate Culture

The pour-plate culture is used to determine approximate number of viable organisms in liquids, such as water or urine. It is used to quantitate bacteria in urine cultures and also to estimate the viable bacterial count in a suspension. This method is carried out in tubes, each containing 15 mL of molten agar. The molten agar in tubes is left to cool in a water bath at 45°C. The inoculum to be tested is diluted in serial dilution. Then 1 mL each of diluted inoculum is added to each tube of molten agar and mixed well. The contents of tubes are poured into sterile Petri dishes and allowed to set. After overnight incubation of these Petri dishes at 37°C, colonies are found to be distributed throughout the depth of the medium, which can be counted using a colony counter.

Stroke Culture

Stroke culture provides a pure growth of bacteria for carrying out slide agglutination and other diagnostic tests. It is carried out in tubes usually containing nutrient agar slopes.

Stab Culture

Stab culture is prepared by stabbing the medium in tubes with a long, straight wire and incubating at 37°C.

Liquid Culture

Liquid culture is prepared in a liquid media enclosed in tubes, flasks, or bottles. The medium is inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes and incubating at 37°C, followed by subculture on to solid media for final identification.

A major disadvantage of liquid culture is that it does not provide pure culture of the bacteria and also the bacterial growth does not exhibit special characteristic appearances.

Anaerobic Culture

Obligate anaerobes are bacteria that can live only in the absence of oxygen. These anaerobes are killed when exposed to the atmosphere for as briefly as 10 minutes. Some anaer-obes are tolerant to small amounts of oxygen. Facultative anaerobes are those anaerobes that grow with or without oxygen.

Anaerobic bacterial culture is a method used to grow anaer-obes from a clinical specimen. Culture and identification of anaerobes is essential for initiating appropriate treatment.

The failure to do so may have serious consequences, such as amputation, organ failure, sepsis, meningitis, and even death.

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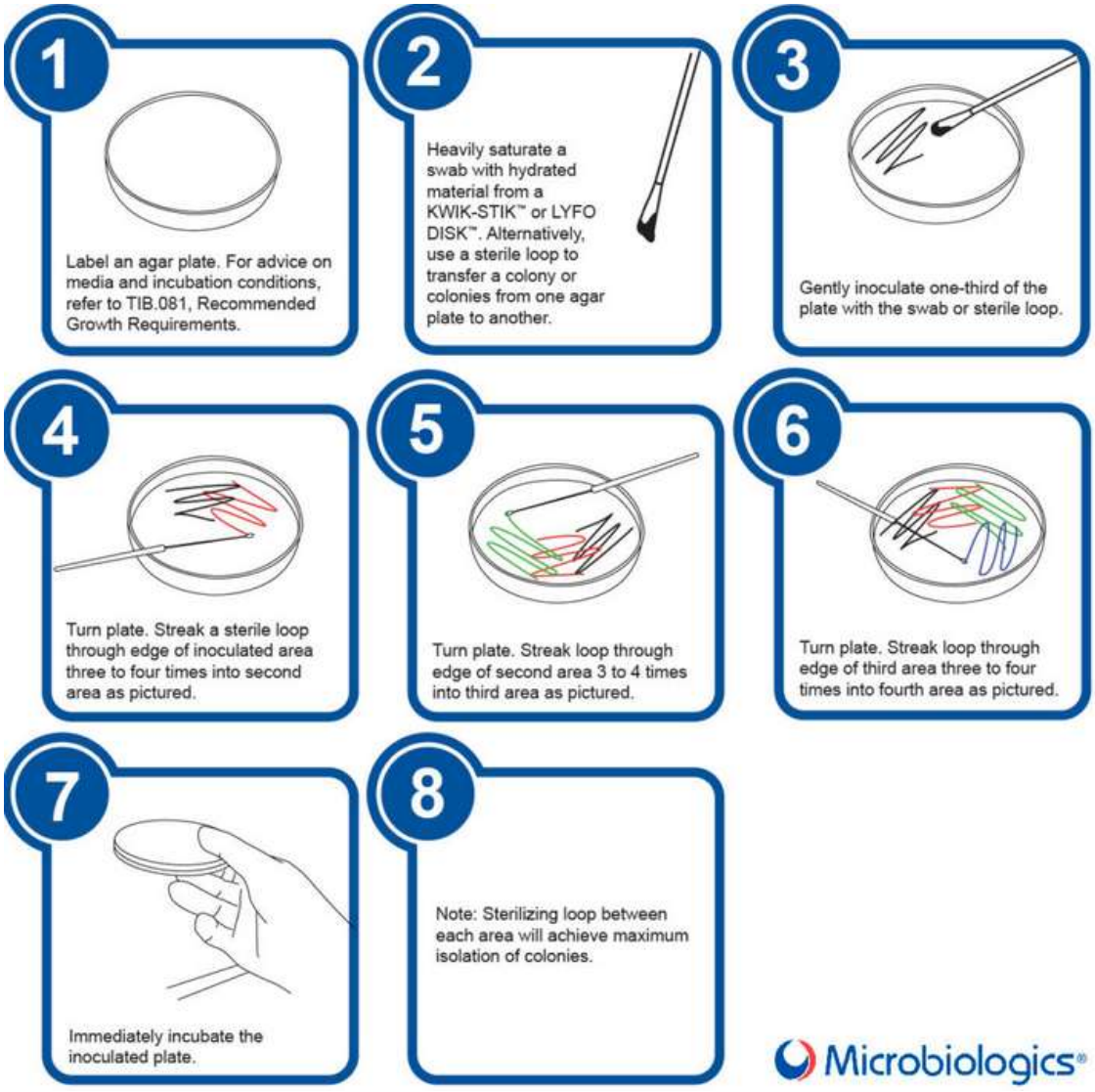
Related Topics

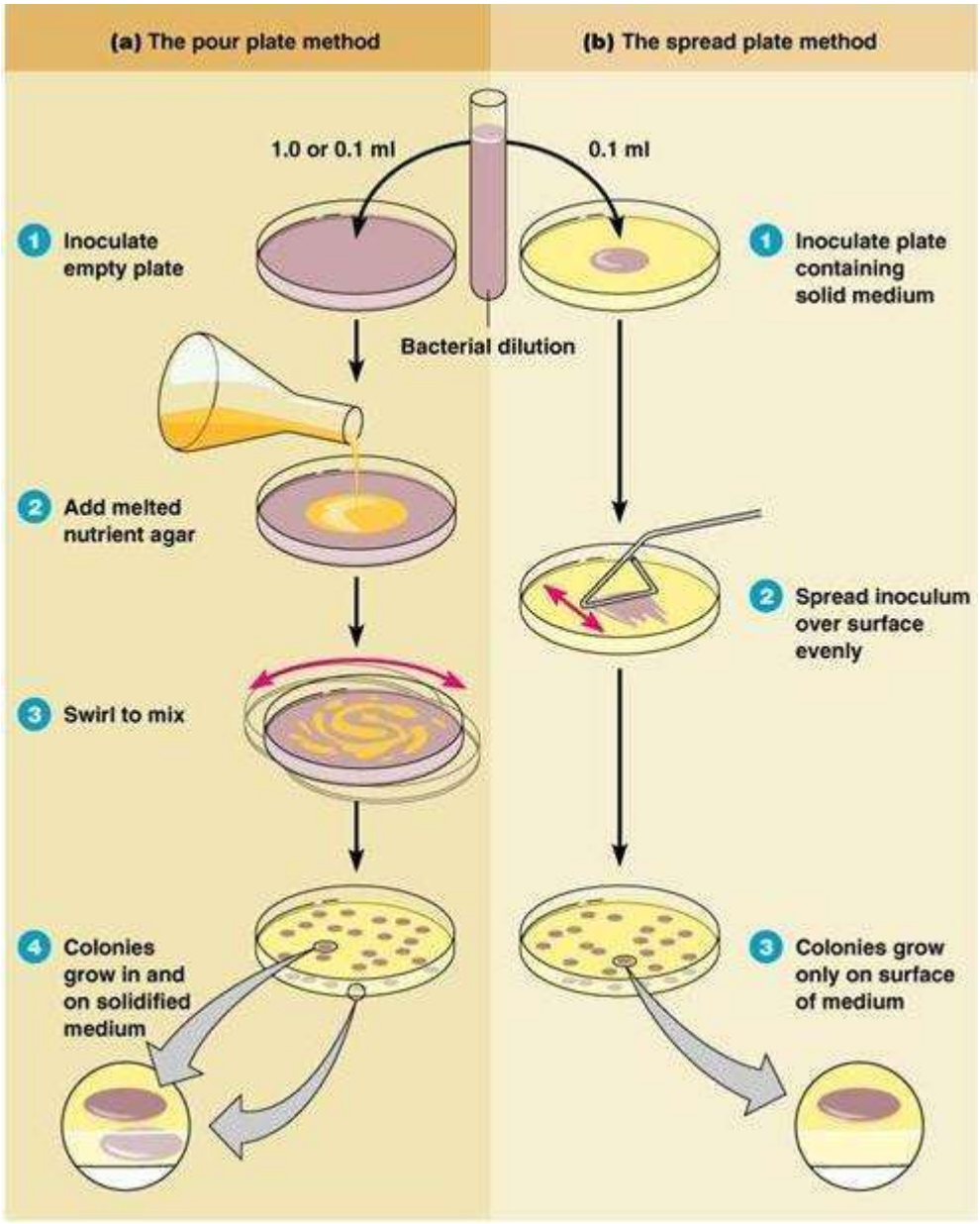
Testing of Disinfectants
Culture Media
Ingredients of Culture Media
Types of Culture Media
Culture Methods
Anaerobic Culture: Specimen, Culture Media, Methods
Identification of Bacteria
Biochemical Reactions - Identification of Bacteria
Typing of Bacterial Strains
Bacterial Taxonomy
Bacterial Classification
Nomenclature of Microorganisms

Lab:4 Culture Methods

Indications for culture -

- Isolate bacteria in pure cultures.
- Demonstrate their properties.
- Obtain sufficient growth for preparation of antigens & for other tests.
- Typing bacterial isolates.
- Antibiotic sensitivity.
- Estimate viable counts.
- Maintain stock cultures.





Methods of Isolation:

- Streak culture or surface plating
- Lawn or carpet culture
- Stroke culture
- Stab culture
- Pour plate method
- Anaerobic methods of culturing bacteria

1. Streaking:

- Routinely employed for isolation
- Platinum / Nichrome loops

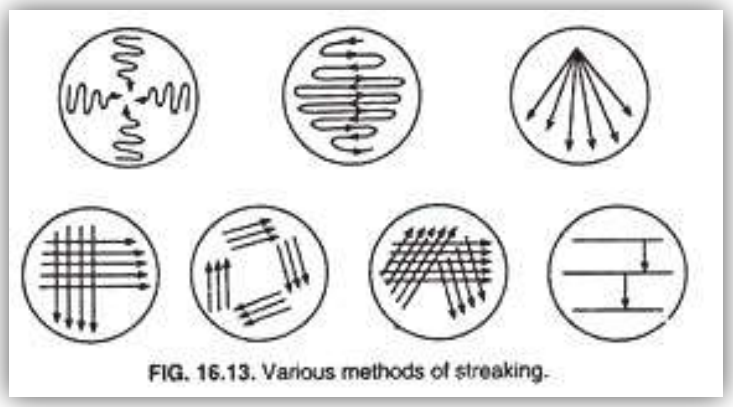
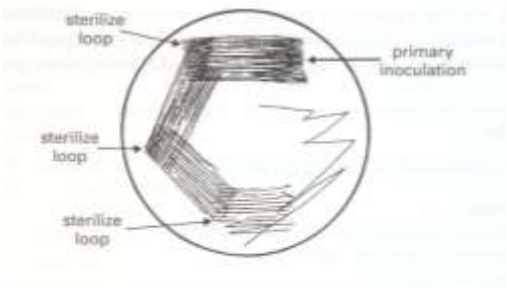


FIG. 16.13. Various methods of streaking.

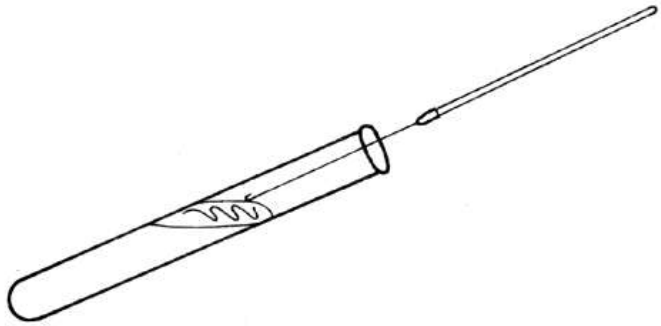


2. Lawn or Carpet Culture

- Uniform surface growth
- Bacteriophage typing
- Antibiotic sensitivity testing
- Preparation of bacterial antigens & vaccines

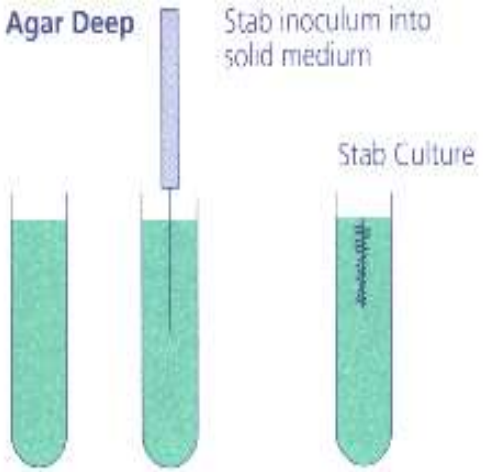
3- Stroke Culture

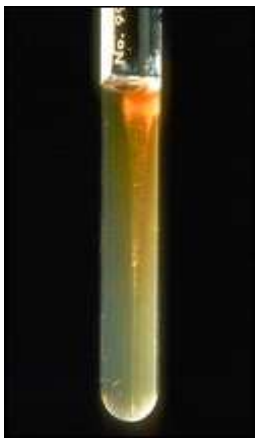
- Tubes containing agar slopes
- For slide agglutination & other diagnostic tests.



4- Stab Culture

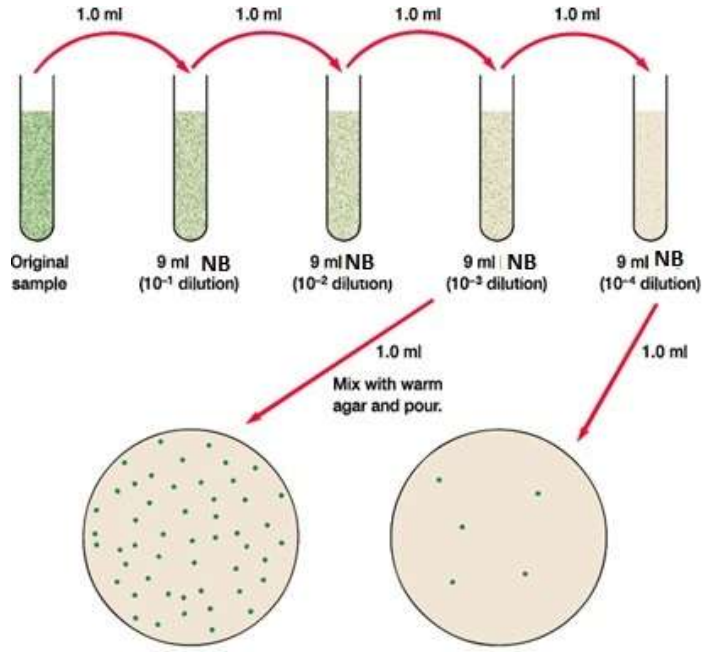
- By puncturing a suitable medium with a long, straight charged wire.
- For gelatin liquefaction, stock cultures & motility

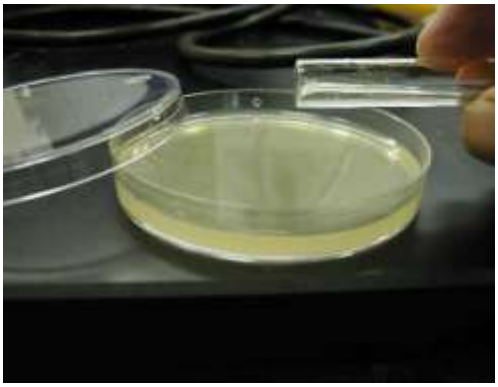




5- Pour Plate Method

- 1 ml of appropriately diluted inoculum is added to 15 ml of molten agar and poured on petridish.
- Colonies appear through out the depth of medium.
- Used to estimate viable count, recommended method for quantitative urine cultures.





Broth/Liquid Culture

- Inoculated by a charged loop, pipette or syringes.
- For blood cultures & sterility testing

6. Anaerobic Culture Methods

Anaerobic condition can be achieved by:

- Cultivation in vacuum
- Displacement of oxygen with other gases
- Chemical or biological methods
- By displacement and combustion of oxygen
- By reducing agents
- Anaerobic chamber

Displacement Method

- Displacement of O_2 with gases like H_2 , N_2 , He or CO_2 .
- Rarely produces complete anaerobiosis.

e.g. Candle jar

Chemical or Biological Methods

- Alkaline pyrogallol (pyrogallic acid in NaOH) absorbs O_2
- Yellow phosphorous
- Rosenthal method - Mixture of chromium & sulphuric acid
- Gaspak

Biological Methods

Absorption of oxygen from small closed systems has been attempted by incubation along with

- Aerobic bacteria EXAMPLE:- *Pseudomonas aeruginosa*
- Anaerobiosis produced by this method is slow and ineffective.

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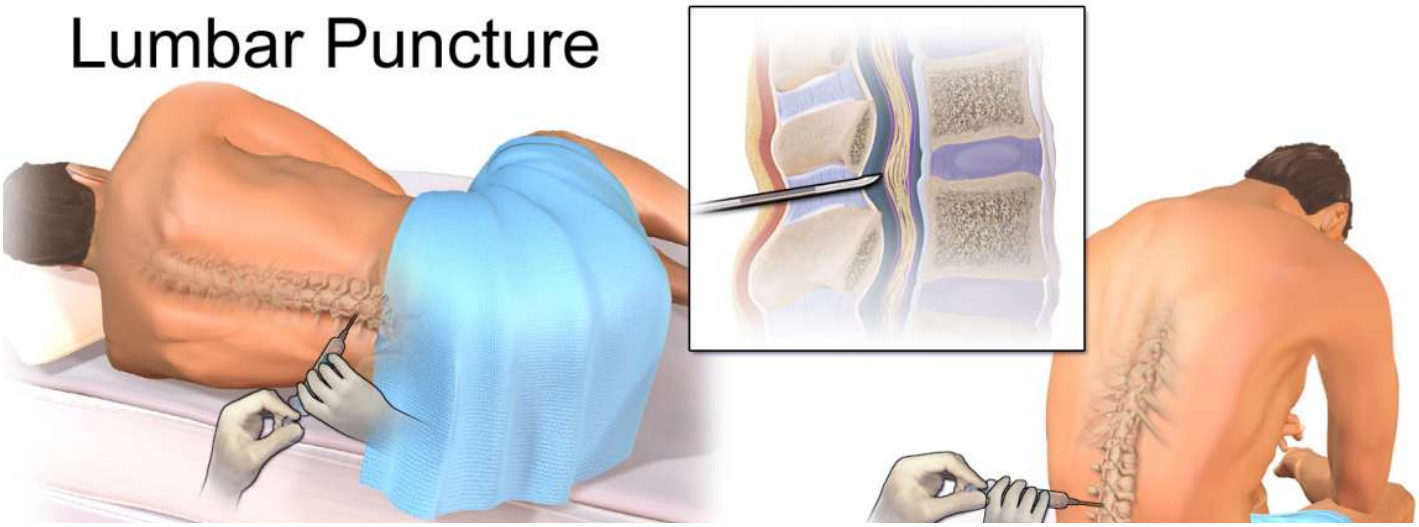
CLINICAL PATHOLOGY

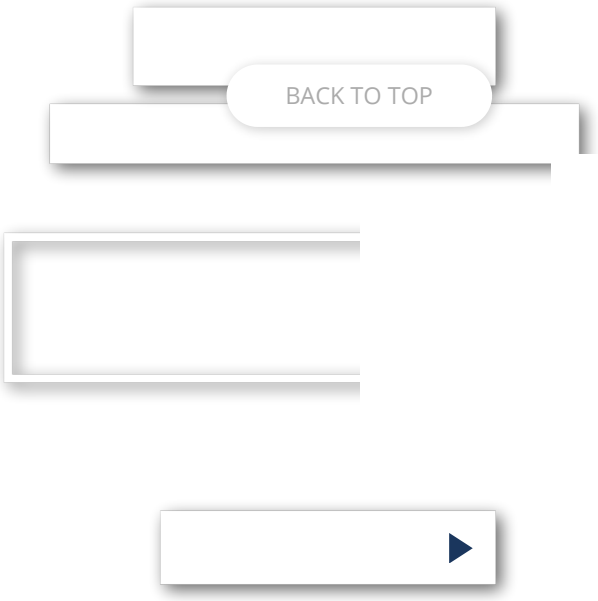
Examination of Cerebrospinal Fluid (CSF)

By Dayyal Dg.
Published: Friday, 10 August 2018
Updated: Tuesday, 22 January 2019 07:36



Lumbar Puncture: (A) Lying position (B) Sitting position

It is a clear, colorless fluid formed in the ventricles of the brain mainly by choroid plexus (an interlaced structure or meshwork of tiny small blood vessels in the lateral third and fourth ventricles). It is mainly an ultrafiltrate of plasma.



Cerebrospinal fluid (CSF) is contained within the cerebral ventricles, the spinal canal and the subarachnoid space (space between arachnoid externally and pia mater internally) surrounding the brain and spinal cord (Figure 1182.1). Cerebrospinal fluid (CSF) is reabsorbed into the blood through the arachnoid villi of dural venous sinuses.

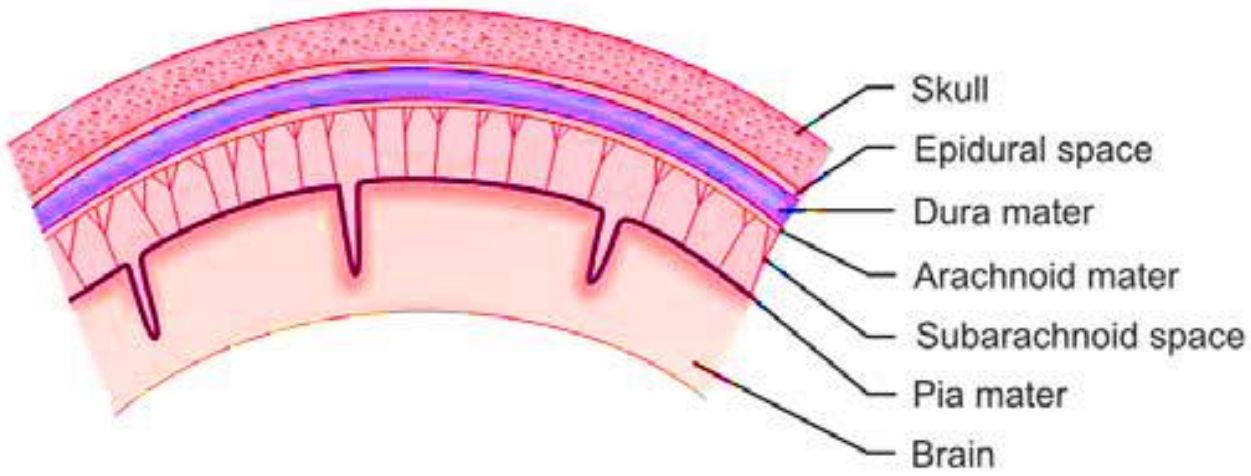


Figure 1182.1: Cranial meninges

COMPOSITION OF NORMAL CEREBROSPINAL FLUID IN ADULTS

- **Total volume:** 100-150 ml (10-60 ml in the newborn
- **Color:** Colorless
- **Appearance:** Clear
- **Clot:** Absent
- **Viscosity:** Similar to water
- **Opening pressure:** 60-180 mm of water (10-100 mm in infants and young children)

- **Cells:**
  – ***Adults:*** 0-5 cells/cmm
  – ***Infants:*** 0-30 cells/cmm
  – ***1-4 years:*** 0-20 cells/cmm
  – ***5-18 years:*** 0-10 cells/cmm
- **Glucose:** 45-80 mg/dl. (Normally CSF glucose is 60% or 2/3rds of blood glucose)
- **Proteins:** 15-45 mg/dl. (Normally CSF proteins are 1% of plasma proteins)
- **Bilirubin:** Absent
- **Chloride:** 120-130 mEq/L (20 mEq/L more than serum level)
- **Oligoclonal bands:** Negative

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FUNCTIONS OF CEREBROSPINAL FLUID

1. It acts as a shock absorber and protects the brain and spinal cord from injury.
2. To serve as a medium between blood and brain for the supply of nutrients to and removal of waste products from the brain.

COLLECTION OF CEREBROSPINAL FLUID

Some diseases produce characteristic alterations in the composition of the cerebrospinal fluid, thus providing the basis for examination of cerebrospinal fluid. The first lumbar puncture or LP was performed in 1891 by Quincke for the collection of the sample of cerebrospinal fluid. For the collection of cerebrospinal fluid from the subarachnoid space (Figure 1182.2), spinal or LP needle is passed between 3rd and 4th or between 4th and 5th lumbar vertebra (L3-L4 or L4-L5). LP is carried out at these levels in order to prevent injury to the spinal cord (spinal cord ends at about T12, below which are cauda equina or nerve roots).

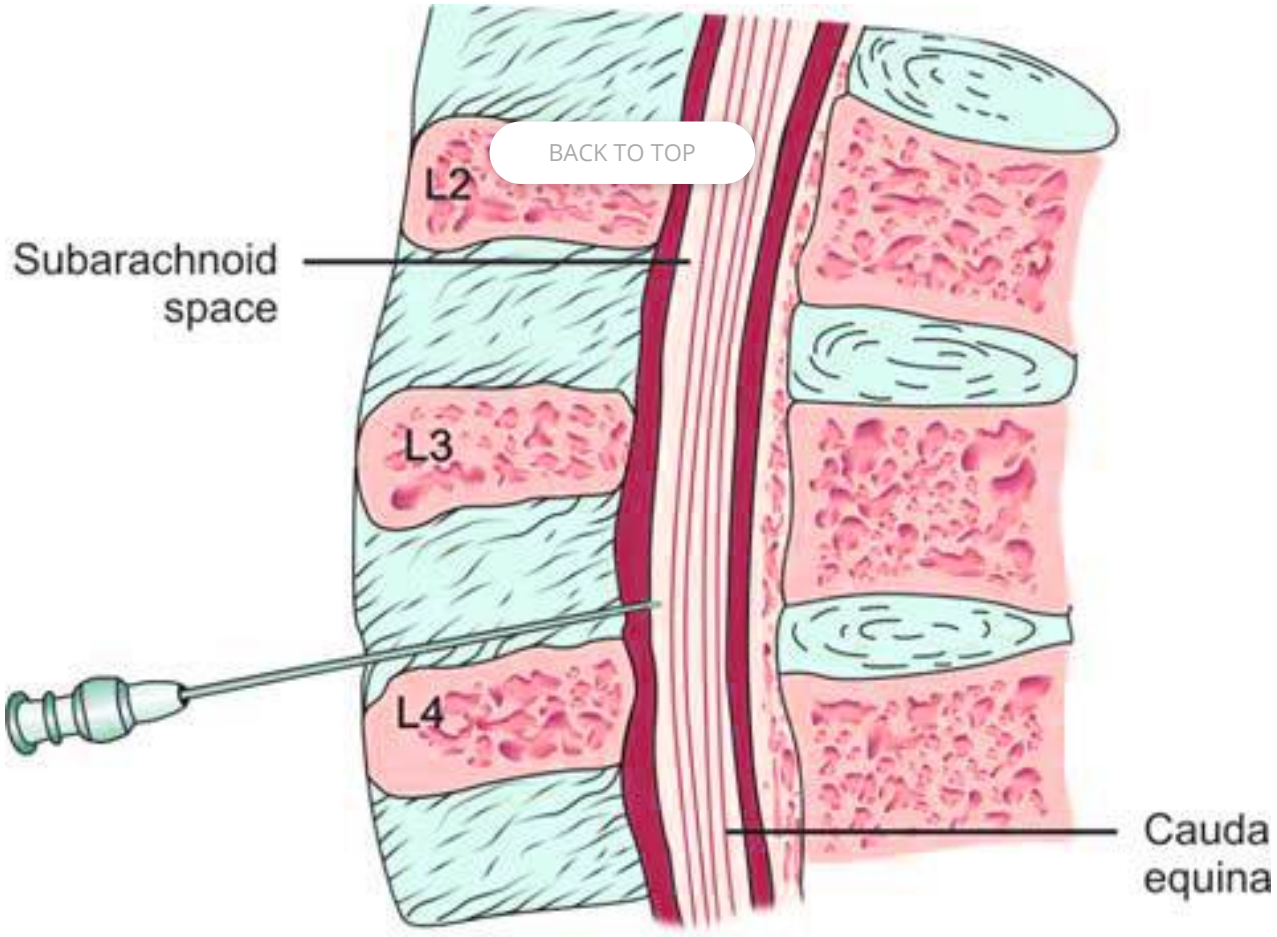

Figure 1182.2: Site of lumbar puncture. Structures through which needle passes are skin/superficial fascia, ligaments, epidural space, dura mater, subdural space, arachnoid mater, and subarachnoid space

The patient is in a side-lying (lateral recumbent) position with his back absolutely vertical and at the edge of the bed, with the knees drawn up and the head flexed onto his chest. This position increases the space between the lumbar vertebrae. Alternately, the patient may be in a sitting position.

The selected site is disinfected with the chlorhexidine-containing solution or povidone-iodine, covered with sterile drapes, and after injecting a local anesthetic, a sterile lumbar puncture needle, preferably 22 gauges (Figure 1182.3), is inserted slowly. An increased resistance is

withdrawn slowly. When the drops of cerebrospinal fluid appear, a pre-assembled manometer is attached to the needle in order to record the opening pressure of cerebrospinal fluid (CSF). The patient should be in the lateral recumbent position for the measurement of opening pressure.

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Figure 1182.3: Lumbar puncture needle

With the help of three-way stopcock in an appropriate position, cerebrospinal fluid (CSF) is collected in sterile plain tubes as follows (Figure 1182.4):

- **Tube 1:** Chemistry (For the glucose, protein and other serology tests)
- **Tube 2:** Microbiology (Gram's staining, bacterial culture, and sensitivity)
- **Tube 3:** Hematology (Total cell count and differential count)
- **Tube 4:** Cytology, special studies

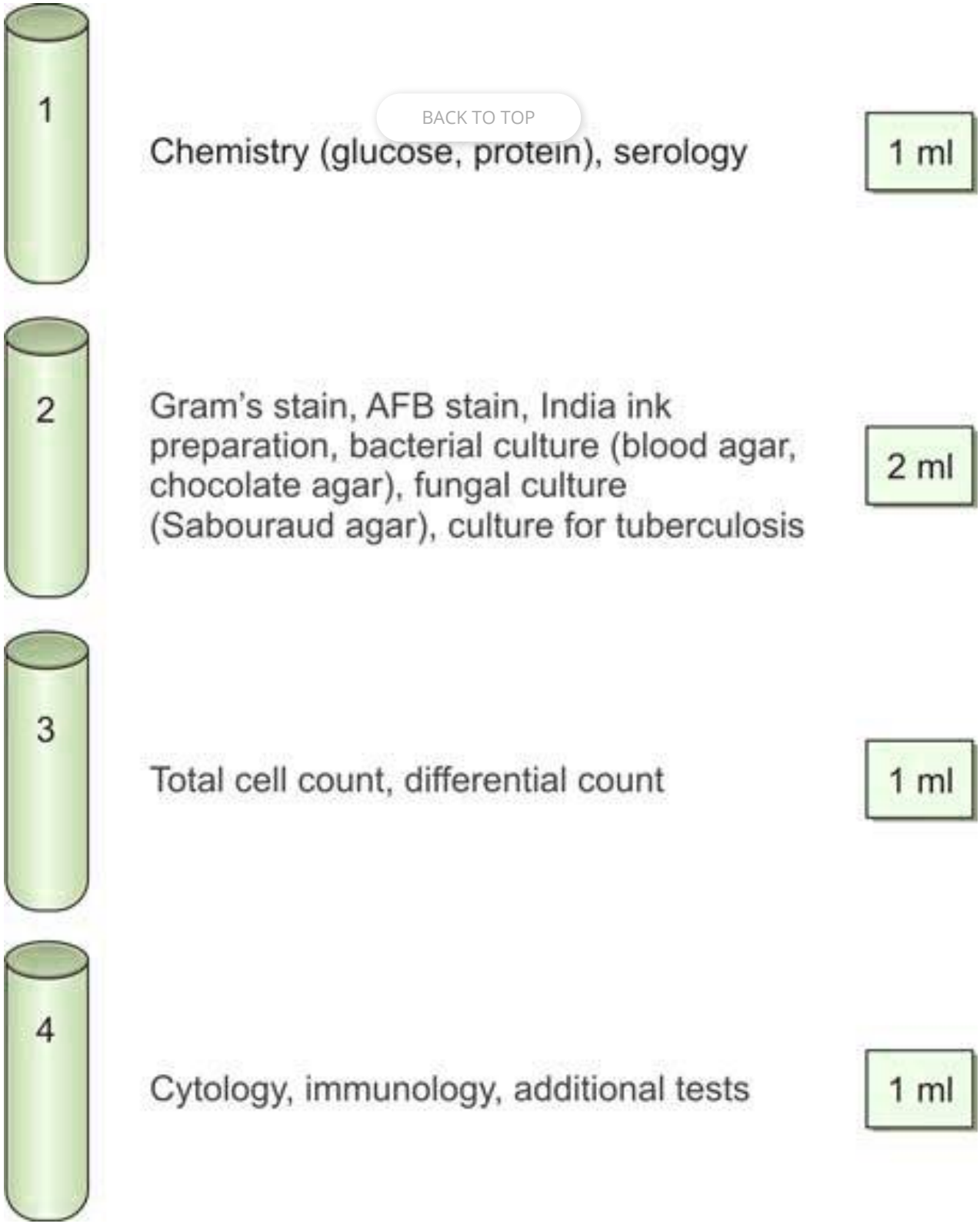

Figure 1182.4: Laboratory tests for evaluation of CSF in different tubes

Usually, 3-5 ml of CSF is collected. If the opening pressure was high, the closing pressure is recorded after the collection of cerebrospinal fluid (CSF).

The needle is withdrawn after replacing the stylet, and a sterile dressing is applied. A venous blood sample is also obtained for the determination of glucose level in blood.

INDICATIONS FOR LUMBAR PUNCTURE

1. Examination of cerebrospinal fluid (CSF) is done if the patient is suspected with the following diseases:
   • Meningeal involvement by leukemia or malignancy
   • Infections in the central nervo BACK TO TOP especially meningitis (inflammation of leptomeninges) and encephalitis
   • Subarachnoid hemorrhage (if CT scan is not available)
   • Inflammatory diseases, e.g. Guillain-Barré syndrome, multiple sclerosis (for diagnostic gammaglobulin findings)
   • Neoplasms of the central nervous system (CNS).
   Indications for emergency lumbar puncture are shown in Table 1182.1.
2. Administration of medications:
   • Antibiotics (eg. amphotericin B in fungal meningitis)
   • Anesthetic agents
   • Anticancer drugs (e.g. methotrexate in acute lymphoblastic leukemia)
3. To reduce the pressure of cerebrospinal fluid (CSF) in benign intracranial hypertension (pseudotumor cerebri)
4. Introduction of radiographic contrast media for myelography
5. For Queckenstedt's test: If the opening pressure of cerebrospinal fluid (CSF) is normal and clinically subarachnoid block or a tumor or a sinus thrombosis is suspected, then Queckenstedt's test may be performed. However, the test is contra-indicated if the pressure of cerebrospinal fluid (CSF) is raised since it can precipitate herniation of the brain. There is a direct correlation between cerebrospinal fluid (CSF) pressure and pressure in the jugular vein (because of continuity with dural venous sinuses in which arachnoid villi project). In Queckenstedt's test, both jugular veins are compressed and then released, and subsequent changes in CSF pressure are observed. Normally, upon compression of jugular veins, CSF pressure rapidly rises and with the release of pressure on jugular veins, CSF pressure rapidly falls. In the presence of a spinal block, there is no rise of CSF pressure with jugular vein compression (or a pressure rise is low or delayed). This test is positive in about 80% of patients with cord compression. With the advent of myelography, Queckenstedt's test is rarely performed.

Table 1182.1 Indications for emergency lumbar puncture

- Suspected meningitis
- Suspected subarachnoid hemorrhage
- Suspected meningeal involvement by leukemia

COMPLICATIONS OF LUMBAR PUNCTURE

1. Post-puncture headache: This is the most common side effect and results from leakage of CSF from puncture site at a rate faster than the rate of CSF production. With the use of a large bore needle, greater CSF leak occurs. Use of a smaller bore needle (22 G) for LP and keeping the patient flat after the procedure for 2-3 hours reduces the risk of headache.

2. Introduction of infection in spinal canal if aseptic precautions are not observed, if septicemia is present, or if infection is present at the site of LP.
3. Subdural hematoma with resultant neurologic deficit in patients with bleeding diathesis.
4. Failure to obtain CSF (dry tap) wh[BACK TO TOP]o the incorrect positioning of the patient or spinal block.
5. Herniation of brain through tentorium (uncus of temporal lobe) or foramen magnum (cerebellar tonsils), if intracranial pressure is high. This can damage the brainstem. This risk has led to the reduced use of lumbar puncture over the last few years.
6. Subarachnoidal epidermal cyst (due to traumatic implantation of a skin plug in subarachnoid space) may develop after a few years if LP is preformed without a stylet.

CONTRAINDICATIONS TO LUMBAR PUNCTURE

1. Raised intracranial pressure due to a spaceoccupying lesion (e.g. brain abscess, posterior fossa tumor, subdural hematoma, epidural abscess): These patients usually have headache, altered pupillary response, absent Doll's eye reflex, abnormal respiratory pattern, papilledema, bradycardia, hypertension, and decerebrate or decorticate posturing. Lumbar puncture in such cases can lead to herniation of brain. If a mass lesion is clinically suspected, cranial computerized tomography (CT) or magnetic resonance imaging (MRI) should be obtained first.
2. Cardiorespiratory compromise
3. Bleeding diathesis that has not been corrected
4. Local infection at the site of lumbar puncture

LABORATORY EXAMINATION OF CEREBROSPINAL FLUID

After collection, specimen of CSF should be transported immediately to the laboratory and examined without delay. This is because (i) cells disintegrate rapidly, and (ii) reduction of glucose level occurs due to glycolysis.

At the latest, CSF should be examined within 1 hour of collection, and CSF cell counts are always done within 30-60 minutes of collection. Glass tubes should not be used for collection since cell adherence to glass reduces the cell count. Specimen for bacterial culture should not be refrigerated as fastidious organisms (Hemophilus influenzae, Neisseria meningitidis) do not survive in the cold temperature.

CSF chemical examination results should always be compared with those in plasma since any change in plasma is reflected in CSF.

Examination of CSF includes:

1. Opening pressure
2. Appearance
3. Total and differential cell counts
4. Chemical examination
5. Microbiological examination
6. Special investigations

1. Opening Pressure

After attaching the manometer to the hub of the spinal needle, patient's legs should be gently extended and neck returned to neu[BACK TO TOP] CSF pressure is then measured. CSF pressure is directly related to jugular and vertebral venous pressures. Patient should be relaxed since tension, straining, or breath-holding will increase the CSF pressure, while hyperventilation will lower the opening pressure.

The normal opening pressure of CSF is:

- 60-180 mm of water in adults in lateral recumbent position
- 10-100 mm of water in children less than 8 years

Causes of increased CSF pressure:

- Tense and anxious patient
- Intracranial mass lesion (e.g. neoplasm, abscess, hemorrhage)
- Meningitis
- Cerebral edema
- Subarachnoid hemorrhage
- Congestive cardiac failure
- Benign intracranial hypertension (pseudotumor cerebri).

Causes of decreased CSF pressure:

- Leakage of spinal fluid following trauma or previous lumbar puncture
- Complete spinal block (obstruction of spinal subarachnoid space due to tumor, abscess, adhesions, herniated intervertebral disk).

A large difference between opening and closing pressures usually indicates presence of a partial or complete spinal block.

If opening CSF pressure is >200 mm, no more than 1-2 ml of CSF should be removed.

2. Gross Appearance of Cerebrospinal Fluid (CSF)

Normal CSF is clear and colorless like distilled water, and does not clot. Abnormal CSF may appear turbid, blood-mixed, xanthochromic, or viscous (Figure 1182.5). Clot formation in CSF is abnormal and indicates increased proteins.

- *Turbid CSF may be due to the presence of:*
  – Leukocytes >200 cells/cmm
  – Red cells >400 cells/cmm
  – Microorganisms like bacteria, fungi, or amebae
  – Radiographic contrast media
  – Aspiration of epidural fat during LP
  – Raised proteins.

- ***Blood-mixed CSF:*** Blood-stained CSF may result from traumatic tap (due to injury to venous plexus in spinal wall) or subarachnoid hemorrhage. Distinction of traumatic tap from subarachnoid hemorrhage is vitally important. Differences between the two are given in Table 1182.2.

- ***Xanthochromia:*** This refers to yellow discoloration of CSF. For its detection, CSF is centrifuged and the supernatant is compared with another tube of same size filled with distilled water. Causes of xanthochromia are:

  – Subarachnoid hemorrhage (12 hours after bleeding episode): In subarachnoid hemorrhage, bleeding occurs in subarachnoid space usually due to rupture of a cerebral aneurysm. Patient presents with severe bursting headache of sudden onset in occipital region that may be followed by loss of consciousness. Red blood cells in CSF are hemolyzed with release of oxyhemoglobin. About a week after bleeding, macrophages and other cells of leptomeninges convert oxyhemoglobin to bilirubin. This produces yellowish discoloration of CSF supernatant (See Figure 1182.5). The investigation of choice, if available, for diagnosis of subarachnoid hemorrhage is CT scan to detect blood in basal cisterns. When CT scan is negative or equivocal and xanthochromia cannot be appreciated visually, spectroscopic examination of CSF is helpful for diagnosis of subarachnoid hemorrhage. It will show absorption peaks of oxyhemoglobin and bilirubin.

  – Jaundice, when serum bilirubin is >6.0 mg/dl

  – CSF protein >150 mg/dl.

  Froin's syndrome is a combination of xanthochromia, excess proteins in CSF, and spontaneous formation of a coagulum in CSF on standing. It results from complete block of subarachnoid space.
- ***Other abnormal colors of CSF:*** These are:

  – Pink: Red cell lysis and hemoglobin breakdown

  – Brownish: Meningeal metastatic melanoma

  – Orange: High carotene ingestion
- ***Clot formation:*** Pellicle (thin membrane or scum on the surface of CSF) or clot formation (after 10 minutes of collection) indicates increased proteins (>150 mg/dl). It occurs in tuberculous meningitis (fine cobweblike clot after 12-24 hours), purulent meningitis (pellicle forms early followed by a large clot), spinal block (complete clotting of CSF), and traumatic LP. Clot formation does not occur in subarachnoid hemorrhage.
- ***Thick viscous CSF:*** This is seen cryptococcal meningitis, meningeal metastatic mucinous adenocarcinoma, severe meningitis, and release of nucleus pulposus fluid in CSF due to needle injury to the intervertebral disk.

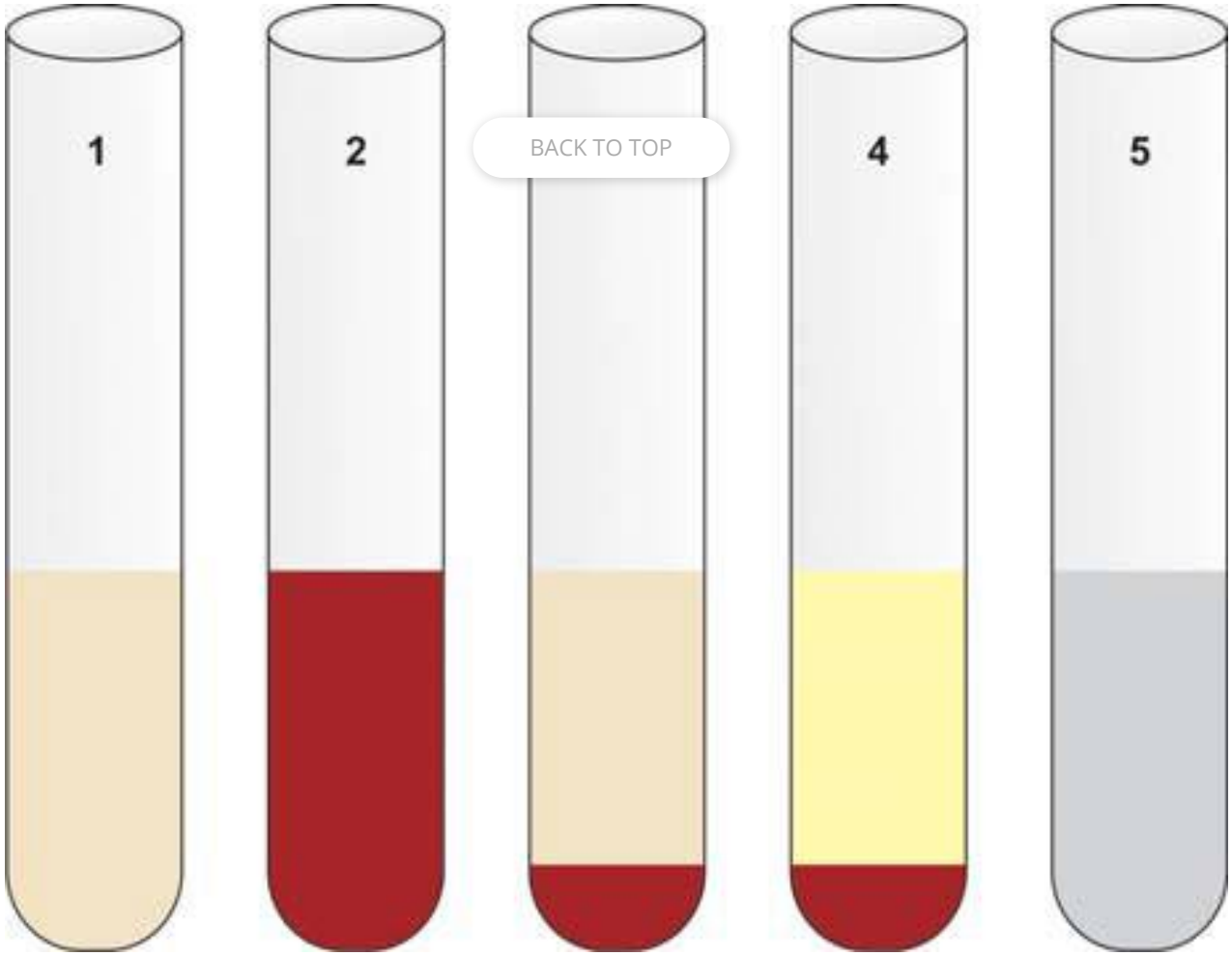

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Figure 1182.5: Appearance of CSF. (1) Normal CSF is clear and transparent. (2) Bloody CSF (either due to traumatic LP or subarachnoid hemorrhage). (3) After centrifugation, bloody CSF due to traumatic LP shows clear supernatant, while that due to subarachnoid hemorrhage shows, (4) yellowish supernatant (xanthochromia) (5) Turbid CSF

Table 1182.2 Differences between traumatic lumbar puncture and subarachnoid hemorrhage

Cerebrospinal fluid finding	Traumatic lumbar puncture	Subarachnoid hemorrhage
1. Gross appearance	Blood more in initial tubes as compared to later tubes; Blood clots on standing.	Blood uniform in all tubes; Blood does not clot on standing
2. Supernatant after centrifugation within 1 hour of collection	Clear	Pink or yellow (xanthochromia); yellow xanthochromia develops 12 hours after hemorrhage
3. Microscopy	Progressive decrease of red cell counts in later tubes	Red cell counts uniform in all tubes; Hemosiderin-laden macrophages present
4. Latex agglutination test for D-dimer*	Negative	Positive
5. Cerebrospinal fluid pressure	Normal	Increased
6. Cerebrospinal fluid protein	Normal	Increased

**D-dimer: A cross-linked fibrin degradation product*

3. Cell Counts in Cerebrospinal Fluid

(A) Total Leukocyte Count:

Cell count on CSF is done manually on the undiluted sample in a counting chamber.

Total leukocyte count increases in various disorders and along with differential count provides important diagnostic information. An increase in cell count in CSF is called as pleocytosis.

It is essential to do a microscopic examination of all CSF samples since white blood cell (WBC) count up to 200/cmm and red cell count up to 400/cmm are associated with the clear appearance of CSF.

For correct results:

- Cell count should be done as soon as possible after collection of CSF since cellular disintegration occurs rapidly. Cells also adhere to the walls of the glass tubes.
- CSF specimen collected in tube 3 should be used.
- No dilution of CSF is usually required. A diluent should be used only if CSF is cloudy and likely to contain increased leukocytes.

Method:

i. CSF sample should be properly mixed. If CSF is clear, it is not diluted. If CSF appears cloudy or turbid, 1:20 dilution is made using 0.05 ml of CSF and 0.95 ml of Turk solution. (Composition of Turk solution: Glacial acetic acid 4 ml, methylene blue solution 10 drops, and distilled water to make 200 ml).
ii. The counting chamber is covered with the coverslip provided.
iii. The counting chamber is filled with the fluid and allowed to stand for 2 minutes for cells to settle.
iv. For counting cells in CSF, Fuchs-Rosenthal counting chamber is preferred because its depth is twice that of improved Neubauer chamber. In this, cells are counted in 5 large squares (4 corner squares and one central square).
v. If undiluted CSF is used, total number of cells counted in 5 squares represents total count per cmm of CSF. If CSF is diluted, the number of cells counted is multiplied by the dilution factor (i.e. 20).

Causes of increased cell count in CSF:

- Meningitis and other infections of CNS
- Intracranial hemorrhage
- Meningeal infiltration by malignancy
- Repeated lumbar punctures

- Injection of foreign substances (e.g. radiographic contrast media, drugs) in subarachnoid space.
- Multiple sclerosis

Presence of blood in CSF due to traumatic tap or subarachnoid hemorrhage artefactually raises the leucocyte count by 1 WBC per 1000 red cells. This correction factor should be used if patient's hemogram is normal. If significant anemia or leukocytosis is present, then leukocyte count in CSF should be corrected as follows:

Corrected WBC count in CSF = WBC count in CSF (cells/cmm) – [(WBC count in blood × Red cell count in CSF) ÷ Red cell count in blood]

(B) Differential Leukocyte Count (DLC):

This provides information about relative proportion of various leukocytes.

If CSF contains only a few cells, then it is centrifuged at high speed (3000 g) for 10 minutes and a smear is made from the sediment. If CSF contains many cells, then a smear is made directly from the uncentrifuged sample. After staining with a Romanowsky stain, smear is examined under the microscope (Figure 1182.6).

Simple centrifugation of CSF often causes cell breakage and distortion. Cytospin preparation with cytocentrifuge (high speed centrifugation to concentrate cells on a slide in a uniform monolayer) has been recommended as it improves the cell yield and preserves the cell morphology well.

In normal adults, differential count shows 70% lymphocytes and 30% monocytes. In young children, a higher proportion of monocytes (up to 70%) are present. Table 1182.3 shows causes of increase in different types of leukocytes in CSF.

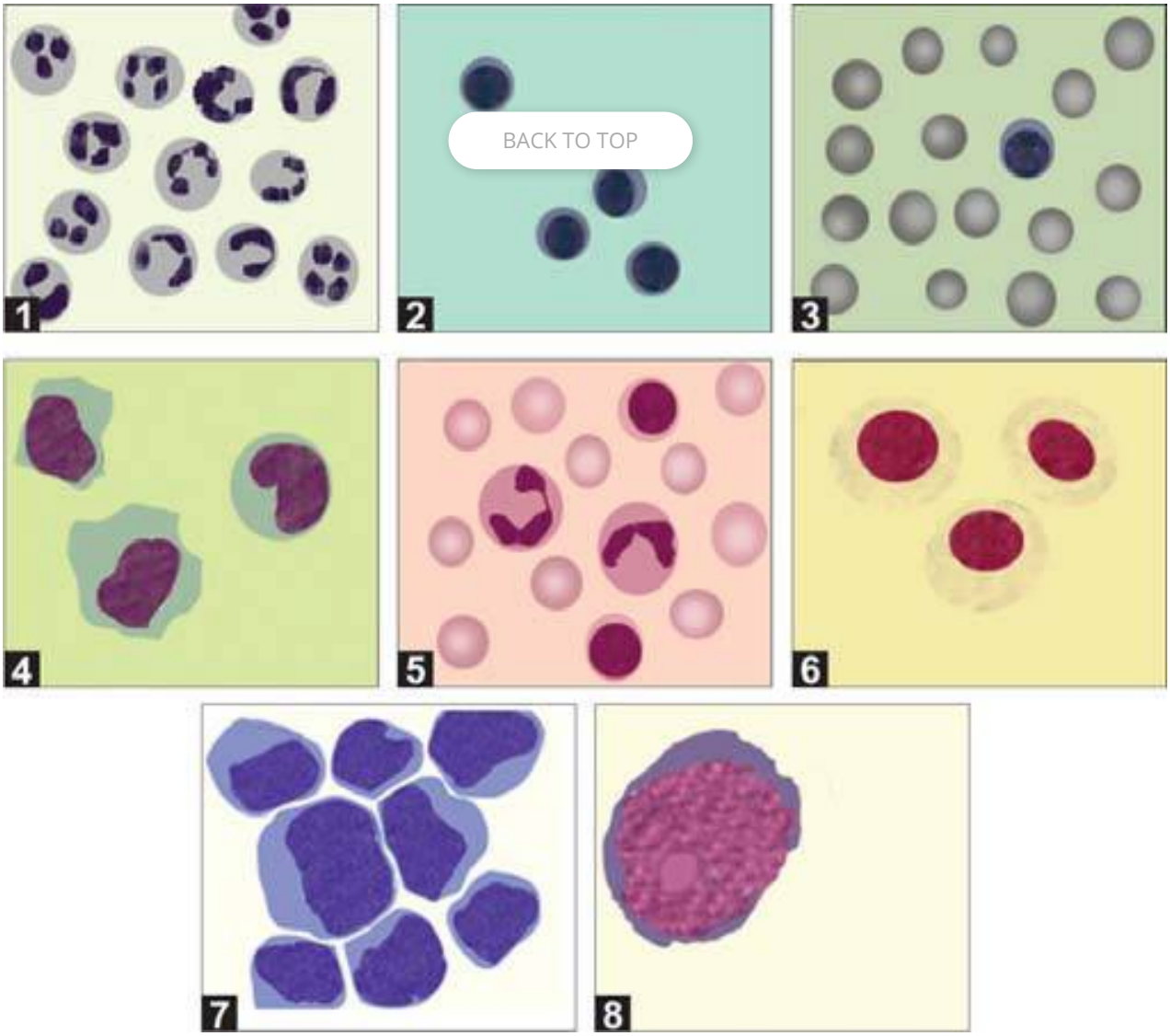

Figure 1182.6: Cells in CSF: (1) Many neutrophils in bacterial meningitis, (2) lymphocytes seen in normal CSF in adults, (3) Many red cells and a small lymphocyte (traumatic tap), (4) Increased monocytes in CSF, (5) Neutrophils, lymphocytes, and red cells in CSF, (6) Lining cells of pia and arachnoid in CSF, (7) Blast cells in CSF, (8) a malignant cell in CSF

Predominant neutrophils	*Predominant lymphocytes*	*Mixed cell pattern (neutrophils, lympho-cytes, monocytes)*	*Predominant eosinophils*
1. Meningitis: bacterial, early viral, fungal, early tuberculous 2. Subarachnoid hemorrhage 3. Repeated lumbar punctures 4. Introduction of anticancer drugs or contrast media in subarachnoid space 5. Meningeal metastasis	1. Meningitis: viral, tuberculous 2. Incompletely treated bacterial meningitis 3. Cysticercosis, toxoplasmosis 4. Multiple sclerosis 5. Subacute sclerosing panencephalitis	1. Tuberculous meningitis 2. Fungal meningitis 3. Chronic bacterial meningitis	1. Parasitic and fungal infections 2. Reaction to foreign material (e.g. shunts)

Table 1182.3: Differential cell count in CSF

(C) Other Cells:

Apart from mature blood cells, CSF may contain immature hematopoietic cells, tissue cells (ependymal cells, pia arachnoid mesothelial cells) and malignant cells. CSF examination is

commonly carried out in acute lymphoblastic leukemia to detect involvement of CNS. Increased WBC count (>5/µl) with lymphoblasts is evidence of CNS involvement.

4. Chemical Examination of Cer BACK TO TOP id

Routine chemical examination of CSF consists of estimation of proteins and glucose. CSF from tube 1 is used for chemical examination.

(1) Estimation of proteins in CSF:

Normal CSF protein level in adults is 15-45 mg/dl. An increase in CSF protein is a sensitive but non-specific indicator of CNS disease. CSF proteins may be normal during early stages of meningitis. Significant elevation (>150 mg/dl) occurs in bacterial meningitis.

There are various methods for estimation of CSF proteins. Turbidimetric method using trichloroacetic acid for precipitation of proteins is commonly used. In principle, trichloroacetic acid, when added to CSF, causes precipitation of proteins and a turbid solution is obtained. Amount of turbidity is compared with the turbidity of a known (standard) concentration of protein in a photoelectric colorimeter.

If a sample is contaminated with blood while doing the lumbar puncture (traumatic tap), false elevation of proteins will occur. This can be corrected by deducting 1 mg/dl of protein for every 1000 red cells per cmm. For this correction to be accurate, red cell count and proteins should be estimated in the sample from the same tube of CSF.

If facilities for estimation of CSF proteins are not available in the laboratory, then Pandy's test for globulins may be performed. In this test, CSF is added to saturated solution of phenol. If cloudiness develops immediately, it indicates presence of increased globulins and the test is reported as positive. If no cloudiness develops, the test is reported as negative (Figure 1182.7).

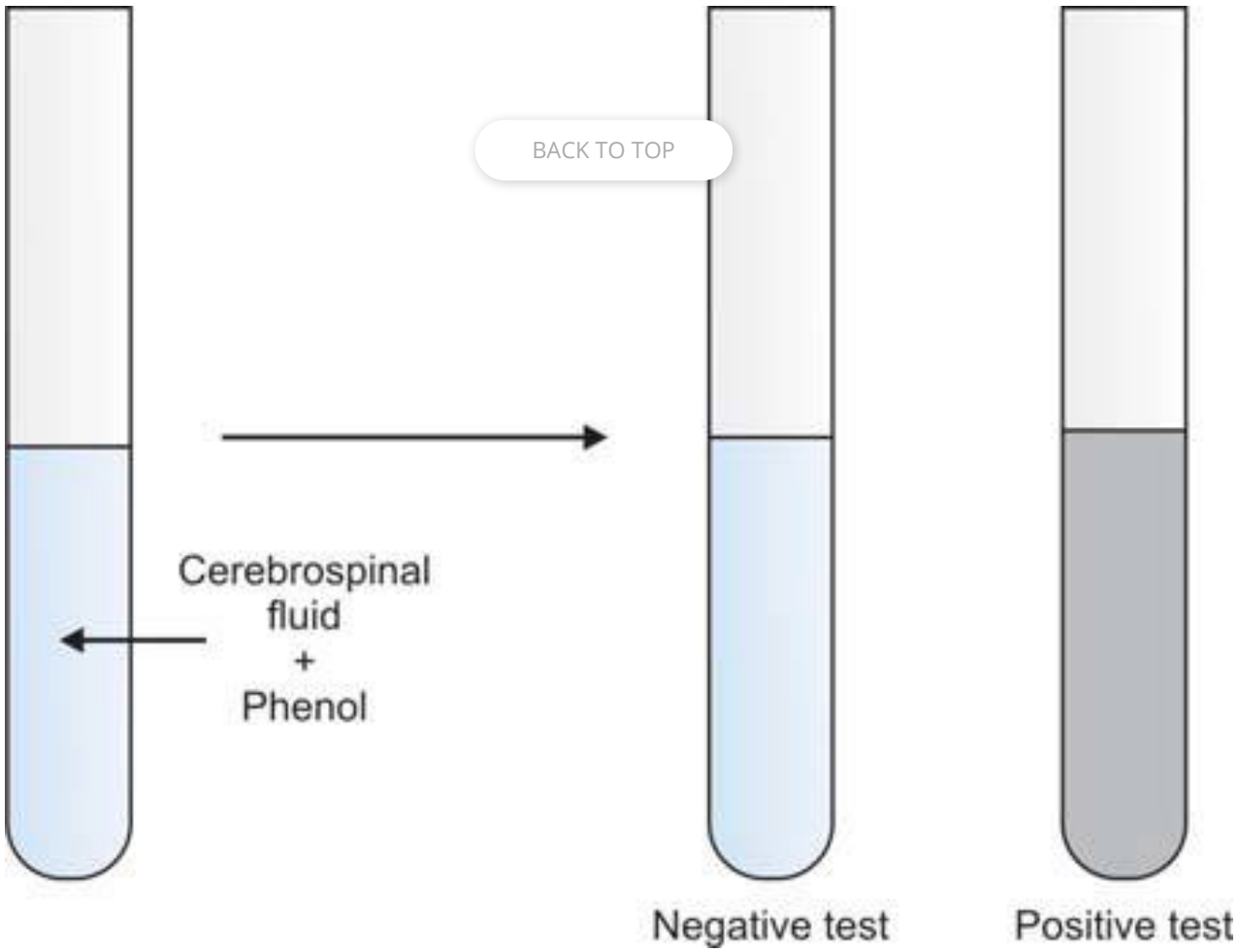

Figure 1182.7: Pandy's test for globulins

Along with CSF proteins, it is necessary to simultaneously measure serum proteins for proper interpretation of results.

CSF proteins are elevated in following conditions:

- **An increased capillary permeability of blood-brain barrier:** Meningitis
- **Mechanical obstruction to the circulation of CSF (causing increased fluid reabsorption due to stasis):** Spinal cord tumor
- **Increased local (intrathecal) immunoglobulin (IgG) production:** Multiple sclerosis, neurosyphilis, subacute sclerosing panencephalitis
- **Both increased capillary permeability and increased local immunoglobulin (IgG) production:** Guillain-Barré syndrome
- **Hemorrhage in CSF:** Traumatic tap, subarachnoid hemorrhage.

Marked elevation (>500 mg/dl) is noted in complete spinal block by a tumor, bacterial meningitis, and bloody CSF.

Differential diagnosis of elevated proteins in CSF (increased capillary permeability of blood-brain barrier vs. increased intrathecal synthesis of immunoglobulin G) can be made from parameters shown in Table 1182.4. Albumin is neither synthesized nor metabolized in CNS. Therefore, increased CSF albumin/serum albumin ratio indicates increased permeability of blood-brain barrier. Immunoglobulin G (IgG) can be synthesized in CNS. Therefore, increased CSF IgG/serum

IgG ratio indicates either increased permeability of blood-brain barrier or increased intrathecal synthesis of IgG. Increased CSF IgG/albumin index indicates local IgG production.

CSF/Serum albumin ratio	CSF/Serum IgG	n index	Causes
1. Increased	Increased	Normal	Increased permeability of blood-brain barrier
2. Normal	Increased	Increased	Increased intrathecal synthesis of proteins

Table 1182.4: Differentiation of causes of elevated proteins in cerebrospinal fluid

(2) Estimation of glucose in CSF:

Normal CSF glucose is 2/3rds of blood glucose (CSF to blood glucose ratio is 0.6). A sample for blood glucose should be drawn 1 hour before LP for comparison with CSF glucose. After collection, CSF sample should be immediately processed for glucose estimation because falsely low result due to glycolysis may occur.

CSF glucose is measured by glucose oxidase method. Normal range is 45-80 mg/dl. CSF glucose <40 mg/dl is abnormal.

CSF glucose is decreased due to utilization by bacteria (pyogenic or tuberculous), leucocytes, or cancer cells in CSF.

Decreased CSF glucose occurs in following conditions:

- Acute bacterial meningitis
- Tuberculous meningitis
- Fungal meningitis
- Meningeal involvement by malignant tumor (meningeal carcinomatosis)
- Hypoglycemia

CSF glucose is normal in viral meningitis.

5. Microbiological Examination

Microbiological tests which can be carried out on CSF sample are—

- **Direct wet mount of CSF:** in suspected cases of cryptococcosis, amebic meningoencephalitis, Candida infection, and trypanosomiasis
- **Gram's smear:** should be done if CSF is turbid and neutrophils are increased.
- **Ziehl-Neelsen smear:** if tuberculous meningitis is suspected.
- **Latex agglutination tests:** for detection of bacterial and cryptococcal antigens.
- **Serologic tests** for syphilis
- **Culture** for bacteria and *Mycobacterium tuberculosis*
- **Polymerase chain reaction** for *Mycobacterium tuberculosis* and viruses.

(A) Direct Wet Mount of CSF:

One drop of CSF deposit (obtained after centrifugation) is placed on a glass slide, covered with a cover glass, and examined under the microscope with reduced illumination. Observe for motile

trypanosomes or sluggishly moving amebae (*Naegleria fowleri*). *N. fowleri* is a free-living ameba in water which enters through the nose and reaches central nervous system. It causes a fatal type of hemorrhagic meningoencephalitis. *Candida albicans* may be seen in unstained wet mount; it appears as oval budding forms and as

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When cryptococcal meningitis is clinically suspected, the wet mount is examined by dark-field microscopy. Alternatively, a drop of India ink is added to the drop of sediment on a glass slide, a coverslip is placed, and examined under the microscope using ×40 objective. *Cryptococcus neoformans* appears as spherical, budding yeast forms, 2-10 μ in diameter and surrounded by a large unstained capsule (Figure 1182.8). Cryptococci can be demonstrated by India ink preparation in 50% cases of cryptococcal meningitis.

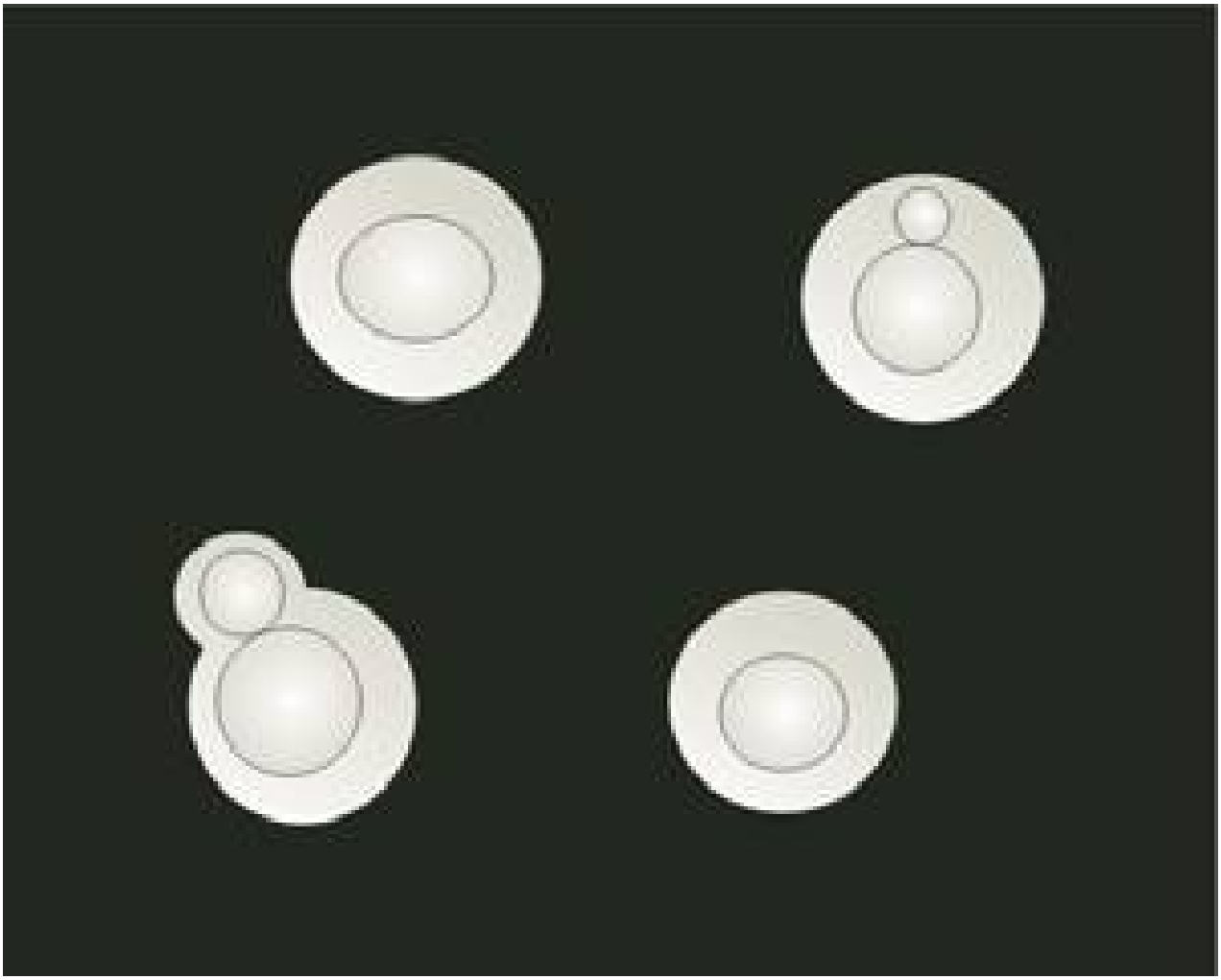

Figure 1182.8: India ink preparation of CSF showing single and budding yeast cells of Cryptococcus neoformans

(B) Gram's Smear:

This must be done if CSF is purulent and neutrophils are increased. Gram's smear is positive in 80% untreated cases and 60% partially treated cases of bacterial meningitis. Therefore, absence of bacteria on Gram's smear does not rule out bacterial infection.

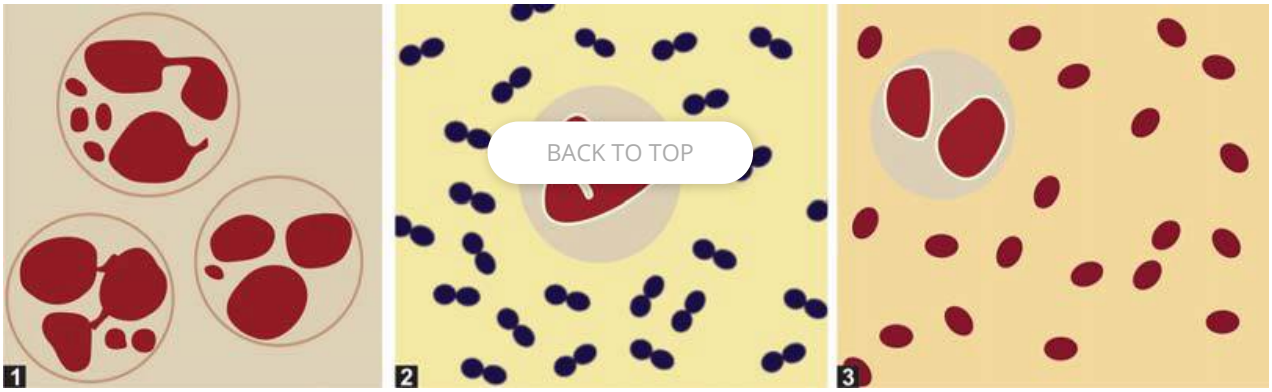

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Figure 1182.9: Gram-stained smears of CSF showing (1) Neisseria meningitidis, (2) Streptococcus pneumoniae, and (3) Haemophilus influenzae

CSF is centrifuged and a smear of the deposit is made on a glass slide. (If CSF is purulent, smear is made directly without centrifugation). Smear is air-dried, stained with Gram's method, and examined under the oil-immersion lens for bacteria (Figure 1182.9). Bacteria which commonly cause meningitis are:

- **Meningococci:** Gram-negative diplococci located inside neutrophils.
- **Pneumococci:** Gram-positive diplococci surrounded by an unstained capsule.
- ***Hemophilus influenzae*:** Gram-negative coccobacilli.
- ***Escherichia coli*:** Gram-negative rods.

In all the above types of meninigitis, the accompanying cells are polymorphonuclear neutrophils.

Detection of typical bacteria on Gram-staining suggests the etiologic agent of meningitis. However, definitive diagnosis requires the culture of CSF.

There is an association between age of the patient and the causative organism of meningitis.

- **0 to 6 months:** Group B streptococci, *Escherichia coli*, *Listeria monocytogenes*
- **6 months to 6 years:** *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Hemophilus influenzae* type B, Enteroviruses
- **6 to 60 years:** *Neisseria meningitides*, *Streptococcus pneumoniae*, Enteroviruses, herpes simplex virus
- **>60 years:** *Streptococcus pneumoniae*, gram-negative bacilli, *Listeria monocytogenes*

(C) Ziehl-Neelsen Staining for *Mycobacterium tuberculosis*:

In tuberculous meningitis, number of tubercle bacilli is usually low in CSF. Ziehl-Neelsen or AFB staining is not a very sensitive method for detection of *M. tuberculosis* in CSF. AFB smears are negative in about 70% of cases of tuberculous meningitis. Fluorescent auramine stains have better sensitivity than Ziehl-Neelsen stain.

(D) Latex Agglutination Tests:

Latex agglutination tests for bacterial antigens are available commercially and are sensitive, rapid, and simple to perform. Currently available tests can detect *N. meningitidis* (groups A, B, C, Y, and W135), *H. influenzae* (capsular type B), *S. pneumoniae* and *S. agalactiae*. These tests are

expensive and their sensitivity is similar to that of Gram's smear. Owing to the occurrence of false-positive results, these tests are not recommended for routine diagnosis of meningitis. These tests are particularly useful in patients who have been partially treated and in whom Gram's stain and culture are negative.

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Latex agglutination tests for cryptococcal antigens are also available and have sensitivity of 90%. These tests have largely replaced India ink preparation for diagnosis of cryptococcal meningitis.

(E) Limulus Lysate Assay for Endotoxin Produced by Gram-negative Bacteria:

Limulus amebocyte lysate assay is a rapid, sensitive, and specific test for the presence of endotoxin. Endotoxin is produced by gram-negative bacteria like *N. meningitides*, *H. influenzae* type b, *E. coli*, and *Pseudomonas*. This test is particularly useful as a rapid test in newborns in whom these infections are common.

(F) Serologic Tests for Syphilis:

If fluorescent treponemal antibody with absorption (FTA-ABS) test is positive in serum, Venereal Disease Research Laboratory (VDRL) test should be done on CSF if neurosyphilis is suspected. VDRL test is highly specific but lacks sensitivity. Therefore, a positive test rules in but does not rule out the diagnosis of neurosyphilis. Other serological tests for syphilis are not suitable for the diagnosis of neurosyphilis in CSF.

> Combination of positive FTA-ABS test in serum and reactive VDRL test in CSF is diagnostic of active neurosyphilis

(G) CSF Culture:

Culture of CSF is indicated if bacteria are seen on Gram-stained smear, or leukocytes or proteins are increased. Culture remains the gold standard for diagnois of bacterial meningitis. For culture, CSF collected in tube 2 is used. Sensitivity of culture for identification of bacteria is about 90%. In incompletely treated cases, sensitivity is less. Usually CSF sample is inoculated on chocolate (heated blood) agar and blood agar. In newborn infants, sample is also inoculated on McConkey's agar.

In tuberculous meningitis, culture for *M. tuberculosis* is positive in about 56% of cases. If a larger volume of CSF is used (i.e. 10 ml) for inoculation, sensitivity for detection increases.

In cryptococcal meningitis, culture is positive in 95% of cases.

(H) Polymerase Chain Reaction (PCR):

PCR is a highly specific and sensitive tool for diagnosis of infections of CNS. It uses probes to detect genes specific for the infecting organism. The test is rapid and requires only a small amount of CSF. High cost and availability only in a few specialist laboratories are major limitations. CSF PCR is mainly useful for diagnosis of viral infections of CNS (e.g. herpes simplex, enteroviruses, herpes zoster, etc.) and of tuberculous meningitis. CSF findings in different types of meningitis are shown in Table 1182.5.

	Condition	Appearance	Leukocytes	Proteins in mg/dl	Glucose in mg/dl	Additional investigations
1.	Normal	Clear, colorless	<5/µl (mostly lymphocytes)	15-45	45-80	–
2.	Acute pyogenic meningitis	Turbid or purulent	Increase[illegible] mostly neutrophils	Increased; 50-1500	Decreased; <40	Gram's stain; culture; Latex agglutination test
3.	Tuberculous meningitis	Clear or cloudy	Increased (100-600/µl); mostly lymphocytes or both lymphocytes and neutrophils	Increased; 45-300	Decreased; 10-45	AFB stain; culture; polymerase chain reaction
4.	Viral meningitis	Clear or cloudy	Increased (6-300/µl); lymphocytes	Increased	Normal	Polymerase chain reaction

Table 1182.5: Cerebrospinal fluid findings in different types of meningitis

6. Special Investigations

(A) CSF Protein Electrophoresis:

Protein electrophoresis of normal CSF differs from normal serum in (i) presence of a prominent transthyretin band and (ii) an extra transferrin band (called β_2-transferrin or tau protein). Protein electrophoresis of CSF is used:

- For identification of oligoclonal bands, and
- To determine whether fluid submitted for examination is CSF.

1. ***Oligoclonal bands:*** Agarose gel electrophoresis of concentrated CSF is used for detection of oligoclonal bands. These are two or more discrete bands in the gamma region. Presence of oligoclonal bands on CSF protein electrophoresis that are absent on concurrently run serum protein electrophoresis is indicative of intrathecal syntheis of immunoglobulins (Figure 1182.10). Oligoclonal bands have been identified in majority of patients (90%) with multiple sclerosis; however, they are not specific for this disorder. They are also seen in subacute sclerosing panencephalitis, viral CNS infections, neurosyphilis, and Guillain-Barré syndrome.
2. ***CSF leakage:*** Occasionally, clear fluid leaking through the nose or ear after trauma or surgery is submitted for examination to determine whether it is CSF. Due to the risk of recurrent meningitis, accurate identification of such fluid as CSF is essential. Recommended test for this purpose is protein electrophoresis with immunofixation for transferrin. Protein electrophoresis of CSF shows an extra transferrin band (called as 'tau' protein), which is absent in other body fluids and secretions. Tau protein is an enzymatically modified transferrin that moves just behind the unaltered transferrin in β region. Identification of two isoform bands of transferrin on protein electrophoresis is a highly sensitive and specific test for identification of fluid as CSF (Figure 1182.11). Other body fluids or secretions do not show the second isoform band.

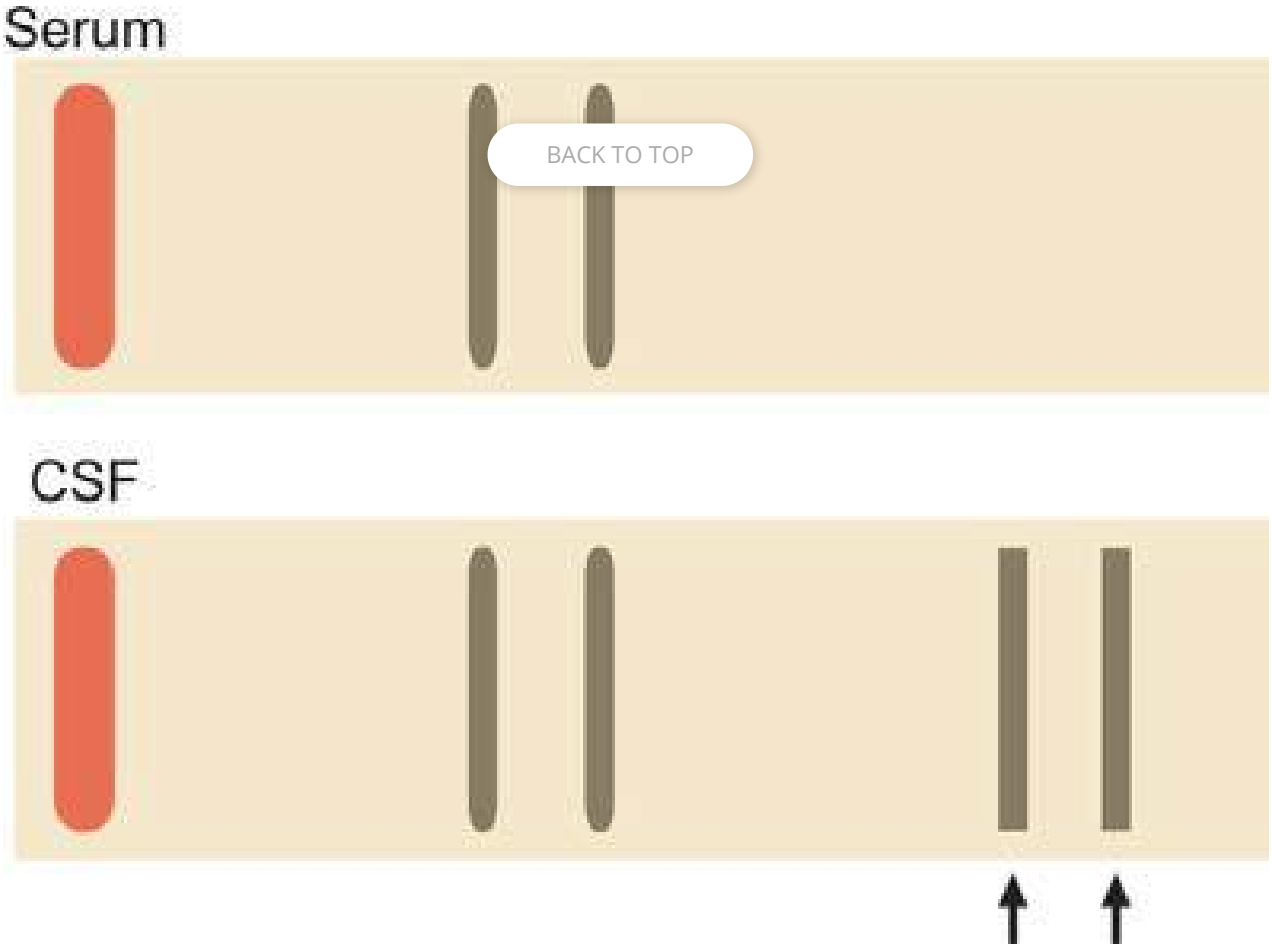

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Figure 1182.10: Oligoclonal bands in CSF in gamma region (arrows) in a case of multiple sclerosis

Figure 1182.11: An example of positive tau protein: Lane 1: Normal CSF; Lane 2: Serum; Lane 3: Nasal fluid. Lane 3 (nasal fluid) shows two bands in the same position as normal CSF confirming CSF leakage from nose

(B) Measurement of Albumin and Immunoglobulin G (IgG):

Comparison of IgG and albumin CSF/plasma ratio can be helpful for the diagnosis of multiple sclerosis (high ratio due to intrathecal synthesis of IgG).

REFERENCE RANGES

Reference ranges are given at the beginning of this article under "Composition of normal cerebrospinal fluid in adults".

CRITICAL VALUES

- Cells: >10/cmm
- Glucose: <45 mg/dl
- Proteins: >45 mg/dl
- Detection of pathogens on Gram stain, latex agglutination tests, Ziehl-Neelsen stain, or India ink preparation
- Positive culture
- Detection of blast cells or malignant cells
- Positive polymerase chain reaction for herpes simplex

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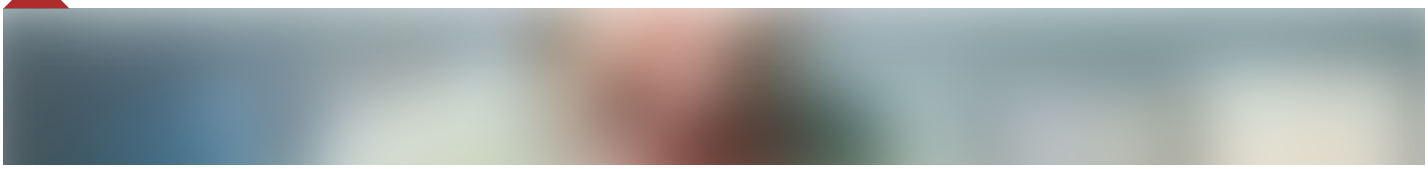

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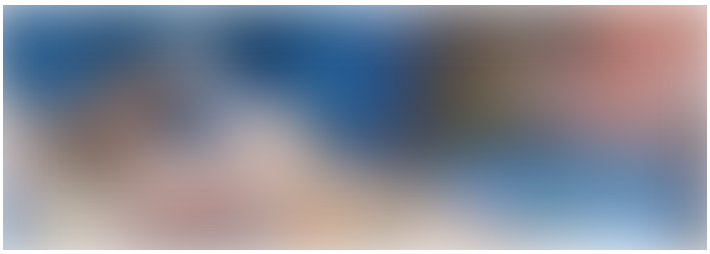

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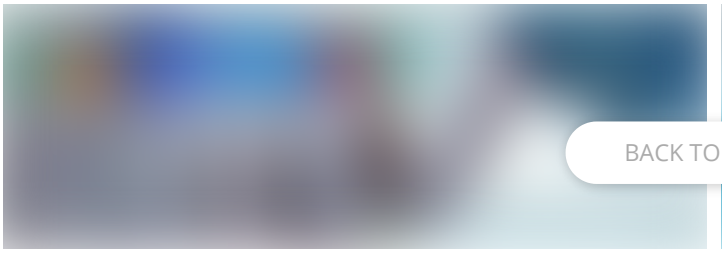

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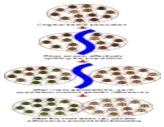

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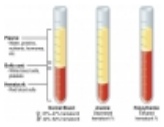

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Semen analysis

Introduction

- A semen analysis measures the amount of semen a man produces and determines the number and quality of sperm in the semen sample.

- A semen analysis is usually one of the first tests done to help determine whether a man has a problem fathering a child (infertility).

- A problem with the semen or sperm affects more than one-third of the couples who are unable to have children (infertile).

Physiology

- Sperm is the male gamete, that is, the male sex cell, which has the capacity to fertilise an egg. Sperm are produced in the seminiferous tubes of the testes. ***Germ cells*** for the production of ***spermatozoa***
- Specialized Sertoli cells provide support and nutrients for the germ cells as they undergo mitosis and meiosis (spermatogenesis).
- When spermatogenesis is complete, the immature sperm (nonmotile) enter the epididymis.
- In the epididymis, the sperm mature and develop flagella.
- They remain stored in the epididymis until ejaculation.
- The ejaculatory ducts receive both the sperm from the ductus deferens and fluid from the seminal vesicles.

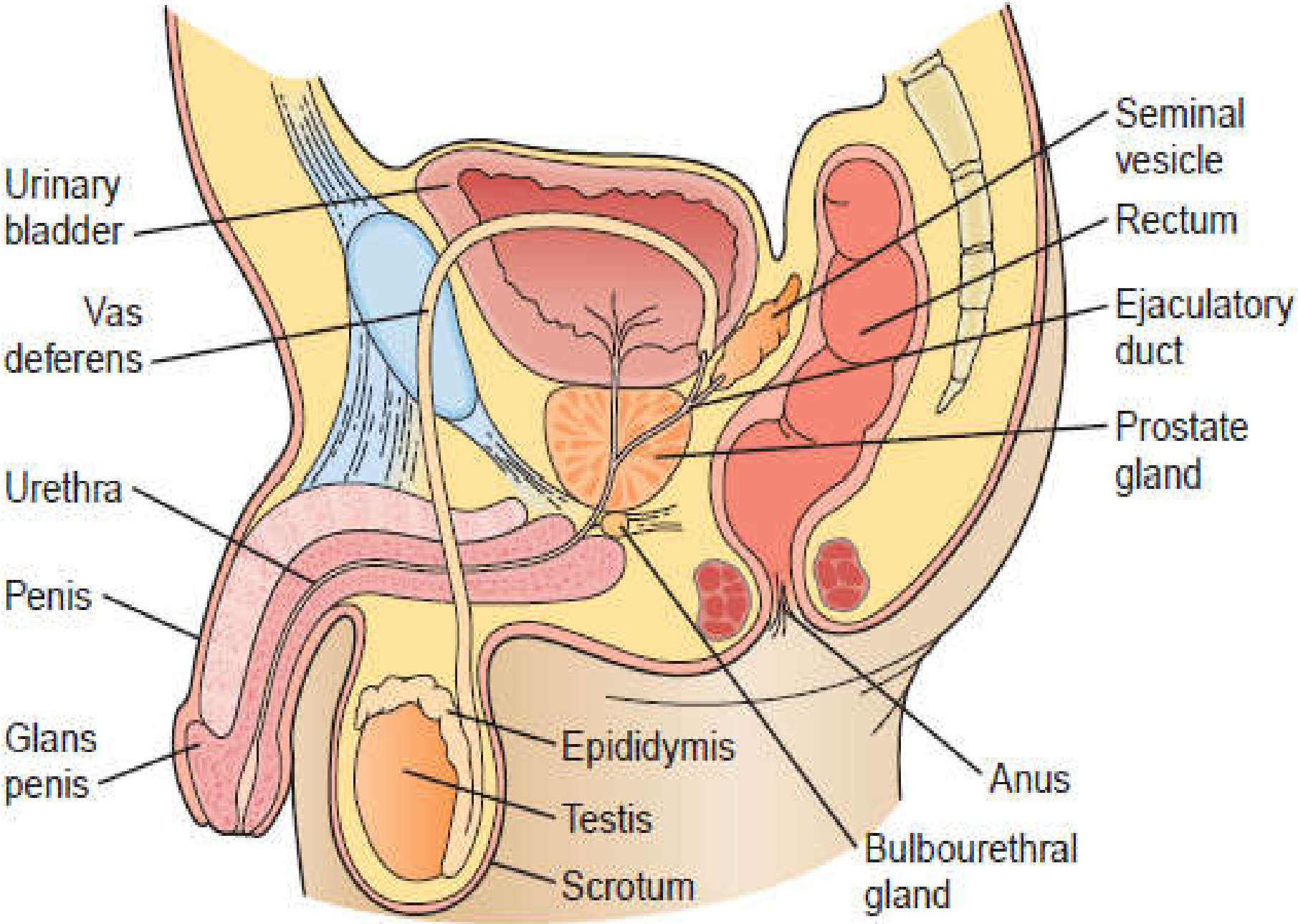
Fluid Fractions

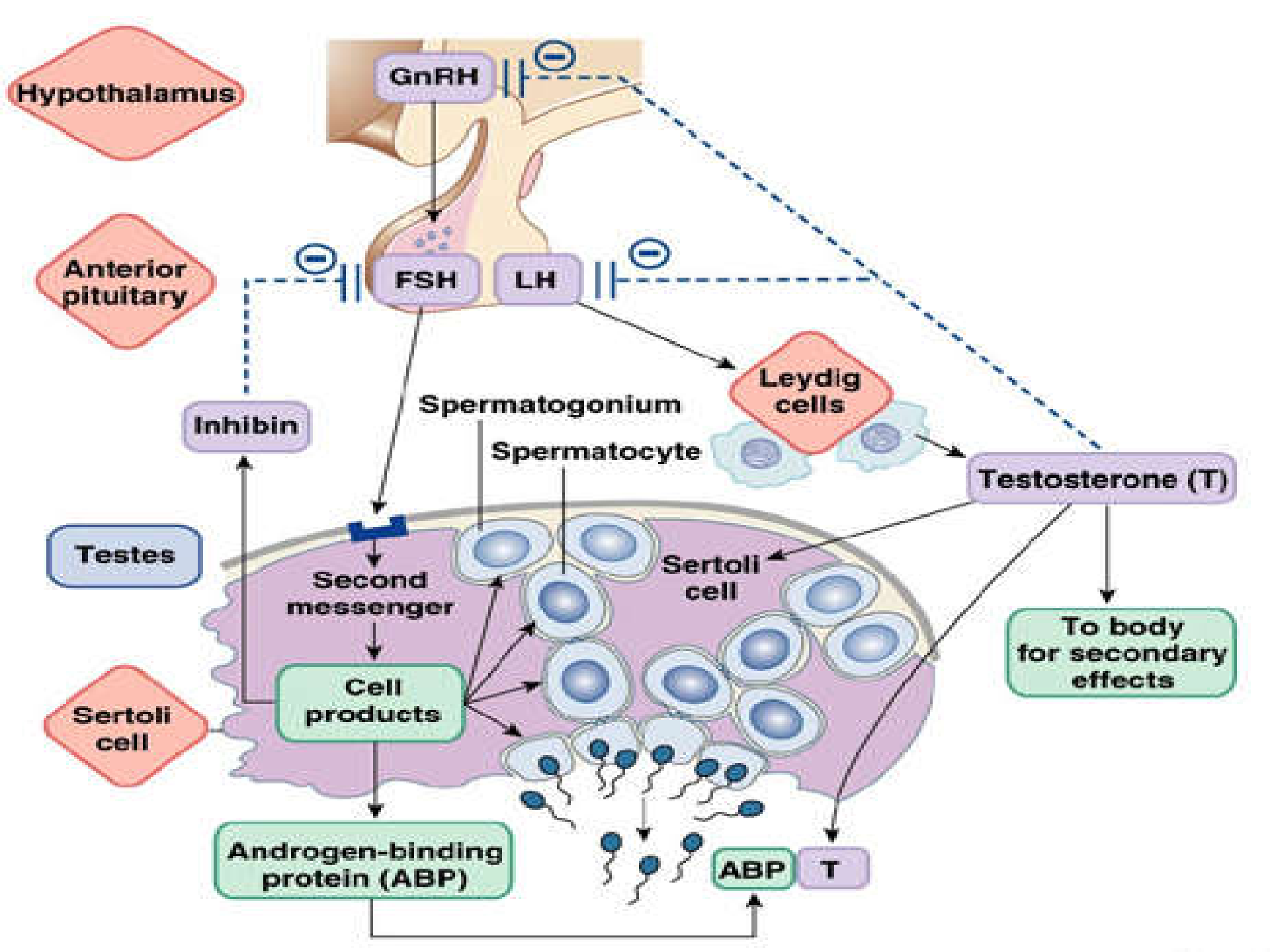
1. ***Urethral glands*** (2-5%) are very small mucus secreting glands.

2. ***Prostate****:* Approximately 20% to 30% of the semen volume is acidic fluid produced by the prostate gland, the secretion contains acid phosphatase and proteolytic enzymes that act on the fluid from the seminal vesicles, resulting in the coagulation and liquefaction of the semen.

3. ***Seminal vesicles*** (produce about 46-80 % of the fluid volume of semen) viscous, yellowish secretion is rich in fructose, vitamin C, prostaglandin, and other substances, which nourish and activate the sperm passing through the tract.

4. ***Testis & Epididymis***: (5%) Spermatozoa are produced in the testis under the influence of testosterone, and then the epididymis (is the first part of the duct system) provides a temporary storage site for the immature sperm that enter it from testis. This fraction still in the inactive form until ejaculation.

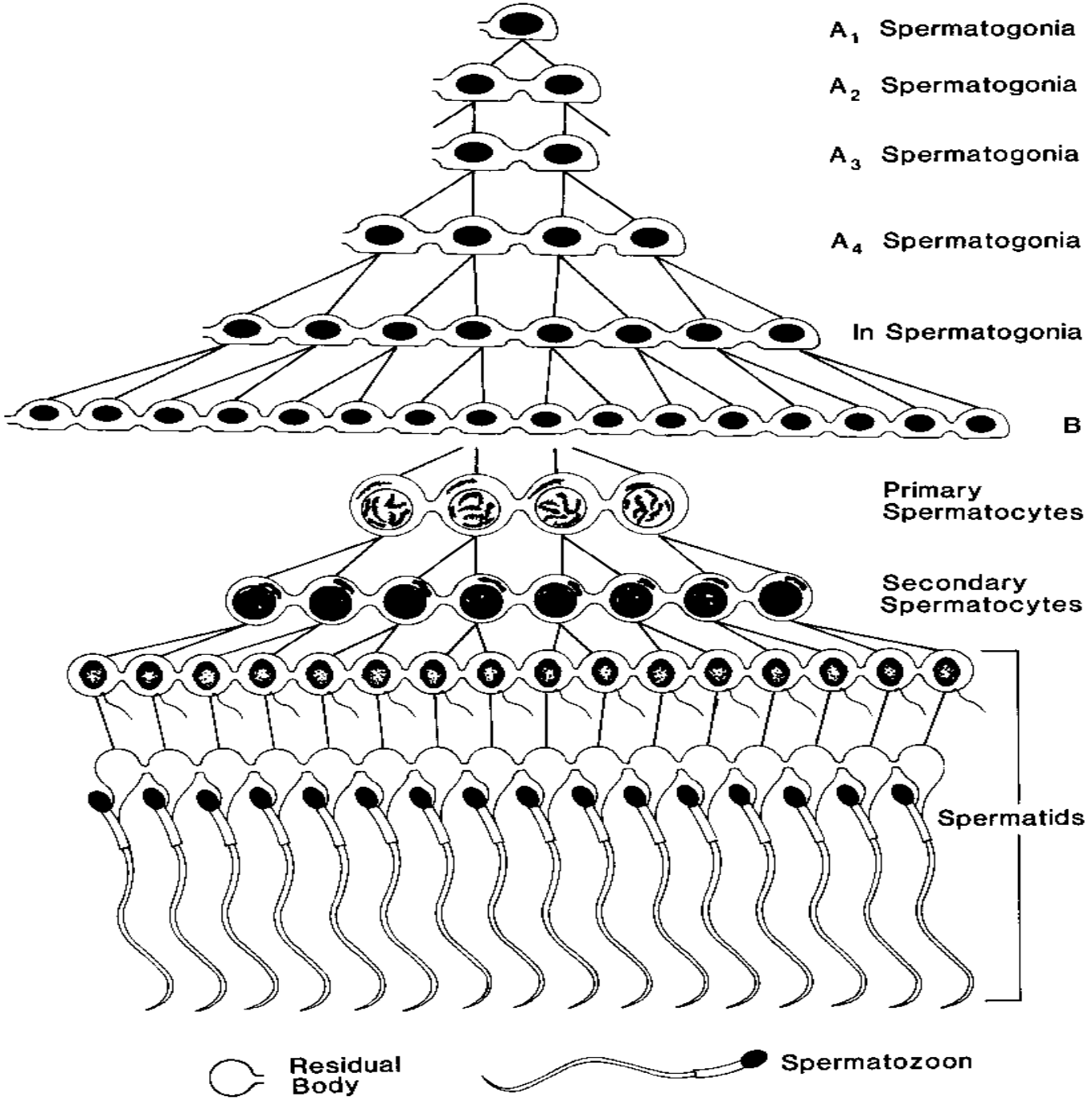

Urinary bladder
Vas deferens
Urethra
Penis
Glans penis
Seminal vesicle
Rectum
Ejaculatory duct
Prostate gland
Epididymis
Testis
Scrotum
Anus
Bulbourethral gland


Hypothalamus
GnRH
Anterior pituitary
FSH
LH
Inhibin
Spermatogonium
Spermatocyte
Leydig cells
Testosterone (T)
Testes
Second messenger
Sertoli cell
Cell products
Sertoli cell
To body for secondary effects
Androgen-binding protein (ABP)
ABP
T

Formation of the sperm cell

- Spermatogenesis is a cascade of cell divisions:
  - Mitosis: spermatogonia to primary spermatocytes
  - First meiotic division: secondary spermatocytes
  - Second meiotic division: haploid spermatids
- This process takes 70 ± 4 days in the human.

- ***Spermiogenesis*:** differentiation of the round spermatid into a spermatozoon

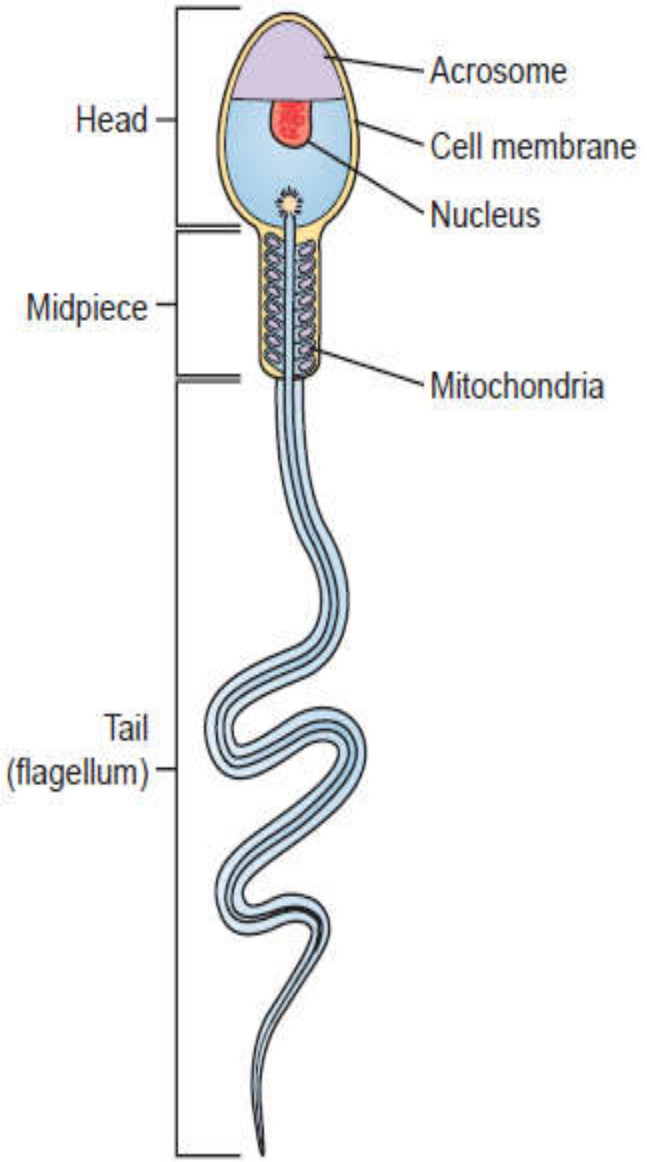

A1 Spermatogonia
A2 Spermatogonia
A3 Spermatogonia
A4 Spermatogonia
In Spermatogonia
B
Primary Spermatocytes
Secondary Spermatocytes
Spermatids
Residual Body
Spermatozoon

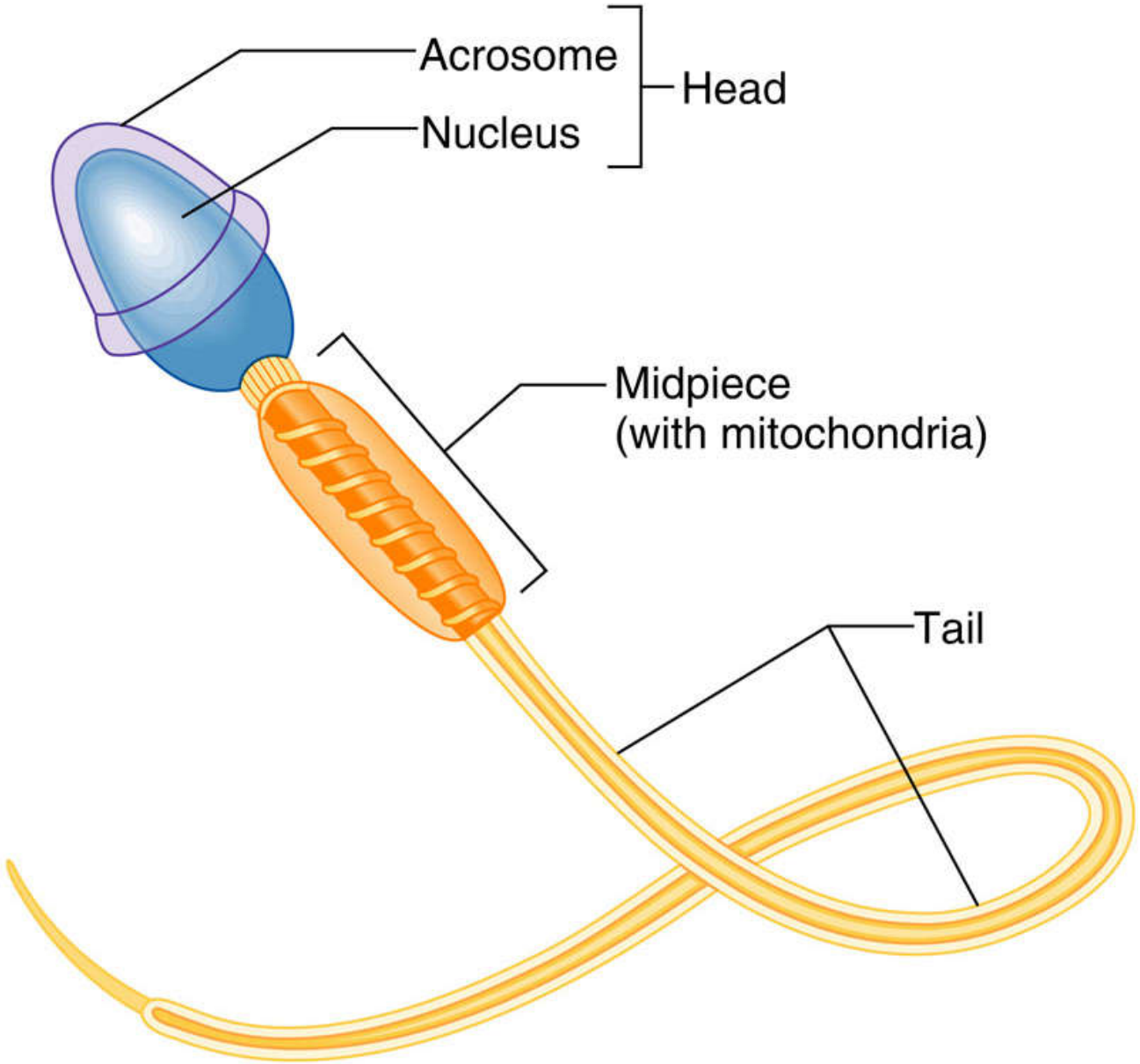
Sperm transport and seminal plasma

- "Testicular sperm" need to undergo more maturation steps before they are ready to fertilize.
- Transported from the testes to the epididymis, where they mature, and acquire the ability to swim.
- Then moved to the vas deferens, for storage.
- At ejaculation, the sperm are transported out of the vas and mix with accessory gland secretions:
  - prostatic fluid (pH slightly alkaline to neutral; contains citric acid and zinc).
  - seminal vesicle fluid (pH strongly alkaline; contains fructose).

What the spermatozoon

- The human sperm cell is about 70 μm long.
- The nucleus is in the head – contains the 23 chromosomes.
- It is the head which binds to the egg at fertilization.
- Mid-piece: the energy for motility is generated.
- Tail: (motility the beat is initiated just behind the mid-piece, and then propagated along the tail).




Acrosome
Nucleus
Head
Midpiece
(with mitochondria)
Tail

What the purpose of the test

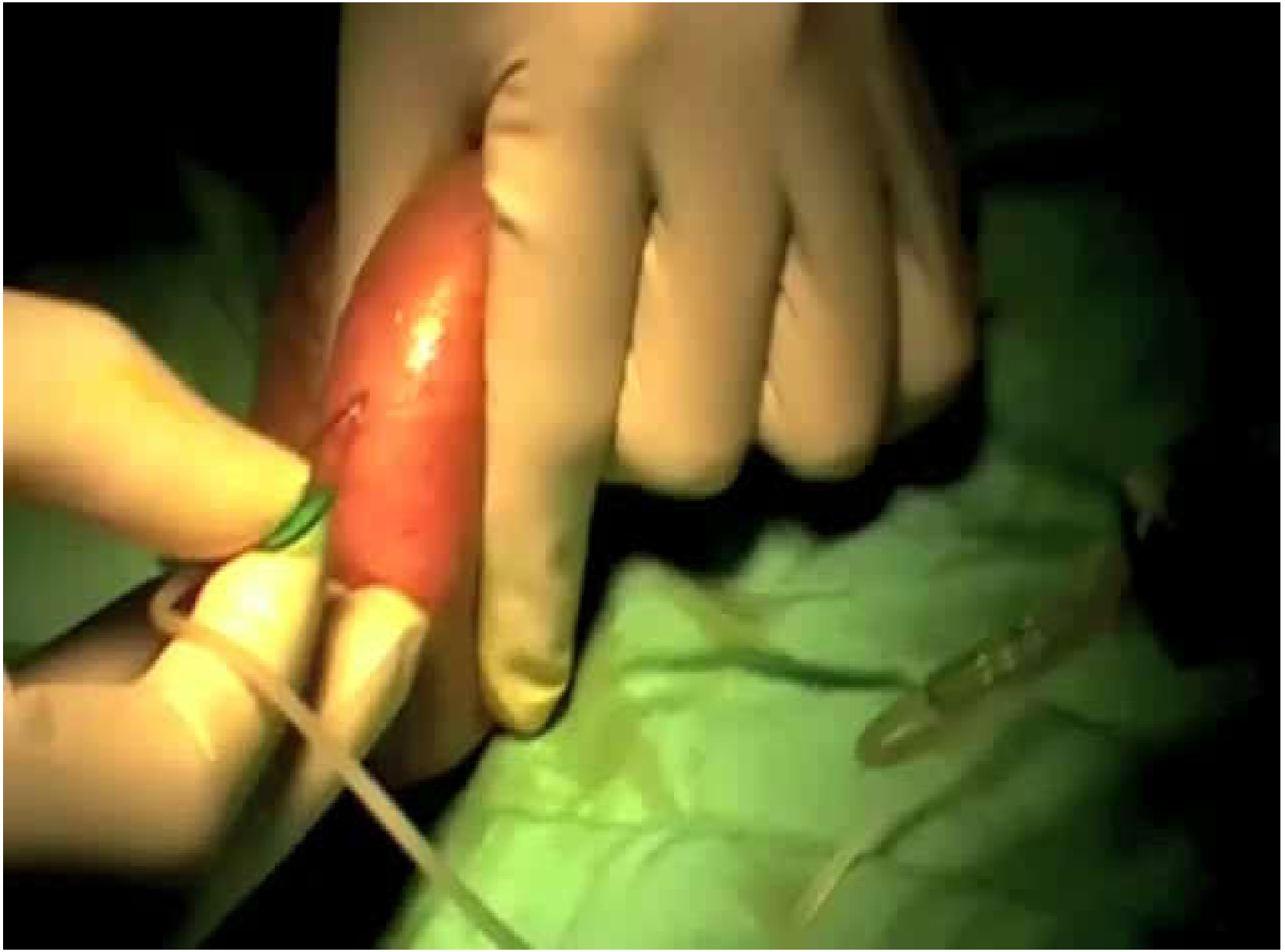
- Investigation of fertility
- Identify treatment options
  - ✓ Surgical treatment.
  - ✓ Medical treatment.
  - ✓ Assisted conception treatment.
  - ✓ Determine the suitability of semen for artificial insemination or IVF.

Sample collection

- Specimen should be collected into pre warmed (21^{o}C), sterile, non-toxic, wide-mouth container, after a couple has abstain from sexual activity for 2-3 days to not longer than 5 days.
- Specimens collected following prolonged abstinence tend to have higher volumes and decreased motility.
- When performing fertility testing, two or three samples are usually tested at 2-week intervals, with two abnormal samples considered significant.
- The specimen should be delivered to the laboratory within 1 hour of collection and the laboratory personnel must record the time of specimen collection and specimen receipt.
- The sample must be kept at 37°C until analysis, which begins ideally within 30 min, but absolutely within 60 min, of ejaculation

Methods of collection

1. **Masturbation** (the method of choice for all seminal fluid tests).
2. **By condom**: it is not recommended for fertility testing because the condoms may contain spermicidal agents.
3. **By coitus interrupts**: (withdrawal method).
4. **TESA**: Testicular sperm extraction (TESE)- Open Testicular Biopsy: is a highly invasive, open surgical procedure performed under general anaesthetic. The scrotum and testes are cut open, before testicular tissues are cut away and examined for sperm, which, if present can be extracted.



Label of sample

- Patient name
- Clinic number
- Date and time
- Laboratory request form

- ***The following should be recorded on the laboratory analysis form:***
  - The period of abstinence (in days).
  - If sample collection was complete or incomplete.
  - The time interval from collection to analysis

Macroscopic examination

- There are several macroscopic evaluations which give useful diagnostic information about the sample:

  - ✓ Appearance
  - ✓ Odour
  - ✓ Liquefaction
  - ✓ Volume
  - ✓ Viscosity
  - ✓ pH

Table 11–2 Normal Values for Semen Analysis[4]

Volume	2–5 mL
Viscosity	Pours in droplets
pH	7.2–8.0
Sperm concentration	>20 million/mL
Sperm count	>40 million/ejaculate
Motility	>50% within 1 hr
Quality	>2.0 or a, b, c in Table 11–3
Morphology	>14% normal forms (strict criteria) >30% normal forms (routine criteria)

Liquefaction

- A fresh semen specimen is clotted and should liquefy within 30 to 60 minutes after collection; therefore, recording the time of collection is essential for evaluation of semen liquefaction.

- Analysis of the specimen cannot begin until after liquefaction has occurred.

- If after 2 hours the specimen has not liquified proteolytic enzymes such as alpha-chymotrypsin may be added to allow the rest of the analysis to be performed.

Macroscopic examination

- ✓ ***Volume***: normal is (2-5 mL). Using disposable volumetric pipette .
- WHO criteria specify that any volume greater than 2.0 mL is normal. Low volume may indicate partial or complete blockage of the seminal vesicles, or that the man was born without seminal vesicles.

- ***Viscosity:*** Estimate the viscosity of the semen by aspirating the semen into the measuring pipette and allowing the semen to drop by gravity and will not appear clumped. Observe the length of the thread.

pH

- The normal pH of semen is alkaline with a range of 7.0 to 8.0.
- Increased pH is indicative of infection within the reproductive tract.
- A decreased pH is associated with increased prostatic fluid.

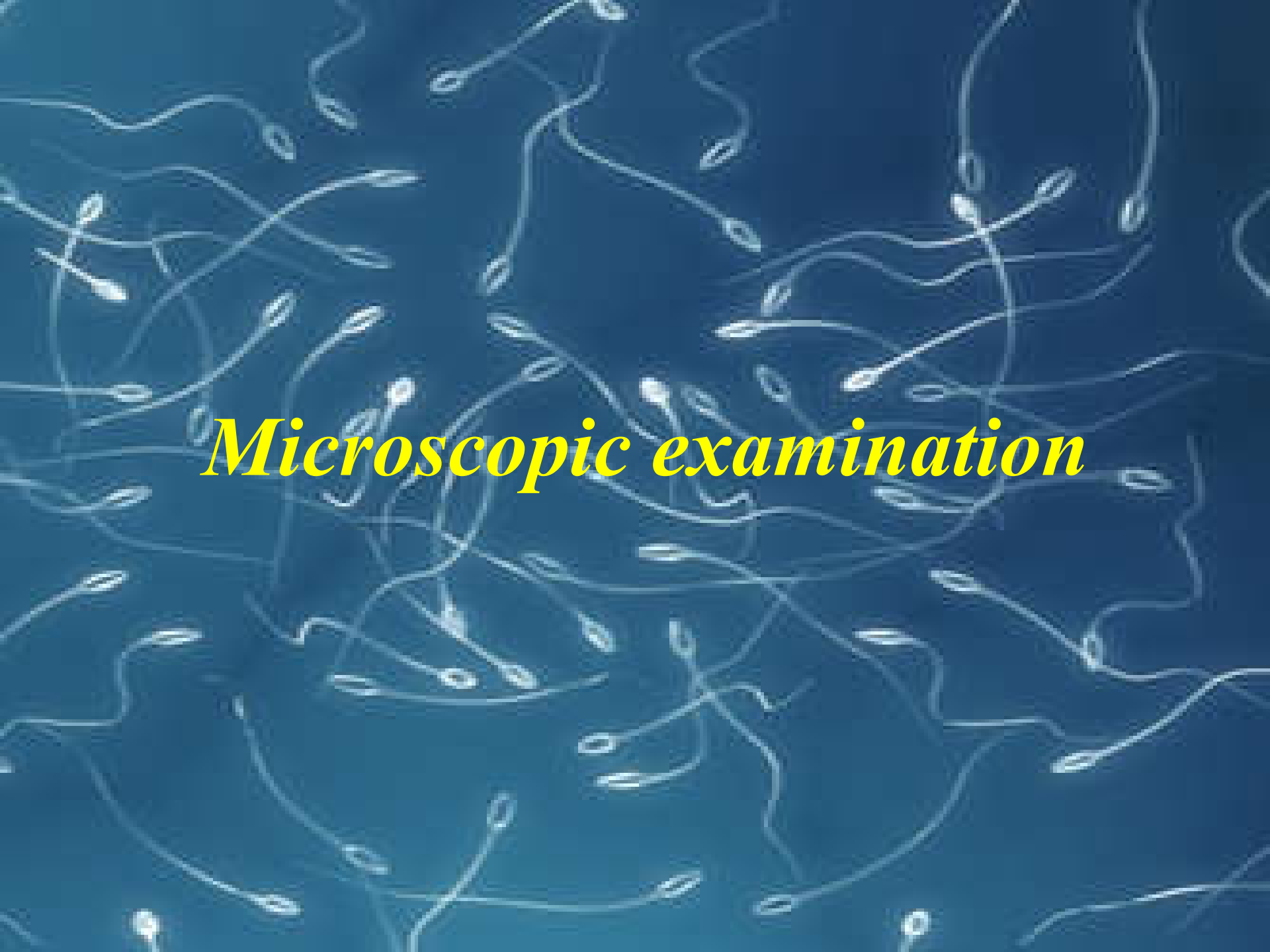
Microscopic examination

- ***The characteristics assessed are:***

  - ✓Motility.
  - ✓Sperm aggregation (random clumping): “some” is normal, but large clumps (each with hundreds of sperm) is abnormal.
  - ✓Spermagglutination (between specific sites): could suggest the presence of antisperm antibodies.
  - ✓Epithelial cells: usually present in small numbers
  - ✓Erythrocytes: should not be present
  - ✓Bacteria and protozoa: presence indicates infection

Normal Semen Analysis

Semen volume	2ml or more (usually 2-4 milliliters per ejaculation)
Semen pH	Semen pH of 7.2-8.0
Liquefaction time	20-30 minutes after collection
Sperm count	40 million spermatozoa per ejaculate or more
Sperm morphology	More than 30% of the sperm have normal shape and structure.
Sperm motility	More than 50% of the sperm show progressive movement or 25% or more with rapid progressive movement.
Vitality	75% or more live, i.e., excluding dye
White blood cells	Fewer than 1 million WBCs/ml


Microscopic examination

Microscopic examination

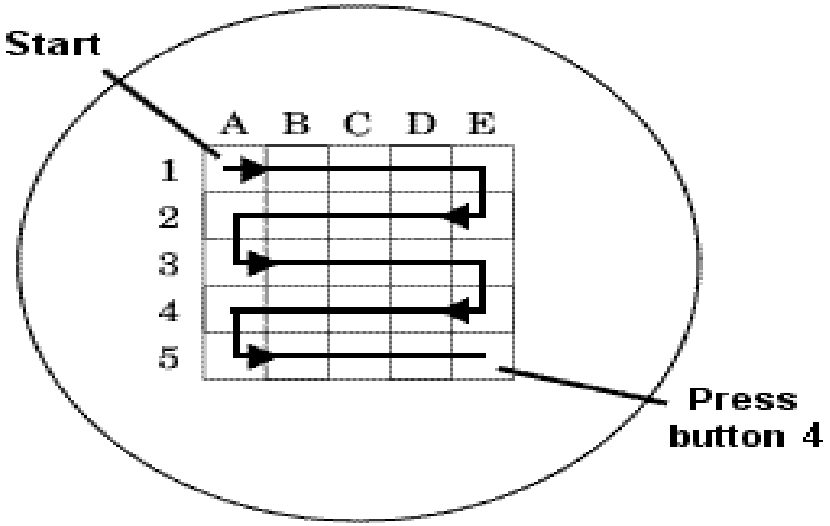
- Normal values for sperm concentration are commonly listed as greater than 20 million sperm per milliliter, with concentrations between 10 and 20 million per milliliter considered borderline.

- The total sperm count for the ejaculate can be calculated by multiplying the sperm concentration by the specimen volume.

- Total sperm counts greater than 40 million per ejaculate are considered normal (20 million per milliliter 2 mL).

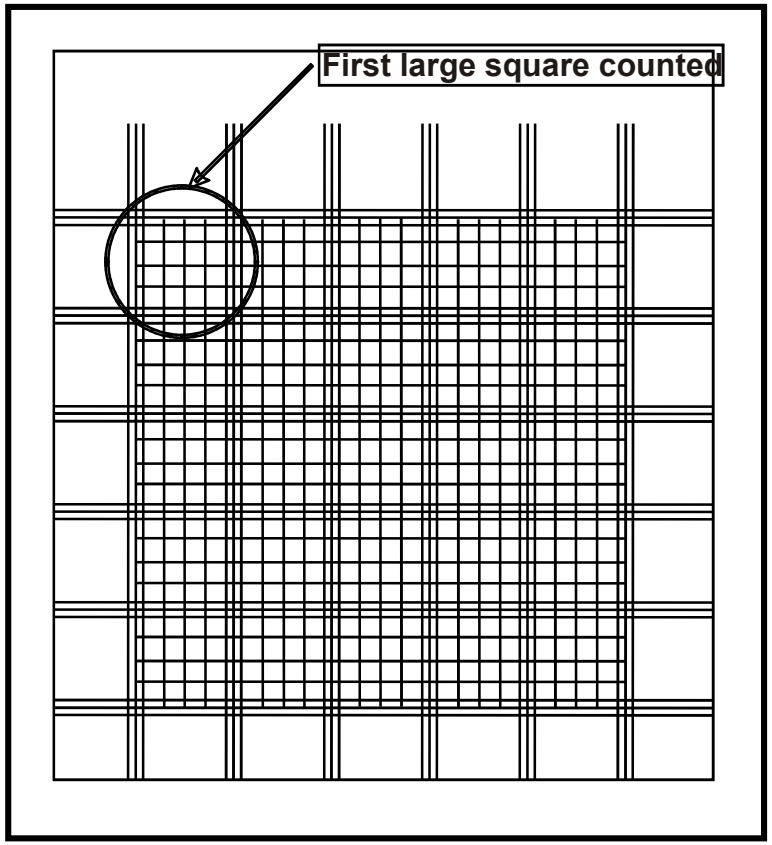
Methods of measuring sperm concentration

- ***Hemacytometer:*** Sperm can be counted by make dilution 1:20 in ***WBC pipette*** or by automatic pipette (which is more accurate) with a solution containing sodium bicarbonate (5g) and formalin (1ml) (immobilize & preserve the spermatozoa), tap water (100 ml) will suffice as a diluent.



Sperm concentration - haemocytometer

- The number of squares assessed depends on the number of sperm counted in the first large square:
  - If < 10 counted, the whole grid is assessed
  - If 10-40 counted, 10 squares are assessed
  - If > 40 counted, 5 squares are assessed



large central square.
This square is ruled into 25 small squares, each of which is further divided into 16

Sperm concentration - calculations

- If the counts of the two chambers are *not* within 5% of their average discard, remix the sample, and set it up again
- If the two counts are in agreement, then the sum of the two counts is divided by the correction factor:
  - If 2 × 25 squares counted, divide their sum by 10
  - If 2 × 10 squares counter, divide their sum by 4
  - If 2 × 5 squares counted, divide their sum by 2
- This gives the sperm concentration in millions per ml
- Sperm count = concentration × total volume

Calculations

- Using a 1:20 dilution and four large WBC's squares counted
- The sperm concentration/ml = No of sperms counted x 50,000
- Using a 1:20 dilution and five small RBC's squares counted
- The sperm concentration /ml = No of sperms counted x 1,000,000

Sperm concentration – interpretation

- The WHO Reference values for:

  Sperm concentration is $\geq 20 \times 10^6$ sperm/ml

- Counts of less than 20 million per milliliter (<20 million/ml) are considered sub-fertile

- If a man has a sperm concentration $< 5 \times 10^6$ sperm/ml, the WHO recommends assessment for numerical and structural abnormalities of sex chromosomes

Direct smear or Wet preparation

Place 10µl of thoroughly mixed, liquefied semen on a clean glass slide under a lightly applied glass cover slip will allow visualization of the sperm in a specimen of semen under HPF.

Motility

- The World Health Organization has a value of 50% and this must be measured within 60 minutes of collection.

- A man can have a total number of sperm far over the limit of 20 million sperm cells per milliliter, but still have bad quality because too few of them are motile.

- The other way around, a man can have a sperm count far less than 20 million sperm cells per millilitre and still have good motility, if more than 60% of those observed sperm cells show good forward movement.

Motility of sperm are divided into four different grades

- ***Grade 4:*** Sperm with progressive motility. These are the strongest and swim fast in a straight line. Sometimes it is also denoted motility a.

- ***Grade 3:*** (non-linear motility): These also move forward but tend to travel in a curved or crooked motion. Sometimes also denoted motility b.

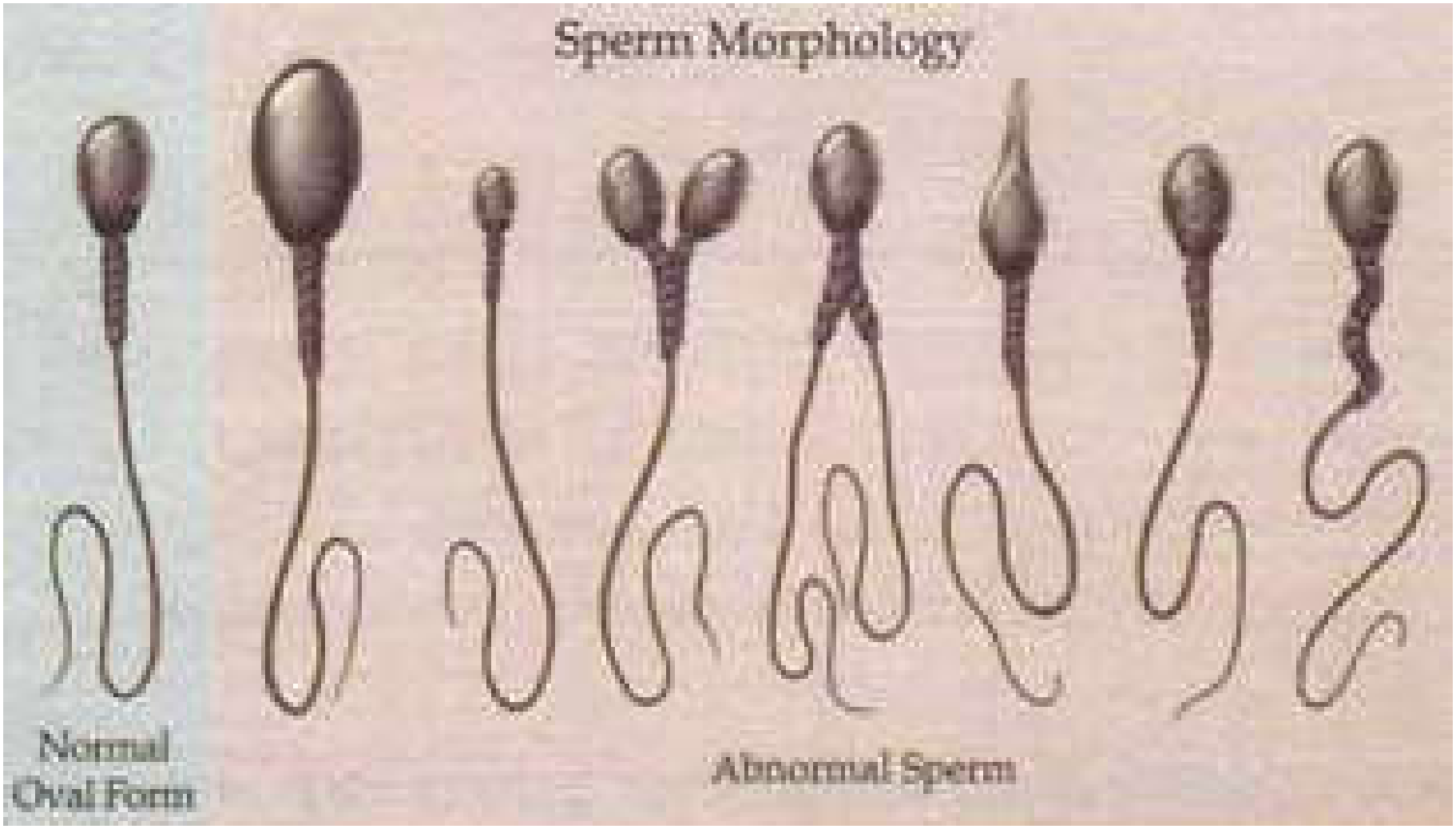
- ***Grade 2:*** These have non-progressive motility because they do not move forward despite the fact that they move their tails.

- ***Grade 1:*** These are immotile and fail to move at all.

Sperm morphology

- Morphology is even more important than motility and concentration
- Because of the small size of the human sperm head, must use an air-dried smear which has been stained
- Prepared samples are assessed using a 100× oil-immersion objective under bright field optics
- The WHO recommends that 200 spermatozoa are counted per sample
- Fields for counting must be selected at random
- When counting, remember about the normal distribution

Abnormal morphology



Normal

Double head

Giant head

Amorphous head

Pinhead

Tapered head

Constricted head

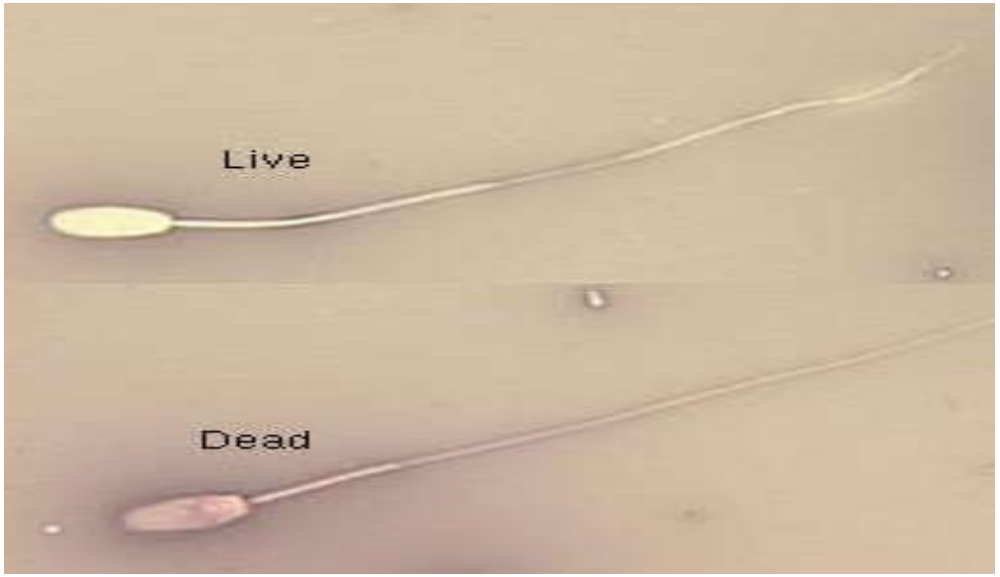
Double tail

Coiled tail

Spermatid

Sperm Vitality

- Usually performed using a vital stain, such as eosin Y, with a counterstain (nigrosin) to differentiate live (unstained) and dead (stained) spermatozoa.



Agglutination

- Agglutination of spermatozoa means that motile spermatozoa stick to each other, head to head, mid-piece to mid-piece, tail to tail, or mixed, e.g. mid-piece to tail.

- The adherence of either immotile or motile spermatozoa to mucus threads, to cells other than spermatozoa, or to debris is not considered agglutination and should not be recorded as such.

- The presence of agglutination is suggestive of an immunological factor of infertility.

Other cells in semen

- ***Leukocytes:*** normally (1-4/HPF), increase number (leukocytospermia) indicates reproductive tract infection
- ***Epithelial cells:*** normally (1-2/HPF)
- ***Spermatocytes:*** (Immature germ cells) 1-2/HPF.
- ***Erythrocytes:*** (1-2/HPF). Increased number may indicate a reproductive tract infection or damage to a small capillary during sample production.
- ***Note:*** bacteria and protozoan such as Trichomonas vaginalis are uncommon in human semen but their presence is indicative of possible male reproductive tract infection and should be reported to the referring doctor for further evaluation.

Semen biochemistry

- ***Acid phosphatase:*** marker for prostatic function
- ***Citric acid:*** can indicate prostatic function – low levels may indicate dysfunction or a prostatic duct obstruction
- ***Zinc:*** marker for prostatic function – *colorimetric assay (WHO)*
- ***Fructose:*** marker for seminal vesicle function, and is a substrate for sperm metabolism – *spectrophotometric assay (WHO)*

Terms

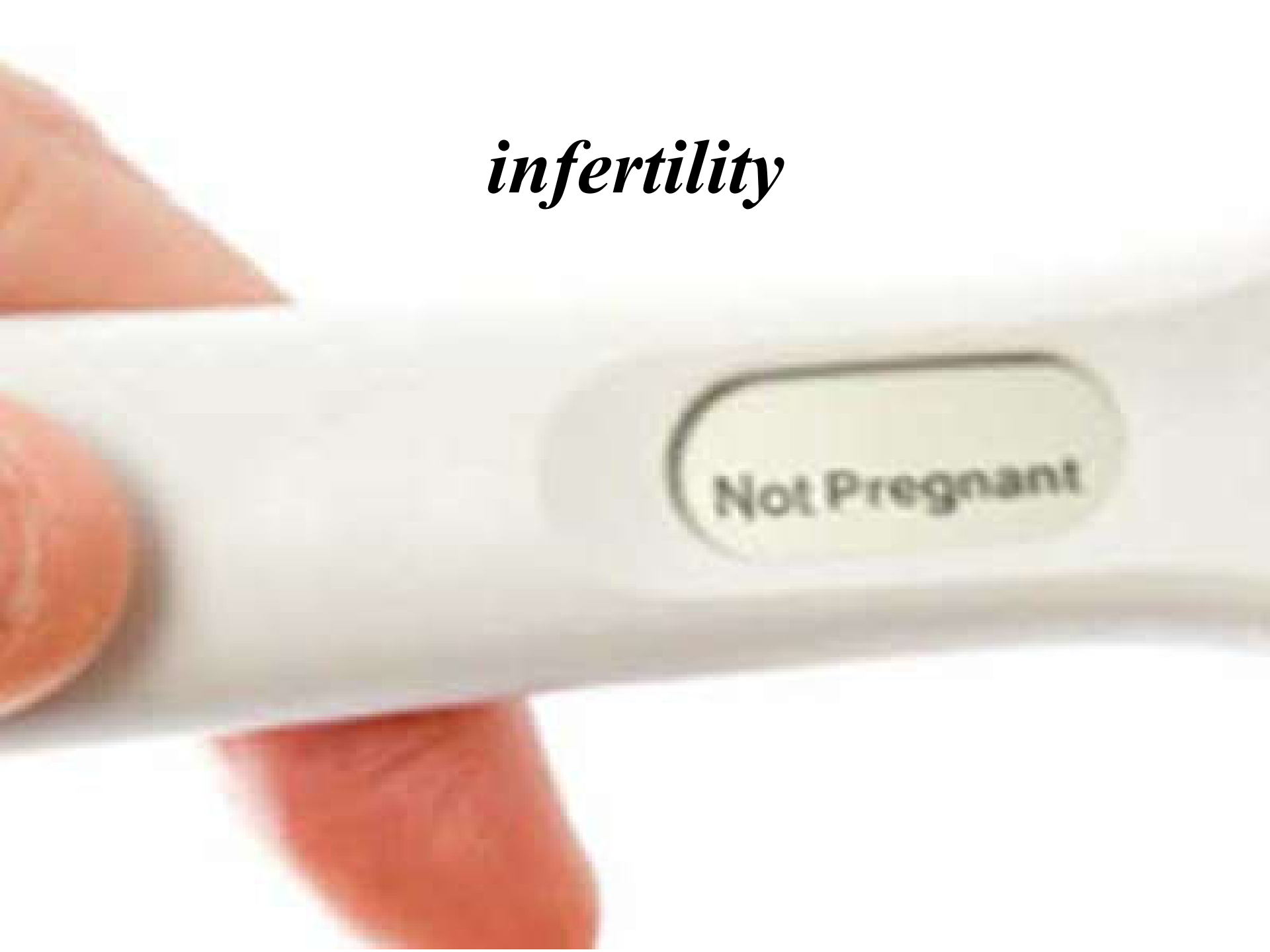
- ***Aspermia:*** absence of semen
- ***Azoospermia:*** describe a total absence of spermatozoa in semen. (After centrifuge sperm count is zero/HPF).
- ***Oligozoospermia:*** refers to a reduced number of spermatozoa in semen and is usually used to describe a sperm concentration of less than 20 million/ml. Sperm count 5-10 sperm/HPF.
- ***Severe oligospermia:*** sperm count 1-2 sperm/HPF.
- ***Polyzoospermia:*** denotes an increased number of spermatozoa in semen and is usually refers to a sperm concentration in excess of 350 million/ml.

- **Asthenozoospermia:** refers to a man who produces a greater proportion of sperm which are immotile or have reduced motility, compared to the WHO reference values.
- **Teratozoospermia:** sperm carry more morphological defects than usual

TZI

- The ***Teratozoospermic Index*** is an expression of the average number of abnormalities per abnormal sperm
- Each sperm cell is assessed for an abnormality in the head, neck/mid-piece, or tail, and for a cytoplasmic droplet
- If it does not have any of these abnormalities, it is “normal”
- If it does have an abnormality, it is “abnormal”, and we score each abnormality. So, if a cell has an abnormal head and tail, it is counted as 1 cell, and 2 abnormalities
- Then, (total # abnormalities) / (total # sperm) = TZI
- A TZI > 1.80 has been associated with poor sperm fertilizing ability

infertility



What is infertility?

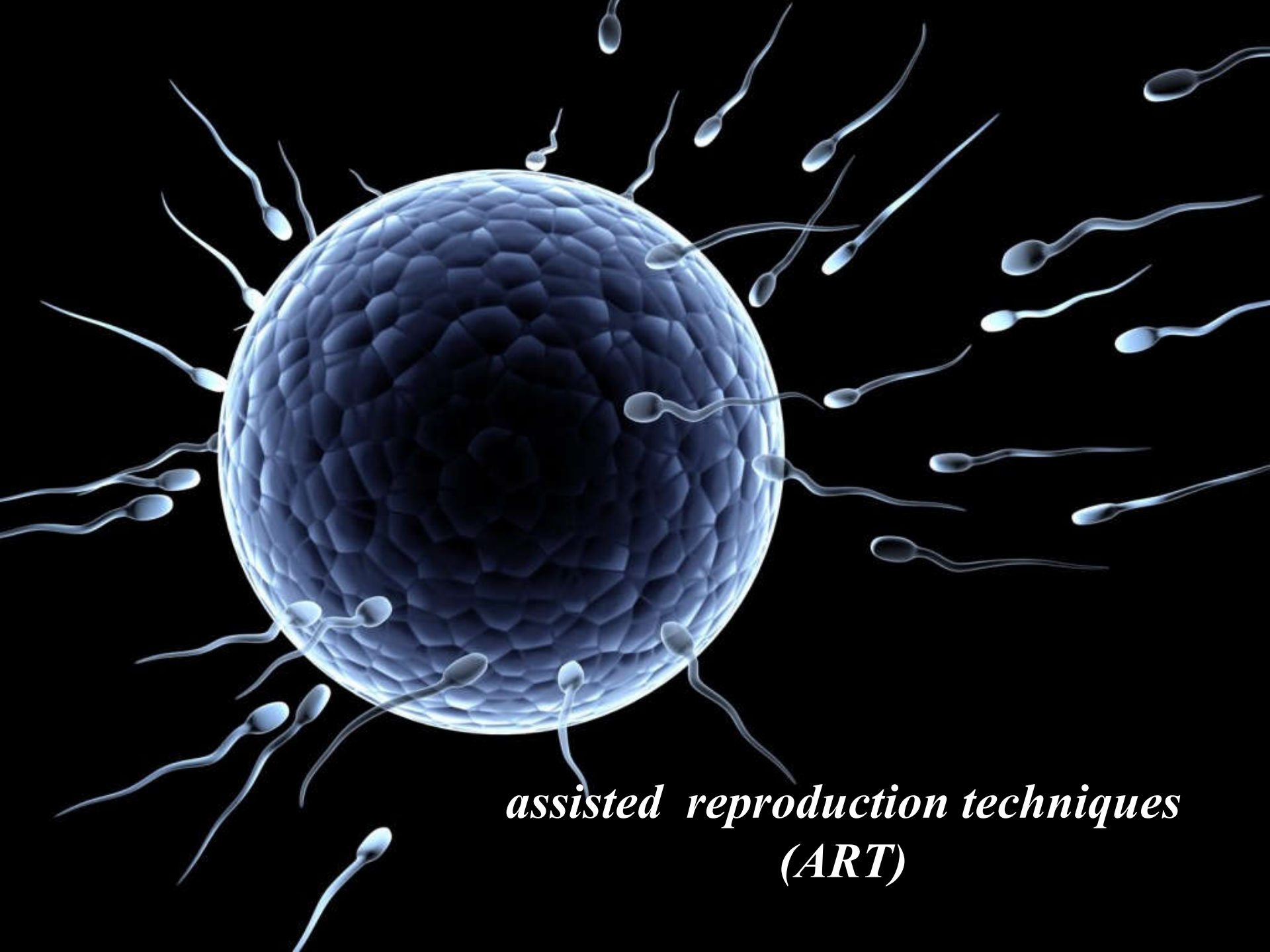
- Infertility means not being able to get pregnant after one year of trying. Or, women who can get pregnant but are unable to stay pregnant may also be infertile.
- **Pregnancy:** is the result of a process that has many steps. To get pregnant:
  - ✓ A woman must release an egg from one of her ovaries (ovulation).
  - ✓ The egg must go through Fallopian tube toward the uterus (womb).
  - ✓ A man's sperm must join with (fertilize) the egg along the way.
  - ✓ The fertilized egg must attach to the inside of the uterus (implantation).
  - ✓ Infertility can happen if there are problems with any of these steps.

What increases a man's risk of infertility?

- **High temperature of testicles (testes)**. Sperm are made in the testes which are in the scrotum. This is the body's way of keeping the testes slightly cooler than the rest of the body, which is best for making sperm.
- **Smoking can affect the sperm count**. If you smoke, you should stop completely for optimum sperm production.
- **Alcohol:** equivalent eight pints of normal strength beer or sixteen small glasses of wine, may interfere with optimum fertility**.**
- **Medicines and drugs**. Most do not interfere with sperm production but some may do. These include: tetracyclines, , colchicine,allopurinol.

What things increase a woman's risk of infertility?

- ✓ Age
- ✓ Stress
- ✓ Poor diet
- ✓ Being overweight or underweight
- ✓ Smoking
- ✓ Excess alcohol use
- ✓ Sexually transmitted infections (STIs)
- ✓ Health problems that cause hormonal changes, such as polycystic ovarian syndrome and primary ovarian insufficiency



assisted reproduction techniques (ART)

- ART includes all fertility treatments in which both eggs and sperm are handled. In general, ART procedures involve surgically removing eggs from a woman's ovaries, combining them with sperm in the laboratory, and returning them to the woman's body. or procedures in which a woman takes medicine only to stimulate egg production without the intention of having eggs retrieved.

- ***The different types of assisted reproductive technology (ART)?***

1. ***In vitro fertilization*** **(IVF)** means fertilization outside of the body. IVF is the most effective ART. It is often used when a woman's Fallopian tubes are blocked or when a man produces too few sperm.

2. ***Zygote intrafallopian transfer*** **(ZIFT):** is similar to IVF. Fertilization occurs in the laboratory. Then the very young embryo is transferred to the Fallopian tube instead of the uterus.
3. ***Gamete intrafallopian transfer*** **(GIFT)** involves transferring eggs and sperm into the woman's Fallopian tube. So fertilization occurs in the woman's body.
4. ***Intracytoplasmic sperm injection (ICSI)*** is often used for couples in which there are serious problems with the sperm. Sometimes it is also used for older couples or for those with failed IVF attempts. In ICSI, a single sperm is injected into a mature egg. Then the embryo is transferred to the uterus or Fallopian tube

Sperm preparation

- **Discontinuous density gradient:** is a technique in which all sperm in the seminal fluid are separated by centrifugation.
- **Swim up:** is a technique in which the most motile sperm are selected from the ejaculate. Fresh semen is covered with a medium and placed in a cylinder which is laid at a 45^{o}C angle. Motile sperm in the ejaculate will then swim up to the top of the tube, leaving immotile sperm and debris in the lower part of the cylinder.

UNIT - V

GENERAL PARASITOLOGY

LEARNING OBJECTIVES

At the end of this section the student is expected to:

• Discuss the various types of parasites and hosts.

• Explain the relationship between a parasite and the host and their effects.

• Discuss in detail the classification of medically important parasites.

• Explain the difference between the Cestodes, Nematodes, Trematodes and protozoa

INTRODUCTION

Man and other living things on earth live in an entangling relationship with each other. They don't exist in an isolated fashion. They are interdependent; each forms a strand in the web of life. Medical parasitology is the science that deals with organisms living in the human body (the host) and the medical significance of this host-parasite relationship.

EFFECT OF PARASITES ON THE HOST

The damage which pathogenic parasites produce in the tissues of the host may be described in the following two ways;

(a) Direct effects of the parasite on the host

• Mechanical injury - may be inflicted by a parasite by means of pressure as it grows larger, e.g. Hydatid cyst causes blockage of ducts such as blood vessels producing infraction.

• Deleterious effect of toxic substances- in Plasmodium falciparum production of toxic substances may cause rigors and other symptoms.

• Deprivation of nutrients, fluids and metabolites -parasite may produce disease by competing with the host for nutrients.

(b) Indirect effects of the parasite on the host:

Immunological reaction: Tissue damage may be caused by immunological response of the host, e.g. nephritic syndrome following Plasmodium infections. Excessive proliferation of certain tissues due to invasion by some parasites can also cause tissue damage in man, e.g. fibrosis of liver after deposition of the ova of Schistosoma.

BASIC CONCEPTS IN MEDICAL PARASITOLOGY

In medical parasitology, each of the medically important parasites are discussed under the standard subheadings of morphology, geographical distribution, means of infection, life cycle, host/parasite relationship, pathology and clinical manifestations of infection, laboratory diagnosis, treatment and preventive/control measures of parasites. In the subsequent section some of these criteria are briefly presented.

Morphology - includes size, shape, color and position of different organelles in different parasites at various stages of their development. This is especially important in laboratory diagnosis which helps to identify the different stages of development and differentiate between pathogenic and commensal organisms. For example,
Entamoeba histolytica and Entamoeba coli.

Geographical distribution - Even though revolutionary advances in transportation has made geographical isolation no longer a protection against many of the parasitic diseases, many of them are still found in abundance in the tropics. Distribution of parasites depends upon:

a. The presence and food habits of a suitable host:

• Host specificity, for example, Ancylostoma duodenale requires man as a host where Ancylostoma caninum requires a dog.

• Food habits, e.g. consumption of raw or undercooked meat or vegetables predisposes to Taeniasis

b. Easy escape of the parasite from the host- the different developmental stages of a parasite which are released from the body along with faeces and urine are widely distributed in many parts of the world as compared to those parasites which require a vector or direct body fluid contact for transmission.

c. Environmental conditions favoring survival outside the body of the host, i.e. temperature, the presence of water, humidity etc.

d. The presence of an appropriate vector or intermediate host – parasites that do not require an intermediate host (vector) for transmission are more widely distributed than those that do require vectors.

Once we are clear about the geographical distribution and conditions favoring survival in relation to different parasites, effective preventive and control measures can more easily be devised and implemented.

Life cycle of parasites - the route followed by a parasite from the time of entry to the host to exit, including the extracorporeal (outside the host) life. It can either be simple, when only one host is involved, or complex, involving one or more intermediate hosts. A parasite's life cycle consists of two common phases one phase involves the route aparasite follows inside the body. This information provides an understanding of the symptomatology and pathology of the parasite. In addition the method of diagnosis and selection of appropriate medication may also be determined. The other phase, the route a parasite follows outside of the body, provides crucial information pertinent to epidemiology, prevention, and control.

Host parasite relationship - infection is the result of entry and development within the body of any injurious organism regardless of its size. Once the infecting organism is introduced into the body of the host, it reacts in different ways and this could result in:

a. Carrier state - a perfect host parasite relationship where tissue destruction by a parasite is balanced with the host's tissue repair. At this point the parasite and the host live harmoniously, i.e. they are at equilibrium.

b. Disease state - this is due to an imperfect host parasite relationship where the parasite dominates the upper hand. It can result either from lower resistance of the host or a higher pathogenecity of the parasite.

c. Parasite destruction – occurs when the host takes the upper hand.

Laboratory diagnosis – depending on the nature of the parasitic infections, the following specimens are selected for laboratory diagnosis:

a) Blood – in those parasitic infections where the parasite itself in any stage of its development circulates in the blood stream, examination of blood film forms one of the main procedures for specific diagnosis. For example, in malaria the parasites are found inside the red blood cells. In Bancroftian and Malayan filariasis, microfilariae are found in the blood plasma.

b) Stool – examination of the stool forms an important part in the diagnosis of intestinal parasitic infections and also for those helminthic parasites that localize in the biliary tract and discharge their eggs into the intestine. In protozoan infections, either trophozoites or cystic forms may be detected; the former during the active phase and the latter during the chronic phase. Example, Amoebiasis, Giardiasis, etc. In the case of helmithic infections, the adult worms, their eggs, or larvae are found in the stool.

c) Urine – when the parasite localizes in the urinary tract, examination of the urine will be of help in establishing the parasitological diagnosis. For example in urinary Schistosomiasis, eggs of Schistosoma haematobium are found in the urine. In cases of chyluria caused by Wuchereria bancrofti, microfilariae are found in the urine.

d) Sputum – examination of the sputum is useful in the following: • In cases where the habitat of the parasite is in the respiratory tract, as in Paragonimiasis, the eggs of Paragonimus westermani are found. In amoebic abscess of lung or in the case of amoebic liver abscess bursting into the lungs, the trophozoites of E. histolytica are detected in the sputum.

e) Biopsy material - varies with different parasitic infections. For example spleen punctures in cases of kala-azar, muscle biopsy in cases of Cysticercosis, Trichinelliasis, and Chagas' disease, Skin snip for Onchocerciasis.

f) Urethral or vaginal discharge – for Trichomonas vaginalis Indirect evidences – changes indicative of intestinal parasitic infections are:

a. Cytological changes in the blood – eosiniphilia often gives an indication

of tissue invasion by helminthes, a reduction in white blood cell count is an indication of kala-azar, and anemia is a feature of hookworm infestation and malaria.

b. Serological tests – are carried out only in laboratories where special antigens are available.

Treatment – many parasitic infections can be cured by specific chemotherapy. The greatest advances have been made in the treatment of protozoal diseases. For the treatment of intestinal helminthiasis, drugs are given orally for direct action on the helminthes. To obtain maximum parasiticidal effect, it is desirable that the drugs administered should not be absorbed and the drugs should also have minimum toxiceffect on the host.

Prevention and control - measures may be taken against every parasite infectiving humans. Preventive measures designed to break the transmission cycle are crucial to successful parasitic eradication. Such measures include:

Reduction of the source of infection- the parasite is attacked within the host, thereby preventing the dissemination of the infecting agent. Therefore, a prompt diagnosis and treatment of parasitic diseases is an important component in the prevention of dissemination.

Sanitary control of drinking water and food.

Proper waste disposal – through establishing safe sewage systems, use of screened latrines, and treatment of night soil.

The use of insecticides and other chemicals used to control the vector population.

Protective clothing that would prevent vectors from resting in the surface of the body and inoculate pathogens during their blood meal.

Good personal hygiene.

Avoidance of unprotected sexual practices.

CLASSIFICATION OF MEDICAL PARASITOLOGY

Parasites of medical importance come under the kingdom called protista and animalia. Protista includes the microscopic single-celled eukaroytes known as protozoa. In contrast, helminthes are macroscopic, multicellular worms possessing welldifferentiated tissues and complex organs belonging to the kingdom animalia. Medical Parasitology is generally classified into:

• **Medical Protozoology** - Deals with the study of medically important protozoa.

• **Medical Helminthology** - Deals with the study of helminthes (worms) that affect man.

• **Medical Entomology** - Deals with the study of arthropods which cause or transmit disease to man.

GENERAL CHARACTERISTICS OF MEDICALLY IMPORTANT PARASITES

Medically important protozoa, helminthes, and arthropods, which are identified as causes and propagators of disease have the following general features. These features also differ among parasites in a specific category.

(1) PROTOZOA

Protozoan parasites consist of a single "cell-like unit" which is morphologically and functionally complete and can perform all functions of life. They are made up of a mass of protoplasm differentiated into cytoplasm and nucleoplasm. The cytoplasm consists of an outer layer of hyaline ectoplasm and an inner voluminous granular endoplasm. The ectoplasm functions in protection, locomotion, and ingestion of food, excretion, and respiration. In the cytoplasm there are different vacuoles responsible for storage of food, digestion and excretion of waste products. The nucleus also functions in reproduction and maintaining life.

The protozoal parasite possesses the property of being transformed from an active (trophozoite) to an inactive stage, losing its power of motility and enclosing itself within a tough wall. The protoplasmic body thus formed is known as a cyst. At this stage the parasite loses its power to grow and multiply. The cyst is the resistant stage of the parasite and is also infective to the human host.

Reproduction – the methods of reproduction or multiplication among the parasitic protozoa are of the following types:

1. Asexual multiplication:

(a) Simple binary fission – in this process, after division of all the structures, the individual parasite divides either longitudinally or transversely into two more or less equal parts.

(b) Multiple fission or schizogony – in this process more than two individuals are produced, e.g. asexual reproduction in Plasmodia.

2. Sexual reproduction:

(a) Conjugation – in this process, a temporary union of two individuals occurs during which time interchange of nuclear material takes place. Later on, the two individuals separate.

(b) Syngamy – in this process, sexually differentiated cells, called gametes, unite permanently and a complete fusion of the nuclear material takes place. The resulting product is then known as a zygote.

(2) HELIMINTHS:

The heliminthic parasites are multicellular, bilaterally symmetrical animals having three germ layers. The helminthes of importance to human beings are divided into three main groups with the peculiarities of the different categories described in table

(3) ARTHROPODS

Arthropods, which form the largest group of species in the animal kingdom, are characterized by having a bilaterally symmetrical and segmented body with jointed appendages. They have a hard exoskeleton, which helps enclose and protect the muscles and other organs. An open circulatory system, with or without a dorsally situated heart pumps the blood (hemolymph) via arteries to the various organs and body tissues. Blood is returned to the heart through body spaces known as hemocoeles. In addition, respiratory, excretory, and nervous systems are present.

Arthropods affect the health of humans by being either direct agents for disease or agents for disease transmission.

The arthropods of medical importance are found in Classes Insecta, Arachnida, and Crustacia which have their own distinguishing features. In Class insecta the body is divided into head, thorax, and abdomen, with one pair of antennae. Diseases like malaria, yellow fever, onchocerciasis, and trypanasomiasis are primarily transmitted by insects.

Plasmodium malariae

In contrast with P.vivax and P.ovale, P.malariae can infect only mature erythrocytes with relatively rigid cell membranes. As a result, the parasite's growth must conform to the size and shape of red blood cell.

This requirement produces no red cell enlargement or distortion, but it results in distinctive shapes of the parasite seen in the host cell, "band and bar forms" as well as very compact dark staining forms. The schizont of P.malariae is usually composed of eight merozoites appearing in a rosette.

Entamoeba histolytica

INTRODUCTION

Amoebas primitive unicellular microorganisms with a relatively simple life cycle which can be divided into two stages:

• Trophozoite – actively motile feeding stage.

• Cyst – quiescent, resistant, infective stage.

Their reproduction is through binary fission, e.g. splitting of the trophozoite or through the development of numerous trophozoites with in the mature multinucleated cyst. Motility is accomplished by extension of pseudopodia ("false foot")

Morphological features

(a) Trophozoites

Viable trophozoites vary in size from about 10-60μm in diameter. Motility is rapid, progressive, and unidirectional, through pseudopods. The nucleus is characterized by evenly arranged

chromatin on the nuclear membrane and the presence of a small, compact, centrally located karyosome. The cytoplasm is usually described as finely granular with few ingested bacteria or debris in vacuoles. In the case of dysentery, however, RBCs may be visible in the cytoplasm, and this feature is diagnostic for E.histolytica.

(b) Cyst

Cysts range in size from 10-20μm. The immature cyst has inclusions namely; glycogen mass and chromatoidal bars. As the cyst matures, the glycogen completely disappears; the chromotiodials may also be absent in the mature cyst.

Life cycle

Intestinal infections occur through the ingestion of a mature quadrinucleate infective cyst, contaminated food or drink and also by hand to mouth contact. It is then passed unaltered through the stomach, as the cyst wall is resistant to gastric juice. In terminal ileum (with alkaline pH), excystation takes place. Trophozoites being actively motile invade the tissues and ultimately lodge in the submucous layer of the large bowel. Here they grow and multiply by binary fission.

Trophozoites are responsible for producing lesions in amoebiasis.

Invasion of blood vessels leads to secondary extra intestinal lesions.

Gradually the effect of the parasite on the host is toned down together with concomitant increase in host tolerance, making it difficult for the parasite to continue its life cycle in the trophozoite phase.

A certain number of trophozoites come from tissues into lumen of bowel and are first transformed into pre-cyst forms.

Pre-cysts secret a cyst wall and become a uninucleate cyst. Eventually, mature quadrinucleate cysts form. These are the infective forms.

Both mature and immature cysts may be passed in faeces. Immature cysts can mature in external environments and become infective.

ASCARIS LUMBRICOIDES

These are common roundworms infecting more than 700 million people worldwide.

Morphology:

Male adult worm measures 15-20 cm in length. The posterior end is curved ventrally. The female worm measures 20-40 cm in length. Its posterior end is straight.

Infective stage and modes of infection:

The egg containing larva when ingested with contaminated raw vegetables causes ascariasis.

Life cycle:

Ingested eggs hatch in the duodenum. The larvae penetrate the intestinal wall and circulate in the blood. From the heart they migrate to the lungs, ascend to the trachea, descend to the esophagus and finally reach the small intestine to become adult. The female pass immature eggs which pass to the soil and mature in 2 weeks.

TAENIA SOLIUM (PORK TAPEWORM)

The adult worms of T. solium reside or inhabit the upper jejunum. Infection has worldwide distribution.

Morphology:

Adult worm measures about 3 meters in length. The globular scolex has rostellum with 2 rows of hooklets. There are <1000 proglottids. Gravid proglottid liberates about 30,000-50,000 eggs.

Life cycle

Embryonated eggs passed with stool are ingested by pig and the embryo is released. It penetrates the intestinal wall and is carried by vascular channels to all parts of the body. After a period of 2-3 months of development the encysted larval stage called cysticerci or bladder worm occurs in

the striated muscles of the tongue, neck, trunk brain, eye, and the nervous system. The cysticercus survives for 5 years. Humans become infected by eating pork containing larvae, cysticercus cellulosae. When improperly cooked cysticercus infected meat is eaten by man, the scolex remains undigested and attaches itself to the intestinal wall and chain of proglottids begin to grow to adult worm.