“Apparatus & equipments used in microbiological laboratories”

**Benzen burner**: used to sterilize the loop for preparation of bacterial smear, also for fix bacterial smear on slide & other uses.

**Loop**: It is made of platinum wire with circle end, it is used to inoculate bacterial culture from one plate to another & to prepare bacterial smears & other uses.

**Slide**: A small rectangle glass piece, used in preparation of smears & doing different investigation in the labolatory microscopic examination.

**Cover slide**: A small delicate (thin) plastic or glass piece used to cover smears (e.g. blood, bacteria, urine etc.) to prevent contamination of microscope & in preparation of permanent microorganisms smears.

**Petri-dish**: A glass or plastic dish with cover, used for growing of bacteria after pouring the media in it.

**Swab**: It is a wooden applicator ended, with a small piece of cotton at one end, it is placed in a test tube & plugged with another piece of cotton, then sterilized by oven. Swabs are used to take different inoculums from patients (e.g. throat swab, eye swab).

**Incubator**: To obtain a proper atmospheric conditions (heat & air) for growing of bacteria, mostly 37°C used for growing bacteria within 18-24 hrs.

**Oven**: Used for sterilization by dry heat, such sterilization requires higher degree of temperature & longer time than autoclaving. A good standard sterilizing is by using 160 – 180°C for 1 - 1/2 hrs. It is important to leave adequate spaces between the shelves to allow for free circulation of air. All glass wares which used in lab are sterilizing by oven, swabs are also sterilized by oven.

**Autoclave**: Depend on moist heat sterilization by using steam under pressure inside the autoclave. The standard autoclaving procedure requires apressure of 15 pound / inch square at a temperature of 121°C for 20 min. Most of the routine media & also solutions which used in lab. are sterilizing by autoclave, Stock cultures of bacteria media are also may be sterilized by antoclaving to destroy & kill all the microorganisms in the culture.

**Centrifuge**: used to separate & precipitate solution samples (e.g. blood, urine, etc) depending on certain round & time e.g 3000 rpm (round / minute).

**Refrigerator**: used to keep chemicals, sterile media, and samples in at 2-4°C.

**Balance**: used for weigh different chemical powders & substances in lab.
"Culture media"

**Media**: is the substances that used to support the growth of microorganism or other cell. On culture media we can study:

- the characters of colony.
- the biochemical reactions of bacteria.

**Agar**: it is a substance obtained from some sea plants (Algi), and it contains along chains of polysaccharides, small amount of protein and inorganic salt. It is used only to solidifying the media. Agar is Melting at 95 – 98 c and solidifying at 35 – 40 c.

**Classification of media according to the state (solidity)**:

1. **Solid media**: the concentration of agar 1.5 - 2 %. The advantages:
   - characters of colony can be studied
   - mixture of bacteria can be isolated
   e.g: 1. Nutrient agar 2. Maconky agar 3. Blood agar

2. **Semi-solid media**: it is between solid media and liquid media, the concentration of agar 0.2 - 0.5 % it is used for motility of bacteria.
   e.g: Gelatin media

3. **Liquid media**: no agar. The advantage is: the bacteria grow faster due to the free medium.
   e.g: 1. Pepton water 2. Nutrient broth 3. Glucose broth

**Types of media according to the function**:

1. **Basal (simple)media**: it is the media which contain most of the nutrient that required for growth of bacteria. e.g Nutrient agar e.g Nutrient broth

2. **Enriched media**: many substances such as (blood, serum) is added to the basal media for fulfillment the growth of some microorganisms, this is known as enrich media. e.g blood agar.

3. **Selective media**: it contain some chemical substances which inhibits the growth of most microorganisms other than the selective one. e.g Lowenstein Jensens media (malachitgreen inhibits the growth of bacteria other than the Mycobacterium).

4. **Differential media**: the media which containing substance or indicator which will differentiate some spp. of microorganisms from another spp. e.g maccokey agar in which the lactose fermenter spp. show as red colonies while non lactose fermenter spp. as pale co
"preparation of smear"

A- From liquid media:
   1- prepare a clean slide.
   2- sterelize the loop and lake a loopful from the liquid media.
   3- spread it by the loop to make a thin film in the centre of the slide.
   4- leave the film to dry in the air (do not using heat).
   5- fix the smear by passing the slide rapidly many times and highly over the
      flame (this will killed bacteria and fix them).
   6- stain the film by according to the stain method (if you want to stain by
      simple method ,cover the film with methylene blue – keep for 2-3 min).
   7- wash the slide, dry the film, then examine by microscope under low power,
      then high power, and finally under oil-immersion objective lens.

B- From solid meadia:
   1- prepare a clean slide.
   2- sterelize the loop and add a loopful of D.W in the center of the slide.
   3- sterelize the loop and take bacteria from one colony on the culture.
   4- emulsify the bacteria by mixing with the water on the slide until getting
      a thin haemogenous film.
   5- dry, and fix the film.
   6- stain the film.
   7- dry, then examine the film by microscope.

“Stains and staining methods”

Bacteria are so transparent when they are examined in the living condition,
therefor it is necessary to stain them with dyes to make it visible in order to
identify and classify them.

staining methods of bacteria are divided into two groups:

A- Posative stains:
   1- Simple stain methods: are the techniques in which we used only one
      Stain or (dye), the resulting smear will stained uniform for all spp. with the
      same color. e.g. methylen blue, safranine, crystal violet.

   2- Differential stain methods: are the techniques in which more than one
      stain are used, then we can differentiate the groups of bacteria.
      e.g: Grams stain method, and Ziehl-Neelsen stain method (acid fast stain).

Gram's stain technique: is differentiate bacteria into two groups:
   (Gr +ve) Gram positive bacterial → appear violet color.
   (Gr -ve) Gram negative bacteria → appear red or pink color.
Stains and chemical solutions which used in Gram stain technique:

a - Basic stain → Crystal violet → 1 min
b - Iodine solution → 1 min
c - Decolorizer solution → 95 % Ethanol
d - Counter stain → Safranine → 1/2 min

Steps of staining (Gram’s stain):
1. Prepare a smear, dry, heat to fix.
2. Add crystal violet (Basic stain) on the film and leave it for 1 min.
3. Wash with tap water, then add iodine and leave for 1 min.
4. Add drops of ethanol (decolorizer).
5. Wash with tap water, then add (counter stain) safranin for 1/2 min.
6. Wash the film, dry, and examine under the microscope.

The result is: 
G +ve appear violet color.
G -ve appear red color.

Ziehl-Neelsen stain (Acid fast stain) technique:

It is differentiate bacteria into two groups:
Acid fast bacteria (Mycobacterium) → appear red or pink color.
Non acid fast bacteria → appear blue color.

Acid fast bacteria e.g. (Mycobacterium tuberculosis) are very difficult to be stained by ordinary stains because of their waxy cell wall, therefore they require a strong basic stain (Carbol fuchsin) which resists the decolorization by strong acid solution, so they called Acid fast bacilli.

Stains and chemical solutions which used in Ziehl-Neelsen stain technique:

a - Basic stain → strong carbol fuchsin.
b - Decolorizer solution → Acid alcohol.
c - Counter stain → Methylene blue.

Steps of staining Acid fast stain or (Ziehl-Neelsen stain):
1. Prepare amear and fix by heat.
2. Cover the smear with carbol fuchsin stain and heat until the vapour is appear from the stain, continue for 5 min. (do not allow stain to dry by adding additional stain continuously).
3. Wash with tap water.
4. Decolorize with acid alcohol or 20% H2SO4 till pink colour is disappear.
5. Cover the film with methylene blue stain for 7 min, wash with tap water, dry and examine under oil immersion.

The result is: Tuberculosis bacilli or acid fast bacilli → Red color
Non acid fast bacilli (all other bacteria) → blue color.

3- Special stain methods: These techniques are used for staining one of the structures of bacteria e.g.

a - Capsule stain
b - Spores stain
c - Flagella stain

B - Negative stain: Which stain the background, while leave the bacteria unstained e.g. Negrosin.