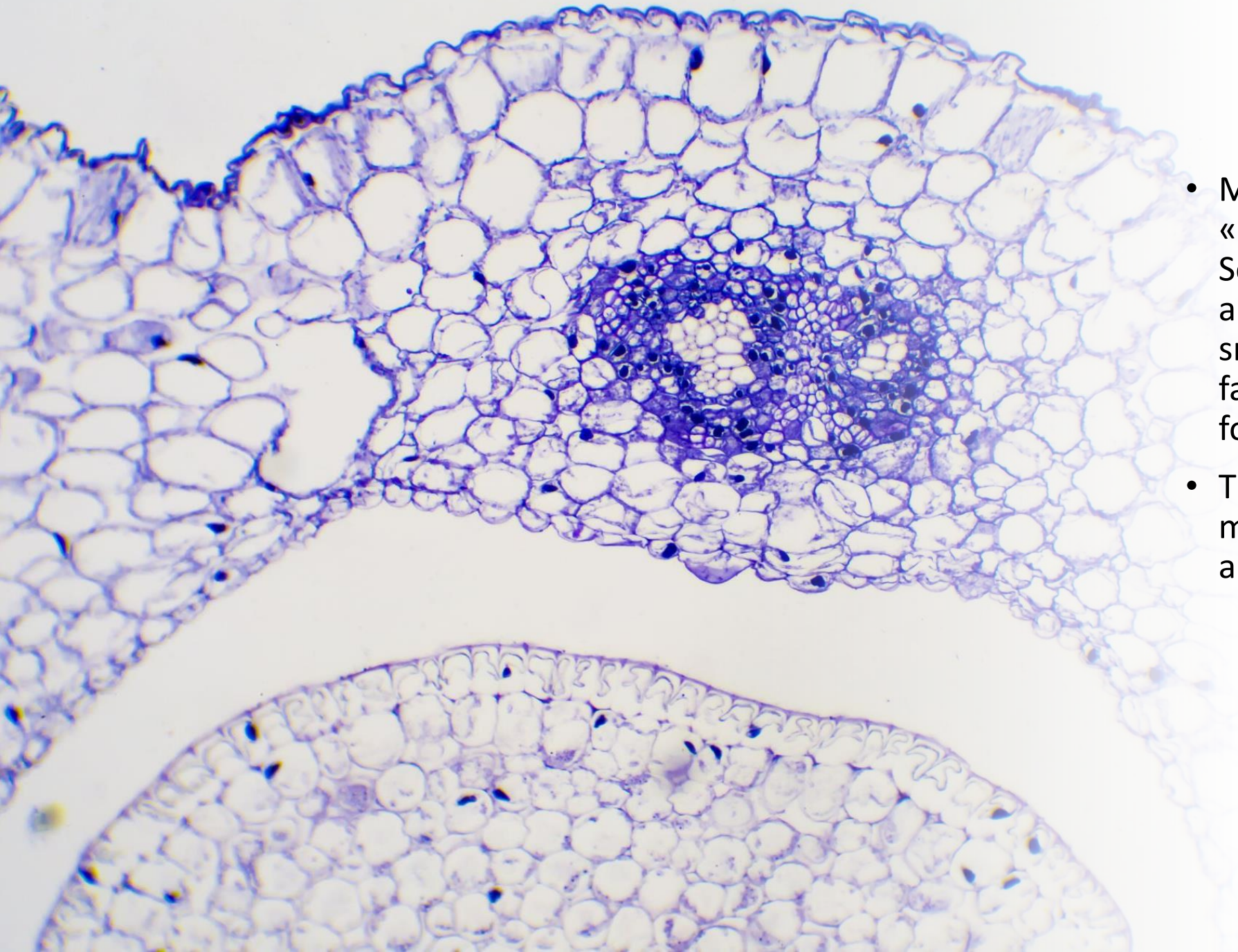
Practical lesson 1

Medical microbiology and immunology, its aims and objectives, historical development. Systematics and classification of microorganisms. Morphology and classification of bacteria. Microbiological laboratory, working regime of microbiological laboratory. Methods of microbiological investigation. Microscopic method. Microscopes. Rules of the immersion microscopy. Preparation of smears from pathological material and pure culture of microbes. Aniline dyes. Simple staining method



- Microbiology – «mikros»-small, «bios»- life, «logos»-the study. Science of pattern, life activity and ecology of the microbes – smallest life forms of flora and fauna origin, which are invisible for the naked eye.
- The size of microbes are measured in micrometers (mm) and nanometers (nm).

- Medical Microbiology

■ Aims – The study of pathogens for humans microorganisms and also diseases which are caused by them, pathogenesis of those illnesses, their laboratory diagnosis, treatment and prevention.

■ Objectives: -Further study of roles of particular species of pathogen microbes in the etiology and in the pathogenesis of different human diseases. -Study of mechanisms of immunity formulation; -Development of diagnostics methods. -Method development of specific prevention and treatment.

The role of microbiology in the activity of the pharmacist:

- Understanding the principles of microbiology and human cell mechanisms allows pharmacists to discover antimicrobial drugs that would prevent an escalating number of communicable diseases. Pharmacists and microbiologists work synergistically to ensure that drug therapies target the opportunistic microbes without harming its human host
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Microbiology began to develop as a science from the second half of the 19th century. The history of the development of microbiology is divided into several stages:

- Theoretical stage
- Morphological stage
- Physiological stage
- İmmunological stage
- Molecular- genetic stage

Classification of microorganisms.

The modern classification

There are 2 upper kingdom of living mater: procariotes and eucariotes.

Procariotes contain: Cianibacterium, Archebacterium, Eubacterium (true bacteria).

Eucariotes contain: animals, plants, fungi (micota).

In this group for microbiology are important:

From animals - class Protozoa

From plants – unicellular water-plants – Algae

From fungi – all microscopical representatives.

Main classification of bacteria is **Bergey's Manual**, which include two divisions:

Cyanobacteria (cyanophyta)

True bacteria which include 19 parts.

Main of them represents:

Bacteria (rod-like, cocci), (aerobic and anaerobic), (endospore forming or no);

Spirochetes and spiriles;

Vibrions;

Actinomycetes (important as producents of antibiotics);

Obligate intracellular parasites (Ricketsia and Chlamidia).

Viruses are grouped in an independent kingdom:

Are genetic parasites

No cell structures and protein synthesis systems

On animals, insects, plants, bacteria (phages) and human

Contain DNA or RNA

Are not visible with the light microscope.

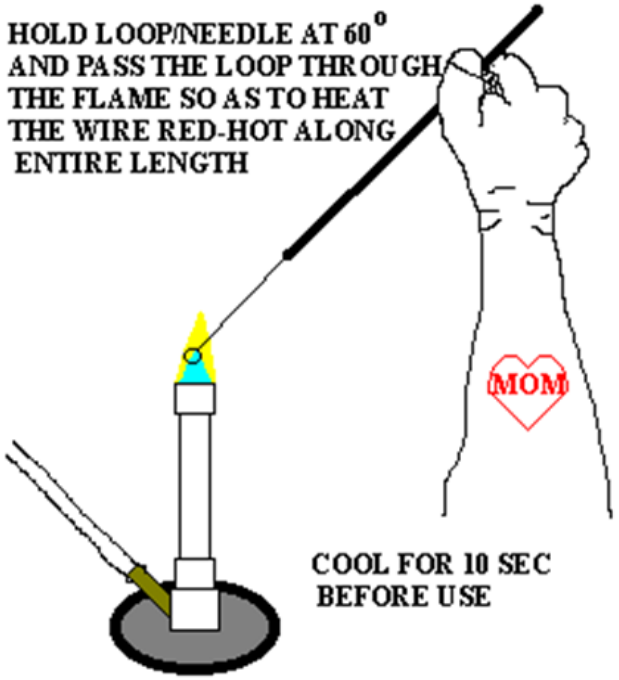
- The microscope is an instrument used to view the micro world. The microscope has multiple lens to pick up the image from the specimen and magnify it.
- Microscopes can be separated into optical theory microscopes (Light microscope - bright-field microscope, dark-field microscope, phase contrast microscope. Fluorescence and confocal microscopes are specialized instruments, used for research, clinical, and industrial applications.), electron microscopes and scanning probe microscopes (SPM). Optical microscopes function through the optical theory of lenses in order to magnify the image generated by the passage of a wave through the sample or reflected by the sample. The waves used are electromagnetic (in optical microscopes or electron beams (in electron microscopes). Types are the compound light, stereo and the electronic microscope. Light microscope produces a dark image against brighter background. Commonly used to view stained cells. **Simple microscopes** have single magnifying lens (like a magnifying glass). **Compound microscopes** have **two sets of lenses** for magnification.

Smear preparation

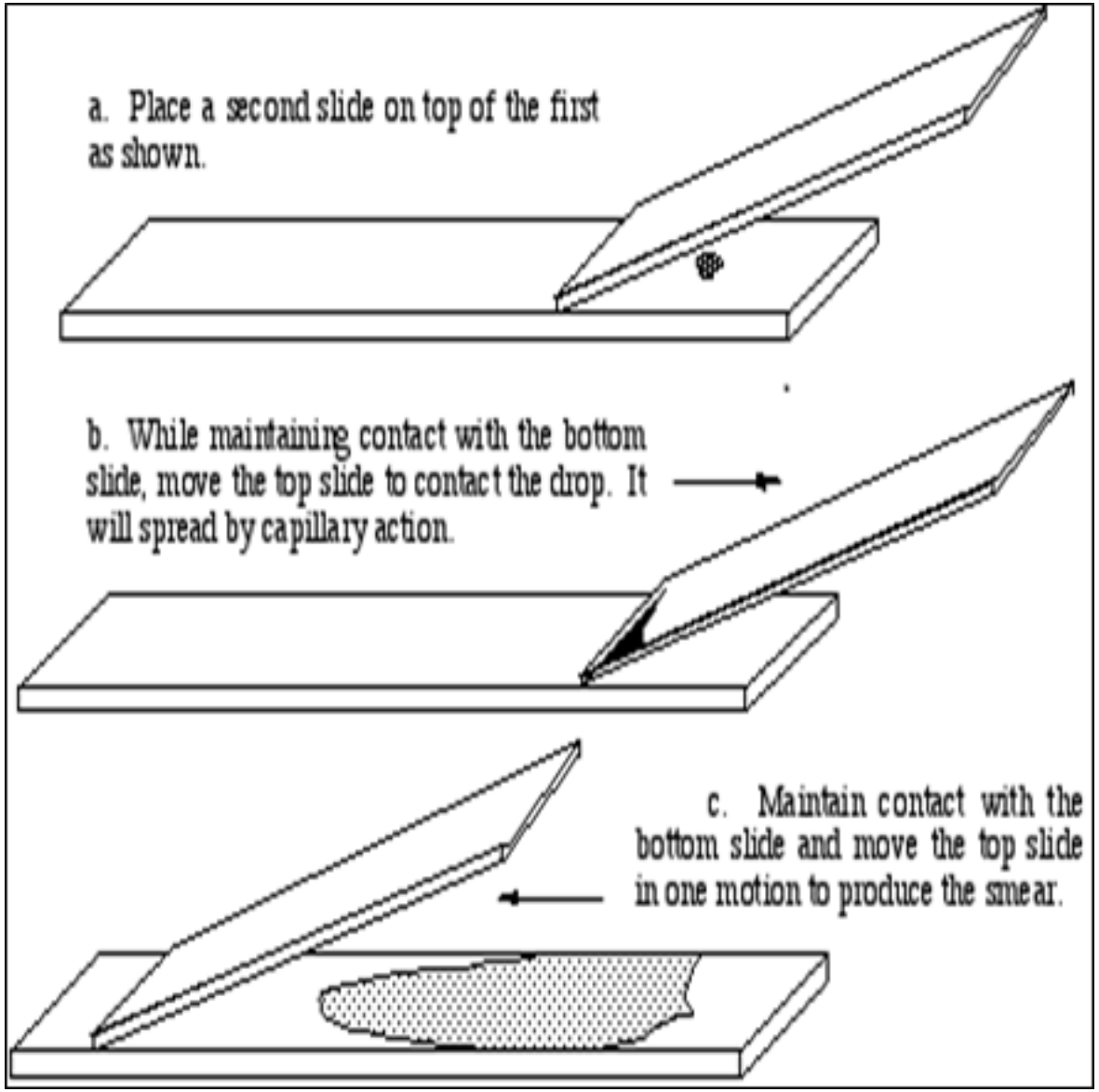
- The first step in preparing a bacteriological smear differs according to the type of medium from which the organisms are removed. To prepare bacteria for staining a sample of bacteria in liquid (broth, milk, saliva, urine, etc.) is spread as a thin layer or smear on a clean glass slide. Make a thin film of the material on a clean glass slide, using a sterile loop or swab for viscous specimens.
- Place the loop in flame (Bunsen burner) starting at the loop and move it through the flame so that the wire becomes red-hot along 4 to 6 cm of its length (Fig.1). Allow the wire to cool for about 10 seconds. Pick up the sample with the cooled loop and distribute it. Repeat the flaming - cooling procedure before laying the loop down on the desk.

Procedure from Liquid Media

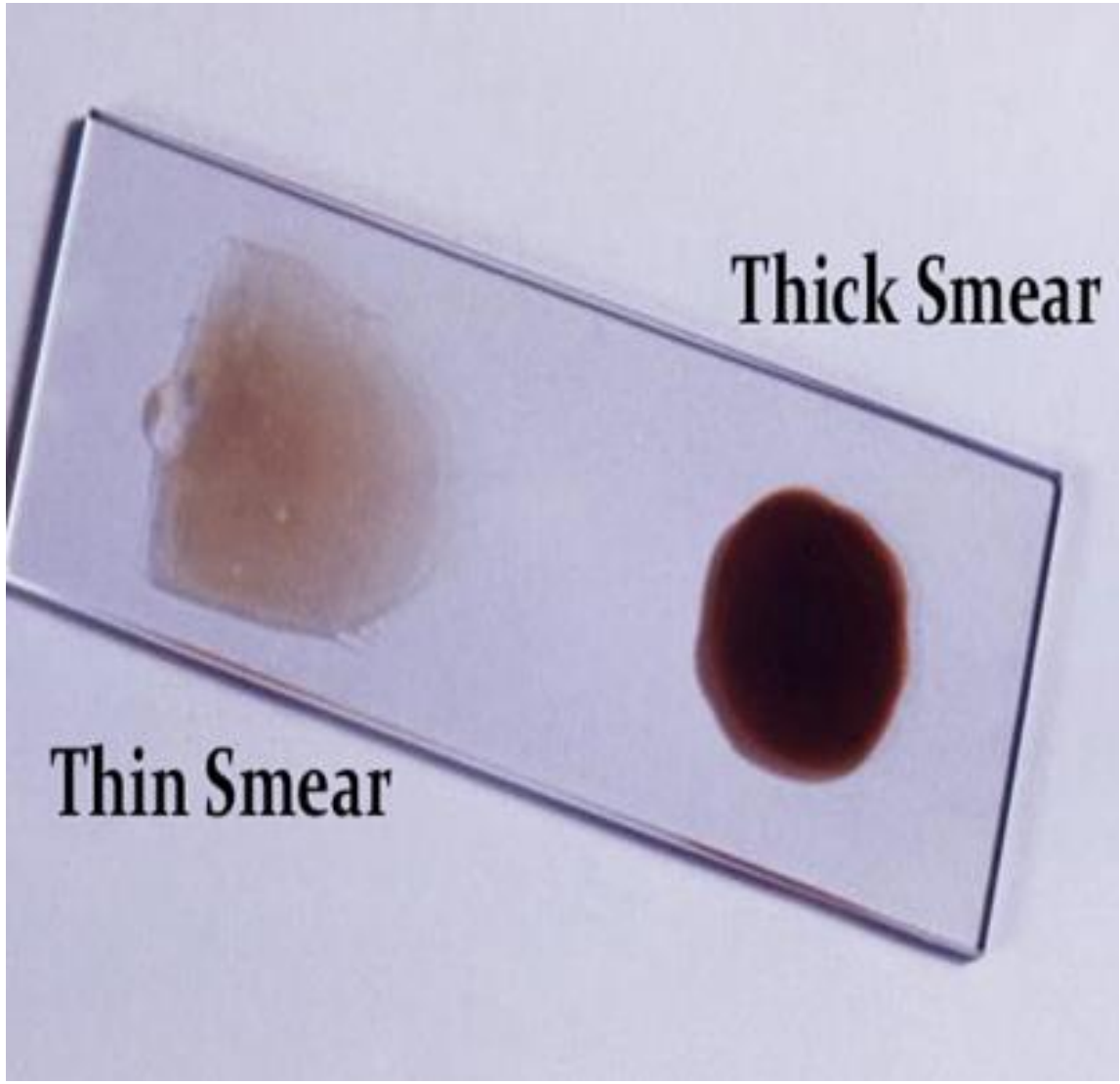
- *Materials:* microscope slides, inoculating loop, Bunsen burner, marking pencil)
- 1. Wash a slide with soap and removing all dirt and grease. Handle the clean side by the edges (Fig.18)
- 2. Write the initials of the organism or organisms on the left-hand side of the slide with a marking pencil.
- 3. To provide a target on which to place the organisms, make a one-two cm
- circle *on the bottom side of the slide*, centrally located, with a marking pencil.
- 4. Shake the culture vigorously and transfer two loopfuls of organisms to the center of the slide over the target circle (Fig.19). *Be sure to sterilize the loop after it has touched the slide.*
- 5. Spread the organisms over an area about one-two cm in diameter.
- 6. Allow the slide to dry by normal evaporation of the water.
- 7. After the smear has become completely dry, pass the slide over a Bunsen burner flame to heat-kill and fix the organisms to the slide.



- Two types of smears are prepared from the peripheral blood – one thin smear and the other thick smear. Thick film examination is about 20 times more sensitive than thin film examination for parasite detection. Thin film examination is done for finding out the species of *Plasmodium*. Prepare the thin and thick blood smears in the following way:
- For thin blood smear take a clean, dust free, grease free slide and take 3 drops of the blood 1 cm from the edge of the glass slide. Take another drop of blood one cm from the first drop of blood (Fig.20). Take another clean slide with smooth edges and use it as a spreader. Make thin smear by bringing in contact the spreader with the drop of blood at an angle of 30-45° from the horizontal and pushing the spreader steadily down the surface of the slide drawing the blood behind till the smear is formed.

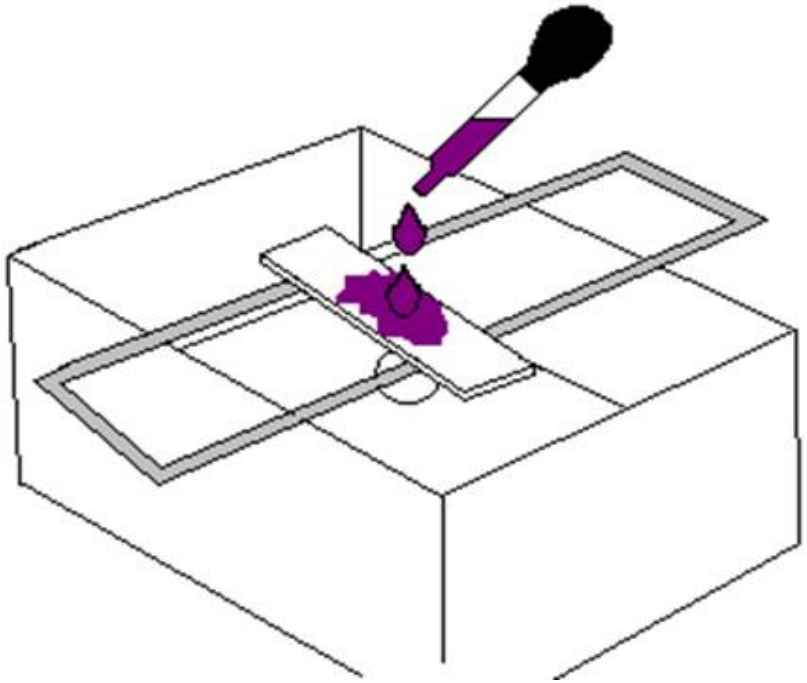


- Make thick smear by joining the 3 drops of blood and spreading it in an area of 10 mm diameter (Fig.21). For thick film preparation, a small drop of blood is placed on a glass slide and spread to approximately 4 time its original surfaces. After extensive drying, best done at 50°C to 60°C for 7 to 10 minutes, the slides can be stained. The cell will wash off slide if insufficiently dried.



Simple staining

- The use of a single stain to color a bacterial organism is commonly referred to as **simple staining**. Some of the most commonly used dyes for simple staining are methylene blue, basic funchsin, and crystal violet. All of these dyes work well on bacteria because they have color bearing ions (**chromophore**s) that are positively charged (cationic). The fact that bacteria are slightly negatively charged produces a pronounced attraction between these cationic chromophores and the organism. Such dyes are classified as **basic dyes**. The basic dye methylene blue (methylene^{+}chloride^{-}) will be used in this exercise. Those dyes that have ionicchromophores are called **acidic dyes**. Eosin (sodium^{+}eosinate^{-}) is such a dye.
- The staining times for most simple stains are relatively short, usually from 30 seconds to 2 minutes, depending on the dye affinity. After a smear has been stained for the required time, it is washed off gently, blotted dry, and examined directly under oil immersion. Such a slide is useful in determining basic morphology.



- **Staining Procedure**
- 1. Prepare a smear on clean grease free slide.
- 2. Air dry and heat fix the smear.
- 3. Stain the smear by flooding it with one of the staining solutions and
- allowing it to remain covered with the stain for the time designated
- below :
- Methylene blue - 1 minute
- Crystal violet - 30 seconds
- Carbol fuchsin - 20 seconds
- 4. Wash the slide with water, air dry and observe under oil immersion
- lens.