كلية المستقبل الجامعة الاهلية
قسم طب الاسنان
المرحلة الثالثة

Simple Staining Procedure

Preparation
assistant

teacher
Fatima Hakim

Lesson objectives

After the end of the lecture, the student will be able to:-

**It is known as Simple Staining Procedure.

Preparation of a smear and heat fixing

1- Using a sterilized inoculating loop, transfer loopful of liquid suspension containing bacteria to a slide (clean grease free microscopic slide) or transfer an isolated colony from a culture plate to a slide with a water drop.

2- Disperse the bacteria on the loop in the drop of water on the slide and spread the drop over an area the size of a dime. It should be a thin, even smear.

3- Allow the smear to dry thoroughly.

4- Heat-fix the smear carefully by passing the underside of the slide through the burner flame two or three times. It fixes the cell in the slide. Do not overheat the slide as it will distort the bacterial cells.

Staining

1- Cover the smear with methylene blue and allow the dye to remain in the smear for about one minute (Staining time is not critical here; somewhere between 30 seconds to 2 minutes should give you an acceptable stain.

2- Using distilled water wash bottle, gently wash off the excess methylene blue from the slide by directing a gentle stream of water overt he surface of the slide.

3- Wash off any stain that got on the bottom of the slide as well.

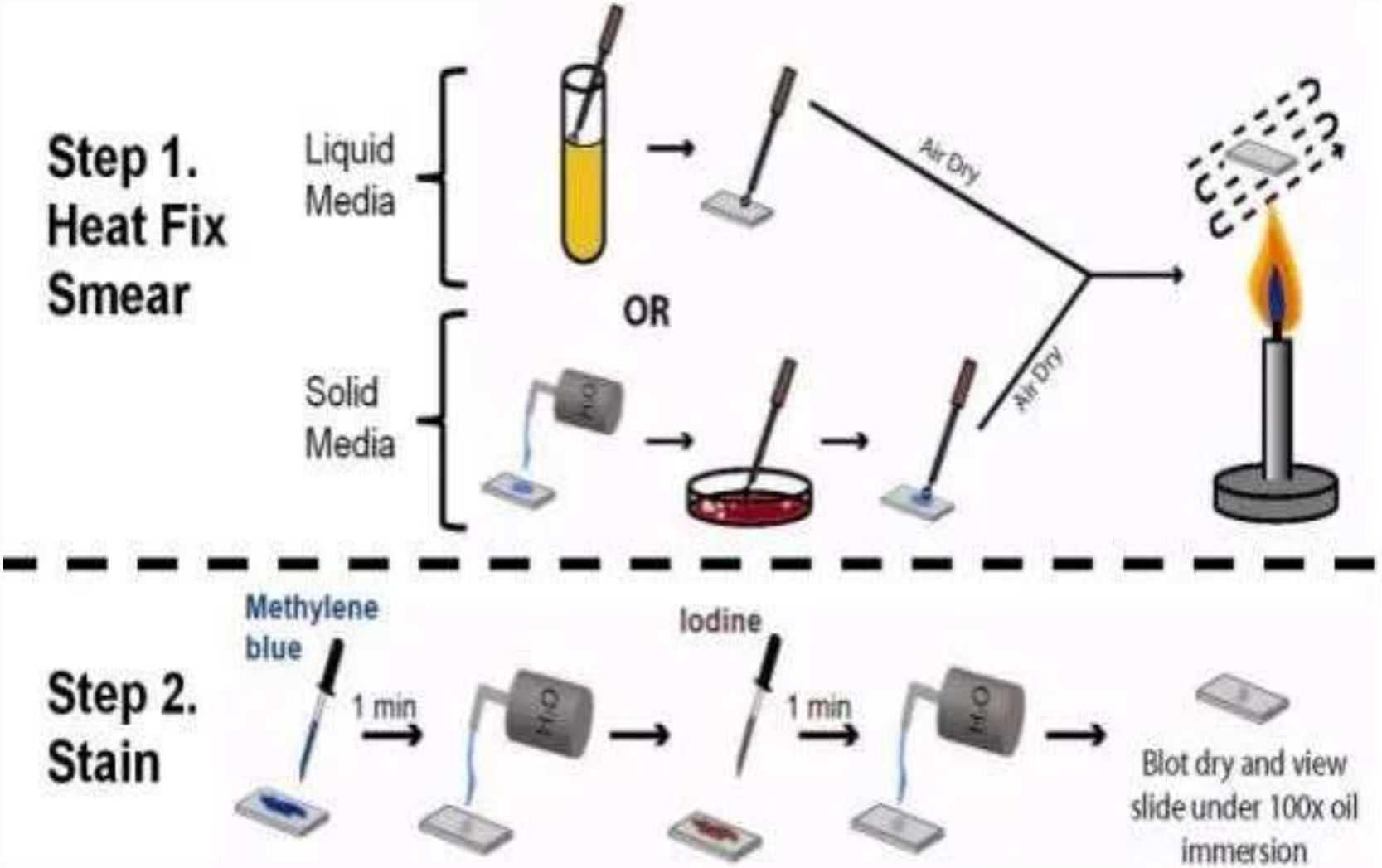
4- Saturate the smear again but this time with Iodine. Iodine will set the stain.

5- Wash of any excess iodine with gently running tap water. Rinse carefully.

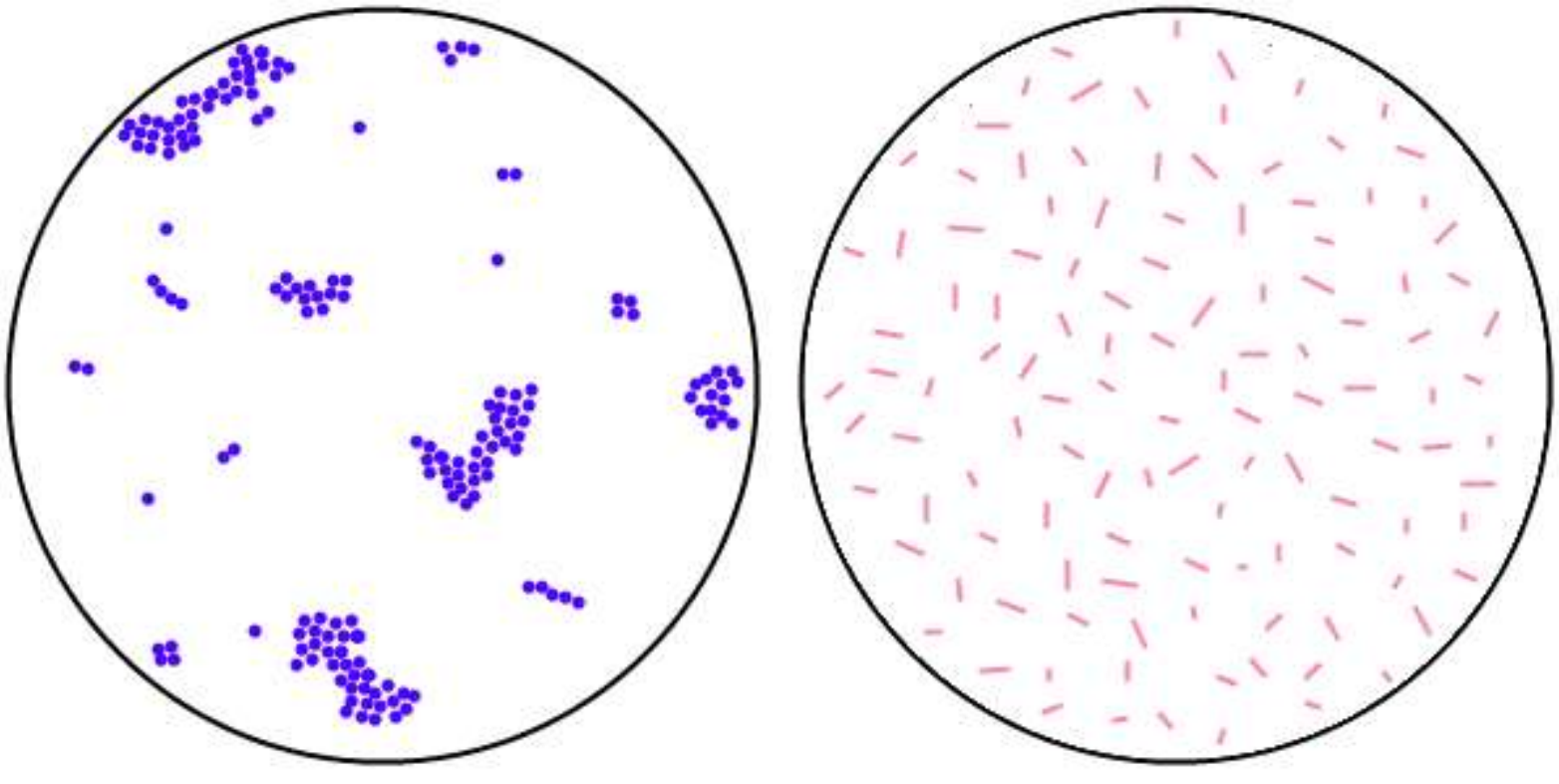
6- Wipe the back of the slide and blot the stained surface with bibulous paper or with a paper towel.

7- Place the stained smear on the microscope stage smear side up and focus the smear using the 10X objective.

8- Choose an area of the smear in which the cells are well spread in monolayer. Center the area to be studied, apply immersion oil directly the smear, and focus the smear under oil with the 100X objective


Step 1.
Heat Fix
Smear
Liquid
Media
Solid
Media
OR
Air Dry
Air Dry
Step 2.
Stain
Methylene
blue
1 min
Iodine
1 min
Blot dry and view
slide under 100x oil
immersion

Results



Uses

- :

1- To differentiate bacteria from yeast cells: When endocervical swab culture is done in Blood agar both Staphylococcus spp and yeast cells may give similar looking colonies in Blood agar (a common error for new technologist or microbiologist with less skill).

2- To identify the bacterial isolate Due to their ubiquitous presence, Bacillus spp may present as a contaminant in the culture plates. In some circumstances (e.g. growth in Blood Agar but no growth in MacConkey Agar), identifying the shape of the bacteria (rod or cocci) may help to eliminate the isolate as possible contaminants (e.g., Bacillus pp) or further process as potential pathogen (cocci).

Thank you for listening