

كلية المستقبل الجامعة الأهلية

قسم طب الأسنان

المرحلة الثالثة

Gram Stain

Preparation

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Lesson objectives

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

****It is known as Gram Stain .**

Gram Stain

Gram staining is a common method used to distinguish two large groups of bacteria based on their different cell wall components. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram-positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Otherwise, Gram-negative bacteria stain red, which is recognized to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process.



Gram staining involves three processes: staining with a water-soluble dye called crystal violet, decolonization, and counterstaining, usually with safranin. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram-negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolonization process, while Gram-negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process.

1. Clean a microscope slide with alcohol.
2. Sterilize an inoculation loop.
3. Using a sterile pipet, add a drop of sterile water so there is a film/bubble across the loop.
4. Transfer the water to the center of the slide.
5. Sterilize the loop.
6. Transfer bacteria from a plate to the slide. Touch the colony lightly to prevent from transferring too much bacteria to the slide. If you can see the colony on your loop, you probably have too much.
7. Sterilize the loop.

8.heat fix the bacteria to the slide by quickly passing the slide through the flame 3 times. The flame should touch the slide for about 1 second for each pass. Turn off the flame of the alcohol lamp before continuing.

9.Cover the fixed bacterial smear with a few drops of crystal violet solution and allow to stand for 1 minute.

10.Rinse off the crystal violet with distilled water.

11.Add a few drops of iodine solution to the slide, and allow to stand for one minute.


12.Rinse off the iodine solution with distilled water.

13. Add decolorizer to the slide by holding the slide over a cup of water and add the decolorizer drop by drop and let it drip into cup until the flow into cup 3 is clear. Finish by rinsing the slide in this cup of water. There should be very little color left on the slide at this point.

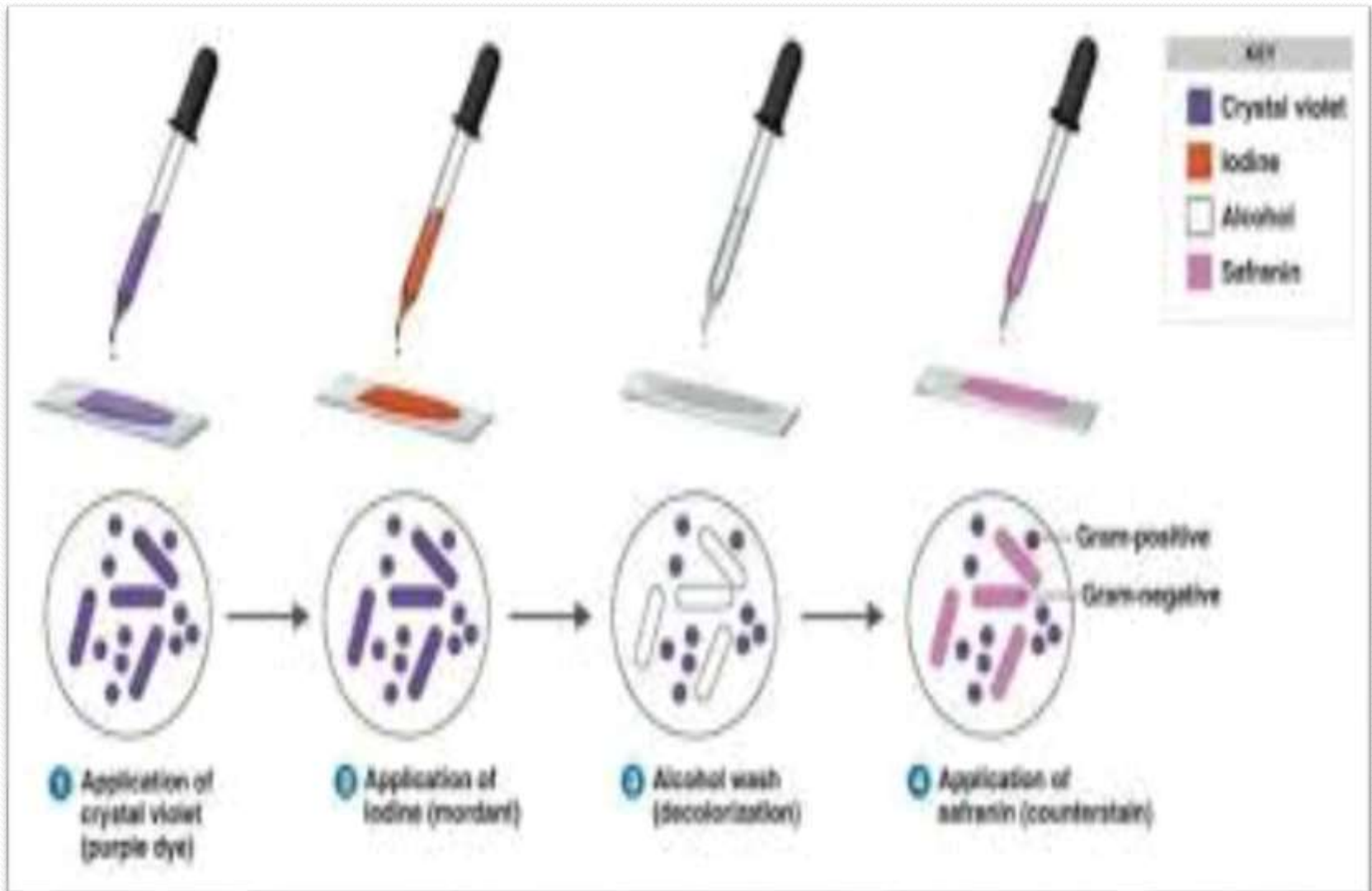
14. Add a few drops of counterstain (safranin) to cover the bacteria and allow to stand for 1 minute.

15. Rinse off the safranin with distilled water.

16. Once the slide is completely dry, it is now ready for viewing under the microscope at 400X. Place cover slip on bacteria before viewing.



See several fields on the slide for bacterial organisms.
Describe the gram reaction of any organisms seen.
Gram-positive bacteria stain deep violet to blue and
gram-negative bacteria stain pink to red.



GRAM-POSITIVE

GRAM-NEGATIVE



Fixation



Crystal Violet



Iodine Treatment



Decolorisation



Counter stain with Safranin



Thank you for listening