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Loveland, CO 80538

JorVet™ J-323 Gram Stain Kit

Directions for Use

HISTORY

The Gram Stain permits the differentiation of two groups of organisms - one of them "gram positive", the other, "gram negative". With Gram Staining methods, the "gram-positive" organisms stain purple, while the "gram-negative" organisms stain red.

The technique dates back to 1884 when Gram employed a safranin counter-stain after staining with crystal violet, and discovered that after alcohol dehydration certain bacteria stained blue-black while other tissues were decolorized. Following this, came many modifications differing mainly in the preparation of the crystal violet staining solution and the type of counterstain used.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS:

Organisms being stained by the Gram method are usually taken from a solid or liquid medium on (in) which they have been cultured from their original source (e.g. wounds, throat swabs, sputum, etc.). An aqueous suspension is made, in the case of the solid medium, but taking a small amount of material and suspending it in a drop of distilled water on a microscope slide. Care should be taken not to make the smear too thick. In the case of a liquid medium, a drop is used directly from the culture container. However, due to the solids from the medium, this method is not always satisfactory. The suspension made by either method is air dried then "fixed" by passing rapidly through a Bunsen burner flame two or three times. Allow the smear to cool before staining. (See SOURCES OF ERROR section.)

Step by Step Procedure:

1. Allow smears to air dry thoroughly (until moisture is no longer visible) then "fix" by passing rapidly through a Bunsen burner flame two or three times. (See SOURCES OF ERROR section.)
2. Place the "fixed" smears on a staining rack and cover completely with Crystal Violet (Reagent #1) for 30 -60 seconds.
3. Wash off the stain with distilled water.
4. Cover the slide with Iodine Solution (Reagent #2) for 30 seconds.
5. Wash off with distilled water.
6. Decolorize (Reagent #3) for 10 - 15 seconds.
7. Wash thoroughly with distilled water.
8. Replace the slide on the stain rack and cover completely with Safranin Counterstain (Reagent #4) for 30 - 60 seconds.
9. Wash with distilled water, air dry and examine under immersion oil. Label clearly. Gram Positive organisms stain a dark purple; Gram Negative organisms stain red.

STABILITY OF FINAL REACTION:

Stained smears, after being properly mounted with mounting medium, have been observed to retain their staining characteristics for at least two months.

SOURCES OF ERROR:

1. Overheating (burning) during fixation can be avoided by just touching the back of the slide to the back of the hand each time the smear has been passed through the flame.
2. Do not stain smears which have only been air dried. Smears *must* also be “fixed”.
3. Smears should not be too thick. After air drying, examine under a microscope. If there are no areas of bacteria separation, more water should be added to dilute the smear. Repeat air drying and “fixing” as in step #1 of the *Step by Step Procedure* section.
4. After staining, it is essential that the back surface of slide is wiped clean.
5. If washing with distilled water is not done adequately, crystallization of stain may appear on the slide.
6. The manufacturer recommends that a known Gram positive and Gram negative control be stained at the same time as the test culture as an additional measure of quality control.
7. Staining times may vary to suit the individual.
8. In steps #3, #5 and #7 of the *Step by Step Procedure* section slides should be removed from the staining rack and held at an angle while washing.

Gram positive organisms such as cocci appear dark blue or black, and Gram negative organisms, such as the coliforms and pseudomonads, appear pink. The nuclei of leukocytes stain pink. All fungi are Gram positive.

REAGENTS:

FOR IN VITRO DIAGNOSTIC USE

- #1. Crystal Violet: Crystal Violet 0.5% w/v in denatured alcohol.
 - a. POISON: Do not take internally. Avoid contact with eyes. Vapor harmful. FLAMMABLE.
 - b. Store at room temperature.
- #2. Iodine Solution: PVP Iodine 1.9% w/v; Potassium Iodide 13% w/v.
 - a. POISON: Do not take internally. Avoid contact with eyes. Avoid breathing vapor.
 - b. Store at room temperature.
- #3. Decolorizer: Specially Denatured Alcohol/Acetone
 - a. Stable indefinitely.
 - b. POISON: Do not take internally. Avoid contact with eyes. Avoid breathing vapor.
 - c. Store at room temperature.
- #4. Safranin Counterstain: Safranin 0.5% in denatured alcohol w/v.
 - a. No special handling precautions.
 - b. Store at room temperature.