

MICROBIOLOGICAL METHODS FOR DISEASE DIAGNOSIS

Bacterial diseases can be identified by various microbiology methods. The samples are taken from the fish under aseptic condition and they are first grown in non-selective media, then the various microbial diagnostic methods are used. The different microbiological diagnostic methods are

1. GRAM'S STAINING

The Gram staining technique is the most important and widely used microbiological differential staining technique. It was developed by Dr. Christian Gram in 1884, and categorizes bacteria according to their Gram character (Gram positive or Gram negative). In addition this stain also allows determination of cell morphology, size, and arrangement. It is typically the first differential test run on a specimen brought into the laboratory for identification. In some cases, a rapid, presumptive identification of the organism or elimination of a particular organism is possible.

PRINCIPLE OF GRAM STAINING

The structure of the organism's **cell wall** determines whether the organism is gram positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolorization while others get decolorized by a decolorizer. Those bacteria which retain the primary stain are called Gram positive and those bacteria which get decolorized and then get counterstained are called Gram negative.

Requirements:

1. Primary Stain : Crystal violet

Solution A :

1. Crystal violet = 2 gm
2. Ethyl alcohol= 20 ml

Solution B :

3. Ammonium oxalate = 0.8 gm
4. Distilled water = 80 ml

Mix solution A and B. Keep for 24 hours and filter. Store in an amber colored bottle.

Mordant : Gram's Iodine

0. Iodine = 1 gm
1. Potassium iodide = 2 gm
2. Distilled water = to 100 ml

Mix and Store in an amber colored bottle.

Decolorizer : 95% Ethanol or 1:1 acetone with ethanol

0. Acetone = 50 ml

1. Ethanol (95%) = 50ml

Counterstain: safranin

0. Safranin O = 0.34 gm

1. Absolute alcohol = 10ml

2. Distilled water = 90ml

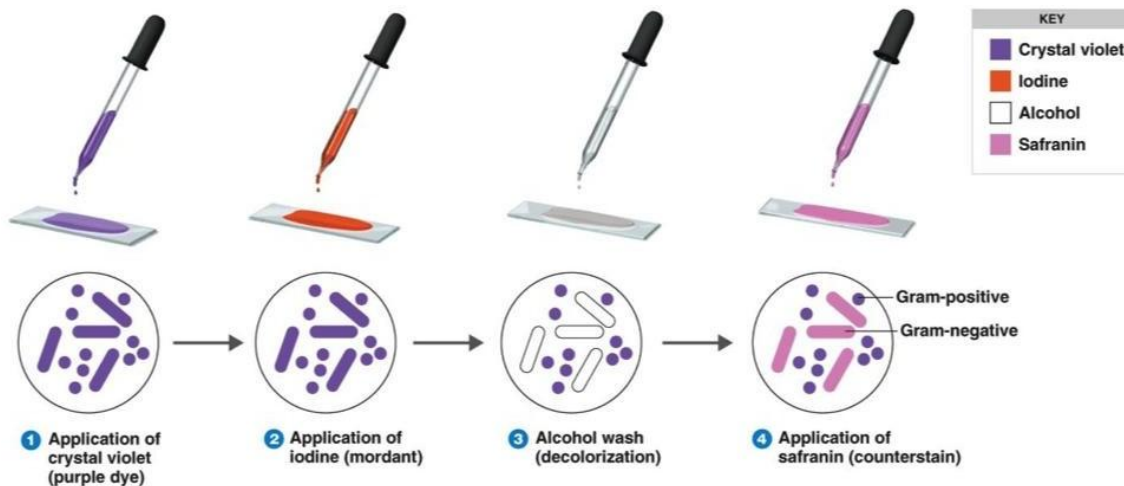
Mix, filter and store in ambered colored bottle.

Procedures:

Smear preparation :

1. Take a grease free dry slide.
2. Sterilize the inoculating loop on a flame of a Bunsen burner.
3. Transfer a loopful of culture (or the specimen) by sterile loop and make a smear at the center. Smear should not be very thin or very thick.
4. Allow the smeat to dry in the air.
5. Fix the dry smear by passing the slide 3-4 times through the flame quickly with the smear side facing up.

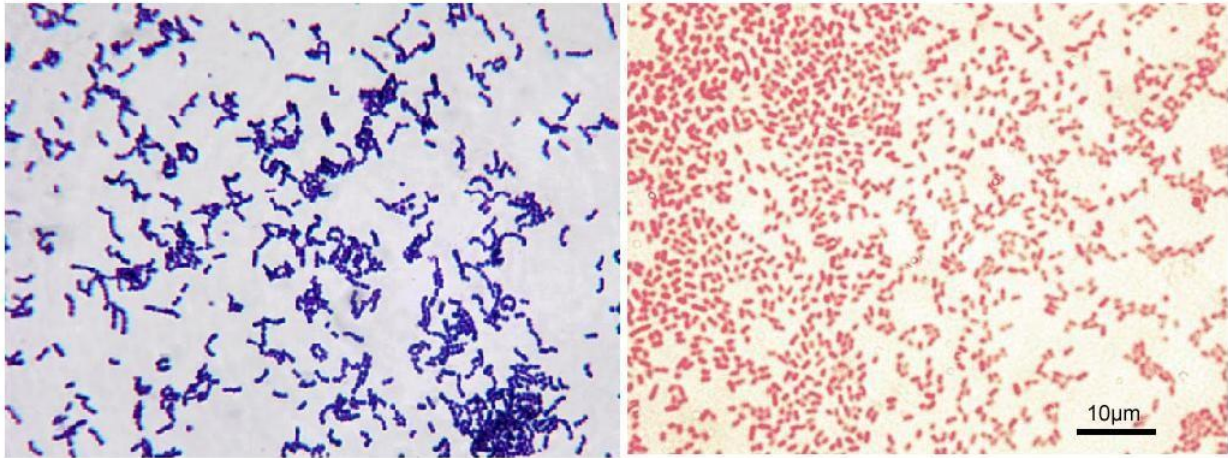
Gram Staining :



1. Place the slides on the staining rods.
2. Cover the smear with crystal violet stain and leave for 1 minute.
3. Wash carefully under running tap water.
4. Flood the smear with Gram's iodine solution and leave for 1 minute.
5. Drain off the iodine Wash the slide for the again in a gentle stream of tap water.
6. Flood the slide with the decolorizing agent then wait for 20-30 seconds. This can also be done by adding a drop by drop to the slide until the decolorizing agent running from the slides runs clear.
7. Gently wash the slide under running tap water and drain completely.
8. Counterstain with safranin for and and wait for about 30 seconds to 1 minute.

9. Wash slide in a gentle and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.
10. Observe under microscope.

INTERPRETATION OF GRAM STAINING



Gram Positive Bacteria

Gram Negative Bacteria

The staining results of gram stain are as follows :

- **Gram Positive** : Dark purple
- **Gram Negative** : Pale to dark red
- **Yeasts** : Dark purple
- **Epithelial cells** : Pale red

Examples of Gram Positive Organisms

Bacillus, Nocardia, Clostridium, Propionibacterium, Actinomyces, Enterococcus, Corynebacterium, Listeria, Lactobacillus, Gardnerella, Mycoplasma, Staphylococcus, Streptomyces, Streptococcus etc

Examples of Gram Negative Organisms

Escherichia, Helicobacter, Hemophilus, Neisseria, Klebsiella, Enterobacter, Chlamydia, Vibrio, Pseudomonas, Salmonella, Shigella

RESULTS: