Staining method of detecting Gram positive and Gram negativebacteria in tissue

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Abstract

Both acute and chronic wounds are frequently plagued by bacterial infections. Antibiotic resistance is a growing problem, thus treating bacterial illnesses should wait until a positive diagnosis.Currently, lengthy culture procedures that might not even detect the presence of germs postpone diagnosis.In order to treat bacterial infections promptly and effectively, a simple and affordable diagnostic tool would be helpful. More sophisticated and Researchers are looking at high-cost methods of identifying bacteria. Although Hematoxylin and Eosin (H&E) and Gram stains have been usedin histology, their non-specific staining means that they are not the best options for the examination of tissue samples.The aim of the present work was to develop a modified Gram stain, which would give a better contrast between the bacteria and the host.

Keywords: tissue, histology, hematoxylin, bacteria, eosin

Introduction:-

The H&E and Gram stains of the serial biopsy were then contrasted with the modified Gram stain, which reveals the collagen present in the tissue sections. Modified Gram stain provided superior contrast between bacteria and non-viable burns when compared to H&E or Gram stain alone, which had trouble distinguishing between Gram-positive and Gram-negative bacteria. Additionally, this technique demonstrated efficacy in identifying bacterial morphology in host tissue when it was applied to the analysis of surgical specimens obtained from patients who had undergone burn debridement. The skin's immune and defensive functions, in addition to its metabolic capabilities, help to separate the body's internal and external environments. Beginning with Skin tissue serves as a physical barrier to prevent microbes from entering beneath-surface host tissues[1]. Bacteria havethe chance to colonize tissues when this barrier is broken (for example, after a burn damage), which ultimately results in the formation of an infection. Bacterial infection can spread to a wide range of both acute (such as bites and scratches) and chronic (such as burns and diabetic foot ulcers) wound types. Clinical signs of infection have not consistently demonstrated to be sufficient for a rapid assessment of the presence of bacteria, which is required for the effective management of infected wounds. Traditional culture-based approaches are limiting and time-consuming for microbial identification.[1][2]

Gram-positive organisms:-

Many bacteria's cell walls are made of peptidoglycan, which is present in thick layers in the cell walls of gram-positive bacteria. In gram-positive bacteria, the peptidoglycan makes up around 90% of the cell wall. Under a Gram stain, they appear blue to purple as a result.[3]
Gram-positive organisms include Staphylococcus species, Streptococcus species, Corynebacterium species, Clostridium species, Listeria species.

Gram Negative Organisms:-

Gram-negative bacteria have thin peptidoglycan layers (10% of the cellwall) and substantial lipid (fatty acid) layers in their cell walls. As a result, When stained with a Gram stain, they show up red to pink.
Gram-negative organisms include Neisseria Gonorrhoeae and Neisseria meningitidis, Moraxella species Escherichia coli (E. coli), Pseudomonas species, Klebsiella species.[4][5]

What diseases can be identified with the aid of Gram stains?

In order to diagnose and treat particular bacterial disorders such as urinary tract infections (UTIs) and bacterial pneumonia, medical practitioners frequently request gramme stains. Doctors might not always request a Gram stain to aid in the diagnosis of bacterial diseases. To diagnose food poisoning, for instance, they frequently do not utilize Gram staining, rathera stool (poop) sample might be used instead.[6]

When is a Gram test performed?

When medical professionals suspect you have a bacterial infection—or occasionally a fungal infection—they frequently order a Gram stain in addition to a bacteria culture.It's one of the most popular methods for immediately determining whether your body is infected with bacteria.[7]

A Gram stain test is carried out by who?

Depending on the kind of infection you could have, different healthcareproviders may collect a sample from you for a Gram stain test. Agynecologist might take a swab to screen for gonorrhea, and apulmonologist might take a sample of your mucus from coughing or spittingto check for bacterial pneumonia. Your provider then places the sample in asterile container and sends it to a lab for analysis. Several stains are addedafter applying the sample to microscope slides. Then a medical labprofessional examines the material under a microscope. They put together a report and give it to your doctor.[8]

How is a Gram stain test performed?

A Gram stain test involves three standard processes, including:

- Acquiring the sample
- Preparation of the sample.
- Looking at the sample.
- Taking a sample for the Gram stain

Your healthcare professional must take a sample from the probable infection site for a Gram stain test. A provider may obtain samples for Gram stain tests in several ways,such as:[9]

- Removing tissue by brushing or scraping it off the surface of a physical component.
- Collecting samples of your body's liquid or waste.
- obtaining a fluid sample from an area of your body via fine-needle aspiration.
- Sites where a swab with brushing and scraping can capture include: throat, genitalia, skin injuries.
- Samples that can be taken straight into a sterile container are asfollows:
  -Spit (sputum)
  -Urine (pee)
  -Stool (poop)

Your body may need to be aspirated with a fine needle in the followingplaces:

- Your joints contain synovial fluid.
- Fluid in your chest cavity (pericardial fluid).
- Your lungs' surrounding fluid (pleural fluid).
- Your spinal cord is surrounded by cerebrospinal fluid (CSF).

Your provider then puts the sample in a sterile container and sends it to alaboratory for testing.[10]

Procedure:-

- Prepare a thin slide.
- Then Fixed cells are stained with crystal violet for 30 to 40 seconds.Wash the crystal violet with water.
- Add gram's iodine to the smear and wait for 1 minute.
  Then wash with water.
- Decolourise with 95% Ethyl Alcohol for not more the 30 seconds.Wash with water.
- Add safranin to the smear then wait for 1 minuteObserve
  the slide under the microscope.

Result:-

Gram positive cells retain the purple colourGram
negative cells appear in pink colour

Discussion: -

The most common coloring method in bacteriology is the Gram stain. Because it distinguishes between Gram-positive and Gram-negative microorganisms, it is known as a differential stain. When bacteria are stained using the Gram staining method, those that stain purple are referred to as Gram-positive, while those that stain pink are referred to as Gram-negative. The words positive and negative simply denote two different morphological groups of bacteria and have nothing to do with electrical charge.

Conclusion:-

Based on the various staining capabilities of bacterial cell walls, the Gram stain distinguishes between distinct types of bacteria. Bacteria with a densepeptidoglycan layer stain blue to purple and are considered gram positive, whereas those with a thin peptidoglycan layer stain red to pink and are considered gram negative.

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