ORIGINAL RESEARCH

To identify various organisims present within mouth ulcers.





Agna Azmin , Meenakshi V P , Namshi Nazar , Rizwa Nourin , Thanveera Thasneem

Under Graduate students, Department of General microbiology, Royal Dental College, Palakkad, Kerala

Access this article online

Website : royaldentalcollege.in/recline/

Address for Correspondence:
Mr. Sandeep P M,
Royal Dental College,
Palakkad, Kerala

INTRODUCTION

The oral cavity is colonized by wide range of micro organisms which may be harmless or harmful to respective individuals based on the level of immunity. Recurrent oral ulceration is a common condition , experienced by most of the people which causes transient soreness and consequently may lead to prolonged ulceration in the mouth, It causes difficulty.

Many patients with recurrent oral ulceration maintain a good health but some may have pre-existing medical problems which may be of relevance that includes anaemia, blood dyscrasias, autoimmune disease and diabetes. The medical history will include ascertaining

medication taken by the patient. “Ulcer is a complete breach of the epithelium”, this becomes covered by fibrin slough and it appears to be a yellow /white lesion surrounded by erythema . It is more common in women than men of 10 to 40 years of age. Mouth ulcer is more common in individuals who are under the pressure of physical or emotional stress for example: during exams, also occurs due to Trauma during dental procedure , aggressive tooth cleaning, eating more spicy food, biting of tongue or cheek accidentally , due to deficiency of vitamin B12 or folic acid , due to some hormonal changes andin eating and speaking.sodium lauryl sulfate found in toothpaste also causes ulceration. Prolonged ulceration may leads to aphthous ulcer.

Aphthous ulcer are canker sores occurring in the

mucous membrane of the mouth including gums, tongue and throat which may vary insize from 1-2mm to 1cm. It will be very painful , open mouth sores are in white or yellow colour with bright red surrounding area. It occurs in individuals of poor immune system

Selection of Patients

The study was conducted in the Department of Microbiology. A total of 10 samples were collected from mouth ulceration in outpatients royal dental college .The patients

Conflict of Interest: None declared

Source of Support: Nil

presented with pain in the ulcer and redness of the surrounding area of the ulcer. They were registered at the General out patients department and their personal records: name, age, sex, and other relevant information were taken.

Collection of Samples

The patients were informed about the project and the swabs were taken with their consent. Mouth ulcer swabs were collected aseptically by a sterile swabs. The samples were sent to the Microbiology Laboratory immediately after collection of sample for analysis. Swabs were collected aseptically using sterile Evepon swab sticks. All the specimens collected were properly labelled with patient's number and date.

Examination of Samples

Ulcer swabs collected from mouth of the selected patients were examined microbiologically using culture technique and direct microscopy as describedby Cruickshank et al., (1985) and Chesbrough (1998).

ALBERT STAINING

Special stains have been developed over time for identifying bacteria species, differentiating them morphologically, and even characterizing there very special features. The most common stain being Gram Staining, Acid-fast staining, endospore staining.

Albert stain is no different. Its application aim at identifying bacteria that contain special structures known as metachromatic granules. Albert stain distinctly identifies metachromatic granules that are found in Corynebacterium diphtheriae.

Corynebacteria are gram-positive, non-spore forming, non-motile bacilli that contain metachromatic (Volutin) granules

Metachromatic granules

Metachromatic (Volutin) granules which are intracellular inclusion bodies, found in the cytoplasmic membrane of some bacterial cells for storage of complexed inorganic polyphosphate (poly-P) and enzymes. When these granules are subjected to stain with methylene blue dye, they appear reddish-purple color and not the blue dye.

Objective

To stain and observe metachromatic granules from a Corynebacterium diphtheriae culture.

Materials Required

1.Albert stain.

2.slide Rack and slides

3. Cotton swab to collect the specimen

Collection of specimen using cotton swab


4.oil immersion microscope

Principle

Albert staining technique aims at detecting the presence of metachromatic granulated bodies of Corynebacterium diphtheriae. Albert stain is made up of two staining solutions; designated as Albert Solution 1 and Albert Solution 2, their compositions being;

Albert Solution 1:

· toluidine blue, malachite green, glacial acetic acid, and alcohol

Albert solution 2:

· Iodine and Potassium iodide in water

To use Albert's staining solutions, each of the two solutions must be prepared effectively with the right percentages of components in order to demonstrate the granules with the right color after staining.

Albert staining solution 1 acts as the staining solution while Albert solution 2 acts as the mordant, i.e an ion element that binds and holds a chemical dye, to make it stuck on the micro-organism.

Procedure

A. Staining:

1. Aseptically, take a loopful culture of Corneybacterium diphtheriae
2. Make a smear at the center of a clean sterile glass slide
3. Heat fix the smear, gently
4. On a staining rack, place the smeared glass slide.
5. Add Albert staining Solution 1 into the smear and leave it for 3-5 minutes
6. Wash the smeared slide with gently flowing tap water

B. Mordanting

1. Add Albert staining solution 2 and leave it for 1 minute
2. Wash the slide with gently flowing tap water.
3. Blot to dry the smeared glass slide
4. Add cedarwood oil on the smear
5. Then observe under a microscope by oil immersion at 000x

Observation :

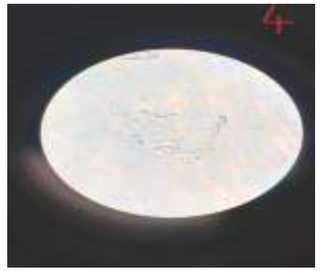
We have collected samples from 10 subjects using a cotton swab which was dipped in saline. The collected specimen was heat fixed and observed under the microscope scope.the observation is as follows:

Subject 1 : Albert stain negative. No metachromatic granule was found.

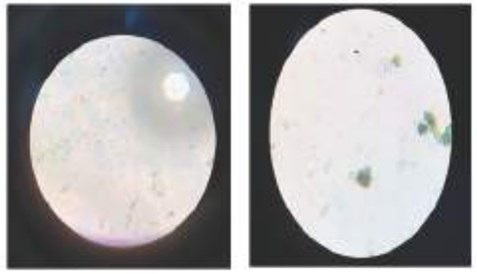
Subject 2: Albert stain positive .Metachromatic granules we're found.

Subject 3: Albert stain negative. No metachromatic granules.

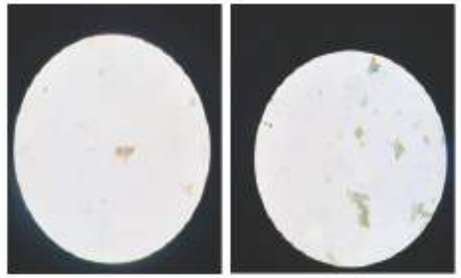
Subjects 4:Metachromatic granules was suspect full



Subject 5: Albert stain positive. Subject 6: Albert stain positive



subject 7: Albert stain positive Subject 8: Albert stain positive



Subject 9: Albert stain positive

10.subject 10 :Albert stain negative.No Metachromatic granules were found

Interpretation

Corynebacterium diphtheriae cytoplasmic membrane contains volutin granules, also known as metachromatic granules, which are a characteristic feature of this bacteria. The staining by Albert solutions, stains the granules making the appear as round-shaped blue-black dots at the bottom of L-shaped or V-shaped green Bacilli.

Limitations of Albert Staining

It can only be used to stain the metachromatic granular bodies and not any inclusions in the cytoplasmic membrane.

Result

The metachromatic granules stain bluish black while the rest of the microbial cell stains green

GRAM STAINING

Gram staining is a differential bacterial staining technique used to differentiate bacteria into Gram Positive and Gram Negative types according to their cell wall composition.

It is the most widely used and the most important staining technique in bacteriology, especially in medical bacteriology. It is generally the first test performed on bacteria during their identification and observation process.

This staining technique uses two stains; crystal violet as primary stain and safranine as a counterstain.Those bacteria with Gram-positive cell walls will retain primary stain and appear violet or purple. These bacteria are termed Gram-Positive bacteria.

The other group of bacteria with Gram-Negative cell wall will lose primary stain andtake up the counterstain and appears pink or red under the microscope. These bacteria are called Gram-Negative bacteria.

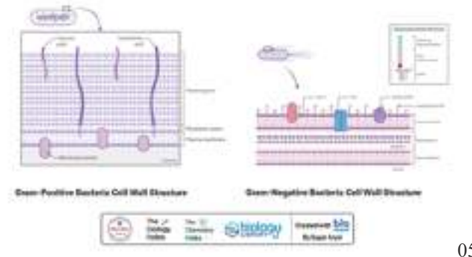
Using this staining technique, bacteria can be differentiated into two groups hence; it is called the differential staining technique.

Objectives

- To differentiate bacteria into Gram-Positive and Gram-Negative.
- To study the morphological structure of bacteria.

Principle

- Gram staining and differentiation are based on the differences in cell wall structure and composition of bacteria. Bacteria having cell walls with a thick layer of peptidoglycan will resist decolorization of primary stain and appear violet or purple.
- Bacteria having a thin peptidoglycan layer with lesser cross-linkage lose primary stain during decolorizing and gain counter stain appearing pink or red.
- In an aqueous solution of crystal violet dye, their molecules dissociate into CV+ and Cl– ions. These ions easily penetrate the cell wall components of both positive and negative bacteria.
- When Gram's Iodine is added as mordant, the iodine interacts with CV+ ion and forms CV-I complex within cytoplasm and cell membrane and cell wall layers.
- When decolorizing solution (ethanol or a mixture of ethanol and acetone) is added it interacts with lipids in the cell wall. The outer membrane of the Gram-Negative bacterial cell wall is dissolved exposing the peptidoglycan layer.
- Whereas in Gram-Positive bacteria, there is no outer membrane, and the peptidoglycan layer is also thick with higher cross-linkage. So, the decolorizing solution dehydrates the peptidoglycan layer trapping all the CVI complexes inside the cell wall and bacteria retain the purple or violet color of crystal violet.
- The peptidoglycan layer is thin with less cross-linking in the Gram-Negative cell wall, hence becoming leaky. This causes cells to lose most of the CVI complexes.
- When counterstain, positively charged safranin, is added, it interacts with the free negatively charged components in Gram-Negative cell wall and membrane and bacteria becomes pink/red.
- Whereas, there is no space to enter inside the dehydrated Gram-Positive cell wall due to CVI complex and dehydration. Hence, safranin can't stain them red or pink and Gram-Positive bacteria reveal the purple or violet color.



Materials Required

1.Gram staining Reagents.

2.Glass slide

3.cotton swab

collection of specimen

4.Microscope with 100x objective lens

Gram Stain Reagents

Gram staining procedure uses different chemicals and dyes that can be grouped such as

1. Primary Stain (Crystal Violet)

· It is an intensely purple-colored organic compound chemically called triphenylmethane dye. It is also known as hexamethyl pararosaniline chloride or methyl violet 10B or gentian violet.

· Its color depends on the pH of the dissolving medium such as,

· at pH -1.0 or below-appears yellow

· at acidic pH of 1 to 2 -appears green

· at neutral pH-appears purple (deep blue-violet)

· at highly basic pH -appears colorless.

In Gram Staining, it is used as a basic dye.It provides violet color to Gram-Positive bacteria.

2. Mordant (Gram's Iodine)

· It is an aqueous solution of iodine and potassium iodide used as mordant in Gram staining. It interacts with CV+ and forms a CVI complex which gets trapped in the dehydrated peptidoglycan layer of the Gram-Positive cell wall.

3. Decolorizing Solution

· It is either acetone or ethanol (95%) or a mixture of acetone and ethanol in ratio 1:1 by volume. The decolorizing solution dissolves the lipid content in the outer membrane of the Gram-Negative cell wall and increases its permeability.

· In the Gram-Positive cell wall the decolorizer dehydrates the peptidoglycan layer and traps the CVI complex within the cell.

4. Counter Stain (Safranin)

· It is a red-colored counterstain used to stain decolorized Gram-Negative cells in the Gram Staining technique. It is a basic dye that interacts with negatively charged components of the cell wall and membrane.

· Besides safranin, dilute carbol fuchsin solution is also used as a counterstain.

Procedure of Gram Stain Slide Preparation

1. Take a clean, clear, grease-free glass slide
2. Sterilize the inoculating loop by flaming and transfer a loop full of bacterial culture suspension in the middle of the glass slide.
3. Spread the suspension with the sterile inoculating loop to prepare a thin smear. The smear must not be too thin or too thick.
4. Let the smear air dry and fix it by passing over the flame. Fixing should be done over a gentle flame. Slide must be moved up and down or circularly over the flame to prevent from overheating. Flaming will fix the bacterial cells on the slide and prevent them from washing out.
5. Heat fixation

Gram Staining Protocol

1.Flood crystal violet solution over fixed smear.

2. After 30 – 60 seconds, pour off the CV solution and rinse with gentle running water.
3. Flood the Gram's Iodine solution over the smear.
4. Leave the iodine solution for 30 – 60seconds and pour off the excess iodine and rinse with gentle running water.
5. Shake off the excess water over the smear
6. Decolorize the smear by passing the decolorizing solution till the solution runs down in clear form. Alternatively, add a few drops of decolorizing solution and shake gently and rinse with distilled water after 5 seconds.

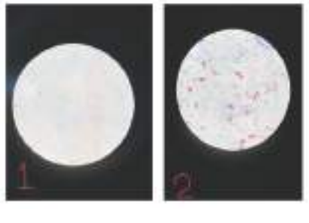
7. Rinse with distilled water to wash decolorizer
8. Shake off the excess water over the smear
9. Pour counter stain over the smear

10. Leave for 30 – 60 seconds and wash w ith gentle running water
11. Air dry or blow-dry the smear

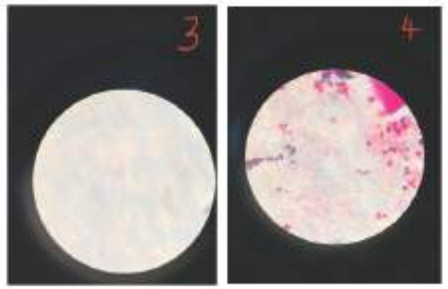
Observation

we have collected 10 samples from stomatitis patients.These specimens are stained by grams iodine and observed under microscope.The observation is as follows:

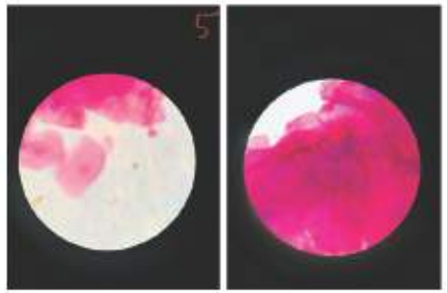
specimen 1:Gram negative bacilli. specimen 2:Gram positive cocci



specimen 3:Tissues.No bacteria isspecimen 4:Gram positive cocci found

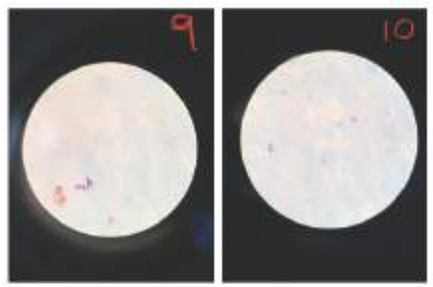


specimen 5:Gram negative cocci Specimen 6:No bacteria is found and Gram positive cocci



specimen 7:Gram positive coccispecimen 8:No bacteria is found and Gram negative bacilli

specimen 9:Gram positive cocci.
specimen 10:Capsules of gram positive cocc



Result and interpretation

Gram staining reveals two categories of bacteria ie,gram positive and gram negative bacteria.

Gram positive bacteria appear violet or purple.

Gram negative bacteria appear pink or red.

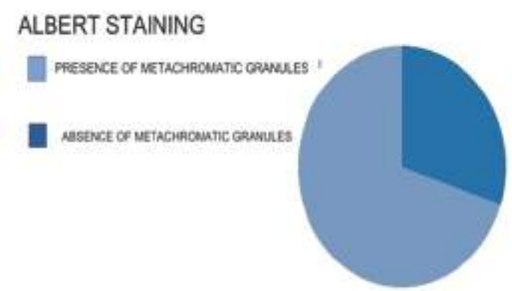
Limitations of Gram Staining

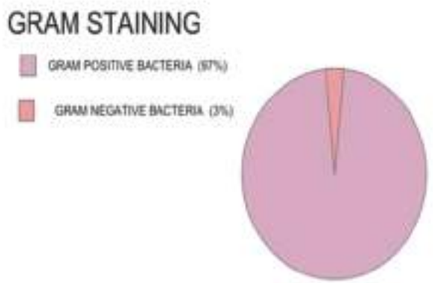
- Can't stain Acid Fast Bacilli and bacteria without cell wall like Mycoplasma.
- Unsuitable for minute bacteria like Ricktessia., Chlamydia., etc.
- Require multiple reagents.
- Over-decolorization may result in the identification of false gram-negative results, whereas under-decolorization may result in the identification of false gram-positive results.
- Smears that are too thick or viscous may retain too much primary stain, making the identification of proper Gram stain reactions difficult. Gram-negative organisms may not decolorize properly.
- Gram stains from patients on antibiotics or antimicrobial therapy may have altered Gram stain reactivity due to the successful treatment.

From this study, the 97 % were Gram Positive bacteria and 3%were Gram Negative for Gram staining . And for Albert staining out of 10 swabs collected 70% show presence of Metachromatic Granules while 30% were futile

STATISTICS OF THE STUDY

A Total of 20 swabs were taken from 10 patients. 10 for each Gram staining and Albert staining were enrolled in this study .The mean age of the patients were 18 to 23 year old . We did two staining Techniques on the swabs collected , the GRAM STAINING and





REFERENCES

- http://dx.doi.org/10.22192/ijarbs.2016.03.10.025
- Formation of volutin granules in Corynebacterium glutamicum Srinivas Reddy Pallerla, Sandra Knebel, Tino Polen, Peter Klauth, Juliane Hollender, Volker F. Wendisch, Siegfried M. Schoberth
- Bhumbla, Upasana. (2018). Albert's Staining. 10.5005/jp/books/14206_10.
- Murphy JR. Corynebacterium Diphtheriae. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 32.