



Safranin, 0.5% w/v

S027

Intended Use:

Safranin, 0.5% w/v is used as Gram's counterstain staining solution.

Composition**

Ingredients

Safranine O	0.500 gm
Ethyl alcohol	10.000 ml
Distilled water	100.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

- 1) Prepare a thin smear on clear, dry glass slide.
- 2) Allow it to air dry and fix by gentle heat.
- 3) Flood with Gram's Crystal Violet (S012) for 1 minute. (If over staining results in improper decolourization of known gram-negative organisms, use less crystal violet).
- 4) Wash with tap water.
- 5) Flood the smear with Gram's Iodine (S013). Allow it to remain for 1 minute.
- 6) Decolourize with Gram's Decolourizer (S032) until the blue dye no longer flows from the smear. (Acetone may be used as a decolourizing agent with caution, since this solvent very rapidly decolourizes the smear).
- 7) Wash with tap water.
- 8) Counter stain with 0.5% w/v Safranin (S027). Allow it to remain for 1 minute.
- 9) Wash with tap water.
- 10) Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective.

Principle And Interpretation

Safranin is used as counter stain in Gram staining procedure to differentiate between gram positive and gram negative organisms. The Gram stain is a differential staining technique most widely applied in all microbiology disciplines laboratories. It is one of the most important criteria in any identification scheme for all types of bacterial isolates. Different mechanisms have been proposed to explain the gram reaction. There are many physiological differences between gram-positive and gram-negative cell walls (1). Ever since Christian Gram has discovered Gram staining, this process has been extensively investigated and redefined. In practice, a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain (2). A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then the counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized. The gram-positive organisms or cells have more mucopeptide in their cell walls as compared to gram-negative ones. Gram-negative bacteria have more content of polysaccharides and lipo-proteins in their cell walls. The polymers of glycerol or ribitol phosphate called as teichoic acids are also found in the cell walls of gram-positive organisms but are very less or almost not present in gram-negative organisms. In a properly stained smear by gram staining procedure, the gram-positive bacteria appear blue to purple and gram negative cells appear pink to red.

Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4, 5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1, 3).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. (2)

Generally the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Use results of Gram stains in conjunction with other clinical and laboratory findings. Use additional procedures (e.g., special stains, inclusion of selective media, etc.) to confirm findings suggested by gram-stained smears(8)
2. Careful adherence to procedure and interpretive criteria is required for accurate results. Accuracy is highly dependent on the training and skill of microscopists (9)
3. Additional staining procedure, such as acridine orange are recommended for purulent clinical specimen in which no organisms are observed by gram stain method.
4. Gram stain positive, Gram stain -negative specimens may be the results of contamination of reagents and other supplies, presence of antimicrobial agents or failure of organisms to grow under usual culture condition (medium, atmosphere, etc.)
5. False Gram stain results may be related to inadequately collected specimens or delay in transit.

Performance and Evaluation

Performance of the stain is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Red coloured solution.

Clarity

Clear without any particles.

Microscopic Examination

Gram staining is carried out where Safranin is used as counterstain and staining characteristic of organisms are observed under microscope by using oil immersion lens.

Results

Gram-positive microorganisms : violet

Gram-negative microorganisms: pinkish red.

Storage and Shelf Life

Store between 10 - 30 °C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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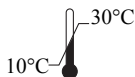
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In vitro diagnostic medical device



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Storage temperature



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