# Gram's Iodine

**S013**

## Intended use

Grams's Iodine is used as mordant in Gram's staining method.

## Composition**

| Ingredients | |
|---|---|
| Iodine | 1.0 ml |
| Potassium iodide | 2.0 ml |
| Distilled water | 300.0 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 decolourized the smear).
7. Wash with tap water.
8. Counter stain with 0.5% w/v Safranin (S027) for 20 seconds and rinses off with water.
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

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 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 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. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin. 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.

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## 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
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.
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. For professional 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.
2. Proper smear preparation is key to obtaining good gram staining results. Avoid excessive material or thick smears which may interfere with the passage of light and lead to distortion of images.
3. Overheating slides during heat fixation can distort the appearance of the organisms.
4. Only fresh cultures and specimens should be gram stained since cell wall integrity of older cells may give improper gram staining characteristics. Gram positive organisms that have lost cell wall integrity because of old age or antibiotic treatment may appear pink.
5. The decolorization step is the most important step in the gram-staining process. Over decolorization results in an abundance of bacteria that appear gram negative, while under decolorization results in too many bacteria that appear to be gram-positive.
6. The procedure given is based on an ideal thin smear of cells. Staining and decolorization times may vary depending on the sample and its thickness.
7. False Gram stain results may be related to inadequately collected specimens or delay in transit.

## Performance and Evaluation

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

## Quality Control

- **Appearance :** Yellow to dark brown coloured solution.
- **Clarity :** Clear without any particles.

- **Microscopic Examination :** Gram staining is carried out where Gram's Iodine is used as one of the stains and staining characteristics of organisms are observed under microscope by using oil immersion lens.
- **Results :** Gram-positive microorganisms : violet
  Gram-negative microorganims : 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 .

## Reference

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Storage temperature

Do not use if package is damaged

IVD

In vitro diagnostic medical device

CE Marking

HiMedia Laboratories Pvt. Limited,
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