



GRAM IODINE PVP

INTENDED USE

Remel Gram Iodine PVP is a reagent recommended for use in qualitative procedures to differentiate gram-negative from gram-positive organisms.

SUMMARY AND EXPLANATION

The Gram stain method was developed in 1884 by the Danish bacteriologist, Christian Gram, to differentiate bacterial cells from infected tissue.¹ Later it was discovered that identification of bacterial cell wall composition could be used to separate bacteria into two groups based on color. Since then, there have been many modifications of the original technique.^{2,3} Traditional formulations of Gram Iodine are made with aqueous KI-I₂ which loses its effectiveness upon exposure to air, light, and increased temperature. Gram Iodine PVP, a modification of the traditional formulation, contains the organic iodine complex, polyvinylpyrrolidone-iodine (PVP iodine) which is more stable than aqueous KI-I₂.⁴

PRINCIPLE

Crystal violet, the primary stain, is a basic dye taken up by all bacteria due to its ability to rapidly permeate the cell wall and stain the protoplast purple. Gram Iodine PVP is a mordant which complexes with the crystal violet in the protoplast of bacterial cells. In gram-positive bacteria, the crystal violet-iodine complex is retained due to decreased cell wall permeability. In gram-negative cells, the complex is removed by the decolorizer due to an increase in the cell wall permeability. The counterstain used is safranin, which stains gram-negative cells red.

REAGENTS (CLASSICAL FORMULA)*

Polyvinylpyrrolidone-Iodine Complex (CAS 25655-41-8)..... 100.0 g
Potassium Iodide (CAS 7681-11-0)..... 19.0 g
Demineralized Water (CAS 7732-18-5)..... 1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

CAUTION! May cause allergic skin reaction. May cause eye, skin, and respiratory tract irritation. May cause reproductive and fetal effects.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information on reagent chemicals.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at room temperature until used.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed from yellow-red, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.⁵⁻⁷

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Absolute Methanol (REF R40121) (optional), (7) Microscope, glass slides, (8) Staining rack, (9) Gram Crystal Violet (REF R40052), (10) Gram Decolorizer (REF R40054), (11) Gram Safranin (REF R40058).

PROCEDURE

1. Make a thin smear of the material for study and air dry. Fix with heat or by flooding with methanol. Allow methanol to evaporate.
2. Place the slide on a staining rack and overlay with Gram Crystal Violet for 1 minute.
3. Wash thoroughly with water and overlay with Gram Iodine PVP for 1 minute.
4. Flood with Gram Decolorizer until the solvent flows colorless from the slide (10-30 seconds).
5. Rinse with water and overlay with Gram Safranin for 30 seconds.
6. Rinse with water and allow the slide to air dry.
7. Examine microscopically under oil immersion lens (1000 X magnification).

INTERPRETATION

Gram-positive organisms stain purple.
Gram-negative organisms stain red.

QUALITY CONTROL

All lot numbers of Gram Iodine PVP have been tested and found to be acceptable. Positive and negative control slides should be tested following established laboratory procedures. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. Controls should be tested with each new lot number of Gram Iodine PVP and each week of use to verify the iodine solution is providing the proper mordant activity and that decolorization is performed correctly.⁷
2. When staining organisms from clinical specimens, it is recommended that growth from an 18-24 hour culture on a non-selective medium be used to achieve the most reliable results.⁷
3. When fixing smears, do not overheat the slide. Excessive heating will cause atypical staining.⁶
4. Gram-positive organisms contained in a specimen may appear gram-negative if the patient is on antimicrobial therapy.⁵




BIBLIOGRAPHY

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5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfalter. 2007. *Manual of Clinical Microbiology.* 9th ed. ASM Press, Washington, D.C.
6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. *Bailey and Scott's Diagnostic Microbiology.* 12th ed. Mosby Elsevier, St. Louis, MO.
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PACKAGING

REF R40061, Gram Iodine PVP 250 ml/Btl, 5 Btl/Pack
REF R40222, Gram Iodine PVP 1 Gallon

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

Manufactured for Remel Inc.
CAS (Chemical Abstracts Service Registry No.)

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