624-71 with 65092A, 65092B, 65092E

Microscopy

Harleco® Gram Stain with Lugol's Iodine Solution

IVD

In Vitro Diagnostic Medical Device

Intended Use

Harleco® Gram stain is used to qualitatively assess the differential staining of bacteria from cultures and human specimens by the modified Gram stain method.
Lugol's Iodine Solution is intended for use in the Harleco Gram Stain Procedure 65092-93.

Principle

Differential staining of bacterial cells in tissue by the Gram method was first mentioned in a treatise by Cad Freidlander,[3] in 1883. In 1884, Christian Gram[4] published a detailed account of his staining procedure. Three of the various modifications were found to give superior results: Hucker,[1,2,5] Burke,[6] and Kopeloff and Beerman.[7]

Our method uses the Gram technique except that the ethanol decolorizer solution has been replaced by an isopropanol/acetone decolorizer solution. The function of the isopropanol in the acetone is to decrease the rate of decolorization.[2] Both Gram-positive and Gram-negative cells take up the primary crystal violet stain. The addition of iodine causes the formation of an iodine-crystal violet complex within the cell wall.

Upon the application of decolorizer, lipid is extracted from the cell walls of Gram-negative bacteria, and the cell wall porosity is increased.[8,9] The iodine-crystal violet complex then diffuses from the cell.

Simultaneously, Gram-positive bacterial cells are dehydrated, cell wall pore size is decreased, and the iodine-crystal violet complex is trapped within the cell.[8,9]

The increased porosity of the Gram-negative cells allows the safranin counterstain to enter the bacteria.

Sample material

Immediately upon obtaining specimen, prepare slide as indicated. Once prepared, the specimen may be stored indefinitely at room temperature in appropriate containers e.g., slide boxes.

Cultured Specimens

Liquid Media:

1. Sterilize wire loop by flaming with a Bunsen burner; allow to cool.
2. Dip loop into suspension and spread on clean glass slide.
3. Air dry smear and heat fix by flaming with a Bunsen burner; allow slide to cool before staining.

Solid Media:

1. Sterilize wire loop by flaming with a Bunsen burner; allow to cool.
2. Place loop of ultrapure water on a clean glass slide.
3. Sterilize wire loop by flaming with a Bunsen burner; allow to cool.
4. Touch needle to colony, suspend in water droplet, and spread evenly on slide.
5. Air dry smear and heat fix by flaming with a Bunsen burner; allow slide to cool before staining.

Human Specimens:

1. Complete explanation of collection of specimens from patients can be found in the Manual of Clinical Microbiology.[10]
2. Obtain specimen with sterile loop, swab or pipette.
3. Smear specimen on a clean glass slide, air dry, and heat fix; allow slide to cool before staining.

Reagents

Cat. No. 65092A

Harleco® Crystal Violet Solution Gram 500 mL

Crystal violet, 100% P.D.C.* (3.5 g/L)

SDA-3A Alcohol, 95% Ethyl, 5% Methyl Alcohol (200 mL/L) Preservative

WARNING! CAUSES EYE IRRITATION. Avoid contact with eyes. Wash thoroughly after handling. **COMBUSTIBLE**. Keep away from heat and open flame.

FIRST AID: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.

Cat. No. 65092B

Harleco® Safranin Stain 500 mL

Safranin O, 100% P.D.C. (6.04 g/L) SDA-3A Alcohol 95% Ethyl, 5% Methyl Alcohol (200 mL/L)

WARNING! COMBUSTIBLE. Keep away from heat and open flame.

Cat. No. 624-71

Harleco® Lugol's Iodine Solution 500 mL

Diluted iodine, potassium iodide aqueous solution

WARNING! CAUSES EYE IRRITATION. Avoid contact with eyes. Wash thoroughly after handling. FIRST AID: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.

Cat. No. 65092E

Harleco® Decolorizer 500 mL

Acetone (200 mL/L) Isopropyl Alcohol 99% (800 mL/L)

DANGER! EXTREMELY FLAMMABLE.

CAUSES EYE IRRITATION: Keep away from heat, sparks, and open flame. Keep container closed. Use with adequate ventilation. Avoid contact with eyes. Wash thoroughly after handling.

FIRST AID: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician. *Pure Dye Content

Also required:

Cat. No. 64969

Harleco® Krystalon™ Mounting Medium

Cat. No. 104699

Immersion oil for microscopy

Sample preparation

The sampling must be performed by qualified personnel. All samples must be clearly labeled. Suitable instruments must be used for collecting and preparing samples. Follow the manufacturer's instructions for application/use.

Reagent preparation

All solutions provided in the kit are ready-to-use.

Staining Procedure

Reagent Materials Required

65092A Crystal Violet Solution Gram

65092B Safranin Stain

624-71 Lugol's Iodine Solution

65092E Decolorizer

Materials Required But Not Provided

Wire loop or inoculating needle

Bunsen burner

Glass slides

Cover slips

Timer accurate to 15 seconds per minute

Absorbent paper

Immersion oil

Mounting media

Microscope, with oil immersion lens

Procedure: Flood Slide Method

1. Flood prepared slide with Crystal Violet Solution Gram. Allow to stand one minute.
2. Rinse gently with distilled water.
3. Flood slide with Lugol's Iodine Solution: allow to stand one minute.
4. Rinse gently with distilled water.
5. Decolorize specimen by rinsing with Decolorizer for 10 seconds. Decolorization is complete when solution runs colorless from slide.
6. Wash gently, but thoroughly, with distilled water.
7. Flood slide with Safranin Stain; allow to stand 1 minute.

8. Rinse gently with distilled water.
9. Blot with absorbent paper.
10. Examine prepared specimen under oil immersion lens.

Stability of Prepared Slide

Once the Gram stain has been performed, the resulting slide is stable indefinitely, particularly if covered with a glass coverslip and mounting medium.

Quality Control

To check the stability of the reagents and stains, perform Gram stains on an 18 to 24 hr. old culture of *S. aureus* and *E. coli*. *S. aureus* yields Gram-positive, purple cocci, and *E. coli* yields Gram-negative, pink to red rods. If these results are not obtained the following checks should be made:

1. Stain a prepared slide of 18 to 24 hr. old cultures of *S. aureus* with Crystal Violet Solution Gram. Rinse gently with cold water. (Gram-positive, purple cocci should be observed; if not, discard stain.) 2. Stain a prepared slide of 18 to 24 hr. old cultures of *E. coli* with Safranin Stain. Rinse gently with cold water. (Gram-negative, pink to red rods should be observed; if not, discard stain.)

3. Stain a prepared slide of 18 to 24 hr. old cultures of *S. aureus* with Crystal Violet Solution Gram. Rinse gently with cold water. Flood slide with Lugol's Iodine Solution and allow to stand for 1 minute. Decolorize by rinsing with Decolorizer for 10 seconds or until solution runs colorless. At this point, the specimen should be purple; if not, the Iodine Solution is not forming an iodinecrystal violet complex and should be discarded.

Results

Gram positive – purple
Gram negative – pink to red

Application Notes:

The microscope used should meet the requirements of a medical diagnostic laboratory.
staining systems, please follow the instructions
The freshly prepared staining solutions should be filtered before use.

Remove surplus immersion oil before filing.

Diagnostics

Diagnoses are to be made only by authorized and trained personnel. Valid nomenclature must be used.
Further tests must be selected and implemented according to recognized methods. Suitable controls should be conducted with each application to avoid an incorrect result.

Storage

The staining reagents, decolorizer and Lugol's Iodine Solution should be stored in their original containers at room temperature (15-25 °C). Storage must not exceed expiration date on box label. Precautions should be taken to minimize exposure to air and heat because the Lugol's Iodine Solution is relatively unstable and may show a decrease in iodine concentration over time. It is therefore important that controls be included in the daily work regime to ensure that the iodine solution is providing the proper mordant activity.

Indications of Deterioration

Some precipitation may occur in the Crystal Violet Solution Gram. If it appears to affect staining results, warm in a 37 °C incubator, then shake until precipitate is dissolved. Filter before use.

Refer to Quality Control section for additional information.

Shelf-life

The Harleco Crystal Violet Solution Gram, Harleco Safranin and Harleco Lugol’s Iodine Solution for microscopy can be used until the stated expiry on the packaging.

The Harleco Decolorizer Solution can be used until the stated expiry on the packaging.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at 15-25 °C.

The bottles must always be kept tightly closed.

Additional instructions

For professional use only.
The application must be carried out by qualified personnel only.

National guidelines for work safety and quality assurance must be followed.

Microscopes equipped according to the standards must be used.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines. Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines.

Auxiliary reagents

Cat. No. 64969	Harleco® Krystalon™ Mounting Medium	50 mL, 500 mL
Cat. No. 104699	Immersion oil for microscopy	50 mL, 500 mL

Hazard classification

Please observe the hazard classification printed on the label and the information given in the safety data sheet. The safety data sheet is available on the website and on request.

Replacement items for this staining procedure:

65092A-95	Crystal Violet Solution Gram	4x500 mL
65092B-95	Safranin Solution	500 mL
624-71	Lugol's Iodine Solution	500 mL
65092E-95	Decolorizer	500 mL
65092A-85	Crystal Violet Solution Gram	4 L
65092B-85	Safranin Solution	4 L
65092E-85	Decolorizer	4 L

Literature

1. Hucker, G.J. J. Bacteriol. 6:395; 1921
2. Hucker, G.J. and Conn, N.J., NY Agr. Exp. Sta. Tech. Bull. 128:1, 1927
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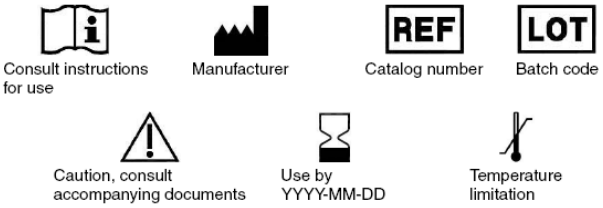

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