PRO-LAB DIAGNOSTICS

GRAM STAINS

(for *in vitro* diagnostic use)

CE

INTENDED USE

For use in the Gram's Staining method for the initial differentiation of Gram Positive and Gram Negative bacteria.

SUMMARY AND EXPLANATION

The Gram stain was originally devised by Christian Gram in 1884. The standard Gram's staining method can be used to differentiate intact, morphologically similar bacteria into two groups. This is based on cell wall colour after employing the staining method. In addition, cell form, size and structural details are evident. This preliminary information can provide initial clues to the type of organism(s) present.

PRINCIPLE

A Crystal Violet-Iodine complex forms in the protoplast of all organisms stained using the above procedure. After decolorizing, those organisms that are able to retain this dye complex are classified as Gram positive. Those organisms that are decolorized and take up the counterstain are classified as Gram negative.

Upon disruption or removal of the cell wall, the protoplast of Gram positive as well as Gram negative cells can be decolorized, and hence the Gram negative attribute lost. Therefore, the mechanism of the Gram stain appears to be related to the presence of an intact cell wall able to act as a barrier to decolorization of the primary stain. Generally, the cell wall is non-selectively permeable. It is theorized that during the Gram stain procedure, the cell wall of Gram positive cells is dehydrated by the alcohol in the decolorizer and loses permeability, hence it retains the primary stain. In the case of the cell wall of the Gram negative cells, due to a higher lipid content, the cell wall becomes more permeable when treated with alcohol, hence the primary stain is lost, allowing for the later counterstain to be taken.

REAGENTS

Ready to use stains.

PL.7000	Crystal Violet	500 ml
PL.7001	Crystal Violet	1 litre
PL.7002	Crystal Violet	2 litres
PL.7000/25	Crystal Violet	250 ml
PL.7003	Gram's Iodine	500 ml
PL.7004	Gram's Iodine	1 litre
PL.7005	Gram's Iodine	2 litres
PL.7003/25	Gram's Iodine	250 ml
PL.7006	Gram's Differentiator	500 ml
PL.7007	Gram's Differentiator	1 litre
PL.7008	Gram's Differentiator	2 litres
PL.7006/25	Gram's Differentiator	250 ml
PL.7009	Neutral Red	500 ml
PL.7010	Neutral Red	1 litre
PL.7011	Neutral Red	2 litres
PL.7009/25	Neutral Red	250 ml
PL.7012	Safranin	500 ml
PL.7013	Safranin	1 litre
PL.7014	Safranin	2 litres
PL.7012/25	Safranin	250 ml
PL.7015	Dilute Carbol Fuchsin	500 ml
PL.7016	Dilute Carbol Fuchsin	1 litre
PL.7017	Dilute Carbol Fuchsin	2 litres
PL.7015/25	Dilute Carbol Fuchsin	250 ml
PL.7052	Lugol's Iodine	500 ml
PL.7053	Lugol's Iodine	1 litre
PL.7053-2	Lugol's Iodine	2 litres
PL.7056	Iodine Acetone	500 ml
PL.7057	Iodine Acetone	1 litre
PL.7058	Iodine Acetone	2 litres
PL.7101	Basic Fuchsin / Neutral Red	500 ml
PL.7102	Basic Fuchsin / Neutral Red	1 litre
PL.7103	Basic Fuchsin / Neutral Red	2 litres
PL.7073	C. Violet - Ammonium Oxalate	500 ml
PL.7074	C. Violet - Ammonium Oxalate	1 litre
PL.7075	C. Violet - Ammonium Oxalate	2 litres
PL.7110	Sandifords Stain	500 ml
PL.7111	Sandifords Stain	1 litre
PL.7112	Sandifords Stain	2 litres
PL.7113	Methyl Violet	500 ml
PL.7114	Methyl Violet	1 litre
PL.7115	Methyl Violet	2 litres
PL.7116	Safranin / Neutral Red	500 ml
PL.7117	Safranin / Neutral Red	1 litre
PL.7118	Safranin / Neutral Red	2 litres
PL.7206	Grams Differentiator (Acetone)	500 ml
PL.7207	Grams Differentiator (Acetone)	1 litre
PL.7208	Grams Differentiator (Acetone)	2 litres
PL.7306	Grams Differnetiator (IMS)	500 ml
PL.7307	Grams Differentiator (IMS)	1 litre
PL.7308	Grams Differentiator (IMS)	2 litres

Concentrated Stains. Dilute to 1 litre with distilled water before use.

PL.8000	Crystal Violet	100 ml
PL.8001	Gram's Iodine	100 ml
PL.8002	Neutral Red	100 ml
PL.8003	Safranin	100 ml
PL.8004	Dilute Carbol Fuchsin	100 ml
PL.8010	Lugol's Iodine	100 ml
PL.8011	Methyl Violet	100 ml

Concentrated Stains. Dilute to 4 litres with distilled water before use.

PL.8000-4.0	Crystal Violet	400 ml
PL.8001-4.0	Gram's Iodine	400 ml
PL.8002-4.0	Neutral Red	400 ml
PL.8003-4.0	Safranin	400 ml
PL.8004-4.0	Dilute Carbol Fuchsin	400 ml
PL.8010-4.0	Lugol's Iodine	400 ml
PL.8011-4.0	Methyl Violet	400 ml

Concentrated Stains. Dilute to 5 litres with distilled water before use.

PL.8000-5.0	Crystal Violet	500 ml
PL.8001-5.0	Gram's Iodine	500 ml
PL.8002-5.0	Neutral Red	500 ml
PL.8003-5.0	Safranin	500 ml
PL.8004-5.0	Dilute Carbol Fuchsin	500 ml
PL.8010-5.0	Lugol's Iodine	500 ml
PL.8011-5.0	Methyl Violet	500 ml

Staining Kits (Ready to use)

PL.8055/25 Gram Staining Kit - Crystal Violet 250 ml, Gram's Iodine 250 ml, Gram's Differentiator 250 ml, Safranin 250 ml.

PL.8056/25 Gram Staining Kit - Crystal Violet 250 ml, Gram's Iodine 250 ml, Gram's Differentiator 250 ml, Neutral Red 250 ml.

PL.8057/25 Gram Staining Kit - Crystal Violet 250 ml, Gram's Iodine 250 ml, Gram's Differentiator 250 ml, Dilute Carbol Fuchsin 250 ml.

Immersion Oil (Reduced hazard - DBP free)

PL.396 Immersion Oil 50 ml

SAFETY PRECAUTIONS

1. Gram stains from Pro-Lab Diagnostics are offered as an *in vitro* material and are in no way intended for a curative or prophylactic purpose.
2. During and after use, handle all materials in a manner conforming to Good Laboratory Practices and consider at all times that material under test should be regarded as a potential biohazard.
3. The device poses no environmental hazard in excess of those posed by the clinical specimens used with the device. Safety precautions should be taken in handling, processing and discarding all clinical specimens as a pathogenic organism may be present. Environmental impact exists and is adequately addressed through proper disposal.

STABILITY AND STORAGE

Room Temperature. Away from sources of ignition. Away from direct sunlight. Stored under these conditions, reagents may be used up to the date of expiry on the label.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES

Refer to a standard microbiology text.

MATERIALS REQUIRED BUT NOT PROVIDED

Clean glass slides, sterile loop, flame / hot air, staining rack, tap water, immersion oil, microscope, blotting paper or equivalent substitute.

PROCEDURE

1. Prepare a thin, uniform smear of specimen and air dry.
2. Heat fix and allow to cool.
3. Flood the slide with Crystal Violet or Methyl Violet, stand for 1 minute. Rinse with water.
4. Flood the slide with Gram's or Lugol's Iodine, stand for 1 minute. Rinse with water.
5. Gently decolorize with Differentiator for approximately 10 seconds or Iodine Acetone for 1 minute. Rinse with water.
6. Flood the slide with counterstain, stand for 30 – 60 seconds.
7. Rinse well with water, gently blot dry.
8. View using oil immersion microscopy.

QUALITY CONTROL

The age of the cultures and the pH of the medium in which the bacteria are grown can markedly affect their reaction to the Gram stain. Use fresh cultures up to 24 hours old.

Recommended QC cultures;

- *Escherichia coli* NCTC 10418 (Pink to Red Gram Negative Bacilli)

Canada ☎ 800 268 2341 Fax 905 731 0206
20 Mural Street, Unit #4, Richmond Hill, ON, L4B 1K3

U.S.A. ☎ 800 522 7740 Fax 800 332 0450
21 Cypress Blvd., Suite 1070, Round Rock, Texas, 78665-1034

U.K. ☎ 0151 353 1613 Fax 0151 353 1614
3 Bassendale Road, Bromborough, Wirral, Merseyside, CH62 3QL

- Oxford *Staphylococcus aureus* NCTC 6571 (Blue to Purple Gram Positive Cocci)
- Haemolytic *Streptococcus* Group A NCTC 8198 (Blue to Purple Gram Positive Cocci)

INTERPRETATION OF RESULTS

Gram Positive organisms – Blue to Purple.
Gram Negative organisms – Pink to Red.

LIMITATIONS

1. False Gram Negative and Gram Positive staining results can be seen due to cellular debris being stained by the technique. e.g. – The nuclei and protoplasm of white blood cells and epithelial cells are stained with counterstain. Solid particulate matter may also be stained by the Crystal Violet.
2. The Gram stain provides preliminary identification information only and is not a substitute for specimen culture.

REFERENCES

1. Manual of Clinical Microbiology. Lennette.
2. The Practice of Medical Microbiology. 12th Edition. V2. R. Cruickshank, J. P. Duguid, B. P. Marmion, R.H.A. Swain.