REF: HS001 4 x 250ml

CYPRESS DIAGNOSTICS

CE IVD

Gram PVP

Intended use
The Gram stains are used to perform the staining of microorganisms, cultures or samples using the Gram differential method.
For *in vitro* diagnostic use only.
For professional use only.

Clinical significance
The Gram staining provides information about the structure of the cell wall and the microbial morphology. This way, essential clues are obtained about potential pathogens present in the patient's sample, allowing their further characterization.

Principle
The Gram stain is a system with two simple successive staining steps separated by a decoloring step. The bacteria that retain the primary dye appear as blue (Gram positive) whereas the ones that lose it are stained pink by the counterstain (Gram negative). The differences in the cell wall structure account for this phenomenon. Gram positive bacteria have a much higher proportion of peptidoglycan in their cell wall, which gets dehydrated by the alcohol the decolorizing agent and this way loses its permeability, retaining the primary dye. On the other hand, Gram negative bacteria have a cell wall with a high lipid content, whose permeability increases upon treatment with the alcohol-containing decolorizer and thus release the primary stain.

Reagent composition

A: Crystal Violet (1 x 250 ml):	
Crystal Violet	0.40%
Ethanol	15%
Phenol	<1%
B: Iodine PVP (Lugol) (1 x 250 ml):	
Iodine	1%
Potassium Iodide PVP (stabilizer)	2%
PVP stabilized	
C: Gram Decolorizer (1 x 250 ml):	
Ethanol	70%
Acetone	30%
D: Safranin (1 x 250 ml):	
Safranin	0.40%
Ethanol	20%

Precautions
Crystal Violet: Warning. H226 – Flammable liquid and vapour. P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 – Keep container tightly closed. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P370+P378 – In case of fire: Use the means described in point 5 of the Safety Data Sheet. P403+P235 – Store in a well-ventilated place. Keep cool. P501 – Dispose of contents/container according to point 13 of the Safety Data Sheet.
Gram Decolorizer: Danger. H225 – Highly flammable liquid and vapour. H319 – Causes serious eye irritation. H336 – May cause drowsiness or dizziness. P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 – Keep container tightly closed. P261 – Avoid breathing dust/fume/gas/mist/vapours/spray. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P370+P378 – In case of fire: Use the means described in point 5 of the Safety Data Sheet. P403+P235 – Store in a well-ventilated place. Keep cool.
Safranin: Warning. H226 - Flammable liquid and vapour. P210 – Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 – Keep container tightly closed. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P370+P378 – In case of fire: Use the means described in point 5 of the Safety Data Sheet. P403+P235 – Store in a well-ventilated place. Keep cool. P501 – Dispose of contents/container according to point 13 of the Safety Data Sheet.
All waste should be properly disposed of in accordance with the applicable local regulations.

Preparation
All reagents are ready for use.

Storage and stability
The reagents will remain stable until the expiration date stated on the label when stored at 15-30°C protected from light. Containers must always be kept tightly closed.
A light precipitate may form for some reagents over time. This, nevertheless, does not affect their functionality.

Additional equipment required but not provided
- Standard microbiology lab equipment and supplies like microscopy slides, Bunsen burner, inoculation loop and filter paper
- Device for staining (manual or automated)
- Microscope with immersion lens

Samples
Smears of bacterial cultures. Samples of various body fluids: sputum, lung fluid, urine sediment, cerebrospinal fluid, tissue, etc.
Spread the sample with an inoculation loop onto a slide to obtain a uniform and thin smear. Air dry and heat-fix by passing the slide through a low flame 2 or 3 times. Leave to cool before performing the staining.
Handle the samples with care due to their potentially infectious nature.

Procedure
1. Cover the smear with Crystal Violet (Reagent A). Let stand for 1 min.
2. Remove excess by rinsing with tap water.
3. Cover with iodine PVP solution (Reagent B) and allow to stand for 1 min.
4. Decant and rinse with tap water. Decant excess water.
5. Decolorize with the Gram Decolorizer (Reagent C) until waste is colourless, around 15-30 seconds depending on the thickness of the smear.
6. Rinse with tap water and decant excess water.
7. Finally, counterstain with Safranin (Reagent D) for 1 min.
8. Rinse with tap water and air dry.
9. Examine under the microscope with an immersion lens.

Interpretation of the results
Gram-positive bacteria: dark violet.
Gram-negative bacteria: pink – red.

Notes
The results of the staining should be treated as a guide and must be confirmed with additional tests. The technique outlined above may be modified in accordance to the technician's preferences in order to obtain variations in the staining intensity. This entails modifications in the staining, de-staining and rinsing times.
Using cultures that are 18-24 hours old maximum and fresh smears is recommended, since old cultures and preparations may give erroneous results.
It is important to control the heat fixation process in order not to alter the microbial cell wall structures.
Antibiotic treatment previous to sample collection may produce alterations in the staining, even causing Gram negative bacteria to get stained as Gram positive bacteria.
If running tap water is used for the rinsing, please be aware that strongly chlorinated water may weaken the counterstaining.

Quality Control
The use of QC samples is recommended in order to assess the appropriate staining of the sample components. Each laboratory should establish its own QC scheme and corrective actions if the controls do not fulfil the established criteria.

We recommend following the QC practices defined by the CLSI. For this purpose carry out controls with ATCC microorganisms or other previously characterized control strains.

Bibliography

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3. CLSI Guidelines and Standards, CLSI, Wayne, P.A
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05.2020, Rev. 6.0

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