SP410 HISTO GRAM STAIN KIT GCC

Differentiation of microorganisms in smears and sections

General & Useage information
The Gram Stain procedure differentiates bacterial cells into 2 groups. Those cells that retain primary dye (Crystal Violet) are termed Gram Positive, those which loose the primary dye after the decolourisation step and take up the counterstain are termed Gram Negative.
This kit contains the reagent required to carry out the Gram Stain technique:

Kit contents

Crystal violet (Primary)	250ml	Iodine solution	250ml
Gram Differentiator	250ml	Counterstain solution	250ml
Picric acetone	250ml	Pipettes	

Storage
Keep all kit reagents tightly closed in the box provided and store in a darkened cupboard at room temperature.

Set up
Make a hole in the top of the dispenser nozzle or cut off the top 3mm on the reagent bottles so that the nozzle produces a small flow of reagent when gently squeezed.
Picric acetone in the interests of safe storage is supplied in T/E sealable bottles - use disposable pipettes to apply the reagent to the slides or a coplin jar or similar. Wipe up spills of this reagent to prevent the buildup of dry picric acid on the bottle or any other surface. Risk of ignition and explosion.

Additional reagent required – Xylene for de-waxing & mounting, alcohols for re-hydration to water. DPX for mounting coverslips if required.

Controls
Ensure a known positive slide is run in the reagent at the same time as the sample to ensure that the reagent system is working correctly. Cataloge CODE: CS 22 Gram Control Tissue pack of 5 or 25.

Method
Wet Procedure.

1. De-wax and rehydrate sections through graded alcohols to water.
2. Flood the slide with crystal violet-oxalate solution for 1- 2 minutes.
3. Wash off the excess dye by swirling the slide gently in a beaker of deionised water .
4. Flood slide with iodine solution, drain off the excess, again flood the slide with iodine solution for 1 minute.
5. Wash the slide in deiomised water, overwashing in difficult at this stage. Blot dry the but not the tissue.
6. Decolourise by running the acetone differentiator over the tissue until the blue-violet dye stops running from the slide. Do not over-differentiate as you will remove dye from Gram Positive organisms. Under-differentiation will fail to remove the violet dye from Gram Negative organisms. After correct differentiation Gram Positive organisms should be purple-black and the background tissue clear. Check microscopically if needed before proceeding to the next step. Rinse immediately in running water to stop the action of the differentiator. Blot dry the slide but not the tissue.
7. Flood slide with counterstain for 1 - 2 minutes.
8. Rinse gently to remove excess counterstain. in deionised water .WARNING - Excessive washing at this stage may remove the counterstain from Gram negative organisms.
9. Rinse slide for 2 seconds with acetone differentiator , drain off excess and apply Picric acetone until section has pink- yellow (salmon) colour. At this stage check control slide for correct differentiation. (Return to picric acetone step if required.
10. Rinse with acetone differentiator, air dry or rinse briefly in 50/50 actone xylene, then xylene and mount with DPX or similar.

The method given in this leaflet is designed as a general guide only in using this kit for Gram stain procedures. The laboratory should modify this method to suit its requirements.

Results

Gram Positive organisms	-	Purple blue-black
Fibrin, Paneth cell granules, Keratohyalin, keratin, some fungi	-	Shades of blue
Gram Negative microorganisms	-	Shades of red
Nuclei	-	Red
Other tissue elements	-	Yellow

Saftey Data.
Crystal violet reagent poses no significant risk in normal use. Do not consume.
Gram differentiator contains Acetone is Highly Flammable. Keep away from sources of ignition. Use in a ventilation hood. Do not breathe vapour. Do not consume.
Grams iodine reagent is very dilute and poses no significant risk in normal use. Do not consume. May irritate eyes and sensitive skin. If in contact with eyes use eye-bath for 5 minutes. If soreness persists seek medical attention.
Gram fuchsin poses no significant risk in normal use. Do not consume.
Picric acetone is Flammable and Toxic. Dry picric acid is explosive and capable of ignition by heat and friction. It forms dangerous explosive compounds with metals. Keep away from metallic components and containers. Although the risk maybe small- be aware of this danger. Keep locked away safe and well away from sources of heat and ignition.
All reagents except Gram differentiator will stain skin and other tissue. If in contact wash with soap & water. For use as an *In-Vitro* diagnostic laboratory reagent only.
When using this kit wear suitable eye/ skin protection and use in a well ventilated area or hood.
H203 Explosive, fire, blast hazard
H224 Extremely flammable liquid and vapour
H301+311 Toxic if swallowed and in skin contact
H332 Harmful if inhaled
H320 Causes eye irritation
H400 Very Toxic to aquatic life
P210 Keep away from heat and sources of ignition
P233 Keep tightly closed
P250 Do not subject to shock or friction
P260 Do not breathe vapour
P262 Do not get in eyes, on skin or clothing
P281 Use personal protective equipment
P314 get medical attention if you feel unwell or symptoms persist.

IF SWALLOWED – rinse mouth with water several times.
IF IN EYES – rinse with eye-bath.
IF ON SKIN – wash with soap and water.

For more complete information on Health & Safety, Storage, Fire-fighting , Transport & Spillage please read the MSDS for this product.

Accidental spillage & Waste Disposal.
Gram differentiator and Picric acetone reagent are Highly Flammable. They are volatile in air so spillage vapours may present an explosion hazard in small spaces if mixed with air.
Picric acetone - Mop up spillage immediately using cloths/ tissue and place in large volume of water to dilute the solvent and keep material wet . It is essential that no picric acid residues remain and dry as they pose an explosion risk. Picric acid contaminated cloths should be disposed as hazardous waste. Small liquid volumes can be run to public sewer (local regulations should be consulted beforehand) diluting greatly with running tapwater. Acetone be evaporated to atmosphere. For all other reagents in this kit clean the spillage area with detergent & water and run the waste to sewer drains.

Unsatisfactory performance.
As part of our duty to monitor product performance and part of our policy of continual improvement. Please report to us any unsatisfactory performance you may experience with this product. If any reagent degrades before expiry of shelf life we will replace that reagent free of charge.

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