

IVD dispositivo medico-diagnostico in vitro

Crystal violet solution for Gram Hucker technical's information's
 Technical card 09-312
 Product code 09-312
 Pack 200-500 ml or on request
 Stability of product properly conserved at 15-20°C 24 months
 CND code W01030799

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For emergency contact your nearest anti-poison unit.

Principle

DDKItalia Gram stain reagents are intended for use in the delineation of Gram-Positive and Gram-Negative organisms in films and tissue. Gram Stain reagents are for "In Vitro Diagnostic Use". Gram stain is used clinically to delineate two distinct groups of microorganisms. Those which retain primary dye (crystal violet) are called Gram positive. Those which lose the primary dye during a decolorization step are called Gram negative. The mechanisms whereby Gram positive organisms retain the primary stain are unknown, although the chemistry and structure of cell walls are most certainly involved. Numerous modifications of the original Gram method have been described. This procedure is based on the work of Hucker utilizing a crystal violet-ammonium oxalate solution that aids in differentiation and is quite stable.

Specimen. 10% neutral buffered formalin. Standard paraffin wax sections.

Procedure & notes. Care should be taken to avoid over decolorization of the slides with the Decolorizer solution. Even Gram positive organisms will become colourless and appear red if the Decolorizer solution is left on the slide for excessive periods of time. The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

Films

- | | |
|---|--|
| 1. Flood film with crystal violet solution, for | 1 minute. |
| 2. Rinse thoroughly in deionised water. | |
| 3. Flood film with Gram's iodine solution, for | 1 minute. |
| 4. Rinse thoroughly in deionised water. | |
| 5. Flood with decolorize solution, for up to | 10 seconds until colour stops running. |
| 6. Rinse thoroughly in deionised water. | |
| 7. Flood film with safranin O solution, for | 1 minute. |
| 8. Rinse thoroughly in deionised water. | |
| 9. Air dry film and examine microscopically. | |

Tissue

- | | |
|---|----------------|
| 1. Deparaffinize sections and hydrate to deionised water. | |
| 2. Place slides on staining rack and cover sections with crystal violet solution, for | 1 minute. |
| 3. Drain off crystal violet solution and rinse thoroughly in deionised water. | |
| 4. Mordant in Gram's iodine solution, for | 5 minutes. |
| 5. Rinse in deionised water and blot sections. | |
| 6. Differentiate in absolute alcohol or acetone. | |
| 7. Rinse in deionised water. | |
| 8. Cover slides with safranin O solution, for | 30-60 seconds. |
| 9. Rinse in deionised water and blot sections. | |
| 10. Cover sections with tartrazine solution, for | 5-10 seconds. |
| 11. Blot off excess stain. | |
| 12. Rinse in 2 changes absolute alcohol. Clear in xylene. Mount with DdMount | |

Result

Gram positive organisms	purple
Gram negative organisms	red

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References

Gram C: Ueber die isolierte färbung der schizomyceten in schnitt und trockenpräparaten. Fortschr Med 2:185, 1884. Hucker GJ, Conn HJ: Further studies on the methods of Gram staining. NY Agric Exp. Stn Tech Bull No. 128, 1927. Histopathological Technic and Practical Histochemistry, 3rd ed., RD Lillie, Editor. McGraw-Hill,

* Notes.

Distilled water or tap water can be used for rinsing and moisturizing.

Always check the pH of your tap water and chlorine levels before proceeding with any type of biological tissue and stain.

* Technical's note: staining time vary according to age, types of solutions, thickness of sections, et. When Gill (code 09-178) modified solution is used, get the best result, staining time (maximum 1-5 minutes), for best change in color, wash quickly in tap water, and then in Scott acidulated solution, (code 00-136). For sections fixed in Bouin, we recommend the use of haematoxylin modified acid AB (code 09-183).

** Please note the alcoholic loses eosin stain with the use, of the days are stretched over time colouring. If you are using purified eosin, check the time, and possibly diluted in ethyl alcohol 96°C, if the cytoplasmic staining was too strong. Before use, filter the following solutions; alcoholic eosin, eosin phloxine; Harris haematoxylin, Gill's haematoxylin. The acidified aqueous solution of eosin is prepared by slowly adding glacial acetic acid. Follow normal precautions for laboratory reagents. Dispose of waste according to regulations at the local, regional or national level. Refer to Data Sheet. Material Safety Data for updated information on risks, hazards and safety associated with the use of these products.

* Risk and Safety Statements outside the EU.

The eosin solution in alcohol is flammable and harmful. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible). Target organs: eyes and nerves. Eosin in aqueous solution. Caution: substance not yet fully tested. Avoid contact and inhalation of the solution of Harris haematoxylin. Organs: heart and nerves. Solutions based hemallum are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing. Organs affected: liver and kidneys. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

* Risk and Safety Statements (U.E.)

The eosin solution in alcohol is highly flammable and harmful. Highly flammable. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible). Eosin in aqueous solution. Caution: Substance not yet fully tested. Solution of hemallum. Do not breathe vapors. Avoid contact with skin and eyes. Gill haematoxylin Solutions are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing.

* The microscope used should meet the requirements of a medical diagnostic laboratory. Carefully follow the instructions for the fixative. If an automated tool was used for staining, follow the instructions of the equipment and software. Remove surplus immersion oil before storing.

* Sample preparation

All samples must be treated according to the technology. All samples must be marked so as to be easily identified. Tools should be used for sampling and sample preparation, which must be observed strictly to manufacturer's instructions about the application and instructions.

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* Diagnostics

The diagnosis should be performed only by authorized and trained persons. Valid nomenclatures must be used. Further tests must be selected and implemented according to recognized methods.

* Conservation. The staining solution should be stored at a temperature between +15°C to 20°C, the dye at +5°C to 30° C. Store at 4-6 °C all kit containing silver solutions and Schiff reagents. The solution and dyes must be used before the expiration date. Stability. After first opening the bottle, the dye solution and the dyes are stable until the expiration date when stored at the temperature requested. Always keep the bottles tightly closed.

* Instructions for use

To avoid errors, the staining process must be performed by qualified personnel. For professional use only. Must observe the National guidelines for work safety and quality assurance. Microscopes are used according

to the standard. Protection against infection. Must be taken with laboratory guidelines for the protection against infection.

* Instructions for disposal

The solutions used and those have expired must be disposed of as special waste according to local regulations regarding disposal of waste.

Endnotes

1 The timing suggested in the leaflet are approximate and may vary according to your specific needs. If they are used intensively, for staining solutions may lose their dyes, so it is necessary to extend the time of staining solutions, or replace with new products.

2. Include positive control slides in each session.

3. Some hydraulic systems deliver acidic water, unsuitable for use for the part of the procedure for the blue coloration. If tap water is acidic, instead using a dilute alkaline solution, for example, water buffered by Scott.

4. The presence of purple or red-brown nuclei a blue color indicates unsatisfactory.

5. If you over-eosin staining, nuclear staining may be masked. If done correctly, with eosin staining shows a three-tone effect. To increase the differentiation of eosin, extend the time of immersion in alcohol, or use a first alcohol with a higher water content. You can adjust the times of immersion in alcohol to obtain an adequate eosin staining.

6. We do not recommend the addition of stock solution in the working solutions of haematoxylin and eosin.

7. Avoid excessive drag (carryover) of water solutions in alcoholic eosin.

8. The data generated by this procedure are to be used only to support the diagnosis and should be evaluated in conjunction with other tests and diagnostic data

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