DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Methylene blue Mallory solution technical information
Technical card code 09-123
Product code 09-123
Stability of product properly conserved at 15-25°C 24 month.
Pack 500-1000 ml or on request

Produce in Italy by:
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in case of emergency UE number	☎	112
in case of emergency UK number	☎	999
en cas d'urgence Suisse number	☎	145

Principle

Haematological methods
The typical colour of cell nuclei, namely purple, is due to molecular interaction between eosin y and the methylene blue azure B-DNA complex.
The staining result can be influenced by several factors as pH of the solutions and buffer solution, buffer substances, fixation, staining time.
The haematological staining technique is used for visualisation of blood parasites protozoans in blood. The nuclei of blood parasites protozoans appear red under the microscope.

Bacteriological methods

Mycobacteria are difficult to stain because of the high proportion of lipid and wax in their cell walls. Up to now, in order to carry out the classical Ziehl-Neelsen staining, the test material has to be heated with carbol fuchsin solution to produce the mycolic acid fuchsin compound.
Once stained, acid fast mycobacteria keep their colouring even after treatment with strong decolourizing solutions as HCl-ethanol.
They remain red after counterstaining with methylene blue, whereas the microorganisms susceptible to acid take on the blue.

Sample material

Haematology.
Air-dried blood smears
Bacteriology
Heat-fixed smears of sputum, FNAB, lavages, imprints, body fluids, exudates, pus, liquid and solid cultures, histological sections.

Bacteriology

1. Fixation
Fixation is carried out over the flame of a Bunsen burner (2-3 times, avoiding excessive heating).
It is also possible to fix the smears in an oven at 100-110 °C for 20 minute.
2. Löffler's methylene blue solution

Staining procedure

Haematology
Fixation is carried out in the first staining step with undiluted May-Grünwald solution
Staining rack
1. Onto each fresh, dried film, pipette just enough May-Grünwald solution to cover the blood film (usually 10 drops or more) and let react for 3 minute.
2. Add an equal amount of distilled water, mix and stain for 1 minute.
3. Pour off fluid and without washing add about 10 drops of diluted buffered Giemsa solution, stain for 5 - 60 minute (try first for 10 -15 minute).
4. Rinse with buffer solution.
5. Dry and examine under the microscope.

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Bacteriology

Staining rack
1. Flood specimens completely with carbol-fuchsin solution. Carefully heat 3 times from below with a bunsen burner to steaming and keep hot for 5 minute. Do not allow the stain to boil.
2. Wash with tap water until no further colour is given off.
3. Cover completely with decolourizing solution and, depending on the thickness of the specimen, allow to stand for 15 – 30 second.
4. Wash immediately with tap water.
5. Counterstain by flooding for 30 second with Mallory modified methylene blue solution.
6. Wash well with tap water.
7. Dry
Allow the specimens to dry and, if necessary, mount with DdMount
Dehydrate histological specimens (ascending alcohol series) and mount with DdMount.

Result

Haematology

Nuclei	red to violet
Lymphocytes	plasma blue, azure granules purple to red
Monocytes	plasma dove-blue
Neutrophilic granulocytes	granules light violet
Eosinophilic granulocytes	granules red to grey-blue
Basophilic granulocytes	granules dark violet
Thrombocytes	violet
Erythrocytes	red
Blood parasites	nuclei bright red

Bacteriology

Mycobacteria	red
Background	light blue

A positive finding is reported as:

"acid fast bacteria detected"
and a negative finding is reported as:
"acid fast bacteria not detected".
It is not possible to state whether there are tuberculosis bacteria or other "atypical" bacteria. It is also impossible to state whether these mycobacteria are still capable of reproduction or are already dead. When acid-fast bacteria are found in the material examined, further investigations in a special laboratory are indicated.

Notes.
Distilled water or tap water can be used for rinsing and hydration. Always check the pH of your tap water and the levels of chlorine 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.

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* 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 haemalum. 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.

* 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 +25 ° 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.

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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