DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Field's fast stain for malaria and helicobacter technical information
Technical card code 12-129
Product code 12-129
Pack 1kit. Number of tests 100 or on request
Stability of product properly conserved at 15-20°C 24 months

Produce in Italy by
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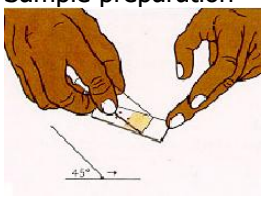
Application.

This method was originally recommended for staining malaria parasites in thick blood films, but it has also been used as a quick Romanowsky stain for thin blood films and marrow smears.
On thin sections of stomach it demonstrates Helicobacter pylori well.
The definitive diagnosis of malaria infection is still based on finding malaria parasites in blood films. In thin films the red blood cells are fixed so the morphology of the parasitized cells can be seen. Species identification can be made, based upon the size and shape of the various stages of the parasite and the presence of stippling (ie bright red dots) and fimbriation (ie. ragged ends). However, malaria parasites may be missed on a thin blood film when there is a low parasitaemia.
Therefore, examination of a thick blood film is recommended. With a thick blood film, the red cells are approximately 6 - 20 layers thick which results in a larger volume of blood being examined. Field's stain method for thick blood films. The method recommended for staining thick blood films by the Hospital for Tropical Diseases is Field's stain, which is made from two components.

Sample preparation



Tissue sections

3µ paraffin sections of formalin fixed tissue are suitable. Many others may be used.
Before staining, bring sections to water with xylene and ethanol.
Blood smears. Remember that fresh blood is a health hazard.

Thick smears

Make a smear by placing 2-3 drops of blood at the centre of a slide and spreading it out to an area about 2 cm in diameter. The smear should be thicker than a regular blood smear, but not so thick that it cracks and peels as it dries. Lay flat and allow to dry. Do not fix as the erythrocytes must be unfixed to be lysed during staining.

Thin smears

Make a blood or bone marrow smear of the usual type and allow to dry.
Fix in methanol for a minute or two before staining.

Method

Place into solution A for	1-2 second.
Rinse with distilled water.	
Place into solution B for	1-2 second.
Rinse with distilled water.	

Air dry.
Coverslip with a resinous medium, or examine without a coverslip.

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Expected results

Bacteria, including H. pylori	deep blue
Cellular elements	similar to a Romanowsky stain
Malaria chromatin	purple red to deep red
Malaria cytoplasm	blue

Reagent:

Fixative	2x30 ml
Field solution 1	2x30 ml
Field solution 2	2x30 ml

Notes

Times in each solution should be adjusted in each laboratory to obtain the colour contrast wanted.
Remember that solution B will tend to remove the staining by solution A.
For H. pylori thin sections show the organism best.
The slide is drained vertically and left to dry. Microscopic examination of the Field's stained thick blood film. The end of the film at the top of the slide when it was draining should be looked at. The edges of the film will also be better than the centre, where the film may be too thick or cracked. In a well stained film the malaria parasites show deep red chromatin and pale blue cytoplasm. White cells, platelets and malaria pigment can also be seen on a thick film. The leucocyte nuclei stain purple and the background is pale blue. The red cells are lysed and only background stroma remains. The occasional red cell may fail to lyse. Schizonts and gametocytes, if present, are also easily recognisable. A thick film should be examined for at least 10 minutes, which corresponds to approximately 200 oil immersion fields, before declaring the slide negative.

Difficulties in the examination of thick blood films

As a result of haemolysis of the red blood cells due to staining of an unfixed film, the only elements seen are leukocytes and parasites, the appearance of the latter being altered. Consequently: the young trophozoites appear as incomplete rings or spots of blue cytoplasm with detached chromatin dots.
The stippling of P. vivax and P. ovale may be less obvious although occasionally ghost stippling may be seen.
The cytoplasm of late trophozoites of P. vivax and P. ovale may be fragmented.
Caution should be exercised when examining thick blood films as artefacts and blood platelets may be confused with malaria parasites.
Conclusion
A thick blood film is recommended for routine diagnosis of malaria in addition to the thin film and is particularly valuable in instances of low parasitemia.
However, in order to correctly speciate the parasite, examination of a thin film is required. If the thick film is negative, it is unlikely that parasites will be found in the thin film.

* 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.

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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.

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