IVD dispositivo medico-diagnostico in vitro

CE

SWK for the examinations of parasites in stools using two techniques' technical's information's
Technical card code 12-104-SWK
Product code 12-104-SWK
Stability of product properly conserved at 15-20°C 18 months

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Principle

G. Swierczynski introduced two biphasic concentrations techniques for examinations of parasites: parasitic concentrations is achieved by two normal non miscible phases, one is aqueous (M.F. solution or aceto-acetic buffer Bailenger), and the other is organic (ethyl acetate based). Both phases permit the partition coefficient of each faecal particle to be assessed and the concentration of the parasitic elements at the bottom. The concentration technique according to Blagg particularly recommended for the detention of the must fragile parasites (trophozoites), cysts and eggs (schistosoma eggs and non-fertilized ascaris eggs)

Procedure (MIF)

In a 30 ml sedimentation tube, prepare extemporaneously the mixture in the following order:
Mix 4 drop of Lugol PVP stabilized and 15 ml of merthiolate-formalin (M.F. solution)
Add 2 to 3 gr of stools (or 2-3 ml if liquid), to the previous mixture, triturate until complete homogenized and allow sediment for a maximum of two minutes (not longer).
Pour 5 ml of supernatant in a 10 ml centrifugation tube, add 4-5 ml of organic phase ethyl acetate based.
Emulsify by shacking vigorously either manually or with a vortex, then degas.
Centrifuge at 1600 rpm (150-200 g), for two minutes to concentrate the parasites at the bottom.
(if the ring appearing between both aqueous and organic phases is thick, scrape it from the wall of the tube with a Pasteur pipette or a loopful)
Get rid of the supernatant by turning the tube upside down. If trace of the ring remain, clean the tube with cotton swab. Mix the sediment with some drop of physiological water (suspensions)
Examine a drop of suspension under microscope between slides and cover-slip

Procedure (Bailenger)

In a 30 ml sedimentation tube, mix 2 to 3 gr of stools (or 2-3 ml if liquid), with 15 ml of Bailenger buffer
Triturate until complete homogenized and allow to sediment for a maximum of two minutes (not longer).
Pour 5 ml of supernatant in a 10 ml centrifugation tube, add 4-5 ml of organic phase ethyl acetate based.
Add 2 to 3 gr of stools (or 2-3 ml if liquid), to the previous mixture, triturate until complete homogenized and allow sediment for a maximum of two minutes (not longer). Emulsify by shacking vigorously either manually or with a vortex, then degas. Centrifuge at 1600 rpm (150-200 g), for two minutes to concentrate the parasites at the bottom.
(if the ring appearing between both aqueous and organic phases is thick, scrape it from the wall of the tube with a Pasteur pipette or a loopful)
Get rid of the supernatant by turning the tube upside down. If trace of the ring remain, clean the tube with cotton swab. Mix the sediment with some drop of physiological water (suspensions). Examine a drop of suspension under microscope between slides and cover-slip
For staining. Mix extemporaneously one drop of Lugol PVP stabilized with two ml of merthiolate-formalin (M.F. solution). Add one drop of this mixture to the suspension. Examine a drop under the microscope between slides and cover-slip

Results

With out staining	parasites are observed by their refringency
With staining	
Cysts, eggs, parasites:	green yellowish brown.
Cytoplasm and nuclear membrane:	dark red.
Nuclei chromatin:	colorless.

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Cysts, eggs, parasites, are colored, yellow or light brown, dark green. After two hours, the initial color of the iodine solution is replaced from eosin. The nuclear membrane becomes dark red to black. The cytoplasm is red, and the chromatin is not stained and appears in refraction.

Reagent	
Lugol PVP stabilized	50 ml
Merthiolate formaldehyde	150 ml
Bailenger buffer	150 ml
Organic phase ethyl acetate	150 ml
Wooden spatula	24 pcs
Sedimentation tube 25 ml	24 pcs
Centrifugations tube 12,5 ml	24 pcs

References

Bailenger J., Coprologie parasitaire et fonctionelle, Drouillard, Bordeaux 3ème éd. (1973). Barret F. Application de la technique du M.I.F. en coprologie parasitaire, Microbia (1975) 2. Golvan Y.J., Drouhet E., Techniques en parasitologie et mycologie, Flammarion, Paris (1972).

* 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.

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* 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 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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