

**IVD** dispositivo medico-diagnostico in vitro

Ziehl-Neelsen Fite for acid fast bacteria technical information  
 Technical card code 14-138  
 Product code 14-138  
 Pack 1kit. Number of tests 100 or on request  
 Stability of product properly conserved at 15-20°C 24 months  
 CND code W01030799

Produce in Italy by  
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For emergency contact your nearest anti-poison unit.

Application. To show pathogenic mycobacteria (especially Koch's bacillus and Hansen's bacillus) in histology sections, sputum smears and culture smears.

Principle. This stained involve carbol fuchsin solution as the staining agent and differentiation in acid. It is used typically for the detection of acid-fast bacilli (stained red); methylene blue is used as the counterstain. A modified version of Ziehl-Neelsen stain with prolonged staining at high temperature is used in the detection of certain lipofuscins in neuronal ceroid-lipofuscinoses (Batten disease), and also to detect nuclear inclusion bodies in chronic lead or bismuth poisoning.

As the causative agents for Leprosy's (*Mycobacterium leprae*) and Nocardiasis (*Nocardia asteroides*) are much less acid and alcohol fast than *Mycobacterium tuberculosis* bacilli, a more gentle dewaxing and minimal exposure to organic solvent is required for adequate staining.

#### Method.

Heat slides in slides dryer to facilitate dewaxing. Take section to water.  
 Drain slide and place in a slide mailer with 15 ml of carbol fuchsin.  
 Fill slide mailer (-15 ml volume), so that fluid comes up to slides frosting.  
 Place mailer into microwave in a beaker, in case of spillage, leave cap of mailer open.  
 Microwave; on full power. First time, 10 second full power. Second time, 7 second full power.  
 (Check slides each time. Caution halt if solution boil). Allow to stand for 2-5 minutes.  
 Wash well in running water; wipe away excess stain 5 minutes  
 Differentiate individually with sulphuric acid, control microscopically 1-3 minutes.  
 Filter on working methylene blue 20 second. Avoid over staining as some staining bacterial staining can be replaced by methylene blue  
 Wash with water. Drain and air dry thoroughly  
 Dip slides in xylene and mount with DdMount

#### Result

Acid fast bacilli:	red ( <i>Leprae</i> bacilli are short rods)
Nocardia:	red (nocardia organism are long, thin and filamentous)
Erythrocytes:	pale pink
Background:	blue

1 - Periodic acid solution	2x30 ml
2 - Carbolfuchsin solution	2x30 ml
3 - Acid differentiation buffer	2x30 ml
4 - Methylene blue solution	2x30 ml

#### \* 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

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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.

**\* 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.

**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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