



Neisser and Gram for polyphosphates technical information  
 Technical data code 12-141  
 Product code 12-141  
 Stability of product properly conserved at 15-20°C 12 month.  
 Pack 1 kit 100 test or on request

Produce in Italy by  
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### Intended use

Staining according to Neisser is a test for the presence of polyphosphates stored in the cells (= storage materials). This method is an indispensable aid to the identification of certain strains of filamentous bacteria. Furthermore, this staining method can make the Bio-P bacteria, responsible for biological phosphate removal, visible.

### Staining procedure

1. Prepare a fixed smear.
2. Place a freshly made mixture of 2 parts of methylene blue and 1 part solution of crystal violet into the slide for a contact period of 10-15 seconds.
3. Afterwards, allow the excess dye to run off the slide.
4. Cover the slides with basic orange 2 for a contact period of 45 seconds.
5. Rinse the slide with tap water (with the flow against the back of the slide).
6. Allow the slide to dry and then view with a 100x bright field objective. Drying can be speeded up by removing most of the water carefully with filter paper.

### Results

Neisser negative cells stain hardly or not at all (slightly brown or yellow). Three main groups of Neisser positive bacteria can be distinguished.

- 1 Filamentous bacteria which stain completely grey-violet. This usually applies to *Nostocoida limicola* or Type 0092.
- 2 Filamentous bacteria which contain blue-black coloured polyphosphate globules. Without staining, these globules cannot be clearly observed with a light microscope. They are indeed clearly visible if a much higher magnification (electron microscopy) is used. These globules, which are present in pairs, are an important identification characteristic for *Microthrix parvicella*.
- 3 Colonies of blue-black coloured cells. These are comprised of Bio-P bacteria. There are some variations in the manner in which these types of colonies stain with Neisser. The shade is sometimes much lighter, or only a part of the cell stains darkly.

### Reagent

Methylene blue solution	30 ml
Crystal violet solution	30 ml
Basic orange 2 solution	30 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 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.

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\* 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 haemallum 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 haemallum. Do not breathe vapours. 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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