DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Nigrosine solution for microbiology technical information
Technical card code 09-258
Product code 09-258
Stability of product properly conserved at 15-25°C 24 month.
Pack 100-200 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

The World Health Organization (WHO) Laboratory manual for the examination of human semen recommends 2 main techniques that use eosin Y as the active stain. The first technique uses eosin Y alone, and negative phase-contrast optics is used to obtain the requisite dark field during evaluation. Two variants of the eosin Y–alone technique are given the first variant includes 30 seconds of exposure to eosin Y, followed by examination as a wet preparation; the second variant runs with a 60-second exposure to eosin Y, after which smears are prepared and air dried. The second recommended technique, subtitled a modification of Blom's technique, is a 2-step eosin-nigrosin technique that uses nigrosin to obtain the dark background for increased contrast and yields reliable evaluations using ordinary light microscope optics. In 1985, Mortimer published a simplified 1-step eosin nigrosin technique. In a recent study, a slightly modified version of this 1-step technique, in which the dyes were dissolved in saline (9 g/L of sodium chloride) instead of distilled water, was evaluated on sperm in semen samples using data from 1235 consecutive routine samples.

The Eosin-Only Protocol

The negative phase-contrast optics required for this technique are not easily obtained, which diminishes its usefulness. However, irrespective of this, there are a number of questions regarding the protocol recommended by the WHO manual in relation to the original protocol. This technique, as introduced by Eliasson and Treichl for human spermatozoa, used a 5-g/L eosin Y solution in 0.15 M phosphate buffer at pH 7.4. The protocol was later tested on a further 20 semen samples. However, in the first edition of the WHO manual, the dilution medium for the eosin was given as "distilled water"; in the second edition, it was given as "physiological saline"; and in the following editions, it was given as 0.9% (9 g/L) aqueous sodium chloride solution, indicating a series of small editorial mistakes or changes not supported by experimental data. The validation of the 20 semen samples was referenced in all 4 WHO editions. The original protocol included mixing equal parts of the eosin-alone solution and semen; after a wait of 1–2 minutes, a smear was made from a drop of this mixture on a microscope slide. After air drying, the smear was assessed using negative phasecontrast microscopy. This procedure was recommended in the second and third editions of the WHO manual. However, editorial changes in the fourth edition provided the option of using 2 variants during assessment: 1) direct assessment using a wet preparation after 30 seconds, or 2) later assessment under oil immersion using an air-dried smear made after 1 minute of incubation. Thus, neither the recommended solvent (physiological saline) nor the technique to assess sperm vitality for a wet preparation is declared in the stated reference.

The Eosin-Nigrosin Protocols

In our previous validation of the 1-step eosin-nigrosin protocol, we obtained results that indicated this technique was at least as good as the eosin-only protocol as well as the 2-step eosin-nigrosin protocols. Moreover, the 1-step protocol appears to yield more reliable results for tests in which low percentages of stained sperm are found.
With this background, we were interested in performing a direct comparison of the 2-step eosin-nigrosin protocol with the 1-step protocol. We used 42 semen samples from routine laboratory work. Vitality smears were made simultaneously with both techniques within 30–60 minutes after ejaculation; these were then coded and assessed blindly. The proportions of dead spermatozoa were consistently higher with the WHO 2-step protocol. Evaluating the protocols by using the sum of proportions of dead and motile sperm showed that the WHO 2-step protocol yielded erroneous results by demonstrating that the sum of dead and motile sperm was more than 100% of their combined total, while the 1-step protocol was less than 100% in most samples. As discussed below, a number of factors may have contributed to these errors: the availability of eosin and the dilution of semen, the type of solvent, the osmolarity of the staining solution, and the time in the staining solution.

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Bibliography

Eliasson, R. & Treschl, L. (1971) Supravital staining of human spermatozoa. *Fertility and Sterility*, 22: 134-7.
Eliasson, R. (1981) Analysis of semen. In *The Testis*, ed. H. Burger & D. de Kretser, pp. 381-99. New York: Raven Press. Jeyendran, R.S., Van der Ven, H.H., Perez-Pelaez, M., Crabo, B.G. & Zaneveld, L.J.D. (1984) Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *Journal ofReproduction and Fertility,* 70: 219-28.

Reagent	
Nigrosine solution ready to use	30 ml
Eosine solution ready to use	30 ml

* 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 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.

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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