DDK Italia

**IVD** dispositivo medico-diagnostico in vitro

CE

Eosin nigrosin (fast staining for spermograms) technical information
Technical card code 13-101
Product code 13-101
Pack 2x50 ml or on request
Stability of product properly conserved at 15-20°C 24 months

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|---|---|---|
| in case of emergency UE number | ☎ | 112 |
| in case of emergency UK number | ☎ | 999 |
| en cas d'urgence Suisse | ☎ | 145 |

## Principe

This one-step staining technique uses nigrosin to increase the contrast between the background and the sperm heads, which makes them easier to discern. It also permits slides to be stored for re-evaluation and quality-control purposes (Björndahl et al., 2003).
For estimates of sperm motility, or vitality by eosin alone and for the hypo-osmotic swelling (HOS) test, prepare fresh replicates from new aliquots of semen. For estimates of vitality from eosin–nigrosin smears and sperm morphology, reassess the slides in replicate

## Method

1. Mix the semen sample well
*(Before removing an aliquot of semen for assessment, mix the sample well in the original container, but not so vigorously that air bubbles are created.*
*This can be achieved by aspirating the sample 10 times into a wide-bore (approximately 1.5 mm diameter) disposable plastic pipette (sterile when necessary).*
*Do not mix with a vortex mixer at high speed as this will damage spermatozoa).*

2. Mix one drop of sperm and one drop of eosin – shake in a porcelain spot plate well or test-tube, for 30 seconds.
3. Add two drop of nigrosin, remix.
4. Make a smear and let it dry
5. Examine under a 40x objective.

## Result

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| Dead spermatozoa: | pink |
| Living spermatozoa: | colourless (nigrosin show up their outlines) |
| Background: | dark blue |

## Asses the percentage of dead spermatozoa and living spermatozoa

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| After 3 hours: | 75 to 85% of living form |
| After 6 hours: | 55 to 65% of living form |
| After 24 hours: | 25 to 40% of living form |

*(Preparation of semen smears. Rapid addition of fixative to semen does not permit adequate visualization of spermatozoa, as they are obscured by denatured seminal proteins. For morphological analysis, it is customary to prepare semen smears that are air-dried before fixation and staining. However, such a process leads to morphological artefacts, since air drying of semen smears is associated with:*

- *changes in sperm dimensions: dried, fixed and stained spermatozoa are smaller than live spermatozoa visualized in semen (Katz et al., 1986);*
- *expansion of immature sperm heads (Soler et al., 2000); and*
- *loss of osmotically sensitive cytoplasmic droplets (Abraham-Peskir et al., 2002; Cooper et al., 2004), although large amounts of excess residual cytoplasm are retained.*

*Two or more smears should be made from the fresh semen sample in case there are problems with staining or one slide is broken. Assessment is performed in replicate, preferably on each of the two slides, because there may be significant between-slide variation in sperm morphology and allow it to dry in air).*

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*Errors in estimating numbers. The precision of the estimate of sperm number depends on the number of spermatozoa counted. In a Poisson distribution, the standard error (SE) of a count (N) is its square root (√N) and the 95% confidence interval (CI) for the number of spermatozoa in the volume of semen is approximately N ± 1.96 × √N (or N ± approximately 2 ×√_N). If 100 spermatozoa are counted, the SE is 10 (√100), and the 95% CI is 80–120 (100 ± 20). If 200 spermatozoa are counted, the SE is 14 (√200), and the 95% CI is 172–228 (200 ± 28). If 400 spermatozoa are counted, the SE is 20 (√400) and the 95% CI is 360–440 (400 ± 40).*
*The sampling errors can be conveniently expressed as a percentage of the count (100×(√N/N)).*

| Reagent | |
|---|---|
| Eosin | 2x50 ml |
| Nigrosin | 2x50 ml |

*References*

*WHO laboratory manual for the examination and processing of human semen, 5th edition, WHO, 2010*

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