DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Shorr for sperm morphology technical information
Technical card code 13-104
Product code 13-104
Pack 2x100 ml or on request
Stability of product properly conserved at 15-20°C 24 months

Made in Italy by
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in case of emergency UE number	☎	112
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Preparation of semen smears

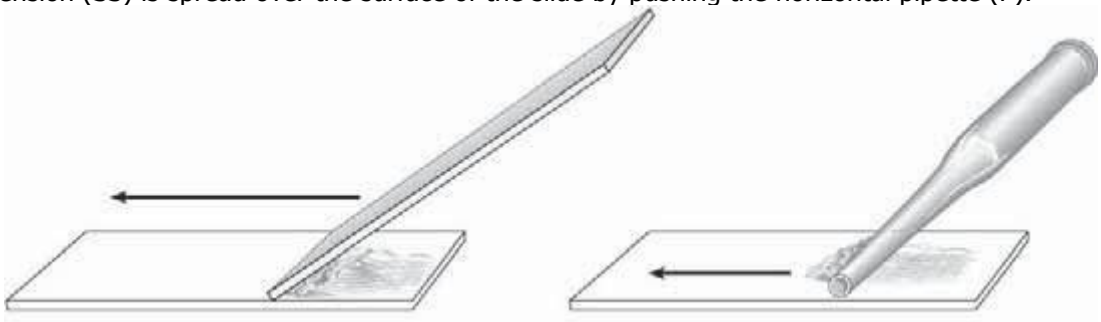
Rapid addition of fixative to semen does not permit adequate visualization of spermatozoa, as they are obscured by denatured seminal proteins. For morphological analysis, it is customary to prepare semen smears that are air-dried before fixation and staining. However, such a process leads to morphological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

Method

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.

Remove an aliquot immediately, allowing no time for the spermatozoa to settle out of suspension. Remix the semen sample before removing replicate aliquots.

Semen smearing methods for sperm morphology
(a) "Feathering" method for undiluted semen. The semen drop (S) spreads along the back edge of the angled slide and is pulled forwards over the slide to form the smear. (b) Pipette method for washed samples. A drop of the sperm suspension (SS) is spread over the surface of the slide by pushing the horizontal pipette (P).



Normal semen samples
Clean both surfaces of the frosted slides by rubbing vigorously with lint-free tissue paper.
Label the frosted portion with identifying information (e.g. identification number, date) using a pencil. Apply a 5-10 µl aliquot of semen, depending on sperm concentration, to the end of the slide. Use a second slide to pull the drop of semen along the surface of the slide. If the dragging slide is non-frosted, the edges of both ends of the slide can be used to make four different smears. Allow the slides to dry in air and stain

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Method

Fixing the air-dried semen smear

Immerse slides in acetic ethanol or 75% ethanol for	1 hour.
Sequentially immerse the slides in:	
1) Running tap water	12-15 dips.
2) Harris haematoxylin	1-2 minutes.
3) Running tap water	12-15 dips.
4) Ammoniacal ethanol	10 dips.
5) Running tap water	12–15 dips.
6) Ethanol 50%	5 minutes.
7) Shorr stain	3–5 minutes.
8) Ethanol 50%	5 minutes.
9) Ethanol 75%	5 minutes.
10) Ethanol 95% (	5 minutes.

*One dip corresponds to an immersion of about 1 second.
Note: The slides can be viewed unmounted or mounted.

Result

Red-stained sperm flagella are stained in samples of good and bad motility.
Both red- and blue-tailed spermatozoa are stained.
Shorr-stained slides without a cover slip revealed the presence of red and blue-stained sperm tails, but after cover slipping, significantly fewer blue-stained sperm tails can be observed.

Reagent

Harris haematoxylin	100 ml
Shorr	100 ml

References

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