Spermac Stain

Staining method for human spermatozoa
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IVD CE

For in vitro diagnostic use - Reagent for professional use only

INTENDED USE

Spermac Stain is a qualitative diagnostic kit for staining human spermatozoa. The purpose of staining spermatozoa is to be able to differentiate morphologically normal from abnormal sperm cells.

GENERAL INFORMATION

The definition and criteria for normality have been largely based on studies done on sperm recovered from the female reproductive tract (especially in post coital cervical mucus) which is considered to be normal. Still different criteria have been proposed, the main ones being the WHO criteria[1] and the Tygerberg (or strict) criteria[2,3].
Spermac Stain is an aid in evaluating morphology as it helps distinguish the different parts of the sperm cell (head, acrosome, equatorial region, mid-piece, tail), making it easier to differentiate between a normal and an abnormal spermatozoon [4,5].
Spermac Stain may help in assessing the diagnosis and the management of male infertility.

MATERIAL INCLUDED WITH THE TEST

- Stain A: red stain - 50ml or 250ml
- Stain B: pale green - 50ml or 250ml
- Stain C: dark green - 50ml or 250ml
- Fix: fixative - 50ml or 250ml
- The certificate of analysis and MSDS are available upon request

MATERIAL NOT INCLUDED WITH THE TEST

Glassware, Coplin jars, microscope (1000x magnification), immersion oil, warm plate at 37°C, tap or distilled water.

STORAGE AND STABILITY

Spermac Stain should be stored in closed Coplin jars or the original bottles, at 2-25°C. The reagents are stable for 36 months after date of manufacture if unused. However, staining removes constituents and introduces contaminants, and thus stains should be replaced when adequate staining is no longer achieved. Filter stains if deposit is noted.

METHOD

We recommend to watch our demonstration video (download via link on our website, or scan barcode):

PREPARATION

Pour the reagents in Coplin jars, make sure the fluid level is high enough to cover the area that is to be stained. Only fill the fixative jar when the slides have been prepared, dried, and are ready for staining. Fill a fifth Coplin jar, or any other recipient that can contain a complete object glass, with tap water (for washing the slides between the different dyes). If the tap water is alkaline (pH > 7), then use distilled water for washing. Clean, wash in alcohol and dry slides before use.

STAINING PROCEDURE

1. Allow a thin feathered-edge smear of fresh, undiluted, preferably liquified semen to air dry for about 5 minutes on a warm plate at 37°C.
   Note: Do not make or dry smears close to the open bottle of fixative, as the fixative vapour (even in very small amounts) interferes with the staining.
2. Fix the smear by immersing the slide for a minimum of 5 minutes in a Coplin jar containing the fixative. Longer fixation is acceptable but not necessary.
3. Remove slide from fixative, briefly place vertically on absorbent paper to drain excess fixative. Do not touch the specimen with the paper. Let the slide dry by placing it on a warm plate at 37°C for 15 minutes.
4. Wash by gently dipping 7 times in tap or distilled water. If staining slides in a cradle containing 5 or more slides, ensure that the washing container is large enough to ensure complete washing of the fixative off the slides. If the washing container is small (e.g. Coplin jar) then repeat the washing procedure with fresh water. Briefly drain excess water off by touching end of slide onto absorbent paper.
5. Stain 2 minutes in stain A. When introducing the slide into the stain solution, dip slide 7 times slowly (about 1 dip per second) in and out of the stain, to ensure complete contact of the sample with the stain. Then leave undisturbed for the rest of the staining period. Wash as above, by dipping 7 times in fresh tap water. Briefly drain excess water onto absorbent paper.
6. Repeat the washing in fresh water. This double washing step after Stain A is important. Briefly drain off excess water onto absorbent paper.
7. Stain 1 minute in stain B. Dip 7 times initially to ensure complete contact of stain with the specimen. Wash as above in fresh water.
8. Stain 1 minute in stain C, dipping 7 times initially. Wash as above in fresh water.
9. Allow smear to air dry.
10. Observe staining under a light microscope (1000x) using oil immersion:
    - acrosome = dark green
    - nucleus = stained red
    - equatorial region = pale green
    - midpiece and tail = green

INTERPRETATION

- Count at least 100 and preferably 200 spermatozoa and classify them as either normal or abnormal, specifying which defects are most common
- Only include identifiable sperm cells in the count.
- The criteria for classifying sperm cells as either normal or abnormal depends on the classification method used in the lab (WHO, 2010)
- According to the WHO, using 2010 WHO criteria, a sample is considered normal if at least 4% of spermatozoa show normal forms[1].

By the strict application of certain criteria of sperm morphology, relationships between the percentage normal forms and various fertility endpoints (time-to-pregnancy, pregnancy rates in vivo and in vitro) have been established, which may be useful for the prognosis of fertility (WHO, 2010).

MOUNTING SLIDES

If slides are mounted staining will fade under mounting medium (after weeks). Therefore, do not mount slides if you want to refer back later. Gently blot off immersion oil, which also fades the staining. It is preferable to make duplicate slides for future reference if necessary, or photographic or video records.

WARNINGS AND PRECAUTIONS

- All semen samples should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or Hepatitis.
- Fix contains Formaldehyde: Toxic by inhalation, in contact with skin and if swallowed. May cause irritation to mucus membranes. Listed as a carcinogen. Possible risks of irreversible effect. May cause sensitization by skin contact.
- All other ingredients have not been established as toxic.

REMARKS ON USE

- Proteinaceous or gelatinous samples and frozen samples must be diluted 1:1 with 3% sodium citrate prior to smearing.
- A stained slide should be transparent with only a very slight hint of green hue. If the slide is dark green, then the slide was exposed to fixative vapours before fixing.
- For transport prior to staining, slides may be prepared, fixed, washed, and dried. Protect against abrasion during transport. When ready to stain, begin the process at the fixative (Step 2), i.e. the slides receive a double fixation. This is important as the fixative contains buffers that ensure that subsequent staining occurs correctly.

REFERENCES

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

[2] Menkveld R, Kruger TF (1991). Atlas of human morphology, Williams and Wilkins, Baltimore.

[3] Menkveld R, Stander FSH (1990). The evaluation of morphological characteristics of human spermatozoa according to stricter criteria, Human Reproduction 5(5): 286-92

[4] Oettlé EE(1986). An improved staining technique which facilitates sequential monitoring of the acrosome state, Development, Growth and Differentiation (Suppl.): 28

[5] Chan PJ, Corselli JU, Jacobson JD, Patton WC, King A (1999). Spermac stain analysis of human sperm acrosomes. Fertility and Sterility 72 (1): 124-128.

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