

SPERMAC STAIN™

Staining method for human spermatozoa
For *in vitro* diagnostic use only

INTENDED USE

Spermac is a 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 criteria¹ and the Tygerberg (or strict) criteria^{2,3}. These methods differ mainly in the fact that so-called borderline normal spermatozoa, according to WHO¹, are classified as abnormal by strict criteria³. Spermac stain is an aid in evaluating morphology in a way that helps distinguish the different parts of the sperm cell (head, acrosome⁴, equatorial region, midpiece, tail), making it easier to differentiate between a normal and an abnormal spermatozoon.

MATERIAL INCLUDED WITH TEST

Reagent A: red stain - 50ml or 200ml
Reagent B: pale green - 50ml or 200ml
Reagent C: dark green - 50ml or 200ml
Fixative: Fix - 50ml or 200ml

MATERIAL NOT INCLUDED WITH TEST

Glassware, Coplin jars, Microscope, Immersion oil, Tap water

STORAGE AND STABILITY

Spermac stain should be stored in closed Coplin jars or the original bottles, at 15-25°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.

PREPARATION

Pour the reagents in Coplin jars; make sure the fluid level is high enough to cover the area that is to be stained.
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).
Clean slides, wash in alcohol and dry slides before use.

METHOD

1. Allow a thin feathered-edge smear of fresh, undiluted semen to air dry for a **maximum** of 5 minutes.
2. Fix the smear by immersing the slide for a minimum of 5 minutes in a Coplin jar containing the fixative.
3. Wash by gently dipping 6-7 times in tap or distilled water. Briefly drain excess water off by touching end of slide onto absorbent paper. Do not touch the specimen with the paper.
4. Stain 1-2 minutes in stain A. Wash as above.
5. Stain 1 minute in stain B. Wash as above.
6. Stain 1 minute in stain C. Wash as above.
7. Allow smear to air dry.

8. Observe staining under a light microscope (100x) using oil immersion:
- acrosome = dark green
 - nucleus = stained red
 - equatorial region = pale green
 - midpiece and tail = green

INTERPRETATION OF THE RESULTS

- 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.
- According to WHO, using WHO criteria, a sample is considered normal if at least 30% of spermatozoa show normal forms¹.
- According to strict criteria the rate of fertilization per cycle (in IVF) drops dramatically when the percentage of normal forms is below 15%^{2,3}.

MOUNTING SLIDES

If slides are mounted staining will fade under mounting medium (after weeks). 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 effects. May cause sensitization by skin contact.
- All other ingredients have not been established as toxic. Full Material Safety Data Sheet is available on request.

REMARKS ON USE

- Proteinaceous or gelatinous samples and frozen samples must be diluted 1:1 with 3% sodium citrate prior to smearing. However, if the smear is immersed in the fixative as soon as the smear is dry, this citrate dilution step is unnecessary.
- 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 dried too long prior to fixing.

REFERENCES

¹ WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 3rd edition, Cambridge University Press, Avon, 1992, pp.107

² Menkveld R, Kruger TF, et al, Atlas of human morphology, Williams and Wilkins, Baltimore, 1991

³ Menkveld R, Stander FSH, et al, The evaluation of morphological characteristics of human spermatozoa according to stricter criteria, *Human Reproduction*, 1990: 5, 5, pp. 286-92

⁴ Oettlé EE, An improved staining technique which facilitates sequential monitoring of the acrosome state, *Development, Growth and Differentiation (Suppl.)*, 1986, p28

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**CONCEPTION
TECHNOLOGIES**

858.824.0888/888.995.8081
6835 Flanders Drive, Ste 500
San Diego, CA 92121
www.conceptiontechnologies.com