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REF **Art.-No. ZR10050**
200 applications

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1000 applications

IVD ***in vitro* diagnostics**

CE

Manual

PLEASE READ CAREFULLY

SemenStain

(Semen staining, spermiogram)

Professional Use Only

Application

SemenStain is a quick-staining-method to assess the morphology of sperms (spermiogram). This method is composed of a staining kit which allows differential staining of the sperm parts due to their different basophilic, eosinophilic and neutrophilic properties.

Principle

The sperms are fixed. Here, the succedan-staining is used, which means, three dyes are used one after the other and it results to differentiate staining of different tissues with individual dyes.

Storage and stability

15-25°C

36 months from date of manufacture

Content

▪ Reagent 1	1x 50 or 250 ml
▪ Reagent 2	1x 50 or 250 ml
▪ Reagent 3	1x 50 or 250 ml
▪ Reagent 4	1x 50 or 250 ml

Necessary utensils (not included)

- Native ejaculate or washed sperms (5-10 µl)
- Staining cuvettes (8x) or tubes (50 ml, 8x)
- Gloves
- Tweezers
- Paper towels
- Slides
- Slides rack (if more than five slides are to dye)
- Immersion oil
- Microscope

Procedure (see also scheme)

1. Apply 5-10 µl sperms per slide, grease the sperms with a coverslip and let dry. We recommend preparing 2 slides per patient.
2. Fill the staining cuvettes with reagent 1, reagent 2, reagent 3 and reagent 4, respectively. Fill 4 other empty cuvettes with water. Place the cuvettes side by side. Label them from 1 to 8.
3. Immerse slides 3 minutes by repeated immersion in cuvette 1 (reagent 1) to fix the preparation. Wash slides 3 minutes in cuvette 2 (water). Then place the slides vertically on paper towels to remove excess water.
4. Color the slides 1 minute by repeated immersion in cuvette 3 (reagent 2). Wash the slides in cuvette 4 (water). Change the water several times until water stays clear; remove with paper towel the excess water of the slides.
5. Color the slides 1 minute by repeated immersion in cuvette 5 (reagent 3), wash the slides according to step 4 in cuvette 6 (water) and remove from the slides with paper towel the excess water.
6. Color the slides 1 minute by repeated immersion in cuvette 7 (reagent 4), wash the slides according to step 4 in cuvette 8 (water) and remove from the slides with paper towel the excess water.
7. Dry the slides on free air.

Evaluation

Evaluate the sperms with immersion oil at 1000x magnification on the side of the slide with lower sperm density. Here the sperms are better to assess individually.

The criteria for classification of sperms by their morphology can be found in the WHO laboratory manual (2010).

Sperm cell parts	Staining/color
Head - Nucleus	red
Head - Acrosome	dark green
Middle part	pale green
Tail	green

After the evaluation the immersion oil can be gently removed from the slide with a paper towel. Then the slide can be immersed in reagent 1 for 5 min, dried and stored. It is also possible to produce preparations with a coverslip and glue for long term storage.

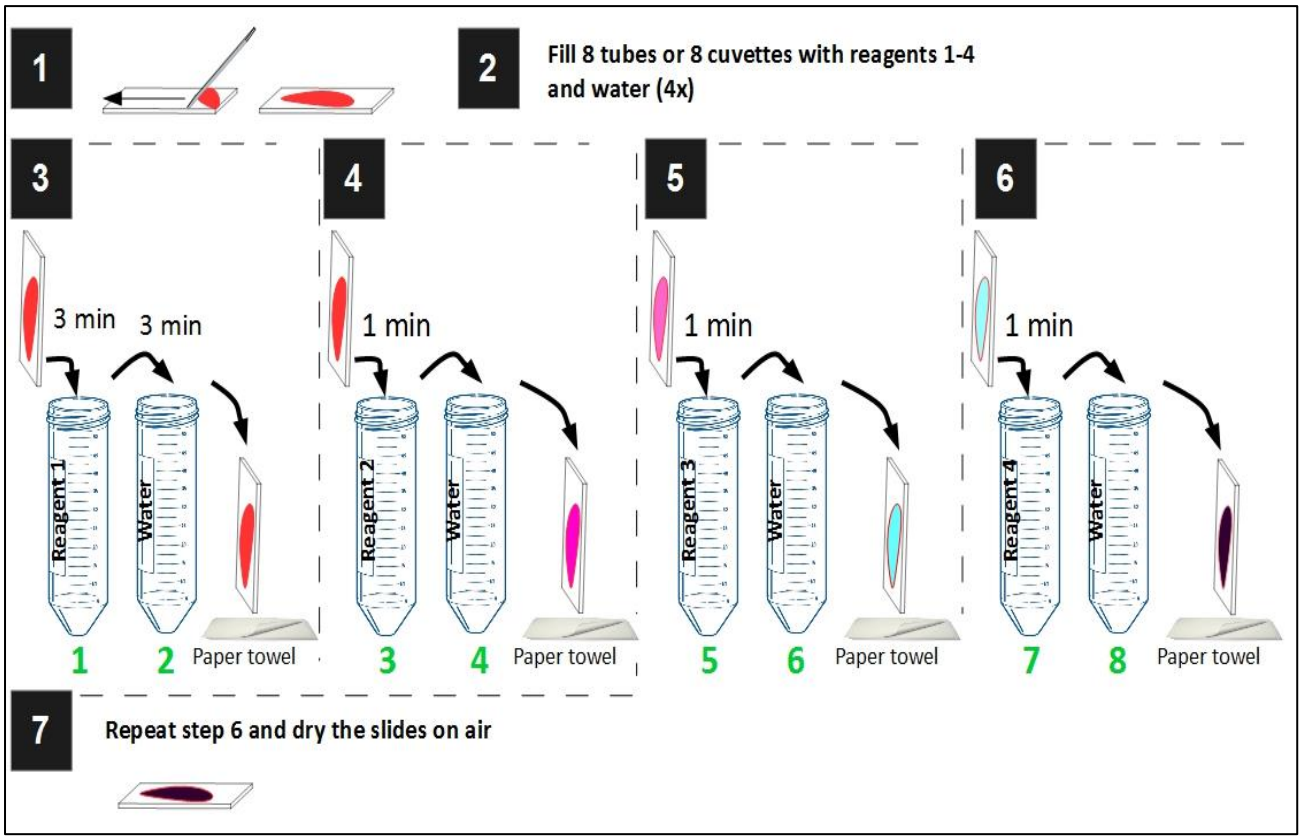
Safety information / precautions

- All semen samples should be considered potentially infectious.
- Handle with all samples like HIV or hepatitis infected material.
- When working with samples and reagents wear always protective clothing (gloves, gowns, eye / face protection).
- Reagent 1 is containing methanol: toxic by inhalation, skin contact or ingestion. May cause organ damage. There is a risk of irreversible damage.
- All other ingredients are not classified as toxic

References

1. **Andersen AG *et al.*** (2000) High frequency of sub-optimal semen quality in an unselected population of young men. *Human Reproduction,* 15:366-372
2. **Auger J, Eustache F** (2000) Standardisation de la classification morphologique des spermatozoïdes humains selon la méthode modifiée de David. *Andrologia*, 10:358-373
3. **Behre HM *et al.*** (2000) Diagnosis of male infertility and hypogonadism. In: Nieschlag E, Behre HM, eds. Andrology, male reproductive health and dysfunction. *Springer*: 92ff.
4. **Cooper TG *et al.*** (2010) World Health Organization reference values for human semen characteristics. *Human Reproduction Update,* 16:231-245
5. **Cross NL** (1995) Methods for evaluating the acrosomal status of human sperm. In: Fenichel P, Parinaud J, eds. Human sperm acrosome reaction. Paris, John Libbey Eurotext (Colloques INSERM): 277-285
6. **Kruger TF *et al.*** (1987) A quick, reliable staining technique for human sperm morphology. *Archives of Andrology,* 18:275-277
7. **WHO Press, (2010)** Laboratory manual for the examination and processing of human semen. 5th edition

Scheme (see also chapter procedure)



REF Article number
consult instructions for use
IVD *in vitro* diagnostics
Temperature limitation
LOT Lot number