SPERM STAIN

3 Steps fast staining. For "in vitro" diagnostic

MICROPTIC
AUTOMATIC DIAGNOSTIC SYSTEMS

Principle

The **SPERM STAIN** constitutes a system to differentiate the morphological structures of the spermatozoa for a perfect functional assessment. It can also be used for the differential staining of blood cells in semen. The stains which make up the **SPERM STAIN** combine polychromy and the quality of classic cytology staining methods (May-Grünwald, Giemsa, Wright) with a very quick execution (time of just 15 seconds). The technique is performed by immersion in the staining solutions.

Product characteristics

The **SPERM STAIN** system is based on the original Romanowsky staining method for differential staining of several cellular structures.

Reagents

Kit 3 x 100 ml. (Ref.99 03 85). Content:

1. **SPERM STAIN NR. 1 1 x 10 ml. (Ref. 99 03 95).**

Hexamethyl-p-roseniline methanolic solution. Handle with care.

2. **SPERM STAIN NR. 2 1 x 100 ml. (Ref. 99 03 96).**

Xanthene buffered solution.

3. **SPERM STAIN NR. 3 1 x 100 ml. (Ref. 99 03 97.**

Thiazine buffered solution

Working reagents

1. **Working reagent Nr. 1**
   Dilute the vial of SPERM STAIN NR 1 to 1000 ml. with Methanol (we recommend a minimum purity of 99.5% and a water content lower than 0.1%)
2. **Working reagent Nr. 2**
   Dilute the content of SPERM STAIN NR 2 with 900 ml of deionised water. Please, use high quality deionised water, no tap water.
3. **Working reagent Nr. 3**
   Dilute the content of SPERM STAIN NR 3 with 900 ml of deionised water. Please, use high quality deionised water, no tap water.

Storage and stability

The components of the kit, stored at room temperature (15 - 25º C) will remain stable until the expiration date stated on the label. The working reagents are stable at room temperature (15 – 25 º C) for a minimum of 3 months when reagents contamination or excessive evaporation are avoided.

Caution

Reagent nº 1, as a methanolic solution is flammable and toxic by contact, inhalation and ingestion. Handle with care. Waste products must be handled attending your local regulations.

Sample preparation

Sperm samples: prepare the smear with 15 µl of fresh sperm on a standard glass slide. Leave it for air drying at least 10 minutes. It is recommended to make a thin and homogeneous spreading of the sample for the best fixation of the dyes and avoiding blurring hypercolorations.

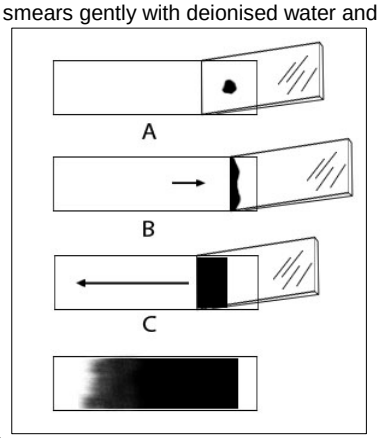
Staining procedure

1- Fix the smear by immersion in the Working Reagent Nr. 1 ; 5 times, 1 second each. Let drain.

2- Stain the smear by immersion in the Working Reagent Nr. 2 ; 5 times, 1 second each. Let drain again.

3- Stain the smear by immersion in the working Reagent Nr. 3 ; 5 times, 1 second each.

Rinse the smears gently with deionised water and leave them



for air-drying.

In order to prevent deterioration of the smears by the immersion oil coversliping is recommend with an appropriate mounting media such as DPX, Eukitt ®. Use two drops and a standard glass coverslip (50 x 22 mm).

Remarks

Staining intensity can be modified by varying the number of the immersions in solutions 2 and 3, depending on what colour is preferred to be emphasized.

Curettes with stain shall always be stored capped, specially 1 in order to avoid undesirable evaporations that could promote colour deviations from the usual stainings.

Results

Sperm head: Dark violet

Sperm acrosome: Pale violet, clearer then the head colour

Midpiece and tail: dark violet

Background: Pale pink

Red blood cells: Pale or deep pink

Neutrophils: Dark blue nucleus. Pink cytoplasm with red-violet granulations

Eosinophils: Blue nucleus. Blue cytoplasm with red or red-orange granules

Basophils: Dark blue or purple nucleus. Purple, almost black, granules

Lymphocytes: Violet nucleus. Sky blue cytoplasm

Monocytes: Very pale violet nucleus. Sky blue cytoplasm

References

Gurr,E. (1965) "The rational use of dyes in Biology", p. 115. Leonard Hill, London.

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Maree, L.; du Plessis, S.S.; Menkvels, R. and van der Horst, G. Human Reproduction, 25 (6), 1369 – 1382 (2010).

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