Morphology slides SCA Protocol

PRE-STAINED SLIDES TO ANALYSE MORPHOLOGY OF SPERM

Principle:

The pre-stained slides for morphology combine conventional microscope slides and isosmotic SpermBlue stain into a ready to use device for morphology assessment of sperm samples. These pre-stained slides are designed for using with automated systems like Sperm Class Analyzer® (SCA®) or SCA SCOPE.

Product characteristics:

1 Box content: 50 slides/ 50 tests

Material required:

Deionized water

Mounting media

Pipette and tips

Coverslips 0.13-0.16 thick, preferably of 24x50 / 24x60mm

Storage and stability:

The slides must be stored protected from light and at room temperature (15 – 25°C). They can remain stable for at least one year since manufacturing date.

Sample preparation/Staining procedure:

1. Do a simple semen washing with PBS.

This step is recommended for removing the debris and mucus strands from seminal plasma that could difficult the automatic analysis of sperms. Likewise, it allows to adequate the sperm concentration in order to optimize the time spent for doing the morphology assessment:

- Take an aliquot of semen sample from about 400µl* and add in 800µl of PBS (twice the volume aliquoted). Homogenize it.

  ** The sample volume to be used may vary depending on the quantity available. The minimum sample volume in order to obtain pellet after centrifugation is 100 µl. It is important that the volume of PBS is twice the volume of the sample used. For Oligospermic samples, the smear can be prepared directly with the pellet to obtain an acceptable concentration.*

- Centrifuge aliquot at 300g for 10 min.

- Discard supernatant.

- Break the pellet (gently tapping the tube with the finger) and add the required volume of PBS to get a final sperm concentration from about 50 M sperms/ml. Use the formula:

$$V_{spz\ washed} = \frac{C_{semen} \times V_{semen}}{50}$$

(Where C_{semen} corresponds to the original concentration of sperms in semen (M/mL), V_{semen} the volume of semen used for washing (before adding the PBS) and $V_{spz\ washed}$ the volume of PBS to add after centrifugation).

- Resuspend pellet with the volume of PBS calculated.

2. Prepare a smear according to the WHO 6th ed. Guidelines:

Pipette 10µl of sperm sample on the edge of the slide and drag the drop with a second slide following a 45º angle (see figure 1).

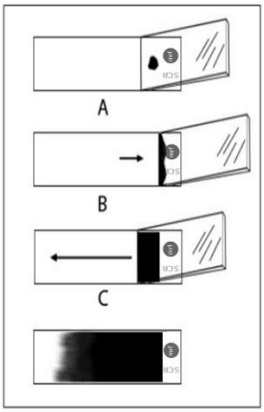

Figure 1: Smear preparation

3. Wait for 20-30 seconds at room temperature.

4. Drop the slide into deionized water twice (1 second/dip).

5. Locate the slide on a vertical position and leave it to air dry at room temperature.

6. Once completely dried, mount slide with Eukitt (or equivalent synthetic media) and a coverslip (see figure 2). Mounting preparations facilitate the correct focusing of some sperm features (e.g. vacuoles) that are omitted at high microscope magnifications when not mounted. The steps to follow are:

1. Put 2 drops of Eukitt on the coverslip.

2. Then place the slide, smear-slide down, onto the coverslip.

3. Press on the slide spread the mountant.

4. Allow the mounted smear to dry horizontally, coverslip side up.

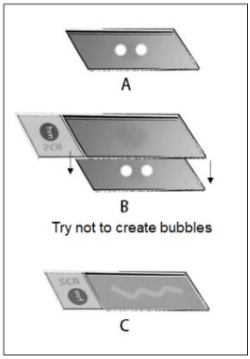

Figure 2: Mounting with Eukitt.

Manufactured by:
Microptic S.L.
Av. Josep Tarradellas, 8, 1º 6ª – 08029 Barcelona (Spain)
Phone: +34 93 419 29 10 Fax +34 93 419 94 26
www.micropticsl.com

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