Reproductive Biology Enterprises

SpermBlue® Protocol

STAIN FOR SPERM MOROPHOLOGY ASSESSMENT IN HUMAN AND ANIMAL SPECIES

Background:

SpermBlue® has been developed to stain all components of sperm (acrosome, head, midpiece, principle piece and end piece of tail) differentially in different intensities of blue (van der Horst & Maree, 2009). The staining procedure is very simple and only involves two main steps, fixing/staining in one medium (less than a minute) and dipping in water for three to six seconds.

Contents of kit:

The SpermBlue® package includes one bottle, containing 250 mL of staining solution. This solution will provide at least 500 smears for morphology assessment and last for at least two years if stored at 4 °C. Take note of expiry date.

Requirements:

- Coplin jars (plastic or glass) or similar standardized containers
- Distilled water
- Mounting medium such as DPX or Eukitt

Staining procedure:

For optimal results, it is recommended to wash semen samples before starting procedure. For example, mix 200 µL raw semen with 1 mL PBS and centrifuge at 300 *g* for 10 min. Remove supernatant and dilute the pellet with PBS to have a working concentration of 4-8 million sperm/mL.

Step 1: Make duplicate sperm smears using 10 µL semen or 10-15µl of swim-up sperm/washed sperm (adapt volume to concentration of sperm) and allow to air dry. If sperm concentration in semen is less than 20 million/mL, use 15 µL semen for preparing smear. The ideal angle of slide which is used to make smear is about 45°. If sperm concentration is low, decrease angle of slide to about 20°. A larger volume of sperm will accordingly be dragged behind moving slide resulting in more sperm on slide.

Step 2: Add SpermBlue® fix/stain solution at 20-25°C to a Coplin jar. Carefully immerse dried smears vertically into the staining tray and stain for 45-50 seconds.

Step 3: Slowly remove slides from Coplin jar and hold it at an angle of 60- 80° to drain off excess staining solution.

Step 4: Slowly immerse stained smears in Coplin jar containing distilled water. Hold slide still for six seconds. Remove slide from water and let excess fluid run off on paper. Make sure angle of slide on paper is about 60° to ensure all stain is not "sucked" from slide by paper.

Step 5: If blue colour is not intense enough, stain for another 5-10 seconds. If blue is consistently too intense, stain for 40 seconds only and dip in water for 6 seconds (performing very soft circular movements with the Coplin jar to drain excess of stain).

Step 6: Ensure slides are completely dry before mounting slide with DPX or equivalent synthetic medium for making permanent slides.

Evaluation procedure:

Step 1: When mounting medium is dry (best to leave overnight), view slides under a light microscope using the 100x oil immersion objective for human, monkey, dog and horse sperm but for CASA the 60x objective. Use preferably the 60x objective for bull and ram, boar, rat and mouse but for CASA the 40x objective.

Step 2: With human and non-human primate, horse and dolphin sperm, acrosome stains light blue and sperm head dark blue. Midpiece stains distinctly dark blue, rest of tail slightly lighter blue and end piece even lighter blue.

In domestic animals such as bull, boar and ram, acrosome stains dark blue, post acrosomal area and equatorial zone stains light blue. Midpiece stains darker blue and rest of tail slightly less dark blue.

Suitable for SCA® automatic morphology analysis (Microptic S.L., Barcelona, Spain).

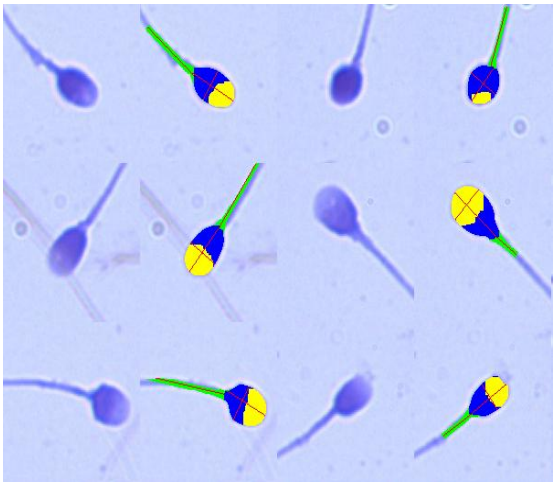
Important comments:

Initial staining results may suggest either too little staining of some sperm as well as differences in staining intensity on the same slide. Each researcher has to experiment to optimize her/his results in this context. Try and adapt staining times at temperature conditions between 20-25°C.

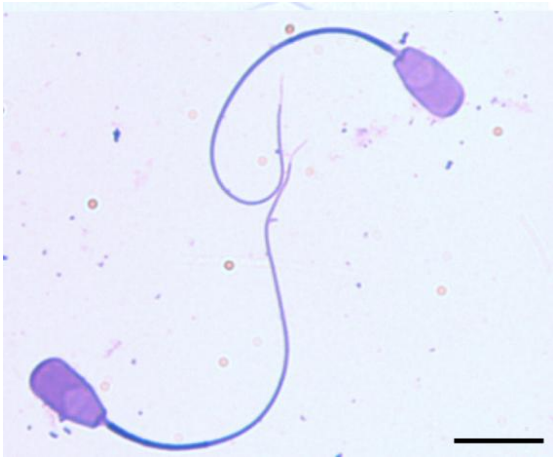
Many existing sperm staining techniques rely on "sperm painting" which is not cytologically acceptable. **SpermBlue®** clearly differentiates all sperm components and is particularly good in the identification of the sperm acrosome (van der Horst & Maree, 2009).

Examples:

Human semen (SCA® analysis)



Boar sperm



Reference:
van der Horst G, Maree L (2009). SpermBlue: A new universal stain for human and animal sperm which is also amenable to automated sperm morphology analysis. *Biotechn Histochem* 84, 299-308

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