Rapid SpermBlue® Protocol

STAIN FOR SPERM MORPHOLOGY ASSESSMENT IN HUMAN AND ANIMAL SPECIES

Background:

The stain has been developed to stain all components of sperm (acrosome, head, midpiece, principle piece of tail and end piece) differentially in different intensities of blue. 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 New Rapid SpermBlue®:

All New Rapid **SpermBlue®** packages contain one bottle with 250ml combined fixative and stain (dark blue staining solution marked Stain) sufficient to stain about 500 sperm smears on slides.

It is recommended that the staining of smears is performed in standardized containers, e.g. plastic/glass Coplin jars.

If **SpermBlue®** is stored at 4°C it will last for at least one year or longer. Room temperature storage (20 – 25°C) not guaranteed but normally lasts one year. Take note of expiry date.

Staining Procedure:

For getting optimal results, we recommend washing semen samples before starting procedure (ex: Mix 200 µl raw sample with 1ml PBS and centrifuge at 300g for 10 min. Remove supernatant and dilute the pellet with PBS to have a working concentration of 4 to 8 million sperm/ml).

Step 1: make duplicate sperm smears using 10µl of semen or 10 to 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 15µl of semen for smear. Ideal angle of slide which is used to make smear is about 45°. If sperm concentration is low, decrease angle of slide which is used to make smear to about 20°.

A larger volume of sperm will accordingly be dragged behind moving slide resulting in more sperm on slide. Ensure sperm smear is totally dry before next step.

Step 2: carefully place dried smears vertically into staining tray (Coplin-type jar) containing New Rapid **SpermBlue®** fixative/stain. Take care to slowly immerse slides in fix/stain solution at 20 to 25°C.

Reference staining times for different species:

Human/Primate	50 secs
Boar/Horse/Rat	1 min
Bull/Dog/Ram	2 min

Note that these staining periods could vary according the laboratory temperature, humidity or pH of the deionized water used for washing slides. Find best times for your lab.

Step 3: carefully remove slides from staining tray and hold it at an angle of 60° to 80° to drain off excess fixative/stain.

Step 4: dip slowly in container/Coplin jar containing distilled water, dip two times for three seconds/each and let excess fluid run off on paper. Make sure angle of slide about 60° and ensure all stain is not "sucked" from slide by paper.

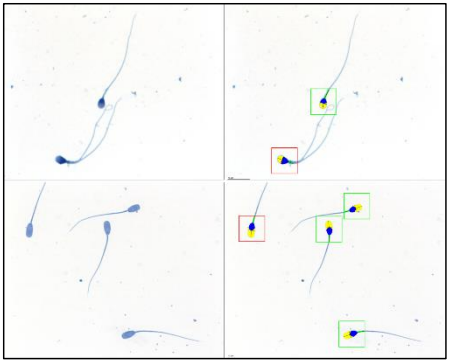
Step 5: if blue colour is not intense enough, stain for another 5-10 seconds. If blue is consistently too intense, stain for 40 seconds only and dip in deionized water for 3 seconds (performing very soft circular movements with the coplin jar to drain excess of colorant).

Step 6: ensure slide is entirely dry and then mount slide with DPX or equivalent synthetic medium for making permanent slides.

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 and 25ºC.

Many existing sperm staining techniques rely on "sperm painting" which is not cytologically acceptable. **SpermBlue®** clearly differentiates all sub-divisions of the sperm accurately and is particularly good in the identification of the sperm acrosome (van der Horst and Maree, (2009) **SpermBlue®**: A new universal stain for human and animal sperm which is also amenable to automated sperm morphology analysis, Biotechnique and Histochemistry 84:299-308).



Example:

With human and sub-human primate, horse, dolphin sperm the acrosome stains light blue and the 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 particularly the equatorial zone stains light blue. Midpiece stains darker blue and rest of tail slightly less dark blue.

Safety Datasheet for SpermBlue®:

SpermBlue® contains toxic components like all cytological stains but is not hazardous. The main active component is a slight skin, oral/nasal irritant and staining should preferably take place in a fume hood. If skin contact has occurred, wash affected area thoroughly with water.

Precautions:

All cytological stains are toxic and have to be handled with care. Always work with gloves and preferably in a fume cupboard. Only stain when sperm are fixed (dead). Do NOT use for live unfixed cells.

Example:

On the left, Human (above) and Boar (below) sperms stained with **SpermBlue®**. On the right, corresponding fields analyzed with the Sperm Class Analyzer (SCA).

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