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 (1 to 2 minutes) and dipping in water for three to six seconds. It works equally well for smears of "raw" semen as well as swim-up/Percoll/PureSperm gradient preparations, using most tissue culture media.

Please note that only fixed/dead sperm to be stained. Not for use with live unfixed cells in any procedure such as in tissue culture.

Contents of New Rapid SpermBlue®:

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

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

If fixative/stain is stored at 4ºC it will last for at least one year or longer. Room temperature storage (20 – 25ºC) not guaranteed but normally lasts one year. Take note of expiry date.

Staining Procedure:

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

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 for 1 to 2 minutes. Take care to slowly immerse slides in fix/stain solution at 20 to 25ºC.

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

Step 4: dip slowly in container/Coplin jar containing distilled water and hold still for three to six seconds and remove and let excess fluid run off on paper. Make sure angle of slide about 60º and ensure all stain is not "sucked" from slide by paper (Hint: find best times for your lab – our lab 1 or 2 min fix/stain and six seconds in distilled water – do not agitate slide in water).

Step 5: ensure slide is entirely dry and then mount slide with DPX or equivalent synthetic medium for making permanent slides. When the mounting medium is dry view under oil immersion x1000 for human, monkey, dog and horse sperm and preferably at x600 for bull and ram, boar, rat and mouse.

Step 6: if staining is not intense enough, stain for another 20 seconds. Try to avoid washing for longer than six seconds if stain is too intensely stained.

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 30ºC.

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 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.

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

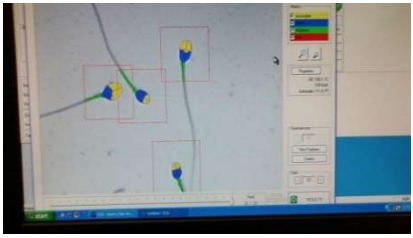
In domestic animals such as bull, boar and ram: Acrosome stains dark blue, post acrosomal area and particularly the equatorial zone stains light blue. Midpiece stains darker blue and rest of tail slightly less dark blue.

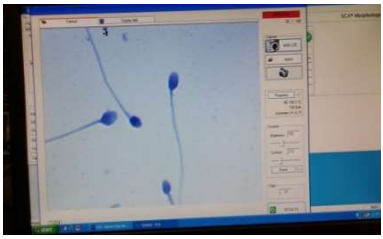
Safety Datasheet for SpermBlue®:

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.





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