SPERMAC - BD

Sperm-Morphology Stain

PRINCIPL :

The method is designed to give sharp nuclear staining, transparency of cytoplasm and good differential coloring of acidophilic and basophilic materials.

REAGNTS :

Reagent 1	Fixative	100 ml
Reagent 2	Nuclear Stain	100 ml
Reagent 3	Cytoplasmic Stain	100 ml
Reagent 4	Cytoplasmic and nucleolar Stain	100 ml

PREPARATION OF SMEARS:

Slides for morphology smears should be completely free from grease to ensure that the smears stick to the glass. If smears fall off the slides during fixation and staining, of the slides being used should be pre-cleaned with 95% (or absolute) ethanol to allow the smears to be attached firmly to the slides.

If a smear is made from a sperm suspension prepared by washing and swim-up technique, it is difficult to make an even and firmly attached smear on the slide if the solution does not contain proteins. In these cases, use slides, which have been pre-coated with albumin or add albumin to the sperm solution (final concentration 1% w/v)

TO MAKE A SMEAR

To make a smear 6 µL aliquot of semen is placed on the slide. The aliquot is then pulled out into a smear with a second slide or a coverslip. This must be done with minimal force otherwise the sperm tails might be broken. Two smears are made from each sample. If the sperm concentration is judged to be below 20 million/ mL then 10-20 µL of semen are used.

Let the smears air-dry. As soon as the moisture of the smear has evaporated, the smear should be fixed.

FIXATION OF SMEARS

Fixation: 15 minutes in Reagent (1). Smears could be kept for days in this Reagent until staining.

One (of the two) smears from each sample is kept as reserve; the other is stained and assessed.

RESULTS:

Nuclei: Blue
Acidophilic: Red
Basophilic: Blue – green

N.B

This procedure is only a guide and it is preferable if each laboratory established his own conditions.

STAINING OF SMEARS :

Fill staining jars with the different solutions (see the sequence below).
Place the microscope slides into each solution according to the following schedule (one "dip" corresponds to immersion of about 1 second):

Stain or reagent	Exposure	comments
Ethanol 50%	10 dips or 10 seconds	**Rehydration:** • Smears transferred directly from a 95% ethanol fixation solution (without drying) must be transferred through at least one container with 50 % ethanol. • Air-dried smears. Rehydration of air-dried smears need longer time, 2-3 minutes in 50% ethanol if "dry" time has been long (days or weeks).
Distilled water	10 dips	
Reagent 2	3 minutes	**Nuclear Staining:** Fixed and dried smears can be transferred directly to R2 container, but the incubation time usually must be increased. R2 is a nuclear stain. If nuclear staining is weak, exposure time can be increased, or preferably, a fresh stain solution used.
Running tap water	5 minutes	Removal of unbound R2.
Acid-ethanol (0.25% HCL in 70% ethanol)	2 dips	**Diffrentiation:** After staining, the stain is bound over the entire cell. Acid treatment removes unspecific staining. After acid treatment the slides should be put under tap water immediately (the intensity of the staining can be checked here, before continuing the staining procedure If the nuclei appear too dark this step (acid-ethanol) can be repeated).
Running tap water	5 minutes	**Cytoplasmic Staining:** To stain cytoplasmic components (see below), dehydration of cells using a series of increasing strength ethanol solutions, is necessary because the staining solutions (R3 and R4) are only soluble in alcohol.
Distilled water	1 dips	
Ethanol 50%	10 dips	
Ethanol 70%	10 dips	
Ethanol 95%	10 dips	
Reagent 3	2 minutes	Cytoplasmic staining
Ethanol 95%	20 dips	
Reagent 4	5 minutes	Cytoplasmic and nucleolar staining.
Rise in three changes of 95 per cent alcohol.		
Complete dehydration in absolute alcohol and clear in xylene.		
Mount in DPx		

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

SPERMAC - BD

Sperm - Morphology Stain
+15 to +25°C
For In Vitro Diagnostic Use

CAT. No. SP 27 28

REAGENTS

R1	**100**	**ml**
R2	**100**	**ml**
R3	**100**	**ml**
R4	**100**	**ml**

CONTACTS

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