



Vitality slides SCA Protocol

VITALITY IN BRIGHTFIELD ASSESSMENT FOR HUMAN SPERM

Rationale of test: the above test has been developed to measure two functional aspects of sperm, firstly the actual live/vital sperm in a sample, and secondly the quality/intactness of the cell membrane or the ability of the cell membrane to withstand osmotic swelling without bursting.

To measure vitality, BrightVit, a nigrosin eosin (NE) based solution is used and “dead cell membranes /compromised cell membranes” will allow eosin (Vital dye) to enter the cell and stain it pink. Alive cells will remain white. The nigrosin serves as a background stain to provide contrast.

The above BrightVit solution has been made up in a hypo-osmotic medium and accordingly at the same time hypo-osmotic swelling is measured. Intact cells/cell membranes will swell but burst cell membranes will show thin straight tails and no signs of swelling. It is accordingly not surprising that there will be often a good positive correlation between live cells (white – no staining) and swollen sperm.

Requirements: Small 0.5mL Eppendorf tubes, Pipette (2 to 50uL) and pipette tips to fit, slides. Mounting medium such as DPX or Eukitt and 20 x 50 x 0.13-0.16 mm cover slips are optional (for slide preservation).

Recommendations: Ensure that all disposables and solutions are at the same temperature (37°C) to avoid temperature shock.

BrightVit remains stable for one year if kept in dark and cool place <20°C.

This kit consists of: 8mL of BrightVit solution and 200 Vitality slides SCA.

Procedure

Step 1: Add 40µL of BrightVit solution (NE) in a small Eppendorf tube (30 µL for very low sperm concentrations 1.5 million and less per mL).

Step 2: Pipette 10µL of semen in the above Eppendorf containing the BrightVit solution, mix well for 15 to 20 seconds and leave for 5-10 minutes at 37°C.

Step 3: Put 20 µL of semen- BrightVit mix in middle of one vitality slide SCA. Put a normal slide horizontally on top of the slide containing the drop and allow the semen stain mixture to spread over the entire surface of both slides. For high sperm concentration samples (more than 10 million sperm/mL) put a drop from about 10-15 µL of semen- BrightVit mix. For low sperm concentration samples (less than 4 million sperm/mL) use about 20-25 µL of semen- BrightVit mix.

Step 4: Move the slides in opposite directions, you now have two slides that can be evaluated. Leave slides at room temperature to air-dry completely.

Mounting slides with Eukitt, DPX or any good synthetic medium is recommended when preservation of sample is required. Once

mounted, put slides in a dark box – eosin is light sensitive and, if left for several days to a few weeks in light, all sperm may become pink.

Step 5: Analyze the slide with SCA system. Score at least 200 sperm, and if the number cannot be reached or there is some problem with first slide, use the replicate. White sperm are scored as alive and pink as dead.

Express results as a percentage.

It is important to know that % vitality (live white cells) should be either the same as % motile or more than the percent motile.

Special notes: Hyperviscosity of semen is often a problem in fertility clinics. In these cases, it is strongly recommended washing semen with a conventional sperm washing medium or even PBS (ex: Mix 200 μ L raw sample with 1 ml of 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).

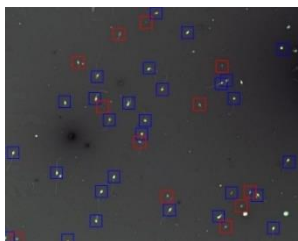


Fig. 1: Red boxes showing pink – dead sperm.
Blue boxes live sperm.

Also, aspiration of the viscous specimen into a Luer-lock syringe, forcing its ejection through a G18 needle, minimize the viscosity of the seminal plasma.

Interpretation of results

Lower reference limit: At least 54% of sperm should be vital for BrightVit stain (white sperm with swollen tails).

See this page for Fig. 1 showing vitality analyzed in automatic mode: BrightVit, and Fig. 2 at higher magnification showing all white cells swollen – showing hypo-osmotic swelling test (HOS). These tests are of great importance in IVF.



Fig. 2: White sperm show osmotic swelling of tails –usually folded back in a loop as it contains a swelling droplet at that point. In contrast, the pink sperm have thin straight tails – burst. So here HOS = Percent vitality.

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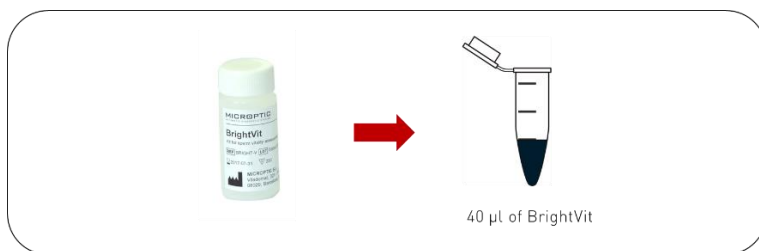
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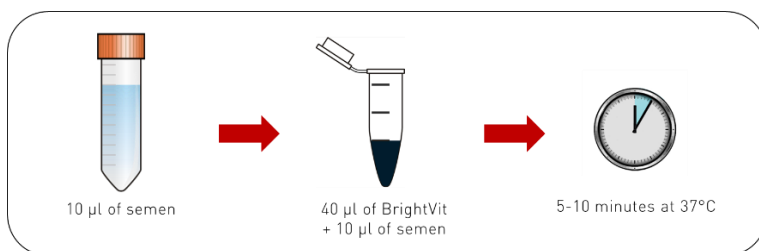
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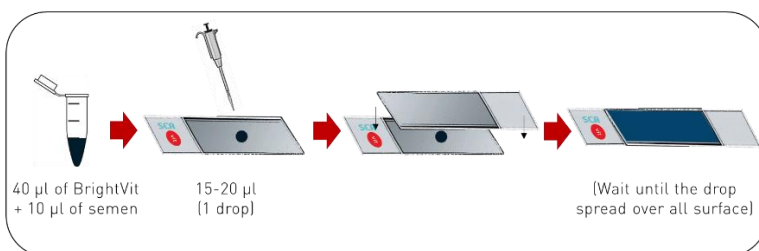
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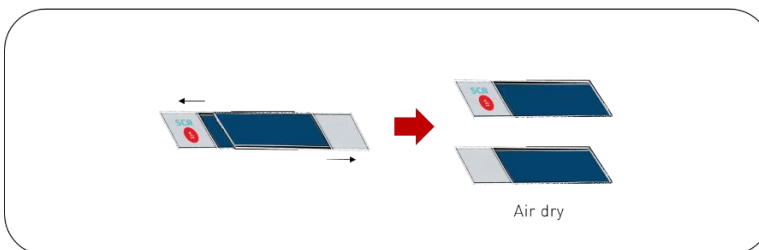
Step 2:



Step 3:



Step 4:



Step 5: Analyze with the system.