BrightVit Protocol

VITALITY IN BRIGHTFIELD ASSESSMENT FOR HUMAN SPERM AND MOST ANIMAL SPECIES

Rationale of test: the above test has been developed to measure two functional aspects of sperm namely 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. Live cells will remain white. The nigrosin serves as a background stain to provide contrast.

The above **BrightVit** solution has been made up in 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 often be a good positive correlation between live cells (white – no staining) and swollen sperm.

Requirements: Microptic **BrightVit** kit, small 0.5mL Eppendorf tubes, Pipette (2 to 20uL) pipette tips to fit, slides and 20 x 50 mm cover slips. Mounting medium such as DPX or Eukitt. Ensure that all disposables and solutions are at the same temperature to avoid temperature shock.

BrightVit kit consists of: 10mL of NE solution in dark bottle will provide about 250 tests (one year if kept in dark and cool place <20°C).

Procedure:

For getting optimal results, we recommend washing semen samples before starting procedure (ex: Mix 200µL 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).

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 and mix well for 15 to 20 seconds and leave for 5-10 minutes at 37°C.

For some animal species (Boar and Bull) incubate the sperm-BrightVit mix at 37°C for 15-20 minutes.

Step 3: put 15-20 µL of semen- **BrightVit** mix in middle of one slide. Put another 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 at room temperature to dry completely. Mount coverslip with DPX or any good synthetic medium.

Once dried, 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: use 20x objective of microscope with Brightfield optics and set critical and Köhler illumination correctly. Even background illumination is required.

Preferably select those areas on slide that are quite darkly stained and avoid the light stained areas.

Score at least 200 sperm on different parts of slide manually or do Automatic SCA **BrightVit** analysis forming part of the Vitality module.

White sperm are scored live and pink as dead.

Express results as a percentage.

Important is 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 washing medium or even PBS. 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 (For more information see ViscoFlux Protocol).

Semen from most animal species present a very high sperm concentration. Thus, dilution with specific non-organic extenders is required before mixing sperm sample with **BrightVit**. Note that some organic extenders (skim milk/egg yolk) could generate artifacts that difficult the differentiation between live/dead cells.

Hypo-osmotic swelling test:

Using the same slides count **manually** the number of swollen sperm compared to burst (straight thin tails) sperm 20x or 40x objective using bright field optics and express as a percentage.

Interpretation of Vitality and HOS:

Lower reference limit: at least 58% of sperm should be vital for **BrightVit** NE stain (white sperm) and 58% for swollen sperm.

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.

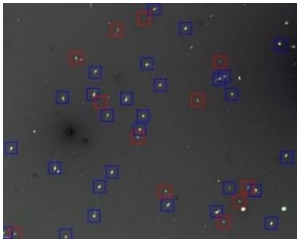

Fig. 1: Red boxes showing pink – dead sperm. Blue boxes live sperm (x20 objective)

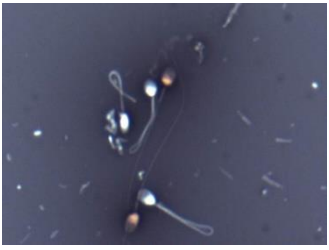

Fig. 2: White sperm show osmotic swelling of tails –usually folded back in a loop as it contains a swelling droplet at that point. In contrast, the pink sperm have thin straight tails – burst. So here HOS = Percent vitality.

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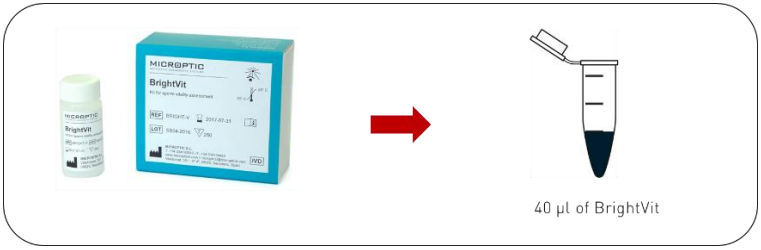
Step 1:



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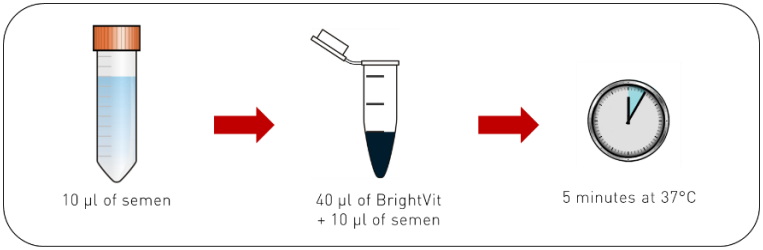

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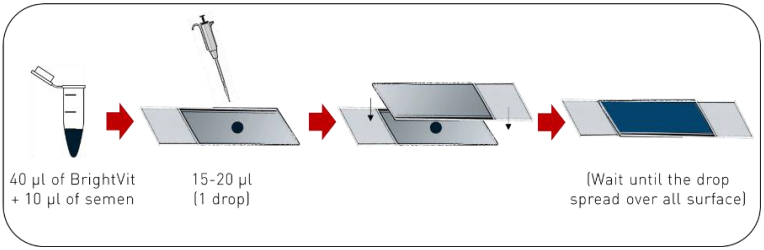

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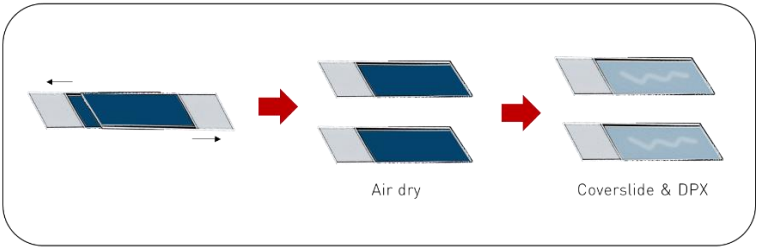

Step 5: