Vitality Staining

Sperm VitalStain™

Background

Sperm vitality should be determined in semen samples with 50% or more immotile spermatozoa according to the WHO laboratory manual for the examination of human sperm.

Sperm VitalStain™ uses the eosin-nigrosine technique in a one-step method to establish the percentage of live spermatozoa. It is based on the principle that dead cells (i.e. those with damaged plasma membranes) will take up the eosin and stain red. Nigrosine provides the background to facilitate visualisation of the unstained (white) live cells. (Ref. 12)

Reagents and Equipment

- Light microscope (40 – 100 x magnification)
- Microscope slides
- Pipette
- Test tube

Procedure

1. Shake the bottle of Sperm VitalStain™ before use.
2. Take an equal amount of Sperm VitalStain™ and the sperm sample (eg. 50 µL SVS + 50 µL sample). Use for example an eppendorf tube.
3. Mix well.
4. Leave for 30 seconds at room temperature.
5. Prepare a slide using your conventional method or use the method recommended by Nidacon.
6. Transfer a 20 µL drop onto a labelled microscope slide with a pipette, making a string/line of fluid in the middle of the slide.
7. Cover this slide with a second microscope slide and, when the drop is evenly spread between the two slides, pull them apart from each other horizontally. You then have two good slides.
8. Air dry the two slides and examine. If you want to store for later use, mount the slides with DPX or equivalent mountant, and a cover slide.
9. Examine using a bright field 40 x objective or a 100 x objective under oil immersion.
10. Count 200 sperm, the white (unstained) are classified as alive and the red or pink are classified as dead. Sperm coloured only at the neck region are classified as alive.

Tips

- The 100x objective with immersion oil will give you a very clear picture of stained versus unstained sperm.