

Standard Operating Procedure-III

Product Name--CRYO-VITALITY- E (SPERM VITALITY STAINING KIT)

The sperm vitality is reflected in the proportion of spermatozoa that are “alive”. It is measured by assessing the ability of sperm plasma membrane to exclude extra-cellular substances like dyes. Sperm vitality should be determined in semen samples with less than fifty percent motile spermatozoa. Vitality assessment also provides check on the accuracy of motility assessments; as the percentage of live spermatozoa should be slightly exceed the total percentage of motile spermatozoa.

Eosin-Nigrosin staining is used for assessing vitality. The technique is based on the principle of dead cells will take up the eosin, and as a result stain pink. The Nigrosin provides a dark background, which makes it easier to assess the slides. The assessment can be carried out at any time and slides also can be preserved for future assessment and record.

Plain eosin staining also can be used to assess vitality in wet smears. This provides quick assessment at the same time of count and motility assessment.

MATERIAL INCLUDED IN THE KIT

Cryo-Vitality E 1 ml

STORAGE

Store reagents at 2-8°C

EQUIPMENT REQUIRED

Light microscope (1000X magnification)

Microscope slides pre-cleaned

Cover slips

Pipettes

Test tubes

METHOD

Cryo-Vitality E

- 1 Mix equal volumes of well-liquefied semen and Cryo-Vitality E (small drops) on a pre-cleaned microscopic slide. Cover with a 22x22 mm cover-slip if there is excess mixture of semen and stain seen blot lightly with tissue paper. Allow 5 minutes for settling and staining of the sperms.
- 2 Examine under high power using high quality non-phase-contrast objective and correctly adjusted bright-field optics.

INTERPRETATION

Spermatozoa that are white (unstained) are counted as live and those showing any degree of pink or red are dead.

LIMITATIONS OF THE METHOD

Spermatozoa stained with this kit can not be used for any further procedures. To differentiate live spermatozoa from dead spermatozoa for use in ICSI (low viability samples), the HOS (hypo-osmotic swelling) test should be used.

WARNINGS AND PRECAUTIONS

All human, organic material should be considered potentially hazardous.

Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens.