Eosin-Nigrosin Stain for Sperm Vitality – Technical Memo

SOLUTIONS: 500 ml
- Eosin Y Stain 0.5%, Aqueous NaCl Part 1068B
- Nigrosin Stain 10%, Aqueous Part 1272B

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Eosin-Nigrosin Stain for Sperm Vitality procedure provides ready-to-use solutions for permanent slide preparation and evaluation of sperm vitality. The Nigrosin Stain provides a dark background for easier recognition of both viable (membrane-intact) and non-viable (membrane-damaged) spermatozoa that will be unstained or stained with the Eosin Y solution.

METHOD:

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

STAINING PROCEDURE:

1. Assess sperm vitality as soon as possible after liquefaction of the semen sample; approximately 30 minutes after collection.
   a. See Procedure Note #1.
2. Mix sample with thorough swirling before aliquoting.
3. In a small vial, centrifuge tube, well plate or slide, combine;
   a. Fresh semen One drop
   b. Eosin Y Stain 0.5%, Aqueous NaCl Two drops
   c. Wait 30 seconds
   d. Add Nigrosin Stain 10%, Aqueous Three drops
   e. Gently swirl to mix
   f. See Procedure Note #2.
4. Place one drop of mixture on microscopic slide(s) and prepare a film type smear.
   a. Repeat for as many smears needed.
5. Allow slide(s) to air-dry.
6. Coverslip air-dried slides with compatible mounting medium.
7. Examine microscopically.

PROCEDURE NOTES:

1. There is a 30 to 60 minute window from time of collection in which slide preparation for sperm vitality assessment should occur to avoid compromising the sample.
2. Use separate droppers/pipettes for each solution and step to avoid sample and reagent contamination.

RESULTS:

Viable sperm White/faint pink heads
Non-viable sperm Red/dark pink heads
Background Dark

REFERENCES:

5. Modifications developed by Newcomer Supply Laboratory.