Eosin-Nigrosin Stain for Sperm Vitality – Technical Memo

SOLUTIONS:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosin Y Stain 0.5%, Aqueous NaCl</td>
<td>500 ml</td>
<td>Part 1068B</td>
</tr>
<tr>
<td>Nigrosin Stain 10%, Aqueous</td>
<td></td>
<td>Part 1272B</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Viable sperm</th>
<th>Non-viable sperm</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>White/faint pink heads</td>
<td>Red/dark pink heads</td>
<td>Dark</td>
</tr>
</tbody>
</table>

REFERENCES:

5. Modifications developed by Newcomer Supply Laboratory.