Vitality-I

evaluation of sperm membrane structural integrity

REF SP/SFT/V-003-A
Turnaround time for test: 15-18 min

Store at: 2°C - 8°C after receiving
Sperm **membrane integrity** (structural & functional) when questionable or inadequate may compromise sperm fertilizing capacity (by affecting motility, capacitation, acrosome reaction & binding of sperm on Zona Pellucida).

*Membrane integrity is evaluated through two tests:*

- Sperm **Vitality (Structural Integrity)** - Supravital **Dye Exclusion**
- **HOS** [Hypo-osmotic Swelling] (Functional Integrity)

Sperm **vitality** is a reflection of live **membrane intact spermatozoa**. It is **tested** based on the ability of cell membrane to *exclude vital stains* (Eosin - Nigrosin dye) from entering the **spermatozoa & permeate** into its nucleus. When physically **damaged** or **broken**, the eosin Y dye is able to **stain sperm**. If membrane is **intact**, the dye is **unable** to do so.

**Vitality** thus **evaluates viability** of cell i.e.

- **Live (Viable)** - Unstained
- **Dead (Non-viable)** - Stained

**NOTE:** Sperm Vitality is also 'Cross – Verification – Quality – Check' for sperm motility evaluation as number of Live (Viable) sperm (as determined by supravital stain) is supposed to be always same or greater than the percent of motile sperm.
Specimen Preparation

• Semen sample is collected with:
  - Abstinence period of 2-7 days.
  - Ideal collection through masturbation in sterile container.
  - Non-spermicidal polyurethane semen collection pouch (Sperm Collect™) can be used when required.

• Semen sample is allowed to liquefy and then well mixed for performing test.

• Ideally test is to be performed within 30 to 60 min of collection.

Special Instructions:

• Hyperviscous semen sample should be processed to bring towards normal viscosity. (Viscosity-CH™ or Viscosity-BR™ kit can be used)

• Severe oligospermic semen sample (i.e. sample with Sperm Concentration less than 5 millions/mL) should be processed to concentrate the sperm concentration to around 8-10 millions/mL before performing test.

• Frozen semen plasma must be thawed at 37°C (with Sperm Warmer™) before performing test.
Kit Contents

- Eosin - Nigrosin Solution : 2.5 mL

Other Reagents (Required But Not Provided In Kit):
- Distilled Water
- Xylene (Neoclear)
- Mounting Solution
- Immersion Oil

Content Box Diagram:

![Content Box Diagram](image)

Storage Conditions:
- The kit should be stored in dark at 2°C - 8°C after receiving.
- Bring all the reagents to room temperature before use.
- Once opened, store reagents in the fridge protected from light.
- Expiry date is printed on the outside of the box.

Equipments

REQUIRED BUT NOT PROVIDED IN KIT
- Microscope
- Controlled Temperature 37°C Dry bath (Sperm Warmer™ / Water bath)
- Pipettes Set
- Slide Warmer™
- Stop watch
- Semen Analysis Chamber (Sperm Meter™)
- Microtip Box
- Glass Slide Stand

Disposable Materials

REQUIRED BUT NOT PROVIDED IN KIT
- Hand gloves
- Semen Collection Container
- Non-spermicidal Semen Collection Pouch (Sperm Collect™)
- Microtips
- Pasteur Pipettes
- Test Tubes
- Glass Slides
- Coverslips
- Microtubes / Storage Vials
- Filter Papers
Procedure

Step 1: Label plastic ware & disposable material with appropriate Patient ID & Sample ID.

Step 2:
- Take 10 µL of undiluted, well mixed liquefied semen.
- Add 50 µL of Eosin-Nigrosin dye solution in a microtube.
- Mix the dye & sample thoroughly with the help of pipette tip & wait for 30 sec.

Step 3: After 30 sec, place 10 µL of sample from Step 2 on a clean Glass-slide & prepare a smear.

Step 4: Allow the smear to air dry. (Use Slide Warmer™)

NOTE: Examine the dry smear as soon as possible.

Quick Glance

Take 10µL Liquefied Semen sample

Add 50µL Eosin - Nigrosin Solution & mix well

Wait 30 secs

Take 10µL sample & prepare smear.

Air dry

Examine under 100x (objective lens) as soon as possible
Examination

- **Put** a drop of **oil immersion** on dry smear.
- **Examine** smear under microscope with help of **100x** lens.
- **Examine** at least **200 sperms** & count the following:
  - **Unstained / White** Sperm (Indicative of **Live** Sperm)
  - **Red / Dark Pink** Sperm (Indicative of **Dead** Sperm)

**NOTE 1**: “Leaky necks” sperm stained only in neck region (Heads remain unstained) are considered as live sperm.

**NOTE 2**: Red / dark pink color is more prominent in post acrosomal area of head.

**NOTE 3**: The finding of a high percentage of viable (Live) sperm in the presence of extremely low motility strongly suggests the presence of an ultra structural (cytoskeleton) sperm defect.

**NOTE 4**: The Supravital Stains are not appropriate for assessing the vitality of the cryo preserved spermatozoa because Glycerol interferes with the stain.

**NOTE 5**: The sum of percentage (%) of Dead sperm & motile sperm should not exceed 100%.

Reference Image:
CASA with Auto & innovative Expert Mode

Individual test module - Morph/Vitality is also available.

- Result interpretation is supported with -

CASA with Auto &
innovative Expert Mode

- This test provides presumptive quantitative information of sperm vitality.
- This parameter should be analyzed by a specialist.
- The result should be evaluated taking into account all clinical & laboratory findings related to the same sample.

<table>
<thead>
<tr>
<th>Result</th>
<th>Reference Value For Live Sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Of Sperm Evaluated : _____</td>
<td>Normal : &gt; 58%</td>
</tr>
<tr>
<td>No. Of Unstained Sperm (Live) : _____</td>
<td>Equivocal : &gt; 55% &amp; ≤ 63%</td>
</tr>
<tr>
<td>No. Of Stained Sperm (Dead) : _____</td>
<td>Abnormal : &lt; 55%</td>
</tr>
</tbody>
</table>

(As per fifth edition of WHO laboratory manual for examination and processing of human semen).
Permanent Stained Slide:

- Dip dried stained - slide into Xylene (Neoclear) solution just prior to coverslipping.

- Place the mounting media on the slide.

- Place the coverslip on to the slide as quickly as possible to avoid air-drying & air bubbles.

Precautions:

- All patient samples & reagents should be treated as potentially infectious & the user must wear protective gloves, eye protection & laboratory coats when performing the test.

- The kit should be discarded in a proper biohazard container after testing.

- Do not eat, drink or smoke in the area where specimens & kit reagents are handled.

- Do not use beyond the expiration date which appears on the package label.

- It is recommended to use of gloves & face mask.

Safety & Environment:

- Do not release the products used into the environment. Follow centre guidelines for the storage & disposable of toxic substances.

- Biological samples must be handled as potentially infectious.
Description of Symbols

- 📚 consult instructions of use
- 📌 product reference
- 📅 lot number
- ⏳ use by
- 🧪 manufacturer
- 🏥 health surveillance device for in-vitro diagnostic
- ⬇️ contains sufficient for ‘n’ tests
- 🧪 temperature limitation
- ☔️ keep dry
- ⚪️ CE mark (Conformité Européene)

Accreditations & Registered Certificates

- **ISO 13485 : 2003** Certified
- [CE] Certified
- **GMDN** Registered
- **US FDA** Registered

For more information & procedure videos

- [www.spermprocessor.com/sft-vitality.html](http://www.spermprocessor.com/sft-vitality.html)
- [www.youtube.com/watch?v=Xvp1YP1kM6k](http://www.youtube.com/watch?v=Xvp1YP1kM6k)

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