

Sperm Vitality

Concept:

Sperm **membrane integrity** (structural and functional) when questionable or inadequate may compromise sperm fertilizing capacity (by affecting motility, capacitation, acrosome reaction, and binding of sperm on Zona Pellucida).

Membrane integrity is evaluated through two tests:

1. **Sperm Vitality** (Structural Integrity) – Supravital **Dye Exclusion**
2. **HOS** (Hypo-Osmotic Swelling) [Functional Integrity]

Sperm Vitality reflects the number of live **membrane intact spermatozoa**. It is **tested** based on the ability of cell membrane to **exclude vital stains** (Eosin – Nigrosine dye) from entering the **spermatozoa** and **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. For example:

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

Note: Sperm Vitality is also an additional quality check for sperm motility evaluation since the number of Live (Viable) sperm, as determined by vitality staining, should be the same or greater than the percent of motile sperm.

Specimen Preparation:

- Semen sample is collected with:
 - **Abstinence period of 2 to 7 days.**
 - **Ideal collection** through **masturbation** in sterile container.
 - **Non-spermicidal polyurethane semen collection pouch** can be used when required.
- Semen sample should liquefy and then mixed before testing.
- Ideally, the test should be performed within **30 to 60** minutes of the sample collection.

Special Instructions:

- **Hyper-viscous** semen sample should be processed to bring towards normal viscosity. (BIOSCREEN **Viscosity** kit can be used to help lower sample viscosity).
- Severe **oligospermic** semen sample (i.e., sample with Sperm Concentration less than **5** million / mL) should be processed to concentrate the sperm concentration to around **8 to 10** million / mL before performing the test.
- **Frozen semen** plasma must be thawed at **37°C** before performing the test.

Kit Contents: 2.5mL of Eosin-Nigrosin Solution.

REQUIRED BUT NOT PROVIDED IN KIT:

- Distilled Water
- Xylene
- Mounting Solution
- Immersion Oil

Storage Conditions:

- The kit should be stored in dark at 2°C to 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.

Procedure:

- **Step 1. Label** plasticware and disposable material with appropriate **Patient ID** and **Sample ID**.
- **Step 2.**
 - **Take 10µL** of undiluted, well-mixed **liquefied semen**.
 - **Add 50µL** of **Eosin-Nigrosin** dye solution in a microtube.
 - **Mix the dye and sample** thoroughly with the help of **pipette tip** and **wait for 30** seconds.
- **Step 3. After 30** seconds, **please 10µL** of sample from **Step 2** on a clean glass-slide and prepare a **smear**.
- **Step 4.** Allow the smear to **air dry** or use a **slide warmer**.

Note: Examine the dry smear as soon as possible.

Examination:

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

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

Red / dark pink color is more prominent in post acrosomal area of head.

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.

The Supravital Stains are not appropriate for assessing the vitality of the cryo preserved spermatozoa because Glycerol interacts with the stain.

The sum of percentage (%) of Dead sperm and motile sperm should not exceed 100%.

Reference Image:



Result:

- Number of Sperm Evaluated: _____ • Number _____ of
Unstained Sperm (Live): _____ • Number of **Stained**
Sperm (Dead): _____
- **Normal Value for Live Sperm.**
 - **Normal.** >58%
 - **Equivocal.** $\geq 55\%$ and $\leq 63\%$
 - **Abnormal.** <55%

Limitations:

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

Cover Slipping Stained Slides:

- **Permanent Stained Slide.**
 - Dip the stained slide in Xylene solution prior to cover slipping.
 - Place the mounting media on the slide.
 - Place the coverslip onto the slide as quickly as possible to avoid air drying and air bubbles.

Precautions:

- All patient samples and reagents should be treated as potentially infectious and the user must wear protective gloves, eye protection, and 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 and kit reagents are handled.
- Do not use beyond the expiration date which appears on the package label.
- It is recommended to use gloves and a face mask.

Safety and Environment:

- Do not release the products used into the environment. Follow guidelines for the storage and disposal of toxic substances.
- Biological samples must be handled as potentially infectious.