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Vitality-I

evaluation of sperm
membrane
structural integrity

 SP/SFT/V-003-A



User Manual

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Turnaround time for test: 15-18 min



Store at: 2°C - 8°C after receiving

Concept

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 **2-7days**.
 - **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 wellmixed 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 5millions/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.

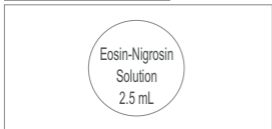
Kit Contents

- 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 :



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 out side 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 μ L** of undiluted, well mixed **liquefied semen.**
- Add **50 μ L** 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 μ L** 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 **10 μ L** Liquefied **Semen** sample



Add **50 μ L** Eosin - Nigrosin Solution & mix well



Wait **30 secs**



Take **10 μ L** 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.

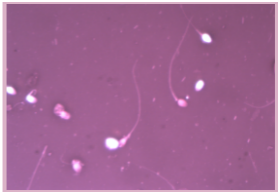
NOTE 2 : Red / dark pink color is more prominent in post acrosomal area of head.

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 :





- Result interpretation is supported with -

sperm soft

CASA with **Auto** &
innovative **Expert Mode**

- Individual test module - **Morph/Vitality** is also available.

- No. Of Sperm Evaluated : _____
- No. Of **Unstained** Sperm (Live) : _____
- No. Of **Stained** Sperm (Dead) : _____

Reference Value For Live Sperm :

- Normal : **>58%**
- Equivocal : **≥ 55% & ≤ 63%**
- Abnormal : **< 55%**

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

Limitations :

- 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.

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.

- 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 & disposal 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



 www.youtube.com/watch?v=Xvp1YP1kM6k

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