



www.spermprocessor.com



Sperm Processor Pvt. Ltd.

6, Welcome Nagar, Garkheda,
Aurangabad (MS) - 431005, India.

Ph.: +91 240 6603800

Fax: +91 240 2341694

Email: info@spermprocessor.com

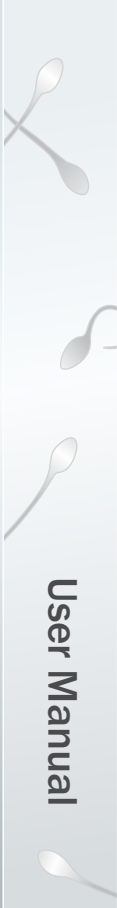


HOS

evaluation of sperm membrane
structural & functional integrity

REF SP/SFT/H-004

IVD



User Manual

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product



Turnaround time for test: 45 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**)

Hypo-osmotic swelling (**HOS**) test evaluates the functional integrity of plasma membrane by its ability to maintain equilibrium between the sperm cell & the environment. *It is based on principle that under hypo-osmotic stress there is influx of fluid causing normal sperm tail to coil & balloon or swell.*

A **dead spermatozoa** exhibits **uncontrolled swelling** to the degree of membrane rupture resulting in **tail straightening**.

The HOS test **identifies live spermatozoa without killing** them, allowing utilization of these spermatozoa for therapeutic procedures, such as Intra Cytoplasmic Sperm Injection (ICSI).

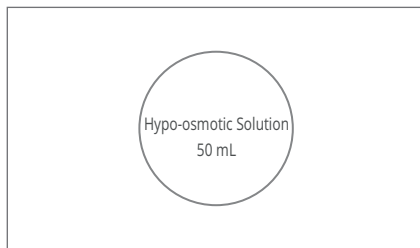
- Semen sample is collected with :
 - **Abstinence period of 2-7days.**
 - **Ideal collection** through **masturbation** in sterile container.
 - **Non-spermicidal polyurethane semen collection pouch (Sperm Collect™)** can be used when required.
- Semen sample is allowed to liquefy and then well mixed 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 5millions/mL) should be processed to concentrate the sperm concentration to around 8-10 millions/mL before performing the test.
- **Frozen semen** plasma must be thawed at 37°C (with Sperm Warmer™) before performing test.

- Hypo-osmotic Solution : 50 mL

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.

REQUIRED BUT NOT PROVIDED IN KIT

- Microscope
- Controlled Temperature 37°C Dry bath (Sperm Warmer™ / Water bath)
- Pipette Set
- Slide Warmer™
- Stopwatch
- Semen Analysis Chamber (Sperm Meter™)
- Microtip Box
- Glass Slide Stand

REQUIRED BUT NOT PROVIDED IN KIT

- Hand gloves
- Semen Collection Container
- Non-spermicidal Semen Collection Pouch (Sperm Collect™)
- Microtips
- Pasteur Pipettes
- Glass Slides
- Coverslips
- Microtubes / Storage Vials
- Filter Papers

Step 1 :

Label plastic ware & disposable material with appropriate **Patient ID** & **Sample ID**.

Step 2 :

- **Incubate 1 mL** of **HOS** solution in a micro tube.
- **Keep** at **37°C** for **5-10** min.

Step 3 :

Add 0.1 mL of semen (well mixed & liquefied) to **Step 2** HOS solution & **mix** gently.

**Step 4 :**

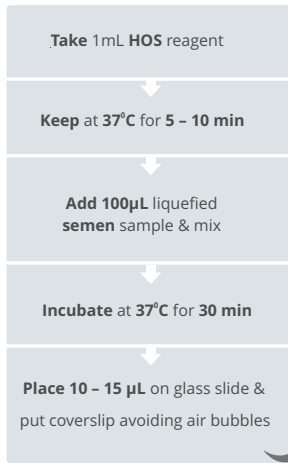
Incubate the **Step 3** solution for **30 min** at **37°C**. (Max 2 hrs)

Step 5 :

- **Mix** the **Step 4** solution gently & place **10-15 µL** on clear glass slide.
- **Put** the **coverslip**.
- Avoid air bubbles.



Quick Glance



Examine
under microscope with **40x** magnification
(Prefer Phase Contrast)

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Examination

- Examine the sample under microscope at **40x** magnification.
- **Evaluate** at least **200 sperm** and **observe sperm tail** for **curling** (swelling) or **non-curling** (straight).

• Following sperm curling (swelling) patterns are commonly observed.

=> **Tip swelling :**

Only **tip** of the tail is **swollen**, whereas **rest** of the tail is **normal**.

=> **Hairpin swelling :**

Tail swelling is observed at the **junction** of main piece and mid-piece junction. Swelling of the tip may or may not occur in these sperm.

=> **Shortened & Thickened tail :**

Sort of Ring pattern.

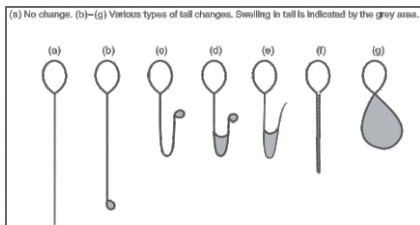
=> **Partly or completely enveloped sperm tail :**

Tail "balloons" due to swelling.

NOTE 1 : Prior to HOS test, observe for spontaneous tail swelling or coiling in liquefied sample.

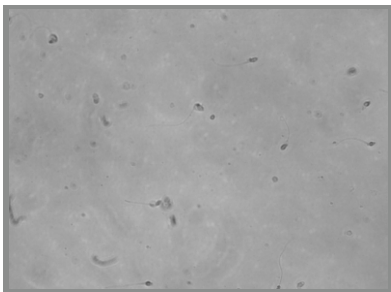
NOTE 2 : For accurate reporting subtract such number from the final score of sperm with curled tail.

Schematic Representation :



(Fifth edition of WHO laboratory manual for examination and processing of Human Semen).

Reference Image :



NOTE 3 : The sperm tail seem to be particularly sensitive to swelling & curling, since the membrane is more loosely attached to the underlying structure in this region than the membrane from the sperm head area.

NOTE 4 : Swelling indicates the membrane integrity & the pattern of tail curling reflects skeletal integrity of tail, which may vary from sperm to sperm & may not depend upon pattern of motility.

NOTE 5 : No tail swelling in majority of sperm in combination with extremely low motility suggest that Non-motile Sperm are Dead sperm.

Use of HOS Test to select the viable (Live) sperm for ICSI Procedure.

Unlike the dye exclusion vitality assays, the HOS assay does not kill the sperm, hence this assay has recently been utilized in a therapeutic manner in ICSI.

- Expose the semen sample to HOS reagent only for Five (5) minutes.
- The sperm that exhibits curling, can be used for ICSI Procedure.

- No. of Sperm Evaluated : _____
- No. of **HOS +ve** Sperm : _____
- No. of **HOS -ve** Sperm : _____

Reference Value For HOS Postive :

- 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.
- 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.

- 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.

- 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-hos.html



 www.youtube.com/watch?v=DGPpBTqwisY

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