HOS
evaluation of sperm membrane structural & functional integrity

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Turnaround time for test: 45 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)

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).
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 the test.

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

Kit Contents

- Hypo-osmotic Solution : 50 mL

Content Box Diagram:

- Hypo-osmotic Solution 50 mL

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

<table>
<thead>
<tr>
<th>REQUIRED BUT NOT PROVIDED IN KIT</th>
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<tbody>
<tr>
<td>• Microscope</td>
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<tr>
<td>• Controlled Temperature 37°C Dry bath (Sperm Warmer™ / Water bath)</td>
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<td>• Pipette Set</td>
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<td>• Slide Warmer™</td>
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<tr>
<td>• Stopwatch</td>
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<tr>
<td>• Semen Analysis Chamber (Sperm Meter™)</td>
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<td>• Microtip Box</td>
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<td>• Glass Slide Stand</td>
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## Disposable Materials

<table>
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<tr>
<td>• Hand gloves</td>
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<tr>
<td>• Semen Collection Container</td>
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<tr>
<td>• Non-spermicidal Semen Collection Pouch (Sperm Collect™)</td>
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<tr>
<td>• Microtips</td>
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<td>• Pasteur Pipettes</td>
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<td>• Glass Slides</td>
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<tr>
<td>• Coverslips</td>
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<tr>
<td>• Microtubes / Storage Vials</td>
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<td>• Filter Papers</td>
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## Procedure

**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.
**Examining the Sperm Sample**

1. **Take** 1mL HOS reagent
2. **Keep** at 37°C for 5 – 10 min
3. **Add** 100µL liquefied semen sample & mix
4. **Incubate** at 37°C for 30 min
5. **Place** 10 – 15 µL on glass slide & put coverslip avoiding air bubbles

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

**Examine**

- **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.
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.

Reference Value For HOS Positive:

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

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.
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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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