**GENERAL INFORMATION**

The hypo-osmotic swelling test (HOS Test) is an in vitro diagnostic (IVD) for professional use to evaluate the vitality of the spermatozoa in a semen sample. In contrast to dead spermatozoa, living cells have intact cell membranes which allow regulated water transport in hypo-osmotic conditions which will result in swelling or curling of the sperm tail upon incubation in HOST medium [1].

**INTENDED USE**

The HOS Test is a semi-quantitative, non-automated diagnostic test to evaluate the vitality (membrane function) of spermatozoa in a semen sample. The HOS Test may help in assessing the diagnosis and management of male infertility. The HOS Test should not be used for the selection of sperm in ART procedures such as intra-cytoplasmatic sperm injection (ICSI).

The HOS Test is designed so that 5x20 tests can be performed with 1 kit.

**TEST PRINCIPLE**

When exposed to hypo-osmotic conditions, the spermatozoon will attempt to reach an osmotic equilibrium. Consequently, water will enter the spermatozoon, thereby increasing the sperm volume. The sperm tail seems extremely susceptible to this process. The swelling of sperm is identified under a microscope (preferably phase-contrast) as changes in shape of the tail (see method section).

**MATERIAL INCLUDED WITH THE TEST**

Product code: HOST (5x 20ml Hypo-osmotic Swelling Test medium)

A certificate of analysis and MSDS are available on request or can be downloaded from our website (www.fertipro.com).

**MATERIAL REQUIRED, BUT NOT PROVIDED**

Microscope object glasses, cover glasses, (phase-contrast) microscope, pipettes and fresh pipette tips, small reagent tubes or Eppendorf tubes, water bath or heat block

**METHOD**

Scan barcode (or follow link on www.fertipro.com) to view the demonstration video:

1. **Specimen collection and preparation**
   - Standard semen collection containers should be used, typically in polypropylene and sperm survival/spERM mobility tested, when semen is collected by masturbation. Non semen-toxic plastic condoms should be used when semen collection by masturbation is not possible. Keep the semen collection container at room temperature before adding the semen sample in order to avoid large changes in temperature that may affect spermatozooa.
   - The HOS Test should be performed on fresh, untreated human semen samples, preferably within one hour after ejaculation.

2. **Reagent preparation**
   - Do not use the product if seal of the bottles is opened or defect before first use.
   - Do not use the product if it becomes cloudy, or shows any evidence of microbial contamination. Reagent is ready to use. Allow to adjust to room temperature before use.

3. **Sample preparation**
   - Let semen liquefy and keep the sample warm at 37°C.
   - In some samples the tails of the spermatozoa may be deformed before performing the test. We advise to determine the percentage of spermatozoa with curled or swollen tail before the test.

4. **Method HOS Test**
   - Transfer 1ml of HOST solution to an Eppendorf tube, preferably using a sterile syringe to avoid contamination in the HOST solution and mix gently with the pipette.
   - Add 100µL of liquefied, warmed semen to the 1mL HOST solution and mix gently with the pipette.
   - Keep at 37°C for at least 30 minutes (but not longer than 120 minutes).
   - Evaluate 200 spermatozoa by microscopy at 200x or 400x magnification (preferably with phase-contrast microscope) and observe the swelling of the tail. Swelling of sperm is identified as changes in shape of the tail, as shown in the figure [2].
   - Discard after each individual test, all used reagents and materials.
**INTERPRETATION**

1. Calculate the percentage of spermatozoa with swollen or curled tails, i.e. the vital spermatozoa, following incubation with HOST medium.
2. Subtract the percentage of spermatozoa with deformed tails observed in the sample before the test.
3. According to the WHO, a semen sample is considered normal if ≥ 58% of the spermatozoa are alive [2]. Together with the input from peer-reviewed literature, we recommend to use the following classification:
   - Viability <50%: abnormal semen sample
   - Viability 50-60%: grey zone
   - Viability >60%: normal semen sample

It is clinically important to know whether immotile spermatozoa are alive or dead. Viability results should be assessed in conjunction with motility results from the same semen sample. The presence of a large proportion of vital but immotile cells may be indicative of structural defects in the flagellum; a high percentage of immotile and non-viable cells (necrozoospermia) may indicate epididymal pathology.

**LIMITATIONS OF THE METHOD**

As the HOS Test is an IVD, spermatozoa treated with the HOS Test cannot be used in any further ART procedures.

**PERFORMANCE CHARACTERISTICS**

Repeatability and reproducibility:
- CV<sub>intra</sub> < 15%
- CV<sub>inter</sub> < 15%

**STORAGE / DISPOSAL**

- HOS Test is stable for 12 months from date of manufacture
- Use within 7 days after first opening
- Store at 2-8 °C
- Suitable for transport or short term storage at elevated temperatures (up to 5 days at 37°C)
- Do not freeze
- Keep away from (sun)light
- The reagents need to be disposed in accordance with the local regulations for disposal of medical devices
- Do not use after expiry date

**WARNINGS AND PRECAUTIONS**

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens.

Any serious incident (as defined in the European In Vitro Diagnostic Medical Device Regulation 2017/746) that has occurred should be reported to FertiPro NV and, if applicable, to the competent authority of the EU Member State in which the user and/or patient is established.

**BIBLIOGRAPHY**