

HOS Test

DIAGNOSTIC KIT FOR THE DETERMINATION OF HUMAN SPERM VITALITY

HOS Test

For *in vitro* diagnostic use only.
Reagent for professional use only.

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 intracytoplasmic 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:



Specimen collection and preparation

Standard semen collection containers should be used, typically in polypropylene and sperm survival/sperm motility 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 spermatozoa.

The HOS Test should be performed on fresh, untreated human semen samples, preferably within one hour after ejaculation.

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.

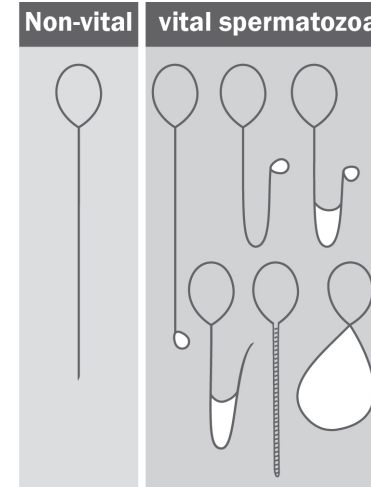
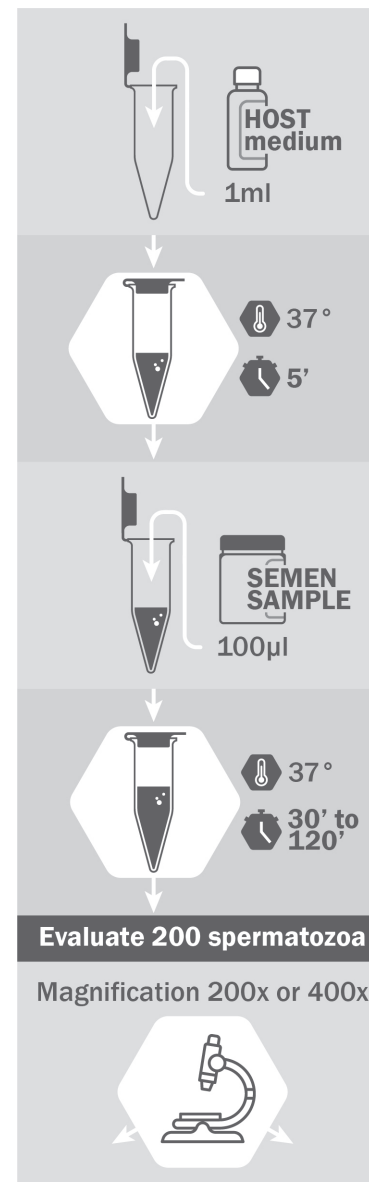
Sample preparation

- 1 Let semen liquefy and keep the sample warm at 37 °C.
- 2 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.

Method HOS Test

- 1 Transfer 1ml of HOST solution to an Eppendorf tube, preferably using a sterile syringe to avoid contamination in the HOST solution and work hygienically (fresh needle/tip). Keep the closed Eppendorf tube with HOST solution at 37 °C for about 5 minutes.
- 2 Add 100µL of liquefied, warmed semen to the 1mL HOST solution and mix gently with the pipette.
- 3 Keep at 37 °C for at least 30 minutes (but not longer than 120 minutes).
- 4 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].
- 5 Discard after each individual test, all used reagents and materials.

Graphic presentation of the protocol:



IVD

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MATERIAL INCLUDED

Catalogue number

HOST

5x 20ml Hypo-osmotic Swelling Test medium

CUSTOMER-TECHNICAL SUPPORT

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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 $\geq 58\%$ 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. Vitality 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:

$CV_{intra} < 15\%$

$CV_{inter} < 15\%$

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


- 1 Jeyendran, R.S., et al., Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J Reprod Fertil*, 1984, 70(1): p. 219-28.
- 2 WHO laboratory manual for the examination and processing of human semen, sixth edition. Geneva: World Health Organization; 2021.

SYMBOLS GLOSSARY

Symbols as defined in ISO 15223


 Catalogue number

 Batch code


 Consult instructions for use

 Manufacturer


 In Vitro Diagnostics

 °C
Temperature limit

 Use-by date

 Keep away from sunlight

Symbol as defined in IVDR 2017/746

 CE marking by Notified Body 2797



Other languages can be downloaded on our website (www.fertipro.com)