halotech®

oxisperm®

Kit REF HT-OS20

for 20 determinations

	Consult instructions of use
REF	Product reference
LOT	Lot number
	Use by
	Manufacturer
Σ	Contains sufficient for "n" test
2°C 8°C	Temperature limitation
	Keep dry

Principle of the method

Presence of destructive reactive oxygen species (ROS) occurs when natural antioxidant defences are unable to block active ROS. The net result is that cellular damage at different levels is produced. Somatic and germ line cells can be putative targets of ROS. When affecting germ-line cells, an excess of oxidative stress has a direct impact on male fertility (Aitken and De Iuliis, 2010).

A number of sensitive assays for the assessment of different levels of ROS and molecules responsible for producing oxidative stress are now well-established, but they are not frequently used by clinical andrology laboratories. OxiSperm provides the clinician with an easy, reliable and well established assay to measure a possible excess of superoxide anions (02-) present in the ejaculate. The test is based onthe nitro blue tetrazolium assay (NBT), in the form of a reactive gel (RG) in the Oxisperm kit. The NBT assay is based on the capacity of the in water soluble tetrazolium salt which is converted by the action of superoxide anions into an in water insoluble blue crystal, known as formazan (Baehner et al., 1976). In sperm, the products of this reaction become attached to the membranes of the sperm and can be easily visualized under bright field microscopy (Figure 1). These crystals produce an increasing colour intensity in the RG (from yellow to different levels of purple-blue, see diagram) which can be easily and comparatively quantified by eye through the use of a colour scale. Alternatively a colorimeter can be used to measure the absorbance of wavelengths ranging from 530 nm to 630 nm. The intensity of the colour is related to the level of oxidative stress (excess of superoxide anions) in the sample (see the colour palette).

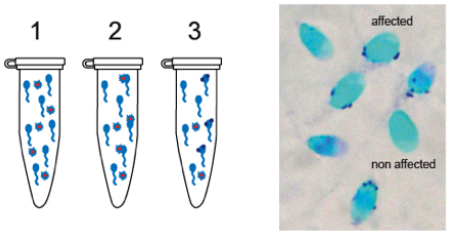

Figure 1. A diagrammatic representation of the oxidative reaction and how the sperm is transformed after the transformation of the NBT to formazan. The photograph on the right shows spermatozoa that were affected by the 02- and other where the effect is null. As the number of molecules deposited on the sperm surface increases the colour intensity of the RG increases.

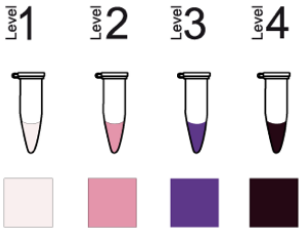

Figure 2.- Diagrammatic representation of the colour intensity level according with the capacity of the sperm sample to produce the colorimentric reaction

Protocol

1. Place the tube with the RG, using the float in a precipitation glass, and heat for 1 minute in a microwave at maximum potency. Alternatively, the RG can be liquefied in a water bath at 90ºC for approximately 5 minutes.
2. Reduce the temperature of the RG to 37°C using a water bath or in an oven at 37ºC.
3. Mix the RG with the semen sample in one of the eppendorf tubes included. Try to avoid bubles in the resulting mix.
4. Gelify the mix at 4ºC for 5 min
5. Incubate for 45 minutes at 37°C and compare the colour of the sample with the colour scheme.

Four levels (L) of intensities have been pre-classified: L1: low; L2: low-medium; L3: medium L4: high.

How to calculate the sample necessary

Divide 1000 between the concentration of spermatozoa. The resulting outcome is the volume that has to be mixed with an identical volume of RG (Proportion 1:1; Semen-GR).

Example: for a concentration of 32 millions of spermatozoa:
1000 / 32 = 31.25
31.25 µl of semen + 31.25 µl of RG.

In order to make the calculations easier it is possible to multiply the volumes by a common factor up to a maximum of 100 µl for both of the parts of the mix.

In our example this would be:
31.25 x 3 = 93.75 µl of semen + the same quantity (93.75 µl) of RG.

In this case a factor x3 has been used.
To obtain the best results, the test must be done using fresh semen samples and just after liquefaction. The samples must be tested for oxidative stress 60 min post-ejaculation. False positives may be obtained after longer delays. A temperature close to 37ºC must be maintained when mixing the semen sample with the RG, otherwise the mix will jellify.

NB: it is very important to have a precise count of the sperm concentration. High sperm concentrations may produce high colour intensities because acceleration of oxidative stress pressure by sperm collision followed by membrane damage. !

Precautions

1. All patient samples and reagents should be treated as potentially infectious and the user must wear protective gloves, eye protection and laboratory coats when performing the test.
2. The test should be discarded in a proper biohazard container after testing.
3. Do no eat, drink or smoke in the area where specimens and kit reagents are handled.
4. The use of gloves and face mask is recommended.
5. Material Safety Data Sheet is available on request.

Storage conditions

Store at room temperature (2-8ºC).

Aitken RJ, De Iuliis GN. On the possible origins of DNA damage in human spermatozoa. Mol. Hum. Reprod. 2010;16:3-13.
Baehner, R. L., Boxer, L. A. & Davis, J. (1976) The biochemical basis of nitroblue tetrazolium reduction in normal human and chronic granulomatous disease polymorphonuclear leukocytes. Blood 48, 309–313.

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