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MiOXSYS®

System Package Insert

For use with the MiOXSYS Analyzer (Catalog #100229) and the MiOXSYS Sensors (Catalog #100283)

For the Qualitative Measurement of Static Oxidation Reduction Potential (sORP) as an Aid to Assess Quality of Human Semen Samples.

Description

The measurement of static Oxidation Reduction Potential (sORP) of a human semen sample by the MiOXSYS System is performed by applying a semen sample to a MiOXSYS Sensor and inserting into the MiOXSYS Analyzer. The MiOXSYS System provides for a rapid table top *in vitro* diagnostic semen analysis utilizing electrochemical technology for the qualitative measurement (millivolts [mV]) of static Oxidation Reduction Potential (sORP) in human semen.

Intended Use

The MiOXSYS System is a rapid *in vitro* diagnostic qualitative semen analysis test for professional use only to assess semen quality in males aged 21-45 years who are undergoing fertility analysis, as an aid in conjunction with established WHO semen analysis parameters (ejaculate volume, sperm concentration, total sperm number, total motility, progressive motility, and morphology) in academic, hospital, reference laboratory, and clinical settings. The MiOXSYS System is not intended for point-of-care use.

Summary and Explanation

The measurement of oxidative stress is indicative of an imbalance between the systemic manifestation of reactive oxygen species (ROS) and other oxidants, and the biological system's ability to detoxify these oxidants or to repair the damage which can result. Disturbances in the normal reduction-oxidation (redox) state of cells may cause toxic effects on cells including but not limited to protein oxidation, lipid peroxidation, and DNA fragmentation.

Oxidation Reduction Potential (ORP) is described as an integrated measure of the balance between total oxidant activity (i.e. reactive oxygen species [ROS], oxidized thiols, superoxide radicals, hydroxyl radicals, hydrogen peroxides, nitric oxides, peroxynitrites, transition metal ions, etc.) and total reductant activity (i.e. free thiols, ascorbates, α-tocopherols, β-carotenes, uric acids, etc.). [1]

The MiOXSYS System provides a convenient and rapid measure of oxidative stress by measuring static oxidation-reduction potential (sORP). The amount of oxidative stress in comparison to the amount of reductive stress (redox balance) present in a human semen sample may be measured with a sORP electrode using the MiOXSYS System. The MiOXSYS test result is intended to be used in conjunction with standard semen analysis parameters (ejaculate volume, sperm concentration, total sperm number, total motility, progressive motility, and morphology) as an aid to assess semen quality.

Studies have demonstrated an inverse relationship between reactive oxygen species (ROS) and sperm concentration, motility, and volume[1-3]; however, ROS is only one contributor to oxidative stress and can be difficult to measure. Analysis of the seminal semen proteome can detect changes in both antioxidants and oxidants are present.[4] The advantage of the MiOXSYS test result is that it takes into account both oxidant and antioxidant activity, providing a total measure of oxidative stress with no sample preparation.

Principles of the Procedure

The MiOXSYS System is based on electrochemical technology, which uses a platinum-based electrode sensor with an Ag/AgCl reference cell, and a galvanostat-based analyzer, which completes the circuit. A human semen sample is applied to the sensor, which is then inserted into the analyzer. The sample is allowed to flow across the working electrode and to fill the reference cell, thereby completing the electrochemical circuit.

After the wetting of the electrode surfaces, the voltage is measured between the reference cell and working electrode every 0.5 seconds (or 2 Hz), while the counter is set to a voltage sufficient to achieve a 1 nA oxidizing current. The resulting sORP measurement displayed reflects the average of the final ten (10) seconds (or twenty [20] readings) of the run. The sample analysis is completed in approximately three (3) minutes. Displayed sORP values above the normal range imply an imbalance between oxidants and antioxidants (elevated oxidants) and signal the presence of oxidative stress in the specimen. Used in conjunction with standard semen analysis parameters (ejaculate volume, total sperm, sperm concentration, total motility, progressive motility, and morphology), this indicator can aid in the assessment of semen quality.

Materials and Equipment

Materials Provided

1. MiOXSYS Sensors (10 Sensors per box).

Materials or Equipment Provided Separately

1. MiOXSYS Analyzer.
2. MiOXSYS Analyzer Calibration Verification Key (CVK) and Calibration Verification Card.
3. MiOXSYS External Control Solutions (Low and High).

Materials or Equipment Needed But Not Provided

1. Disposable powder-free latex gloves or equivalent.
2. Sterile collection containers capable of holding ≥100µL of sample.
3. Vortex mixer.
4. Aerosol resistant micropipette tips capable of delivering 30 L volume.

Warnings and Precautions

1. For professional use only.
2. MiOXSYS Sensors must be used with the MiOXSYS Analyzer.
3. Performance characteristics of the MiOXSYS System have been established only on human semen samples.
4. Proper sample collection, storage and transport of human specimens are essential for accurate results.
5. External Control Solutions must be kept at room temperature 20-28°C (68-82°F)). **Do not freeze**.
6. High and Low Control Solutions are to be used to evaluate the performance of the MiOXSYS System and are not to be used in the testing of patient samples.
7. Universal precautions must be followed when handling specimens and MiOXSYS System materials and equipment.
8. Wear disposable gloves while handling specimens and thoroughly wash hands after specimen handling.
9. Follow Biosafety Level 2 and Good Laboratory Practices prior to and during testing. Treat all specimens and used MiOXSYS Sensors as capable of transmitting infectious diseases.
10. Quality Control Programs for CLIA Moderately Complex Testing Laboratories should be employed.
11. Each MiOXSYS Sensor is sealed in an airtight pouch and is intended for single use only. The protective pouch should remain sealed until use.
12. Dispose of used MiOXSYS Sensors immediately after processing pursuant to the proper disposal of biological fluids guidelines.
13. Do not eat, drink or smoke in areas where specimens, sensors or External Control Solutions are handled.

Storage and Stability

Stability for MiOXSYS Sensors has been established at 15-30°C (59-86°F). Sensors must be disposed of after the expiration date indicated on the labeling.

Quality Control

1. Good Laboratory Practices recommend the use of external quality control checks. Users should follow the appropriate federal, state, and local guidelines concerning running external controls.
2. MiOXSYS External Control Solutions are supplied separately (cat# 100279) and new lots should be identified for use.
3. The MiOXSYS External Control Solutions contain known sORP values and are used to confirm that the MiOXSYS Sensor and MiOXSYS Analyzer are functioning properly together.
4. Two levels, Low Control and High Control, are provided. The Low Control produces a value representative of an abnormally high result.
5. A separate MiOXSYS Sensor must be used for each quality control test.
6. The MiOXSYS System test results should not be relied upon if the external controls do not produce accurate results.

Controls should be employed:

- For first use of the analyzer or for training purposes.
- If the analyzer's function is suspect.
- If the sensor is suspect.
- If test results are not within the expected range.
- As part of scheduled metrology quality control checks.
- To comply with a laboratory's internal quality control and accreditation requirements.
- When a new user is performing the test.
- When a new sensor lot is being employed.
- When the storage and handling of sensors deviate from the conditions specified by the manufacturer (room temperature 5-30°C [41-86°F]).

When unacceptable quality control values are obtained, all test results should be considered invalid. Refer to the package insert for the MiOXSYS External Quality Control Solutions for further instructions.

Specimen Collection and Handling

Semen samples should be collected in accordance with published guidelines for collection and liquefaction for semen analysis and be tested within one (1) hour of liquefaction.

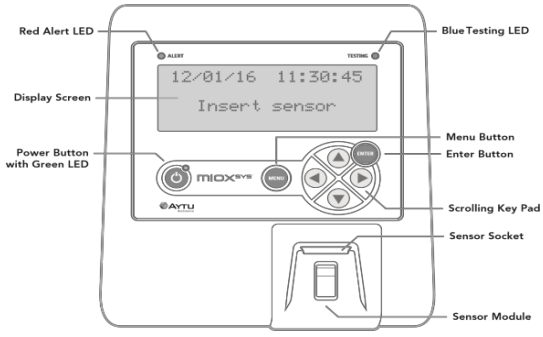
Samples that will not be tested within one (1) hour of liquefaction should be flash frozen immediately after liquefaction and stored at -80° C until tested. Specimens may be frozen and thawed one time only.

Test Procedure

A. MiOXSYS Analyzer Setup

1. Place the MiOXSYS Analyzer on a flat, level surface.
2. Prior to testing, semen samples should be brought to room temperature 5-30°C (41-86°F).
3. Press the power button on the MiOXSYS Analyzer. The green power LED on the power button will illuminate to indicate the unit is ON. If using AC power, the display screen will be backlit.
4. "MiOXSYS" and the date and time will appear on the display screen for 3 seconds.
5. When the MiOXSYS Analyzer is ready, "Insert sensor" will appear on the display screen (Figure 1).

Figure 1. MiOXSYS Analyzer



Specimen Testing

A. Sensor Insertion

1. Unseal an individual MiOXSYS Sensor.
2. Holding sensor at front side edges (Figure 2), insert the MiOXSYS Sensor face-up with the sensor electrodes facing the MiOXSYS Analyzer. Align the socket insertion end with the sensor socket on the MiOXSYS Analyzer. Make sure the sensor is fully inserted before continuing the test procedure.

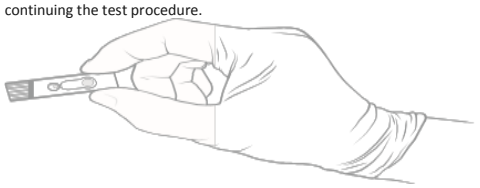


Figure 2. View of MiOXSYS

3. Once the MiOXSYS Sensor is inserted properly, "Waiting for sample" will appear on the display screen and a 2-minute sample detection countdown timer will begin.

B. Sample Application

1. The semen sample used for sORP analysis can be either fresh or frozen, but must be at room temperatures 5-30°C (41-86°F) when tested and must be tested within one (1) hour of liquefaction.
2. 30ul of the sample is required for each test and the sample must be applied using an aerosol resistant micropipette tip.
3. Apply sample to the Sample Application Port on the inserted MiOXSYS Sensor. Make sure that the entire port is covered (Figure 3).

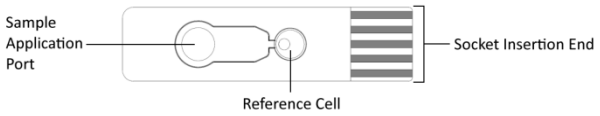


Figure 3. View of sensor showing the application port for loading of sample.

C. Sample Run

1. When the sample reaches the Reference Cell of the sensor, the testing automatically begins. Proper functioning of the test is indicated by the blinking of the blue testing LED light.
2. Once the test is initiated, the display screen will show "Processing sample" and the time remaining for the analysis.
3. **IMPORTANT NOTE:** Do not press any buttons or remove the sensor while testing is in progress.
4. If an error occurs during testing, an error code will appear on the display screen and the red alert LED light will illuminate. Please make a note of the error reading for your records. Follow the instructions on the screen to clear the error.

D. Test Results

1. Audible beeps indicate completion of the test.
2. Test results will appear on the display screen in the following order: 1) Date 2) Time 3) sORP (in millivolts or mV).
3. **IMPORTANT NOTE:** Record the date, time, and sORP value in your records prior to removing the sensor from the analyzer.
4. Remove sensor from the sensor socket after the data is recorded.

PRECAUTION: Discard the used MiOXSYS Sensor observing the proper disposal of biological fluids guidelines.

5. Once the used MiOXSYS Sensor is removed, additional samples may be tested repeating these steps, after inserting a new Sensor.
6. Once sample testing is complete, the MiOXSYS Analyzer can be switched "OFF" by pushing and holding the power button down.

NOTE: If the MiOXSYS Analyzer is "ON" but inactive, the MiOXSYS Analyzer will automatically turn "OFF." A 15-second timeout warning appears on the display with a warning beep emitted every second. The timeout clock can be reset by pressing any button.

To Check Calibration:

Calibration verification with the Calibration Verification Key (CVK) must be performed by the user upon installation of the MiOXSYS Analyzer and at monthly intervals thereafter to verify that the instrument is properly calibrated.

1. Press the power button on the MiOXSYS Analyzer. The green power LED on the power button will illuminate to indicate the unit is ON. If using AC power, the display screen will be backlit.
2. "MiOXSYS" and the date and time will appear on display screen for 3 seconds.
3. Insert the CVK into the sensor slot with the A-side facing up. The MiOXSYS Analyzer will indicate that a calibration check is being performed on the A-side.
4. When the verification is complete, the results will be displayed as:

   Side A:
   ORP = Range 99.0 mV - 101.0 mV
   ICell = Range [-101.0 nA] - [-99.0 nA]

   NOTE: Before removing the CVK, record the date, time, and results; compare them to the acceptable ranges listed on the Calibration Verification Card.
5. Repeat the procedure after inserting the CVK into the sensor slot with the B-side facing up.

   Side B:
   ORP = Range 295.8 mV - 304.2 mV
   ICell = Range [-30.4 nA] - [-29.6 nA]
6. If the MiOXSYS Analyzer is out of calibration, please discontinue further use and call Aytu BioScience, Inc. at 720.437.6580.

Calculation of Results

The sORP measurement displayed reflects the average of the final ten (10) seconds (or twenty [20] readings) of the run. The sample analysis is completed in approximately three (3) minutes. sORP values above the normal range indicate a change in the balance between oxidants and antioxidants in favor of the oxidants, and signify the presence of oxidative stress in the specimen.

NOTE: A typical example of calculating and norming sORP values to sperm concentration is illustrated below:

Number	Sample	Date	Time	sORP (mV)
1	Patient A	5/29/2015	10:13am	76.8

Sperm Concentration = 62.6 x 10^ /mL ; Patient sORP =76.8 mV;
Normed sORP = 76.8/62.6 x 10^6 mL = 1.22 mV/10^6 sperm mL

Performance Characteristics

	Abnormal Quality		Normal Quality	
	MiOXSYS/ Fails Quality Criteria	Sensitivity (95%CI)	MiOXSYS/ Meets Quality Criteria	Specificity (95%CI)
Int'l Site (n=365)	205/324	63.3% (57.8-68.5)	36/41	87.8% (73.8-95.9)
US Site (n=93)	48/74	64.9% (52.9-75.6)	17/19	89.5% (66.9-98.7)
Total (n=458)	252/398	63.4% (58.5-68.1)	53/60	88.3% (77.4-95.2)

The MiOXSYS test was designed to be a highly specific tier-one test with an overall predictive value of 97.3% (CI =94.5-98.9). This means that there is a 97% chance that a semen sample with an ORP value greater than (>) 1.38mV/10^6/mL will have abnormal quality as determined by the semen parameters outlined in the current edition of the WHO manual for semen analysis.

TESTS FOR INTERFERING SUBSTANCES

The following substances, at the specified saturated solvent/diluent concentrations, do not interfere with test results: WBC (1x$10^{6)}$ Saliva, Whole Blood (10%). Whole Blood at concentrations greater than 10% interferes with the MiOXSYS System.

Additional testing was performed with abnormal and normal semen samples specimens (per the latest W.H.O criteria inoculated bodily hormones to a final concentration of 20µg/mL. The following substances do not interfere with test results: 19-norethindrone acetate, testosterone, β-estradiol, Noregestrel.

The following antioxidant properties, at the saturated solvent/diluents concentrations indicated, do not interfere with test results: ascorbic acid (<168µM), folate (84nM), and selenium (27.5µg/mL).

CROSSREACTIVITY

Crossreactivity studies were performed with abnormal and normal semen samples specimens (per the latest W.H.O criteria) inoculated with bacterial or fungal organisms to a final concentration of 1.2 x 10^6 CFU/mL and 3.1 x 10^4 CFU/mL. None of the following organisms reacted with the MiOXSYS System: Escherichia coli, Corynebacterium diphtheria, Neisseria gonorrhea, and Chlamydia trachomatis.

Limitations

1. Performance characteristics have not been established for samples tested more than one (1) hour after liquefaction.
2. This test is intended for use with semen samples greater than or equal (>) to 1 million sperm concentration.
3. Viscous samples and liquefaction issues may affect sample flow and interfere with the sample migrating properly to the reference cell of the sensor.
4. Repeated centrifugation may lead to an artificial increase in sORP values due to the shearing forces generated by centrifugation.
5. Semen samples must be brought to room temperature 5-30°C (41-86°F) before testing.
6. sORP values are intended to be used in conjunction with standard semen analysis parameters (ejaculate volume, sperm concentration, total sperm number, total motility, progressive motility, and morphology) as an aid to assess semen quality.
7. Performance characteristics have not been established for patients less than 21 years of age and greater than 45 years of age.
8. Performance characteristics have not been established for samples that have undergone more than one (1) freeze-thaw cycle.
9. Performance characteristics have not been established for samples collected in lubricants or stored in cryoprotectant.

References

1. Pons-Rejraji, H., et al., [Role of Reactive Oxygen Species (ROS) on Human Spermatozoa and Male Infertility]. *Gynecol Obstet Fertil,* 2009. 37(6): pp. 529-35.
2. Agarwal, A., et al., Characterizing Semen Parameters and Their Association with the Reactive Oxygen Species in Infertile Men. *Reprod Biol Endocrinol,* 2014. 12:p. 33.
3. Du Plessis, S.S., et al., Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. *J Assist Reprod Genet,* 2015. 32(4): p. 509-20.
4. Intasqui, P., et al., Differences in the seminal plasma proteome are associated with oxidative stress levels in men with normal semen parameters. *Fertil Steril,* 2015.

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Glossary of Symbols

Manufacturing Date	Read Usage Instruction
In vitro Diagnostic Medical Device	This product meets the requirements of 98/79/EC of in vitro diagnostic medical devices
Underwriters Laboratory	Catalogue Number
Serial Number	Biological Sample
Re-use not Allowed	Caution, Consult Document

Authorized representative for European Community

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