To: Valued Customers

From: Technical Operations

Re: General Questions and Suggestions for Using the MiOXSYS System

Date: 5/2/2018

Technical Tips:

1. **What is sSORP?**

Oxidative stress has a negative effect on sperm function by disrupting the integrity of the DNA as a result of concurrent damage to proteins and lipids present in the sperm-cell plasma membrane, therefore affecting cell membrane fluidity and permeability. ROS generation is based upon the ability of unsaturated fatty acids to inhibit complex I of the electron transport chain, with minor inhibition of complex III, which results in electron leakage from complex I and ultimately transfers electrons to O2 molecules, therefore, posing a major threat to human spermatozoan physiological function.

sSORP is a real time electro chemical measure of oxidative stress that takes into account the complete picture of oxidants and reductants in a biological sample. Unlike other specific measures, such as MDA, DNA, isoprostane or glutathione, cysteine, alpha tocopherols, thioredoxin, sORP measures the total redox balance, and indicates when an imbalance is present.

sORP measures the voltage 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 sSORP measurement displayed reflects the average of the final ten (10) seconds (or twenty [20] readings) of the run.

2. **Has the Precision of MiOXSYS been evaluated?**
    - Intra-observer variability CV = 8.39%
    - Inter-observer variability CV = <5%

3. **Has sORP been studied with inflammation levels?**

sORP has been studied with other markers of inflammation and has been found to be associated with TLR-4 and COX-2, which were studied in leukocytospermic and non-leukocytospermic samples.

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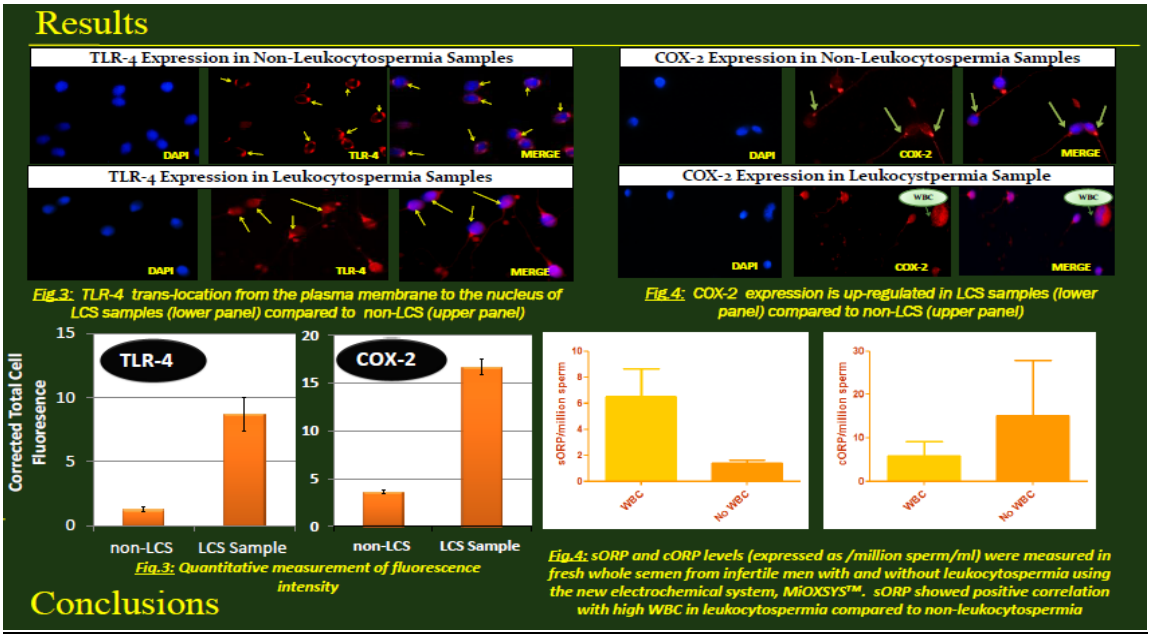

4. Why is sORP the most useful/informative sperm parameter according to one of the studies.

A multi-center study conducted at nine participating andrology centers evaluated the sORP measure in conjunction with the WHO 5th edition semene paramters. A logistic regression model was performed on all measures (six semen analysis parameters and sORP measure) in order to determine the predictability of identifying abnormal / normal semen quality within the sample.

Measures were categorized according to overall contribution and significance. sORP ranked the highest (beta 2.88, p = 0.01) in terms of predicting abnormal /normal semen quality, followed by progressive motility (beta 2.29, p= 0.00), and total motility (beta .494, p= 0.001).

5. How do I handle a very viscous sample?

For samples that will not liquefy under normal circumstances, 5% of a trypsin enzyme or 1 gram per liter of bromelin can be added to the sample for further break down (5-10 minutes). Sigma catalog T4549

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6. Has sORP been tested in donors over a duration of time?

Yes. sORP has been studied in known donor samples for greater than 90 days.

> 45 days of time interval

Donor 380	12/5/2017	12/14/2017	12/19/2017	4/26/2018
ORP (mV/10^6 spem/mL)	0.22	0.17	0.18	0.30
Time interval	9 days			
		5 days		
			128 days	

Donor 461	2/23/2016	21/7/16	4/25/2018
ORP (mV/10^6 spem/mL)	0.45	0.8	0.39
Time interval	47 days		
		839 days	

Donor 378	12/5/2017	12/8/2017	4/19/2018
ORP (mV/10^6 spem/mL)	0.38	0.25	0.14
Time interval	3 days		
		132 days	

Donor 511	2/23/2016	6/30/2016	4/24/2018
ORP (mV/10^6 spem/mL)	0.21	0.36	0.37
Time interval	128 days		
		663 days	

Do I need to perform duplicate Testing? - We ran duplicates on all analytical and initial validation studies to determine that duplicate testing was not necessary.

However, if you prefer to perform duplicate testing, then two measures within a 5-10mV difference should be averaged and recorded. If there is a greater difference than 10 mV, then you can either: 1. run another set of duplicates and use this new data if both points are within 5-10mV of each other; 2. run a 3rd replicate and use the average of the two that are within 5-10mV. If there is still a discrepancy between the readings then we discard the sample from further use.

Do I need to perform the test within a specific amount of time?- we recommend that performing sORP measurements within one hour after liquefaction at room temperature. Alternatively, you may store the post liquefied samples in a clean vial containers at -80°C freezer for future testing within 30 days.

Do I need a specific amount of sample volume?- Our test sensors require 30µl semen that has undergone liquefaction. It is very important that you use the same volume for all samples.

How should I defrost frozen samples?- when running frozen (post thaw) samples, they should be brought to room temperature before adding them to the test sensor.

What do I when I experience a sensor error? – For sensor errors, please refer to the page 10 of the MiOXSYS User Manual for troubleshooting analysis.