TECHNICAL BULLETIN: SAMPLE MIXING, SEMEN LIQUEFACTION, AND VISCOSITY
For SQA-V GOLD, QwikCheck GOLD and SQA-Vision Systems | Wednesday, January 14th, 2015

BACKGROUND:

Incomplete semen liquefaction, high viscosity, decreased viscosity and insufficient mixing can all impact semen test results. The WHO 5th edition laboratory manual for the examination and processing of human semen provides guidelines for handling semen samples to promote accurate testing and reliable results.

THOROUGH MIXING AND REPRESENTATIVE SAMPLING (WHO 5th Edition, section 2.4.1):

It is difficult to obtain a representative sample of semen from a liquefied ejaculate if the sample is not well mixed. In fact, two separate aliquots may show marked differences in sperm motility, vitality, concentration, and morphology. To obtain consistently reproducible results, the sample should be thoroughly mixed before aliquots are taken for assessment (see Box 2.3 below):

Box 2.3 Thorough mixing of semen

Before removing an aliquot of semen for assessment, mix the sample well in the original container, but not so vigorously that air bubbles are created. This can be achieved by aspirating the sample 10 times into a wide-bore (approximately 1.5 mm diameter) disposable plastic pipette (sterile when necessary). Do not mix with a vortex mixer at high speed as this will damage spermatozoa.

LIQUEFACTION & VISCOSITY (WHO 5th Edition, sections 2.3.1 - 2.3.2):

Immediately after ejaculation into the collection vessel, semen is typically a semisolid coagulated mass. Within a few minutes at room temperature, the semen usually begins to liquefy (becomes thinner). The entire sample usually liquefies within approximately 15 minutes at room temperature, although rarely it may take up to 60 minutes or more. Occasionally samples may not liquefy even after 60 minutes which makes the accurate assessment of the sample difficult. In these cases, additional treatment, mechanical mixing or enzymatic digestion is advised.

In contrast to a partially liquefied sample, a viscous semen sample exhibits homogeneous stickiness and this consistency will not change over time. After liquefaction, the viscosity of the sample can be estimated by observing the drops or length of the thread created by gently aspirating the sample into a wide-bore (approximately 1.5 mm diameter) plastic disposable pipette and allowing the semen to drop by gravity. A normal sample leaves the pipette in small discrete drops. If viscosity is abnormal, the drop will form a thread more than 2 cm long.

Methods to reduce viscosity are the same as those for delayed liquefaction. Samples observed to have abnormally high viscosity or excessive liquefaction time (greater than 1 hour) should be treated with the *MES QwikCheck™ Liquefaction Kit* and tested after a 15 minute waiting period to ensure accurate results.

MANUFACTURER'S RECOMMENDATION FOR RUNNING LOW (DECREASED) VISCOSITY SAMPLES:

In rare cases, samples with decreased viscosity can affect your Sperm Concentration results. A possible indicator of this situation is a test result with lower than observed Sperm Concentration and higher than observed Motility %. Another indicator might be a test result of <2 M/mL for Sperm Concentration but a Motility result of >50%. If the sample is observed to be excessively "watery" (decreased viscosity) or "clear" in opacity and the results match either of the scenarios outlined above, the sample should be re-run on the instruments **WASHED** mode or prepared as a washed sample and run on the instruments **WASHED** mode.

Washing Low (Decreased) Viscosity Semen Samples:

- Measure the sample before washing and place the entire sample into a 15ml centrifuge tube
- Add up to 10ml of *QwikCheck™Dilution* media
- Centrifuge at 220g (1200 rpm) for 10 minutes
- Remove the supernatant
- Re-suspend the pellet with *QwikCheck™Dilution* media to the original sample volume
- Run the sample on the WASHED mode of the SQA-V and receive a FULL report