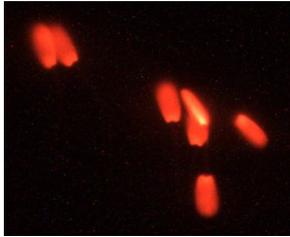


Sperm Concentration Assay

Assessment of sperm concentration using the flow cytometer combines the unique characteristics of size, shape and DNA-specific staining to differentiate sperm from debris. Large numbers of spermatozoa (5,000+) can be included for each sample, providing greater precision than CASA or hemacytometer assessment.



Sperm stained with Propidium Iodide for positive identification

Flow Cytometry: What is it and how is it beneficial in semen quality assessment?

Flow cytometers are precision instruments that direct fluid suspensions of fluorescently labelled cells between lasers and detectors. These labels allow rapid and precise quantitative measurements of a variety of sperm attributes, both inherent to the sperm and the environment in which they have been stored or exposed. These assays are sensitive tools for evaluating the structural and functional status of sperm beyond traditional CASA and microscopic techniques.

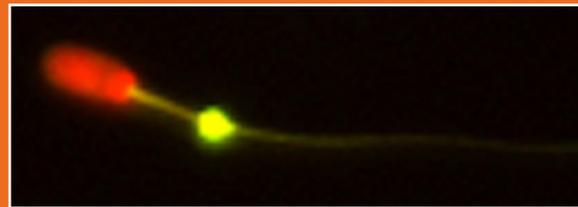
KRC led the way in making this technology accessible to clients by installing a flow cytometer in 2010. Utilizing the next generation of micro-fluidic design, this high-throughput system with a motorized stage for 96-well plates automates analysis for most assays, making this service affordable for commercial AI studs.

All assays can be run on fresh extended semen so submission is as simple as shipping regular doses as processed for AI customers or routine QA submissions.

Coming Soon...

- Bacterial concentration
- Ubiquitin
- Dynamic Response: Acrosome reaction induction under capacitation conditions
- Oxidation (expanded ROS detection)
- Membrane fluidity with apoptotic marker (combined)
- Concentration & Viability (combined)
- Others: to be announced*

*The flexibility of the flow cytometry platform makes it an ideal instrument for adapting new assays. As such, new protocols are continuously being investigated and custom applications may be accommodated on client request.



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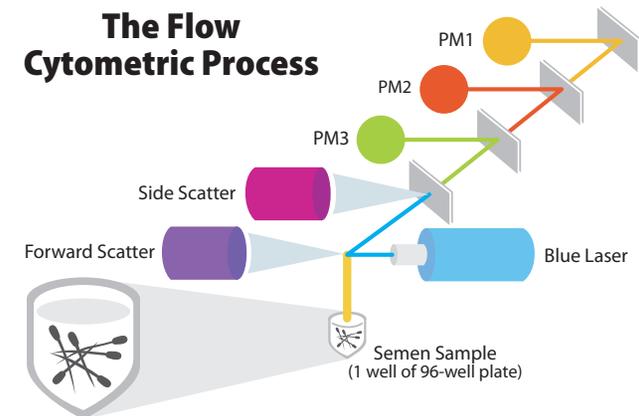
Kuster Research and Consulting, Inc.

...from concept to conception...

Advanced Semen Quality Assessment by Flow Cytometry



The Flow Cytometric Process



Dedicated Flow Cytometric Assays for Advanced Semen Analysis

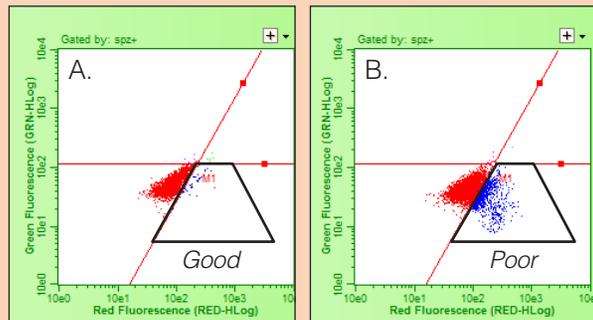
Available today at KRC*

CompDNA Assay

Measures the ability of the sperm chromatin to maintain structural integrity after undergoing acid stress. The risk of subfertility increases along with the proportion of susceptible sperm in the ejaculate. Both conception rate and litter size are affected by chromatin defects.

Chromatin instability is considered a non-compensable defect, since affected sperm may retain the ability to fertilize, effectively wasting oocytes by blocking access from unaffected sperm better able to maintain normal development.

Abnormalities can be permanent or transient; while each male tends to have a unique pattern over longer periods of time, this parameter is susceptible to environmental stressors.



Example of a boar semen sample with: A) a low level and B) high level of chromatin instability.

Note: Fresh or frozen semen can be used in the CompDNA assay; please contact KRC for further instructions if frozen semen submissions are preferred.

Sperm Viability and Acrosome Integrity Assay

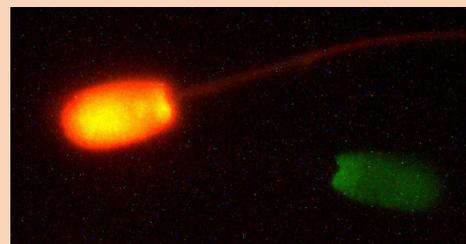
Combines three fluorescent stains to simultaneously measure both the membrane viability and acrosome integrity in the same sperm, while excluding debris from affecting the results. Retaining acrosome integrity during storage is essential for sperm to penetrate the oocyte during the process of fertilization. Sperm viability is discussed in the Sperm Viability Assay.



Sperm with disrupted acrosome Dead spermatozoa

Sperm Viability Assay

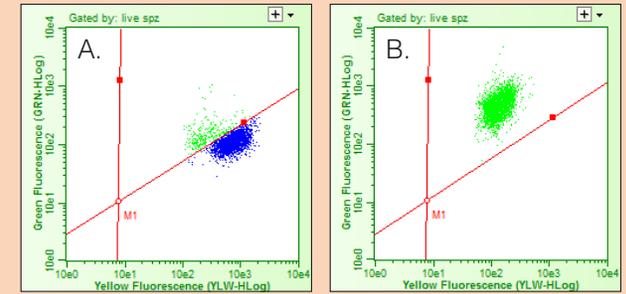
Measures the percentage of viable spermatozoa with an intact membrane. Sperm require a viable plasma membrane to survive in the female reproductive tract prior to ovulation. Although considered a compensable trait, viability is a key determinant of sperm quality and becomes increasingly important when reduced sperm numbers are utilized for AI.



Dead Live

Oxidation Assay

The oxidation assay measures the intracellular level of reactive oxygen species (ROS) or free radicals in spermatozoa. Elevated levels of these ROS are harmful to sperm membranes and DNA, predisposing to sub-fertility.



Example of the oxidation status of a boar semen sample. A) basal and B) induced.

Membrane Fluidity Assay

Changes in phospholipid arrangement and membrane fluidity are an early indicator of spermatozoan stress, including toxin or environmental exposure which can lead to compromised fertility. This assay detects changes in membrane fluidity providing sensitive early detection of stress in sperm prior to complete loss of membrane viability.



Normal Spermatozoa



Membrane phospholipid disorder

Mitopotential Assay

Analyzes mitochondrial membrane potential to determine the percentage of spermatozoa with normal mitochondrial function. Mitochondria are the energy production organelles for sperm. They are essential for maintaining motility and other functions necessary to reach the oocyte and penetrate for fertilization.



Low potential



High potential