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Fast and Precise Semen Analysis with the NucleoCounter® SP-100™

The NucleoCounter® is maintenance and calibration free, offering extreme ease of use and reliable operation for the preparation of insemination products for animal husbandry.

Introducing the NucleoCounter® SP-100™

ChemoMetec A/S has developed and patented a novel technology which addresses the problems of conventional routine semen analysis. The integrated fluorescence microscope in the NucleoCounter® SP-100™ is designed to detect signals from individual sperm cell nuclei. This result of the NucleoCounter® SP-100™ represents direct cell concentration of the sample which offer considerable accuracy compared to indirect methods.

NucleoCounter® SP-100™ is intended for use in semen collection centers and processing facilities offering accurate, simple and reliable semen analysis as means for product optimization and quality control.

Key benefits of the NucleoCounter® SP-100™ are 45 sec. analysis time, high accuracy and simple integration into any work environment.

Simple as 1-2-3

The system consists of the NucleoCounter® SP-100™ fluorescent microscope, the SP1-Casssette™ containing immobilized dye and Reagent S100 for dilution and sample preparation. The SemenView™ PC software application for optional documentation, image viewing and data processing is delivered with the instrument, however NucleoCounter® SP-100™ can also be operated as a stand-alone instrument. Furthermore the NucleoCounter® SP-100™ is available in a configuration which allows direct connection to an optional printer for documentation.

NucleoCounter® SP-100™ is suited for the analysis of semen from several species such as boars, bulls and stallions.

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The NucleoCounter® SP-100™ - Cell Counting System

The core of the system is a novel integrated fluorescence microscope, comprising an excitation light source of light emitting diodes (LED´s), optics (including lenses, excitation and emission filters), and a charged coupled device (CCD) camera. The fluorescent microscope is optimized to excite the nuclei staining dye, Propidium Iodide (PI), with intense green light and subsequently detect the red light emitted from DNA in the sperm cells with a CCD camera. Detected signals are correlated to direct sperm count, which is presented to the user in the built-in display. Optionally, the image and result can be transferred to a PC for viewing and documentation using the SemenView™ software application which is included.

Handling the NucleoCounter® SP-100™

Compared to indirect methods for semen analysis the NucleoCounter SP-100 is fast, efficient and reliable. The determination of direct sperm cell count with the NucleoCounter SP-100 involves sample preparation and sample analysis. During sample preparation the sample is diluted and the plasma membrane of the sperm cells disrupted, thus exposing the DNA of the individual cells to the nuclei staining dye. The cell lyzate is subsequently loaded into an SP1-Cassette where the DNA of the nuclei is stained. The SP1-Cassette is then placed into the NucleoCounter SP-100 instrument for analysis. During analysis the fluorescent signal from the PI bound to DNA of the individual cell nuclei is detected and the cells counted in the built-in computer. The sperm cell count of the sample is presented in the NucleoCounter SP-100 display as millions of cells per ml (mill/ml).

Sample preparation - Time: 10 seconds

- 1. Pipette a representative semen sample (50.0 µl) into a sample vial. Add a volume of reagent (10.00 ml). This dilutes the semen sample and prepares the cells for staining (201 fold dilution).

Sampling - Time: 5 seconds

- 2. Load the SP1-Cassette with the cell lyzate by immersing the tip of the cassette into the cell lyzate and aspirate the sample.

Sample analysis - Time: 30 seconds

- 3. Place the SP1-Cassette in the NucleoCounter SP-100 instrument and press the “Run” button. After aproximately30 seconds the total cell concentration appears in the display. The data is optionally presented on a PC using the SemenView software application.

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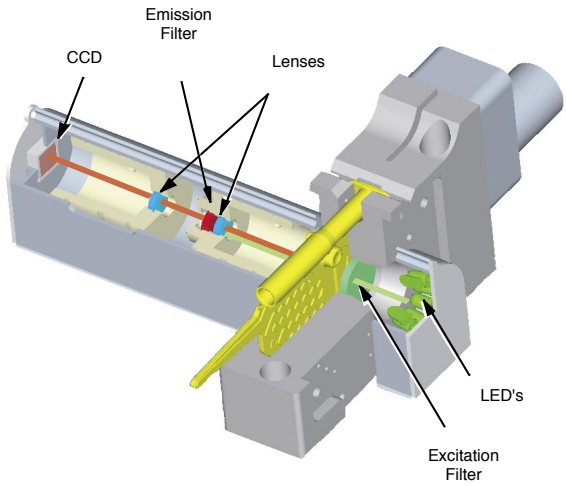
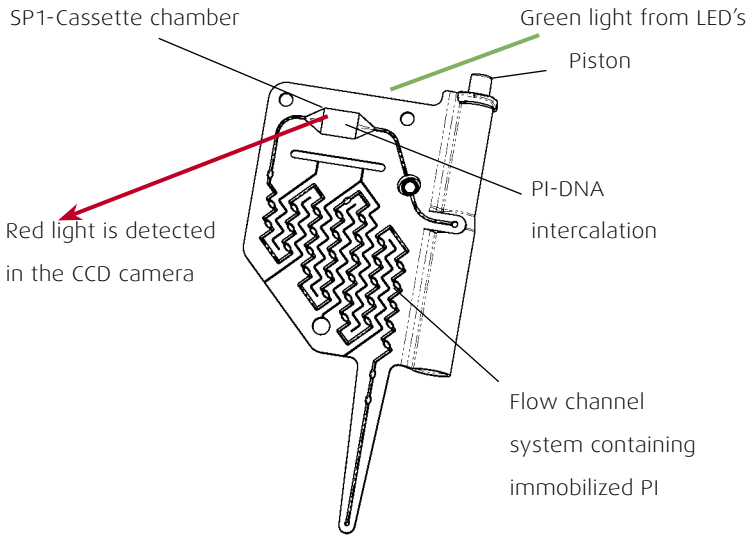
Integrated Fluorescence Microscope

The interior of the NucleoCounter is a fluorescence microscope. The SP1-Cassette is placed into the fixture. LED's emit green light in order to excite the PI-DNA intercalation. The excitation filter sorts the green light and the emission filter sorts the PI-DNA emitted red light. The CCD camera placed to the left registers the red light and the signals are correlated to the cell count. The magnification is approximately x1.3.

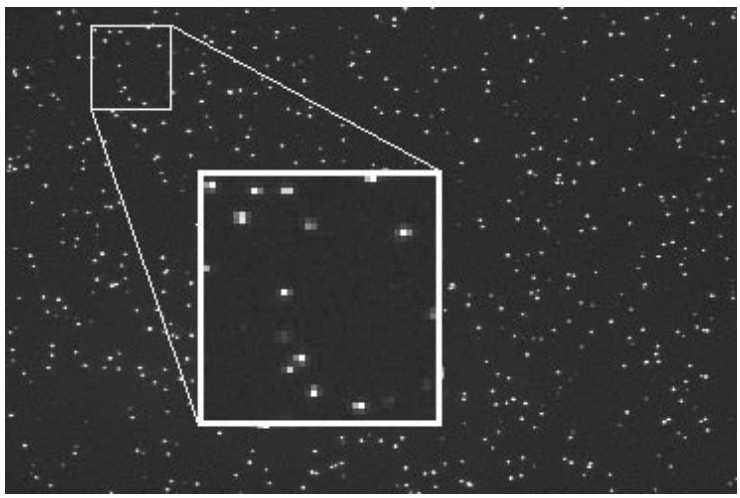


The cell nuclei are stained within the SP1-Cassette. After placement in the fixture of the NucleoCounter SP-100 instrument, the stained mixture is transferred to the SP1-Cassette chamber.

Green light excites the PI-DNA intercalation and the red light emitted is registered in the CCD camera for correlation into individual cell count.



The patented method of low magnification microscopy offer several advantages compared to other methods for particle counting. The large view area allows a large sample volume to be analyzed in a single image. The limited size of the imaged cells, relative to the size of the image elements (enlarged section in the illustation) demands only limited quality of the imaging system and allows cells to be imaged for a long time since slight movement of the cells during exposure is not reflected in the image.



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The NucleoCounter® SP-100™- Accurate and Precise Cell Counting

Accuracy

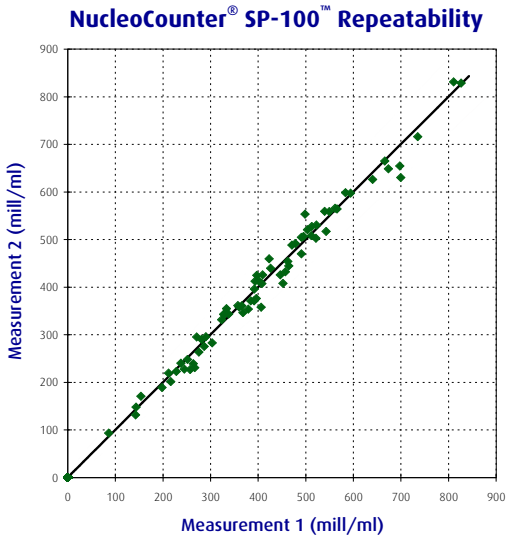
The counting of sperm cells in the SP-1 Cassette with the NucleoCounter SP-100 system is operator independent. The NucleoCounter technique assures that the volume of the sperm cell lyzate analyzed using the SP1-Cassette is accurately known for each measurement. Each NucleoCounter SP-100 instrument is carefully calibrated during production assuring optimal accuracy. The construction of the NucleoCounter SP-100 instrument offers great stability rendering NucleoCounter SP-100 maintenance and calibration free. Thus the NucleoCounter SP-100 offers unique long-term accuracy compared to other direct and indirect methods for semen analysis.

Precision

Approximately 1 µl of sperm lyzate is analyzed in one measurement. Thus a high number of sperm cells are counted in each analysis assuring high precision compared to many other methods.

Repeatability

An illustration of the superb repeatability of the NucleoCounter SP-100 is given in the repeatability graph. A total of 75 boar semen samples were measured in duplicate and the results are plotted against each other. The thin lines adjacent to the diagonal line represent the theoretical limits of 95% significance of the difference between duplicate measurements (Poisson distribution).



Reproducibility

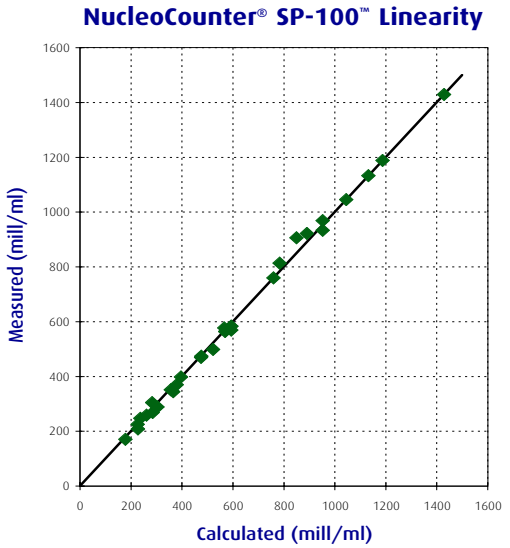
For the investigation of reproducibility 15 standard NucleoCounter SP-100 instruments where compared to a master instrument. Variance analysis (ANOVA) of repeatability and differences between instruments showed no significant effect between instruments (p=0.88). This suggests that the reproducibility of the NucleoCounter SP-100 is solely determined by the repeatability error.

Precision of NucleoCounter® SP-100™ is equal Reproducibility. Typical values when measuring boar sperm count (200 fold dilution).

Cell Count (mill/ml)	Repeatability CV (%)	Reproducibility CV (%)
200	3.8	3.8
400	3.1	3.1
800	2.7	2.7

Linearity

The response of the NucleoCounter SP-100 is linear over an extensive range. This is illustrated in the linearity graph which shows results obtained from the measurement of accurately diluted boar semen samples. Each point on the graph is the average of 2 measurements.



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The NucleoCounter® SP-100™- Key Benefits

- **Simple and fast**
  Analysis takes less than 30 seconds. The procedure is simple and easy to learn by all laboratory staff. The NucleoCounter SP-100 requires no daily cleaning or calibration and is maintenance free.

- **High Accuracy**
  The NucleoCounter SP-100 offers accurate count of individual sperm cells. Reproducibility is substantially equal to repeatability.

- **High Precision**
  The precision of NucleoCounter SP-100 is high. The large number of cells counted generally renders precision of about 3% (one standard deviation).

- **Integration**
  The small size and simple and fast operation makes the NucleoCounter SP-100 ideal for integration in any existing production environment.

- **Direct Cell Counting**
  The results of the NucleoCounter SP-100 are direct estimates of cell concentration as opposed to methods based on indirect determination.

- **Calibration free**
  The optical system of the NucleoCounter SP-100 is calibrated during production. This system is not altered during the life-time of the instrument. Each SP1-Cassette is calibrated individually during production

- **Maintenance free**
  The NucleoCounter SP-100 is constructed using durable components such as Light Emitting Diodes (LED's) as light source and Charge Coupled Device (CCD) as detector.

- **Safe sample disposal**
  The SP1-Cassette is disposable and can be disposed of safely as laboratory waste.

- **Great reliability**
  Great consideration has been given to reliability in the development of the NucleoCounter SP-100 instrument.

- **User-safety**
  The potentially hazardous propidium iodide is safely enclosed in the SP1-Cassette thereby providing optimal safety for the operator.

- **Specific and established method**
  By using the DNA binding fluorescent propidium iodide the cell count is based on a very specific signal.

- **Small loading volume**
  Only 50 µl semen sample are required to perform an analysis.

- **Documentation**
  The included SemenView software application is ideal for data processing and documentation purposes.

- **Compact instrument**
  The NucleoCounter SP-100 fits into any laboratory facility due to its small size (38x26x22 cm) and low weight (3kg).

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Technical Specifications

The NucleoCounter® SP-100™

Specificity
The NucleoCounter SP-100 counts sperm cell nuclei stained with the DNA specific fluorescent dye, propidium iodide.

Analysis time
After pressing "Run" on the instrument the result will be displayed within 30 seconds.

Measurement range (ejaculates)
Working range is 50 – 1,4000 mill/ml,
optimal range is 200 – 800 mill/ml
(at 200 fold dilution).

Operation
Menu-controlled by means of keyboard and LCD display.

Physical data

Weight	3 kg
Height	26 cm
Width	38 cm
Length	22 cm

Power
External 11-13 VDC Power Supply

Power consumption

Peak	25 W
Ready mode	2.5 W
Standby	2 mW

Operation conditions
For indoor use. Maximum relative humidity (RH) 80% for temperatures up to 31°C decreasing linearly to 65% RH at maximum temperature of 35°C; minimum temperature 15°C.

USB
USB, version 1.1.
Note: Support of USB Hubs varies, dependent on model.

The NucleoCassette™

Reagent
Each SP1-Cassette contains approximately 2.8 µg propidium iodide (PI).

Storage
Store the SP1-Cassettes in a sealed bag at max. 30°C.

Stability
Stable for minimum 12 months.

SemenView™ software

System requirements
Windows 2000 operating system.
Windows 2000 compatible computer.
USB 1.1 port.

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