

Application Note No. 101

Determination of Sperm Cell Density in Boar Semen Doses for AI

Application This Application Note describes how sperm cell density in boar semen doses for Artificial Insemination may be determined by the NucleoCounter® SP-100™ system.

The system is intended to be used for quality control of semen doses at boar stations and semen collection centers. The NucleoCounter SP-100 system determines the sperm cell density in semen doses with a very high precision and accuracy. The system can also be used for the determination of the sperm cell density in ejaculates, which have been pre-diluted with extender.

Semen doses diluted with the most widely used extenders (also egg yolk containing extenders) can be analyzed with the NucleoCounter SP-100.

The NucleoCounter SP-100 system consists of an instrument (NucleoCounter SP-100), a single use, disposable cassette (SP1-Cassette), a dilution- and lyzing buffer (Reagent S100) and various accessories (please see the Materials and Equipment section below).

Measurement data (sperm cell densities and images) may optionally be transferred to a PC using the SemenView™ software package or a printer.

Principle Please refer to the users manual for the NucleoCounter SP-100 and Package Inserts for Reagent S100 and SP1-Cassettes.

Materials and Equipment

- NucleoCounter SP-100 with instrument software version 1.22 or later and settings for boar semen (CM part no. 900-0100)
- SP1-Cassettes (CM part no. 941-0006)
- Reagent S100 (CM part no. 910-0100) with bottle holder and a mounted sample cup-holder (part no. 929-0003)
- 1-10 ml bottle-top dispenser, Brand Dispensette® III Variabel with filling tube fitted for the Reagent S100 container (CM part no. 911-0003)
- 100-1000 µl automatic Finnpiptette (ThermoLabsystems no. 4500-120) with tips (ThermoLabsystems no. 9401-100). An equivalent pipette can also be used.
- 20 ml sample cup of polypropylene with a polyethylene screw cap (CM part no. 911-0004)
- Waste container (e.g. 25L plastic drum or container with lid)
- PC with SemenView (CM part no. 950-0100) installed connected to the NucleoCounter SP-100 (recommended)

ChemoMetec A/S requests the customers to buy the sample cups from:

In Vitro A/S

Kratbjerg 336, DK-3480 Fredensborg
Phone +45 4847 5070, Fax +45 4847 5775,

e-mail in-vitro@in-vitro.dk

In-Vitro part no. EN-481-8: 20 ml PP sample cup with PE lid

Adjustment The dispenser should be adjusted to dose exactly 10,00 ml of Reagent S100. It is

and usage of dispenser	<p>recommended, that this is being done using a balance with a 0.01-gram resolution.</p> <p>The specific gravity of Reagent S100 at 25°C is 1,005 g/ml (1,006 g/ml at 20°C). At 25°C, the dispenser shall be adjusted to give a volume of an average weight of 10,05 g.</p> <p>The dispenser should be controlled and if necessary adjusted at appropriate intervals.</p> <p>Please also refer to the manufactures manual regarding dispensing and maintenance of the dispenser.</p>
Adjustment and usage of pipette	<p>Please, be aware that semen has a sticky character and a minor part of the sample will always remain in the pipette tip after pipetting. Therefore, it is important to calibrate the pipette using semen samples instead of water.</p> <p>The pipette should be adjusted to dose exactly 1000 µl of sample if the dilution factor (DF) is 11 or 500 µl of sample if the DF is 21. The specific gravity of a boar semen dose with 30 millions cell per ml is approx. 1,015 g/ml at 25°C. Hence, the pipette should be adjusted to give an average semen dose of 1015 mg with DF=11 or 508 mg with DF=21. For this adjustment a balance with a 1 mg resolution shall be used.</p> <p>Please also refer to the manufactures manual regarding pipetting and maintenance of the pipette. Please note that the applied pipetting principle has a substantial influence on the volume that is dispensed. Always apply the same principle.</p> <p>The pipette should be controlled and if necessary adjusted at appropriate intervals.</p>

Determination of Dilution Factor

All samples have to be diluted with Reagent S100 prior to measurement. How much the sample must be diluted depends on the sperm cell concentration of the sample.

In semen doses from boars, the sperm cell density is normally within the range of 20-70 million cells per ml.

The NucleoCounter SP-100 system has the highest precision, when the cell density in a Reagent S100 diluted semen sample is in the range 0,5-7,0 million cells per ml dilution. This is marked with the gray zones on the table below. The zone with the darkest shading shows the optimal area in which the cell density in the S100 diluted sample is in the range of 1,0-5,0 million cells per ml.

As seen from the table below, a dilution factor of 11 or 21 is a good estimate as a general dilution factor, since the cell density in the majority of the semen doses lies within the optimal range (marked by gray shading).

If the semen doses in average contain 25-35 million sperms per ml a DF of 11 is recommended.

If the semen doses in average contain 40-60 million sperms per ml a DF of 21 is recommended.

Use the table below to obtain an appropriate DF for samples containing other sperm cell concentrations (e.g. pre-diluted ejaculates). Use a DF of 201 for ejaculates, refer to Application Note No. 100.

Sperm cell density in the semen sample before dilution with Reagent S100	Choose the dilution factor which best covers the sperm cell density of the semen sample (refer to the left column)				
	DF = 201	DF = 101	DF = 51	DF = 21	DF = 11
5 mill./ml					
8 mill./ml					
10 mill./ml					
15 mill./ml					
20 mill./ml					
25 mill./ml					
30 mill./ml					
35 mill./ml					
40 mill./ml					
45 mill./ml					
50 mill./ml					
60 mill./ml					
70 mill./ml					
80 mill./ml					
90 mill./ml					
100 mill./ml					
125 mill./ml					
150 mill./ml					
175 mill./ml					
200 mill./ml					
250 mill./ml					
300 mill./ml					
400 mill./ml					
500 mill./ml					
600 mill./ml					
700 mill./ml					
800 mill./ml					
900 mill./ml					
1000 mill./ml					
1100 mill./ml					
1200 mill./ml					
1300 mill./ml					
1400 mill./ml					

How to obtain
a certain
Dilution Factor

The Dilution Factor, DF, is calculated as:

$$\text{Dilution Factor (DF)} = \frac{\text{Number of parts of semen sample} + \text{number of parts of Reagent S100}}{\text{number of parts of semen sample}}$$

The table below specifies how a number of standard dilutions may be obtained, using a constant amount of Reagent S100 of 10 ml.

Please note, that dilution factors below 11 should only be used with care, due to the fact, that the high pH value of the Reagent S100 is difficult to maintain if the relative amount of sample in the mixture increases.

	Dilution Factor (DF)				
	DF = 201	DF = 101	DF = 51	DF = 21	DF = 11
Semen Sample	50 µl	100 µl	200 µl	500 µl	1000 µl
Reagent S100	10 ml	10 ml	10 ml	10 ml	10 ml

Procedure

1. Key in the Sample ID (optional)
2. Check that the Dilution Factor (DF) is correct.
3. Aspirate a representative volume of 1000 µl (DF=11) or 500 µl (DF=21) of semen dose using the automatic pipette mounted with a new pipette tip. Transfer the sample to the center area of the bottom of a 20 ml sample cup. (*cf. figure 1*)
4. Place the sample cup in the cup-holder and dispense 10,00 ml of Reagent S100 into the cup using the dispenser. Dispense the reagent directly onto the semen sample at the bottom of the cup. Use a firm, consistent plunger pressure, so that the plunger moves smoothly. (*cf. figure 2*)
5. Immediately after the dispensing, a portion of the mixture shall be aspirated into an SP1-Cassette. The tip of the cassette should be immersed below the surface of the sample during aspiration. Apply a consistent pressure to the piston and press the piston all the way until it reaches the cassette. Avoid touching the window (clear area) of the measurement chamber. (*cf. figure 3*)
6. Once the sample has been aspirated into the SP1-Cassette, open the lid and insert the cassette in the NucleoCounter SP-100. Close the lid and press the "Run" key on the instrument in order to initiate the analysis. (*cf. figure 4*)
7. After approximately 30 seconds the analysis is completed, and the result is shown on the LCD-display (in millions cells/ml, see *figure 5*) and on the PC (in SemenView) or on the printer if such are connected.
8. Open the lid and remove the used cassette from the NucleoCounter SP-100. Now, the instrument is ready for a new analysis.
9. The used cassette and the used sample cup shall be disposed of. The screw cap of the sample cup should be applied before the cup and sample is disposed of.

Item 4 describes a mixing step, which comprise an addition of Reagent S100 to a semen sample. As long as at least 10 ml of Reagent S100 is dispensed onto the sample there is no need for further mixing, since the sample and the reagent are thoroughly mixed during the dispensing. If less than 10 ml reagent is added then further mixing is necessary by putting on the lid and inverting the cup 5 times.

Handling and
storage

For handling and storage of Reagent S100 and SP1-Cassettes, refer to the individual packing labels and Packing Inserts.

Warnings and precautions	For safe handling and disposal of the reagent and cassettes refer to the packing labels, Packing Inserts and the user's guides for the NucleoCounter SP-100, the dispenser and the pipette.
Limitations	<p>The system is not for human semen diagnostic.</p> <p>Refer to the NucleoCounter SP-100 user's guide for instructions and limitations.</p> <p>The results presented by the NucleoCounter SP-100 system depend on correct use of the reagents, SP1-Cassettes and the NucleoCounter SP-100 instrument.</p>
Disclaimer	ChemoMetec A/S reserves the right to introduce changes in the product to incorporate new technology. This application note is subject to change without notice.
Trademarks	NucleoCounter® SP-100™ and SemenView™ are trademarks of ChemoMetec A/S. Dispensette® is a registered trademark of Brand GmbH, Germany. Finnpiquette® is a registered trademark of ThermoLabsystems Oy, Finland
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Appendix

Illustrations of procedure steps

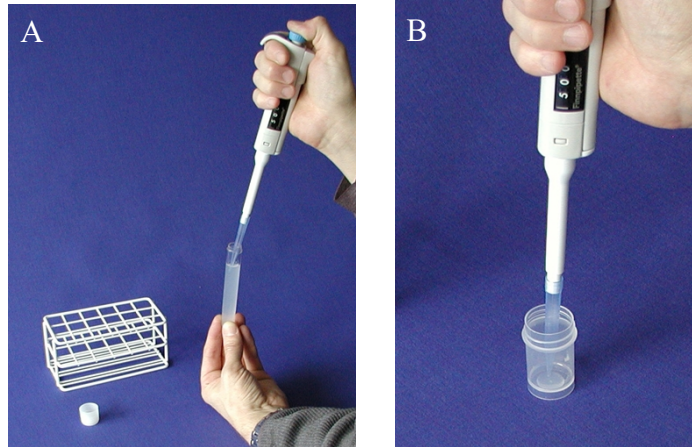


Figure 1. (A) Pipetting of 1000 µl / 500 µl of semen dose and (B) transferring sample to center area of bottom of sample cup

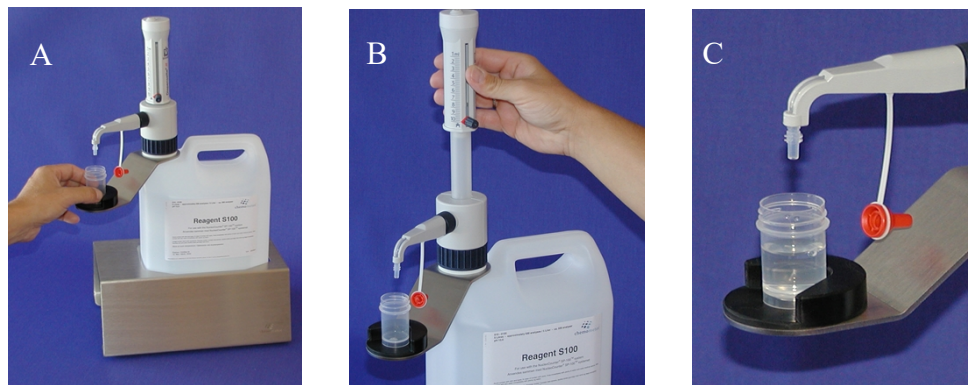


Figure 2. (A) Placing the sample cup in the cup holder; (B) Dispensing 10 ml of Reagent S100; (C) Sample cup with lyzate mixture



Figure 3. Loading the cassette with the lyzate mixture



Figure 4. (A) Insertion of cassette; (B) Closing the lid; (C) Pressing the Run button

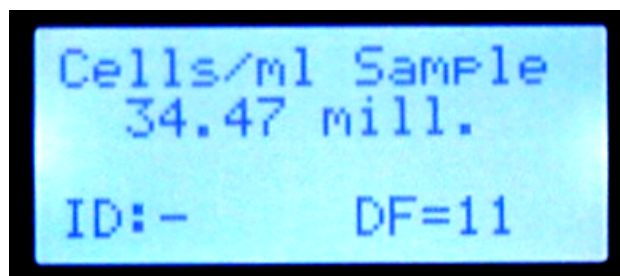


Figure 5. Result of the analysis shown in the LCD display (in this case the DF was 11).