NucleoCounter® SP-100™

User's Guide

Revision 1.5

Technology that counts

chemometec

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NucleoCounter® SP-100™

Cell Counter For Mammalian Semen

Manual No. 991-0100 (English)

Revision 1.5

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Caution!

This equipment must be operated as described in this User' s Guide. Please read the entire guide before attempting to use this unit. Please pay attention to that gloves or protective clothing are not worn on the illustrations/pictures shown in this User's Guide. However, ChemoMetec A/S does recommend that the user wear suitable protective clothing etc.

Contacting support

Technical information including product literature, answers to questions regarding the operation of the NucleoCounter® SP-100™ not covered in this document and information on software upgrade is available through the following:

- For Email support, send questions to NucleoCounter® SP-100™ Technical Support on the address **support@chemometec.com**
- To speak with a Technical Support Specialist, call (+45) 48 13 10 20.

Please note the NucleoCounter® SP-100™ serial number and have it available when contacting ChemoMetec A/S for support. The NucleoCounter® SP-100™ serial number is found on the label affixed to the bottom of the instrument and in the display upon start-up.

Sales and ordering information

For sales assistance with NucleoCounter® SP-100™ or the SemenView™ software, to place an order for a NucleoCounter® SP-100™ or consumables, call (+45) 48 13 10 20, fax (+45) 48 13 10 21, or send e-mail to **sales@chemometec.com**

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ChemoMetec A/S reserves the right to make changes in the product design without reservation and without notification to its users.

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Declaration of Conformity

Name of product: NucleoCounter® SP-100
Type: Mammalian Semen

Other identifying data: Part no. 900-0100

The product complies with requirements of the following directives:

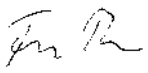
89/336/EEC - Electromagnetic Compatibility (EMC)

89/392/EEC - Machinery Directive

Harmonized standards, which have been used:

EN61326: 1997+A1: 1998 (Class B) +A2: 2000, Electrical equipment for measurement, control and laboratory use – EMC requirements (emission and immunity). Annex B and C from A1: 1998 is used.

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Date: 2005-11-04 Signed: 

Name: Frans Ravn

Position: Chief Technology Officer

Name and address of manufacturer:

ChemoMetec A/S
Gydevang 43
DK-3450 Allerod
DENMARK

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WEEE directive information – Europe only

Correct Disposal of This Product
(Waste Electrical & Electronic Equipment) - Europe only

This marking shown on the product or its literature, indicates that it should not be disposed together with other household wastes at the end of its working life. To prevent possible harm to the environment or human health from uncontrolled waste disposal, please separate this from other types of wastes and recycle it responsibly to promote the sustainable reuse of material resources.

Business users should contact their supplier and check the terms and conditions of the purchase contract. This product should not be mixed with other commercial wastes for disposal.

This information is listed in “Chapter 13 WEEE directive information in more EU languages”.

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Introduction and intended use

The NucleoCounter SP-100 is intended for use in connection with production of semen doses for artificial insemination within the field of animal husbandry.

The NucleoCounter SP-100 is designed to count sperm cells in semen from mammalian animals

The instrument is part of the NucleoCounter SP-100 system, which also comprises a disposable SP1-Cassette, Reagent S100 (lysis/dilution buffer) and PBS (dilution buffer).

The NucleoCounter SP-100 system allows reliable, fast and objective cell counting to be carried out based on an automated method of fluorescence microscopy. The system also enables the user to stain and count sperm cells without being exposed to potentially hazardous DNA dyes.

The optimal firmware settings of the NucleoCounter SP-100 vary from species to species.

Semen from most species, e.g. bulls, stallions, dogs and bucks (rabbit), can be tested with the same instrument settings.

However, semen from Boars requires different instrument settings due to weaker fluorescense signals from boar sperm cells compared to sperms from most other species.

If the instrument firmware version is 1.23 or lower, the type of species is set from the factory and this setting cannot be changed by the operator.

If firmware version 1.24 – 1.30 is installed in the instrument, the species can be selected using the F11 command. However, the F11 command does only affect the settings used for a Total Count. The settings for the Non-Viable Count is not changed by the F11 command and therefore the factory settings is being used. Contact ChemoMetec for more information about changing to the correct Non-Viable settings.

If firmware version 1.31 or higher is installed in the instrument, the species can be selected using the F11 command. Furthermore, the F11 command will in addition to selection of the correct settings for a Total Count, also select the correct settings for a Non-Viable Count.

The NucleoCounter SP-100 is developed as a stand-alone instrument but optionally it may be connected to a computer via an USB-interface or to a printer via RS-232 printer port. When connected to a computer the SemenView software offers various features such as documentation of the obtained results. Read more about the software in the SemenView User's Guide.

The NucleoCounter SP-100 part number is 900-0100. Refer to Section 11.1 with respect to other part numbers of the NucleoCounter SP-100 system.

The NucleoCounter SP-100 system is ***not*** intended for human diagnostic purposes.

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Warnings and precautions

Wherever the ⚠ symbol appears on the NucleoCounter SP-100 instrument, it indicates that the manual must be consulted for precautions and warnings.

Power and Cables

Use the shielded USB cable supplied with the NucleoCounter SP-100 to ensure that you maintain the appropriate EMI classification for the intended environment.

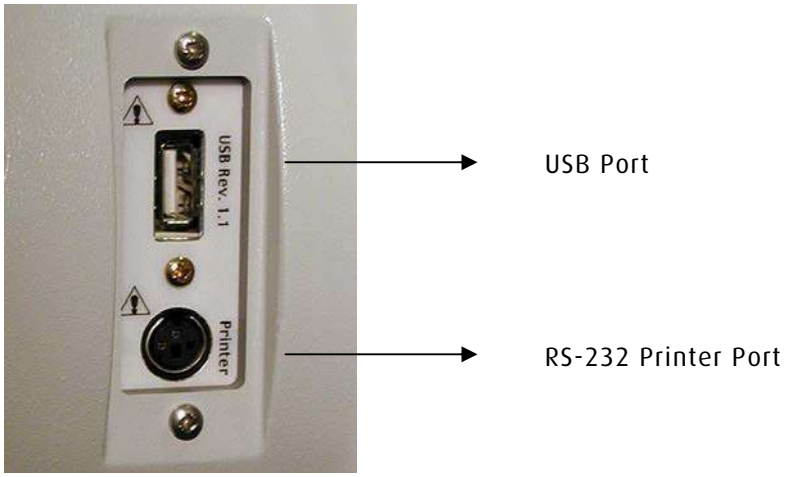

***Figure 1**. USB Port & Printer Port*

⚠ The USB interface connector of the NucleoCounter SP-100 must only be connected to SELV circuits. External computing devices connected to the USB interface connector of the NucleoCounter SP-100 has to comply with the standards, UL 1950 and IEC/EN 60950.

***Table 1**. Description of the NucleoCounter SP-100 USB interface connector*

Pin no.	Name	Maximum Voltage level[1]
1	+5V	+5 VDC
2	D-	+3.5 VDC
3	D+	+3.5 VDC
4	DGND	0 VDC
Metal enclosure	Shield (connected to DGND)	0 VDC

[1] In normal operation mode (refers to Pin no 4)

The NucleoCounter SP-100 is equipped with a RS-232 printer port. It is located just below the USB connector on the rear side of the instrument.

Table 2 *Description of the NucleoCounter Printer Output connector*

Pin no.	Name	Maximum Voltage level[2]
1[3]		
2	Rx	±10 VDC
3	Tx	±10 VDC
Metal Enclosure	DGND	0 VDC

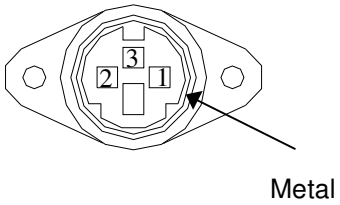

Figure 2. *Printer Output connector seen from the cable entry side.*

For connection of a printer (CM no 939-0006) please refer to Technical Note no. 100 (CM no. 994-0102).

NucleoCounter SP-100 is powered by an external 12VDC power supply. For safe use, please follow the instructions for connecting the power supply.

⚠ The NucleoCounter SP-100 shall only be used with one of the following external power supplies:

- Power supply Class I, Proton Electronic Industrial Co. Ltd., model SPN-260-12, input rated 100-240 Vac, 50-60Hz, 1.6 A. Output rated 12 Vdc, 4.3 A.
- Power Supply Class I, Powerbox Europe AB, model EBH 03 131, input rated 100-240 Vac, 47-63 Hz, 0.8A. Output rated 11-13 Vdc, 2.7 A.
- Power supply Class II, Celetron USA Inc., model ZVC40LT12E, input rated 100-240 Vac, 50-60Hz, 1.2 A. Output rated 12 Vdc, 3.3 A.

[2] In normal operation. Refers to DGND Metal Enclosure. Taken from the datasheet for MAX202ECSE.

[3] There is no signal connected to pin 1.

⚠ The detachable power supply cord set and appliance inlet of the external power supply are considered as the disconnecting device.

⚠ The Mains supply cord and plug of the external power supply shall comply with any national regulations.

⚠The user shall be made aware of that, if the NucleoCounter SP-100 and the external power supply is used in a manner not specified by the manufacturer, the protection provided by the NucleoCounter SP-100 and the external power supply may be impaired.

Electromagnetic interference

Note! This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment to an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

Caution! Changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Cassettes, Reagents and Dispensers

Please refer to appropriate package inserts for the usage and handling of cassettes, lysis/dilution buffers and dispensers.

Caution! When using a bottle-top dispenser: To protect against accidental splashes protective clothing, eye protection and gloves must be worn when using potentially hazardous liquids.

General

Any biological specimen should be handled as if it is capable of transmitting infectious disease and disposed of with proper precautions according to federal, state and local regulations.

Avoid specimen contact with skin or mucous membranes.

Never pipette by mouth.

Table of Contents

1 Basics of the SP-100 system

This section describes the basic principles of the NucleoCounter SP-100 system. In the section the attention is primarily paid to performing a **Total Count**. With respect to performing a **Non-Viable Count**, please refer to "*Viability Testing with NucleoCounter SP-100, Addendum to User's Guide* (part no. 991-0104).

1.1 NucleoCounter SP-100 system

The NucleoCounter SP-100 system is based on staining the DNA from mammalian sperm cells using a fluorescent dye, propidium iodide (PI). When PI is added to a suspension of chemically pre-treated sperm cells, the dye will specifically bind to the nuclei of the cells. If the cell suspension subsequently is illuminated with green light, the dye bound to the cell nuclei will emit red fluorescense light. When exposing an image using microscopic technique each nucleus can be observed as dots on a dark background.

The NucleoCounter SP-100 system is comprised of the NucleoCounter SP-100 instrument, a disposable SP1-Cassette and Reagent S100, which is a lysis/dilution buffer. *Figure 3* shows the system and the accessories and additional equipment needed to perform a Total Count.

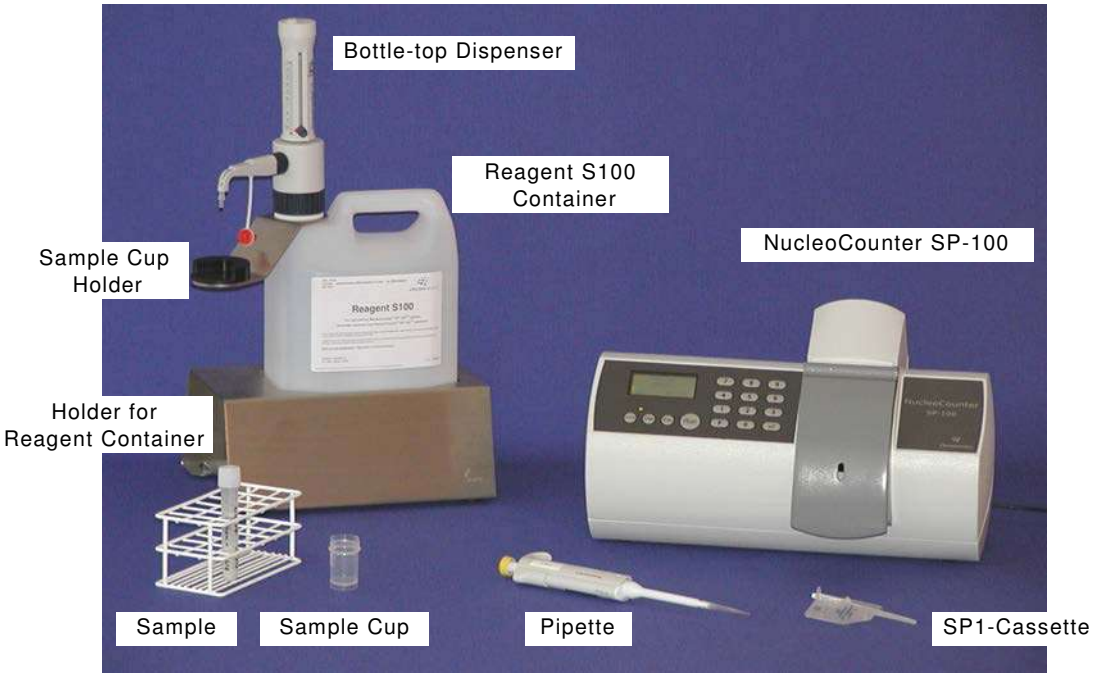

Figure 3. *NucleoCounter SP-100 system with accessories and equipment needed to perform a Total Count on a Semen Ejaculate.*

1.1.1 Lysis/dilution buffer - Reagent S100

A lysis/dilution buffer, Reagent S100 (*Figure 4*), is always added to the semen sample prior to performing a Total Count. The addition of Reagent S100 buffer has two

purposes. First, the Reagent S100 is used for disruption of the plasma membranes, rendering the nuclei of the sperm cells susceptible to staining with PI. Secondly, the reagent is used for dilution of the semen sample. The mixture of the semen sample and Reagent S100 is called a ***lyzate mixture*** *or* ***sample/reagent mixture***.

In ejaculates from boars, the sperm cell density is normally within the range from 100 to 1400 million cells per ml. In order to obtain an accurate and precise sperm count with the NucleoCounter SP-100 it is recommended to dilute the boar ejaculate 201 times (50 µl of ejaculate + 10 ml of Reagent S100). This corresponds to a sperm cell density in the range from 0,5 to 7,0 million per ml in the lyzate mixture. Approximately 1 µl of the lyzate mixture is counted in the SP1-Cassette, corresponding to a total of 500-7000 sperm cells per analysis.

Ejaculates from each animal species have its own optimal dilution factor depending on the typical sperm cell concentrations for the species (refer to Application Notes).

Semen doses for AI can also be analyzed with the NucleoCounter SP-100. The sperm cell concentration in these samples is most often reduced compared to the ejaculates. Therefore, the dilution factor is lower for these samples than for the corresponding ejaculates. The samples should be diluted so that the final cell concentration in the lyzate mixture is in the range from 0,5 to 7,0 million per ml. Refer to Application Notes for the optimal dilution factor.

An appropriate dispenser (e.g. Dispensette® III, Brand, Germany) is mounted on the top of the Reagent S100 container. A sample cup holder is also mounted on the container. For safe handling of the reagent, the container is placed in a holder.

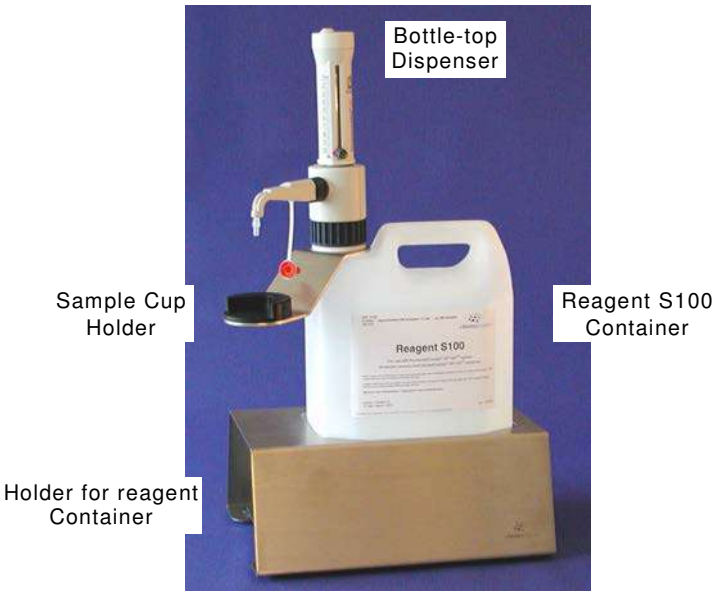

Figure 4. *Reagent S100 container placed in a holder and equipped with a bottle-top dispenser and a sample cup holder.*

1.1.2 SP1-Cassette

The SP1-Cassette is a small custom made disposable plastic device designed for optimal sample handling and safe disposal. The cassette is shown in *Figure 5*. The main features of the cassette are the piston, the flow system containing the fluorescent dye (PI), and the measurement chamber with the measurement window.

The SP1-Cassette is loaded by gently pressing the piston rod, thereby creating a partial vacuum through the flow system. Approximately 60 µl of the lyzate mixture is loaded into the cassette.

The PI is immobilized in the first three lanes of the flow system. As the lyzate mixture is loaded into the SP1-Cassette and transported through the flow system towards the measurement chamber (the clear window), the immobilized PI is dissolved and mixed with the lyzate mixture. PI intercalates with DNA and stains the nuclei of the cells. Due to the fluorescent nature of PI it absorbs green light and then emits red fluorescence light, which is used for detection of the stained nuclei. The actual cell count is performed in the measurement chamber of the SP1-Cassette, using the NucleoCounter SP-100 with its integrated fluorescence microscope and automatic image analysis (refer to Section 1.1.3).

The total volume of the measurement chamber is approximately 3 µl. As mentioned in Section 1.1.1, the analysis volume is approximately 1 µl, i.e. one third of the total measurement chamber volume is analyzed.

In connection with the production of the SP1-Cassettes each cassette is marked with a black dot code, which specify the precise depth of the measurement chamber of the cassette. The dot code is read and decoded in the instrument during analysis and the volume is obtained by multiplying the area to be analysed with the depth of the cassette. The area analysed is only dependent on the optics of the instrument. Therefore, the area is constant and specific for each instrument.

Caution! In order to avoid contaminating the measurement window it is important not to touch the window when handling the cassettes.

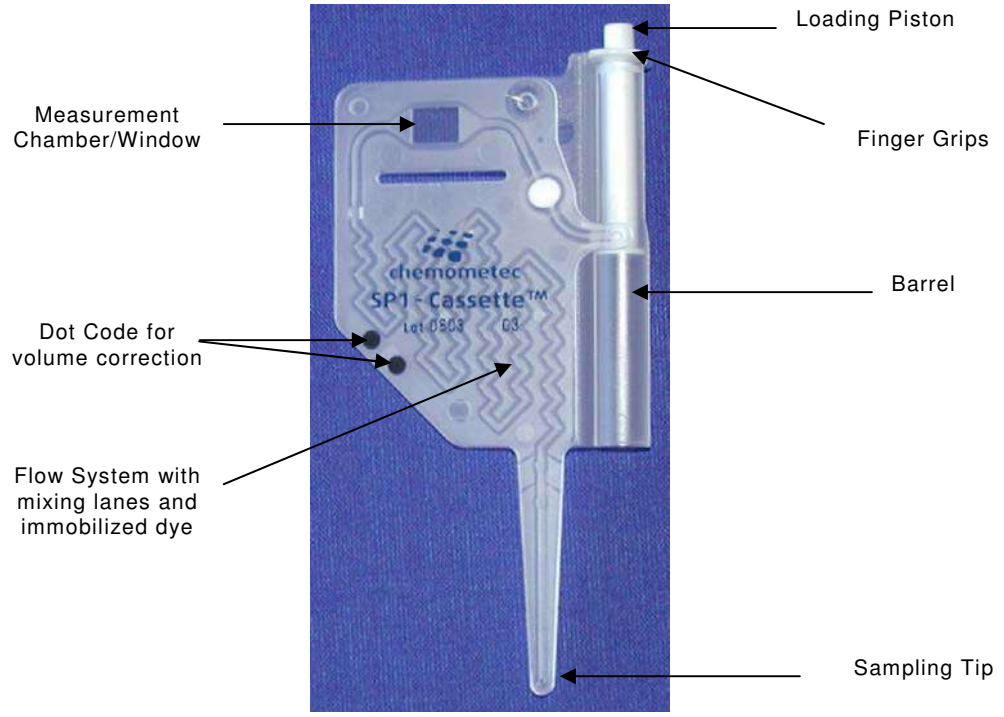

Figure 5. *Ready-to-use SP1-Cassette. The PI is immobilized in the first three lanes of the flow system.*

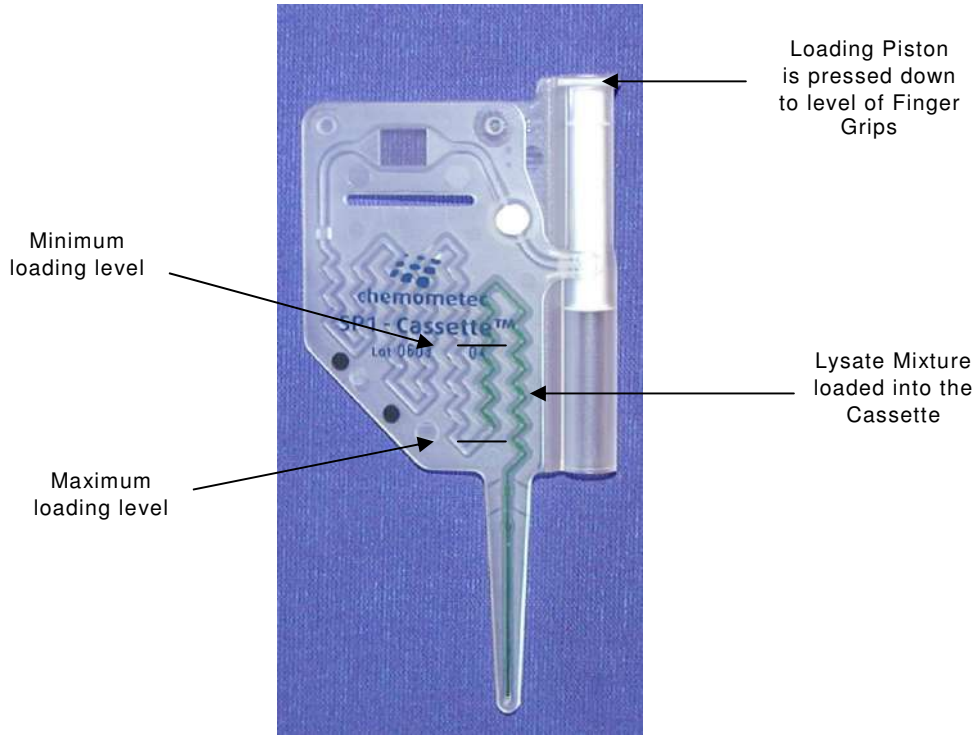

Figure 6. *Loaded SP1-Cassette. The lyzate mixture is loaded into the second lane of the cassette, between the minimum and the maximum loading level.*

1.1.3 NucleoCounter SP-100 with an integrated fluorescence microscope

The NucleoCounter SP-100 is shown in the figure below.

Figure 7. *NucleoCounter SP-100 instrument*

Refer to Section 5 with respect to the operation of the instrument.

The compact fluorescence microscope integrated in the NucleoCounter SP-100 comprises LED's (light emitting diodes) as excitation light source, excitation and emission filters, optics (lenses) and a CCD (charged coupled device) camera. Furthermore, the NucleoCounter SP-100 contains advanced software for image processing. The fluorescence microscope, with a cassette inserted, is shown in *Figure 8*.

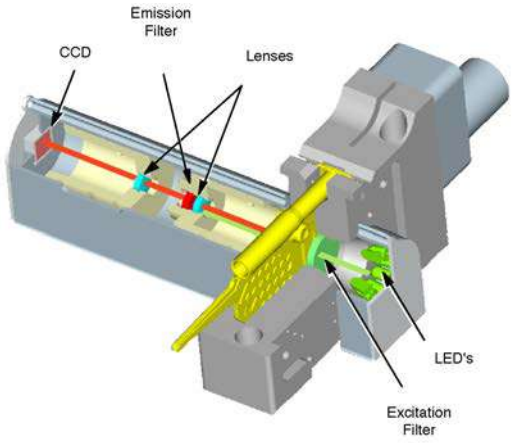

Figure 8. *The NucleoCounter's compact fluorescence microscope*

Before the NucleoCounter SP-100 fluorescence microscope is activated a SP1-Cassette is loaded and inserted into the insertion slit of the instrument. The lid is closed in order to

exclude external light from interfering with the analysis. The measurement is activated, by pressing the "**Run**" button.

When "**Run**" is activated, the actuator moves the piston rod down through the barrel of the cassette. Hereby, the stained lyzate mixture is transported through the flow system of the cassette. The form and dimensions of the flow channels facilitate effective mixing of lyzate mixture and stain. Sensors monitor liquid flow, and when the stained lyzate mixture has reached the measurement chamber of the cassette, the actuator stops the piston movement.

A standing still situation is obtained after a few seconds. Then the stained mixture of the measurement chamber is illuminated by green excitation light from eight LED's. The green light is passed through an excitation filter before it reaches the measurement chamber. The excitation filter allows only green light of the appropriate wavelengths for the excitation of PI to pass.

The green light will excite the PI bound to DNA of the sperm cells and in return PI emits red fluorescence light. Some of the green light will pass through the measurement chamber together with the red fluorescence light. This green light is now absorbed in an emission filter, which only allows the red light to pass through. Using an optical lens system the red light is finally focused on the CCD chip of the camera.

When the red light reaches the CCD, the photons are transformed into electrical signals. A fluorescent image of the content of the measurement chamber is recorded from the electrical signals and an example of such an image is shown in *Figure 9*. Each spot in the image represents a sperm cell nucleus stained with PI. The spots are red but they are represented as white dots in the image because the camera has a black and white CCD.

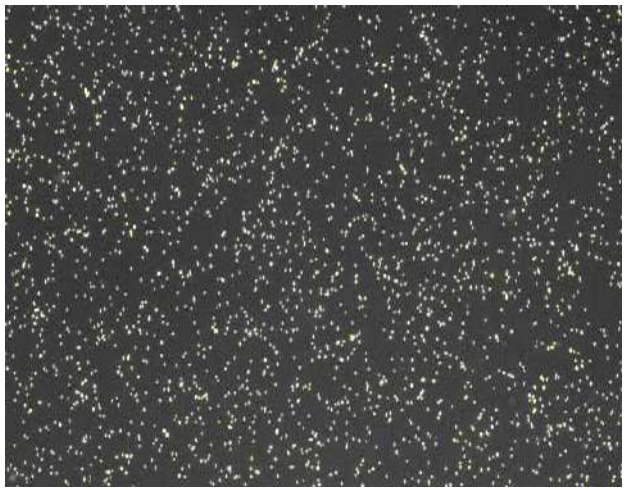

Figure 9. *Fluorescent image (from SemenView PC software) of sperm cell nuclei contained in the measurement chamber of an SP1-Cassette*

The NucleoCounter SP-100 is equipped with advanced software for image analysis. This integral software analyzes the recorded image and counts the number of nuclei in the image. As the volume of the cassette measurement chamber and the dilution factor is known, the cell density in the initial sample can be calculated. The concentration of sperm cells in the initial sample (in millions per ml) is presented in the NucleoCounter SP-100 display. If the instrument is connected to a PC via the USB-interface, the results and the recorded fluorescent image can be viewed with the SemenView software (Refer to User's Guide for SemenView). A printer can also be connected to the RS-232 printer port of the instrument. However, only the result and the important parameters and not the fluorescent image can be transferred to the printer. Please refer to *Technical Note No. 100* with respect to the printer application.

The entire process takes approximately 30 seconds (from pressing "**Run**" to presentation of the result). A Non-Viable Counts takes approximately twice the time of a Total Count (i.e. 60 seconds) because a more complex analysis is carried out in the instrument.

Performing a cell count with the NucleoCounter SP-100 is objective and virtually independent of the operator.

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2 Inspection & Unpacking of Equipment

2.1 Inspection of boxes

After you have received your order from ChemoMetec A/S, inspect the box or boxes carefully for any damage that may have occurred during shipping. Report any damage to the carrier and to ChemoMetec A/S immediately.

2.2 Packing List Verification

Unpack your order, saving the packing materials for possible future use. Also be sure to save the User's Guide, for instruction and reference.

Verify your ChemoMetec packing list that you have received the correct and ordered materials, and that nothing is missing.

If any part of your order was damaged during shipping or is missing, or fails to operate, please contact ChemoMetec A/S.

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3 Control Buttons, Keypad and Display

3.1 Interactive Controls

3.1.1 Control Buttons

There are four Control buttons (*Table 3* and *Figure 10*) on the front of the instrument:

Table 3. *Control Buttons on NucleoCounter SP-100*

On/Off	Use this button to turn the instrument on and off
Run	Use this button to start measuring
Esc	Use this button only as instructed in this manual
Del	Use this button only as instructed in this manual

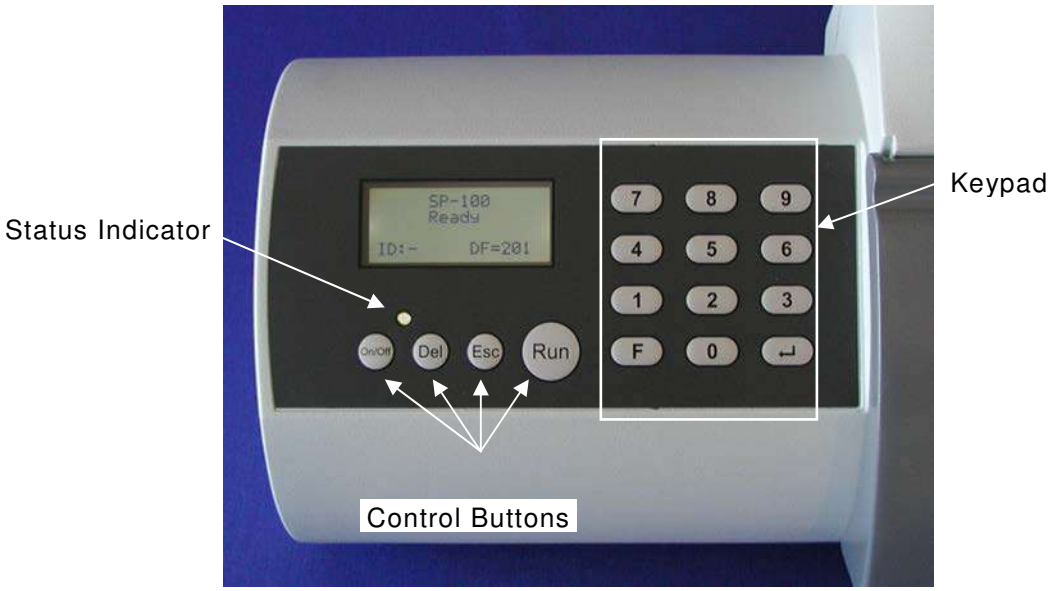

Figure 10. *Control Buttons, Keypad and Status Indicator of NucleoCounter SP-100 instrument*

3.1.2 Keypad

The Keypad is comprised of 12 keys (see *Figure 10*): "**0-9**", "**F**" (function key) and "↵" (Enter). The keypad is used for typing Sample ID, choosing Dilution Factor, language etc.

3.2 Interface Displays

A small Status Indicator light above the "**On/Off**" button (see *Figure 10*) illuminates when the unit is turned on. It is green when the instrument is ready to work or when

using the keypad, red while it is measuring or displaying an error message, and again green when the results are displayed.

The display screen communicates information to the user. It indicates, at various times: unit identification, software version, start-up status, operating functions, cell counts and shutdown status.

4 Installation and Start-up

4.1 Power on, power off

The NucleoCounter SP-100 is powered by a 12VDC external power supply. To connect the power supply to the NucleoCounter SP-100, connect it to the DC socket (see *Figure 11*), and plug the mains power cord of the power supply into a wall outlet.

Figure 11. *Connecting the 12VDC power cord plug to the NucleoCounter SP-100*

The NucleoCounter SP-100 can now be turned on and off by pressing the "**On/Off**" button on the front panel.

Caution! During start-up the NucleoCounter SP-100 performs a self-check. Never insert or remove any cassette during the start-up.

During start-up you will hear a brief noise as the actuator motor is activated for test purposes. This is perfectly normal. While the NucleoCounter SP-100 is starting up the display shows for a short period instrument details as follows:

Display Text Type 1

```
NucleoCounter
   SP-100
v1.22 2004-02-12
S/N: 007-01
```

Figure 12. *The start-up display*

The start-up display shows the instrument type in line 1 and 2 of the display and the version number and date (year-month-date) of the integral software of the instrument in line 3. The serial number of the instrument is shown in line 4 of the display.

After a successful start-up the display changes (*Figure 13*) and the status indicator on the keyboard panel turns green.

Display Text Type 2

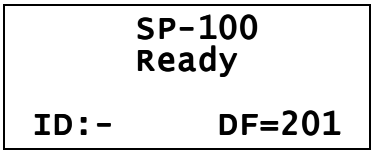

Figure 13. *The NucleoCounter SP-100 is ready for cell counting*

If the instrument has performed as described above, it is ready for use. If the instrument displays an error message at start-up refer to chapter 4.

Pressing the "**On/Off**" button shuts down the NucleoCounter SP-100 and while the instrument shuts down the display will momentarily look as shown in *Figure 14*.

Display Text Type 3

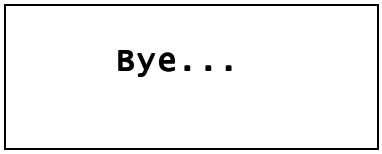

Figure 14. *The shutdown display*.

4.2 Installation of SemenView

Regarding installation of the SemenView PC-software, please refer to the SemenView User's Guide.

4.3 Connection to PC via an USB interface and Installation of SemenView

Connection of the NucleoCounter SP-100 to a PC via the USB interface (see USB port in *Figure 1*) and installation of the SemenView PC-software is described in the *SemenView User's Guide*.

4.4 Connection to Printer via Printer port (RS-232)

Connection of the NucleoCounter SP-100 to a Printer via the Printer port (see RS-232 Printer port in *Figure 1*) and installation of the Printer is described in *Technical Note no. 100*.

4.5 Connection to PC via Printer port (RS-232)

Connection of the NucleoCounter SP-100 to a PC via the Printer port (see RS-232 Printer port in *Figure 1*) and installation of the PC is described in *Technical Note no. 100.*

5 Operation of NucleoCounter SP-100

In this section the operation of the NucleoCounter SP-100 is described.

5.1 Generel remarks

Please pay attention to that a command like **F0** is typed in by pressing the **F**-key (function key) followed by "**0**".

When pressing the **F**-key the following display text appears (if the instrument is in the "**Ready**" status or the "**Result**" status):

Display Text Type 4A

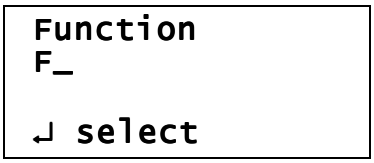

***Figure 15**. The instrument is ready for typing in the function number.*

When typing in "**0**" the display will show:

Display Text Type 4B

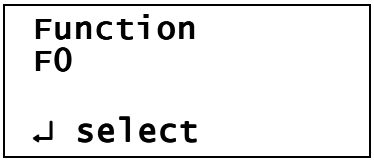

***Figure 16**. The **F0** function can be selected by pressing the "↵" button. **Figure 29** shows what is displayed when "↵" is pressed.*

If the function does not exist the following text is displayed when pressing "↵":

Display Text Type 5

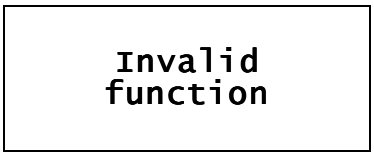

***Figure 17**. The function that has been chosen does not exist. Press any key.*

When a specific setting or a value is saved the display will for a few seconds show **"storing"** or **"saving"** (there is no difference between saving and storing. The expressions are used indiscriminately):

Display Text Type 6

Figure 18*. The function that has been chosen is stored. Wait a few seconds for getting to the "**Ready**" mode.*

Display Text Type 7

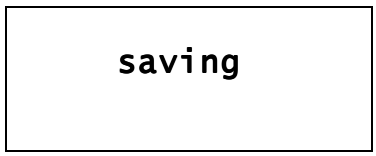

Figure 19*. The function that has been chosen is saved. Wait a few seconds for getting to the "**Ready**" mode.*

If the stores/saved values shall replace the present default values or settings this can be achieved in connection with shutting down the instrument. When a parameter has been changed the instrument will show the following text in the display:

Display Text Type 8

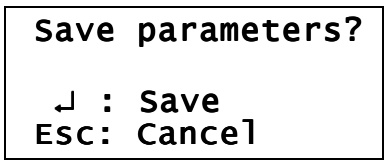

Figure 20. *Save the settings and use these as the new default setting?*

Press "↵" to save the changes as the new default settings or press "**Esc**" in order not to overwrite the existing default settings. When pressing "**Esc**" all changes will be lost and next time the instrument is turned on, the old settings will be used again.

5.2 "Ready" status

After the NucleoCounter SP-100 has started successfully the display should read:

Display Text Type 2

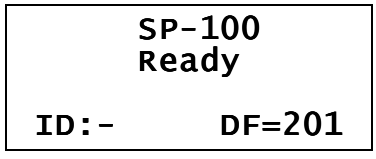

Figure 21. *The NucleoCounter SP-100 is ready for cell counting. No Sample ID is chosen. Dilution Factor is set to 201.*

This display is also shown if the "Esc" button is activated in the result status or any other situation, except during measuring or shutting down.

The Sample ID (see *Section 5.4*) and the Dilution Factor (DF, see *Section 5.5*) is always displayed in the bottom line when the instrument is in the "**Ready**" status or in the "**Result**" status.

5.3 "Result" status

Following a successful analysis, the sperm cell density will be displayed on the display screen. The instrument is in the **"Result"** status.

Figure 22 shows the result from the analysis of an ejaculate, which has a sperm cell density of 455.0 million sperm cells per ml. It is important to notice that it is the cell density of the undiluted sample (initial or raw sample), which is presented (see also *Section 5.5*)

The results are always shown with 4-5 digits.

The number of digits does not reflect the precision or the accuracy of the instrument.

Display Text Type 9A

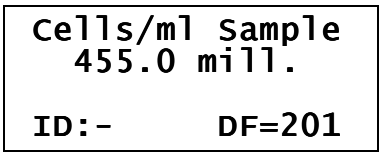

Figure 22. *Cell density of the ejaculate is 455 million per ml.*

5.3.1 Retest flag

In some cases the result is displayed as *Display Text Type 9A* but with a flashing "**Retest**" message added in line no. 3 (*Figure 23*). This message is observed when the image analysis indicates that the image is not a fully normal image. In most cases the results are still valid. However, it is recommended to retest the sample in order to obtain a result with a higher degree of reliability.

Display Text Type 9B

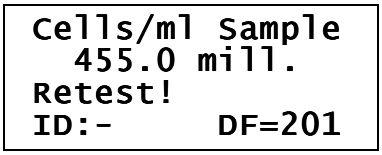

Figure 23. *Cell density of the ejaculate is 455 million per ml. Flashing Retest! flag indicates that the "true" result may deviate from 455 million per ml. A retest of the sample is suggested.*

5.3.2 Presentation of Result when Retest Flag is raised (F32)

The cell count is by default presented in the display even when the Retest flag is raised. The setting can be changed using the **F32** function. Press "**F32** + ↵" and the following display text will appear:

Display Text Type 10

```
Retest res.? On
   1: On
   2: Off
↵ store
```

Figure 24. *The "**Retest res.?**" is "**On**" but can be changed to "**Off**" by pressing "**2**" and back to "**On**" again by pressing "**1**". The display will be updated instantly with the new setting. Press "↵" to store the chosen setting.*

Press "**2**" and the text will change to "**Retest res.? Off**". Then press "↵" and the cell count result will be omitted, when a Retest flag is raised.

Press "**F32 + ↵**" followed by "**1 + ↵**" to get back to the default value. When pressing "**F32 + ↵**" followed by "**Esc**" the NucleoCounter SP-100 will jump back to the "**Ready**" status.

If the default value has been changed the new value will be stored until it is changed again. When shutting down the SP-100 ask if the changes in the settings shall be saved as the new default value:

Display Text Type 8

```
Save parameters?

 ↵ : Save
Esc: Cancel
```

Figure 25. *Save Retest setting as the new default setting?*

Press "↵" to save or "**Esc**" if the present default setting should not be overwritten.

5.4 Sample ID

Prior to the analysis, i.e. before pressing the "**Run**" button, a Sample ID can be typed in. This is done by typing in at least one and a maximum of four digits using the keys from "**0-9**", followed by pressing the "↵" button. When the first digit, e.g. "**1**", is typed in the following display text will appear:

Display Text Type 11

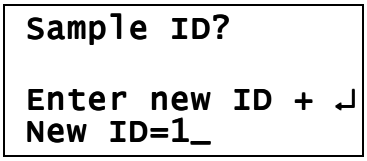

Figure 26. *This display text is shown if the digit "**1**" is typed in when the instrument is in the Ready status or Result status. If the Sample ID is 1234 the display will be automatically updated during the typing in of the remaining digits "**2**", "**3**" and "**4**".*

The ID will appear in the display and if the SP-100 is connected to a PC with SemenView installed the Sample ID will also appear on the PC screen.

Display Text Type 2

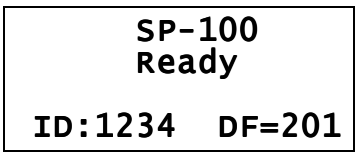

Figure 27. *The NucleoCounter SP-100 is ready. Sample ID is 1234*

Display Text Type 9

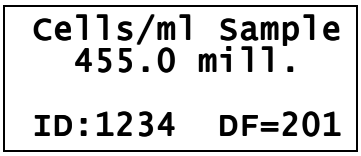

Figure 28. *Cell density of sample with ID 1234*

The Sample ID can only be typed in when the instrument is in the "**Ready**" status or "**Result**" status.

The sample ID is shown in the bottom line of the display both in "**Ready**" status or "**Result**" status (prior to and subsequent to the measurement).

A digit can be deleted by pressing the "**Del**" button.

When only a "**0**" is typed following by pressing the ↵ button the ID will be cleared from the display. If a Sample ID is not typed in or cleared then "**ID:-**" is displayed

After each measurement the Sample ID will automatically be cleared from the memory of the instrument. This means that when performing consecutive measurements on the same sample, the Sample ID must be typed in for each measurement.

5.5 Dilution Factor (F0)

The Instrument will automatically multiply the actual sperm count of a diluted semen sample with the so-called Dilution Factor (DF). When testing a semen ejaculate from a boar it is recommended to dilute the sample 201 times by adding 200 volumes of Reagent S100 to 1 volume of sample. The instrument can compensate for this 201-fold dilution using the DF feature and present the sperm count of the ejaculate.

EXAMPLE - Changing the DF from 201 to 1000

To change the DF press "**F0 + ↵**". If the current DF is 201 the following will be displayed:

Display Text Type 12

```
Dilution factor
DF=201
Enter new DF + ↵
New DF=_
```

Figure 29. *The Dilution Factor can now be typed in*

Then type "**1000 + ↵**" and the DF will be changed to 1000:

Display Text Type 2

```
      SP-100
      Ready

ID:-       DF=1000
```

Figure 30. *Dilution Factor is set to 1000*

The DF must be a 1 to 4-digit whole number in the range from 1 to 9999. A DF value below 11 is not recommended unless it is previously validated that the results are not negatively affected. In particular it should be validated thoroughly if the buffer capacity of the sample is so high that it may impact the overall pH of the lyzate mixture.

If the DF has been changed the new value will be stored until it is changed again. When shutting down the SP-100 ask if the actual DF shall be saved as the new default value:

Display Text Type 8

```
Save parameters?

 ↵ : Save
Esc: Cancel
```

Figure 31. *Shall the DF value be saved and used as the new default value?*

Press "↵" to save or "**Esc**" if the present default value should not be overwritten.

Please note, that the precision and accuracy is dependent on the total number of counts in the cassette. Therefore, in order to obtain a high degree of precision and accuracy it

is important to adjust the Dilution Factor to the sperm cell density of the sample. See *Section 1.1.1* and *Application Notes* for more information on this subject.

Instruments with the Viability Option included

If the instrument is in the viability mode (F31 is On), then **DF** is replaced with **DF1** (Dilution Factor for the Total Count) and **DF2** (Dilution Factor for the Non-Viable Count). With respect to performing a Total Count and a Non-Viable Count in the viability mode, please refer to "*Viability Testing with NucleoCounter SP-100, Addendum to User's Guide* (part no. 991-0104).

5.6 Correction Factor (F10)

The default value of the Correction Factor is 1000. This factor can be changed in order to obtain a higher or lower cell count. The Correction Factor must be a number in the range from 800 to 1200. A Correction Factor of 1050 means that all cell count results will be multiplied with 1050/1000 = 1,050. This corresponds to a 5% increase in the cell count levels.

The NucleoCounter SP-100 is calibrated from the factory but the "true" count of a sample can always be questioned and likewise can it be discussed. Hence, the user may, within certain limits, "correct" the NucleoCounter SP-100 result in order to match a preferred reference method. However, it is recommended not to change the Correction Factor unless there are particularly weighty grounds for doing this.

EXAMPLE - Changing the Correction Factor from 1000 to 1050

To change the Correction Factor press "**F10 + ↵**". If the current Correction Factor is 1000 the following will be displayed:

Display Text Type 13

```
Corr. factor
CF=1000
Enter new CF + ↵
New CF=_
```

Figure 32. *The Correction Factor can now be typed in*

Then type "**1050 + ↵**" and the Correction Factor will be changed to 1050. Or press "**Esc**" to jump back to the "**Ready**" status without affecting the setting.

If the Correction Factor has been changed the new value will be stored until it is changed again. When shutting down the SP-100 ask if the changes in the settings including the actual Correction Factor shall be saved as the new default value:

Display Text Type 8

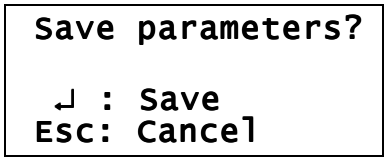

Figure 33. *Shall the Correction Factor value be saved and used as the new default value?*

Press "↵" to save or "**Esc**" if the present default value shall not be overwritten.

5.7 Choosing Animal Species (F11, only firmware v1.24 or higher)

The instrument settings of the NucleoCounter SP-100 vary from species to species.

Semen from bulls, stallions, dogs, rams, he-goats and bucks (rabbit) can be tested with the same instrument settings.

Semen from boars requires different instrument settings due to weaker fluorescense signals from boar sperm cells compared to sperm cells from most other species.

If the instrument firmware version is **1.23** or lower, the type of species is set from the factory and this setting cannot be changed by the operator.

If firmware version **1.24 – 1.30** is installed in the instrument, the species can be selected using the F11 command. However, the F11 command does only affect the settings used for a Total Count. The settings for the Non-Viable Count is not changed by the F11 command and therefore the factory settings is being used. This can produce erroneous results. Contact ChemoMetec for more information about changing to the correct Non-Viable settings.

If firmware version **1.31** or higher is installed in the instrument, the species can be selected using the F11 command. Furthermore, the F11 command will in addition to selection of the correct settings for a Total Count, also select the correct settings for a Non-Viable Count.

With the limitations as mentioned above the species can be chosen using the **F11** command. Press "**F11 + ↵**" and the following display text type will be shown:

Display Text Type 14

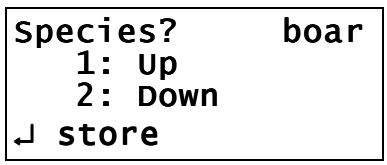

Figure 34. *The animal species can be chosen with the F11 command*

Press "**1**" and the ***Bull*** species can be chosen:

Display Text Type 14

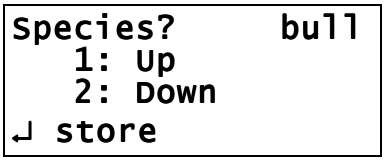

Figure 35. *The **Bull** species type can now be chosen. Press "↵" to choose the species indicated in the display (or "**Esc**" to return to the previous species).*

Press "**1**" again and the ***Stallion*** species can be chosen (only firmware v1.3 or higher).

Display Text Type 14

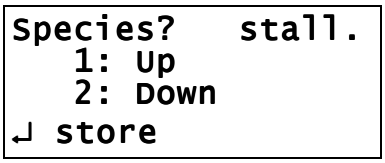

Figure 36. *The **Stallion** species type can now be chosen. Press "↵" to choose the species indicated in the display (or "**Esc**" to return to the previous species).*

Press "**2**" and get stepwise back to ***Bull*** or ***Boar***. If "**2**" is pressed three times then ***Other*** appears in the display:

Display Text Type 14

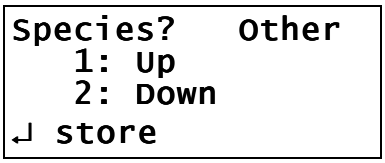

Figure 37. *The **Other** species type can now be chosen Press "↵" to choose the species indicated in the display (or "**Esc**" to return to the previous species).*

For dogs, rams, he-goats and bucks (rabbit) choose ***Bull*** or ***Stalion***. The ***Other*** is reserved to other species than boars, bulls, stallions, dogs, rams, he-goats and bucks (rabbit) and should not be used unless ChemoMetec recommend this.

If the species has been changed the new species will be remembered until it is changed again. When shutting down the SP-100 ask if the actual species shall be saved as the new default species:

Display Text Type 8

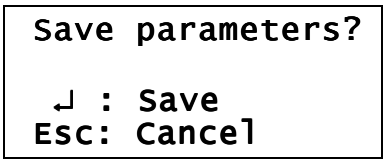

Figure 38. *Shall the actual Species value be saved and used as the new default species?*

Press "↵" to save or "**Esc**" if the present default species type shall not be overwritten.

Contact ChemoMetec or refer to the appropriate applications notes with respect to the instrument settings for the analysis of other types of semen.

5.8 Language (F210)

The messages and the text in the display window can be presented in four languages: English (en), French (fr), Italian (it) or Danish (da). If firmware v1.26 is installed in the instrument only two languages can be chosen: English (en) and Danish (da).

When typing "**F210 + ↵**" it is possible to change the language. Languages are chosen by using the "**1**" and "**2**" keys.

EXAMPLE - Changing the Language from English to French

To change the Language from English to French type "**F210 + ↵**". The following will be displayed in the window:

Display Text Type 15

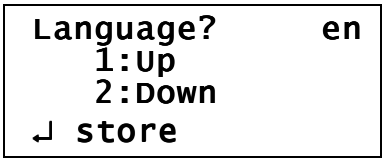

Figure 39. *A new language can now be chosen*

Use the "**1**" and "**2**" keys to toggle between the languages. Press "**1**" to choose French. The following window will now appear:

Display Text Type 15

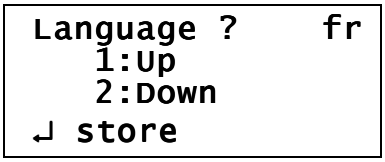

Figure 40. *French is the selected language*

Press "↵" to store the language (or "**Esc**" to return to the previous language):

Display Text Type 2

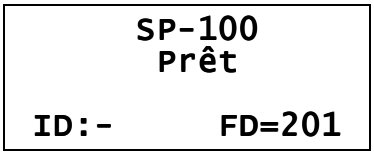

Figure 41. *French is the chosen language*

Press "**F210 + ↵**" followed by "**2 + ↵**" to go back to the English language again.

If the Language has been changed the new Language will be remembered until it is changed again. When shutting down the SP-100 ask if the actual language shall be saved as the new default language:

Display Text Type 8

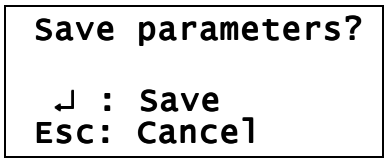

Figure 42. *Shall the actual language value be saved and used as the new default language?*

Press "↵" to save or "**Esc**" if the present default language should not be overwritten.

5.9 LCD contrast (F220)

When typing "**F220 + ↵**" it is possible to adjust the contrast of the LCD display. Use the "**1**" or "**2**" keys in order to increase or decrease the contrast of the LCD-display. The default value is 4. Values in the range from 0-9 can be chosen. The display will be updated with the current value, which is shown in the upper right hand corner of the display.

Display Text Type 16

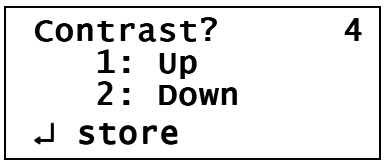

Figure 43. *A new LCD-contrast value can now be chosen*

Display Text Type 16

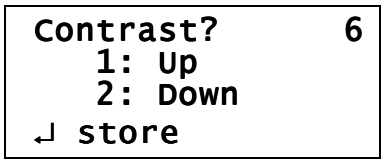

Figure 44. *LCD-contrast value 6 is going to be chosen*

Press "↵" to store the chosen value (or "**Esc"** to keep the previous value and return to the "**Ready**" status).

If the value has been changed the new contrast will be remembered during the session. When shutting down the SP-100 ask if the new value shall be saved as the new default value (Display Text Type 7). Press "↵" to save or "**Esc**" if the present default value should not be overwritten.

5.10 Instrument Info (F100)

When typing "**F100 + ↵**" the Instrument Info will appear in the display as in the Display Text Type shown below.

Display Text Type 17

```
ChemoMetec A/S
NC SP-100
v1.22 2004-02-12
S/N: 007-01
```

Figure 45. *Instrument Info*

The display shows the name of the manufacturer in line 1 of the display and the instrument type in line 2 of the display. The version number and date (year-month-date) of the integral software of the instrument is shown in line 3. The serial number of the instrument is shown in line 4 of the display. The display text is almost identical to the start-up display text (*Display Text Type 1 in Figure 12*).

5.11 Zero Count Check (F50, only firmware v1.3 or higher)

The instrument is able to perform a so-called **Zero Count Check**. This check is carried out with **no** cassette placed in the insertion slit of the instrument and with the lid closed. The check comprises an enumeration of the number of CCD-pixels that shows elevated signal levels. If this number of pixels is above a predefined limit then the cassette insertion slit might be contaminated with an interfering particle, like a dust particle. An error message is shown in the display and a cleaning of the insertion slit is recommended (refer to section 7 Maintenance of NucleoCounter SP-100).

If a PC with SemenView is connected to the instrument a contaminant can often be observed as an object, which is stationary in all images.

To initiate a **Zero Count Check** type in "**F50 + ↵**". Then the following text will appear in the display:

Display Text Type 18

```
0 count check

 ↵  Continue
Esc Abort
```

Figure 46. *Zero Count Check, step 1*

Press "↵" to continue. Now the instrument ask you to remove any cassette from the insertion slit:

Display Text Type 19

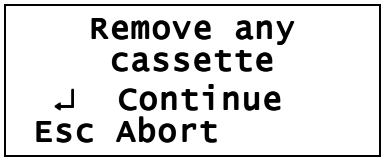

Figure 47. *Zero Count Check, step 2*

Press "↵" to continue. Now the instrument ask you to close the lid in order to avoid false light to enter the insertion slit during the Zero Count Check:

Display Text Type 20

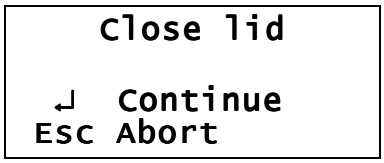

Figure 48. *Zero Count Check, step 3*

Press "↵" to continue. Then a "Wait" message appers in the display:

Display Text Type 21

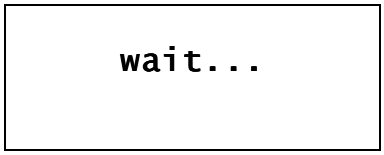

Figure 49. *Zero Count Check, step 3A*

After a few seconds the instrument has performed the Zero Count Check. If no contamination of the insertion slit can be detected by the instrument an "OK" message appears in the display (see figure below). Press any key to get back to the "Ready mode".

Display Text Type 22

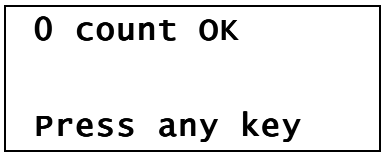

Figure 50. *The result of the Zero Count Check is OK*

If a contamination of the insertion slit is detected an error message is shown:

Display Text Type 23

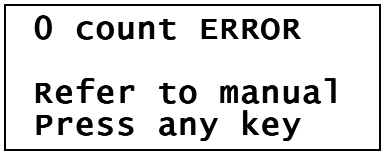

Figure 51. *The Zero Count Check did not pass the test*

Clean the insertion slit, e.g. with compressed, dry air (refer to section 7 Maintenance of NucleoCounter SP-100 and Technical Note No. 004 "*How to clean the NucleoCounter*"). Perform the zero count check again. If the error appears even after several attempts to clean the insertion slit with compressed air and if necessary the NucleoCounter® Clean Kit, contact ChemoMetec A/S or the local distributor.

5.12 Setting the Date (F200, only relevant when printer is connected)

The function, **F200**, is used to set the date. This feature is only used when a printer is connected to the printer port. Please, also refer to *Technical Note No. 100* with respect to setting and using the Date feature.

The date can be set using the **F200** command:

Display Text Type 24

```
Set date?
2005-02-23
20yy-mm-dd
↵ store
```

Figure 52. *Type in the date and press "↵" to save the new date or press the "Esc" button to cancel the operation.*

5.13 Setting the Time (F201, only relevant when printer is connected)

The function, **F201**, is used to set the Time. This feature is only used when a printer is connected to the printer port. Please, also refer to *Technical Note No. 100* with respect to setting and using the Time feature.

The Time can be set using the **F201** command:

Display Text Type 25

```
Set time?
11:02
hh-mm
↵ store
```

Figure 53. *Type in the time and press "↵" to save the new time or press the "Esc" to cancel the operation.*

5.14 Resetting the Counter (F30, only relevant when printer is connected)

The function **F30** is used to reset the Counter. The Counter counts the number of analysis performed by the instrument. The Analysis Number is incremented by 1 each

time an analysis is performed. The Analysis Number can be reset to 0 using the resetting function.

This feature is only used when a printer is connected to the instrument. Please refer to *Technical Note No. 100* with respect to using this feature.

To reset the Counter type "**F30+↵**":

Display Text Type 26

```
Reset counter?

↵  Continue
Esc Cancel
```

Figure 54. *Press "↵" to reset the counter or press the "Esc" to cancel the operation.*

If "↵" is selected the display will shortly show "Resetting..." indicating that the Analysis Number is being reset to 0:

Display Text Type 27

```
Resetting...
```

Figure 55. *The counter is being reset.*

5.15 Print to a PC (F310, only relevant when a PC is connected to printer port)

The function **F310** (Print -> PC) is used to choose between a normal print report to a printer or a short print report designated for a PC. The default setting is "**Print -> PC Off**" and is used when a printer is connected to the instrument. The "**Print -> PC On**" is designated for printing/logging the result of the analysis to a PC via the RS-232 printer port. Please refer to *Technical Note No. 100* with respect using this feature.

Use the F310 command to to choose the type of print report:

Display Text Type 28

```
Print->PC    Off
    1:On
    2:Off
↵ store
```

Figure 56. *Use the "1" and "2" buttoons to toogle between On and Off. Press "↵" to save the chosen setting or press the "Esc" to cancel the operation.*

5.16 Viability mode - F31 (only relevant for instruments with Viability Option included)

If the Viability Option is supplied with the instrument the Viability mode can be activated by the F31 command:

Display Text Type 29

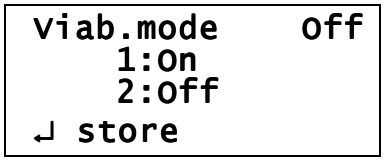

Figure 57. *Use the "1" and "2" buttons to toogle between Viability mode set to* ***On*** *or* ***Off****. Press "↵" to save the chosen setting or press "****Esc****" to cancel.*

With respect to carrying out a viability measurement (in fact the viability is calculated from a Total Count and a Non-Viable Count), please refer to "*Viability Testing with NucleoCounter SP-100, Addendum to User's Guide* (part no. 991-0104).

5.17 Inserting and removing the Cassette

When a cassette has been properly loaded with lyzate mixture, as explained in *Section 1.1.2*, the cassette is inserted into the NucleoCounter SP-100 instrument.

The grey lid covers the cassette insertion area. This lid is designed to protect the area from dust and other potential contaminants, and to keep external light from interfering with the fluorescent image recording. The lid is fitted with magnetic hinges, so it can be easily removed for cleaning purposes.

To insert the cassette:

1. Flip the lid up
2. Grip the cassette on either side of the piston barrel, but taking care not to touch the measurement windows (see *Figure 58*).
3. Insert the cassette, bevelled edge down, into the insertion slit (see *Figure 58*).

After analysis the lid is lifted and the cassette is removed from the insertion slit and disposed properly according to national or local laws or regulations regarding the nature of the mixture it contains.

Unless you are inserting/removing a cassette or cleaning the inserting area or the lid, keep the lid closed at all times.

Figure 58. *Illustration of how to grip and insert the cassette into the insertion slit*

The instrument is delivered with two lids. The largest of the two lids is shown in the figure above. This lid can be used as described above but in order to protect the insertion area from dust and dirt this lid must be placed on the instrument in the closed position when the instrument in not in use. The smallest of the two lids (see the figure below) is more easy to handle and the lid can replace the large lid if it is more convenient during a measurement session. **However, replace the small lid with the large lid when the session is over.**

Figure 59. *NucleoCounter SP-100 with the small version of the lid which can be used when many samples are being analyzed*

5.18 Measuring ("Run" button)

When a cassette has been loaded and inserted into the instrument, the measurement is activated, by pressing the "**Run**" button. The display shows:

Display Text Type 30

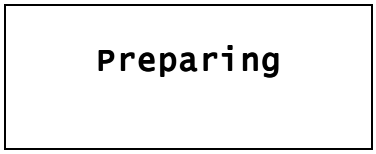

Figure 60. *The instrument is in the "**preparing**" mode*

During "**preparing**", the actuator moves the piston rod down through the barrel of the cassette. Hereby, the sample/dye mixture is transported to the cassette measurement chamber. The form and dimensions of the flow channels facilitate effective mixing of sample and stain. Sensors monitor sample flow. When the sample has been correctly loaded into the measurement chamber, the piston is stopped.

Now the display shows:

Display Text Type 31

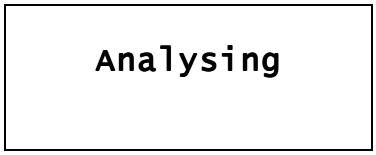

Figure 61. *The instrument is in the "**analysis**" mode*

The analysis will occur after a few seconds and the fluorescent image is recorded. Once recorded, the image is analysed by the instrument's integral software (firmware). Upon completion of the analysis, the result is shown on the display. The entire process takes approximately 30 seconds. If an external computer is connected, the results and the recorded fluorescent image can be viewed using SemenView software.

After each measurement the cassette can be safely removed (see *Section 5.17*). Sometimes, however, the complete process described above may be interrupted. Please familiarize yourself with *Section 8 Troubleshooting, Error messages*.

Instruments with the Viability Option included

If the instrument is in the viability mode (F31 is On), then the following text is displayed when the "**Run"** bottum is activated:

Display Text Type 32

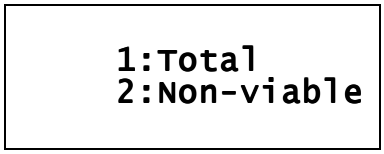

Figure 62. *Use the "**1**" and "**2**" buttoons to choose a "Total Count" or a "Non-Viable count". When "**1**" and "**2**" is activated the instrument will automatically initiate the "**Run**" routine. The "**Run**" routine takes about 30 seconds for a Total Count and approximately 60 seconds for a Non-Viable Count.*

5.19 Overview of Functions

Function	Name	Comment
F0	Dilution Factor	Abbreviated DF, or DF1 and DF2 in the viability mode
F10	Correction Factor	Abbreviated CF
F11	Animal Species	Only available with instrument SW version 1.24 or higher
F30	Reset Counter	Only relevant with printer connected to printer port
F31	Viability Mode	Only relevant if viability option is supplied with the instrument
F32	Retest result	
F50	Zero Count Check	
F100	Instrument Info	
F200	Setting Date	Only relevant with printer connected to printer port
F201	Setting Time	Only relevant with printer connected to printer port
F210	Language	
F220	LCD contrast	
F310	Print -> PC	Only relevant with printer/PC connected to RS-232 printer port

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6 Description of a typical Analysis Procedure (Total Count)

The Application Notes describe the analysis procedures in detail (e.g. *Application Note No. 100* deals with the analysis of boar ejaculates).

Always refer to the latest version of the appropriate *Application Note*.

In this section a brief description of *Application Note No. 100 Version 1* is given. The individual procedural steps are illustrated with pictures.

The following list contains the items required for determing sperm cell density in boar semen with the NucleoCounter SP-100 system.

- NucleoCounter SP-100 with software version 1.04 or higher
- SP1-Cassettes
- Container with Reagent S100 (lysis/dilution buffer)
- 20 ml polyethylene sample cups with polypropylene lids
- Container holder and sample cup holder
- Bottle-top dispenser 1-10 ml, e.g. 1-10 ml Brand Dispensette III Variabel dispenser equipped with a 298 mm filling tube
- Adjustable, automatic pipette, e.g. 10-100 µl Finnpipette adjusted to give 50 µl boar semen ejaculate, and pipette tips
- Optionally the NucleoCounter SP-100 can be connected to a computer via an USB-interface. When connected to a computer the SemenView™ software offers various features such as documentation of the obtained results.

Briefly, the Total Count analysis procedure comprises the following steps:

1. Type in the Sample ID on the NucleoCounter SP-100 and check that the Dilution Factor is correct.

2. Using the automatic pipette mounted with a new pipette tip aspirate 50 µl of the ejaculate. Transfer the sample to the center area of the bottom of a 20 ml sample cup (see *Figure 63*).

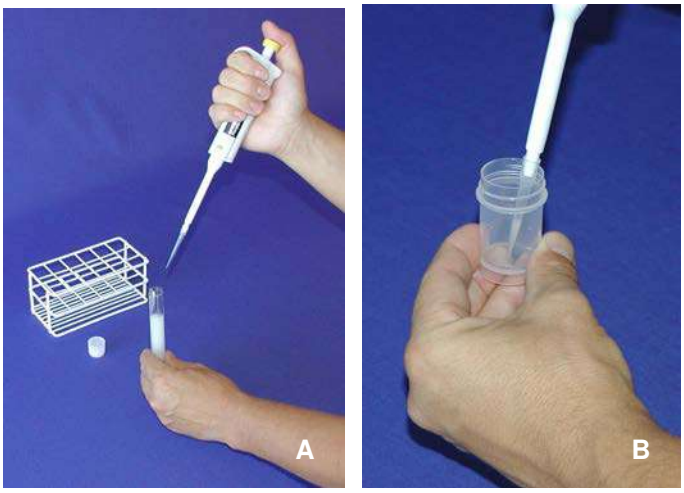

Figure 63. *Pipetting of 50 µl of sample (A) and transferring sample to sample cup (B)*

3. Place the sample cup in the cup-holder and dispense 10.00 ml of Reagent S100 into the cup using the dispenser (see *Figure 64*). Dispense the reagent directly onto the semen sample at the bottom of the cup. Use a consistent plunger pressure, so that the plunger moves smoothly. There is no need for further mixing, since the sample and the reagent are thoroughly mixed during the dispensing of the Reagent S100.

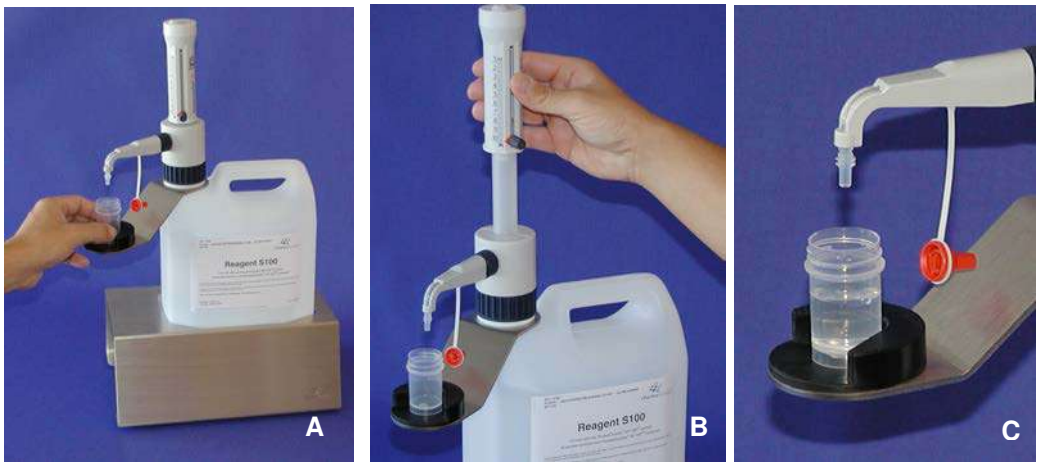

Figure 64. *Placing the sample cup in the cup holder (A), dispensing 10 ml of Reagent S100 (B) and sample cup with lyzate mixture (C)*

4. **Immediately,** i.e. max. 10 seconds after the dispensing step, a portion of the lyzate mixture shall be aspirated into a SP1-Cassette (see *Figure 65*). 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 finger grips of the cassette. **Avoid touching the window (clear area) of the measurement chamber.**

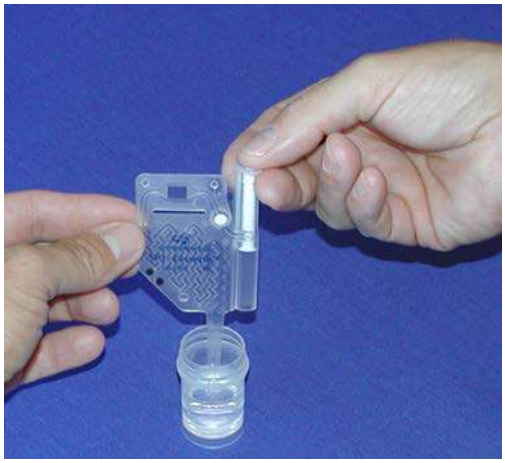

Figure 65. *Loading the cassette with lyzate mixture*

5. Once the sample has been aspirated into the SP1-Cassette, open the lid and insert the cassette in the insertion slit of the NucleoCounter SP-100. Close the lid and press the "**Run**" button on the instrument in order to initiate the analysis (see *Figure 66*).

Figure 66. *Insertion of cassette (A), closing the lid (B) and pressing the "**Run**" button (C)*

6. After approx. 30 seconds, the analysis is completed, and the result (in millions per ml) is shown on the LCD-display (see *Figure 67*) and on the PC (in *SemenView*) if such is connected.

Figure 67. *Result of the analysis shown on the LCD display*

7. Open the lid, remove and dispose of the cassette. Place the lid on the used sample cup and dispose of this, too. The NucleoCounter SP-100 system is ready for a new analysis.

Remember - samples obtained from the sperm cell material must be representative

Dispose of the SP1-Cassette and the sample cup with its content according to national and local laws/regulations regarding the nature of the mixture it contains

7 Maintenance of NucleoCounter SP-100

A regular cleaning of the NucleoCounter SP-100 is recommended in order to protect its surface and assure the quality of the collected images.

Depending on the environment in which the NucleoCounter SP-100 is operated it is suggested that regular cleaning of the cabinet is carried out. When cleaning the cabinet it is recommended to use a soft moist cloth and gently wipe the surface. Any contamination, which does not come off immediately, should be rubbed gently with a cloth wetted with mild detergent. Never use organic solvents or aggressive detergents to clean the exterior of the NucleoCounter SP-100 as this might damage the surface.

The cassette insertion area and the optical parts inside the NucleoCounter SP-100 should be adequately protected against dust and other contaminants. Therefore, great care must be taken to ensure that the lid covering the SP1-Cassette insertion area is closed when SP1-Cassettes are not being loaded into or removed from the NucleoCounter SP-100. If the insertion area becomes soiled it should immediately be cleaned with a clean, dry and dust free cloth. **Always replace the small lid (if such is used, see *Figure 59*) with the large lid when a measurement session is over.**

When cleaning the cassette insertion area, great care must be taken against introducing any liquid or dust into the insertion slit of the NucleoCounter SP-100. Any liquid that enters the interior of the NucleoCounter SP-100 can damage the optical parts and thus compromise the quality of the cell counts.

An object on the surface of an optical component can influence the collected image. A contaminant will normally be visible as a faint object in the image. Since it is also possible that such contamination is on the surface of the SP1-Cassette, ***only objects, which are stationary in all images, are possible contaminations of the optical system.*** An example of a relatively large contamination is given in *Figure 68*.

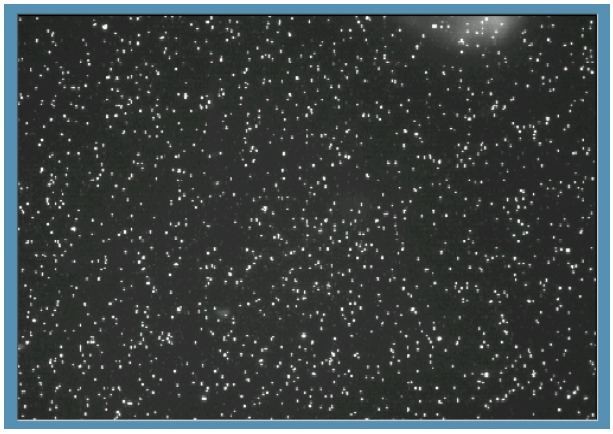

Figure 68. *An image showing a contamination of the optical system, visible as a white cloudy phenomena in the upper right hand corner. If the contamination is stationary it should be removed using compressed air.*

The presence of a contaminant (e.g. dust particles) will normally not influence the counting of cells. The NucleoCounter SP-100 will distinguish between cells and contaminants, since cells are generally significantly smaller than contaminants.

Use the F50 function (refer to section *5.11 Zero Count Check (F50,* only firmware v1.3 or higher)) regularly in order to check for contaminantion of the insertion slit.

Dust and other contaminants deep inside the insertion slit should be removed using dry, compressed air (see Figure 69) and if necessary using the NucleoCounter® Clean Kit (refer to Technical Note No. 004 "How to clean the NucleoCounter"). Use the F50 function to verify the rinsing effect. If the error appears even after several attempts to clean the insertion slit with compressed air, contact ChemoMetec A/S or the local distributor.

Figure 69. *Removal of dust from the insertion slit using compressed air.* ***Never keep the container in a horizontal position and preferable do not keep it in a position that is more angled than shown in the figure.***

If liquid has been spilled into the insertion slit of the instrument, **contact ChemoMetec A/S or the local distributor.**

8 Troubleshooting, Error messages

Under certain conditions the NucleoCounter SP-100 will display error messages during operation. Corrective actions are suggested below but if they do not correct the errors, contact ChemoMetec A/S or your local distributor.

With respect to problems with the transfer of images and results to the PC via the USB, please refer to the User's Guide for the SemenView software.

8.1 No valid cassette

When trying to analyze an SP1-Cassette, which is not valid, the NucleoCounter SP-100 displays the error message shown in *Figure 70*.

Display Text Type 33

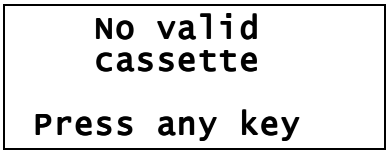

Figure 70. *The error message displayed when the NucleoCounter SP-100 does not recognize the cassette as valid*

This error message is displayed in the following situations:

- The SP1-Cassette has been analyzed previously
- The SP1-Cassette is not inserted properly into the NucleoCounter SP-100.
- There is no SP1-Cassette inserted into the NucleoCounter SP-100.

Press any key to go to the Ready status.

8.2 Analysis aborted

The NucleoCounter SP-100 displays the error message shown in *Figure 71* when the analysis has been aborted because the stained mixture inside the SP1-Cassette has not reached the chamber within a given time.

Display Text Type 34

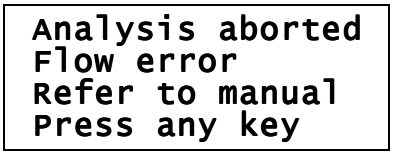

Figure 71. *Error message displayed by the NucleoCounter SP-100 when the analysis has been aborted.*

A common cause of this is that an insufficient volume of lyzate mixture has been loaded into the SP1-Cassette. Load a new cassette and repeat the analysis.

The error will also occur if the SP1-Cassette has not been inserted properly into the NucleoCounter SP-100. Then the instrument cannot press the piston down and the error occurs. Take out the cassette, insert it properly and run the analysis.

Press any key to go to the Ready status.

8.3 Actuator error messages

The actuator moves the piston down into the barrel of the cassette. If the actuator has a failure an actuator error messages will be displayed. This error message is shown in *Figure 72*.

Display Text Type 35

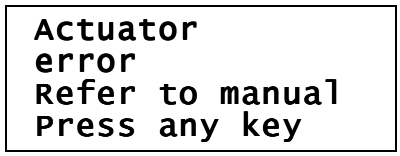

Figure 72. *Actuator error message*

Do the following if the actuator error message appears: First, examine if the SP1-Cassette has been inserted properly. If not, insert the cassette once more and attempt the analysis again. If the cassette was inserted properly turn the NucleoCounter SP-100 off and on a few times. If you hear the actuator motor during start-up it is likely that the error has been corrected.

Press any key to go to the Ready status.

8.4 Sample could not be analyzed

When the NucleoCounter SP-100 cannot determine the concentration of cells during analysis the error message in *Figure 73* is displayed.

Display Text Type 36

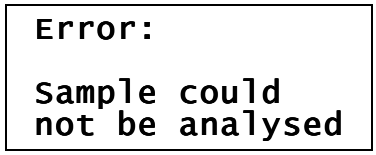

Figure 73. *This error message is displayed when the concentration of cells in the SP1-Cassette cannot be determined*

The error appears if the concentration of stained nuclei inside the SP1-Cassette chamber is far too high, e.g. when the ejaculate is insufficiently diluted with Reagent S100. The

message can also appear if the lid is not closed, which may cause ambient light to interfere with the analysis.

Press any key to go to the Ready status.

8.5 Sensor error

The black dots, which are printed on the SP1-Cassettes are read by sensors inside the NucleoCounter SP-100 each time an analysis is run. The dots code for the volume of lyzate mixture in the SP1-Cassette chamber. If one or more of the sensors has a malfunction an error message will be displayed, see *Figure 74*.

Display Text Type 37

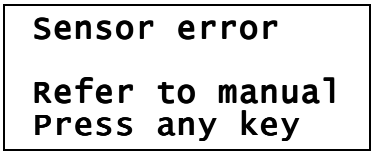

Figure 74. *This error message is displayed if one of the sensors, which read the black dots on the SP1-Cassettes, has a failure*

If the error message is displayed analyses can still be made but the accuracy of the results cannot be guaranteed since the volume of the measurement chamber may not be read correctly.

Press any key to go to the Ready status.

8.6 Power-on failure

If the NucleoCounter SP-100 cannot be turned on, please verify that the power supply is connected to the NucleoCounter SP-100 and a working power plug.

If the NucleoCounter SP-100 still does not turn on, inspect and/or replace the fuses as described below.

The fuse holder is located just above the NucleoCounter´s power plug, see *Figure 75*.

Figure 75. *Location and removal of the fuse holder*

Disconnect the power supply from the NucleoCounter SP-100. Then remove the fuse holder by use of a screwdriver (turn CCW) as indicated in *Figure 75*. Inspect the fuse. If it needs replacement use an UL Listed fuse: 600mA/250Vac (UL 248).

If the NucleoCounter SP-100 does not turn on after replacement of the fuse, or if the fuse was not broken, please contact ChemoMetec A/S or your local distributor for further instructions.

8.7 Instrument is not able to finish the analysis

If a PC is connected to the NucleoCounter SP-100 via a USB cable and the PC is not powered on, then the NucleoCounter SP-100 can have a problem with finishing the analysis (e.g. the display keeps showing "Analyzing", see figure below).

Display Text Type 31

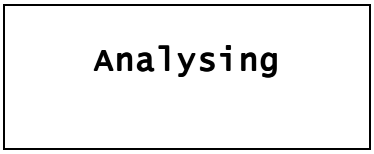

Figure 76. *The instrument is in the analysis mode*

Solution: Remove the USB cable from the instrument or power on the PC.

9 Technical specifications

Specificity

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

Total Count: Counts total number of sperms (using Reagent S100, a lysing/dilution medium)

Non-Viable Counts: Counts only the "dead" sperms (using PBS or an extender as the dilution medium).

Sample types

Mammalian semen, including semen from boars, bulls, dogs, stallions, rams, he-goats, bucks (rabbit) etc.

Semen doses used for Artificial Insemination can also be analyzed with the NucleoCounter SP-100

Sample consumption

Total Count: The sample is mixed, lysed and diluted with Reagent S100 prior to analysis. It is recommended that at least 50 µl of sample is used for this dilution.

Non-Viable Counts: The sample is mixed and diluted with PBS or extender prior to analysis. It is recommended that at least 50 µl of sample is used for this dilution.

Reagent consumption

It is recommended that 10 ml of reagent (Reagent S100 for a Total Count or PBS/Extender for a Non-Viable Count) is used for dilution of a 50 µl sample of a boar ejaculate. This corresponds to a 201-fold dilution of the sample.

When analysing a boar semen dose which contains approximately 30 million sperm cells per ml it is recommended to add 10 ml of reagent to 1 ml of semen dose (dilution factor 11).

The sample consumption and reagent consumption will vary from species to species, please refer to application notes.

Sample/reagent mixture consumption

At least 100 µl of sample/reagent mixture is recommended for SP1-Cassette loading. Approximately 60 µl of the mixture is loaded into the cassette.

Analysis volume

Approximately 1 µl of the sample/reagent mixture is being analyzed in the NucleoCounter SP-100.

Measurement range in Semen Samples

In principle all samples (ejaculates, semen does etc.) containing **> 0.5 million sperms per ml** can be analyzed.

Measurement range in sample/reagent mixture

The sample should be diluted with reagent so that the sample/reagent mixture contains a certain amount of cells in the mixture. The total range, the recommended range and the optimal range is:

Total Range -	50.000 to 7.000.000 sperms per ml mixture
Recommended Range -	500.000 to 7.000.000 sperms per ml mixture
Optimal Range -	750.000 to 5.000.000 sperms per ml mixture

The sample should always be diluted so that the cell density of the resulting sample/reagent mixture is within the recommended range.

Repeatability (CV%)

The repeatability is typical **< 4%** for a **Total Count**, provided that the cell concentration of the sample/reagent mixture is in the Optimal Range (se above).

The repeatability for a Non-Viable Count is normally higher than for the Total Count. The repeatability for a **Non-Viable Count** is typical **< 8%**.

Dilution Factor (DF)

DF in the range from 1 - 9999 can be programmed in the NucleoCounter SP-100. DF below 11 is not recommended without first consulting ChemoMetec.

Presentation of Result

The sperm cell density in the sample/reagent mixture is multiplied with the dilution factor giving the sperm cell density in the sample. The result of the

	analysis is presented in *Millions of Cells per ml* of the initial Sample. The result is always shown on the NucleoCounter display. If connected to a PC with NucleoView installed the result is also displayed on the PC monitor. The viability in percent can also be displayed on the PC-monitor if both a Total Count and a Non-Viable Count are performed.	
Operation	Menu-controlled by means of keyboard and LCD display.	
Analysis time	When pressing "**Run**" on the NucleoCounter SP-100 the result will be displayed within: 30 seconds for a Total Count 60 seconds for a Non-Viable Count	
Physical data	Weight	3 kg
	Height	26 cm
	Width	38 cm
	Depth	22 cm
Power	⚠ See Section 11.5 Power Supply	
	Input	12VDC (11-13VDC)
	Fuse	UL Listed fuse: 600mA/250Vac (UL 248)
Power consumption NucleoCounter	Peak	25 W
	Ready mode	2.5 W
	Standby	2 mW
Operation conditions	Maximum relative humidity 80 percent for temperatures up to 31°C decreasing linearly to 65 percent relative humidity at maximum 35°C; minimum temperature 15°C.	
USB (For image and result transfer to PC)	USB, version 1.1. Note: Does not support USB Hubs	
RS-232 Printer port **(To be used for printer, SW-updating and result transfer to PC)**	Baud rate: Data bits: Parity: Stop bits: Flow control:	19200 8 None 1 None

Other environmental operating conditions for the NucleoCounter SP-100:

Indoor use only	
Altitude	Up to 2000 m
Mains supply	Refer to the specifications for the Power supplies in section 11.5 Power supply
Installation Category	II (Refer to UL 61010A-1)
Overvoltage Category	II (Refer to UL 61010A-1)
Pollution Degree	II (Refer to UL 61010A-1)

10 EMC and EMI safety standards

The NucleoCounter SP-100 complies with EMC/EMI safety standards as follows.

EN61326: 1997+A1: 1998 (Class B) +A2: 2000, Electrical equipment for measurement, control and laboratory use – EMC requirements (emission and immunity). Annex B and C from A1: 1998 is used.

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

The NucleoCounter SP-100 complies with the following Safety standards:

UL 3111-1 (UL61010A-1): Electrical Equipment for Measurement, Control and Laboratory use.

IEC/EN61010-1: Electrical Equipment For Laboratory use.

CAN/CSA-C22.2 No. 1010.1-92: Electrical Equipment for Measurement, Control and Laboratory use.

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11 Equipment and Accessories

11.1 Equipment and Accessories List

The equipment and accessories for the NucleoCounter SP-100 are listed in the table below.

⚠ Ensure that items marked with this symbol are the only equipment and accessories used together with the NucleoCounter SP-100.

Table 4. *Equipment and accessories for NucleoCounter SP-100 system*

Item	Part no.	Description
NucleoCounter SP-100	900-0100	Instrument
SP1-Cassette	941-0006[4]	A disposable device used for performing the cell-count.
Reagent S100, 5L	910-0100[5]	A reagent used for lysis of cells and dilution of sample prior to performing a Total Count
Reagent S100, 500 mL	910-0101	
PBS, 5L	910-0009	A reagent used for dilution of sample prior to performing a Non-Viable Count
PBS, 500 mL	910-0008	
CD-rom containing SemenView™ software and electronic version of SemenView User's Guide	950-0100	PC software used for storing of data and presentation of cell counts and images
NucleoCounter SP-100 User's Guide	991-0100	User's guide for the NucleoCounter SP-100 (English version)
Viability Testing with NucleoCounter SP-100, Addendum to User' s Guide	991-0104	Addendum to User's Guide regarding Viability Testing with NucleoCounter SP-100 (English version)
SemenView User's Guide	991-0103	User's guide for the SemenView software (English version)
Applic. Note 100-GB	994-0101	Sperm density in Ejaculates from Boars (English version)
Applic. Note 101-GB	994-0103	Sperm density in Boar Semen Doses for AI (English version)
Applic. Note 102-GB	994-0104	Sperm density in Ejaculates from Bulls (English version)
Applic. Note 103-GB	994-0105	Sperm density in Bull Semen Doses for AI (English version)
⚠ External USB cable	931-0001	A USB cable used for transmission of image data from the NucleoCounter SP-100 to an external PC.
⚠ Power supply	See section below	A device for generating the DC-voltage for the NucleoCounter SP-100 from the mains supply.

[4] In a box with 10 bags each containing 10 cassettes

[5] In a container with 5 Litres (sufficient for 480-500 analysis of boar ejaculates)

Item	Part no.	Description
⚠ Fuse for 12VDC input	939-0001	A device for protecting the NucleoCounter SP-100 against over current.
Mains Power cord	See section below	A cord for mains supply of the external power supply.
Dispenser 1-10 mL	911-0003	Dispenser for Reagent S100 (Brand Dispensette III, no.4700 140)
Finnpipette 10-100 µL	911-0006	Automatic pipette for sample pipetting (Thermo-Labsystems no. 4500 110)
Finnpipette 100-1000 µL	911-0009	Automatic pipette for sample pipetting (Thermo-Labsystems no. 4500 120)
Finntip 250, 96 pcs.	911-0011	
Finntip 1000, 96 pcs.	911-0012	
Sample Cup 20 mL, 1000 pcs.	911-0004	Sample cup of polypropylene with a polyethylene screw cap for dilution of an ejaculate or a semen dose
Sample Cup 35 mL, 500 pcs.	911-0007	
Eppendorf Tube, 1000 pcs.	911-0014	Tube for dilution of a semen dose
SP-100 Test Kit	912-0004	Fluorescent beads for checking the instrument.
Cleaning Kit NucleoCounter	911-0013	Kit for cleaning of the NucleoCounter cassette insertion slit
Dry, compressed air	See section below	Used for removal of dust from the optical system of the NucleoCounter SP-100
Bottle Stand	929-0001	Stand for two 500 ml reagent bottles (Reagent S100 & PBS)
Container Stand	929-0003	Stand for a 5L reagent container
Short LID Grey	929-0007	Short lid for the NucleoCounter
Starter Kit Impact Prin.	972-0001	External Impact Printer
Starter Kit Thermal Prin.	972-0002	External Thermal Printer
Paper Impact Print., 5 RL	939-0004	Paper for Impact Printer
Ribbon Impact Print., 1pcs	939-0005	Ribbon for Imapct Printer
Paper Thermo Printer, 5 RL	939-0007	Paper for Thermal Printer
Serial PC Cable, 2 Meter	931-0011	Cable for connection of the NucleoCounter to a PC via a serial port. Used for integration of the NucleoCounter to semen production PC-SW.

11.2 External Printer

An external thermo printer may optionally be connected to the NucleoCounter SP-100 for the printing of test results.

For connection of a printer please refer to Technical Note No. 100 (CM no. 994-0102).

Also refer to the section *Warnings and precautions* with respect to the properties of the Printer Port.

11.3 Viability option

The NucleoCounter SP-100 can also be supplied with the viability option, which can be used for the estimation of the number of viable sperm cells in a semen sample. Contact ChemoMetec A/S for further information.

11.4 Dry compressed air

Dry, compressed air (KENAIR Air Duster - CFC Free) is used for removal of dust from the optical system of the NucleoCounter SP-100 (refer to *Section 7*).

The item can be obtained from Kenro Ltd, Greenbridge Road, Swindon SN3 3LH, UK. Tel: +44 (0) 1793 615836, E-mail: sales@kenro.co.uk. Alternatively the item, or a similar item, might be available in your own country through local dealers.

11.5 Power supply

⚠ The NucleoCounter SP-100 shall only be used with one of the following external power supplies:

- Power supply Class I, Proton Electronic Industrial Co. Ltd., model SPN-260-12, input rated 100-240 Vac, 50-60Hz, 1.6 A. Output rated 12 Vdc, 4.3 A.
- Power supply Class I, Powerbox Europe AB, model EBH 03 131, input rated 100-240 Vac, 47-63 Hz, 0.8A. Output rated 11-13 Vdc, 2.7 A.
- Power supply Class II, Celetron USA Inc., model ZVC40LT12E, input rated 100-240 Vac, 50-60Hz, 1.2 A. Output rated 12 Vdc, 3.3 A.

⚠ The detachable power supply cord set and appliance inlet of the external power supply are considered as the disconnecting device.

Contact ChemoMetec A/S for information on order of a specific power supply listed above.

11.6 Mains Power cord

Detachable power supply cord set for the Class I specified power supplies:

For US 125 Vac

UL listed, type SVT, rated min. 60C, 18 AWG, 3 conductors. Provided with molded-on grounding-type (NEMA 5-15P) attachment plug, rated min. 125 Vac, min. 2.5A. Opposite end terminates in molded on, IEC320 style connector, rated min. 125 Vac, min. 2.5 A.

For Europe 250Vac

Cord type min. H05RR-F or min. H03VV-F or min. H03VVH2-F, rated min. 60C, 3x0.75 mm2. Provided with molded-on grounding-type attachment Plug, rated min. 250 Vac, min. 2.5 A. Opposite end terminates in molded on, IEC320 style connector, rated min. 250 Vac, min. 2.5 A.

Detachable power supply cord set for the specified Class II power supply:

For US 125 Vac

UL listed, type SPT-2 or SVT, rated min. 60C, 18 AWG, 2 conductors. Provided with molded-on ungrounding-type (NEMA 1-15P) attachment Plug, rated min. 125 Vac, min. 2.5 A. Opposite end terminates in molded on, IEC320 style connector, rated min. 125 Vac, min. 2.5 A.

For Europe 250Vac

Cord type min. H05RR-F or min. H03VV-F or min. H03VVH2-F, rated min. 60C, 2x0.75mm2. Provided with molded-on ungrounding-type attachment Plug, rated min. 250 Vac, min. 2.5 A. Opposite end terminates in molded on, IEC320 style connector, rated min. 250 Vac, min. 2.5 A.

⚠ The Mains supply cord and plug of the external power supply shall comply with any national regulations.

⚠The user shall be made aware of that, if the NucleoCounter SP-100 and the external power supply is used in a manner not specified by the manufacturer, the protection provided by the NucleoCounter SP-100 and the external power supply may be impaired.

Contact ChemoMetec A/S for information on order of a specific Power supply cord listed above.

12 Display Text Types (overview in en, fr, it, da)

	En	Fr	It	Da
Display Text Type 1	NucleoCounter SP-100 V1.3 2005-04-21 S/N: 007-01	NucleoCounter SP-100 V1.3 2005-04-21 S/N: 007-01	NucleoCounter SP-100 V1.3 2005-04-21 S/N: 007-01	NucleoCounter SP-100 V1.3 2005-04-21 S/N: 007-01
Display Text Type 2	SP-100 Ready ID:- DF=201	SP-100 Prêt ID:- FD=201	SP-100 Pronto ID:- FD=201	SP-100 Klar ID:- FF=201
Display Text Type 3	Bye...	A bientôt...	Arrivederci...	Farvel...
Display Text Type 4	Function? F ↵ select	Fonction? F ↵ choisir	Funzione? F ↵ seleziona	Funktion? F ↵ vælg
Display Text Type 5	Invalid function	Fonction invalide	Funzione non valida	Ukendt funktion
Display Text Type 6	Storing…	Mémorisation	Memorizzazione..	Gemmer…
Display Text Type 7	Saving…	Enregistrement	Salvataggio…	Gemmer…
Display Text Type 8	Save parameters? ↵ : Save Esc: Cancel	Enr. paramètres? ↵ : Enregistrer Esc: Annuler	Salva parametri? ↵ : Salva Esc: Annulla	Gem parametre? ↵ : Gem Esc: Fortryd
Display Text Type 9A	Cells/ml Sample 455.0 mill. ID:- DF=201	Cells/ml échant. 445.0 mio ID:- FD=201	Cellule/ml Camp. 455.0 mil. ID:- FD=201	Celler/ml prøve 455.0 mill. ID:- FF=201
Display Text Type 9B	Cells/ml Sample 455.0 mill. Retest! ID:- DF=201	Cells/ml échant. 445.0 mio Retester! ID:- FD=201	Cellule/ml Camp. 455.0 mil. Rimisura! ID:- FD=201	Celler/ml prøve 455.0 mill. Gentest! ID:- FF=201
Display Text Type 10	Retest res.? On 1: On 2: Off ↵ store	Rés.Retest Oui 1: Oui 2: Non ↵ mémoriser	Ris. misura? On 1: On 2: Off ↵ memorizza	Gentest res.? Til 1: Til 2: Fra ↵ gem
Display Text Type 11	Sample ID? Enter new ID + ↵ New ID=	ID échantillon? Saisir ID + ↵ Nouvelle ID=	ID del Campione? Imm. nuova ID + ↵ Nuova ID=	Prøve ID? Tast ny ID + ↵ Ny ID=
Display Text Type 12	Dilution factor DF=201 Enter new DF + ↵ New DF=	Facteur dilution FD=201 Saisir FD + ↵ Nouveau FD=	Fattore di dil. FD=201 Imm. nuovo FD + ↵ Nuovo FD=	Fortyndingsfakt. FF=201 Tast ny FF + ↵ Ny FF=

	En	Fr	It	Da
Display Text Type 13	Corr. factor CF=1000 Enter new CF + ↵ New CF=	Facteur corr. FC=1000 Saisir FC + ↵ Nouveau FC=	Fattore di corr. FC=1000 Imm. nuovo FC + ↵ Nuovo FC=	Korr. Faktor KF=1000 Tast ny KF + ↵ Ny KF=
Display Text Type 14A	Species? other 1: Up 2: Down ↵ store	Espèce? autre 1: Précédent 2: Suivant ↵ mémoriser	Specie? altro 1: Su 2: Giù ↵ memorizza	Art? andre 1: Op 2: Ned ↵ gem
Display Text Type 14B	Species? boar 1: Up 2: Down ↵ store	Espèce? verrat 1: Précédent 2: Suivant ↵ mémoriser	Specie? verro 1: Su 2: Giù ↵ memorizza	Art? orne 1: Op 2: Ned ↵ gem
Display Text Type 14C	Species? bull 1: Up 2: Down ↵ store	Espèce? taureau 1: Précédent 2: Suivant ↵ mémoriser	Specie? toro 1: Su 2: Giù ↵ memorizza	Art? tyr 1: Op 2: Ned ↵ gem
Display Text Type 14D	Species? Stall. 1: Up 2: Down ↵ store	Espèce? étalon 1: Précédent 2: Suivant ↵ mémoriser	Specie? Stall. 1: Su 2: Giù ↵ memorizza	Art? Hingst 1: Op 2: Ned ↵ gem
Display Text Type 15	Language? en 1:Up 2:Down ↵ store	Langue? fr 1: Précédent 2: Suivant ↵ mémoriser	Lingua? it 1: Su 2: Giù ↵ memorizza	Sprog? da 1: Op 2: Ned ↵ gem
Display Text Type 16	Contrast? 4 1: Up 2: Down ↵ store	Contraste? 4 1: Précédent 2: Suivant ↵ mémoriser	Contrasto? 4 1: Su 2: Giù ↵ memorizza	Kontrast? 4 1: Op 2: Ned ↵ gem
Display Text Type 17	ChemoMetec A/S NC SP-100 V1.3 2005-04-21 S/N: 007-01	ChemoMetec A/S NC SP-100 V1.3 2005-04-21 S/N: 007-01	ChemoMetec A/S NC SP-100 V1.3 2005-04-21 S/N: 007-01	ChemoMetec A/S NC SP-100 V1.3 2005-04-21 S/N: 007-01
Display Text Type 18	0 count check ↵ Continue Esc Cancel	0 contrôler rés ↵ Continuer Esc Annuler	Controllo ris. 0 ↵ Continua Esc Annulla	0 count check ↵ Fortsæt Esc Afbryd
Display Text Type 19	Remove any cassette ↵ Continue Esc Cancel	Retirer toute cassette ↵ Continuer Esc Annuler	Rimuovi ogni cassetta ↵ Continua Esc Annulla	Fjern cassette ↵ Fortsæt Esc Afbryd
Display Text Type 20	Close lid ↵ Continue Esc Cancel	Fermer couvercle ↵ Continuer Esc Annuler	Chiudi Coperchio ↵ Continua Esc Annulla	Luk låget ↵ Fortsæt Esc Afbryd
Display Text Type 21	wait…	attendre…	attendi…	vent…
Display Text Type 22	0 count OK Press any key	0 résultat OK Appui sur touche	ris. 0 OK Premi un tasto	0 count OK Tryk en tast
Display Text Type 23	0 count ERROR Refer to manual Press any key	Défaut 0 résultat Voir manuel Appui sur touche	Errore ris. 0 Vedi manuale Premi un tasto	0 count FEJL Se manual Tryk en tast

	En	Fr	It	Da
Display Text Type 24	Set date? 2005-02-23 20yy-mm-dd ↵ store	Régler la date? 2005-02-23 20aa-mm-jj ↵ mémoriser	Imposta data? 2005-02-23 20aa-mm-gg ↵ memorizza	Indstil dato? 2005-02-23 20yy-mm-dd ↵ gem
Display Text Type 25	Set time? 11:02 hh:mm ↵ store	Régler l'heure 11:02 hh:mm ↵ mémoriser	Imposta ora? 11:02 hh:mm ↵ memorizza	Indstil tid? 11:02:00 hh:mm ↵ gem
Display Text Type 26	Reset counter? ↵ Continue Esc Cancel	RAZ compteur? ↵ Continuer Esc Annuler	Azzerare cont.? ↵ Continua Esc Annulla	Nulstil tæller? ↵ Fortsæt Esc Afbryd
Display Text Type 27	Resetting...	Resetting...	Resetting...	Resetting...
Display Text Type 28	Print->PC Off 1: On 2: Off ↵ store	Impr->PC Non 1: Oui 2: Non ↵ mémoriser	Stampa->PC Off 1: On 2: Off ↵ memorizza	Print->PC Fra 1: Til 2: Fra ↵ gem
Display Text Type 29	Viab.Mode? Off 1: On 2: Off ↵ store	Mode viab. Non 1: Oui 2: Non ↵ mémoriser	Mod. vita? Off 1: On 2: Off ↵ memorizza	Viab.Mode? Fra 1: Til 2: Fra ↵ gem
Display Text Type 30	Preparing	Préparation	Preparazione	Forbereder
Display Text Type 31	Analysing	Analyse	Analisi	Analyserer
Display Text Type 32	1: Total 2: Non-viable	1: Total 2: Non-viable	1: Totale 2: Non vitali	1: Total 2: Non-viable
Display Text Type 33	No valid cassette Press any key	Cassette non valide Appui sur touche	Nessuna cassetta valida Premi un tasto	Ugyldig kassette Tryk en tast
Display Text Type 34	Analysis aborted Flow error Refer to manual Press any key	Analyse annulé Défaut flux Voir manuel Appui sur touche	Analisi fallita Errore di flusso Vedi manuale Premi un tasto	Analyse afbrudt Flow fejl Se manual Tryk en tast
Display Text Type 35	Actuator error Refer to manual Press any key	Actionneur Défaut Voir manuel Appui sur touche	Azionatore errore Vedi manuale Premi un tasto	Aktuator fejl Se manual Tryk en tast
Display Text Type 36	Error: Sample could not be analysed	Défaut: Analyse échant. impossible	Errore: Impossibile ana-lizzare campione	Fejl: Prøve kunne ikke analyseres
Display Text Type 37	Sensor error Refer to manual Press any key	Défaut capteur Voir manuel Appui sur touche	Err. del sens. Vedi manuale Premi un tasto	Sensor fejl Se manual Tryk en tast

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13 WEEE directive information in more EU languages

United Kingdom

Correct Disposal of This Product
(Waste Electrical & Electronic Equipment) - Europe only

This marking shown on the product or its literature, indicates that it should not be disposed together with other household wastes at the end of its working life. To prevent possible harm to the environment or human health from uncontrolled waste disposal, please separate this from other types of wastes and recycle it responsibly to promote the sustainable reuse of material resources.

Business users should contact their supplier and check the terms and conditions of the purchase contract. This product should not be mixed with other commercial wastes for disposal.

Sweden

Korrekt avfallshantering av produkten
(elektriska och elektroniska produkter) - Endast för Europa

Denna markering på produkten och i manualen anger att den inte bör sorteras tillsammans med annat hushållsavfall när dess livstid är över. Till förebyggande av skada på miljö och hälsa bör produkten hanteras separat för ändamålsenlig återvinning av dess beståndsdelar.

Företagsanvändare bör kontakta leverantören samt verifiera angivna villkor i köpekontraktet. Produkten bör inte hanteras tillsammans med annat kommersiellt avfall.

Slovenia

Ustrezno odstranjevanje tega izdelka
(odpadna električna in elektronska oprema) - Samo Evropa

Oznaka na izdelku ali spremljevalni dokumentaciji pomeni, da ga na koncu uporabne dobe ne smemo odstranjevati skupaj z drugimi gospodinjskimi odpadki. Da bi preprečili morebitno tveganje za okolje ali zdravje človeka zaradi nenadzorovanega odstranjevanja odpadkov, izdelek ločite od drugih vrst odpadkov in ga odgovorno reciklirajte ter tako spodbudite trajnostno ponovno uporabo materialnih virov.

Podjetja naj pokličejo dobavitelja in preverijo pogoje nabavne pogodbe. Tega izdelka pri odstranjevanju ne smete mešati z drugimi gospodarskimi odpadki.

Slovakia

Správna likvidácia tohoto výrobku
(Elektrotechnický a elektronický odpad) - Platí len pre Európu

Toto označenie na výrobku alebo v sprievodnej brožúre hovorí, že po skončení jeho životnosti by nemal byť likvidovaný s ostatným odpadom. Prípadnému poškodeniu životného prostredia alebo ľudského zdravia môžete predísť tým, že budete takéto typy výrobkov oddeľovať od ostatného odpadu a vrátite ich na recykláciu.

Priemyselní používatelia by mali kontaktovať svojho dodávateľa a preveriť si podmienky kúpnej zmluvy. Tento výrobok by nemal byť likvidovaný spolu s ostatným priemyselným odpadom.

Portugal

Eliminação Correcta Deste Produto
(Resíduo de Equipamentos Eléctricos e Electrónicos) - Apenas na Europa

Esta marca, apresentada no produto ou na sua literatura indica que ele não deverá ser eliminado juntamente com os resíduos domésticos indiferenciados no final do seu período de vida útil. Para impedir danos ao ambiente e à saúde humana causados pela eliminação incontrolada de resíduos deverá separar este equipamento de outros tipos de resíduos e reciclá-lo de forma responsável, para promover uma reutilização sustentável dos recursos materiais.

Os utilizadores profissionais deverão contactar o seu fornecedor e consultar os termos e condições do contrato de compra. Este produto não deverá ser misturado com outros resíduos comerciais para eliminação.

Poland

Prawidłowe usuwanie produktu
(zużyty sprzęt elektryczny i elektroniczny) - Tylko obszar Europy

Oznaczenie umieszczone na produkcie lub w odnoszących się do niego tekstach wskazuje, że produktu po upływie okresu użytkowania nie należy usuwać z innymi odpadami pochodzącymi z gospodarstw domowych. Aby uniknąć szkodliwego wpływu na środowisko naturalne i zdrowie ludzi wskutek niekontrolowanego usuwania odpadów, prosimy o oddzielenie produktu od innego typu odpadów oraz odpowiedzialny recykling w celu promowania ponownego użycia zasobów materialnych jako stałej praktyki.

Użytkownicy w firmach powinni skontaktować się ze swoim dostawcą i sprawdzić warunki umowy zakupu. Produktu nie należy usuwać razem z innymi odpadami komercyjnymi.

Norway

Korrekt avhending av dette produkt
(Avfall elektrisk og elektronisk utstyr) - Kun Europa

Denne merkingen som vises på produktet eller dens dokumentasjon, indikerer at den ikke skal kastes sammen med annet husholdningsavfall ved slutten av sin levetid. For å hindre mulig skade på miljøet eller menneskelig helse fra ukontrollert avfallsavhending, vennligst atskill dette fra andre typer avfall og resirkuler det ansvarlig for å fremme bærekraftig gjenbruk av materielle ressurser.

Forretningsbrukere bør kontakte sin leverandør og undersøke vilkårene i kjøpekontrakten. Dette produktet skal ikke blandes med annet kommersielt avfall som skal kastes.

Netherlands

Correcte verwijdering van dit product
(elektrische & elektronische afvalapparatuur) - Alleen Europa

Dit merkteken op het product of het bijbehorende informatiemateriaal duidt erop dat het niet met ander huishoudelijk afval verwijderd moet worden aan het einde van zijn gebruiksduur. Om mogelijke schade aan het milieu of de menselijke gezondheid door ongecontroleerde afvalverwijdering te voorkomen, moet u dit product van andere soorten afval scheiden en op een verantwoorde manier recyclen, zodat het duurzame hergebruik van materiaalbronnen wordt bevorderd.

Zakelijke gebruikers moeten contact opnemen met hun leverancier en de algemene voorwaarden van de koopovereenkomsten nalezen. Dit product moet niet worden gemengd met ander bedrijfsafval voor verwijdering.

Latvia

Izstrādājuma pareiza likvidēšana
(nolietotas elektriskās un elektroniskās ierīces) - Tikai Eiropā

Uz izstrādājuma vai tam pievienotajās instrukcijās dotais marķējums norāda, ka to nedrīkst likvidēt kopā ar citiem sadzīves atkritumiem pēc tā ekspluatācijas laika. Lai novērstu videi un cilvēku veselībai iespējamo kaitējumu, kas ir saistīts ar nekontrolējamu atkritumu likvidēšanu, tas jānošķir no citiem atkritumiem un jāpārstrādā, lai sekmētu materiālo resursu atbildīgu atkārtotu lietošanu.

Rūpnieciskajiem lietotājiem jāsazinās ar piegādātāju un jāpārbauda pirkuma līguma nosacījumi. Šo izstrādājumu nedrīkst sajaukt ar citiem likvidējamiem rūpnieciskajiem atkritumiem.

Lithuania

Tinkamas produkto atliekų tvarkymas
(atitarnavusi elektros ir elektronikos įranga) - Tik Europai

Šis ženklas, pateikiamas ant produkto ar jo dokumentacijoje, nurodo, kad pasibaigus produkto tarnavimo laikui, jo negalima išmesti kartu su kitomis buitinėmis atliekomis. Kad būtų išvengta galimos nekontroliuojamo atliekų išmetimo žalos aplinkai arba žmonių sveikatai, ir siekiant skatinti aplinką tausojantį antrinių žaliavų panaudojimą, pašom atskirti jį nuo kitų rūšių atliekų ir atiduoti perdirbti.

Verslo vartotojai turėtų kreiptis į savo tiekėją ir peržiūrėti pirkimo sutarties sąlygas. Šis produktas tvarkant atliekas negali būti sumaišytas su kitomis atliekomis.

Italy

Corretto smaltimento del prodotto
(rifiuti elettrici ed elettronici) - Solo Europa

Il marchio riportato sul prodotto o sulla sua documentazione indica che il prodotto non deve essere smaltito con altri rifiuti domestici al termine del ciclo di vita. Per evitare eventuali danni all'ambiente o alla salute causati dall'inopportuno smaltimento dei rifiuti, si invita l'utente a separare questo prodotto da altri tipi di rifiuti e di riciclarlo in maniera responsabile per favorire il riutilizzo sostenibile delle risorse materiali.

Gli utenti aziendali sono invitati a contattare il proprio fornitore e verificare i termini e le condizioni del contratto di acquisto. Questo prodotto non deve essere smaltito unitamente ad altri rifiuti commerciali.

Hungary

A termék megfelel leadása
(Elektromos és elektronikus készülékek hulladékkezelése) - Kizárólag Európa

A terméken vagy a hozzá tartozó dokumentáción szerepl jelzés arra utal, hogy hasznos élettartama végén a terméket nem szabad háztartási hulladékkal együtt kidobni. Annak érdekében, hogy megel zhet legyen a szabálytalan hulladékleadás által okozott környezet- és egészségkárosodás, különítse ezt el a többi hulladéktól, és felel sségteljesen gondoskodjon a hulladék leadásáról, a hulladékanyagok fenntartható szint újrafelhasználása céljából.

Az üzleti felhasználók lépjenek kapcsolatba a forgalmazóval, és vizsgálják meg az adásvételi szerz dés feltételeit. A terméket nem szabad leadni kereskedelmi forgalomból származó egyéb hulladékkal együtt.

France

Comment éliminer ce produit
(déchets d'équipements électriques et électroniques) - Europe uniquement

Ce symbole sur le produit ou sa documentation indique qu'il ne doit pas être éliminé en fin de vie avec les autres déchets ménagers. L'élimination incontrôlée des déchets pouvant porter préjudice à l'environnement ou à la santé humaine, veuillez le séparer des autres types de déchets et le recycler de façon responsable. Vous favoriserez ainsi la réutilisation durable des ressources matérielles.

Les entreprises sont invitées à contacter leurs fournisseurs et à consulter les conditions de leur contrat de vente. Ce produit ne doit pas être éliminé avec les autres déchets commerciaux.

Finland

Tämän tuotteen turvallinen hävittäminen
(elektroniikka ja sähkölaitteet) - Vain Eurooppa

Oheinen merkintä tuotteessa tai tuotteen oheismateriaalissa merkitsee, että tätä tuotetta ei tule hävittää kotitalousjätteen mukana sen elinkaaren päätyttyä. Hallitsemattomasta jätteenkäsittelystä ympäristölle ja kanssaihmisten terveydelle aiheutuvien vahinkojen välttämiseksi tuote tulee käsitellä muista jätteistä erillään. Jäte on hyvä kierrättää raaka-aineiksi kestävän ympäristökehityksen takia.

Yrityskäyttäjien tulisi ottaa yhteyttä tavarantoimittajaan ja selvittää hankintasopimuksen ehdot. Tätä tuotetta ei tule hävittää muun kaupallisen jätteen seassa.

Estonia

Õige viis toote kasutusest kõrvaldamiseks
(elektriliste ja elektrooniliste seadmete jäätmed) - Ainult Euroopa

Selline tähistus tootel või selle dokumentidel näitab, et toodet ei tohi kasutusaja lõppemisel kõrvaldada koos muude olmejäätmetega. Selleks, et vältida jäätmete kontrollimatu kõrvaldamisega seotud võimaliku kahju tekitamist keskkonnale või inimeste tervisele ning edendada materiaalsete vahendite säästvat taaskasutust, eraldage toode muudest jäätmetest ja suunake taasringlusse.

Firmad peaksid võtma ühendust tarnijaga ning kontrollima ostulepingu tingimusi ja sätteid. Toodet ei tohi panna muude hävitamiseks mõeldud kaubandusjäätmete hulka.

Spain

Eliminación correcta de este producto
(material eléctrico y electrónico de descarte) - Europa solamente

La presencia de esta marca en el producto o en el material informativo que lo acompaña, indica que al finalizar su vida útil no deberá eliminarse junto con otros residuos domésticos. Para evitar los posibles daños al medio ambiente o a la salud humana que representa la eliminación incontrolada de residuos, separe este producto de otros tipos de residuos y recíclelo correctamente para promover la reutilización sostenible de recursos materiales.

Los usuarios comerciales pueden contactar con su proveedor y consultar las condiciones del contrato de compra. Este producto no debe eliminarse mezclado con otros residuos comerciales.

Greece

Σωστή Διάθεση αυτού του Προϊόντος
(Απορρίμματα Ηλεκτρικού & Ηλεκτρονικού Εξοπλισμού) - Μόνον για την Ευρώπη

Τα σήματα που εμφανίζονται επάνω στο προϊόν ή στα εγχειρίδια που το συνοδεύουν, υποδεικνύουν ότι δεν θα πρέπει να ρίπτεται μαζί με τα υπόλοιπα οικιακά απορρίμματα μετά το τέλος του κύκλου ζωής του. Προκειμένου να αποφευχθούν ενδεχόμενες βλαβερές συνέπειες στο περιβάλλον ή την υγεία εξαιτίας της ανεξέλεγκτης διάθεσης απορριμμάτων, σας παρακαλούμε να το διαχωρίσετε από άλλους τύπους απορριμμάτων και να το ανακυκλώσετε, ώστε να βοηθήσετε στην βιώσιμη επαναχρησιμοποίηση των υλικών πόρων.

Οι επιχειρήσεις-χρήστες θα πρέπει να έλθουν σε επαφή με τον προμηθευτή τους και να ελέγξουν τους όρους και τις προϋποθέσεις του συμβολαίου πώλησης. Το προϊόν αυτό δεν θα πρέπει να αναμιγνύεται με άλλα συνηθισμένα απορρίμματα προς διάθεση.

Germany

Korrekte Entsorgung dieses Produkts
(Elektromüll) - Nur Europa

Anzuwenden in den Ländern der Europäischen Union und anderen europäischen Ländern mit einem separaten Sammelsystem) Die Kennzeichnung auf dem Produkt bzw. auf der dazugehörigen Literatur gibt an, dass es nach seiner Lebensdauer nicht zusammen mit dem normalen Haushaltsmüll entsorgt werden darf. Entsorgen Sie dieses Gerät bitte getrennt von anderen Abfällen, um der Umwelt bzw. der menschlichen Gesundheit nicht durch unkontrollierte Müllbeseitigung zu schaden. Recyceln Sie das Gerät, um die nachhaltige Wiederverwertung von stofflichen Ressourcen zu fördern.

Gewerbliche Nutzer sollten sich an Ihren Lieferanten wenden und die Bedingungen des Verkaufsvertrags konsultieren. Dieses Produkt darf nicht zusammen mit anderem Gewerbemüll entsorgt werden.

Denmark

Korrekt affaldsbortskaffelse af dette produkt
(elektrisk & elektronisk udstyr) - Kun Europa

Mærket på dette produkt eller i den medfølgende dokumentation betyder, at produktet ikke må bortskaffes sammen med almindeligt husholdningsaffald efter endt levetid. For at undgå skadelige miljø- eller sundhedspåvirkninger på grund af ukontrolleret affaldsbortskaffelse skal dette produkt bortskaffes særskilt fra andet affald og indleveres behørigt til fremme for bæredygtig materialegenvinding.

Erhvervsbrugere bedes kontakte leverandøren og læse betingelserne og vilkårene i købekontrakten. Dette produkt bør ikke bortskaffes sammen med andet erhvervsaffald.

Czecho-slovakia

Správná likvidace tohoto produktu
(Znièení elektrického a elektronického zaøízení) - Pouze Evropa

Tato značka zobrazená na produktu nebo v dokumentaci znamená, že by neměl být používán s jinými domácími zařízeními po skončení svého funkčního období. Aby se zabránilo možnému znečištění životního prostředí nebo zranění člověka díky nekontrolovanému zničení, oddělte je prosíme od dalších typů odpadů a recyklujte je zodpovědně k podpoře opětovného využití hmotných zdrojů.

Obchodníci by měli kontaktovat své dodavatele a zkontrolovat všechny podmínky koupě. Tento výrobek by se neměl míchat s jinými komerčními produkty, určenými k likvidaci.

The information contained herein is to the best of our knowledge accurate and complete. However sperm cells and sperm cell environments may vary in property. Therefore systematic and/or random deviation between estimates obtained by the NucleoCounter SP-100 and other cell counting methods may occur. As such, nothing contained or stated herein including results obtained from use of the NucleoCounter SP-100 or SP1-Cassette shall be construed to imply any warranty or guarantee. ChemoMetec A/S and affiliated companies shall not be held liable for damages and customers shall indemnify ChemoMetec A/S and affiliated companies against liability flowing from use of potentially inaccurate data generated by the NucleoCounter SP-100. It is recommended that all results obtained with the NucleoCounter SP-100 are validated against appropriate reference methods and/or traditional laboratory methods at regular intervals.