

Viability Testing
with
NucleoCounter[®] SP-100[™]

Addendum to User's Guide

Revision 1.4



Technology that counts



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1 Introduction and intended use

This manual is an addendum to the User's Guide for the NucleoCounter SP-100. In connection with viability testing of semen samples from mammalian animals, this addendum shall always be used together with the User's Guide for the NucleoCounter SP-100.

IMPORTANT!

If firmware version is 1.23 or below:

The factory settings of the NucleoCounter SP-100 that are being used for viability testing vary from species to species.

These settings cannot be changed by the operator.

Contact ChemoMetec with respect to which types of animal semen that can be tested using the viability set-up.

If firmware version is 1.24 – 1.30:

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 preset factory settings are being used.

Contact ChemoMetec for more information about changing to the correct Non-Viable settings.

If firmware version is 1.31 or higher:

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 the Non-Viable Count.

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

1.1 Basics of the Viability test

This section describes the basic principles of the NucleoCounter SP-100 viability test.

1.1.1 Principles of the Viability Test

The NucleoCounter SP-100 viability test comprises two separate tests:

- 1) A Total Count (TOTAL), and
- 2) A Non-Viable Count (NON-VIABLE)

The TOTAL sperm count analysis is in essential identical to a “normal” sperm density analysis as described in the User’s Guide for the NucleoCounter SP-100 and in the Application Notes describing the determination of sperm density in semen samples. The only change is that the analysis is initiated by pressing the “RUN” button and then the “1”-key instead of only pressing the “RUN” button.

The NON-VIABLE sperm count differs from TOTAL in several aspects:

1. First of all, the semen sample is **not** diluted in Reagent S100. The Reagent S100 is designed to break the cell membranes of the sperm cells, and thereby dye and detect all the cells, also the healthy ones. In a non-viable count only the dead cells are to be detected. Thus, the dilution medium does not contain any detergent or any other substance, which may affect the membrane integrity. A phosphate buffered saline or a semen extender normally used for production of semen doses can be applied for the NON-Viable analysis. In the NON-Viable analysis only dead sperm cells with a damaged cell membrane will be colored by the fluorescent dye, propidium iodide. This dye is immobilized in the SP1-Cassette.

The dilution medium may have a minor affect on the Non-Viable Count. It has been observed that the Non-Viable Counts can be somewhat higher for PBS compared to many extenders. Therefore, it is important always to use the same dilution medium in order to be able to compare Non-Viable Counts.

2. Secondly, the dilution factor for the NON-VIABLE test, designated as DF2, may also differ from the dilution factor used for the TOTAL test, DF1. In general, DF2 is equal to or lower than DF1. The reason for this is that the number of dead sperm cells usually is much lower (and never higher) than the total count. Thus, in order to obtain a satisfactory counting statistics it is often necessary to reduce the dilution of the semen sample when performing a NON-VIABLE test compared to a TOTAL test. However, it is recommended that DF2 is at least 50% of the optimal DF1 (if DF1 is 201 then DF2 should be at least 101). Most often customers use identical dilutions factors for the Total Count and the Non-Viable Count.
3. Finally, the actual analysis on the NucleoCounter SP-100 is also different for Non-Viable Count compared to the Total Count. One major difference is the analysis time, which is approximately 50 seconds longer than the TOTAL test. In this period of time the cassette

is analyzed intensively and a Non-Viable Count is generated based on a complex algorithm. This analysis and algorithm is not common for all types of semen. Thus, the parameters of the analysis and the algorithm must be especially chosen for each animal species. The operator cannot key in these parameters via the keyboard/display, and therefore, the instrument must be correctly set-up from the factory. If firmware 1.31 or higher is installed in the instrument the correct viability settings is automatically chosen when the species is chosen (F11 function).

1.1.2 Calculation of the Viability

The viability in percent is calculated from TOTAL count and the NON-VIABLE count as follows:

$$\text{Viability (\%)} = 100\% * (\text{TOTAL} - \text{NON-VIABLE}) / \text{TOTAL}$$

If the NucleoCounter SP-100 is connected to a PC with SemenView installed the viability can be calculated automatically (refer to User Guide for SemenView ver. 1.2 or later).

1.1.3 Precision

The precision of the Total Count (typical <4%) is considerable better than of the Non-Viable Count (typical <8%). This is both due to improved poisson statistics for the Total Count and to improved fluorescence signals from sperm cells that are treated with Reagent S100.

Attention! When testing boar semen the estimated viability is typically in the range from 80-90%. Even if semen samples are stored for several days the estimated viabilities are frequently not significantly reduced. Moreover, the viabilities are often not well correlated to microscopical motility analysis. The reason for this can be that boar sperm cells lose their ability to move much faster than they die.

2 Operation of NucleoCounter SP-100 in Viability Mode

In this section the operation of the NucleoCounter SP-100 in the viability mode is described. Only the additional features and the changes compared to the “normal” set-up are described. The “normal” set-up is defined as set-up of a NucleoCounter SP-100 that only can give information of the total number of sperm cells in a sample and the operation of such an instrument is described in the *User's Guide* for the NucleoCounter SP-100.

2.1 Choosing the Viability Mode (F31)

The viability of a sperm sample can only be estimated when the Viability mode is “On”. With the command “F31+↵” the Viability mode can be chosen, but only if the viability option is included in NucleoCounter SP-100.

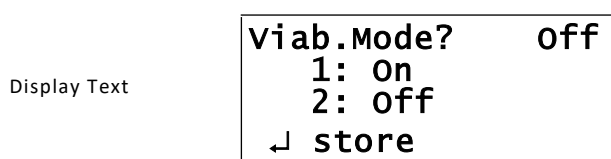


Figure 1. The viability mode can be selected (press “1”) or dropped (press “2”).

The buttons “1” and “2” toggle between the “On” and “Off” mode. The text string in the upper right corner will be updated with the *On* or *Off* text string.

Press “↵” to confirm the chosen setting or press “Esc” to jump back to “Ready” status without doing any changes.

When the Viability mode is set to “Off” the instrument works as described in the *User's Guide* for the NucleoCounter SP-100 and can only be used for counting of the total number of sperm cells in a semen sample.

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

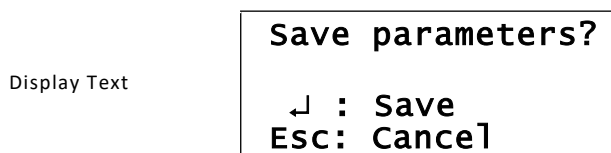


Figure 2. Shall the actual Viability mode be saved and used as the new default mode?

Press “↵” to save or “Esc” if the present default viability mode shall not be overwritten.

2.2 Dilution Factor 1 (DF1), Dilution Factor 2 (DF2) and Dilution Factor (DF)

When the NucleoCounter SP-100 is in the viability mode the instrument makes use of two different dilution factors, Dilution Factor 1 (DF1) and Dilution Factor (DF2). DF1 corresponds to the DF that is used when the Viability mode is Off. Thus DF1 is always used for the determination of the total number of sperm in a semen sample. The DF2 is the dilution factor that is used for the non-viable count.

F31 is Off:

DF can be changed with the F0 command.

Changes to the DF will also change the DF1 because DF is identical to DF1.

When F31 is Off the DF is presented in the displays.

F31 is On:

Now two dilutions factors exist, DF1 and DF2.

DF1 is as mentioned above identical to the DF, and is used for TOTAL counts. Changes to the DF1 will also change the DF.

DF2 is used for the Non-Viable Counts.

DF1 and DF2 can be changed with the F0 command. When the display shows the Ready-display or the Result-display, the DF1 or DF2 is shown in the lower right corner of the display. If the DF1 is shown, it will also be DF1 that is changed by the F0 command. On the other hand the DF2 will be affected with the F0 command only if DF2 is shown in the Ready-display or the Result-display.

To change a dilution factor, first select the dilution factor to be changed. Use the "Esc" key to toggle between DF1 and DF2.

If the "Result" status display is shown the "Esc" command will apart from toggling between DF1 and DF2 also change from the "Result" status to the "Ready" status.

EXAMPLE - Changing the DF1 from 201 to 1000

To change the DF1 press "F0 + ↵". If the current DF1 is 201 the following will be displayed:

Display Text

```
Dilution factor
DF1=201
Enter new DF1+↵
New DF1=
```

Figure 3. The Dilution Factor can now be typed in

Then type "1000 + ↵" and the DF1 will be changed to 1000:

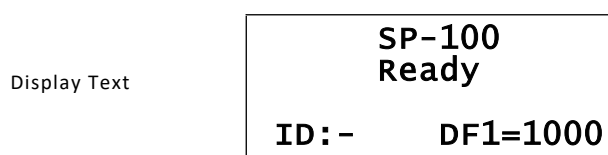


Figure 4. Dilution Factor 1 is set to 1000

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 prior validated. 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 remembered until it is changed again. When shutting down the SP-100 ask if the actual DF shall be saved as the new default value:

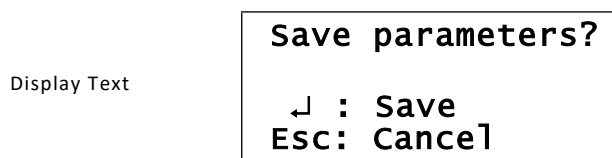


Figure 5. Shall the DF1 value be saved and used as the new default value?

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.

However, it is recommended that DF2 is at least 50% of the optimal DF1 (if DF1 is 201 then DF2 should be at least 101). Most often customers use identical dilutions factors for the Total Count and the Non-Viable Count.

2.3 Changes compared to the “normal” set-up

When the NucleoCounter SP-100 is in the viability mode the display will read this following a successfully start-up:

Display Text	SP-100 Ready
	ID: - DF1=201

Figure 6. The NucleoCounter SP-100 is ready for cell counting. The instrument is in the viability mode and this is indicated by adding the “1” digit to the DF. The Dilution Factor 1 is set to 201.

Display Text	Cells/ml Sample 455.0 mill. T
	ID: - DF1=201

Figure 7. Cell density of the ejaculate is 455.0 millions per ml. DF1 is 201. The “T” indicates that the test was a TOTAL test.

Display Text	Cells/ml Sample 75.10 mill. N
	ID: - DF2=201

Figure 8. Cell density of the ejaculate is 75.10 millions per ml. DF2 is 201. The “N” indicates that the test was a NON-VIABLE test.

2.4 Measuring (“Run” button)

When a cassette has been loaded and inserted into the instrument, the measurement is activated, by pressing the “Run” button followed by either pressing the “1” button or the “2” button.

Display Text	1: Total 2: Non-viable
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Figure 9. After pressing the “RUN” button a TOTAL test or a NON-VIABLE test can be chosen by pressing “1” or “2” respectively.

2.5 Overview of Functions (not all functions are described in this addendum)

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