Evaluation of Stallion semen with the NucleoCounter SP-100.

Make sure that the instrument set-up is for Stallions: Press F11 and enter – if the display does not read Stall. Scroll by pressing 1 or 2 until Stall. appears.

In order to measure % Viability the instrument must be in Viability-mode. Press F31 and select Viability mode On.

By pressing On/Off the instrument will ask if you want to save these parameters. If you press Enter the new parameters will be saved as default parameters and the next time the instrument is turned On it will be with the changed parameters. If you press Esc all changes are "forgotten" next time the instrument is turned on.

Total Cell Count.

The recommended dilution factor (DF) for stallion semen is 101. This DF ensures that the instrument can measure cell counts in the range from 50 mill/ml up to 700 mill/ml and with high accuracy in the range 125-500 mill/ml.

Set the instrument for DF=101 by pressing F0 and type in 101 as new dilution factor. After pressing Enter, the instrument will read DF=101 or DF1=101(if Viability mode is enabled) in the lower right display corner. If Viability mode is enabled the Esc button is used to toggle between DF1 and DF2. Select DF2 in the display and Press F0 again and type in 101 as new DF. Now you will use a dilution factor of 101 for both the Total Cell count and the non-Viable cell count.

Pipet 50 ul of semen into an appropriate sample container. Adjust the dispenser on the Reagent S100 bottle to 5,0 ml and dispense 5,0 ml of Reagent S100 into the sample cup. Make sure that it is well mixed. Immerse the tip of the SP1 cassette in the sample mixture and press down the white piston on the cassette to level the handle of the cassette. Sample mixture is now aspired into the cassette.

Insert the cassette in the slit of the instrument, press RUN (and then press 1 if Viability mode is enabled) and wait 30 seconds for the Total Cell count. When ready it will be displayed on the instrument – and the result is transferred to the SemenView software (if used).

Non-Viable Cell Count.

The procedure for measurement of non-viable cells is exactly the same as for Total Cell count – except the Reagent S100 is NOT used. Instead the sample is diluted in PBS or an extender. If using an extender it MUST be clear. Milky extenders like INRA 96 cannot be used for the dilution due to the opacity of the extender!

However, semen samples extended with INRA 96 or alike can easily be measured since the sample is diluted 100 times with a CLEAR PBS solution.

Pipet 50 ul of semen into an appropriate sample container. Next, pipet 5,0 ml of PBS into the sample cup. Make sure that it is well mixed. Immerse the tip of the SP1 cassette in the sample mixture and press down the white piston on the cassette to level the handle of the cassette. Sample mixture is now aspired into the cassette.

Insert the cassette in the slit of the instrument, press RUN, then press 2 and wait 80 seconds for the non-Viable Cell count. When ready it will be displayed on the instrument – and the result is transferred to the SemenView software (if used). The non-Viable cell count takes longer than the Total Cell count – approx. 80 seconds instead of 30 seconds.

You now have two cell counts – the Total Cell count (T) and the non-Viable cell count (n). The viability can now be calculated using the formula:

$$\frac{T-n}{T} * 100 = \%\ \text{Viability}$$

The SemenView software will calculate the % Viability automatically. It is important though, that the sequence is as described here (T first and then n), since the SemenView expects this sequence.

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SVP