

INSTRUCTIONS FOR THE USE OF THE STANDARD COUNT ANALYSIS CHAMBER

LOADING THE STANDARD COUNT CHAMBER

Using a positive displacement pipet, place approximately 3 to 5 ul of sample into one of the two clear zones labeled SAMPLE A and SAMPLE B.

Allow a few seconds for the sample to completely load into the analysis area, then remove any excess fluid remaining in the loading zone.

Perform the analysis near the center of a large open area of the Standard Count Chamber.

AUTOMATED ANALYSIS

The Standard Count is compatible with all CASA systems. Because there is no counting grid etched into the chamber, you may select fields for analysis from any part of the chamber.

1. Set up your automated analysis system to accept the chamber depth that corresponds to the Standard Count (20 microns). You may want to contact the original equipment manufacturer for specific information regarding instrument adjustment.
2. Select 4 or more fields for analysis from the central area of the Standard Count Chamber. The accuracy of your analysis will be proportional to the total number of sperm counted. This is true for motility as well as concentration evaluations.
3. Proceed with the analysis according to your laboratory's procedures.

MANUAL ANALYSIS

NOTE: If you are currently using a disposable counting chamber, and your microscope has already been calibrated, there is no need to recalibrate. You may use the calibration factors (F) previously established for your microscope to determine sperm concentration (C) using the formula shown below ($C = N \times F$).

A good quality laboratory microscope is recommended for semen analysis. Phase contrast optics with an objective magnification of 10X to 40X are preferred for an easy visualization of the sperm cells. The Standard Count contains no counting grid, so it is necessary to use an eyepiece reticle to define the area being counted. This is best accomplished with a 10 X 10 net pattern that projects 100 boxes over the viewing field. Mid-Atlantic Diagnostics, Inc. can provide a reticle compatible with virtually all major brands of microscopes.

CALCULATE THE SPERM CONCENTRATION by using the following formula: $C = N \times F$

C = sperm concentration

N = average number of sperm per box

F = calibration factor

If the concentration permits, we recommend counting at least 100-200 sperm from a field selected in the center of the Standard Count chamber.

1. **Calculate (N)** by dividing the total number of sperm counted by the number of boxes counted. Obviously, you must keep track of the number of boxes counted in order to perform this calculation.

$$N = \frac{\text{\# of sperm}}{\text{\# of boxes}}$$

2. **The factor (F)** is a calibration factor designed to compensate for the optical variation that is experienced from microscope to microscope, even those of the same model and manufacturer. Once a specific microscope is calibrated and the factor F is derived, you can use that value F for all samples analyzed with the same magnification on that specific microscope.

$$F = \frac{1,000,000}{T \times D^2}$$

Where F = the calibration factor determined for each microscope, magnification and Standard Count chamber depth.

T = the chamber depth (in microns). For the Standard Count, you would use the number 20.

D = the distance across a single box of the reticle (in microns)

3. **Calculate (D)** by using a Stage Micrometer. To achieve this:
 - 1) Fit the reticle into the microscope eyepiece, insuring that it is firmly in place and parallel to the optical plane.
 - 2) Place the stage micrometer on the microscope stage.
 - 3) Line up the stage micrometer such that one of the larger lines is imposed upon the right edge of the reticle matrix. The divisions on the stage micrometer are 100um (distance between the large lines), 50um (distance between the secondary lines) and 10um (distance between the smallest lines).
 - 4) Measure the distance across all 10 boxes of the reticle.
 - 5) To calculate the distance across a single box in the reticle matrix, divide the distance obtained in step #4 by 10.
4. **Incorporate the value (D)** into the formula and derive the factor F. Remember that the value D is squared in the formula to account for width x length. Assuming that the value of D is 25 microns and that you are using a Standard Count with a 20 micron chamber depth, the factor F would then be equal to:

$$F = \frac{1,000,000}{20 \times 25^2}; F = 80$$

DETERMINING PERCENT MOTILITY

Count only the motile sperm in the boxes and record that number.

Recount the same boxes and this time count only the non-motile sperm.

Add the motile and non-motile sperm to obtain the total number of sperm counted

Calculate percent motility as follows:

$$\% \text{ Motility} = \frac{\text{\# of motile sperm}}{\text{total \# sperm counted}}$$



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