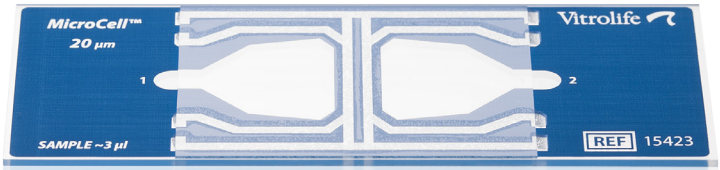
MicroCell
User manual



Vitrolife

CE

IVD — In Vitro Diagnostics

Do not re-use, discard after procedure

Caution: Consult accompanying documents

REF — Catalog number

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EXPIRY

0°C +32°C — Store at room temperature

For professional use

Non sterile

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MicroCell

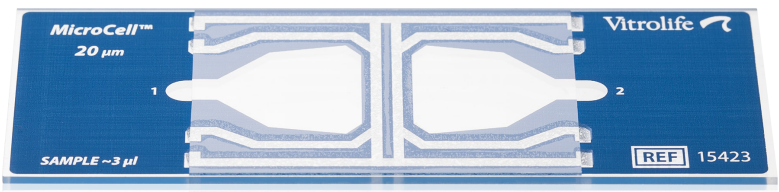
The MicroCell is manufactured within strict tolerances resulting in a counting chamber of uniform and fixed depth. Because of this feature, when the MicroCell is used as part of an automated and/or manual semen analysis protocol, it provides accurate and precise sperm concentration and motility data.

Important Characteristics

Each MicroCell contains two or four independent chambers. It is recommended that they be used for duplicate sampling.

The coverslip of the MicroCell is 0.5mm thick. It is important that the optical properties of the system used for analysis is compatible with a 0.5mm coverslip.

Please read this instruction manual completely prior to using the MicroCell slide.



Neat Sample Preparation

Allow the semen sample to completely liquefy (usually about 30 minutes at room temperature) and thoroughly mix the sample according to the procedure established in your laboratory.

Post Washed Samples

The removal of seminal debris is one of the primary objectives of sperm washing techniques. Unfortunately, these techniques also remove the seminal protein which serves to protect and lubricate the sperm. It is well known and documented that sperm has a tendency of adhering to clean glass if removed from its protein based environment. The incidence of cell adherence varies depending on the wash procedure that is used. The majority of MicroCell users report that they do not have a problem with cell adherence and in all probability this will be your experience as well.

If you do see a significant increase in cell adherence, the addition of protein to the post wash sample (typically 0.3 % HSA) has proven to be very effective in reducing the incidence of the phenomenon.

Loading the MicroCell

1. Using a positive displacement pipette, place approximately 2-5 microliters of the sample to be evaluated (volume depends on which slide is being used) into the clear loading zones. (See Fig 1).

   Approximate fill volume by slide
   a. REF 15423 = 3-5 µL
   b. REF 15424 = 2-3 µL

2. Allow a few seconds for the sample to load into the analysis area, remove excess fluid remaining in the loading zone. **The loaded sample should remain stable for 30 minutes at room temperature.**

3. Perform the analysis in the center of the viewing field of the MicroCell.

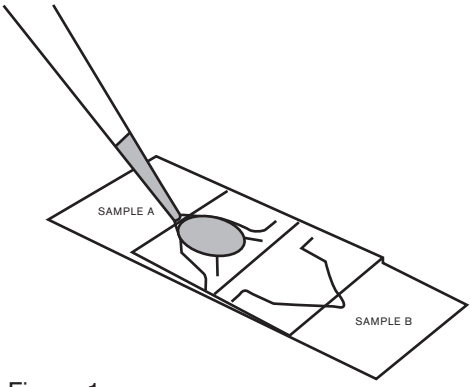


Figure 1.

Semen Analysis

Automated Analysis

The MicroCell is compatible with all Computer Assisted Semen Analysis Systems (CASA).

1. Set up the CASA system to accept the appropriate chamber depth. If necessary, contact the original equipment manufacturer for specific information regarding instrument adjustment.

2. Select 4 or more fields for analysis from the center of the viewing field of the MicroCell chamber. The accuracy of the analysis will be proportional to the total number of sperm counted. This is true for motion parameters as well as concentration; ensure adequate number of motile sperm have been analyzed to provide accurate motility values.

3. Proceed with the analysis according to the instructions provided by the manufacturer of the CASA instrument.

Manual Method

The Microscope

A quality laboratory microscope is recommended for sperm concentration and motility analysis. Phase contrast optics with an objective magnification of 10X to 40X is preferred for easy visualization of the sperm cells. The MicroCell contains no counting grid, so it is necessary to use an eye piece reticle in the microscope to identify the area to be counted. This is best accomplished with a 10 X 10 net pattern that projects 100 boxes over the viewing field. Vitrolife can provide a net reticle for virtually all major brands of microscope. Vitrolife can also provide the stage micrometer for calibration.

Calibration for Manual Analysis

Calculating the F factor

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. The calculation must be performed for each microscope and magnification used.

Once a specific microscope is calibrated and the factor F is derived, use that value F for all samples analyzed with the same magnification on that specific microscope.

The formula for the calculation of the F factor is:

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

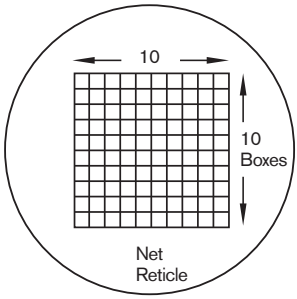
F= The factor determined for each microscope and magnification.

T= The chamber depth (microns, µm). For the MicroCell, T = 20

D= The distance across a single box of the reticle (microns, µm).

How to Calculate the D (distance) by using the Stage Micrometer.

1. Install the reticle in the microscope eyepiece. Ensure the reticle is firmly in place and parallel to the optical plane. Your view through the eyepiece should be as in Figure 2.



VIEW THROUGH EYEPIECE

Figure 2.

2. Place the stage micrometer on the microscope stage. Line up the stage micrometer so that one of the larger lines is to the left edge of the reticle matrix. The distance divisions on the stage micrometer are 100 µm (distance between the large lines), 50 µm (distance between the secondary lines) and 10 µm (distance between the smallest lines).

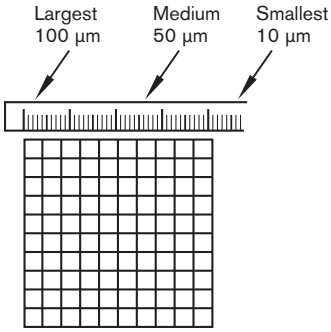


Figure 3.

3. Measure the distance across all 10 boxes of the reticle.

4. To calculate the distance across a single box in the reticle matrix, divide the total distance measured by the number 10. This number is D.

5. Incorporate the value into the formula to calculate F.

   T= 20 for the MicroCell

   D= lenght of one box in the reticle. *Remember that the value D is squared in the formula to calculate the area of the box.*

For example:

D = 25 µm
T = 20 µm

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

If you have any questions regarding the installation of your reticle or use of the stage micrometer, please contact one of our technical support representatives.

Calculating Sperm Concentration

The formula for calculation of sperm concentration is:

C = N x F

Where: C = sperm concentration in millions per mL (10^6 mL^{-1})
N = average number of sperm per box
F = Factor

We recommend counting at least 200 cells and a minimum of 5 fields; if the concentration permits. Select viewing fields for analysis from the center of the MicroCell chamber.

Calculating (N)

To obtain the average number of sperm per box (N), divide the total number of sperm counted by the total number of boxes counted.

$$N = \frac{\text{\# of sperm}}{\text{\# of boxes}} = \text{avg. sperm/box}$$

Determining the Percent Motility

The percent motility of the sample may be obtained using a slight modification of the above procedure.

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

2. Recount the same boxes, this time only counting the non-motile sperm.

3. Add your results from Step 1 and Step 2, which equals the total number of sperm counted.

4. Divide the number in Step 1 (the motile sperm) by the number in Step 3 (total sperm counted) and multiply by 100. This is the percent motility.

$$\% \text{ Motility} = \frac{\text{\# of motile sperm}}{\text{\#of motile + non-motile sperm}} \times 100$$

Important Information

If the magnification is changed, a new F factor must be calculated.
The area delineated by the grid pattern in the eye piece must be calibrated for each magnification used with the MicroCell.

All microscopes must be calibrated separately.
Actual magnification obtained from different microscopes varies, even when identical optics and manufacturers microscopes are used.

If the reticle is replaced, a new F factor must be calculated.

Disposal Instructions
MicroCell loaded with patient specimen should be considered clinical or medical waste. Proper disposal should be according to national or regional regulations per regulated medical waste procedures.

Precautions
MicroCells are intended for single use only and MAY NOT BE REUSED. Reuse may cause contamination and failed procedure.

MicroCells are 100% inspected for defects prior to shipment; however, breakage may occur during transport. Please inspect MicroCell slides before use for broken glass. Do not use broken MicroCells, as this may adversely affect results.

Once MicroCells have been loaded with patient sample they should be considered biohazardous material; therefore universal precaution should be observed to avoid occupational exposure.

Trouble Shooting

Semen Viscosity & Aggregation Problems
If the sample is too viscous to load under laboratory conditions, proceed with a dilution of the sample as specified in your laboratory procedures.

Very Low Sperm Concentration
For very low concentration samples, where it is not possible to count 200 sperm, count all boxes in 5 different fields and divide the total number of sperm counted by 500 to obtain N. It is best to use minimal dilution of the original semen & the lowest magnification to increase the number of sperm in each field.

Remember: Use the correct factor in your calculation after changing these parameters.

High Sperm Concentrations
For very high concentrations, you may want to dilute the sample or use a higher microscope magnification. Use a dilution of 1:5 to 1:20, depending on the sperm concentration. Mix the diluted sample thoroughly.

Remember: Use the correct factor in your calculations after changing these parameters.

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