# MAKLER® COUNTING CHAMBER


SEFI MEDICAL INSTRUMENTS LTD.

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## INSTRUCTIONS FOR USE

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## DESCRIPTION:

The Makler counting chamber is a simple-to-use device for rapid and accurate sperm count, motility and morphology evaluation, from undiluted specimen.

The Chamber is composed of two parts: (Fig. 1).

1. The lower main part has a metal base (A) and two handles (H). In the center of the base there is a flat disc (D) made of optical flat glass on which the sample (S) is placed. Around the disc there are four pins (P). Their tips are 10 microns above the surface level of the disc.
2. The upper part is the cover glass (C) encircled with a metal ring. At the center of its lower surface there is a $1mm^2$ grid, subdivided into 100 squares, each one of 0.1 x 0.1mm. When the cover glass is placed on the four tips, the space bounded in a row of 10 squares is exactly one millionth of mL. Therefore, the number of sperm heads in 10 squares indicates their concentration in million/mL.

## ACCESSORIES:

**1.** Cleaning brush.
2. Lint free lens paper .
3. Chamber Grip - this device should be placed on the stage of the microscope during sperm analysis. It grips the chamber tightly and enables smooth shifting

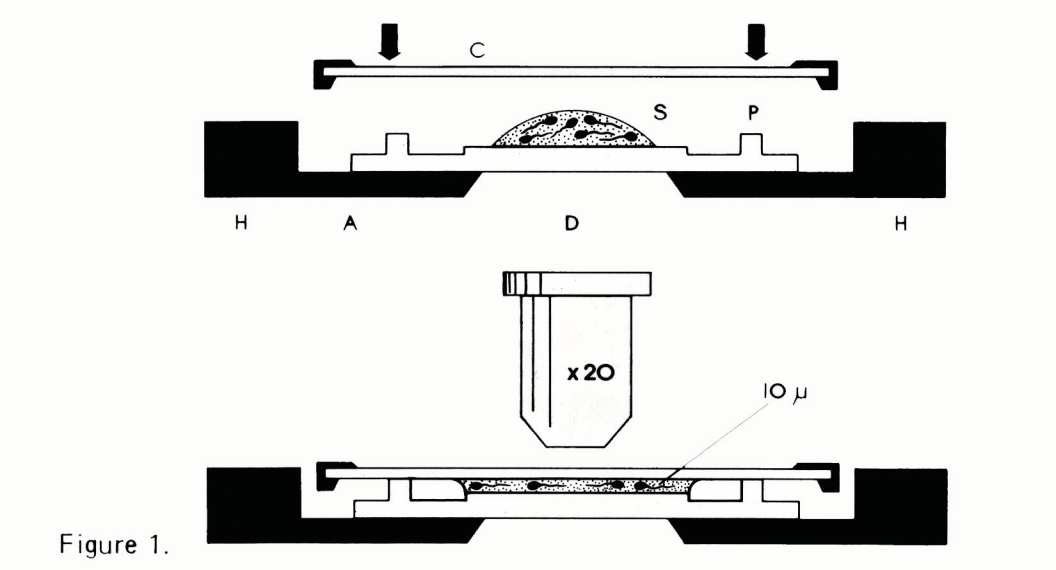


Figure 1.

of the Chamber on the stage.
When the sperm analysis is completed, hold the grip and slide the Chamber out.
Slide the Chamber again into the grip for a new sperm analysis.

**PREPARATION OF THE CHAMBER:**
Before placing the sample on the disc, make certain that the opposed surfaces are absolutely clean and free of dust, since the size of most particles is larger than the very thin space between the glasses. For this purpose, use the lens paper for wiping both surfaces.
The cleanliness can be tested by placing the cover glass on the four tips and looking for color fringes at the four contact points (Newton's phenomenon). They can be best seen against fluorescent light.

**METHOD OF PERFORMING SEMEN ANALYSIS:**
Mix the specimen well, taking care to avoid formation of bubbles. With the aid of a wooden rod or a pipette, place a small drop in the center of the disc area. Grasp the cover glass with your fingers opposite the black dots and immediately place the cover glass on the four pins. Press gently, looking again for the appearance of the color fringes. The drop will spread on the entire area of the disc into a thickness of 10 microns.


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Some surplus does not interfere with proper analysis as long as the tips are not flooded.
Once the cover glass is in place, avoid touching, lifting and covering again, as this may change the uniform spread of sperm within the Chamber.
Lift the Chamber by its handles and place it on the stage of the microscope.
You may use the Chamber Grip to fit it properly.

**IMPORTANT**

Never use a x40 objective with this Chamber. The cover glass may be damaged while trying to focus . Even when using the proper x20 objective, take care not to press on the cover glass. The image is usually seen clearly when the tip of the objective is about 1mm. above the surface. We are not responsible for any damage to the cover glass, resulting from improper use of the microscope.

It is recommended to use a x20 objective and x10 eyepiece with this Chamber.
A x10 objective is not recommended because sperm will be seen too small, unless x20 eyepiece is used. A x40 objective can not be used due to the thickness of the cover glass.

**SPERM COUNT:**
If sperm are too dense and vivid, they should be immobilized first. This is easily done by transferring a part of the specimen into another test tube. The test tube is then inserted into 50$^{O}$C- 60$^{O}$C hot tap water, for about 5 min. A drop from a well-mixed, preheated specimen is placed on the Chamber and covered with the cover glass. The sperm heads within the squares of the grid are counted in the same way blood cell are counted in hemocytometer (Fig. 3).

In the event the number of sperm is substantial, count their number in a strip of 10 squares. This number represents their concentration in millions per mL.
Repeat this count in another strip or two, to determine the average. Alternatively or optionally, it is recommended that the count be made from 2 or 3 other drops of the specimen to increase the reliability of count determination. In the case of oligospermic specimen, it is suggested to count sperm in the entire grid area.
Five zeros are then added to the number counted and the result is the concentration in millions per mL.

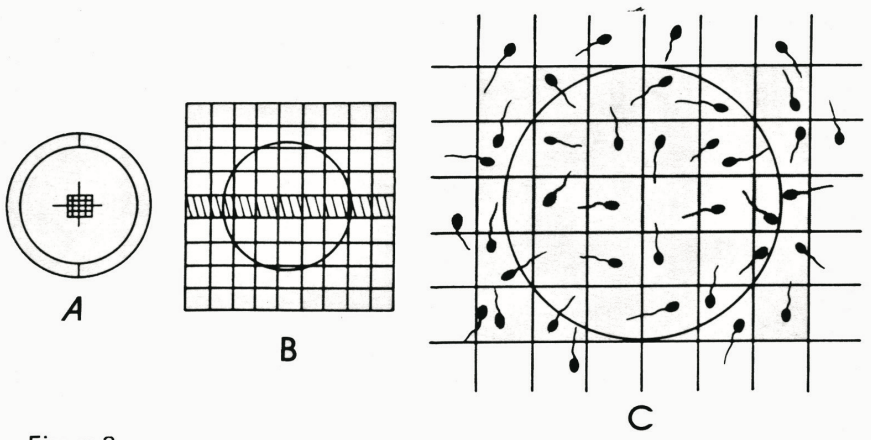
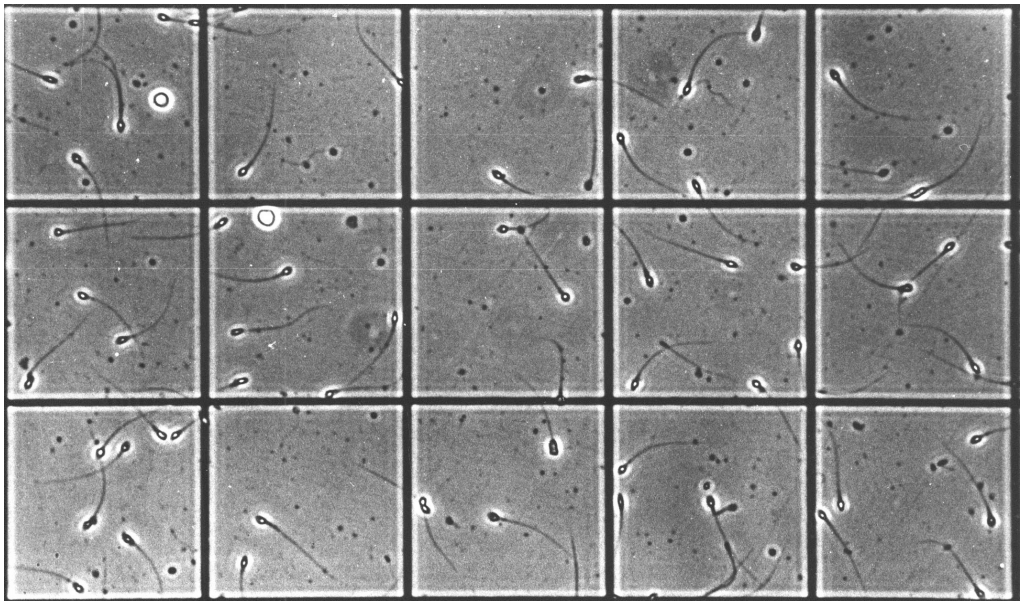



Figure 3.



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After the sperm are brought into focus, move the stage of the microscope and locate the grid in the center of the view area. Then, adjust the Chamber so that the grid lines will appear in vertical and horizontal position.
During this search you will have the opportunity to observe the followings:

a) Are the sperm spread uniformly? If not, the sample was not mixed well enough.
b) Are all sperm seen in one focal plane without blurring? If not, perhaps the surfaces were not clean and large particles have intervened between the two surfaces of the Chamber.
In either case, repeat this brief procedure from the beginning.

**MOTILITY EVALUATION:**

It is suggested to perform motility evaluation within 3-5 minutes after application of the sample to avoid errors due to tendency of sperm to migrate from the periphery. Count all non motile sperm within 9 or 16 squares. Then count the motile sperm in the same area and estimate the grade of motility from +1 to +4. Repeat this procedure in another area of the grid, as well as from another 3 to 4 drops and calculate the average.
This estimation is much more accurate than that performed from ordinary slide where sperm may be compressed by the cover slip and their movement impaired. Makler Counting Chamber provides standard conditions for all analyzed specimen where sperm can move freely in a frictionless horizontal plane.

**MORPHOLOGY**

Rapid evaluation of sperm morphology can be performed out of a wet unstained sample containing immobilized sperm. A phase contrast microscope is preferred for this purpose. Count all normal and abnormal sperm in a certain area of the grid and repeat this procedure from other samples to make a total count of 200.

Obviously, in cases of oligospermic specimen the number of scanned sperm can be lower.

The Chamber is not suitable for morphology determination from stained dried sample.

**SPECIAL CASES:**

**Bubbles:** If bubbles appear in the grid area, it is recommended that the drop be replaced by another one, unless the bubbles are too small to interfere with the analysis.

Large particles of dust, threads, etc., can also interfere with the count by changing the depth of the space and the drop should be replaced.

Major variations in counts between drops of the same specimen occur when samples were not mixed well, in cases of high viscosity, or when the surface area of the Chamber was not clean of particles or dust.

**Clumping**: sometimes preheated sperm will clump within the Chamber if too much time elapses until they are counted. In this case, replace the drop with a properly mixed new specimen. In a few cases, agglutinated clumps may be present within the grid area, and in this case the drop should be replaced.

**Crystals:** Specimens that are left for an extended period may contain crystals which may not interfere with the count, but make it more difficult.
Sometimes, these crystals are too large and may damage the surface area. Therefore, special care should be taken when samples containing crystals are analyzed.

**CLEANING AND PREPARATION FOR REUSE:**
Do not rinse or soak the Chamber in tap water. Dip the brush into water or into noncorrosive antiseptic solution and simply wipe both sides of the glasses. Then, squeeze the brush and sponge off the remaining water. Finally dry the surface with the lint free lens paper.
Avoid touching the tips of the pins as much as possible.
The Chamber is now ready for reuse.
In general there is no need to change the focus once it has been fixed for the examination.
Simply slide the Chamber in or out of the Chamber-grip without raising the objective.

**References:**


1. Makler A. The improved 10 mic. Chamber for rapid sperm count and motility evaluation. Fertil Steril 33:337-338, 1980.

2. Ludwig G. and Frick J. (eds) Spermatology - Atlas and Manual, Springer-Verlog. Berlin, Heidelberg, New York etc. 1990.

3. Anne M. Jequier: Male Infertility, a Guide for the Clinician. Blackwell Science Ltd. Oxford U.K. 2000 p. 9-58.

4. Makler A. Human Seminology. Chapter 7: 115 -130, In: Biotechnology of Human Reproduction, Editors: A. Ravelli, I. Tur-Kaspa, JG Holte, M. Massobrio, Parthenon Publishing Group, 2003.


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