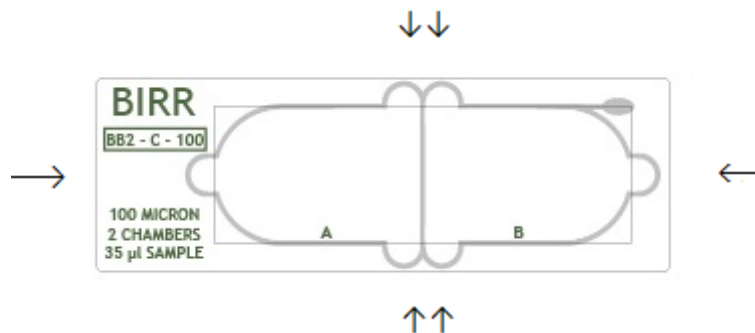


Disposable counting chamber BB2-C-100**Manual****Introduction**

The BIRR disposable counting chamber BB2-C-100 is designed for the microscopic assessment of low concentration of semen and other cell suspension, or for the analysis of semen of animals with large sperm like mouse and rat. BB2-C-100 slide has two chambers for two assessments.

The user is responsible for the methods of disposal. If they are used for the assessment of human semen or for the assessment of infectious material the used slides need to be treated as contaminated waste. Follow the local rules how to treat contaminated waste.

Description of the counting chamber

→ Points to filling area chamber A; ← Points to filling area chamber B; ↓↓ and ↑↑ air outlets.

The chamber is made of a standard microscopic slide and a cover slip. The dimensions of the microscopic slide are $75.0 (\pm 0.2) \times 25.0 (\pm 0.2) \times 0.7 (\pm 0.1)$ mm. The dimensions of the cover glass are $55.0 (\pm 0.2) \times 18.0 (\pm 0.2) \times 0.7 (\pm 0.1)$ mm. Variation in the dimensions of the glass plates do not affect the functionality.

The glass slides are cleaned and coated. With a robot writer a pattern of white resin with spacers is made on the microscopic slide. With a robot arm the cover slip is placed on this pattern, softly compressed and the resin is cured with a light flash.

During the production process the chamber height of each slide is checked.

The absence of toxicity is verified with the help of a survival test with swine semen. Swine semen is very sensitive to toxic substances.

The actual chamber height and the non-toxicity of the slides is depicted in the quality sheet of a specific batch.

Counting chambers with a chamber height of 100 microns are used for specialist applications only. Specialists of BIRR are willing to assist with the development with the user specific manuals for the local application.

If the chambers are used for the assessment of low cell numbers it is advised to stain the cells with fluorescent dyes and to use a fluorescence microscope

For the assessment of low cell numbers for clinical purposes, like check after vasectomy, it is advised to use the BB2-V-20 slides.

Disposable counting chamber BB2-C-100

NB. Calibrate your microscope before initial use.

If you use the BIRR slides in combination with a CASA system (computer aided semen analysis): follow the instructions for calibration of the CASA manufacturer.

However, keep in mind the depth of vision, only low magnification objective lenses like 4x can be used together 100-micron height chambers.

Manual assessments**First use**

Place a 10x10 eyepiece reticle (10 x 10 mm) in one of your eyepieces. This eyepiece reticle is divided in 10x10= 100 blocks each 1x1 mm

This eyepiece has to be purchased from the microscope company. Sometimes special tools are needed to place the eyepiece reticle and a specialist has to perform the job.

Most microscopes have magnification conform the figures depicted on the lenses. A 10x eyepiece magnifies linearly 10x and a 10x objective lens magnifies linearly also 10x.

NB. Some microscopes have in between lenses; one has to take into account the factor of these lenses.

Using a 10x objective lens and a 10x10 mm eyepiece reticle, the field of vision of the reticle equals 1,000x 1,000 microns. Each block equals 100 x 100 microns. The content of the one block equals 100x 100 x 100 microns = $10^6 \mu^3$.

The average counted number per block equals the number of cells in $10^6/\text{ml}$.

Using a 20x objective lens, the reticle equals 500x 500 microns. Each block equals 50 x 50 microns. The content of one block equals 10x 50 x 50 = 250,000 μ^3 .

Multiply the average number per block x 4 to get the concentration in $10^6/\text{ml}$

Using a 40x objective lens, the reticle equals 250x 250 microns. Each block equals 25 x 25 microns. The content of one block equals 100x 25 x 25 = 62,500 μ^3

Multiply the average number per block x 16 to get the concentration in $10^6/\text{ml}$

Fields of vision

Since chambers with a chamber height of 100 microns are often used for the assessment of low cell numbers, one can use the content of one complete microscopic field.

This is just an example, because there are many different diameters of microscopic fields.

Assume that the diameter of the microscopic field is 20 mm. The linear magnification is 20x

(20x objective lens). The diameter of one field equals 1 mm or 1,000 microns, $r = 500\mu$. The

surface equals πr^2 , $3.14 \cdot 500^2 = 785,000 \mu^2$ and the content equals 78,500,000 μ^3 or 0.0785 μl .

This means that 13 fields equal 1 μl .

Stickers with defined opening are available and can be fixed on the slide. A sticker with an opening of 10 mm x 10 mm equals 10 μl .

NB. The depth of vision is much less than 100 microns both for 10x, 20x and 40x objective lenses.

Disposable counting chamber BB2-C-100

Segre-Silberberg effect

This is a phenomenon to be explained by laws of physics, see literature references: Douglas-Hamilton 2005a and 2005b. It means that particle size, viscosity of the fluid and chamber height define flow velocities resulting in not homogeneous cell suspensions; cells are transported to the filling front. The path length of the BB2-C-100 is approximately 26 mm. For 4-micron particles like human and boar semen, a path length of > 20 mm will result in a Segre-Silberberg effect (Douglas-Hamilton et.al. 2005). Since the effect depends also on the dimensions of the cells, it has to be assessed experimentally.

NB. If the 100-micron chambers will be used for the assessment of very low numbers, one does not need to correct for the Segre-Silberberg effect.

Assessment of cell concentration

Keep in mind that human semen can be infectious. Work carefully, use gloves.

Example

Using 20x objective lens and assessing 13 microscopic fields (1 μ l) and zero sperm cells has been found, it does not mean that there will be no cells present. The 99% confidence interval of Poisson distribution with zero events has a mean μ and an interval of $0 \leq \mu \leq 5.3$. Extrapolation to number of cells per ml this means that the 99% interval will be between zero and 5,300. If 26 fields are checked the upper limit will be reduced to 2,600 cell/ml. If one would be sure that there are less than 100 cells/ml (upper value 99% confidence interval) one has to check 673 microscopic fields.

Number of counted cells	Poisson mean and 99% confidence interval
0	$0 \leq \mu \leq 5.3$
1	$0.005 \leq \mu \leq 7.4$
2	$0.1 \leq \mu \leq 9.3$
3	$0.3 \leq \mu \leq 11.0$
4	$0.7 \leq \mu \leq 12.6$
5	$1.0 \leq \mu \leq 14.2$
6	$1.5 \leq \mu \leq 16.6$
7	$2.0 \leq \mu \leq 17.1$
8	$2.6 \leq \mu \leq 18.6$
9	$3.1 \leq \mu \leq 20.0$
10	$3.7 \leq \mu \leq 21.4$

References

Cooper TG, Hellenkemper B, Jonckheere J, Callewaert N, Grootenhuis AJ, Kersemaekers WM, Leung A, Wang C. Azoospermia: virtual reality or possible to quantify. *J Androl* 2006; 27: 483-490.

Douglas-Hamilton DH, Smith NG, Kuster CE, Vermeiden JP, Althouse GC. Particle distribution in low-volume capillary-loaded chambers. *J. Androl.* 2005 Jan-Feb;26(1):107-14.

Disposable counting chamber BB2-C-100

Douglas-Hamilton DH, Smith NG, Kuster CE, Vermeiden JP, Althouse GC.
Capillary-loaded particle fluid dynamics: effect on estimation of sperm concentration.
J. Androl. 2005 Jan-Feb;26(1):115-22.

Dutch Society of Clinical Embryologist
Controle na vasectomie, Laboratorium protocol, Versie: 1.0
Ingangsdatum: 1 Augustus 2010 Revisiedatum: 1 Augustus 2015

Rijnders S, Bolscher JG, McDonnell J, Vermeiden JP.
Filling time of a lamellar capillary-filling semen analysis chamber is a rapid, precise, and accurate method to assess viscosity of seminal plasma.
J. Androl. 2007 Jul-Aug; 28(4): 461-5

WHO manual 5th edition, 2010

For the calculation of Poisson intervals see:
<http://www.danielsoper.com/statcalc3/calc.aspx?id=86>