

## Manual

### Introduction

The BIRR disposable counting chamber BB2-V-20 is designed for the assessment of low sperm numbers, like check after vasectomy. In the literature there has been a debate how to perform check after vasectomy, because the paper of Cooper et al (2006) proofed that the other methods are less precise.

Local societies and the WHO have formulated new protocols for the assessment of low numbers, since it is theoretically not possible to assess the presence of zero cells. .

The organization of Dutch clinical Embryologist has also formulated a new instruction on this topic. The cut-off level of a successful vasectomy was defined as the presence of less than 100,000 sperm cell per ml. The minimal volume for the assessment was defined as 1  $\mu$ l.

This manual describes how to assess less than 2600 cell per ml (upper level 99% confidence interval).

After the use of the slides they needed to be treated as contaminated waste. Follow the national and local rules for disposal of contaminated waste.

### Description of the counting chamber



Two chamber A and B. The letters A and B indicate the filling place. The grid 5 x 10 mm. The width of the grid can be seen in one microscopic field using a 20x eyepiece.

The chamber is made of a standard microscopic slide and a cover slip. 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 place on this pattern, softly compressed and the resin is cured with light flash.

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

The dimensions of the microscopic slide are approximately 75.0 ( $\pm$  0.2) x 25.0( $\pm$  0.2) x 0.7( $\pm$  0.1) mm. The dimension of the cover glass are 32.0 ( $\pm$  0.2) x 24.0 (( $\pm$  0.2) x 0.7 mm ( $\pm$  0.1) mm. Variation in the dimensions of the glass plates do not affect the functionality.

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

Each of the two BB2-V-20 chambers contains a grid, dimensions 10,0 x 5,0 mm. The grid is divided in 5 blocks of 1.0 x 10.0 mm. To improve the finding of the grid the lines are extended at both side 1.5 mm. The distance between two lines of the grid, 1 mm, can just be seen using a 20x objective lens. This allows scanning of the width of the chamber in one microscopic field.

**Disposable counting chamber BB2-V-20**

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

The assessment of semen variables, number of cells per ml and motility, can be performed in a one-step procedure without dilutions, without concentrating and without inactivation of the sperm cells. Because of the coating sperm, cells will not adhere to the surface of the slide and motility can be assessed without interference of the surface of the chamber.

It is advised to use a 20x objective lens.

**Because of the presence of a grid, the slide can be used without calibration of the microscope.**

It is not advised to use the BB2-V-20 slides in combination with a CASA system (computer aided semen analysis).

**Segre-Silberberg effect**

The BB2 –V- 20 slide is designed for the assessment of low or very low concentrations. It does not make sense to correct for the Segre-Silberberg effect.

**Assessment of cell concentration and sperm motility**

Assessments can be performed at room temperature. Normally the BB2-V-20 slide is not used for motility assessments.

The number of assessed cells per complete grid equals the number of cells in millions per ml.

Keep in mind that human semen can be infectious. Work carefully, use gloves.  
Note your observations according to the local work instructions.

- Pipette 5  $\mu$ l at the filling place of the chamber A. Do not overfill do not under fill. Remove excess of fluid with a paper tissue.
- Take some time to look at the sample, note the particulars.
- Scan carefully the first block of the grid, using a 20x objective lens. One has to see the upper and lower lines of the block.
- Scan the other four blocks in the same way.
- Repeat the procedure with chamber B.
- Calculate the mean value.

**NB. National professional organizations have their own protocols for the assessment of low sperm numbers and on the cut-off values for successful vasectomies.**

**Example**

The BB2-V-20 is designed for the assessment of low sperm numbers.

If the content of 1 grid (1  $\mu$ l) is carefully assessed 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  $\mu$  and an interval of  $0 \leq \mu \leq 5.3$ . Extrapolation to

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number of cells per ml this means that the 99% interval will be between zero and 5,300. If two grids are assessed the upper limit will be reduced to 2,650 cells/ml (assessed volume has doubled).

If one would to be sure that there are less than 2,600 cells/ml (upper value 99% confidence interval) one has to use another type of sperm analysis slide, like the BB2-C-100, or one has to repeat the assessments with extra BB2-V-20 slides.

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**

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

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