

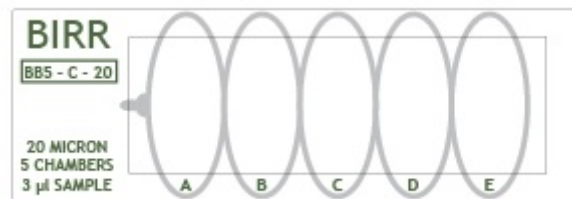
## Manual

### Introduction

The BIRR disposable counting chamber BB5-C-20 is designed for the microscopic assessment of semen. BB5-C-20 slide has five chambers; each can be used for one assessment.

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

### Description of the counting chamber



Five chambers, A, B, C, D and E indicate the filling place of the chamber.

The chamber is made of a standard microscopic slide and a cover slip. The dimensions of the microscopic slide are approximately 75.0 ( $\pm 0.2$ ) x 25.0 ( $\pm 0.2$ ) x 1.0 ( $\pm 0.1$ ) mm. The dimension of the cover glass are approximately 55.0( $\pm 0.2$ ) x 18.0( $\pm 0.2$ ) x 0.7 ( $\pm 0.1$ ) mm. Variations in these dimensions do not affect the functionality of the slides.

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 violet light.

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 are 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 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.

Low concentrations can be assessed with a 10x objective lens and high concentrations with a 40x objective lens. However, using a 40x objective lens, one has to realize that the depth of vision is less than the 20-micron chamber height and one has to focus continuously.

**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.

## 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.

Normally sperm concentration is expressed in millions per ml, or  $10^6$  cells per  $10^{12}$  per  $\mu^3$  (cubic microns) or number of cells per  $10^6 \mu^3$ .

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. 5 blocks equal  $10^6 \mu^3$  and equals the concentration in millions per ml.

Using a 20x objective lens, the reticle equals 500x 500 microns. Each block equals 50 x 50 microns; 20 blocks equal  $10^6 \mu^3$  and equals the concentration in millions per ml.<sup>3</sup>

Using a 40x objective lens, the reticle equals 250x 250 microns. Each block equals 25 x 25 microns; 80 blocks equal  $10^6 \mu^3$  and equals the concentration in millions per ml.

### Segre-Silberberg effect

This is a phenomenon to be explained by laws of physics, see literature references:

It means that particle sizes, viscosity of the fluid and chamber height defines flow velocities resulting not homogeneous cell suspensions. Cells are transported to the filling front.

One has to correct for this phenomenon. For precise correction, see appendix 1. A very handy rule of thumb is: Very viscous semen no correction factor, normal liquefied semen 10% correction factor, cells suspend in culture medium or other watery solution: correction factor 30%.

### Assessment of cell concentration and sperm motility

#### Materials, equipment needed.

- Hot plate or heated microscopic stage, 37°C
- Pipette and disposable pipette point to pipette 3 – 8  $\mu$ l
- Stopwatch (optional)

Use slides that have been warmed to 37°C (on a heat plate). For optimal motility assessments use a microscope with heated stage (37°C)

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

**Disposable counting chamber BB5-C-20**

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Pipette 4 µl at the filling spot of the chamber. Start at the same time the stopwatch  
Stop the stopwatch as soon as the filling front has reach the other side of the chamber.  
Read the filling time, read the Segre-Silberberg correction factor in appendix 1.  
The filling time can be converted into the viscosity of the sample, see appendix 2.

**Alternative**

Estimate the viscosity: very viscous, normal liquefied, watery solution.

Wait till the chamber has been filled fully and remove excess of fluid with a paper tissue.

Take some time to look at the sample, note the particulars.

Assess the motility according to the WHO manual instructions; we advise to use the WHO manual 2010 (PM: progressive motile, M: motile (motile on the spot) and NM: Non-motile)

Perform the assessments in 5 different areas. Count at least 200 cells (if possible).  
Note number of counted cells and number of blocks used for the assessment.

**Assessment of the concentration:**

1. Number of counted cell: N
2. Number of blocks used for the assessment: X
3. Calculate  $N/X*20$  (20x objective lens) *or*
  - i.  $*5$  (10x objective lens) *or*<sup>\*</sup>
  - ii.  $*80$  (40x objective lens)<sup>\*</sup>
4. Multiply by – the Segre-Silberberg correction factor  $S_x$  read in Appendix 1.

**or**

Multiply by - 1.0 very viscous semen  
- 1.10 normal viscous semen  
- 1.30 watery solutions

**\*NB.** These figures are only valid after calibration of your microscope, and if one as ascertained that the actual magnification is according the numbers depicted on the objective lens.

**Semen analyses for diagnostic purposes:**

For diagnostic purposes it is advised to perform the assessments *in duple*. The two assessments have to be performed in the same way; same number of blocks has been used for the assessments.

Take the sum and the differences of the two assessments and read the table:

Disposable counting chamber BB5-C-20

Sum	Acceptable Difference*	Sum	Acceptable Difference*
144–156	24	329–346	36
157–169	25	347–366	37
170–182	26	367–385	38
183–196	27	386–406	39
197–211	28	407–426	40
212–226	29	427–448	41
227–242	30	449–470	42
243–258	31	471–492	43
259–274	32	493–515	44
275–292	33	516–538	45
293–309	34	539–562	46
310–328	35	563–587	47

\*Based on the rounded 95% confidence interval.

This table relates to the actual counted numbers of two separate assessments performed in the same way (same number of blocks counted in each of the assessments). If the difference is larger than the acceptable difference, one has to repeat the assessments. If an assessment has been rejected three times it is advised to use the mean value.

## References

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

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

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**Appendix 1**

filling time seconds	Segre Silberg effect correction factor	filling time seconds	Segre Silberg effect correction factor
1,7	1,32	5,7	1,15
2,2	1,31	5,9	1,14
2,4	1,30	6,4	1,13
2,5	1,29	7,5	1,11
2,6	1,28	8,6	1,10
2,7	1,27	9,6	1,09
2,8	1,26	10,7	1,08
3,0	1,25	11,8	1,08
3,1	1,24	12,8	1,07
3,4	1,23	14-16	1,06
3,6	1,22	17-19	1,05
3,8	1,21	20-26	1,04
4,1	1,20	27-32	1,03
4,3	1,19	33-64	1,02
4,5	1,18	64-128	1,01
4,8	1,17	>128	1,00
5,3	1,16		