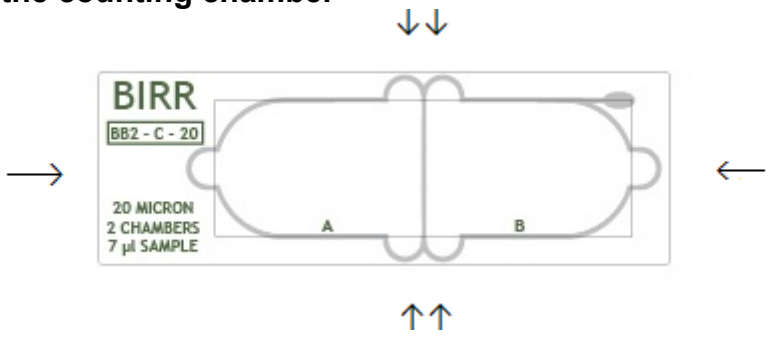
Manual

Introduction

The BIRR disposable counting chamber BB2-C-20 has two chambers for two assessments of one semen sample.

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



Two chambers, A and B. → Point to filling place of chamber A; ← point to filling place of chamber B.
↑↑ , ↓↓ air outlets

The chamber is made of a standard microscopic slide and a cover slip. The dimensions of the microscopic slide are approximately 75.0 (± 0.2) x 25.0 (± 0.2) x 0.7(± 0.1) mm. The dimension of the cover glass are 55.0 (± 0.2) x 18.0 (± 0.2) x 0.7 mm (± 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 place 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.

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 assesses 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 can 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 μ^3 (cubic microns). The actual counts per 10^6 μ^3 equals the sperm concentration in millions per ml.

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 one block equals 20 x 100 x 100 = 200,000 µ3; 5 blocks equal 10^6 μ^3.

Using a 20x objective lens, the reticle equals 500x 500 microns. Each block equals 50 x 50 microns. 20 blocks equal 10^6 μ^3.

Using a 40x objective lens, the reticle equals 250x 250 microns. Each block equals 25 x 25 microns: 80 blocks equal 10^6 μ^3

Segre-Silberberg effect

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

It means that particles seize, viscosity of the fluid and chamber height defines flow velocities resulting in 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 suspended 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 µl
- 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.

Pipette 7- 9 µl at the filling place of the chamber.
- Start the stopwatch at the same time.
- 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.
- Remove excess of fluid with a piece of paper tissue.

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.

Calculation 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**
    ii. *80 (40x objective lens)*
4. Multiply by – the Segre-Silberberg correction factor *Sx* 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:

Sum	Acceptable Difference*
144–156	24
157–169	25
170–182	26
183–196	27
197–211	28
212–226	29
227–242	30
243–258	31
259–274	32
275–292	33
293–309	34
310–328	35

Sum	Acceptable Difference*
329–346	36
347–366	37
367–385	38
386–406	39
407–426	40
427–448	41
449–470	42
471–492	43
493–515	44
516–538	45
539–562	46
563–587	47

*Based on the rounded 95% confidence interval.

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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.
J. Androl. 2005 Jan-Feb;26(1):107-14.

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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
2,5	1,32	6,2	1,16
2,6	1,31	6,6	1,15
2,7	1,30	6,9	1,14
2,9	1,29	7,5	1,13
3,0	1,28	8,7	1,11
3,1	1,27	10,0	1,10
3,2	1,26	11,2	1,09
3,5	1,25	11,3-13,7	1,08
3,6	1,24	13,8-16,1	1,07
4,0	1,23	16,2-18,7	1,06
4,2	1,22	18,8 -23,6	1,05
4,5	1,21	23,7 - 29,9	1,04
4,7	1,20	30,0-37,4	1,03
5,0	1,19	38-75	1,02
5,2	1,18	75-150	1,01
5,6	1,17	> 150	1,00