Manual

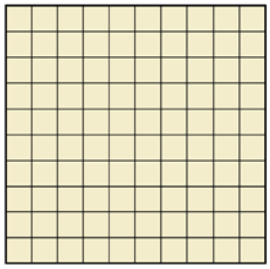
Introduction

The BIRR disposable counting chamber BB2-G-10 is designed for diagnostic microscopic assessment of semen samples. BB2-G-10 slide 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 disposal of contaminated waste.

Description of the counting chamber

A is the filling area of chamber A and B is the filling area of chamber B.
The first spot, small spot, close to the filling area can be used of motility analysis.



The second spot contains a counting grid, 1 mm x 1 mm, squares of 100 µ x 100 µ

The chamber is made of a standard microscopic slide (L x W x H: 75.0 (±0.2) x 25.0 (±0.2) x 1.0 (±0.1) mm) and a cover glass (32.0 (±0.2) x 24.0 (±0.2) x 0.7 (±0.1) mm). Variations in the dimensions of the glasses 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 place on this pattern, softly compressed and the resin is cured with a flash of 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 sperm cells. Swine sperm cells are 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 and other cells will not adhere to the surface of the slide and motility can be assessed without interference of the surface of the chamber.

The surface of the grid is 1,000 x 1,000 = 10^6 µm^2. The height of the chamber is 10 µm; the content of the column above the grid is $10*10^6$ µm^3. The grid is divided in 100 blocks; the content of one block is $0.1*10^6$ µm^3; 10 blocks have a content of $1.0*10^6$ µm^3.
The total number of cells present in 10 blocks equals the number of cells in 10^6/ml.
If all the blocks of one grid is counted, one has to divide this figure by 10 to get the initial concentration in millions per ml (10^6/ml). The final concentration has to be corrected for the Segre Silberberg effect and is the average of two assessments.

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 10-micron chamber height and one has to focus continuously.
It is advised to use a phase contrast microscope.

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

It is not advised to use the Birr BB2-G-10 slides in combination with a CASA system (computer aided semen analysis): CASA systems will perform optimal in chambers without grid, like the Birr BB2-C-20 or BB5-C-20.

Segre-Silberberg effect

This is a phenomenon to be explained by laws of physics, see literature references (Douglas-Hamilton 2005a, 2005b). It occurs in all capillary filled slides, tubes etc.
It means that particle size, viscosity of the fluid and chamber height define flow velocities. This can cause the transport of cells to the filling front. The content of the chamber will not be homogeneous anymore.

One has to correct for this phenomenon. A handy rule-of-thumb is:

- Very viscous semen; no correction factor;
- Normal liquefied semen 15 % correction factor (multiply the initial outcome by 1.15)
- Cells suspended in culture medium or other watery solution: correction factor 40% (multiply the initial outcome by 1.40)

Assessment of cell concentration and sperm motility

Use slides that have warmed to 37°C (on a hot 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 5 µl at the filling place of the chamber (A or B two chamber slide).
Determine the grade of 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.
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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)

Count the total of number of cells per block. Count at least 5 blocks; count if possible at least 200 cells.

Assessment of the concentration:

1. Number of counted cell: N
2. Number of blocks used for the assessment: X
3. Calculate N/X * 10
4. Multiply by -1.0 very viscous semen
    i. -1.15 normal viscous semen
    ii. -1.40 watery solutions

Semen analyses for diagnostic purposes:

Repeat the procedure in chamber B

The two assessments have to be performed in the same way; same number of blocks has been used for each of the assessments.

Take the sum and the differences of the two numbers of cells counted in 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.

If the differences 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 of the three assessments..

If the assessment is accepted, calculate the concentration of each assessment and calculate the mean value.

References

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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.
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