SPERMCOUNT

The concentration of spermatozoa in the semen is determined to calculate the number of doses that can be produced from that volume of semen.

Counting can be done manually, using the Bürker Chamber, or automatically using a colorimeter.

BÜRKER CHAMBER

Using the Bürker chamber, the number of abnormal forms in the sperm, such as proximal or distal cytoplasmic droplets and coiled tails, can be determined.

The procedure is as follows:

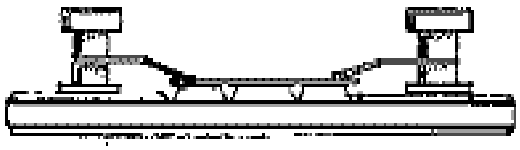
1. Take 1mL of ejaculate and dilute to 100mL with saline formalin in a flask and mix well, but gently (by inverting it two or three times).

2. Then remove a drop of the dilution with a Pasteur pipette and fill the prepared Bürker chamber. The pipette is placed between the cover and the chamber, so that it fills by capillary action. Leave for a couple of minutes then place under a microscope.

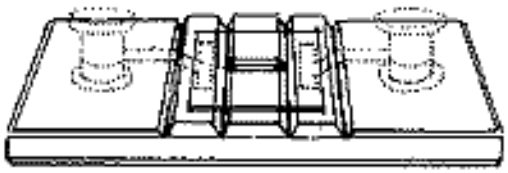
3. Once in the microscope, start with the x10 objective to locate and position the reticule, then move to x40 for the sperm count.

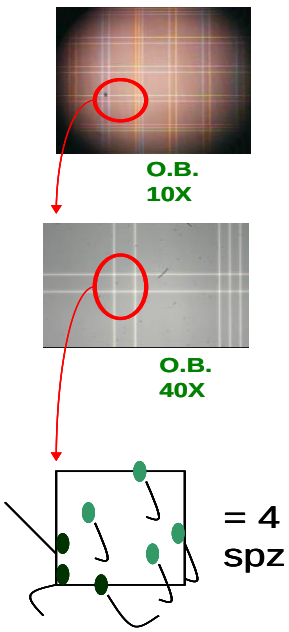
Counting is performed as follows:

- Count the spermatozoa present in **40** small squares.
- Those spermatozoa whose heads are inside the squares, and those touching the top or right-hand edge of the square, along with those in the upper and lower right corners are counted.
- The number of spermatozoa counted multiplied by 10^7 is the concentration of spermatozoa per mL of the ejaculate. Multiplying this value by the volume then gives the total number of spermatozoa in the ejaculate.



Cámara de Bürker





Calculating the doses to prepare:

The **total number of spermatozoa** is the number of normal spermatozoa (ie, those without malformations) counted in 40 frames, multiplied by the volume of the ejaculate. This is divided by the **concentration** of spermatozoa desired for the dose, which is usually 3,000 to 4,000 x 10^6 (depending on the abnormal morphology quantity). The resulting value is the **semen dose** that can be prepared at that concentration.

D – Number of doses to produce
V – Volume of ejaculate obtained (includes the 50-100mL of extender included in the vessel prior to collection)
N – Number of spermatozoa without malformations counted with the Bürker chamber, multiplied by 10^7 (spermatozoa per mL of semen)
C – Concentration required for the dose (in thousands of millions)

Therefore: **D = V x N / C**

For example, 250 mL of ejaculate **(V)**
35 normal sperm counted (in 40 squares) **(N)**
3000 million (3000x10^6) spermatozoa per semen dose **(C)**

D = 250 x 35(10^7) / 3000x10^6 -> (**D** = 250 x 35 / 300)

D = 8750 /300

D = **29** doses, each containing 3000 million spermatozoa, can be produced.

To calculate the volume of extender needed, simply multiply the number of doses to prepare **(D)** by the volume contained in each package used (eg, 90 mL), then subtract the volume of ejaculate we already have **(V).**

When diluting the semen, the following must be taken into account:

- Mix the ejaculate and extender gently.
- The temperature difference between the two should be the least possible. It must not exceed 2°C and the semen and extender must both be about 37°C.

COLORIMETER

Using the colorimeter allows a quicker estimate of the concentration of ejaculated sperm to be made, which is very useful if there are a large number of ejaculates to process.

The system is based on the ability to measure the light passing through a solution and its relationship with the spermatozoa concentration.

The colorimeter emits a light beam which passes through the solution (in this case the ejaculate sample) to a photodetector, which determines the ***transmittance*** (T), ie the amount of Light that has passed through the sample. The "opposite" of the transmittance is the ***absorbance*** (A), which is the light that has been absorbed by the solution, and has therefore not reached the photodetector.

$$A = \log (1/T)$$

The absorbance value is proportional to the sperm concentration.

The problem is the enormous variability in absorbance between different ejaculates due to different protein concentrations and semen plasma compositions. To overcome this difficulty, calibration curves (which are usually actually straight lines) are drawn.