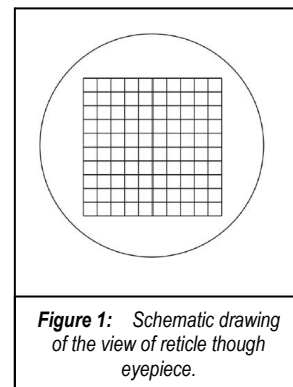




## Manual Sperm Counts using a Leja<sup>®</sup> Slide and Eyepiece Reticle

The below procedure provides basic guidelines on how to determine accurate sperm concentrations using the Leja slide and an eyepiece reticle.

Prior to performing a count, it is required that a correction factor (**F**) is determined for the microscope and objective used for the manual count. This only needs to be done once for a specific microscope and objective combination. The procedure for determining the correction factor (**F**) is also provided on the Leja website: [www.leja.nl](http://www.leja.nl) (downloads ▢ manuals).



### Counting Procedure:

When performing manual counts, you must keep track of two things:

**S** = number of sperm counted (a minimum of 200 cells per chamber is recommended).

**B** = number of boxes counted to achieve **S**, figure 1 shows a reticle image with 100 boxes.

Typically, counting begins in the uppermost left hand side of the reticle and moves from left to right for *Row 1*, right to left for *Row 2*, left to right for *Row 3*, and so on.

To avoid double counting sperm from row to row, the following rules should apply per box:

- Only sperm heads are counted (if only the tail of the sperm is in the box, it is not counted).
- All sperm heads totally within the box are counted.
- For sperm heads which cross the boundary between boxes, at least half of the sperm head should be within the box in order to be counted as part of that box.

### Calculating Concentration

You first must determine the number of sperm counted per box (**N**):

$$N = \frac{S}{B}$$

To calculate the concentration (**C**) in millions per ml ( $10^6 \text{ ml}^{-1}$ ), multiply the number of sperm per box by the correction factor **F**:

$$C = N \times F$$

*Important note:* The concentration above reflects only the concentration of the sample in the chamber. If a dilution was made on the raw sample before loading the Leja slide, this dilution factor must be taken into consideration before calculating the raw ejaculate concentration.