1. **Subject of the protocol**
   Cell counting – Bürker chamber

2. **Safety**
   - Wear a labcoat at all times
   - If you are not sure of your aseptic technique wear gloves, spray hands with disinfectant (e.g. 70% ethanol)
   - Observe the safety and work guidelines stated in general protocols
   - Dispose of waste according to waste disposal protocol
   - In case of any equipment failure inform your supervisor and consult on the action to take. Do not leave the equipment **unattended!**

3. **General information**
   Quantitation in cell culture is required for the characterization of the growth properties of different cell lines for experimental analyses and to establish reproducible culture conditions, for the consistency of primary culture and the maintenance of cell lines.

4. **Chemicals**
   - Proper medium – check the requirement of each specific cell line.
   - PBS – phosphate buffer saline without Mg and Ca ions
   - Trypsine-EDTA – for adherent cell lines
   - Ethanol 70%

5. **Preparation of reagents**
   Depending on the medium

6. **Counting monolayer cells**
   - **Sample the cells.**
     - Trypsinize the monolayer as for routine subculture (consult the proper protocol) total volume of medium 10 ml.
     - Mix the suspension thoroughly to disperse the cells.
   - **Prepare the slide**
     - Clean the surface of the slide with 70% alcohol.
     - Clean the coverslip, and, wetting the edges very slightly, press it down over the grooves. The appearance of interference patterns (“Newton’s rings”—rainbow colors between the coverslip and the slide, like the rings formed by oil on water) indicates that the coverslip is properly attached, thereby determining the depth of the counting chamber.
     - Mix the cell sample thoroughly, pipetting vigorously to disperse any clumps, and collect 20 µL in to the tip of a pipettor.
     - Transfer the cell suspension immediately to the edge of the hemocytometer chamber, and expel the suspension and let it be drawn under the coverslip by capillarity. Do not overfill or underfill the chamber, or else its dimensions may change, due to alterations in the surface tension, the fluid should run only to the edges of the grooves.
     - Mix the cell suspension, reload the pipettor, and fill the second chamber if there is one.
### 5. Counting cells

- Select a 10× objective, and focus on the grid lines in the chamber.
- Count the cells lying within this 1-mm² area, using the subdivisions (also bounded by three parallel lines) and single grid lines as an aid for counting. Count cells that lie on the top and left hand lines of each square, but not those on the bottom or right handlines, to avoid counting the same cell twice. See Picture 2
- Measure concentration applying the equation below,

\[
\text{number of cells in 1ml} = \frac{n \times 10^4}{z}
\]

- \(n\)-the whole number of cells in all the counted squares 1mm²
- \(z\)- the number of counted squares 1mm²

- Number of cells in field shall hold in range of 10-100. In case of a large number of cells prepare a suitable dilution and repeat the counting.
Picture 1. Bürker counting chamber.

Attention: In every square count the cells situated inside the field and connected with the edges: upper and side left. Do not count the cells adjacent to the edge bottom and side right.

Picture 2. The grid