Tips on how to count cells by using the new disposable counting chamber C-Chip

Biochrom AG Information

The disposable plastic counting chamber C-Chip looks like the established Neubauer "improved" counting chamber. It allows for a counting of cells of a wide range of different sizes: leukocytes, thrombocytes and erythrocytes, yet also bacteria, fungal spores and pollen. What makes the C-Chip more favourable than the traditional glass counting chamber is the fact that the user no longer needs to use cover slips or clean the chip. At the same time, a safer handling can be ensured as contact with infectious material is virtually impossible. The depth of the chambers is strictly predetermined, which allows a reproduction of counting at any time.

Minor errors occurring during the cell counting process lead, as a consequence of the dilution processes, to extreme cell count differences and thus distort the test results. Please find below some informative tips on how to use the C-Chip that is integrated in the Neubauer "improved" counting chamber and on how to count different cell types using the C-Chip.

1 C-Chip features

The C-Chip represents a high-precision disposable counting chamber made of high-quality plastics allowing a manual determination of the cell count. It includes two independent counting chambers with separate injection areas for one sample each. Cover slips are no longer required. As the depth of the chambers is strictly predetermined, the disposable counting chamber is extremely precise, with counting being reproducible at any time. The counting chambers match the Neubauer "improved" counting chamber.

The C-Chip has been designed for single use and should be used directly after opening the packing. Due to its single-use feature, there is no need to clean the chip or to interrupt the work flow. The C-Chip can be used for all established automatic counters.

When using the C-Chip the infection risk is reduced due to the minimised exposition to biologically active, infectious material.

fig. 1: individual packing of the C-Chip

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2 Neubauer “improved” counting chamber

2.1 Design of the Neubauer “improved” counting chamber

The counting chamber counting grid includes:

- 9 large squares (3 x 3)
- 4 corner squares L (small boxes no. 1, 3, 7, 9)

The corner squares (L) are divided into 16 squares (4 x 4).

The central square no. 5 is divided into 5 x 5 squares (E).

The squares (E) are divided into 4 x 4 small squares.

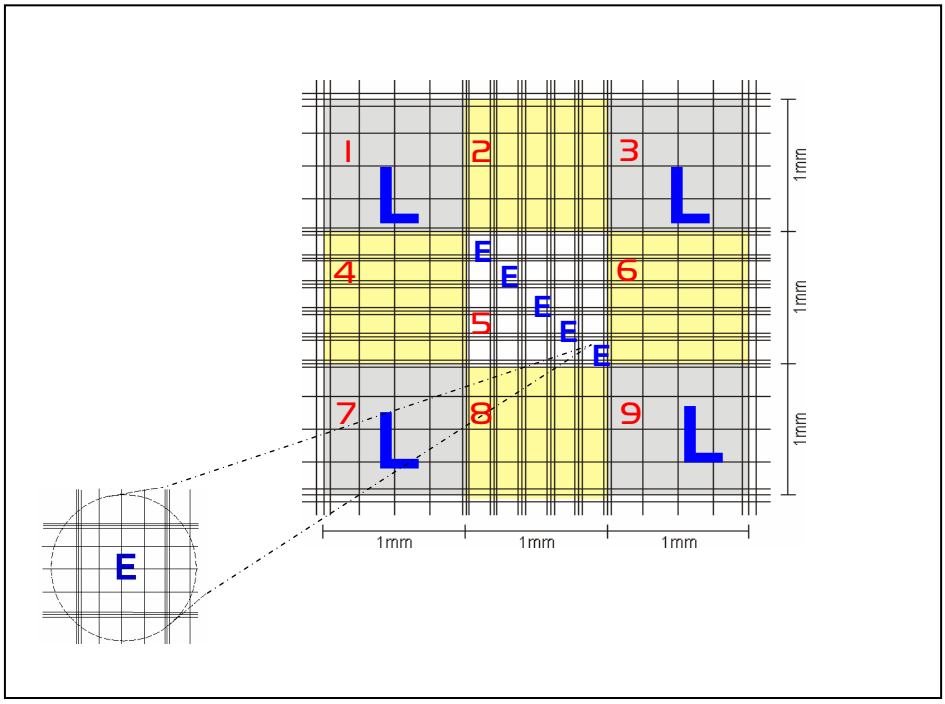

fig. 2: design of the Neubauer “improved” counting chamber

Volume data in the L-squares:

The area of the L-squares results from the length of the edges:
1 mm x 1 mm = 1 mm^2

The chamber depth of 0.1 mm accounts for a volume in the L-squares of 0.1 mm^3
(conversion: 0.1 mm^3 equal 0.1 µl or 10^{-4} ml).

2.2 Counting methods

Depending on the individual cell type that is to be counted the user has to count different groups of squares and to determine the mean value. Cell counting requires thorough knowledge of the counting technique and of how to deal with the boundaries:

- In order not to double-count or neglect cells, cells are counted in a meander-like manner:

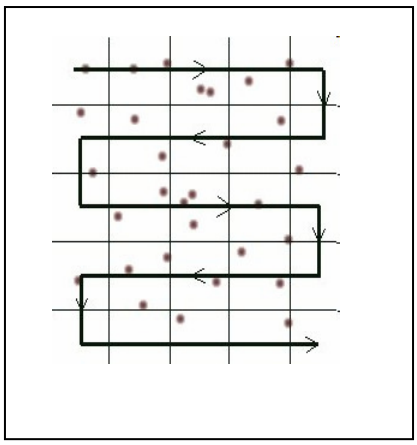

fig. 3: meander-like counting method

- When counting the cells, the user has to respect the boundaries carefully:
  One advantage the Neubauer "improved" counting chamber features is the triple boundaries of the group squares (the central line represents the actual boundary), which makes it easier for the user to determine the exact "position" of the cell. Please note: only cells of two boundaries are being counted, for example at the top and on the left.

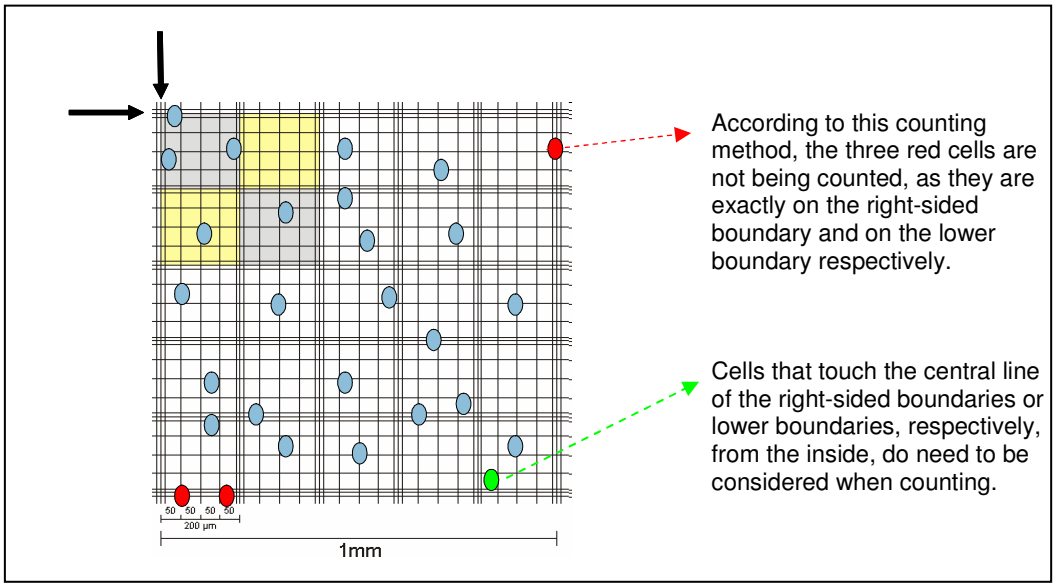

fig. 4: counting of cells

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Neglecting this procedure results in a distortion of the actual cell count in the five- or even six-figure range. A reproduction of the tests is difficult; further qualitative statements are wrongly made.

2.3 Cell count determination using the example of HeLa cells and erythrocytes

2.3.1 HeLa cells

A total of 84 cells are counted in the L-corner squares no. 1, 3, 7, 9.

Determination of the mean cell count per each L-corner square:
84:4=21

HeLa per ml = 21 cells
x 10^4 ml^{-1} (volume factor in one L-corner square)

This results in 210,000 cells/ml.

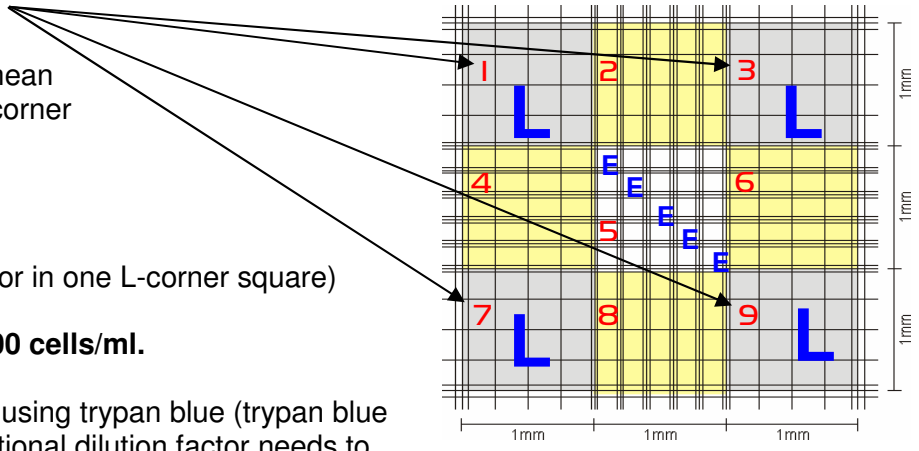

fig. 5: counting of HeLa cells in the four corner squares and of erythrocytes in the central square no. 5

If the cells are stained using trypan blue (trypan blue staining 1:10), an additional dilution factor needs to be included:

HeLa per ml = 21 cells
x 10 (dilution factor)
x 10^4 ml^{-1} (volume factor in one L corner square)

This results in 2,100,000 cells/ml or 2.1 x 10^6 cells/ml.

2.3.2 Erythrocytes

Erythrocytes are being counted in the central square no. 5 (fig. 6), which is divided into 5 x 5 smaller squares (E). Each E-square is divided into 4 x 4 small squares.

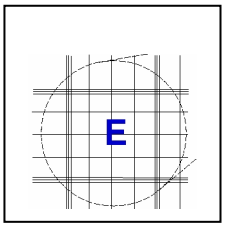

fig. 6: 4 x 4 small squares for the counting of erythrocytes

Calculation example:

cell count in the five E-squares: 420
dilution of the erythrocytes: 1 : 200

erythrocytes per ml = 420 cells
x 5 (projection of the cell count for the entire central square no. 5)
x 200 (dilution factor)
x 10^4 ml^{-1}(volume factor in one L-square)

This results in 4,200,000,000 cells/ml or 4.2 x 10^6 cells/µl.

The following table 1 includes general determination methods for leucocytes, erythrocytes and eukaryotic cells.

table 1: general cell count determination for different cell types and procedures

leucocytes (1 : 20 dilution)	**cell count leucocytes**
1. Dilute blood using accepted laboratory methods. 2. Load 10 µl of the sample into the injection area using a pipette. 3. Count the cells in the 4 large corner squares under a microscope.	leucocytes per ml = (cell count in 4 large squares/4) x 20 (dilution factor) x 10^4 (volume factor)
eukaryotic cells	**cell count eukaryotic cells**
1. Use trypsin-EDTA to dissociate and pelletise adherent cells. Pelletise suspension cells. 2. Carefully remove the supernatant using a pipette (without disturbing the pellet) and set the cells in an appropriate volume medium or PBS, respectively, to reach an approximate density of 5x10^3 to 5x10^6 (ideal range ensuring reliable cell counting). 3. Resuspense the cells carefully using a pipette (until no further clumps or cell agglomerates are visible) 4. Load 10 µl of the sample into the injection area. 5. Count the cells in the 4 large corner squares under a microscope.	cells per ml = (cell count in 4 squares/4) x dilution factor x 10^4 (volume factor)
erythrocytes (1 : 200 dilution)	**cell count erythrocytes**
1. Dilute blood using accepted laboratory methods. 2. Load 10 µl of the sample into the injection area. 3. Count the cells in 5 small squares of the central square under a microscope (in a diagonal manner, as shown in fig. 5 for the E-squares).	erythrocytes per ml = cell count in 5 small squares x 5 x 200 (dilution factor) x 10^4 (volume factor)

3 Use

3.1 Design of the C-Chip

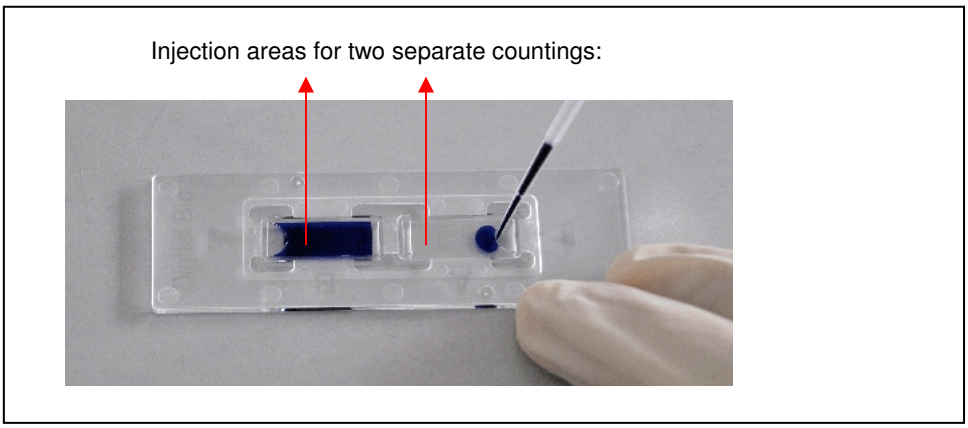

fig. 6: design of the C-Chip and injection of a second sample

3.2 Procedure

The C-Chip is easy to use. In general, the following approach can be adopted:

1. Mix the sample well and dilute if necessary.
2. Load 10 µl carefully into the injection area using a pipette.
3. The sample fills the counting chambers by capillary action.
4. Count the cells as usual (microscope).
   Tip: Prior to the counting, observe the entire counting chamber at low magnification and check whether the cells are spread evenly. Otherwise remix the sample.

To count specific cell types, Biochrom AG recommends to count in accordance with table 1, page 5.

4 Trouble shooting

- Inject the sample carefully and avoid the formation of air bubbles.
- Remove dust particles if present.
- Check the calibration and focussing of the microscope.
- In case of elevated cell density: dilute and consider the new dilution factor for your calculations.
- The difference between the cell counts in the individual squares should not exceed 10. Otherwise, stir the sample and recount.

5 Product details of the C-Chip

parameter	disposable counting chamber C-Chip
cat. no.	P DHC-N01
units	1 box contains 50 C-Chips with 2 tests each
storage	room temperature (RT)
raw material	plastics
use	suitable for the counting of all cell types
note	➢ "ready to use“

- To find these products in our online shop go to: http://www.biochrom.de/en/products/plastic-for-cell-culture/.
- Please contact info@biochrom.de to order your free sample.

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