LO - Laboroptik Counting chamber instructions for use

Introduction

This document covers the following topics:

1. What is a counting chamber and where is it used
2. Construction
3. Design and identification
4. Production and quality
5. Filling the chambers
6. Counting particles
7. Calculations
8. Cleaning instructions

1. What is a counting chamber and where is it used?

The counting chamber is a precision measuring instrument, manufactured from special optical glass.

It is used to determine the particle count by volume of a fluid. The particles are counted by eye under a microscope.

The principle use of counting chambers is in the medical laboratory for blood analysis (counting leucocytes, erythrocytes and thrombocytes) and also for the counting of cells in cerebral-spinal fluid and many other specialities.

Besides that, counting chambers can be used in chemical food laboratories for the enumeration of bacteria and fungal spores, as well as in veterinary and other laboratories.

2. Construction

All counting chambers follow the same construction principle (figure 1).

On a thick base plate made of special optical glass, the size of a microscope slide, there are four longitudinal grooves cut into the middle third. The two major external surfaces are unfinished and used for labeling.

The central bar and the two outer supports are ground and polished. On the central bar (the chamber base) the counting grids are engraved.

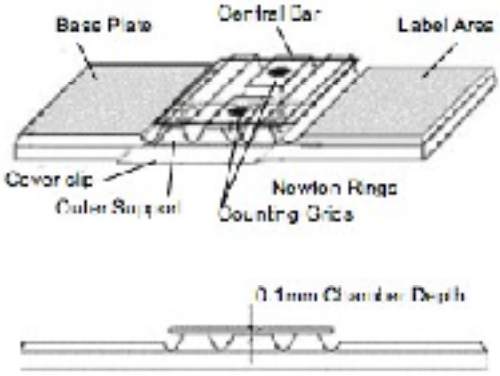


Figure 1

This bar is deeper in relation to the outer supports, typically by 0.1 or 0.2 mm.

When a flat-ground cover glass is placed onto the outer supports there is a gap of 0.1 or 0.2 mm thickness respectively between it and the chamber base. The imaginary vertically projected boundary lines of the counting grids form the lateral volume limit.

3. Design and identification of counting chambers

Counting chamber types:

- Double chamber - central bar divided (two counting nets) - figure 2.
- Single chamber – central bar un-divided (one counting net) - figure 3.

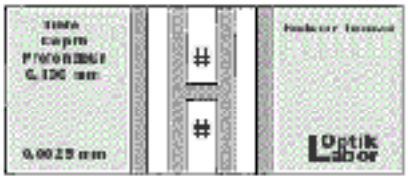


Figure 2

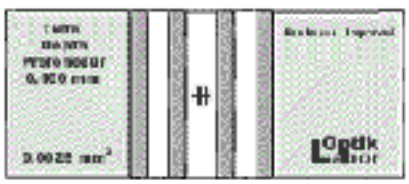


Figure 3

Furthermore, we distinguish between standard and bright-line versions.

- In the standard version, the counting grid is etched directly into the glass.
- In the bright-line version, the chamber base is coated with a very thin metallic mirror. The counting grid is etched into the metalized surface. Using a phase-contrast microscope a colour conversion is possible, so that the counting grid can be seen as either dark or light.

The bright-line design is generally preferred as it is more comfortable to work with.

3.1 Identification

Printed on the two un-worked sides of the counting chamber is:

- The counting grid system
- The name and trademark of the manufacturer
- The chamber depth in mm
- The area of the smallest counting grid square in mm^2.

4. Production and quality

Counting chambers are precision measuring instruments, often used in medical laboratories. In Europe, only CE marked chambers are used. Abroad, non-CE marked counting chambers and cover glasses are available.

This does not mean that there is a first and second choice. In each case there may be people treated on the basis of results of measurements with our counting chambers.

All of the LO-Laboroptik optical laboratory counting chambers are manufactured using the latest methods in accordance with the current weights and measures regulations and DIN standards. We distinguish only between Certified and Certifiable counting chambers.

4.1 Production

The production of counting chambers is described only in general terms.

The process includes several steps, between which are strict controls.

The inner support (chamber base) and the two external supports are machined by grinding and polishing the surfaces glass. The purpose of this process is to bring the two outer support surfaces into the same level plane and to lower the inner central bar (the chamber base) relative to those outer supports by exactly the specified dimension.

After these operations the corresponding counting grid (system) is engraved using a diamond dividing machine.

Lastly comes the printing and baking.

All counting chambers must pass a rigorous final inspection, which is in accordance with the requirements of DIN - based standards and certification regulations. Only then is the distinction made between certified and certifiable counting chambers, with a portion being sent to the office of weights and measures and the others passing directly into sales.

4.2. Quality control requirements

The tolerances are:

- Chamber depth to ± 2% of the nominal value as per the grid requirement.
- For distances of less than 0.4 mm between any net lines ± 0.002 mm of the nominal value.
- For distances of 0.4 mm or more between any net lines ± 0.5% of the nominal value.
- Net division angle ± 1°.

The width of the lines shall not be greater than 0.005 mm.

The flatness tolerances according to DIN 7184 Part 1 are:

- For the chamber base in the counting net 0.002 mm.
- For the cover slip contact surfaces in the region of a counting net 0.002 mm.
- For the cover slip surfaces 0.003 mm.

The cover slips shall meet the requirements of DIN 58884.

5. Filling the counting chamber

5.1. Sliding on the cover slip

The external supports are moistened with distilled water and then the cover glass is pushed with gentle pressure from the front of the counting chamber.

⚠ Caution

The cover glass is fragile!

The formation of interference lines (Newton rings) between the outer webs and cover glass shows that the cover glass is placed correctly (figure 4).

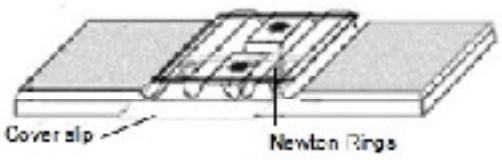


Figure 4

5.2. Feeding

Take a well-mixed pipette from the shaker and discard the first drop.

Wipe the outside of the pipette dry and tilt at an angle until a small droplet is formed at the pipette tip.

This drop is then placed between the cover glass and the counting chamber.

Capillary action fills the gap between cover glass and the chamber base. Before the diluted blood solution reaches the edges of the chamber base, the tip should be pulled away again. If air bubbles are visible or if the liquid spills over the edges into the grooves, the chamber must be cleaned and fed refilled (figure 5).

5.3 Use of blood pipettes


Figure 5

a) Erythrocyte - pipette (red ball)

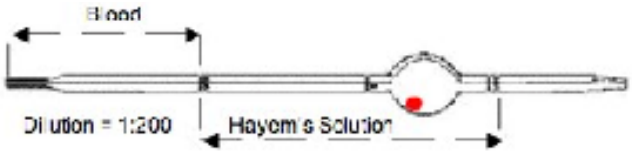


Figure 5.1

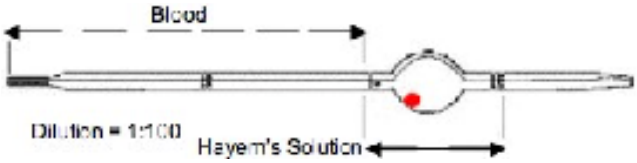


Figure 5.2

b) Leucocyte - pipette (white ball)

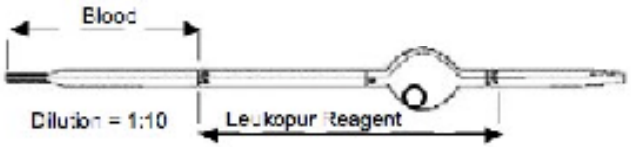
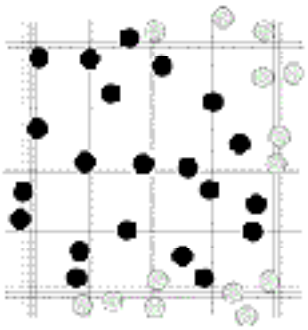


Figure 5.3

6. Counting particles

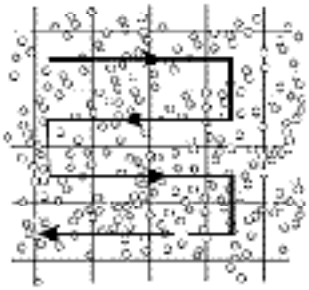
6.1. Counting technique

Cell counting assumes that a user possesses an exact knowledge of the limit lines of the counting chamber grids. An example of this is shown in the following figure (Improved Neubauer - figure 6).
In order to ensure that blood cells that lie on or near the limit lines are not double counted or ignored in the count, one must count using certain rules.



The figure above (figure 6) illustrates all the counted cells defined within the measuring range of the one large square. Cells are counted as being in the large square if they are touching or resting over the left and upper dimension lines or completely within the enclosed smaller squares. The cells to be valid to be counted are marked in black.

One should count the cells within the large squares in the manner shown below in Figure 6.1.



The count starts at the upper left corner of the arrow and continues through the large squares in a back and forth fashion.

6.2. Notes on counting

a) When using a counting chamber, the lens aperture of the microscope should be nearly closed.

b) The variation in counted cell numbers between the large squares included in the group should not exceed 10 cells.

c) All counts should be duplicated. After counting in the upper counting grid, the lower grid should be counted as a control. It is important to make sure that the sample does not dry out. This can be avoided by filling the counting chamber just shortly before the count then performing the count after the sedimentation time has expired.

d) The difference between the sums of the counts for the two counting grids must not exceed 10 cells. The mean of the counts is then used in the calculation formula (or multiplied by the appropriate factor).

7. Calculations

Formula:

$$\frac{\text{Number of cells counted}}{\text{Surface area counted (mm}^2\text{) x Depth of chamber (mm) x Dilution factor}} = \text{Cells per µL of original sample}$$

Example:
Counting chamber: Neubauer improved

a) Leucocytes
1. Counted cells = 156 Leucocytes
2. Surface area counted = 4 large squares, ie 4 mm²
3. Chamber depth = 0.1 mm
4. Dilution = 1:20

$$\frac{156 \times 20}{4.0 \times 0.1 \times 1} = 7800 \text{ Leucocytes/µL}$$

b) Erythrocytes
1. Counted cells = 528 Erythrocytes
2. Surface area counted = 5 small squares, ie 0.2 mm²
3. Chamber depth = 0.1 mm
4. Dilution = 1: 200

$$\frac{528 \times 200}{0.2 \times 0.1 \times 1} = 5.28 \times 10^6 \text{ Erythrocytes/µL}$$

8. Cleaning of counting chambers

Immediately after completing the count, remove the cover glass and clean the counting chamber with water or with 0.7% sodium Mucasol.

⚠ **Note**
At this concentration the Mucasol does not attack the glass surface of the chamber!

Subsequently, the chamber should be dried with a soft cloth.

Alternatively, the counting chamber may be rinsed with acetone after cleaning with water. It will then dry streak-free.

9. A short description of the most commonly used counting chamber:

The various systems used for counting chambers differ in the design of the counting grid and the chamber depth. The counting grid is made up of a square net division which is not visible until it is placed under a microscope (approx. 100 times magnification).

Here is a short description of the most common system:

Neubauer improved
Largest square size: 1 mm²
Group square: 0.4 mm²
Smallest square size: 0.025 mm²
Depth of chamber is 0.100 mm

The net division of these chambers has 3 times 3 large squares each with an area of 1 mm^2.

The four corner squares are used for leucocyte counts.

The large square in the middle is also divided into a five by five group of squares with an edge length of 0.2 mm and an area of 0.04 mm^2 each.

These groups of squares are in turn divided into sixteen very small squares each with an area of 0.0025 mm^2.

Five of these group squares are used for erythrocyte counts.

Special attention should be given to the fact that the chamber has triple lines on all sides, of which the central line is to be regarded as the actual dimension line.

This is important for deciding whether cells in the border area are to be counted or not.

Information and support

Visit www.trajanscimed.com or
contact techsupport@trajanscimed.com

Specifications are subject to change without notice.

CE IVD

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