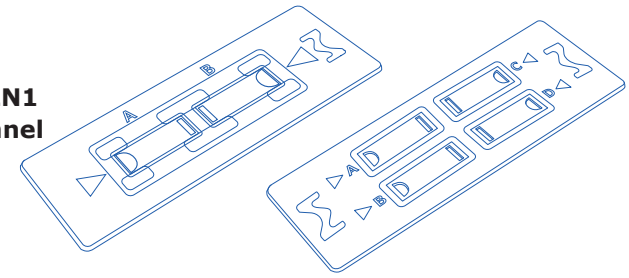
User Guide

Millicell® Disposable Hemocytometer

Neubauer Improved

MDH-2N1
2 Channel



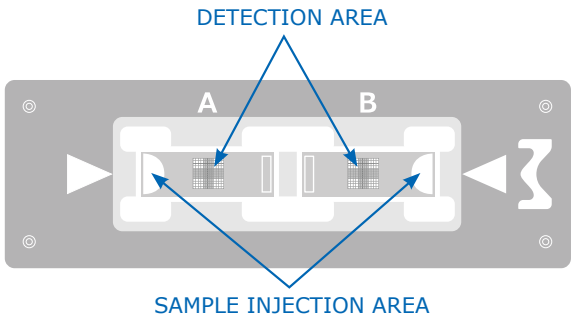
MDH-4N1
4 channel

Introduction

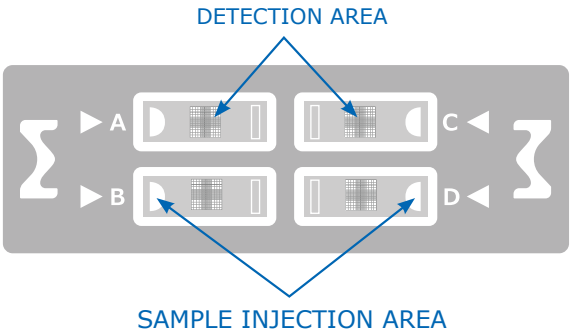
The plastic Millicell® Disposable Hemocytometers (2 and 4 channel) are used for manual cell counting. They consist of surface-patterned enclosed chambers with ports for sample injections (Figure 1).

Figure 1

2 CHANNEL



4 CHANNEL



Safety Precautions

For analyzing hazardous or potential infectious materials:

- Take necessary precautions
- Handle with care
- Dispose in an appropriate way

Long exposure to solvents will cause the slide to warp.

Xylene and toluene based mounting media should be avoided.

Glycerol, gelatin, and other aqueous-based media are recommended.

Safety Symbols

The safety symbols on the Millicell® Disposable Hemocytometer are intended to inform you of potential danger or a particular caution. Before use, please read and the consult the guide for the symbols and their meanings.

LOT	Batch code (Lot number)		Use by
	Do not reuse		Manufacturer

NOTE: The Millicell® Disposable Hemocytometer is for single use only. Do not reuse.

It should be used immediately after unsealing.

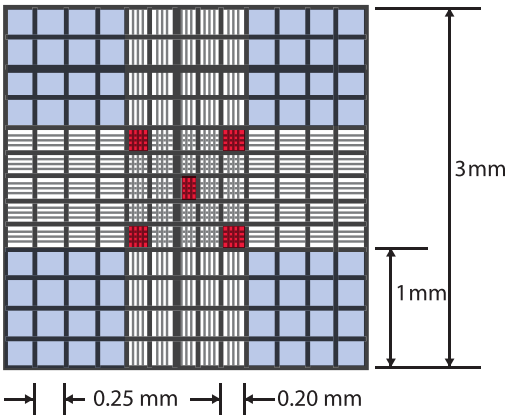
Neubauer Improved Grid Pattern

The Millicell® Disposable Hemocytometer uses a standard Neubauer Improved grid pattern. It consists of 9 large squares, each measuring 1 X 1 mm with a chamber depth of 0.1 mm. Each square has a total volume of 0.1 mm^2 or 10^{-4} cm^3 (Figure 2).

The central square (which can be seen in its entirety with 10X objective) is divided into 25 medium squares (seen in entirety at 40X); each of these is again divided into 16 small squares each with an area of 0.025 mm^2. The four corner squares are divided into 16 smaller squares (Figure 2).

Counting can be done in either the central large square (using the 5 small red squares) or the blue corner squares, depending on the size of cell being studied and estimated concentration in the sample.

Figure 2
Grid pattern of Neubauer Improved



Counting with Millicell® Disposable Hemocytometer

These devices are for single use only and should be used immediately after unsealing.

Once removed from packaging, avoid touching the detection areas as smudges could impair visualization and counting.

Depending on the type of sample, a suitable dilution concentration should be prepared for cell counting. Typically, the concentration range for a cell count with Neubauer Improved Chamber is between 5 X 10^4 and 5 X 10^6 cells/ml. If the sample is too concentrated, the cells will be too crowded and difficult to count. If it is too dilute, the sample size will not be enough to make strong inferences about the concentration in the original mixture.

Adherent Cell Counting

1. Prior to counting, adherent cells must be detached from their growing surface using Trypsin-EDTA or other method.
2. Transfer detached cells to a conical tube and centrifuge to pellet.
3. Carefully aspirate off supernatant without disturbing the pellet.
4. Thoroughly re-suspend the pellet in an appropriate volume of growth media or PBS to ensure a single cell suspension is achieved (no cell clumps or aggregates).
5. It may be necessary to further dilute your sample prior to loading.
6. Load 10 µL sample into the sample injection area outlined in Figure 1. Apply slow and steady pressure to prevent introduction of air bubbles.
7. Count the cells in the 4 large corner squares using 10X objective.

$$\text{Cells per mL} = \frac{\text{cells in 4 corner squares}}{4} \times \text{dilution factor (step 5)} \times 10^4 \text{ (volume factor)}$$

Note: If working with suspension cells, Steps 1-3 above may be omitted. The sample will need to be diluted to an appropriate concentration before loading into the hemocytometer.

Erythrocyte/RBC Counting

1. Erythrocyte concentrations in blood are quite high; a routine starting dilution is 1:200.
2. Load 10 µL sample into the sample injection area outlined in Fig. 1. Apply slow and steady pressure to prevent introduction of air bubbles.
3. Count the erythrocytes in the 5 small red squares of the large central square using 40X objective.

$$\text{RBCs per mL} = \frac{\text{cells in 5 small red squares}}{5} \times 25 \times 200 \text{ (dilution factor)} \times 10^4 \text{ (volume factor)}$$

Note: A similar procedure can be used for counting platelets, certain yeast cells and types of bacteria.

Troubleshooting

In case of poor visibility results:

- If detection area seems smudged or dirty, wipe surface with a Kimwipe® or similar paper fiber optic cleaning wipe.
- Adjust the focus of the microscope. Large cells can be viewed using 10X objective; for smaller cells, use a 40X objective.

Product Ordering

Purchase online at SigmaAldrich.com/products.

Description	Device Count	Chanel	Type	Chamber Depth	Catalogue No.
Millicell Disposable Hemocytometer 2N1	50	2	Neubauer Improved	100 um	MDH-2N1-50PK
Millicell Disposable Hemocytometer 4N1	50	4	Neubauer Improved	100 um	MDH-4N1-50PK

Notice

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