

Most common errors in cell counting.

Most significant errors.



ERROR IN OBTAINING THE SAMPLE

“The sample is not the population”

Usually, a small quantity is extracted from the original sample in order to carry out the counting. This sample will have a different concentration than the original one. Once this is done, the content is pipetted into the counting chamber

How to reduce this error?

In order to reduce this error it is convenient to shake the sample with a shaker, and extract the necessary quantity to carry out the cell counting as soon as possible. Likewise, a pipette can be used to shake the sample, pipetting repeatedly into the recipient where the sample is taken from.

ERROR IN LOADING THE NEUBAUER CHAMBER.

It is common to load the Neubauer chamber with a quantity slightly greater or smaller than 10 microliters. When the quantity is greater than 10 microliters, the coverglass will slightly rise in order to allocate the overload. When the quantity is smaller than 10 microliters, the chamber will not hold enough quantity, leading to an error.

Our estimation is that this error moves around 3-5%.

How to reduce this error?

- 1) Being careful when introducing the sample in the chamber not to overload it.
- 2) Some technicians slightly heat up the coverglass with their breath, before putting it on the counting chamber. Later, they observe that some multicolor rings have been created around the surface of the coverglass, indicating that it has been attached properly.
- 3) Keeping the micro-pipettes perfectly calibrated.



STATISTICAL ERROR

This error is encountered when trying to approximate a population (original sample) to a sample from that population that is too small.

Most of lab-technicians use 4 or 5 grids of the counting chamber, regardless the concentration.

This error moves around 20-30%.

How to reduce this error?

- 1) In order to reduce this error, the size of the sample should be taken into account when calculating the concentration and the number of samples used.

For a culture of 100ml with a Neubauer Chamber:

Concentration	Number of Samples (1)	Maximum Error Range (confidence. 95%)	Maximum Error Range (confidence. 90%)
1.0 x 10 ⁶ cel/ml	1	43,10%	36,19%
1.0 x 10 ⁶ cel/ml	2	30,49%	25,59%
1.0 x 10 ⁶ cel/ml	3	24,89%	20,59%
1.0 x 10 ⁶ cel/ml	4	21,56%	18,09%

NOTE: The estimation of the error has been carried out presuming a confidence interval for variance of 22% for the samples

(1) A sample is considered when 5 grids of the Neubauer chamber have been counted.

- 1) As a general rule, some laboratories count 80 or 100 cells, regardless the concentration. This sometimes implies loading some counting chambers to calculate a concentration, if this is very small.

ERROR IN THE ALLOCATION OF THE CELLS AROUND THE NEUBAUER CHAMBER.

Occasionally, the sample does not distribute equally around the counting chamber, due to its inclination or manipulation



How to reduce this error?

- 1) Shake the sample before introducing it into the counting chamber.
- 2) Make sure that the counting chamber lies completely flat when introducing the sample

HUMAN ERRORS

There are various types of errors that are made by the person who carries out the cell counting, directly or indirectly.

- **Bad visualization of the sample:** if the person who carries out the cell counting does not visualize the sample properly, some of the cells may be left out. Moreover, other elements might be taken for cells, when they are not.

How to reduce this error?: using an optimal microscope in good state. Carrying out the manipulation of the microscope properly and using the appropriate optic for the size of the cells.

- **To lose count:** if the person who carries out the cell counting loses count, he/she should start again.

How to reduce this error?: using an automated counter. It is also important that the person who carries out the cell counting is always the same.

- **Error when applying the formula:** if the *counting* formula is applied in the wrong way, wrong results will be obtained. This is one of the main sources of confusion and error when carrying out the cell counting.

How to reduce this error? Using an automated counter and the same counting chamber. Check the size of the chamber and make sure that the formula applied makes sense.

- **Error in counting the borders:** it occurs when the person who carries out the cell counting can't tell whether a cell is in or out of the counting. It is estimated that this error is smaller than 1%.

How to reduce this error? Using an automated cell counter. Using a clear criteria when selecting the borders

ERROR IN DILUTION

Any error made when using the liquids to carry out the dilution will translate into an error made in the diluted sample.

How to reduce this error?: to calibrate periodically the pipettes and other measurement instruments.

ERROR IN CULTURING DEAD AND APOPTOTIC CELLS

When culturing cells, it is necessary to know the percentage of dead and alive cells (viability). If we only count the total amount, we will be making an error proportional to the viability of the sample. Only alive cells will grow.

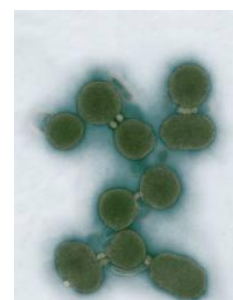
How to reduce this error? to calculate manually or automatically the viability.

ERROR WHEN COUNTING DUE TO AGGREGATES

Under certain circumstances, some types of cells tend to aggregate, making it harder or even impossible to count.

Flow Cytometers are especially problematic when dealing with aggregates, since a group of various cells will be counted as one single cell.

This error moves around 20-30%.



How to reduce this error? to make sure that the sample is in good conditions. Carry out the counting immediately after loading the counting chamber.

REFERENCES

[1] A SIMPLIFIED METHOD OF PHYTOPLANKTON COUNTING, Eva Willen. Br. Phycol. J. 11:235-278, September 1976.

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