Use of haemocytometer to quantify concentration of cells' suspensions V.2

abotero [1]

[1]University of Alberta

2

Dec 16, 2021

1

dx.doi.org/10.17504/protocols.io.b2yvqfw6

Plant pathology

abotero

This protocol presents the procedures to estimate the concentration of cells' suspensions using an hemocytometer

DOI

dx.doi.org/10.17504/protocols.io.b2yvqfw6

abotero 2021. Use of haemocytometer to quantify concentration of cells' suspensions. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b2yvqfw6 abotero

Hemocytometer

protocol ,

Dec 16, 2021

Dec 16, 2021

56053

The lower limit for accurate counting of cells in a hemocytometer is usually considered to be **2.5 x 10^5/ml.**

For cell numbers greater than **2.5 x 10^6/ml,** it is generally recommended that the sample be diluted.

Hemocytometer
Microscope
Vortex (optional)
Micropipette 10-100µl
Pipette tips

:

1 Wash the hemocytometer with a washer bottle and dry it using a paper towel

2 Cover the hemocytometer with the coverslip and place it on a flat surface

3 Vortex briefly the cell suspension whose concentration will be calculated

Citation: abotero Use ofÃÂ haemocytometer to quantify concentration of cells' suspensions
https://dx.doi.org/10.17504/protocols.io.b2yvqfw6

4 Using a micropipette collect 10-100 µl of the previously agitated suspension

5 Place the micropipette tip on the hemocytometer edge near the coverslip

6 Empty the spore suspension slowly into the chamber of the hemocytometer

7 Place the hemocytometer in the microscope's mechanical stage and secure it using the stage clip

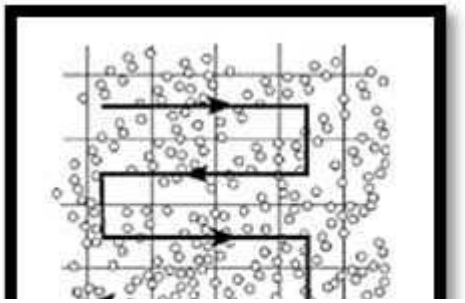
8 Turn on the microscope and focus on the hemocytometer

9 Look out for the first square to be counted

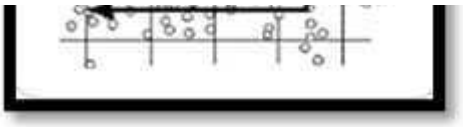
10 Count the number of cells on each square

If the cells are touching the upper or the left lines of the square, cells are to be counted, but if they are touching the lower or right line they should not be counted

11 Count all the squares following a zig-zag



Citation: abotero Use ofÃÂ haemocytometer to quantify concentration of cells' suspensions
https://dx.doi.org/10.17504/protocols.io.b2yvqfw6



12 Write down the number of cells on each square

The lower limit for accurate counting of cells in a hemocytometer is usually considered to be ***2.5 x 10^5/ml.***

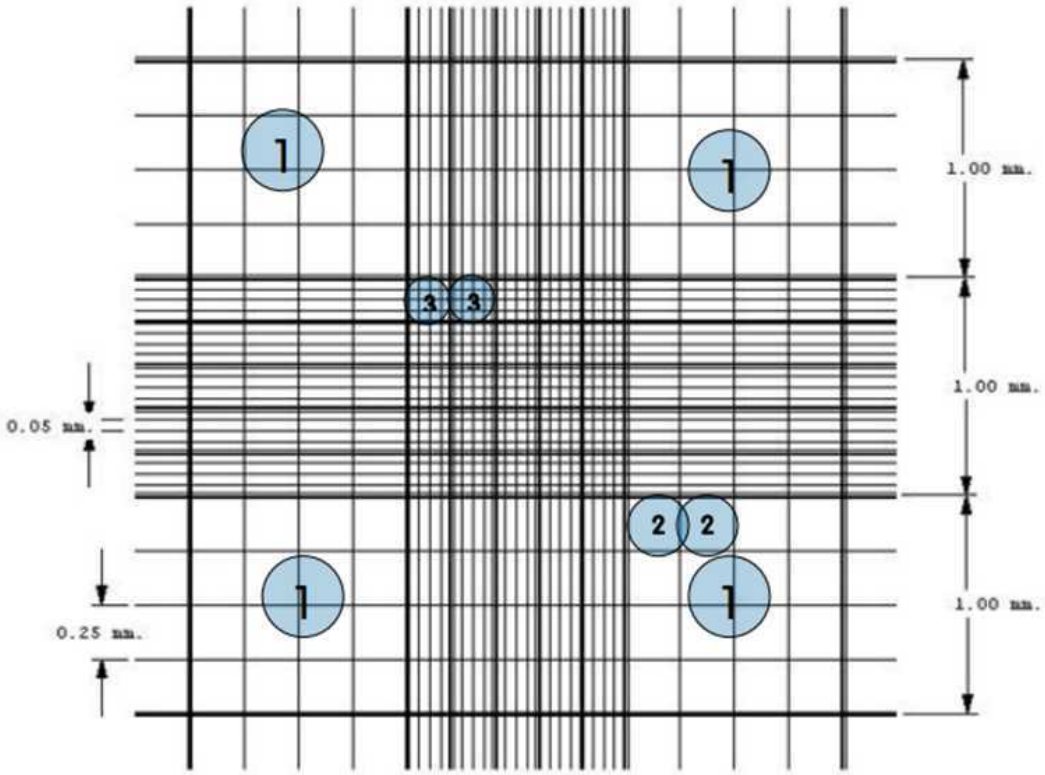
For cell numbers greater than ***2.5 x 10^6/ml,*** *it is generally recommended that the sample be diluted.*

Count the number of cells on at least five squares. Ideally, repeat the procedure twice for higher accuracy

13 Estimation of the concentration

According to the cell's size cells can be counted on squares 1, 2 or 3

Citation: abotero Use ofÃÂ haemocytometer to quantify concentration of cells' suspensions
https://dx.doi.org/10.17504/protocols.io.b2yvqfw6



13.1 If cells were counted on squares of size 1

Concentration (cells/ml)= Number of counted cells*10,000/Number of squares counted

If the cells' suspension has been diluted the formula should be adjusted to:

*Concentration (cells/ml)= Number of counted cells*10,000/Number of squares counted*dilution*

13.2 If cells were counted on squares of size 2

Concentration (cells/ml)= Number of counted cells*160,000/Number of squares counted

If the cells' suspension has been diluted the formula should be adjusted

Citation: abotero Use ofÃÂ haemocytometer to quantify concentration of cells' suspensions
https://dx.doi.org/10.17504/protocols.io.b2yvqfw6

to:

Concentration (cells/ml)= Number of counted cells*160,000/Number of squares counted*dilution

13.3 If cells were counted on squares of size 3

Concentration (cells/ml)= Number of counted cells*250,000/Number of squares counted

This square size is typically used to count *Plasmodiophora brassicae* resting spores

If the cells' suspension has been diluted the formula should be adjusted to:

Concentration (cells/ml)= Number of counted cells*250,000/Number of squares counted*dilution

Citation: abotero Use ofÃÂ haemocytometer to quantify concentration of cells' suspensions
https://dx.doi.org/10.17504/protocols.io.b2yvqfw6