THOMA AND NEUBAUER IMPROVED CHAMBER COUNTING YEASTS (TIRAGE)

In this document it is intended to explain clearly and easily the process of counting and viability of yeasts for the process of transformation of wine into champagne/cava as well as the use of the corresponding formulas for each case. It is an easy process but it requires some patience for not getting wrong and obtain a good results.

**Thoma chamber.** Basic Data:

Big central square (center of the cross, 400 small squares): \(1 \text{mm}^2/400\) squares.
Medium square (formed by \(5\times5=25\) small squares): \(0.25 \text{ mm} \times 0.25 \text{ mm}\)
Small square: \(0.05 \text{ mm} \times 0.05 \text{ mm} = 0.0025 \text{ mm}^2\)
Division lines (in red): 0.025 mm from the extreme of small square

**Neubauer improved chamber.** Basic data (same measures as before except medium square):

Big central square (formed by 400 little squares): \(1 \text{mm}^2/400\) squares.
Medium square (formed by \(4\times4=16\) small squares): \(0.2 \text{ mm} \times 0.2 \text{ mm}\)
Small square: \(0.05 \text{ mm} \times 0.05 \text{ mm} = 0.0025 \text{ mm}^2\)
Division lines: 0.025 mm
The difference between Thoma chamber and Neubauer chamber lies in lateral squares printed with an “L”. These are used for counting other cellular microorganisms. For the case of “tirage” there is no difference between them because it is only used the central square.

![Fig. 3: Neubauer improved chamber](image)

**How chambers are used?**

Sample preparation:

1. Clean the chamber and the coverslip with distilled water and alcohol 96%
2. Dry them well with soft paper.
3. Set the coverslip over the chamber.
4. Homogenize, stirring well, the sample where yeasts lie.
5. Take a sample with a pipette.
6. Set the end of the pipette in one of the two slots of the chamber and, by capillarity, yeasts will distribute in the chamber.
7. If an air bubble appears repeat the operation from the beginning.
8. Set the chamber in the deck of the microscope to carry out the microscopic observation.
9. Wait few minutes before counting in order yeasts lie at the bottom of the chamber.

Microscope preparations:

The focus of the microscope begins with the lower zoom objective which later will be changed to one of higher zoom. It is centered the microscope objective approximately at the center of the cross of the chamber, then the chamber is placed nearly touching the objective and lately the chamber will be moved down slowly till the image appears clearly. It is advised to work at x400.

Counting total yeasts:

It is advised to perform the average of yeasts contained in several groups of squares. Using one chamber or other the procedure is the same. For example according to fig. 4, it is presented to count yeasts contained in groups of 25 and 16 squares respectively depending on the chamber used.
I.e. 1. (Thoma chamber, groups of 25 squares):

- Group 1 (G1) of 25 squares --> 14 yeasts
- Group 2 (G2) of 25 squares --> 15 yeasts
- Group 3 (G3) --> 13 “
- Group 4 (G4) --> 14 “

Arithmetic average is calculated: \(\frac{14+15+13+14}{4} = 14\) average yeasts contained in 25 squares.

And apply the generic formula as follows:

\[
\frac{X \text{ yeasts}}{Y \text{ squares}} \times \frac{\# \text{ chamber squares}}{\text{Chamber volume}} \times \frac{1000 \text{ mm}^3}{1 \text{ cm}^3(0.1 \text{ mL})} = X \text{ millions of yeasts} / \text{mL}
\]

Both chambers have 400 useful squares.
Thoma chamber volume = 1 mm x 1 mm x 0.1 mm (long x wide x deep) = 0.1 mm³
Neubauer improved chamber useful volume = 0.2 mm x 0.2 mm x 0.1 mm x 25 = 0.1 mm³

That is to say, even we use the Thoma chamber or the Neubauer improved one, for both, it is used the same formula in which the variables to use will be the number of yeasts contained in a determined number of squares:

I.e. 2.: If 14 yeasts are found in 25 squares of the Thoma chamber, the calculation would be as follows:

\[
\frac{14 \text{ yeasts}}{25 \text{ squares}} \times \frac{400 \text{ squares}}{0.1 \text{ mm}^3} \times \frac{1000 \text{ mm}^3}{1 \text{ cm}^3(0.1 \text{ mL})} = 2.24 \text{ millions of yeasts} / \text{mL}
\]

Using a Neubauer improved chamber is going to be proved that differences does not exist between both chambers. Let’s convert the 14 yeasts in 25 squares to \(X\) yeasts in 16 squares applying a rule of three:

14 —— 25
\(X——16\)

If there are 14 yeasts contained in 25 squares, how many yeasts will be contained in 16 squares?

\[
X = \left(\frac{16 \times 14}{25}\right) = 224 / 25 = 8.96
\]
So, the Neubauer improved chamber will contain:

\[
\frac{8.96 \text{ yeasts}}{16 \text{ squares}} \times \frac{400 \text{ squares}}{0.1 \text{ mm}^2} \times \frac{1000 \text{ mm}^3}{1 \text{ cm}^3 (0.1 \text{ mL})} = 2.24 \text{ million of yeasts / mL}
\]

It is proven that both chambers work the same way.

A trick exists in order to avoid such calculations. It is faster but at the same time introduces a small error. Form groups of 4 small squares in a diagonal and calculate the arithmetic average as it has been already explained before. Once the arithmetic average is calculated, multiply by one million and you will obtain the millions of yeasts contained per mL. Follow the example:

\[
X \text{ yeasts contained in 4 squares} \times 1.000.000 = X \text{ millions of yeasts / mL}
\]

I.e. 3.: According to fig. 5 If there are 3 groups of 4 little squares with 7 yeasts of average:

Arithmetic average \(7+6+8 = 21 / 3 = 7\) yeasts of average

The final result would be 7 yeasts contained in 4 squares \(\times 1.000.000 = 7\) millions of yeasts / mL

If there are some yeasts contained in some squares, the fast formula can not be used. First it should be transformed by another rule of three:

I.e. 4.: If there are 14 yeasts contained in 25 squares, how many yeasts would be contained in 4 squares?

The following rule of three must be applied:

\[
14 \quad 25 \\
X \quad 4
\]

\[
X = \frac{4 \times 14}{25} = \frac{56}{25} = 2.24
\]

Applying the fast formula:

\[
2.24 \times 1.000.000 = 2.240.000 \text{ yeasts / mL} \quad \text{or} \quad 2.24 \text{ million of yeasts / mL}
\]

It is obtained the same result as applying the generic formula.

Fig. 5: Counting example by groups of 4 in Thoma/Neubauer improved chamber
The final idea is to show that no matter which camera you use if yeasts are counted in 2x2 squares as small squares of both cameras have the same proportions as detailed on the first page, so the volume of one camera is the same as the other. If the volume is a fixed value of 4,000,000 squares / cm³ (mL), if it is divided by 4 as it is the number of squares 2x2 commented we obtain a fixed value of 1,000,000 squares / cm³ (mL) which it is the value we will end multiplying the average number of cells contained in the four small squares (2x2) as seen on i.e. 4.

Counting viable yeasts (% viable yeasts):

1- Mix a little drop of dye with a little drop of must in fermentation in a normal slide.
2- Cover it with a coverslip.
3- It is displayed in a microscope.
4- Stained yeasts are dead and not stained cells are alive.
5- View 3 fields in the diagonal of the coverslip as it is shown in fig. 6. Count all yeasts contained in each field and make a list of dead and alive yeasts. At this point calculate the percentage of viability.
6- The calculation of the % viable yeasts is done as follows: living yeasts / total yeasts x 100 = X % viable yeasts

Note: The dye can be any known. If methylene blue is used, take in mind that it is harder to count yeasts because it stain too much the solution in blue, so it is strongly recommended to dilute the dye before using it with the sample to analyse or, on the other hand, use rhodamine instead. This one has a soft pinkish colour which makes simpler the counting. Both dyes stain dead yeasts.

I.e. 5.:

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<tr>
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\[
\frac{45}{45 + 3} = \frac{45}{48} = 0.93 \quad \text{The 93% are viable}
\]