

Yeast Count & Viability Double Thoma Haemocytometer

- Using the 1ml Pasteur pipette, measure out 1ml of the beer sample into the Sterilin bottle
- Using another Pasteur pipette measure out 1ml of the Methylene Blue solution and mix with the 1ml of beer sample
- Shake for 20 seconds
- Take the haemocytometer out of its case and breathe on the top surface to create condensation
- Firmly press the cover slip down onto the condensation to ensure a good seal is made
- Using the original pipette (the one used for transferring the beer), transfer some of the now mixed beer and MB solution onto the haemocytometer slide
- Just touch the edge of the Pasteur pipette against the cover slip
- Allow the fluid to seep under the cover slip by capillary action
- Place the slide under the microscope and ensure that the correct lens is chosen (x40)
- Once focused you should be able to find the grid shown:

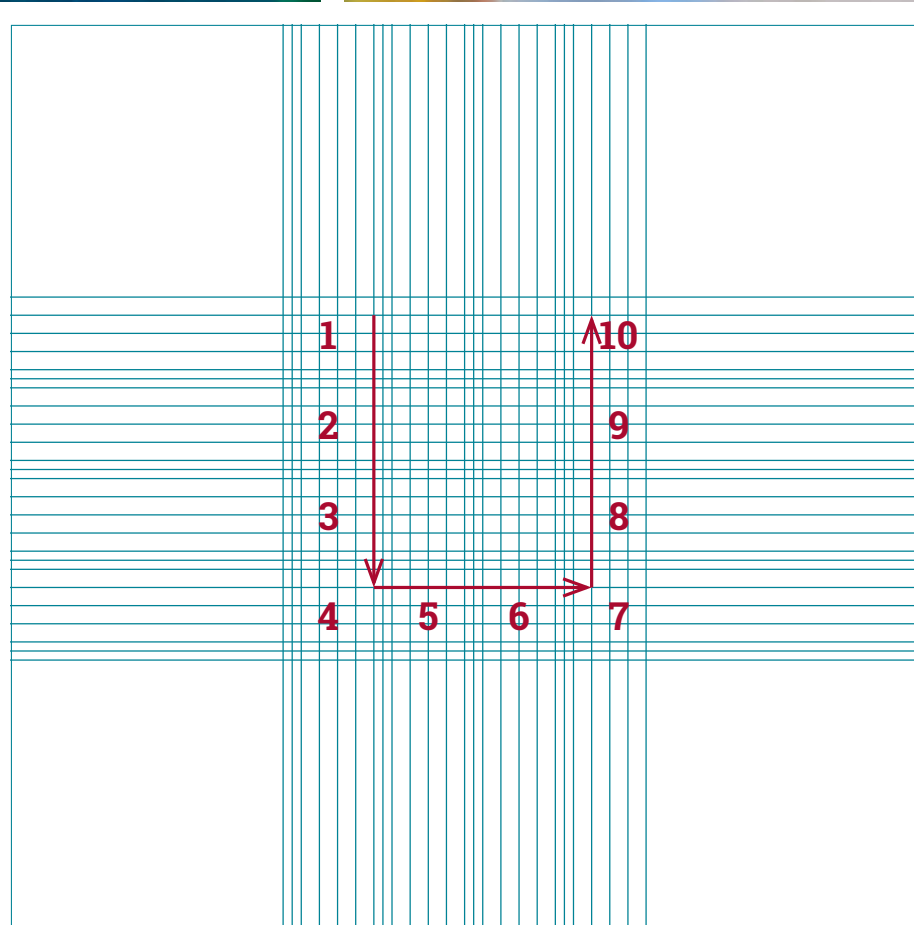


Figure 1

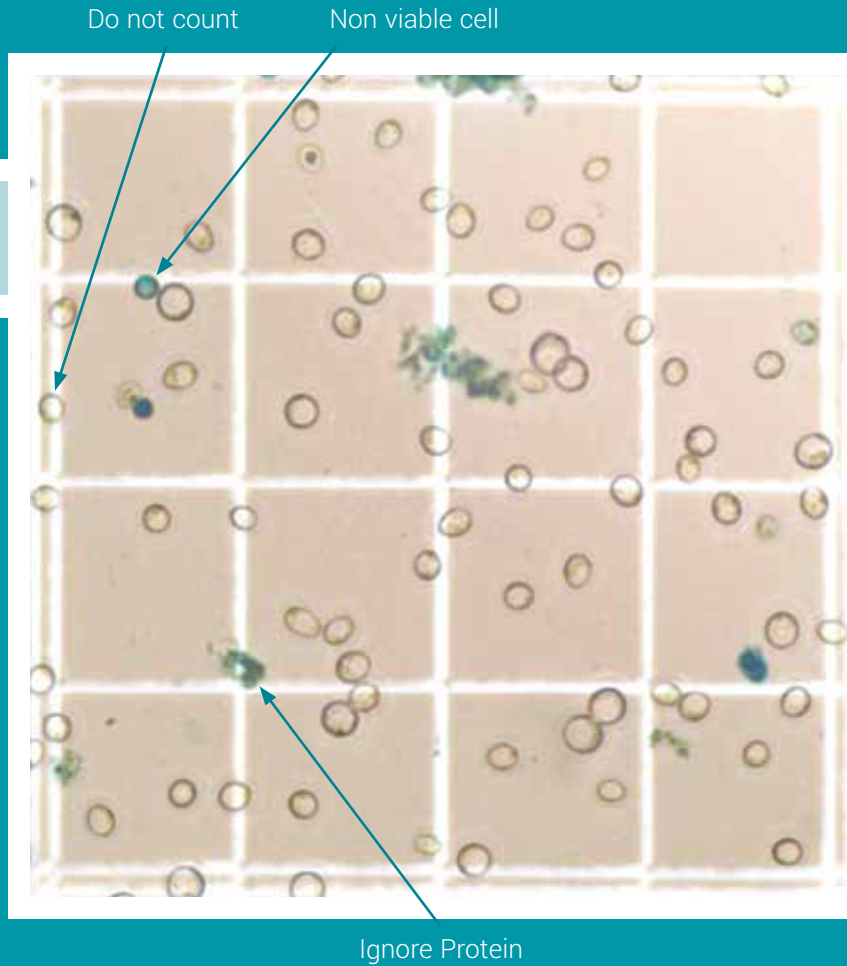


- Now count the cells in each box in Figure 1 as shown, labelled 1 – 10 using the following photo as a guide:

Figure 2



- This photo represents one of the smaller boxes labelled 1 – 10 (Figure 1)
- Count the entire number of cells in this 4x4 grid
- Only count cells that are completely inside the grid
- For cells that are overlapping outer grid lines only count cells that are 50% + inside the grid
- Ignore the fluffy lumps of protein
- Once all cells are counted then count the number of 'non viable' cells which are blue from soaking up the MB dye
- For total count and viability use the working out below:



Total Count



• 62	Total # of cells counted (Example)
• x 2	Dilution factor from methylene blue
• Equation:	$(\text{Number of cells} / 0.04) \times 1000 = \text{Total Number of cells per ml}$
IE:	$(124 / 0.04) \times 1000 = 3,100,000 = 3.10 \times 10^6 \text{ per ml Total Cells}$

Non Viable Cells



• 6	Total # of cells counted that are blue (Example)
• x 2	Dilution factor from methylene blue
• Equation:	$(\text{Number of cells} / 0.04) \times 1000 = \text{Total Number of cells per ml}$
IE:	$(12 / 0.04) \times 1000 = 300,000 = 0.3 \times 10^6 \text{ per ml Total Cells}$

Viability (%)



$(0.3 / 3.10) \times 100$	9.67 % Non Viable Cells
$100 - 9.67$	90.33 % Total Viability



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