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Cell Counting V.2

Laura Sánchez[1]

[1]AEGIS - Madrid iGEM 2019

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AEGIS - Madrid iGEM 2019

Manuela de las Casas
Universidad Complutense de Madrid, AEGIS - Madrid iGEM 2019

ABSTRACT

Bacterial concentration can be measured by several methods. One of them is turbidity. Turbidity, or light scattering, is measured in a spectrophotometer such as a Spectronic 20. This method has the advantage of being the quickest method at the lab bench.

MATERIALS TEXT

Reagents

- Turbid broth culture of E. coli
- Tryptic soy broth (TSB)

Lab equipment

- 21 clean culture tubes (they do not need to be sterile)
- Clean spectrophotometer cuvettes
- 10-1000 ml pipets
- Pipump
- Vortex mixer
- Spectrophotometer

SAFETY WARNINGS

Please note: Be sure to wear gloves during this procedure, to avoid getting culture on your hands while transferring liquid from tubes. Lab coat is also required. If a laminar flow cabinet is available, it is recommended for this procedure.

BEFORE STARTING

Check all working surfaces and materials are clean before start

Making an absorbance curve for Escherichia coli

1 Turn on the spectrophotometer and set the wavelength to 425 nm. Let it warm up for at least 15 min.

2 Label the culture tubes 1 – 21. Using different pipets, add turbid E. coli culture and TSB to each tube in the volumes shown in the table.

Dilution #	*Volume of Turbid Culture, ml*	*Volume of Sterile Broth, ml*	*Dilution Factor*
1	10.0	0.0	1
2	9.5	0.5	0.95
3	9.0	1.0	0.9
4	8.5	1.5	0.85
5	8.0	2.0	0.8
6	7.5	2.5	0.75
7	7.0	3.0	0.7
8	6.5	3.5	0.65
9	6.0	4.0	0.6
10	5.5	4.5	0.55
11	5.0	5.0	0.5
12	4.5	5.5	0.45
13	4.0	6.0	0.4
14	3.5	6.5	0.35
15	3.0	7.0	0.3
16	2.5	7.5	0.25
17	2.0	8.0	0.2
18	1.5	8.5	0.15
19	1.0	9.0	0.1
20	0.5	9.5	0.05
21	0.0	10.0	0

3 Mix each tube thoroughly by vortexing for 1 – 2 sec with a vortex mixer.

4 With no tube in the spectrophotometer, set the 0%T setting.

5 Transfer 3 – 4 ml of broth from tube 21 (TSB with no E. coli) to the spectrophotometer cuvette, carefully wipe the outside of the tube with a kimwipe, place the tube in the machine, and set the 100%T setting (blank the spectrophotometer). Switch the spectrophotometer setting to “Absorbance.”

6 Remove the cuvette and pour the broth back into tube 21. Carefully drain the last few drops from the lip of the cuvette into a kimwipe. Put the contaminated kimwipe in the biohazard bag.

7 Starting with tube #20 and going backwards to tube #1, repeat steps 5-6.

8 Transfer the data to a Microsoft Excel spreadsheet. The spreadsheet should contain the following columns: volume of E. coli culture, volume of broth, dilution factor, and measured absorbance.

9 In the spreadsheet, create an X-Y scatter plot of dilution factor vs absorbance.

* Please note: All charts must be added as separate sheets in the workbook, not pasted into the worksheet with the data columns.

10 Add a second order polynomial trendline to the plot, and set the options on the trendline to show the equation and the correlation coefficient.

11 From the plot, identify the range in which absorbance is proportional to bacterial concentration. Create a second plot, plotting only the linear range of dilution factor vs absorbance.

12 Add a linear trendline to the second plot, and set the options for the trendline to show the equation and the correlation coefficient.