Technical Note: OD_{600} Measurements

Creating Microbial Growth Curves

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Introduction

Strictly speaking optical density measurements of cell cultures at 600 nm (OD_{600}) are of light scattering as a factor of turbidity, not absorption. The degree of light scattering depends on biomass rather than cell number as distinct cell types vary in size and shape (Figure 1) which affects the degree of scattered light. Clumping together of cells will also affect readings, so a lower biomass inferring a lower OD_{600} reading is more reliable. Therefore target OD_{600} values for Exponential (Log) phase induction of cells should be around half of the Stationary phase OD_{600}. When this is achieved varies between cell type, the culture media used, and the growth conditions. Therefore, changes to cell species and strains, culture media, and growth conditions should be supported by new growth curves, observing OD_{600} and colony forming units per millilitre (CFU mL-1) as a function of time.

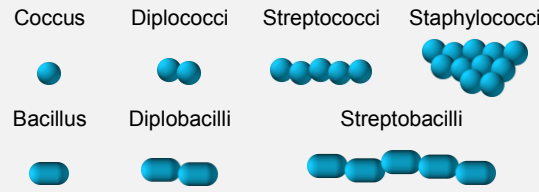

Figure 1: Some examples of common bacterial morphology.

About the Instrument

Different instruments will give different OD values for the same sample. This is due to the amount of scattered light that is 'seen' by the detector, and is influenced by the optical geometries such as slit size, detector size, and the distances between these optical components and the sample position (Figure 2).

A growth curve generated using one instrument cannot be reliably applied to OD_{600} measurements taken with a second instrument. If a sample is measured on the instrument used to create the growth curve ('Instrument 1'), then measured in

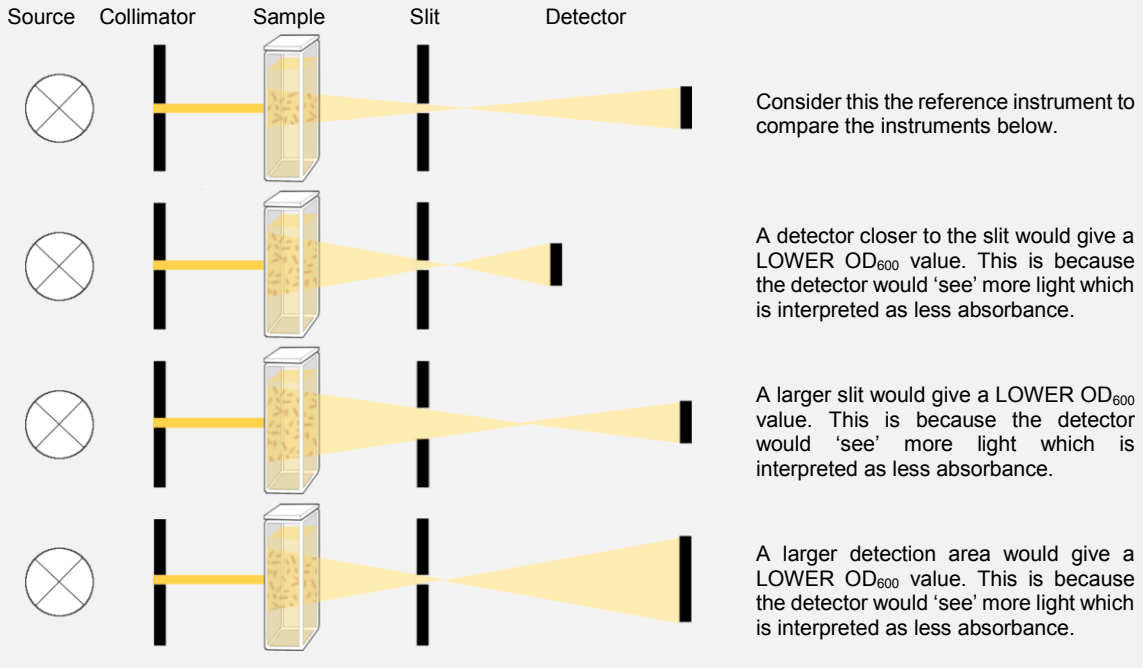

Figure 2: The illustration compares the effect of optical geometry on the measurement of turbid samples.

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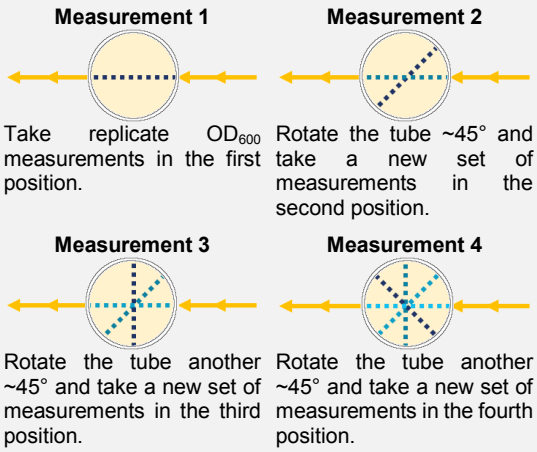

Figure 3: The illustration shows the above view of a test tube and how it can be rotated between replicate measurements to minimise the effects or irregular refraction.

the second instrument ('Instrument 2'), a factor can be determined using:

$$\frac{OD_{600}\ Instrument\ 1}{OD_{600}\ Instrument\ 2} = Factor$$

This multiplication factor can then be applied to subsequent OD_{600} measurements taken with 'Instrument 2' and the value will correspond more closely to the original growth curve.

About the Sample Vial

Various sample vial material and dimensions contribute differently to the degree of scattering. It is still not uncommon to take OD_{600} measurements using cylindrical tubes, such as test tubes. In this case, not only will the material and vial wall thickness have different transmission properties but also different refractive properties when compared the flat boundary surfaces of a cuvette. As a result, when measurements are made in test tubes the tube is rotated several times for each sample and the average is taken (Figure 3).

Just as separate growth curves are required for different cell species and strains, culture media, and growth conditions, new curves should be created for different sample vials types.

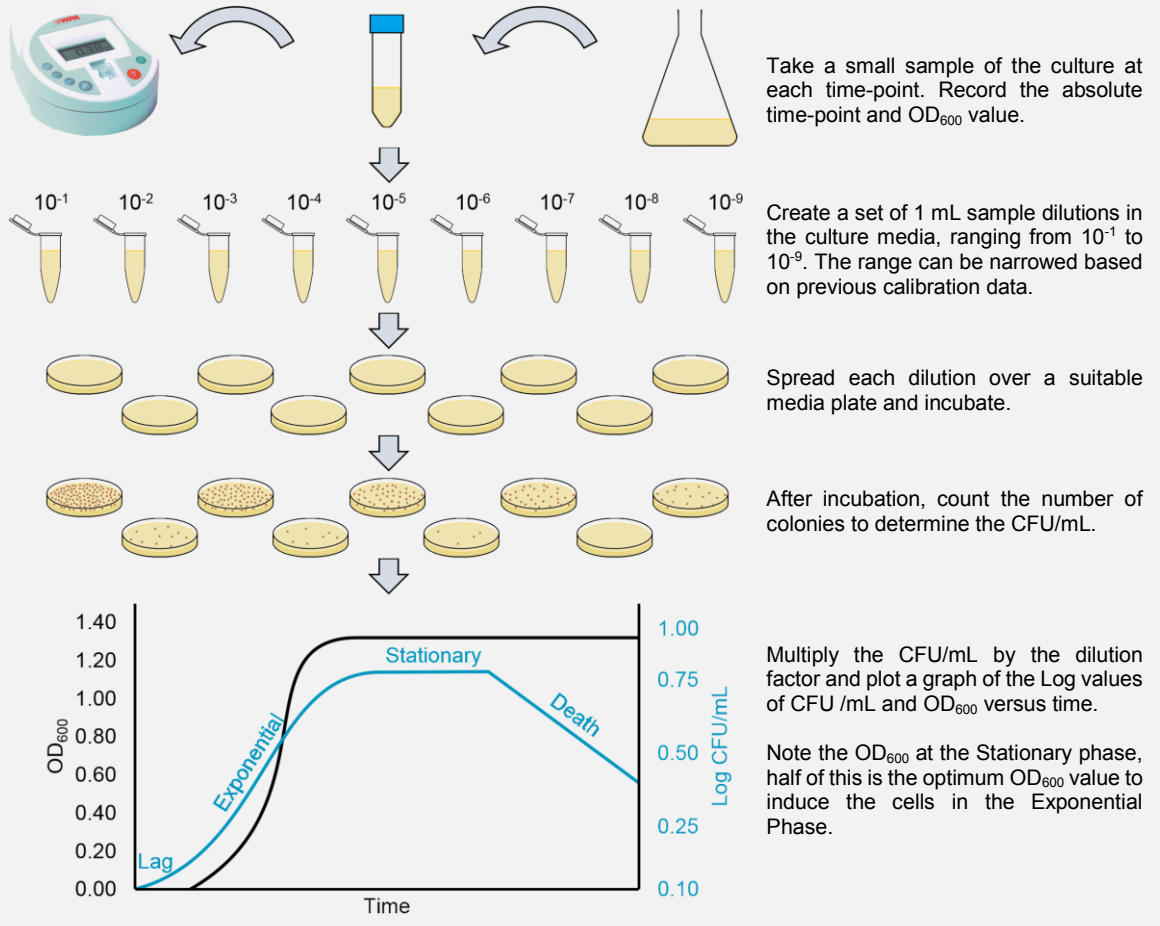

Figure 4: Illustration shows the process of creating a microbial growth curve. Note that when creating a set of dilutions, performing serial dilutions should be avoided as they are inherently inaccurate.

Creating a Growth Curve

From a growth culture, at the periodic intervals take an aliquot and make a set of 1 mL dilutions. Depending on your cell of interest, this time-point intervals can range from minutes to hours, days, weeks, or even months. For *E. coli* cultures this will typically be every 20-30 minutes. Take replicate OD_{600} measurements of each dilution then spread them on media plates. Incubate the plates and count the number of colonies for the plates where they are most distinguishable. Account for each plate's dilution factor and take the mean average. This is the number of colony forming units (CFU) in 1 mL. Plot the CFU per mL, and the mean replicate OD_{600} values against time.

Use this growth curve to highlight the OD_{600} value which correlates to the Exponential (Log) phase, at half the Stationary phase OD_{600}, to optimally induce the cells (Figure 4).

FAQ's

Q1 Can the dry weight of cells be used instead of CFU's?

A1 Yes, but the dry weight will not take into account dead cells.

Q2 Why are measurements taken at 600 nm?

A2 Conventionally measurements are taken at 600 nm because typical growth media is straw-orange in colour. 600 nm light is also orange, so it this eliminates any effects of absorbance on the light scattering.

Q3 Is it normal for OD_{600} measurements to be variable?

A3 Large variations, especially at very low and very high concentrations, are expected. Mean averages of replicate measurements when creating the growth curve and when taking measurements helps to minimise the inherent error in OD_{600} readings.

Q4 If OD_{600} measurements are of light scattering as a factor of turbidity, can I use turbidity standards?

A4 Turbidity standards are used to construct calibration curves to test turbid samples. This is the same process as creating growth curves of CFU and OD, but the turbidity standards will not likely share the same properties as the cell of interest.

Q5 Can I use the drop plate technique to determine CFUs?

A5 Yes, the spread plate technique described within this document is only detailed due to convention and is for illustrative purposes.

Bibliography

General reading

Caldwell, D. (1995). *Microbial physiology & metabolism*. Dubuque, Iowa: Wm. C. Brown.

Harding, S. E. (1986). Applications of Light Scattering in Microbiology. *Biotechnology and Applied Biochemistry*, 8(6), pp.489-509.

Koch, A. (1968). Theory of the angular dependence of light scattered by bacteria and similar-sized biological objects. *Journal of Theoretical Biology*, 18(1), pp.133-156.

Koch, A. (1970). Turbidity measurements of bacterial cultures in some available commercial instruments. *Analytical Biochemistry*, 38(1), pp.252-259.

Naghili, H., Tajik, H., Mardani, K., Rouhani, S. M. R., Ehsani, A., and Zare, P. (2013). Validation of drop plate technique for bacterial enumeration by parametric and nonparametric tests. *Veterinary Research Forum*, 4(3), pp.179-183

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