## Can you see something odd about this data set?

Spectrophotometer exercise section data  Section ID: 2

| | Optical density at 540 nm | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Coomassie Blue (µg/ml) | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 | Group 10 |
| 20 | 0.248 | 0.259 | 0.279 | 0.228 | 0.259 | 0.282 | 0.245 | 0.245 | 0.076 | 0.281 |
| 40 | 0.548 | 0.523 | 0.566 | 0.532 | 0.542 | 0.599 | 0.505 | 0.505 | 0.186 | 0.524 |
| 60 | 0.835 | 0.806 | 0.812 | 0.846 | 0.804 | 0.901 | 0.757 | 0.757 | 0.305 | 0.784 |
| 80 | 1.048 | 1.058 | 1.091 | 1.069 | 1.07 | 1.113 | 1.084 | 1.084 | 0.433 | 1.046 |
| 100 | 1.302 | 1.33 | 1.378 | 1.342 | 1.328 | 1.286 | 1.306 | 1.306 | 0.583 | 1.305 |

Grubb's test for detecting outliers:

$$Z = \frac{|mean - value|}{Standard\ deviation}$$

Outliers have Z value larger than critical Z value.

http://graphpad.com/support/faqid/1598/

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Z value for group 9 at 20 µg/ml = 2.72

The value is larger than the critical Z for sample size of 10 (2.29).

OUTLIER! → Remove the data!

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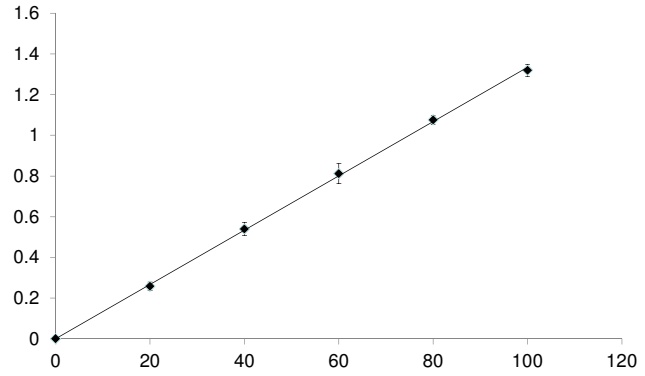
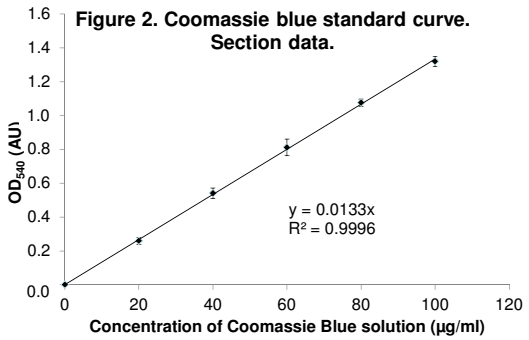
## What's wrong with this figure?




## Example figure




**Student A**

In part C, we want to analyze the activity of β-galactosidase by measuring the OD420, OD550 and OD600. Moreover, we utilize the optical density values along with the time and culture volume at which the assay was performed to calculate the units of β-galactosidase. The anticipated activity of β-galactosidase is shown in table 3.1.1 and we predict that the strains with high activity such as CAG12033+IPTG and ML308 with/without IPTG will show a higher value of β-galactosidase unit compared to the strains with low activities.

**Student B**

In part C of this experiment, the activity of β-galactosidase was analysed by measuring the OD420, OD550 and OD600. In addition, the time and the culture volume was recorded in order to calculate the unit of β-galactosidase. In the table 3.1 and 3.2 from the results had showed that the strains with high activity such as CAG12033+IPTG and ML308 with/without IPTG will show a higher value of β-galactosidase unit compared to the strains with low activities.

Plagiarism

**Wong et al. 2009**

Neurog3 (Neurogenin 3 or Ngn3) is both necessary and sufficient to induce endocrine islet cell differentiation from embryonic pancreatic progenitors. Since robust Neurog3 expression has not been detected in hormone-expressing cells, Neurog3 is used as an endocrine progenitor marker and regarded as dispensable for the function of differentiated islet cells. Here we used [illegible]-dependent lines of Neurog3 knock-in reporter mice and mRNA/protein-based assays to examine Neurog3 expression in hormone-expressing islet cells. Neurog3 mRNA and protein are detected in hormone-producing cells at both embryonic and adult stages. Significantly, inactivating Neurog3 in insulin-expressing cells at embryonic stages or in Pdx1-expressing islet cells in adults impairs endocrine function, a phenotype that is accompanied by reduced expression of several Neurog3 target genes that are essential for islet cell differentiation, maturation, and function. These findings

**Student essay**

In hormone-expressing cells, Neurog3 is used as an endocrine progenitor marker and regarded as dispensable for the function of differentiated islet cells (Wang et al., 2009). Three different functional assays approaches were used to assess the localization, function and [illegible] phenotypic appearance resulting from different types of mutation introduced into this gene. Gene knock-in approach examined *Neurog3* expression in hormone-expressing islet cells. *Ngn3* mRNA and protein [illegible] were detected at embryonic and adult developmental stages. The *lacZ* gene replacing *Ngn3* gene has shown that Ngn3 is expressed in hormone expressing cells e.g. insulin secreting cells. Inactivating *Neurog3* in *insulin*-expressing cells at embryonic stages or in *Pdx1*-expressing islet cells in adults impairs endocrine function, a phenotype that is accompanied by reduced expression of several *Neurog3* target genes that are essential for islet cell differentiation, maturation, and function (Wang et al., 2009).

Plagiarism

- You need to describe and summarize **using your own words** even if you give the citation.
- **COPY AND PASTE is plagiarism**.
- Quotations may be used only in rare occasions such as terminology definitions.
  - **i.e. Quotations should not be used in most occasions.**

## Lab report check list

(Moodle)

- Follow **Biol368 Lab Report Guideline**.
- Follow **Project Grading Scheme**.
- "**Analysis of result**" sections in the Lab manual contain many questions you need to answer as part of lab report.
- Grading schemes will not tell you where questions are. You need to find the questions on your own.

## Lab reports submission

1. Both soft and hard copies must be submitted.
   - Hard copy: submit **before the lab starts**.
   - Soft copy: upload to Moodle by **6 pm**.
   - You don't have to scan hand drawing.
2. If the Moodle server is down at the time of submission, you may e-mail the file to biol368concordia@gmail.com . Use this address for emergency situation only.
3. 5 % late penalty if you miss the hard copy submission on time.

- 10 % if submitted after the midnight of the submission day.
- + 5 % per day after that point.

## Calculations

- You should use Excel to do calculations.
- You need to provide sample calculations. Provide the general formula and give a specific example.
- Sample calculations can be written by hand.

## Today's agenda

- Media making
- Sterilization
- Aseptic techniques
- Single colony isolation
- Bacteria count
- Biosafety levels

## Media making

| Media making plan | | | Abbreviations | |
|---|---|---|---|---|
| Group | Type of media (# of flasks)* | | Min | MinA* glucose agar |
| 1 | Min (1) | MinR (1) | MinR | MinA* glucose agar + argenine |
| 2 | MinL (1) | MinM (1) | MinL | MinA* glucose agar + leucine |
| 3 | Min lac (2) | | MinM | MinA* glucose agar + methionine |
| 4 | Mac lac (1) | | Min lac | MinA* lactose agar |
| 5 | nutrient (2)** | | Mac lac | MacConkey lactose agar |
| 6 | nutrient (2)** | | Nutrient | Nutrient agar |
| 7 | LB tet (2)** | | LB tet | LB agar + tetracycline |
| 8 | LB tet (2)** | | LB | LB agar |
| 9 | LB (2) | | *All MinA media include thiamine. | |
| 10 | LB (2) | | | |
| * 350 mL per flask unless otherwise specified. | | | | |
| ** Make two flasks, each containing 500 mL media. | | | | |

## Today's agenda

- Media making
- Sterilization
- Aseptic techniques
- Single colony isolation
- Bacteria count
- Biosafety levels

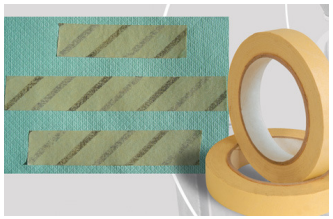
## Sterilization

- Sterilization
- Disinfection

## Sterilization method

## Autoclave

- **Autoclave machine** steam heats materials under a high pressure (>15 psi, 121 oC, > 15 min).
- More effective then dry oven.
- Kills everything!

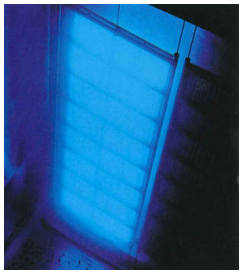


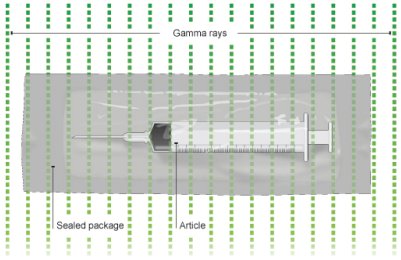
**Autoclave tape**

## Filter sterilization

- Liquid is passed through sterile disposable filter with pore size 0.22 µm or less.
- Removes any bacteria/microbe particles. (Sizes of bacterial cells are 1-10 µm.)

## Gamma ray sterlization






## Sterilization method

- Glassware, metals
  - **Autoclave**

## Sterilization method

- Plastics (heat resistant)
  - **<u>Autoclave</u>**

## Sterilization method

- Plastics (not heat resistant)
  - **Gamma ray (manufacturer)**


## Sterilization of liquids

- Heat resistant components
  - water
  - LB medium
  - Salts
  - Sugar
- **Autoclave**

## Sterilization of liquids

- Heat sensitive components
  - Antibiotics
  - Amino acid
- **Filter sterilization**

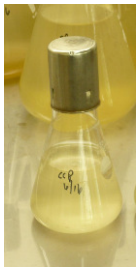
## Sterilization of Biohazard materials

- Biohazard garbage:
  - Bacterial culture, plates, tubes, tips, etc.
  - Any solid materials that came contact with biohazardous materials.

Close lid!

Biohazard bag is autoclaved.

## Sterilization of Biohazard materials



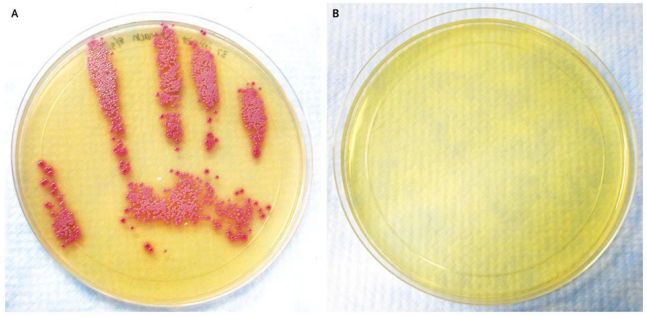
Autoclave and discard the contents.

## Today's agenda

- Sterilization
- Aseptic techniques
- Single colony isolation
- Bacteria count
- Biosafety levels

## Aseptic techniques

- Techniques to prevent contaminations of sterile equipment and pure cultures.




Imprint of the hand of a healthcare worker. Pink colonies are methicillin resistant *Staphylococcus aureus (*MRSA)

The same worker's hand had been washed with alcohol foam.

**Donskey and Eckstein, New England Journal of Medicine 2009 Jan 15 360 (3): e3**
http://content.nejm.org/cgi/content/full/360/3/e3

## Clean environment with a disinfectant

Before and after working:

Hands
- Wash with soap

Lab Bench
- Wipe with 80 % ethanol

## Basic aseptic techniques

- Wash hands with soap.
- Wipe bench with 80 % ethanol.
- Turn on the burner.
  - This will create the upwards airflow and minimize the chance of particles landing on your medium.
  - Open lids only when the burner is on. Minimize the lid opening time.

## Gloves?

- Do not put on gloves when you have burners on for aseptic techniques.
- Must wash hands to minimize contamination.
- Wear gloves if you are working with pathogens or if your materials need to be grown for a long time. In this case, you should working in a biosafety cabinet to provide better aseptic techniques.

## Basic aseptic techniques

- Keep the **burner on** during manipulation.
- Slightly tilt the tubes and bottles before opening.
- Heat briefly the mouth of glass tubes or bottles before and after the transfer of bacteria.


NO GOOD


GOOD

Do not leave fires unattended.

## Today's agenda

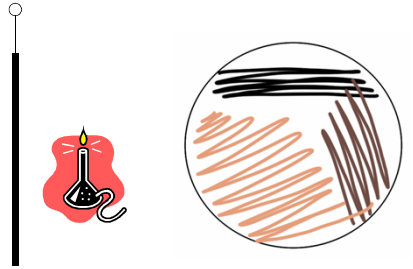
- Sterilization
- Aseptic techniques
- Single colony isolation
- Bacteria count
- Biosafety levels

## Single colony isolation

**Streaking**: Technique to isolate a pure culture on a single plate. (Single, isolated colony is derived from a single cell.)

## Single colony isolation by streaking

http://www.sumanasinc.com/webcontent/animations/content/streakplate.html



If you use a sterile tooth pick, change tooth pick between streaks.

## Put plates UPSIDE DOWN in the incubator.

## Today's agenda

- Sterilization
- Aseptic techniques
- Single colony isolation
- Bacteria count
- Biosafety levels

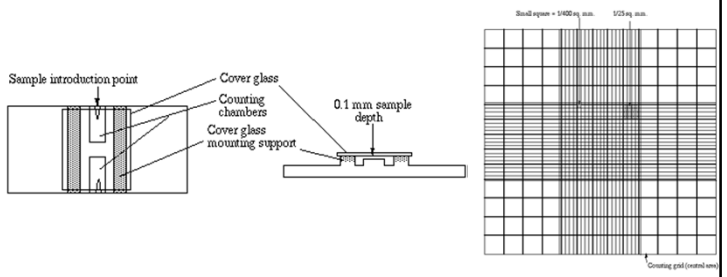
## Bacterial cell count

## Bacterial cell count

1. Direct counting (hemocytometer)
2. Optical density
3. Viable count

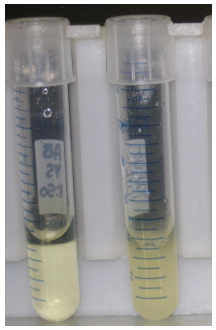
## 1. Direct microscope counts

- Counting chambers (hemocytometer)
- Direct method but it normally counts dead cells as well.
- Cell viability staining can differentiate live cells from dead cells.



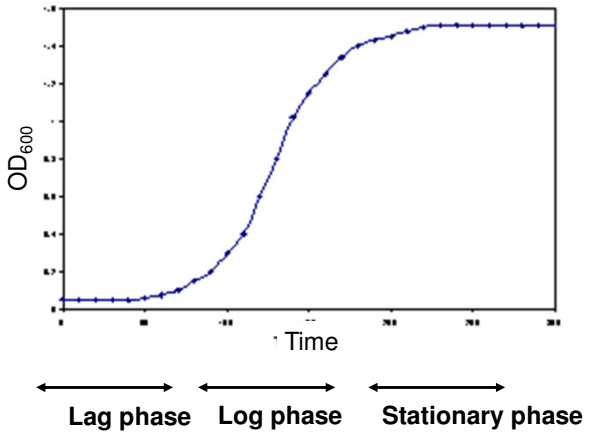
## 2. Optical density ($OD_{600}$)

- Estimate of the cell number by measuring the turbidity.
- For *E. coli*, $OD_{600}$=1 is approximately 5 x $10^8$ cfu/ml.
- Fastest and easiest to estimate bacterial count.
- Does not differentiate viable cells from dead cells.



Broth only | Bacterial culture

## Growth curve ($OD_{600}$)



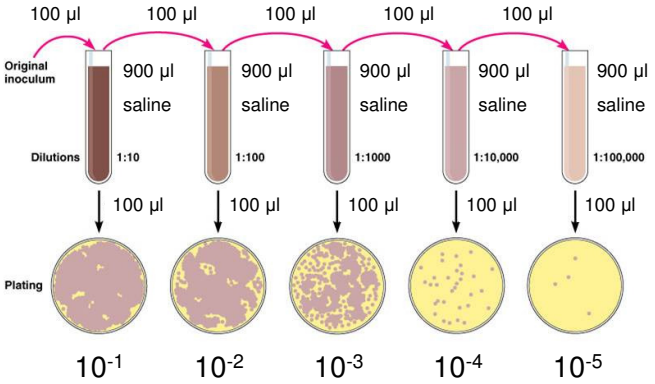

## $OD_{600}$ measurement

- Avoid contaminating the spectrophotometer.
- Tips, tubes and cuvettes used for $OD_{600}$ measurement should be discarded in the biohazard disposal.
- The culture should not be dumped in the sink.

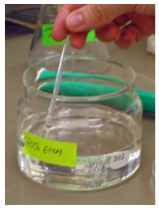
## 3. Viable cell count

- Estimate number of viable cells by the number of colonies the culture can form (**colony forming unit, cfu**).
- To obtain well-separated colonies, **serial dilution** of the bacterial culture is necessary.
- Commonly used for bacterial counts from environmental samples
- As a general role, you should select plates that has 100 – 300 colonies to obtain accurate reading.

## Serial dilution

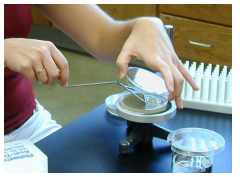



## Plating



Dip spreader in ethanol.

Place the spreader over flame to let ethanol burn out.



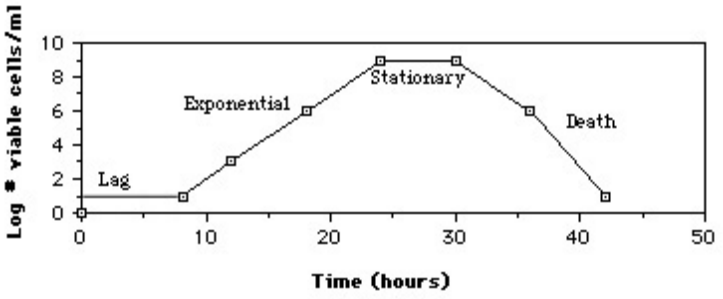
Spread cultures while turning plates.

Fire safety: Do not place ethanol container near the flame.

## After plating, leave plates up until no more liquid is seen on the plate.

## Put plates UPSIDE DOWN in the incubator.

## Growth curve (viable count)




**Lag phase** **Log phase** **Stationary phase**