Quantification of *Escherichia coli* via Analysis of β-glucuronidase Enzyme Concentrations

Final Presentation

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CHEM 498 Research

Under Dr. Sam Mugo

Introduction

- *Escherichia coli* is an aggressive, fast-growing and pathogenic bacteria
- Naturally found in lower intestines of most warm-blooded organisms
- Considered to be one of the most dangerous types of bacterial pathogens
- Can cause hemorrhagic colitis and hemolytic uremic syndrome
- Higher risk for people with poor immune system
- Levels over 1 CFU / 25 g of ingested food are considered dangerous

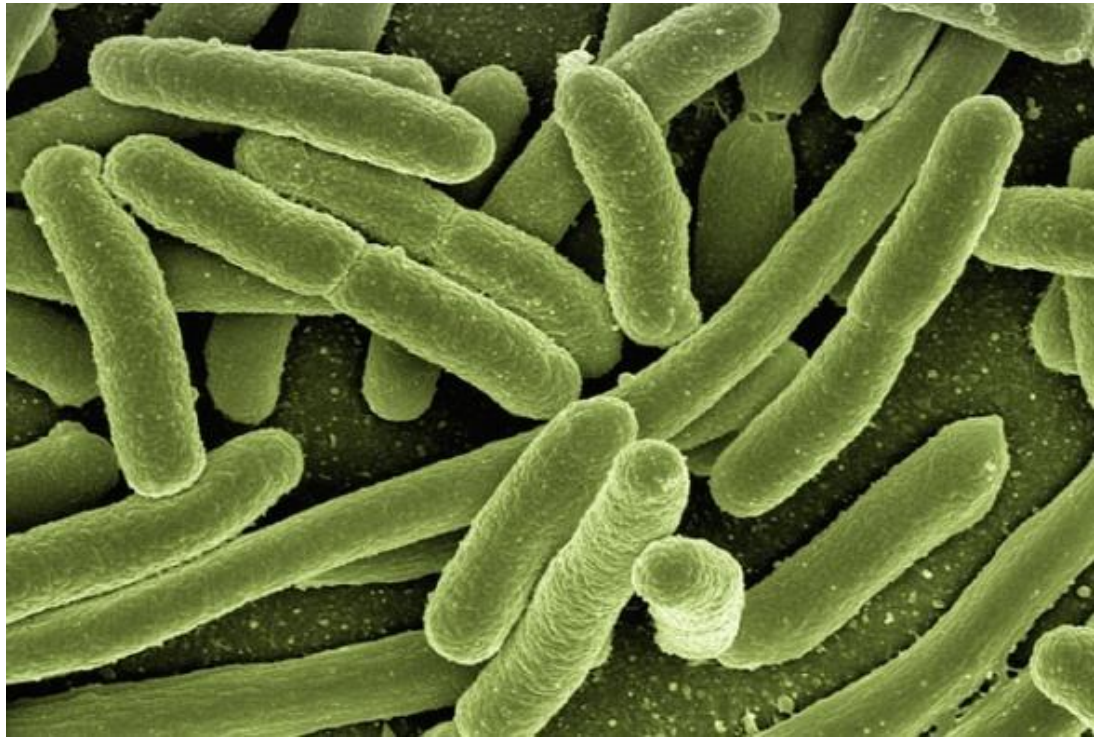


Figure 1: E. coli under SEM

Infections

2006 was prominent year in outbreaks

- September – 276 infected and 3 killed due to E. coli in spinach across 26 states in USA, Canada and Mexico
- December – 71 infected from lettuce from Taco Bell in northeastern USA

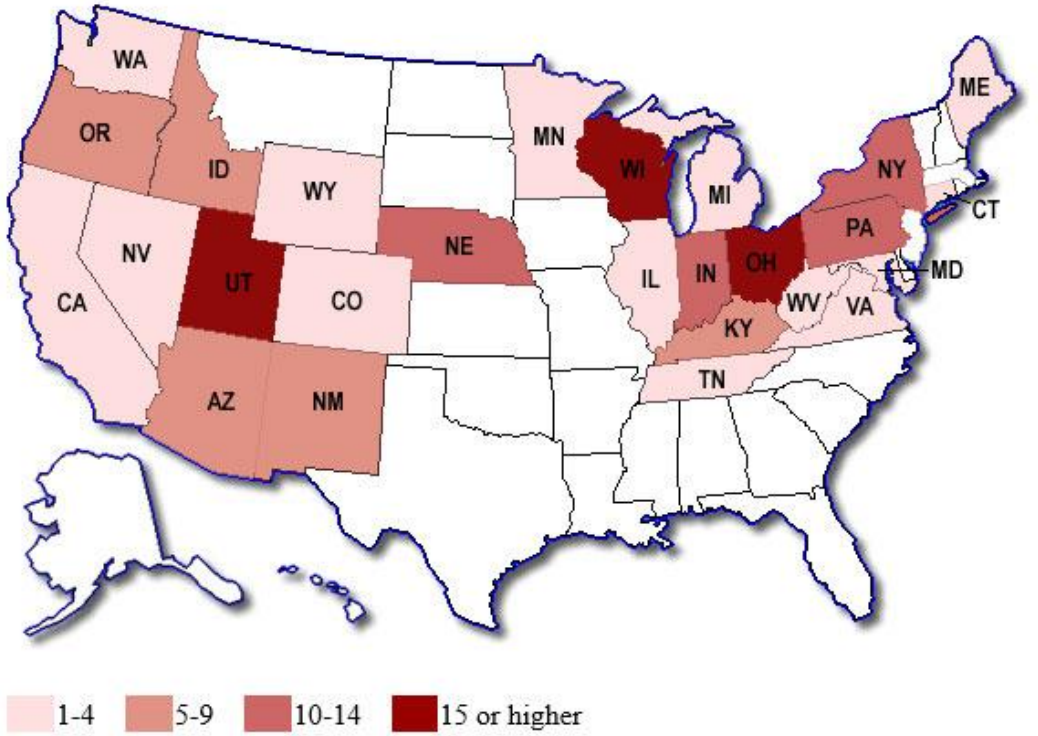


Figure 2: Spinach outbreak of E. coli across USA (CDC)

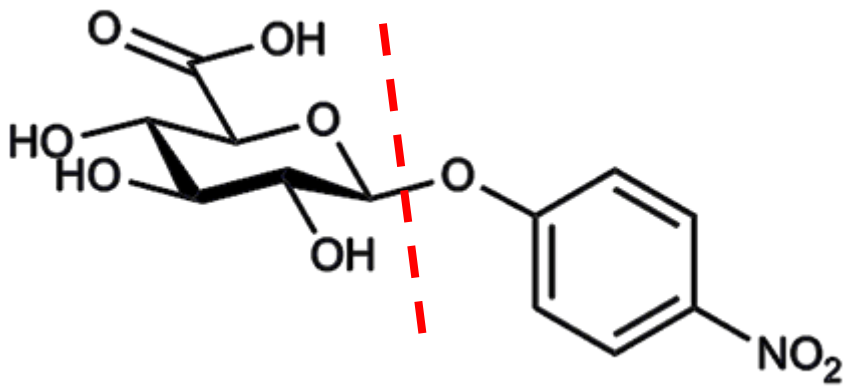
Testing

- Traditional testing requires amplification, or growing cells, to determine strain and concentration
- Four main fields of testing:
  1. Food Industry Quality Control
  2. Clinical Diagnosis
  3. Drinking Water
  4. Sewage Treatment

- Therefore, we need fast and reliable testing for the presence of *E. coli*

Biochemistry of *E. coli*

- Approximately 97% of *E. coli* strains produce a digestion enzyme called β-glucuronidase (β-GUS)
- Intracellular enzyme that cleaves a D-glucuronic acid with the remainder of the molecule
- Detection of the enzyme can be determined by the configuration of the residue attached to the D-glucuronic acid



Growth of *E. coli*

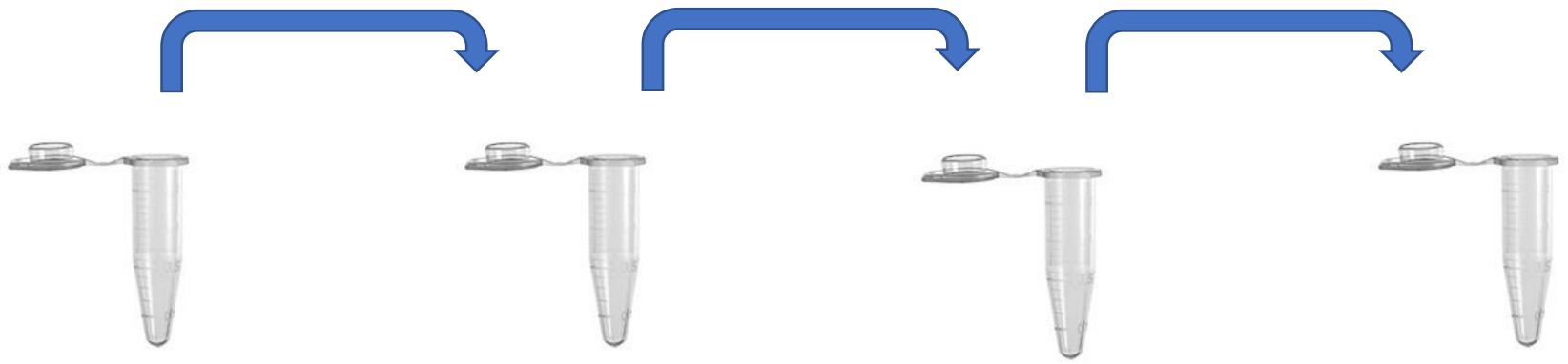
- *E. coli* (KL25 strain) is sampled from existing colony
- Transferred onto agar plate to incubate single colonies
- VWR enrichment broth powder mixed into water at concentration of 13.01 g/L
- Single colony from incubation plate transferred into ~50 mL of enrichment broth
- Broth solution can be quantified and will live for approximately 1 month

Quantification of *E. coli* in Broth by Serial Dilution

- Eight (8) serial dilutions performed at a dilution rate of 1 E. coli stock: 10 water
- Concentration of each serial dilution determined using following formula:

$$[S.D.]_n = [stock]_i \cdot 10^{-n}$$

- Dilutions performed in 2.5 mL centrifuge tubes

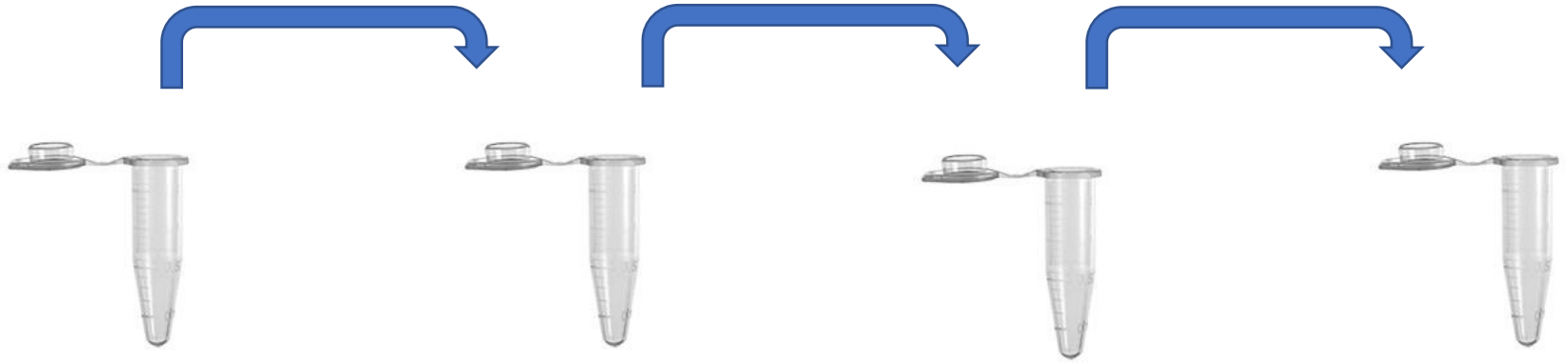


[S.D. 1]
100 µL of stock
900 µL of water

[S.D. 2]
100 µL of S.D. 1
900 µL of water

[S.D. 3]
100 µL of S.D. 2
900 µL of water

[S.D. 4]
100 µL of S.D. 3
900 µL of water

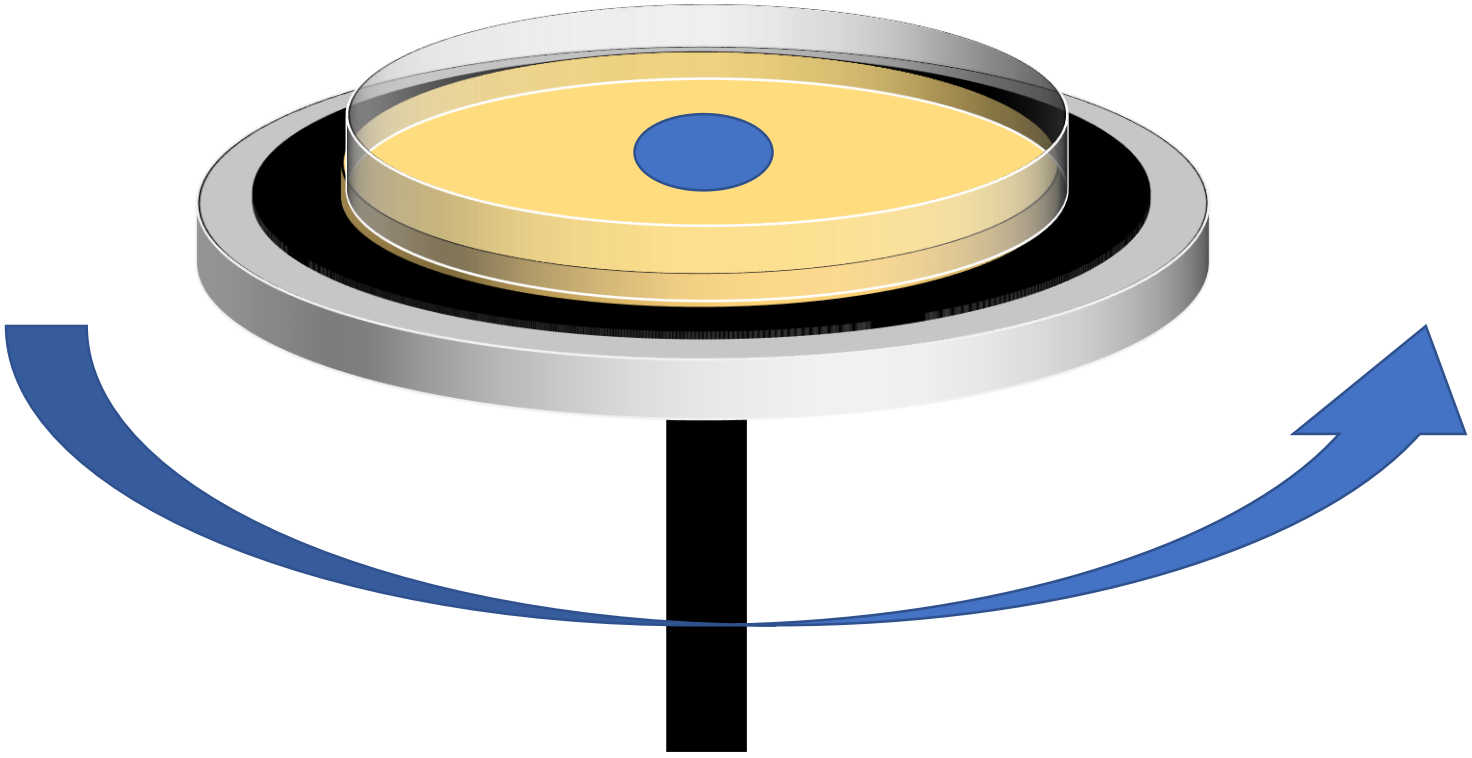


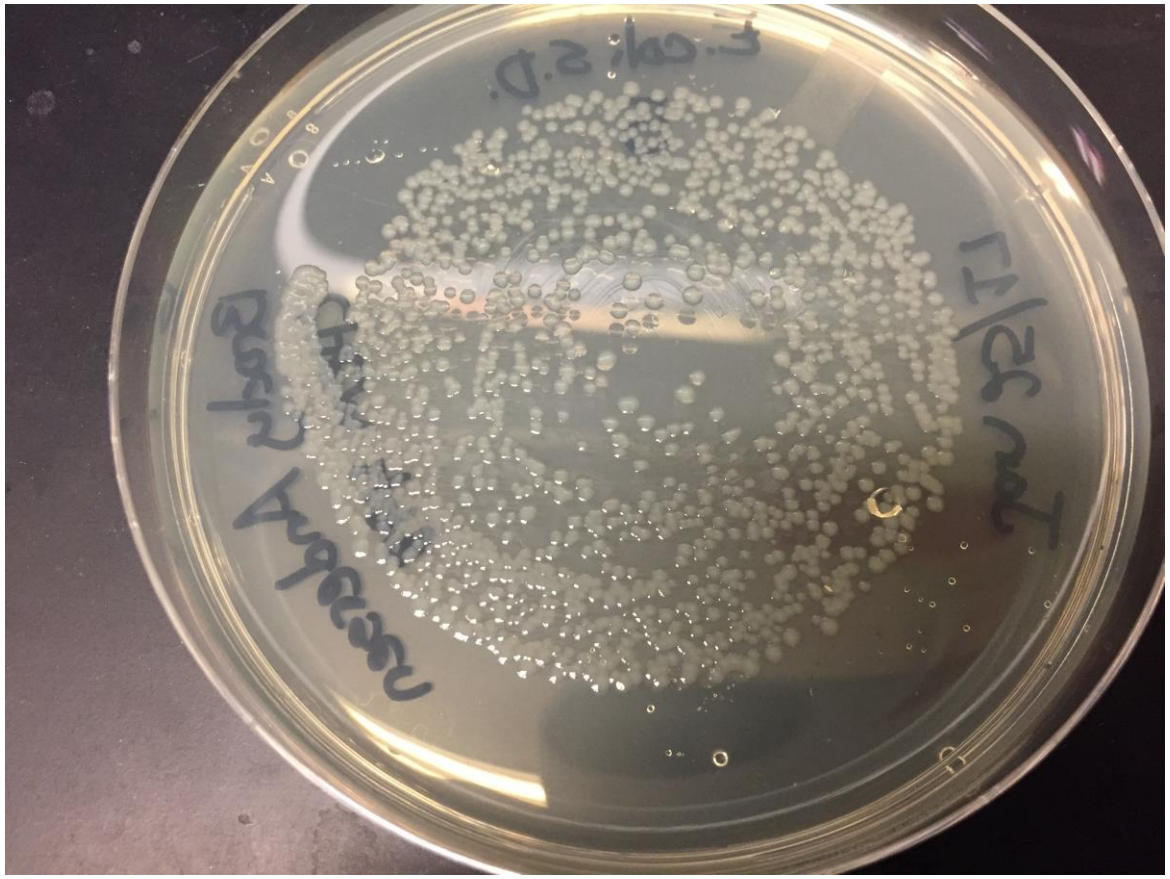
[S.D. 5]
100 µL of S.D. 4
900 µL of water

[S.D. 6]
100 µL of S.D. 5
900 µL of water

[S.D. 7]
100 µL of S.D. 6
900 µL of water

[S.D. 8]
100 µL of S.D. 7
900 µL of water





Serial Dilution 5 (Stock x 10^{-5})

Cannot count the colonies

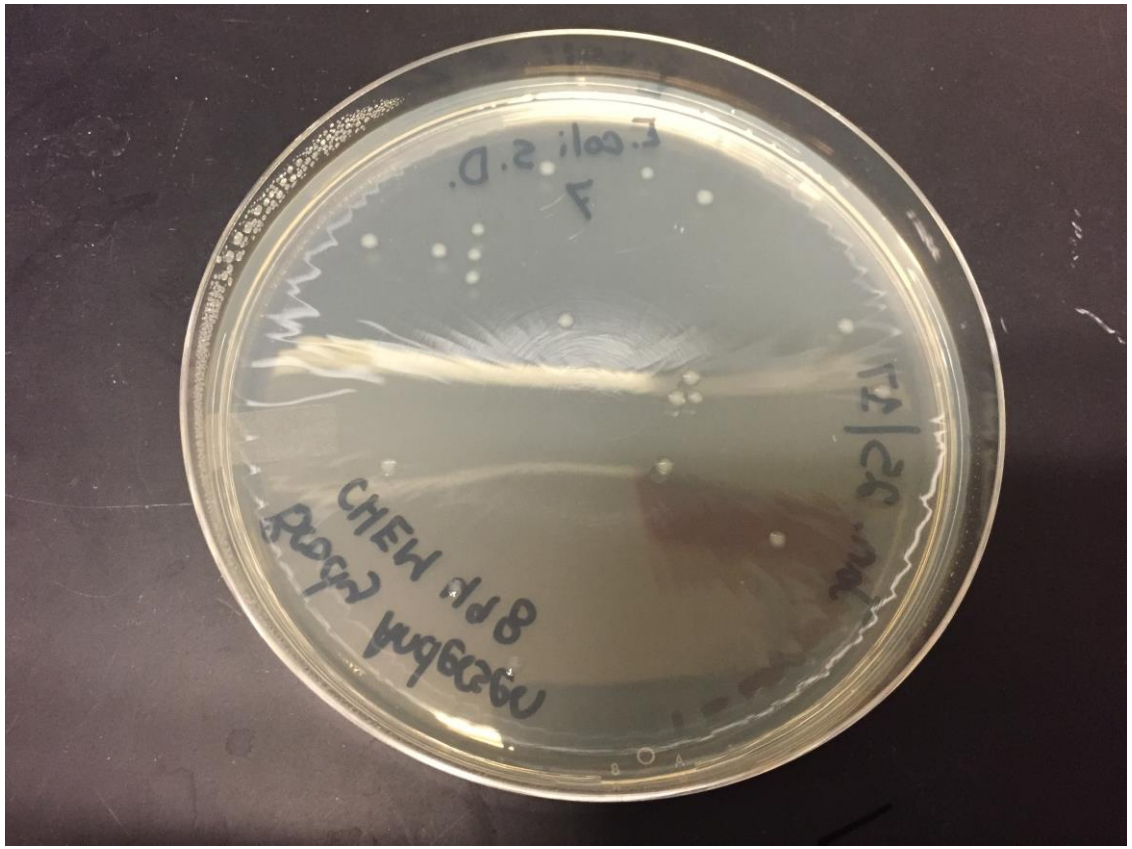
Concentration indeterminate

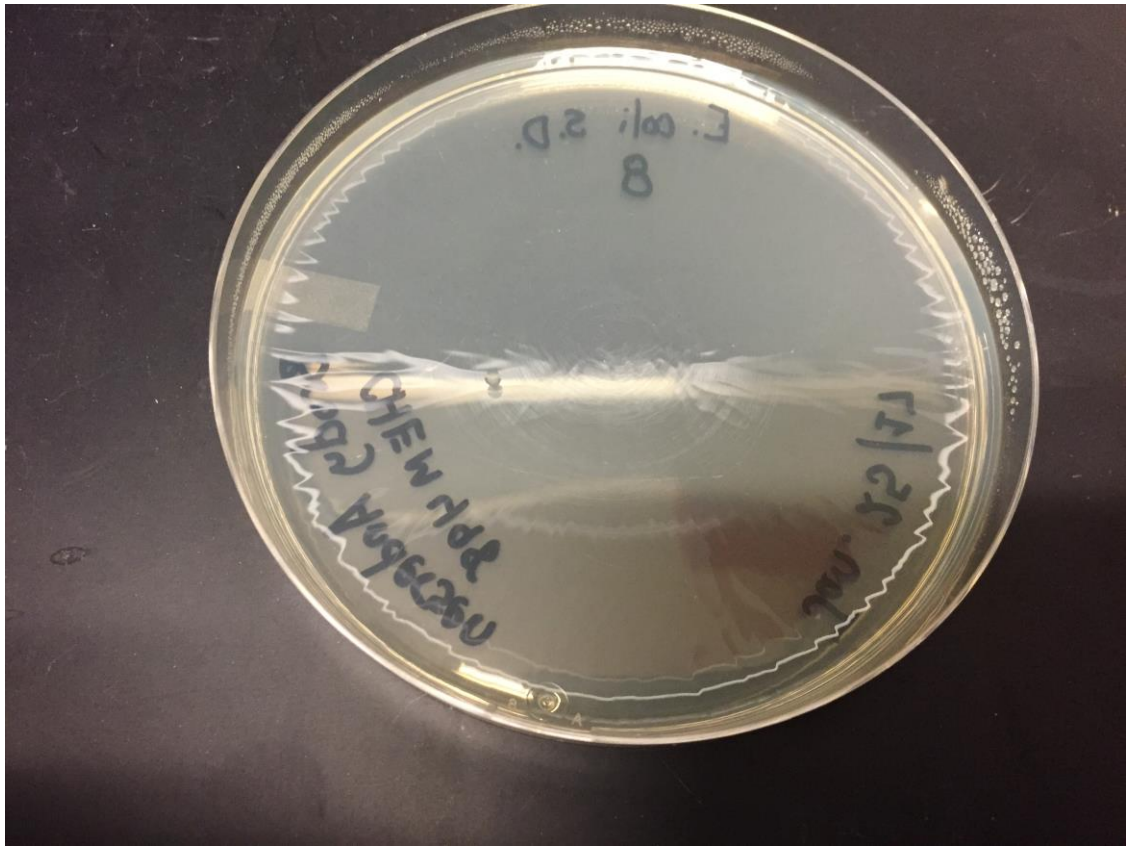
Serial Dilution 6 (Stock x 10^{-6})

160 total colonies

[SD 6] = 1.6 x 10^{8} colonies / volume

[SD 6] = 1.6 x 10^{6} cells / µL





Serial Dilution 7 (Stock x 10^{-7})

19 total colonies

[SD 6] = 1.9 x 10^{8} colonies / volume

[SD 6] = 1.9 x 10^{6} cells / µL

Serial Dilution 8 (Stock x 10^{-8})

1 colony

[SD 6] = 1 x 10^{8} colonies / volume

[SD 6] = 1 x 10^{6} cells / µL

Scope of Research

A Colourimetric Method of *E. coli* Detection

- Using nanocellulose and enzyme-activated dyes to quantify *Escherichia coli* in a sample
- Film created by allowing a mixture of polyethylene oxide (PEO) and crystalline nanocellulose (CNC) in water to dry on an overhead transparency
- Droplet of 4-nitrophenyl β-D-glucuronide (4-NβD_g)dye and droplet of *E. coli* broth sample placed on CNC/PEO film
- Colour change of solution from colourless to blue
- Intensity of colour quantifiable by image digitization
- Intensity proportional to amount of enzyme β-glucuronidase (β-GUS) present and calibration curve developed for *E. coli* concentration

Colorimetric Method of Analysis

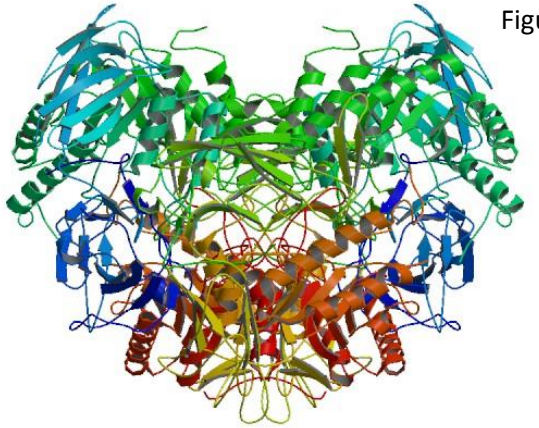


Figure 2

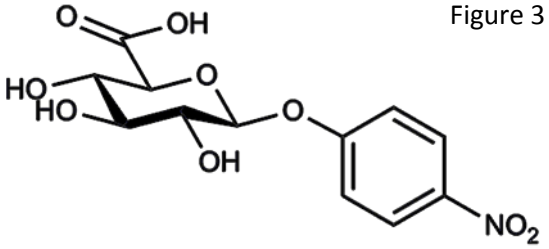
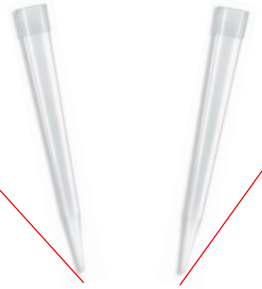


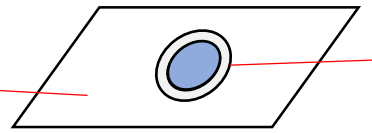
Figure 3

50 µL of *E. coli* sample or β-glucuronidase standard

50 µL of 4-nitrophenyl β-D-glucuronide dye

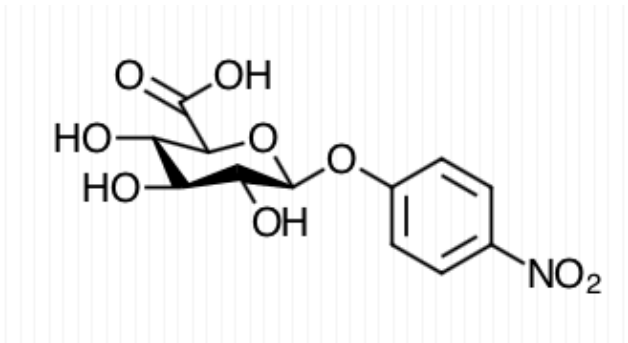


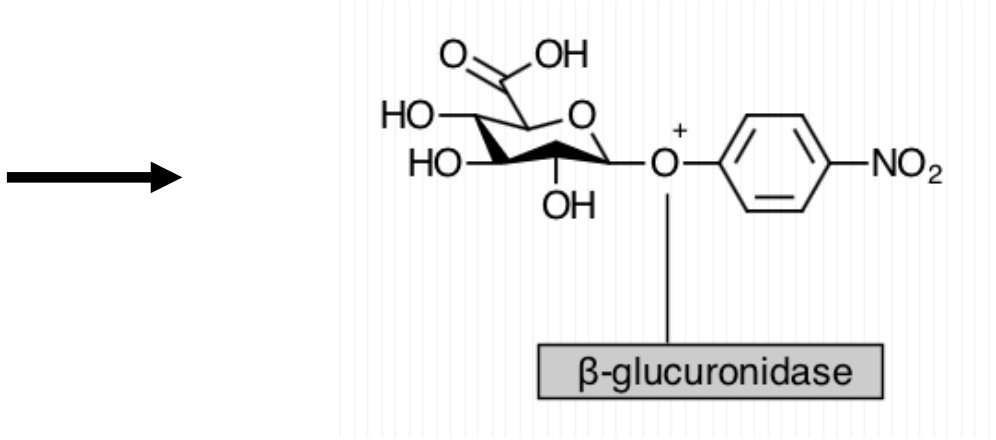
Transparency



70% Polyethylene oxide:
30% Nanocellulose

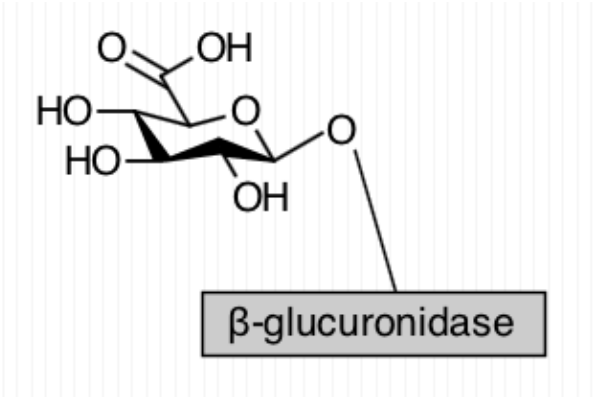
Colourimetric Reaction





Colourless

Colourless



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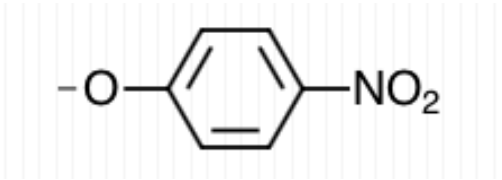
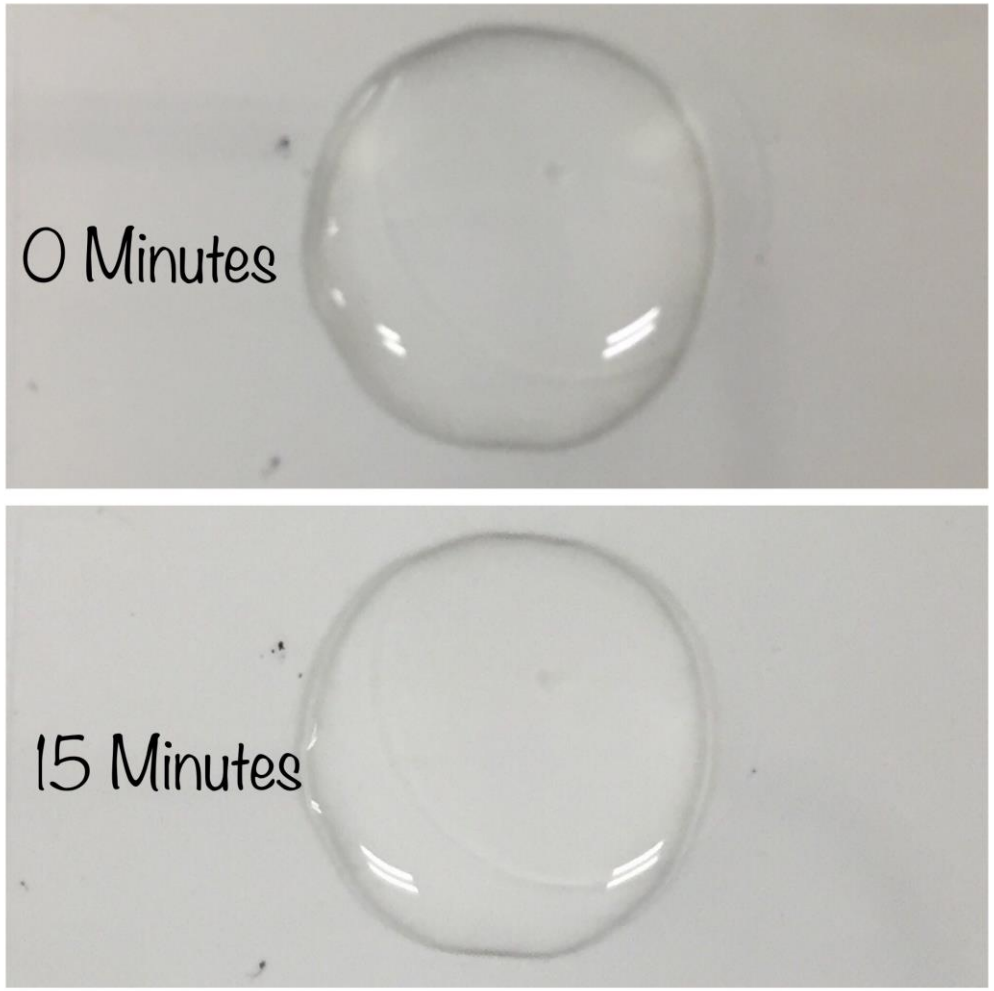


Colourless

Blue

Colourimetric Results

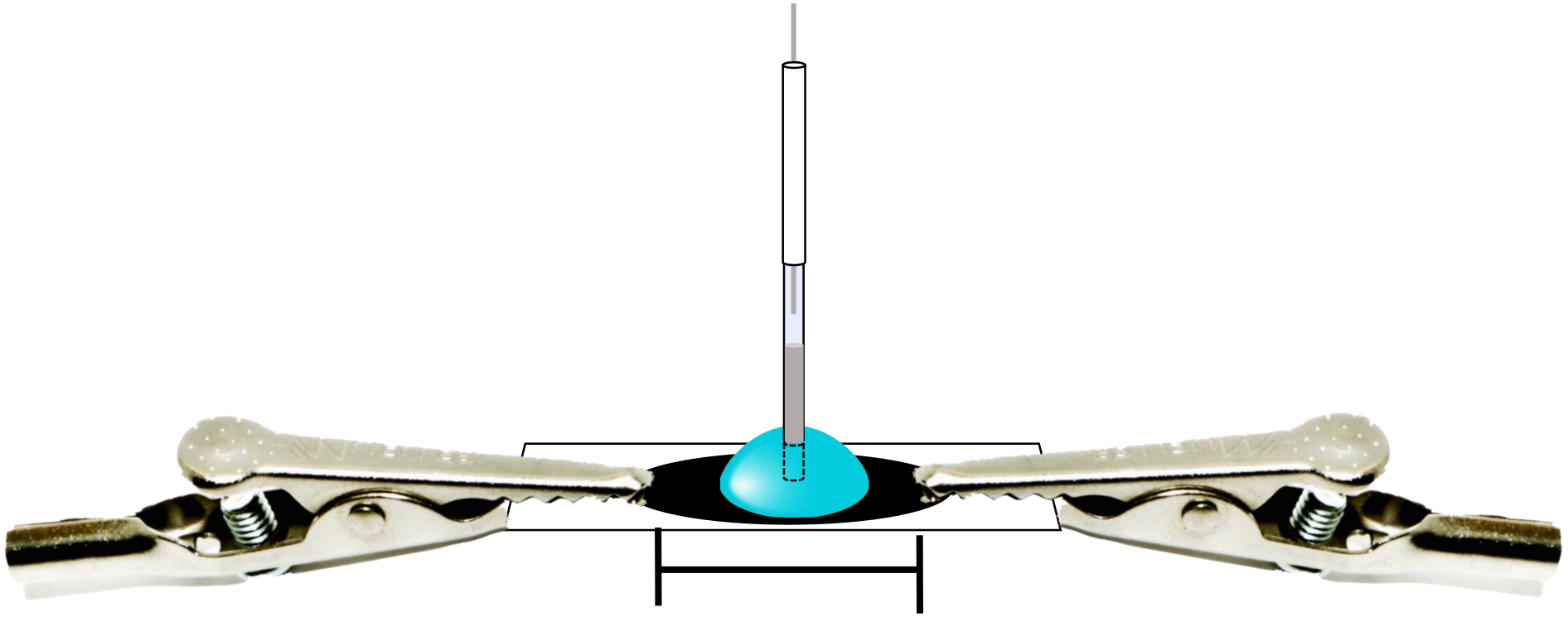


Scope of Research

A Conductometric Method of *E. coli* Detection

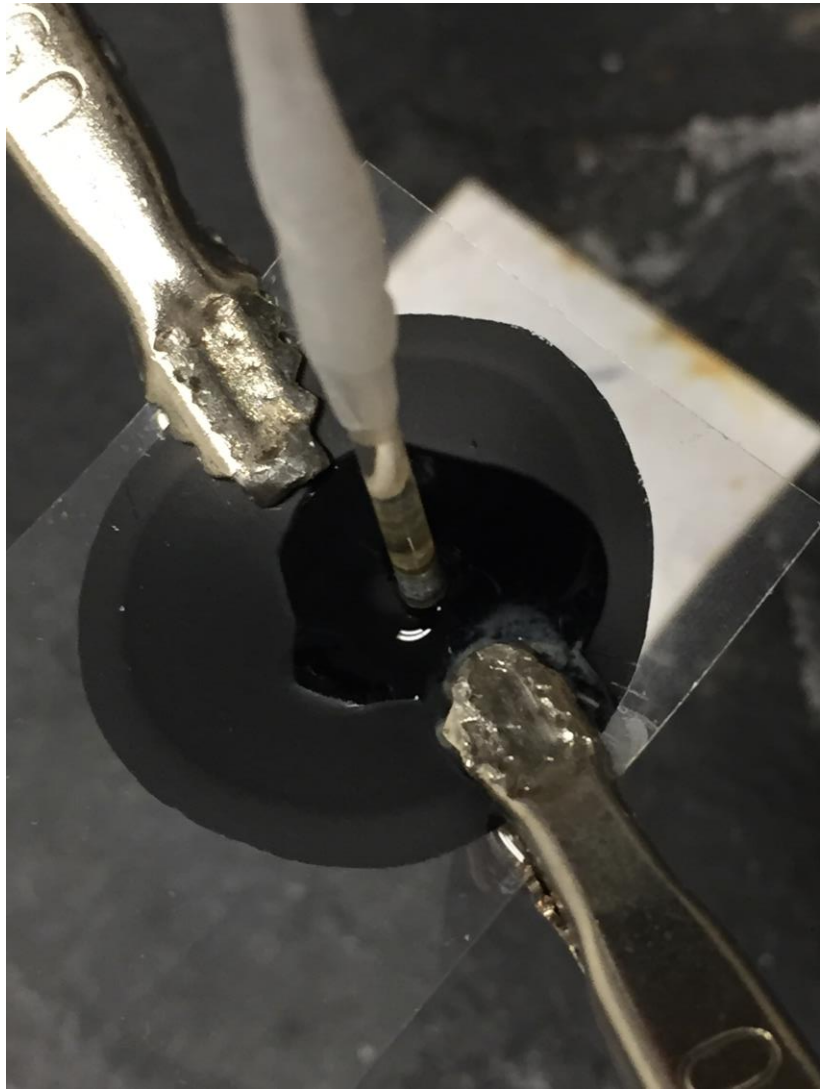
- Using nanocellulose and potentiostat to quantify ***Escherichia*** *coli* in a sample
- Film created by allowing a mixture of polyethylene oxide (PEO), crystalline nanocellulose (CNC) and powdered carbon in water to dry on an overhead transparency; measure cyclic voltammogram of film
- Droplet of 4-nitrophenyl β-D-glucuronide (4-NβD_g)dye and droplet of *E. coli* broth sample placed on CNC/PEO/C film
- Place voltage across the film to obtain cyclic voltammogram
- Change in resistance is due to the detection of 4-nitrophenol in the sample
- Change proportional to amount of enzyme β-glucuronidase (β-GUS) present and calibration curve developed for *E. coli* concentration

Conductometric Method of Analysis

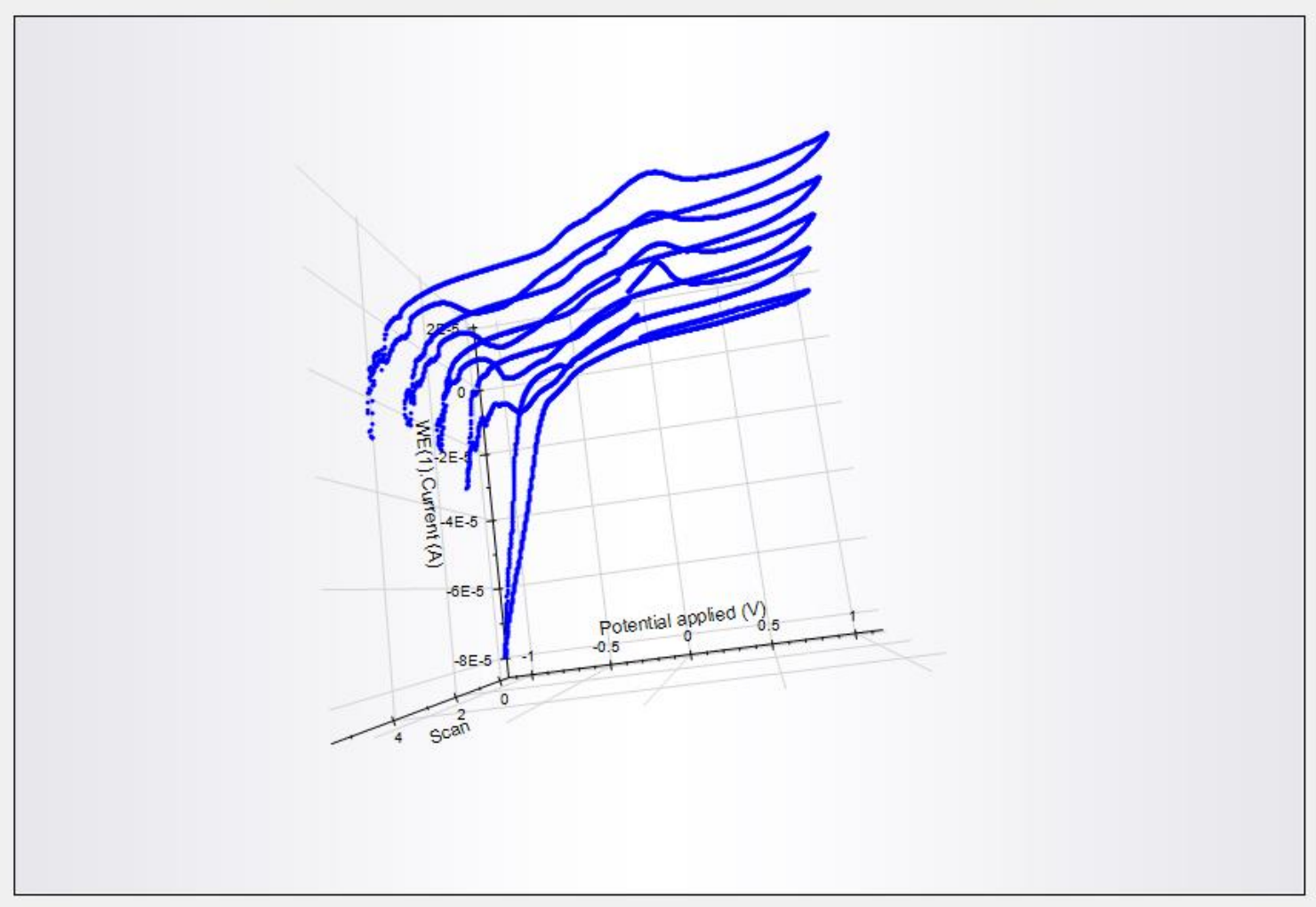


~ 1.5 cm

Conductometric Method of Analysis



Best Result

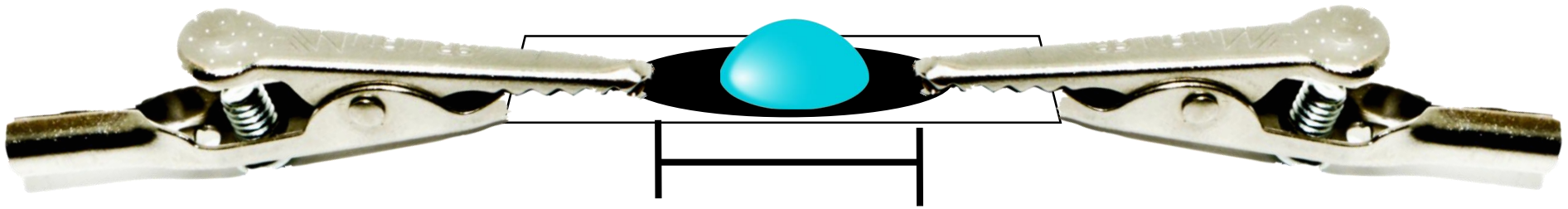

WE(1).Current (A)
0
-2E-5
-4E-5
-6E-5
-8E-5
Potential applied (V)
-1
-0.5
0
0.5
1
Scan
0
2
4

Scope of Research

A Resistometric Method of *E. coli* Detection

- Using nanocellulose and a multimeter to quantify ***Escherichia*** *coli* in a sample
- Film created by allowing a mixture of polyethylene oxide (PEO), crystalline nanocellulose (CNC) and multiwalled carbon nanotube (CNT) in water to dry on an overhead transparency; measure resistance of film
- Droplet of phosphate buffer solution and droplet of *E. coli* broth sample placed on CNC/PEO/C film
- Measure resistance across the film using multimeter
- Change in resistance is due to the detection of bacteria in the sample
- Change proportional to amount of *E. coli* present and calibration curve developed for *E. coli* concentration

Resisometric Method



Results

- Resistance too great for detection
- Distance between electrodes too great, no space of liquid sample
- All results show undiscernible change in resistance
- Resistance may be decrease by the addition of polymerized aniline on the surface of the film
- Unable to test because of time

Further Research

- Testing both colourimetric and conductometric methods using new materials to ensure there is viability
- Incorporating polymerized aniline (PANI) into the hydrogel to decrease the background internal resistance
- Development of image digitization program for colourimetric methods
- Development of film alternatives such as wells and integrated electrodes

References

Figures

- 1 - https://news.liverpool.ac.uk/2015/09/21/scientists-discover-how-e-coli-survives-stomach-acid/
- 2 - https://www.cdc.gov/ecoli/2006/spinach-10-2006.html
- 2 - http://www.rcsb.org/pdb/images/3K46_bio_r_500.jpg?bioNum=1
- 3 - https://secure.megazyme.com/images/sites/1/products/59765676-d88e-441a-bfb3-7af39ceefe34/medium/O-PNPBGA.png

Literature

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