Title: Uncoiling E. Coli

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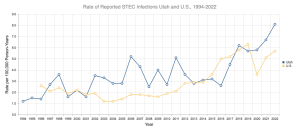
DATE	ACTION	RESOURCES USED	OTHER NOTES
September 19th, 2024	Brainstorm Ideas for Project	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8324482 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10678172/ https://my.clevelandclinic.org/health/diseases/16638-e-coli-infection	Finding an approach to curing a disease - **Malaria** - Pills - Highly effective treatments but not fully - Reduces chance of sickness by 90% - Common - $6 for rapid test diagnostics and between $9 and $90 for uncomplicated or severe malaria - **E Coli.** - No specific treatment - Calgary Case - What about children? Probably won't go away by itself for children, maybe for adults - Early detection or prevention methods - Cures <1 week (average adult time to recover) - Mean and standard deviation (SD) costs of an E. coli BSI including ED visits and

			hospitalizations for 90 days in children were $99,214 ($152,809) **- Autism** - Why it happens - Early detection - Genetics behind autism - What about in families that have no prior history of autism?
September 20th, 2024	Project Decided	N/A	Studying a specific strain of E.Coli (potentially the one involved in Calgary daycare infections) and developing a cost-effective treatment, especially for more vulnerable groups (eg. older persons aged 65+, newborns and young children) and trying to create an early detection/prevention methods for E.Coli (eg. a device that a food inspector can use to test a specific item for the specific E.Coli strain)
September 27th, 2024	Research Started & Project Title Decided	https://www.cbc.ca/news/canada/calgary/ecoli-daycare-meat-loaf-report-1.7279159 https://www.cdc.gov/ecoli/about/kinds-of-ecoli.html	Title - Uncoling E. coli: Detection and Treatment Strain Involved in Daycare Outbreak: STEC

		https://my.clevelandclinic.org/health/diseases/16470-hemolytic-uremic-syndrome	STEC E. coli is Shiga toxin-producing E. coli Information:  - Commonly causes HUS (hemolytic uremic syndrome) - HUS - Damages blood vessels in kidneys - Destroys blood cells and reduces platelets - Largely affects kidneys but can also affect other organs - Typical HUS affects 5%-15% people who have E. coli diarrhea
October 4th, 2024	Research Continued	https://ibis.utah.gov/ibisph-view/indicator/view/FooPoiEcoli.Year.html https://www.mayoclinic.org/diseases-conditions/e-coli/symptoms-causes/syc-20372058 https://www.health.ny.gov/diseases/communicable/e_coli/stec.htm https://www.mayoclinic.org/diseases-conditions/e-coli/diagnosis-treatment/drc-20372064	In the United States, there are around 100000 infections, 3000 hospitalizations and 90 deaths per year “Effective prevention is the best treatment for STEC”  E. coli is commonly transmitted through contact with

			contaminated food such as: - Ground Beef - After cattle are slaughtered and processed, the E. coli present in their intestines can get on the meat. Ground beef also combines different types of animals, increasing risk of contamination - Unpasteurized Milk - Fresh Produce Healthy adults normally recover in a week, however for vulnerable groups, especially children under 5, can develop a form of kidney failure called hemolytic uremic syndrome (HUS). Symptoms of STEC E. coli typically appear 3-4 days after contact with the contaminated product but can appear anywhere between 1-10 days after contact. There is no specific treatment for STEC infections. Antibiotics aren't typically used for treatment as they aren't

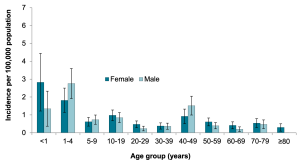
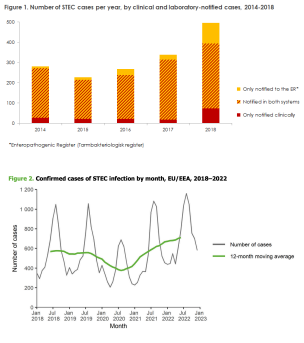
			proven to be effective against STEC infections and can in some cases, increase the risk of HUS.
October 9th, 2024	Renert School Proposal Started	N/A	Information Required: • Title • Background on topic • Why is it important and outstanding? • What issue/concern is it going to solve? • How are you going to find answers (steps)?
October 13th, 2024	Research Continued	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4607253/ https://pubmed.ncbi.nlm.nih.gov/19827953/ https://www.mayoclinic.org/diseases-conditions/e-coli/symptoms-causes/syc-20372058	2,801,000 acute illnesses (illnesses that are usually sudden and severe) annually, leads to 3890 cases of HUS and 230 deaths. These estimates are likely conservative (low). - Link 1 Of 3464 cases of STEC, 218 (6.3%) developed HUS. Highest amount of HUS cases was in children <5 (15.3%). Death occurred in 0.6% in those without HUS 4.6% in those with HUS - Unpasteurized Milk - E. coli on a cow's udder or on milking

			equipment can get into raw milk that is not further pasteurized - Fresh Produce - Runoff from cattle farms can contaminate fields with fresh produce. Specific vegetables are susceptible to this type of contamination (eg. lettuce or spinach)
October 16th, 2024	Renert School Proposal Slideshow Started	Canva	Background - E. coli is a bacteria that is commonly spread through contaminated food - Ground Beef - Unpasteurized Milk - Fresh Produce - Shiga Toxin-Producing E. coli (STEC) is a strain of E. coli that releases a toxin which damages the body's cells - Typically associated with more severe symptoms - Vulnerable groups: people <5 and >65 - Estimated >265,000 cases of just STEC E.coli infections per year in the United States (2018) - Mild to severe symptoms

			- Many cases are not reported as individuals do not seek treatment or receive testing for infection - Estimated 3-5% case-fatality rate (WHO) - No specific treatment for STEC E. coli infections - Antibiotics are not used - Not effective - Can worsen symptoms/increase the risk of HUS - “Effective prevention is the best treatment” Importance - Although STEC E. coli may not cause an extremely large amount of deaths, many people are still infected and affected by the severe symptoms - STEC E. coli infections can be fatal, not only affecting infected individuals, but their families as well. Take the Calgary daycare case, for example

			- The lack of treatment is a concern. For E. coli related infections, there is no treatment either for curing the infection, relieving symptoms or preventing complications - Early detection of E. coli on foods that is potentially going to be distributed can reduce the risk of widespread contamination and prevent the spread of this infection - Early detection can also help minimize damage and reduce the risk of other symptoms or conditions (eg. preventing the use of antibiotics, which can lead to HUS in some cases) - Early detection can also provide doctors or nurses with more time to understand the severity of symptoms, risk of developing HUS or investigating into the

			source of the infection Objectives - My first objective is to conduct research on the topic of STEC E. coli, including symptoms, related bacteria, the composition of the bacteria, HUS, etc. - Study similar bacteria to STEC E. Coli, research on treatments for the bacteria and see if the treatment could potentially be used for STEC E. coli - Finally, my last objective is to use all of the previously used research to create an early detection or prevention method to test for STEC E. coli Approach - Research into various components of STEC E. coli including shiga toxin, chemical composition, interactions with body cells, strains of E. coli in different environments,

			etc. - Studying similar types of bacteria, treatments that have been proven effective and identifying if said treatments can be used for treating STEC E. coli - Use all of previously researched information to aim to create a easy-to-use device that can detect E. coli in samples of food and provide quick results and compare with previous experiments regarding this
October 20th, 2024	Graph Added to Proposal Presentation Slideshow Edited for Less On-Screen Text and Larger Font Size	https://www.gov.uk/government/publications/escherichia-coli-e-coli-o157-annual-totals/shiga-toxin-producing-escherichia-coli-stec-data-2021	
October 22nd, 2024	Slide Added	https://en.ssi.dk/surveillance-and-preparedness/surveillance-in-denmark/annual-reports-on-disease-incidence/annual-report-on-stec-and-haemolytic-uraemic-syndrome	

		https://www.ecdc.europa.eu/sites/default/files/documents/STEC_AER_2022_Report.pdf	Relevance - The number of STEC E. coli cases have been increasing every year - A decline was observed in 2020 - Due to Covid-19 pandemic - No countries in the European Union have reported a decrease in the number of cases from 2018-2022 - Four countries (Australia, France, Malta and Spain) reported a significant increase in the same time period
October 25th, 2024	Slideshow Presented for Renert School Judges	N/A	CYSF Candidates Announced October 30th - This Project was Selected
November 12th, 2024	Research on STEC and Shiga Toxin Specifically Started	N/A	Lives in the stomach of healthy cattle
November 14th, 2024	Research into Shiga Toxin *Note - For the sections labelled “copied”, I have looked into and specifically aimed to	https://www.sciencedirect.com/science/article/abs/pii/S0041010109005522 https://www.sciencedirect.com/topics/medicine-and-dentistry/shiga-toxin	- “Shiga toxin belongs to the group of bacterial and plant toxins that act on cells by binding to cell surface receptors via a binding-moiety, then

	understand at the very least, the base concepts from the notes		the toxins are endocytosed and transported retrogradely to the Golgi apparatus and the endoplasmic reticulum (ER) before an enzymatically active moiety enters the cytosol and exerts the toxic effect." - Moiety: any part of a molecule (does not have to be the full molecule) - Endocytosis: a fundamental cellular process where nutrient uptake, receptor internalization and regulation of cell signaling are moderated - Microorganisms, such as bacteria or viruses, and toxins (including Shiga toxin) use this process in order to enter cells - Golgi apparatus: a cell organelle that processes and packages proteins and lipids for transport outside the cell - Endoplasmic Reticulum: produce proteins required by the

			cell to function (rough ER contains ribosomes which produce these proteins) “The family of Shiga toxins consists of several structurally and functionally similar protein toxins. The prototype of the family is the Shiga toxin (Stx) elaborated by S. dysenteriae type 1; the Shiga toxins expressed by E. coli have a numerical designation following the name of Shiga toxin, e.g., Shiga toxin 1. Shiga toxin 1 (Stx1) differs from Shiga toxin from S. dysenteriae by a single amino acid. Shiga toxin 2 (Stx2) is approximately 50% homologous with Stx1 at the protein level and is immunologically distinct. Other forms of Shiga toxins are listed in Table II. Shiga toxin family proteins inhibit protein synthesis by blocking the elongation factor 1–dependent

			binding of aminoacyl-tRNA to ribosomes. All Shiga toxins known to date are composed of an enzymatically active A subunit and a pentamer of identical B subunits that mediates specific binding activity. The A subunit functions as a glycohydrolase, cleaving a specific adenine from the 28S rRNA and irreversibly inhibiting ribosomal function. This activity is identical to the enzymatic activity observed for the plant toxin ricin [89]. X-ray crystallographic analyses of Shiga toxin and Stx1 B subunits have confirmed the AB5 structure, revealing a pentameric B subunit ring surrounding a C-terminal α-helix of the A subunit [90,91], which is similar to the AB5 structure of cholera toxin and E. coli heat-labile toxin. When the A subunit is nicked

			with trypsin and reduced, an A1 portion of approximately 28 kDa and an A2 peptide of approximately 4 kDa are separated. The A1 fragment contains the enzymatically active portion of the toxin molecule, and the A2 component is required to noncovalently bind the whole A subunit to the B pentamer [92]. The A1 and A2 fragments are linked by a disulfide bond. The A2 fragment may be important in holotoxin assembly [92], and the disulfide bridge between A1 and A2 appears necessary for pentamer formation [93]. The disulfide loop contains the sequence Arg-X-X-Arg, a consensus motif for cleavage by the membrane-anchored protease furin. Cleavage of Shiga toxin at this site, resulting in the formation of A1 and A2 fragments, appears to be important for cellular

			intoxication [94]. Interestingly, Stx2d is activated by intestinal mucus; this characteristic distinguishes Stx2d from other Stx2 family members. The substance from mucus that activates Stx2d has been identified as an elastase that cleaves the C-terminal two amino acids of the A2 subunit [95], but elegant studies with hybrid Shiga toxins have demonstrated that this activation also depends on the structure of the B pentamer [96]. Structural features important to the enzymatic activity of Shiga toxins have been defined. Stx1, Stx2, and Stx2e and the plant toxin ricin, all of which inhibit protein synthesis by the same mechanism of action, share two areas of homology in their A subunits [97]. In area 1, glutamic acid 167 is critical for biologic

			activity of Stx1 [98]. Similar experiments in Stx2 have shown similar results, affirming the importance of this glutamic acid residue in the active site [97]. In area 2, deletion of amino acids 202 through 213 of the Stx2 A subunit still allows holotoxin assembly but not cytotoxicity [97]." - Copied from https://www.sciencedirect.com/topics/medicine-and-dentistry/shiga-toxin
November 25th, 2024	Research into Shiga Toxin *Note - For the sections labelled "copied", I have looked into and specifically aimed to understand at the very least, the base concepts from the notes	https://www.sciencedirect.com/topics/medicine-and-dentistry/shiga-toxin https://pmc.ncbi.nlm.nih.gov/articles/PMC6402506/	Interactions with body cells - Shiga toxin, similarly to ricin and viscumin (plant toxins) removes an adenine from the 28S RNA of the 60S subunit of the ribosome -> inhibits protein synthesis - Adenine: one of the four nucleotide bases and one of the two purine bases, the other being guanine. Adenine and thymine are paired

			together in double-stranded DNA - 28S RNA: a structural ribosomal RNA for the subunit of eukaryotic cytoplasmic ribosomes, making it one of the most basic components of eukaryotic cells. 28S RNA acts as a ribosome and catalyzes the peptide bond formation, when two amino acids join together to form a protein - 60S: a subunit in eukaryotic cells that catalyzes peptide bond formation. - "Ribosomes contain two different subunits, both of which are required for translation. The small subunit ("40S" in eukaryotes) decodes the genetic message and the large subunit ("60S" in eukaryotes) catalyzes peptide bond formation" - Protein synthesis: a biological process in which cells make proteins

			- This ribotoxic effect is followed by apoptosis - Apoptosis: a programmed, type of cell death where a series of molecular steps in a cell lead to the death of the cell. Often used by the body in order to remove abnormal or unnecessary cells, which is why apoptosis is common in early stages of development “Shiga toxins are either phage-encoded (Stx1 and Stx2) or chromosomally encoded (Stx and Stx2e) [99–101]. In the early 1970s it was first reported that lysates of an E. coli O26:H11 strain isolated from an outbreak of infantile diarrhea could transfer enterotoxigenicity to a nonpathogenic E. coli in vitro [102]. This was the first indication that in STEC, Shiga toxins were encoded by bacteriophages. From lysates of this strain, the

			phage known as H-19B subsequently was isolated. This phage was shown to encode Stx1 and to have DNA sequence homology with the phage lambda (?) [103,104]. A second lambdoid phage encoding Stx2, designated 933W, was isolated from a clinical O157:H7 isolate responsible for an outbreak of hemorrhagic colitis in 1982 [10,99,105]. Since these initial observations, it is now appreciated that Stx-encoding phages are lambda-like and that the regulatory components relating to induction and phage gene control appear to be similar to those in lambda. Clinical STEC isolates may contain a single phage or multiple phages. Many clinical O157:H7 isolates harbor distinct Stx1 and Stx2 bacteriophages. Various groups have published

			the nucleotide sequences of the stx genes [106–111]. The different stx operons have a similar structure and are composed of a single transcriptional unit consisting of one copy of the A subunit gene followed by the B subunit gene. It was believed initially that the B subunit had its own promoter [112]; later it was appreciated that a single promoter transcribes A and B subunits; B subunit translation is augmented due to a stronger ribosomal binding site compared with A subunit translation. This results in more B subunit translation, providing more B subunits for the A1:B5 structure of the Shiga toxin holotoxin [113]. Comparison of the nucleotide sequence of the A and B subunits of Stx1 and Stx2 reveals 57% and 60% homology, respectively, with 55%

			and 57% amino acid homology [109]. Despite this degree of homology, Stx1 and Stx2 are immunologically distinct, and neither is cross-neutralized by polyclonal antibody raised to the other toxin. Stx2c is very similar to Stx2; the A subunits are identical, and the B subunits share 97% amino acid homology. Stx2c and Stx2d have identical B subunits; the A subunits share 99% homology [114]. Stx2e has the least similarity to other Stx2 family members. Although the Stx2 and Stx2e A subunits have 93% deduced amino acid homology, the B subunits have only 84% deduced amino acid sequence homology [115]. It is not surprising therefore that the receptor binding specificity of Stx2e is different from that of other Stx2 family

			members. A fifth family member, Stx2f, has been described recently in STEC isolated from populations of feral pigeons, although little is known about this variant [116]. The search for Shiga toxin receptors on mammalian cells began in 1977, when Keusch and Jacewicz reported that toxin-sensitive cells in tissue culture removed toxin bioactivity from the medium, whereas toxin-resistant cells did not [117]. Furthermore, these studies suggested that the receptor was carbohydrate in nature and that the toxin was a sugar-binding protein or lectin. Later, a toxin-binding membrane component was extracted from toxin-sensitive HeLa cells and from rabbit jejunal microvillus membranes (MVMs) [118]. The MVM binding site was shown

			to be globotriaosylceramide (Gb3) [118,119]. Lindberg and colleagues reported that Stx1 bound to the P blood group active glycolipid Gb3, which consists of a trisaccharide of galactose α1-4-galactose-β-1-4-glucose linked to ceramide, and that Gb3 could inhibit biologic activity of Stx1 in cell culture systems [120]. Most Stx2 family members share a preference for Gb3 binding, except for Stx2e, which binds preferentially to globotetrosylceramide (Gb4), another neutral glycolipid, which has a subterminal Gal-α1-4-Gal disaccharide [121–123]. In many cases, the sensitivity of cells to Shiga toxins appears to be related to the number of toxin receptors present on the cell surface. Measures that increase or decrease toxin receptor expression

			directly alter responses to these potent toxins [124]. In addition, toxin activity can be modulated by the fatty acid composition of the lipid ceramide moiety [125], in particular fatty acid carbon chain length, which alters the intracellular uptake pathway of toxin and its biologic activity. This may explain why some cells can express Gb3 but fail to respond to toxin [126]. Arab and Lingwood [127] demonstrated the importance of the surrounding lipid environment on the availability of glycolipid carbohydrate for Shiga toxin binding. The lipid heterogeneity of Gb3 appears to be important in Shiga toxin binding and may define a growth-related signal-transduction pathway used by Shiga toxin [128]. Sensitivity of cells to Shiga toxin also can be affected by

			regulation of receptor expression. For example, rabbit intestinal brush-border membrane Gb3 is both developmentally and maturationally regulated via the biosynthetic Gb3-galactosyltransferase and degradative α-galactosidase enzymes [129]. Gb3 is also maturationally regulated in cultured human intestinal epithelial cells. Gb3 is induced by exposure of villus-like Caco2A cells, but not crypt-like T84 cells, to known regulators of gene transcription, e.g., sodium butyrate [130]. In Caco2A cells, butyrate-induced Gb3 expression coincides with expression of villus cell differentiation markers such as alkaline phosphatase, lactase, and sucrase. Produced by normal resident enteric flora in high concentration, butyrate effects on intestinal

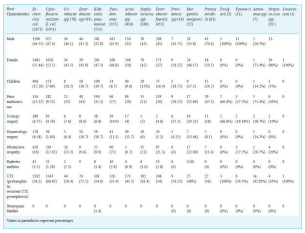
			epithelium may be pertinent in the human colon, the site of STEC infection." - Copied from https://www.sciencedirect.com/topics/medicine-and-dentistry/shiga-toxin
November 27th, 2024	Mentor Meeting	N/A	Mentor Name: Ms. Maja Omerovic Discussion Regarding - Project Overall - Potential Detection or Testing Method - Paper https://www.sciencedirect.com/topics/medicine-and-dentistry/shiga-toxin
December 2nd, 2024	Research into Shiga Toxin	https://link.springer.com/article/10.1007/s00253-015-7236-3	"Shiga toxins are a group of type 2 ribosome-inactivating proteins (RIPs) produced in several types of bacteria. The toxins possess an AB5 structure, which comprises a catalytic A chain with N-glycosidase activity, and five identical B chains and recognize and bind to the target cells with specific

			carbohydrate moieties. In humans, the major molecular target which recognizes the Shiga toxins is the Gb3 receptor, which is mainly expressed on the cell surface of endothelial cells of the intestine, kidney, and the brain. This causes these organs to be susceptible to the toxicity of Shiga toxins. When a person is infected by Shiga toxin-producing bacteria, the toxin is produced in the gut, translocated to the circulatory system, and carried to the target cells. Toxicity of the toxin causes inflammatory responses and severe cell damages in the intestine, kidneys, and brain, bringing about the hemolytic uremic syndrome (HUS), which can be fatal. The Shiga toxin requires a couple of steps to exert its toxicity to the target

			cells. After binding with the target cell surface receptor, the toxin requires a complicated process to be transported into the cytosol of the cell before it can approach the ribosomes. The mechanisms for the interactions of the toxin with the cells are described in this review. The consequences of the toxin on the cells are also discussed. It gives an overview of the steps for the toxin to be produced and transported, expression of catalytic activity, and the effects of the toxin on the target cells, as well as effects on the human body." - copied from https://link.springer.com/article/10.1007/s00253-015-7236-3
December 6th, 2024	Research into Similar Bacteria Started	https://www.mayoclinic.org/diseases-conditions/salmonella/symptoms-causes/syc-20355329 https://penntoday.upenn.edu/news/how-avoid-foo	Salmonella - Common bacterial disease affecting the intestinal tract - Typically live in animal and human intestines

		d-poisoning-e-coli-and-salmonella	and are shed through stool (feces) - Most likely sources of information are contaminated water or food - Higher risk in countries without clean drinking water and/or proper sewage disposal - Causes include: raw meat, poultry, seafood, raw/undercooked eggs, unpasteurized dairy products, fruits, vegetables, improperly handled food, infected surfaces and infected pets/animals - Some people with salmonella infection have no symptoms (can be asymptomatic) - Symptoms include: diarrhea, fever, nausea, vomiting, chills, headache, blood in stool and stomach cramps within 8 to 72 hours after exposure (⅓ - 3 days) - Diarrhea can cause severe dehydration - should receive

			immediate medical attention - Occasionally, individuals can develop typhoid fever, a deadly disease, particularly in developing countries - Healthy people normally recover within a few days to a week without specific treatment - Life-threatening complications can develop if infection spreads beyond the intestines - Connections to E. coli - Both bacteria and fundamentally similar - Salmonella evolved from E. coli 100 million years ago - E. coli is more heterogeneous and lives in the gut of organisms like cows
December 26th, 2024	Research on Shiga Toxin Added	https://pmc.ncbi.nlm.nih.gov/articles/PMC7126671	Essentially, STEC releases Shiga toxin which in turn, damages the intestines, making it harmful to the body

			The STEC secrete Shiga toxins Stx 1 and Stx2, which bind to the enterocytes, the absorptive epithelial cells present on the luminal surface of the small and large intestines - Note: Although we have E. coli in our guts, it is typically a harmless strain that does not affect humans like STEC does. In fact, most strains of E. coli are harmless.
December 30th, 2024	Image Added Research on Similar Bacteria Information about Prevention Added	https://pmc.ncbi.nlm.nih.gov/articles/PMC3573607/ https://emedicine.medscape.com/article/785774-medication?form=fpf https://pmc.ncbi.nlm.nih.gov/articles/PMC3573607/ https://www.frontiersin.org/journals/cellular-and-infection-microbiology/articles/10.3389/fcimb.2020.00169/full	 Treatment - Antibiotics - Can't be used for STEC as in most cases, antibiotics either increase the risk of an individual getting HUS (as it can increase the production of shiga toxin) or have not been shown to have any effect

			- An example of an antibiotic for salmonella is ciprofloxacin - However, in general, around 73% of all E. coli isolates, a culture of microorganisms for study, were found to be resistant to ciprofloxacin. - Not only is it that antibiotics cannot be used for STEC, a major portion of other strains are resistant to various antibiotics. Toxin-Based Vaccines Stx is the main virulence factor associated with the potency of STEC-mediated disease pathology. It is released from the bacterial cell and exposed to the host immune system and has, therefore, long been considered one of the most prudent targets for vaccine strategy. Once released from the bacterial cell, Stx can be detected in the intestinal lumen and its target

			cells in the kidney and brain (Clements et al., 2012). Hence, immune cells primed to recognize Stx by prior vaccination can interfere and respond to the toxin as it travels from the site of release to the distal organs and eliminate it before it reaches its targets (Figure 2A). Of Stx1, Stx2, and the different varieties of each of these subtypes, Stx2 has been at the center of a greater number of vaccination approaches as it is commonly associated with more severe disease outcomes in humans, such as the development of HUS. Several vaccination strategies have been developed and tested that are solely toxin-based. With the active site of Stx known, inactive derivatives of the toxin are easy to make, and present safe alternatives for application. Several

			different studies showed that vaccination of animals with purified inactive Stx derivatives induced the production of neutralizing antibodies against the respective toxin and protected the animals from toxemia or limited shedding or disease after challenge (Gordon et al., 1992; Acheson et al., 1996; Konadu et al., 1999; Marcato et al., 2001, 2005; Ishikawa et al., 2003; Kerner et al., 2015; Schmidt et al., 2018). In addition to vaccines based on the inactive toxin, hybrid subunit vaccine approaches have been tested as the development of a vaccine, which may induce neutralizing antibodies against not only one but both types of Stx, would be ideal.

			Vaccination with a hybrid toxin consisting of an inactive Stx2 A-subunit fused to the native Stx1 B-subunit was able to produce neutralizing antibodies against both Stx1 and Stx2. Mice immunized with this toxin were protected from subsequent lethal challenge with either Stx1 or Stx2, or both toxins (Smith et al., 2006). Injection with a purified fusion protein consisting of the B-subunits of Stx subtypes 1 and 2 (Stx2B-Stx1B; short 2S) generated neutralizing antibodies against both Stxs and increased survival of mice after a challenge with E. coli O157:H7 lysates (Gao et al., 2009). Interestingly, the protective effects of the vaccine were stronger with the Stx2B-Stx1B-subunit fusion than when

			separate B-subunits were used for immunization (Gao et al., 2009). Moreover, a fusion protein comprising the B-subunit of Stx1 and the inactive A-subunit of Stx2 (Stx2Am-Stx1) was constructed. Immunization with this protein resulted in a strong induction of neutralizing antibodies against both types of Shiga toxin and increased the survival rate of mice after challenge with E. coli O157:H7 lysates (Cai et al., 2011). A vector-based DNA vaccine encoding the C-terminal 32 amino acids of the Stx2 A-subunit and the complete B subunit (pStx2ΔAB) also produced neutralizing antibodies against both Stx2 subunits. It further decreased the mortality of mice after lethal challenge with Stx2 (Bentancor et al., 2009).

			Intranasal immunization with the Stx2 B-subunit in combination with a mutant of heat-labile toxin induced neutralizing antibodies against both Stx1 and Stx2 in vivo and protected mice against fatal disease. Interestingly, immunization of mice with the B-subunit of Stx1 only protected against subsequent challenge with Stx1 (Tsuji et al., 2008). A fusion of the Stx B-subunit to the B-subunit of heat-labile toxin has also been assessed for toxin subtype 2e. Here, the ability of the fusion protein to induce neutralizing antibody production was much higher than for the Stx2 B-subunit alone. Furthermore, mice that had been immunized with the Stx2eB-LTB fusion protein were

			protected against challenge with a lethal dose of toxin (Ran et al., 2008). In another study, the B subunit of Stx2 was fused with Brucella lumazine synthase, a protein that forms a dimer of pentamers thereby creating a scaffold for the presentation of the Stx. The resulting fusion protein induced a lasting immune response in mice after three vaccinations and vaccinated mice were protected from intravenous challenge with Stx2. Furthermore, antibodies isolated from vaccinated mice neutralized Stx2 as well as its variants. In addition, weaned mice inoculated with the immune sera were protected against oral infection with EHEC (Mejias et al., 2013).

			Vaccine Approaches Based on LEE-Encoded Proteins While Stx is produced by all strains that are classed as STEC, only some STEC strains encode the locus of enterocyte effacement pathogenicity island (LEE), which encodes for the T3SS. Therefore, the use of T3-secreted proteins in vaccine approaches is valid but limits the specificity of the vaccines to LEE-positive STEC strains. As the most common EHEC strains including those of the O157:H7 serotype are LEE-positive, the T3-S protein-based approaches will target many of the most common serotypes. However, they do not affect other pathotypes such as the O104:H4 strain, which caused the outbreak in Germany in 2011.

			Only a few of the T3-secreted proteins can be used as targets for vaccination strategies, however, as most are translocated from the bacterial cell directly into the host cell cytoplasm and are never exposed to the surrounding environment. A few proteins including EspA, EspB (Figure 2A), Tir, and intimin (Figure 2B) are exposed on the outside of the cell at times as antibodies to these proteins are detectable in humans after an EHEC infection (Li et al., 2000; Asper et al., 2011). These proteins have been assessed as targets for vaccination strategies. The translocated intimin receptor (Tir) inserts into the host cell membrane upon translocation. Its surface-exposed receptor domain then interacts with the bacterial outer membrane protein

			intimin to induce intimate attachment of the bacteria to the host cell surface. Furthermore, protein components of the T3 secretion system such as the sheath protein EspA, as well as EspB and EspD, the proteins required for forming a pore in the host cell membrane, have also been assessed for their immunogenicity (Loureiro et al., 1998; Martinez et al., 1999; Asper et al., 2011; Guirro et al., 2013). Most T3-secreted protein-based vaccines have been assessed using the intranasal immunization route. This vaccination approach promises a needle-free application and has, so far, yielded promising results. Subcutaneous and intranasal immunization of mice

			with T3-secreted proteins showed that while subcutaneous injection was unable to raise an immune response, intranasal vaccination induced the production of anti-Tir and EspA antibodies. This reduced E. coli O157:H7 shedding after infection (Babiuk et al., 2008). Subcutaneous and intranasal immunization with purified Tir showed similar results. Mice immunized intranasally produced neutralizing antibodies, which resulted in reduced fecal shedding of E. coli O157:H7 after infection and increased animal survival (Fan et al., 2012). Intranasal immunization of mice with a purified fusion protein consisting of EspB and the C-terminus of intimin induced neutralizing antibodies against both EspB and intimin and antisera of

			immunized mice had promising anti-hemolytic effects in vitro (Cataldi et al., 2008). A recombinant fusion protein of EspA, intimin, and Tir (EIT) was created and used for immunization of mice. Subcutaneous or oral immunization of mice with the EIT protein resulted in a significant decrease of bacterial colonization and shedding after challenge with EHEC O157:H7 and an increase in anti-EIT IgG and IgA (Amani et al., 2010). In a follow-up study, rEIT was linked to chitosan and used for either intranasal electrospray (Doavi et al., 2016) or oral (Khanifar et al., 2019a) immunization of mice. Intranasal and oral administration both induced specific immune responses and reduced bacterial shedding after challenge with E. coli O157:H7 (Doavi et al.,

			2016). Oral administration additionally helped protect mice against E. coli O157 challenge and reduced damage (Khanifar et al., 2019a). An additional approach was made by encapsulating EIT together with the B-subunit of Stx2 (Khanifar et al., 2019b). Mice were subcutaneously or orally immunized and either infected with E. coli O157:H7 or challenged with a fatal dose of Stx2. While the former mice showed reduced colonization and bacterial shedding, the latter showed increased survival (Khanifar et al., 2019b). A shortened variant of the EIT fusion protein consisting of only EspA and intimin (EI) was recombinantly expressed and its immunogenicity was assessed after subcutaneous injection

			with two subcutaneous boosters and a third booster that was administered i.p. (Rad et al., 2013). This fusion protein, too, induced an immune response and decreased bacterial shedding and histopathological changes in the intestine after challenge (Rad et al., 2013). In addition, the immunogenicity and protective efficacy of a DNA vaccine against a truncated version of the EHEC factor for adherence-1 (Efa-1'; the homolog of LifA in EPEC and C. rodentium) was evaluated in mice. Intranasal immunization with plasmid DNA induced efa-1-specific immune responses and protected mice from subsequent challenge with E. coli O157:H7 (Riquelme-Neira et al., 2015).

			Peptide-based approaches to vaccination include the KT-12 peptide, which is based on a predicted B-cell epitope of intimin conjugated to adjuvant (Wan et al., 2011) and the synthetic peptides CoilA and CoilB, which interact with EspA (Larzabal et al., 2013). KT-12, when used for intranasal immunization, induced the production of neutralizing antibodies and protected mice from challenge with E. coli O157:H7 (Wan et al., 2011). Immunization of mice with CoilA and CoilB was shown to block intestinal damage in mice infected with C. rodentium (Larzabal et al., 2013). A fusion of EspA, the C-terminus of intimin and the B-subunit of Stx2 (EIS), was constructed and assessed for its ability to induce the production of

			neutralizing antibodies. Indeed, antibodies against all three components of the fusion protein were detected, and immunized mice were protected from challenge with E. coli O157:H7 or lysates thereof (Gu et al., 2009). Fusion of the processed, active form of the Stx2A-subunit (Stx2A1) to the N-terminus of EspA induced the production of neutralizing antibodies in immunized mice (Cheng et al., 2009) and a fusion of the B-subunits of Stx1, Stx2, to a truncated version of intimin resulted in increased immune responses and protection of mice after a fatal challenge with E. coli O157:H7 (Gao et al., 2011). Intranasal immunization with a novel EspA-Tir fusion protein (EspA-Tir-M; designating that the

			middle domain of Tir was used) showed high levels of neutralizing antibodies while subcutaneous vaccination had little effect. Additionally, intranasal immunization increased the survival of mice from subsequent challenge with E. coli O157:H7 and reduced organ damage (Lin et al., 2017b). Bacteria-Based Vaccines Attenuated or Vaccine Strains Several vaccination approaches use non-pathogenic bacteria or bacterial vaccine strains as delivery vehicles and to increase immunogenicity. These approaches include genetically modified EHEC, EPEC, and Salmonella strains as well as probiotic strains such as Lactococcus lactis and Lactobacillus acidophilus.

			A non-pathogenic variant of the EHEC O157:H7 86-24 strain was created by deletion of both the gene encoding the transcriptional regulator of the LEE (ler) and the stx gene. These deletions completely abolished cytotoxicity in vitro when compared to EHEC EDL933. A derivative of this strain that expresses the inactive forms of Stx1 and Stx2 from a plasmid also showed highly diminished cytotoxicity in vitro. Injection with either stx/ler deletion mutant or the respective Stx1/Stx2-expressing strain reduced the colonization of E. coli O157:H7 after infection of mice. Furthermore, if mice were immunized when pregnant, they passed the immunity on to their offspring, which were protected against E. coli O157:H7 infection (Liu et al., 2009).

			Enteropathogenic E. coli (EPEC) also presents a vaccine alternative for EHEC as it is less pathogenic and shares the LEE pathogenicity island-encoded virulence genes. Immunization with EPEC raised neutralizing antibodies against EspB and intimin and conferred some protection against an EHEC infection. Vaccinated mice showed only mild disease phenotypes such as slight intestinal damage, while no kidney pathology could be detected (Calderon Toledo et al., 2011). Immunization of mice with a Salmonella Typhimurium vaccine strain expressing an inactive Stx2 variant consisting of the A2- and B-subunit of Stx2 (Stx2ΔAB) resulted in efficient colonization of the Peyer's patches and

			production of neutralizing antibodies. Serum collected from immunized mice was able to neutralize Stx2-mediated toxicity in vitro. However, there was only minimal protection observed when mice were challenged with a lethal dose of Stx2, and no protective effect was seen for kidney health (Rojas et al., 2010). Additionally, another group constructed a Salmonella Typhimurium strain expressing intimin, which was used to immunize mice orally (Oliveira et al., 2012). This immunization resulted in a significant increase in the levels of serum IgG and fecal IgA and reduced fecal shedding after an E. coli O157:H7 infection (Oliveira et al., 2012). A boost vaccination 2 weeks after the initial immunization led to continuously high

			colonization levels of the vaccine strain and dissemination into the underlying tissues such as Peyer's patches and spleen (Oliveira et al., 2012). Oral immunization of mice with attenuated Salmonella expressing a hybrid protein consisting of EspA in combination with the C-terminus of intimin and the Stx2 B-subunit (EIS, also see above) raised neutralizing antibodies against the respective proteins and protected mice from a lethal challenge with EHEC for more than 70 days. This period could be extended by a subcutaneous boost with purified EIS (Gu et al., 2011). Inoculation of mice with a recombinant Mycobacterium bovis BCG (rBCG) vaccine, which was modified to express the Stx2 B-subunit, induced the production of

			neutralizing antibodies against Stx2. Two high-dose intraperitoneal immunizations resulted in decreased colonization and increased survival after fatal challenge with a STEC strain (Fujii et al., 2012). The probiotic lactic acid bacterium Lactococcus lactis is considered a safe vaccine vehicle. Use of a L. lactis strain expressing the Stx2 A1-subunit (the A-subunit missing the 15 C-terminal amino acids) for the immunization of mice resulted in increased levels of fecal and serum IgA. Immunized animals had significantly reduced intestinal and kidney damage. Furthermore, immunized mice showed increased survival after challenge with a lethal dose of Shiga toxin isolated from either E. coli O157:H7 or Shigella

			dysenteriae (Sreerohini et al., 2019). L. lactis expressing the T3-secreted protein EspB did not yield neutralizing antibodies when used to infect mice. After an i.p. boost with recombinant EspB, however, specific IgG and IgA levels increased (Ahmed et al., 2013). In a follow-up study, the L. lactis was modified to secrete EspB after expression, which resulted in an increased production of neutralizing antibodies. Also, mice immunized with this version of the EspB-expressing L. lactis were protected against E. coli O157:H7 colonization (Ahmed et al., 2014). An L. lactis strain expressing the EspA protein has also been designed. However, this strain has so far only been used for the production of recombinant EspA, as described above. Here, too, a system to either

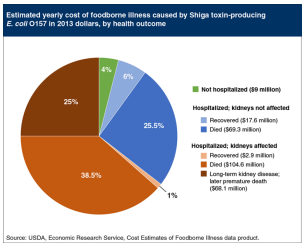
			display the protein at the cell surface or secrete it from the cell will probably be needed but may be worthwhile (Luan et al., 2010). A recombinant L. lactis strain that displays the Stx1 B-subunit via albumin binding domains (single-domain non-immunoglobulin scaffolds) on the bacterial cell surface was recently designed by Zadravec et al. (2016). ELISA and FACS analysis confirmed the ability of this strain to bind Stx1. The immunogenicity and safety of this strain and its ability to protect against challenge were, however, not yet tested in animals. Lastly, a recombinant Lactobacillus acidophilus variant expressing EspA and the Tir central domain (EspA-Tir-M) inhibited A/E lesions formation by EHEC O157:H7 after

			pre-incubation in vitro. Oral immunization of mice induced the production of specific and systemic neutralizing antibodies and reduced EHEC O157:H7 colonization. It also inhibited intestinal A/E lesions and toxin-mediated organ damage (Lin et al., 2017a). Plant-Based Vaccines Plant-based vaccines have been trialed with the idea that they can easily be used for oral vaccination. Toxin-Targeted Plant-Based Vaccines The inactive A-subunit of Stx2 was produced by expression in a tobacco plant cell line (NT-1). Subsequent immunization of mice by feeding or by parenteral immunization with an oral boost resulted in increased Stx2 IgA and IgG levels. It was able to

			protect mice from a lethal challenge with an EHEC strain. Sera of immunized mice further neutralized toxicity in vitro (Wen et al., 2006). For the treatment of porcine edema disease, which is a severe and often fatal disease in pigs that is also mediated by Stx (in this case subtype Stx2e), Hamabata et al. have recently developed stx2eB-transgenic lettuce for immunization. Infection of piglets with STEC after oral vaccination by feeding the lettuce showed decreased levels of pathogenesis in lettuce-fed piglets (Hamabata et al., 2019). Virulence Protein-Targeted Vaccines The immunization capacity of plant-codon optimized intimin expressed in the NT-1

			tobacco cell line was also assessed by either i.p. injection of purified protein, feeding of transgenic plant cells, or a combination thereof. Here, mice immunized by injection and boosted by feeding developed neutralizing antibodies against intimin and reduced the time of bacterial shedding after challenge with E. coli O157:H7 (Judge et al., 2004). In another approach, chimeric protein composed of the LEE-encoded proteins EspA, intimin, and Tir (named EIT and further described above) was codon-optimized for expression in tobacco and canola plants. Using this plant-based expression strategy, recombinant EIT was prepared, and immune responses in mice were assessed after parenteral and oral immunization as well as after a combination. Here, a

			combination of subcutaneous injection and oral gavage yielded the highest immune responses and resulted in significantly reduced fecal shedding of E. coli O157:H7 after challenge (Amani et al., 2011).
December 31st, 2024	Research on Similar Bacteria	https://www.frontiersin.org/journals/cellular-and-infection-microbiology/articles/10.3389/fcimb.2020.00169/full https://www.mdpi.com/2072-6651/13/6/416	Antibiotics (not used) - Antibiotics are not recommended in any case for STEC infections in particular - Reasons include: - No proof that antibiotics can actually help reduce or treat STEC infections - Can increase the risk of developing HUS as shiga toxin production can be increased - In some cases, bacterial SOS response (especially in the quinolone family which includes ciprofloxacin, ofloxacin, etc. - broad spectrum antibiotics) can be initiated, which can lead to mutations in the DNA of the bacteria

			causing them to become more resistant towards antibiotics Treatments for Salmonella - Probiotics - Are live bacteria or yeast that are beneficial to the body, particularly the digestive system - Keep the gut healthy - A few probiotics that are used for salmonella include lactobacilli and bifidobacterium - Lactobacillus inhibits STEC proliferation (an increase in numbers, typically rapid) as it produces hydrogen peroxide, lactic acid, IgA and leukocyte activity
January 2nd, 2025	Graph Added Research on Similar Bacteria	https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=78625	 “While most E. coli bacteria are relatively harmless, a small group

			of E. coli produce a Shiga toxin that can severely damage a person's kidneys. The most well-known Shiga toxin-producing E. coli (STEC), STEC O157, causes less than 1 percent of U.S. foodborne illnesses with an identifiable pathogen cause and only 2 percent of the cost of these pathogens. Yet, because it is a major cause of outbreaks and because it can cause kidney damage, STEC O157 is often in the news. ERS estimates that the 63,000 illnesses caused by STEC O157 (formerly called E. coli O157) each year in the United States impose $271.4 million in economic burden. Most people (97 percent) sickened with STEC O157 recover without being hospitalized. In roughly 2,100 cases of STEC O157 illnesses, however, people are hospitalized; in 15 percent of these cases the kidneys are

			affected—sometimes resulting in death, ongoing dialysis, or a kidney transplant. Cases in which the kidneys are affected account for 64.5 percent of the economic burden from foodborne STEC O157. This chart is based on a chart in the ERS report, Economic Burden of Major Foodborne Illnesses Acquired in the United States, May 2015." - copied from https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=78625 - Typically, individuals are able to recover from STEC without the use of antibiotics or other treatments - In some instances, treatment can also be supportive therapy - Rehydration therapy: used to prevent and treat dehydration, particularly caused by diarrhea

			- Dialysis: treatment to clean blood and remove excess fluid from body when kidneys cannot function (especially from HUS) healthily enough - Can often be time-consuming - EXPENSIVE - the average cost can be upwards of $60,000, something that many people, including the government, cannot afford for long periods of time - Not always the case as vulnerable groups (such as those under 5, older than 65, weakened immune system, etc.) may not be able to recover as they are at a disadvantage essentially
January 3rd, 2025	Research on Similar Bacteria	https://www.sciencedirect.com/science/article/abs/pii/S1567134807001748 https://pmc.ncbi.nlm.nih.gov/articles/PMC375161/	Connections to E. coli - “Salmonella enterica serovar Typhimurium (S. Typhimurium) and certain Escherichia coli are human pathogens that have evolved through the acquisition

			of multiple virulence determinants by horizontal gene transfer. Similar genetic elements, as pathogenicity islands and virulence plasmids, have driven molecular evolution of virulence in both species. In addition, the contribution of prophages has been recently highlighted as a reservoir for pathogenic diversity. Characterization of horizontally acquired virulence genes has several clinical implications. First, identification of virulence determinants that have a sporadic distribution and are specifically associated with a pathotype and/or a pathology can be useful markers for risk assessment and diagnosis. Secondly, virulence factors widely distributed in pathogenic strains, but absent from non-pathogenic bacteria,

			are interesting targets for the development of novel antimicrobial chemotherapies and vaccines. Here, we summarize the horizontally acquired virulence factors of S. Typhimurium, enterohemorrhagic E. coli O157:H7 and uropathogenic E. coli, and we describe their use in novel therapeutic approaches. Commensal bacteria, as Escherichia coli, have adapted to coexist with the human host without causing disease. Pathogenic bacteria have adapted to colonize the human host and have acquired the ability to cause clinically significant pathologies. Salmonella enterica and E. coli are closely related enterobacteria that diverged from a common ancestor 100–150 million years ago (Doolittle et al., 1996). The genomes of the two species are essentially

			superimposable and genome sequencing demonstrated that the median homology between non-pathogenic E. coli and Salmonella enterica serovar Typhimurium (S. Typhimurium) genomes is 80% (Blattner et al., 1997, McClelland et al., 2001). Both species have evolved into intestinal pathogens. In addition, E. coli strains have also evolved as extraintestinal pathogens. The molecular evolution of virulence of human pathogens S. Typhimurium and pathogenic E. coli is driven by the acquisition of multiple genetic elements (pathogenicity islands, plasmids, and prophages) by horizontal gene transfer. These horizontally acquired elements encode virulence factors necessary for colonization and

			replication within the host, neutralization of host defences and spread into new hosts. Main virulence determinants used by S. Typhimurium and/or E. coli include adhesins, type III secretion systems (T3SS) that inject effector proteins into host cells, toxins and iron acquisition systems. Both non-typhoidal S. enterica and pathogenic E. coli are increasingly resistant to multiple antibiotics. For example, the resistance of uropathogenic E. coli strains to trimethoprim–sulfamethoxazole and fluoroquinolones, the drugs of choice for the treatment of urinary tract infections (UTI), has become a major concern both in hospitals and in the community (Gupta et al., 2001). Hence it is urgent to develop better prevention and

			treatments against these infections. The implication of horizontally acquired virulence factors in clinical issues is multiple. First, genes that have a sporadic distribution, associated with specific pathology and/or epidemic features, can be used as markers of infection to improve molecular epidemiology and diagnostic methods. Secondly, genes that are stable in pathogenic strains, and absent from non-pathogenic strains, can provide novel therapeutic targets and novel vaccine strategies. Here, we present a brief overview of horizontally acquired genetic elements from S. Typhimurium and pathogenic E. coli that play a role in virulence and we provide examples of the implications of these studies for the development of novel

			therapeutic approaches." - copied from https://www.sciencedirect.com/science/article/abs/pii/S1567134807001748 Treatments - Probiotics - Bifidobacterium inhibits the production of Stx production (not growth) by increasing the pH of the stomach and increasing the concentration of acetic acid
January 12th, 2025	Rough Idea for Detection Method Created	N/A	- Replicate intestinal mucus as it activates stx - Research into differences between the replicated mucus and actual live mucus and if it interacts with stx differently - Add something that could make the mucus glow a certain colour if stx is activated or if the e coli is detected somehow - Like the synbio thing with the bacteria art - Similar to covid testing kit, e coli is swabbed

			with a q-tip and then put into the mucus liquid and after a few minutes, if it turns x colour, it has e coli and if it turns y colour or doesn't change colours it doesn't have e coli - Test on 5 samples
January 13th, 2025	Looking into Rough Detection Method Idea	https://pmc.ncbi.nlm.nih.gov/articles/PMC9331835/ https://science.ok.ubc.ca/2020/11/12/ubco-research-contributes-to-new-understanding-of-gastro-intestinal-mucus/ https://www.nature.com/articles/s41598-022-11468-2	Background - Intestinal mucus can be replicated, although it seems a little difficult Other papers - Using phage-based assays - Phage: virus that infects bacteria - Assay: lab test to find the amount of a specific substance
January 15th, 2025	Mentor Meeting	N/A	Discussed about creating or looking into previous detection methods Contacted another mentor, Mr. Gustavo Marchani, regarding potential labs to detect E. coli on lettuce

January 17th, 2025	Mentor Meeting	N/A	Meeting with Ms. Maja Omerovic, Mr. Gustavo Marchani and Dr. Iaci Soares to discuss detection methods. New idea to conduct a PCR test on E. coli and use primers. To Research - Primers - PCR - Scientific papers on E. coli that contaminates lettuce - Papers working with primers - Current Protocol
January 18th, 2025	Detection Method	https://pmc.ncbi.nlm.nih.gov/articles/PMC6131372/# https://pmc.ncbi.nlm.nih.gov/articles/PMC7347742/	Research - Study what primers are - Look into textbooks or videos - Ask Dr. B for biochem textbook - Look into PCR - Look for scientific papers on E.coli that contaminates lettuce - Look for papers that have worked with primers - Current protocol

January 19th, 2025	Detection Method	Campbell Biology https://www.idtdna.com/pages/community/blog/post/dna-synthesis-the-basics https://www.nature.com/scitable/definition/primer-305/ https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/primase https://www.sciencedirect.com/topics/neuroscience/dna-polymerase-i https://www.genome.gov/genetics-glossary/Primer https://www.youtube.com/watch?v=a5jmdh9AnS4	- Primer: a short single-stranded nucleic acid that initiates DNA synthesis - "A primer is a segment of new strand (complementary to the template) with a 3′ hydroxyl group to which nucleotides can be added" - Synthetic primers are also known as oligonucleotides - DNA synthesis: the process of creating a new DNA strand from a template strand - Primase is an enzyme that synthesizes RNA primers - Synthesize: produce through biological or chemical processes Required as DNA polymerase cannot initiate polymer synthesis on single-stranded DNA templates - DNA polymerase is an enzyme that forms new DNA molecules through assembling nucleotides

			(which are the basic building blocks of DNA/RNA) - “Primase starts a complementary RNA chain with a single RNA nucleotide and adds RNA nucleotides one at a time, using the parental DNA strand as a template. The completed primer, generally five to ten nucleotides long, is thus base-paired to the template strand. The new DNA strand will start from the 3′ end of the RNA primer.” - Campbell Biology
January 23rd, 2025	Detection Method	https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction-PCR https://medicine.yale.edu/keck/dna/protocols/guidelines/	- Primers dictate the segment of DNA that is to be copied in PCR - Results in millions or billions of copies of the DNA segment in a short time - Amplified DNA can be used in lab procedures Can be used to diagnose or detect infectious diseases, like E. coli - Amplification increases quality size

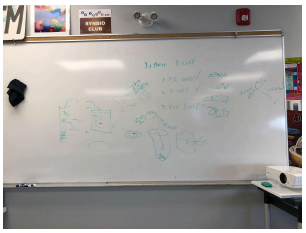
			- “PCR involves using short synthetic DNA fragments called primers to select a segment of the genome to be amplified, and then multiple rounds of DNA synthesis to amplify that segment.” - NHGRI Parameters - Yale - 20-30 nucleotides in length. - 50% G/C content. - G & C "clamps" on the 5' and 3' ends (at least a single G or C residue.) - Avoid multiple Thymidine residues on 3' and 5' ends. - Avoid primers with long runs (more than 4) of a single base. - Avoid primers with secondary structures or that can hybridize to form dimers or hairpins. These can be easily predicted if a primer design program is used such as primer select. - Melting temperature 55o-65oC.

			- Check primers for specificity in annealing to template. - Primers should be located at least 30-40 bases upstream of the area of interest in the sequence read.
January 24th, 2025	Detection Method	https://www.researchgate.net/figure/List-of-primers-used-for-the-detection-of-E-coli-16S-rRNA-gene-stx1-and-stx2-genes_tbl1_305526980 https://www.researchgate.net/figure/Primer-sequences-for-E-coli-STEC-and-six-O-antigen-amplifications_tbl1_333091291 https://www.iss.it/documents/20126/0/EURL-VTEC_Method_02_Rev_1.pdf/b5173cbd-5789-c729-b0c3-81da039e88c7?t=1644309049719 https://journals.asm.org/doi/10.1128/spectrum.03773-23 https://pmc.ncbi.nlm.nih.gov/articles/PMC87549/ https://pmc.ncbi.nlm.nih.gov/articles/PMC139680	Criteria - Addgene - Length of 18-24 bases - 40-60% G/C content - Start and end with 1-2 G/C pairs - Melting temperature (Tm) of 50-60°C - Primer pairs should have a Tm within 5°C of each other - Primer pairs should not have complementary regions Look into previously used primers

January 26th, 2025	Mentor Meeting	N/A	Discuss all other aspects of project with mentor.
January 29th, 2025	Mentor Meeting	N/A	Meeting with mentor and Mr. Gus to discuss primers and detection method for E. coli.
February 2nd, 2025	Detection Method	N/A	Purpose - Detect E. coli in a lettuce sample (more specifically pathogenic E. coli like STEC) - Can be applied wider to ensure that food is safe to consume and to protect public health - Potentially determine if alternative, faster ways could be used (the innovation part) - Eg. colony PCR - would it work, accuracy rate, etc. - Colony PCR can take around 1 hour while normal PCR can take between 6 hours to a day Expected result - This will work and E. coli will be detected in both colony and normal PCR

			- Prediction: normal PCR will have a higher accuracy rate than colony PCR but will take longer (tradeoff)
			
			Materials
			- PCR
			- Thermocycler
			- Primers
			  - Actin Primers
			  - ybbW Primers
			  - stx2 Primers
			
			Procedure
			- Obtain store-bought lettuce
			- Prepare samples
			- Extract DNA
			- Could try colony PCR to speed up the process
			- Setup the PCR
			- Run the PCR
			- Gel electrophoresis

February 3rd, 2025	Detection Method	N/A	Lettuce: romaine, few leaves per test Homogenize: blending Filter: probably Washing: If we're doing a single experiment with only 1 or 2 samples, then I would say that we shouldn't wash the

			lettuce since its more realistic. Only if the accuracy would be severely undermined then it would probably be fine to wash. However, if we do an experiment that, for example, compares the difference between washing and not washing lettuce then that having both would be nice
February 6th, 2025	Meeting	N/A	Meeting with Mr. Gustavo Marchani to discuss detection method further and gain clarification on certain aspects of project 
February 18th, 2025	Research Paper Started Draft Experiment Process Sent to Mr. Gustavo Marchani	N/A	
February 19th, 2025	Add Basic Information into Research Paper	N/A	

February 21st, 2025	Add Information into Research Paper	Previously used sources	
February 22nd, 2025	Work on Research Paper	Previously used sources	
February 23rd, 2025	Work on Research Paper	Previously used sources	
February 28th, 2025	Work on Detection Method Work on Research Paper	Previously used sources	
March 6th, 2025	Work on Research Paper	Previously used sources	
March 7th, 2025	Work on Research Paper	Previously used sources	
March 9th, 2025	Work on Research Paper	Previously used sources	
March 12th, 2025	Work on Detection Procedure	N/A	
March 14th, 2025	Work on Research Paper	Previously used sources	
March 16th, 2025	Work on Research Paper	Previously used sources	Add citations into text and put work in own words

March 17th, 2025	Work on Research Paper	Previously used sources	Understand more complex sections of text or literature
March 18th, 2025	Second-to-final Draft of all Components, Including Paper, Completed Make changes to proposal based on feedback	N/A	
March 19th, 2025	Draft of CYSF Platform Requirements Completed and Sent to Mentor(s) Procedure Completed	N/A	
March 20th, 2025	CYSF Platform Completed Video Recorded Mentor Meeting	N/A	APA citations checked by Ms. Madison Paul and Ms. Adrienne Carr Research paper checked by Ms. Maja Omerovic Mentor Meeting to Discuss Gel Electrophoresis