RESEARCH

Project: Field Ready, Point of Need Detection of Foodborne Pathogens in Irrigation Sources

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DEPARTMENT OF FOOD SCIENCE

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Final report, research overview and summary:

Escherichia coli O157:H7, commonly referred to as *E. coli* O157:H7, is a pathogenic strain of the bacteria *Escherichia coli* that has garnered significant attention in the context of irrigation water due to its potential to cause foodborne illnesses. This strain is known for its ability to survive and thrive in water sources used for irrigation, posing a serious concern for the contamination of fresh produce. When contaminated irrigation water comes into contact with crops, especially leafy greens and vegetables, there is a risk of *E. coli* O157:H7 adhering to the plant's surface or being internalized, making it difficult to eliminate through standard washing or disinfection processes. As a result, *E. coli* O157:H7 in irrigation water has been a focal point for agricultural and public health agencies striving to implement stringent safety measures to protect the integrity of our food supply chain and reduce the risk of foodborne outbreaks associated with contaminated produce.

Testing irrigation water for generic *E. coli* is a critical component of ensuring the safety of agricultural practices and the produce they yield. The requirements for such testing typically involve the collection of representative water samples from various sources within the irrigation system, including reservoirs, wells, or canals. These samples are then analyzed in accordance with established protocols, such as those outlined by regulatory agencies or industry standards. The tests aim to quantify the presence of generic *E. coli* bacteria, which serve as an indicator of fecal contamination and potential pathogens. Stringent guidelines may specify the frequency of testing, acceptable *E. coli* levels, and appropriate corrective actions if contamination is detected. However, current generic *E. coli* irrigation testing requirements have proved ineffective at indicating the presence of foodborne pathogens in water that was subsequently implicated in leafy green foodborne outbreaks.

A rapid field-based assay was developed for the detection of viable (living) *E. coli* O157:H7 in water used to irrigate fresh produce. The assay consists of a rapid filtration step, in which 5 liters of irrigation water are filtered through a reusable cartridge, followed by a 8 hour incubation step in which any concentrated bacteria are enriched in growth media to increase their numbers. Next, the enrichment is tested for the presence of *E. coli* O157:H7 bacterial cells using a reporter bacteriophage assay. Bacteriophages are viruses that specifically infect bacteria, and they can be genetically modified such that, upon infection of bacterial cells, they make the bacteria produce luminescence. The genetically modified bacteriophages are called reporter bacteriophages. The reporter bacteriophages are added to the bacterial enrichment, where they will infect any *E. coli* O157:H7 cells and produce luminescence. After 2 hours a luminescent substrate is added and the sample is read in a hand held luminomenter. The assay is capable of detecting 10^{-1} CFU/ml of *E. coli* O157:H7 within 10 hours. This research could lead to a field-based assay for direct detection of *E. coli* O157:H7 in irrigation water within 10 hours.

Regarding knowledge transfer, this research was presented at the 2023 International Association for Food Protection Annual meeting in Toronto, as follows: C. Chiappe, H. M. Davidson, T. Bakker, and L. Goodridge. 2023. Development of a Rapid, Field-Based Assay for Detection of *Escherichia coli* O157:H7 in Irrigation Water. Abstract P2-228. A manuscript is also in preparation and will be submitted in December, 2023.

Sincerely

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Canadian Produce Marketing Association, Canadian Food Safety Fund Final Report
Field Ready, Point of Need Detection of Foodborne Pathogens
in Irrigation Sources
Lawrence Goodridge
July 25, 2023

i. Abstract
Contaminated irrigation water has been implicated in outbreaks of *Escherichia coli* O157:H7 in which leafy greens were the identified vehicle. A rapid field-based assay was developed for the detection of *E. coli* O157:H7 in water used to irrigate fresh produce. Five liters of tap water was dechlorinated and inoculated with *E. coli* O157:H7 at final concentrations of 10^1, 10^0, and 10^{-1} CFU/ml. Duplicate plate counts were conducted on all inoculated water samples. The water was pumped (0.50 L/min) through Disposable K-Cup paper filters that were housed in a newly developed 3D-printed cartridge. Following filtration, the filters were removed from the cartridge, placed in a 250 ml Flask and enriched in Tryptic Soy Broth (TSB) at 37°C with shaking for 8 and 24 hours. Next, a swab was dipped into the enrichments, which were analyzed for the presence of *E. coli* O157:H7 using the PhageDx *E. coli* O157:H7 Easy Phage assay, which detects bacterial cells based on infection with luciferase reporter bacteriophages. Following completion of the assay, Relative Light Units (RLUs) were read in a Hygiena EnSURE Touch hand-held ATP luminometer. All experiments were repeated several times and each sample was tested in duplicate. All samples tested positive for *E. coli* O157:H7 after both 8 and 24 hours of enrichment. After 8 hours of enrichment, RLUs ranged from an average of 1,619 for the original 10^{-1} CFU/ml sample to 20,000 RLUs for the 10^1 CFU/ml sample. After 24 h of enrichment, all samples produced RLU readings of 20,000. Current generic *E. coli* irrigation testing requirements have not proved effective at indicating the presence of foodborne pathogens in water that was subsequently implicated in leafy green foodborne outbreaks. This research could lead to a field-based assay for direct detection of *E. coli* O157:H7 in irrigation water within 10 hours.

ii. Introduction
Fresh fruits and vegetables have increasingly become responsible for many cases of foodborne illness. For example, In the U.S. and Canada, at least 40 *Escherichia coli* outbreaks were linked to leafy greens between 2009 and 2020 (CDC 2021). Together, these outbreaks resulted in 1,212 illnesses, 420 hospitalizations, 77 cases of HUS and eight deaths. The outbreaks include seven *E. coli* outbreaks since 2017, that have been linked to leafy greens, salad mix or romaine lettuce. These outbreaks have resulted in at least 541 illnesses, 241 hospitalizations, 50 cases of HUS and seven deaths in the U.S. and Canada. In response, the Canadian government outlined a set of strict import rules on all romaine lettuce shipments originating from Salinas arriving in Canada from October 7, 2020 to December 31, 2020 (CFIA 2020). These rules include required proof of origin and product testing.

Irrigation water may play an important role in contaminating vegetables and fruits with foodborne pathogens (Solomon *et al*. 2002). Agricultural water can be contaminated via sewage overflows, polluted storm water runoff, and agricultural runoff. For example, the 2005 multistate *S. enterica* Newport outbreak associated with tomatoes was reported to be related to contaminated irrigation water (CDC 2007). Laboratory testing identified the outbreak strain of *E. coli* O157:H7 in canal water samples taken from the Yuma, Arizona growing region during an outbreak in 2018 that led to eight cases from five Canadian provinces, with one person being hospitalized (FDA 2018). In another outbreak that led to Canadian illnesses in November and December of 2018, the outbreak strain of *E. coli* O157:H7 was found

in one sample collected in the sediment of an agricultural water reservoir at a ranch in California owned by a company implicated in the outbreak (CDC 2019).

Runoff from animal waste can contaminate water that fresh produce growers use for irrigation (Beuchat 1996). After irrigation with contaminated water, the bacteria can adhere and enter into plants, and translocate within infested plants. Therefore, minimizing the risk of contamination by human bacterial pathogens during the pre-harvest period is essential to reducing foodborne illness risks.

Fecal pollution, which may contain foodborne pathogens, is traditionally evaluated with fecal indicator bacteria including fecal coliforms and generic *E. coli* (EPA 2000). Contradictory results have been reported as to the correlation between indicator organisms and the occurrence of human pathogens in surface water (McEgan *et al.* 2013). In the 2018 Californian romaine lettuce outbreak described above, water from the agricultural reservoir in which the *E. coli* O157:H7 outbreak strain was found had previously tested negative for generic *E. coli* (Bloch, 2018).

Clearly, there is an acute need to develop effective solutions to reduce the burden of foodborne disease related to the production of fresh produce. One potential solution to this problem is to test irrigation water for foodborne pathogens (Shogren and Neilson, 2018). This approach is hampered by the fact that there are currently no available point of need, simple to use assays that can be used to test irrigation water for the presence of pathogens. Additionally, the low concentration of pathogens in irrigation water sources means that pathogen tests need to be sufficiently sensitive to detect the target bacteria.

The objective of this project was the development of a low cost, rapid point of need assay to detect foodborne pathogens (*E. coli* O157, *Salmonella* spp*., Listeria monocytogenes*) in irrigation water in 8 hours. Two aims were associated with this project, including the validation of a rapid filtration approach to efficiently concentrate foodborne pathogens from irrigation water sources (Aim 1) and the integration of the concentration step with the PhageDx *E. coli* O157:H7 Easy Phage assay, (for on the farm, point of need irrigation water testing) (Aim 2).

iii. Materials and methods

Aim 1. The rationale for testing liquid (water) samples lies in the fact that the use of water is imperative in the vegetable production process, and testing agricultural water allows for assessment of multiple routes of vegetable contamination. Also, pathogenic microorganisms are likely to be more equally dispersed than in solid (vegetable) samples, and testing liquid samples allows for much easier sample prep (i.e. microorganism isolation and concentration). A potential disadvantage of testing agricultural water is that the level of microorganisms may be less than the level found on the actual vegetables. If pathogens are present on vegetables at very low levels, it is possible that they might be missed in water. A major obstacle in detecting bacteria is the fact that due to the current limits of detection, bacteria must be enriched until a population large enough to become detectable is present. This requirement can delay test results by 24-48 hours depending on the bacteria. An alternative to enrichment is bacterial concentration, during which large amounts of the sample to be tested are concentrated, which effectively increases the bacterial population to the required detection limit. Thus, the first aim of the project was the development of a sensitive, simple, and cost effective approach to concentrate pathogens from irrigation water. We developed a 3D printed cartridge (Figure 1) for rapid concentration of large volumes (5 L) of irrigation water within 10 minutes. The cartridge houses a resusable K-Cup filter, and a disposable K-Cup paper filter is placed within the K-Cup filter. Water is pumped into the cartridge and through the paper filter with the use of a pump. The cartridge was printed using a high temperature resin, which allows for the cartridge to be reused following sterilization.

To evaluate the ability of the filter to concentrate *E. coli* O157:H7, influent wastewater samples were obtained weekly from the Guelph Wastewater Treatment Plant for a period of one year, and 500 ml were pumped through the filter unit. Following filtration, the K-Cup paper filter was removed from the cartridge, and DNA was extracted using a Qiagen DNA isolation kit. Quantitative Real-time PCR was conducted on the samples to detect *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* using the Quant Studio Real Time PCR device and the SureTect™ PCR Assays.

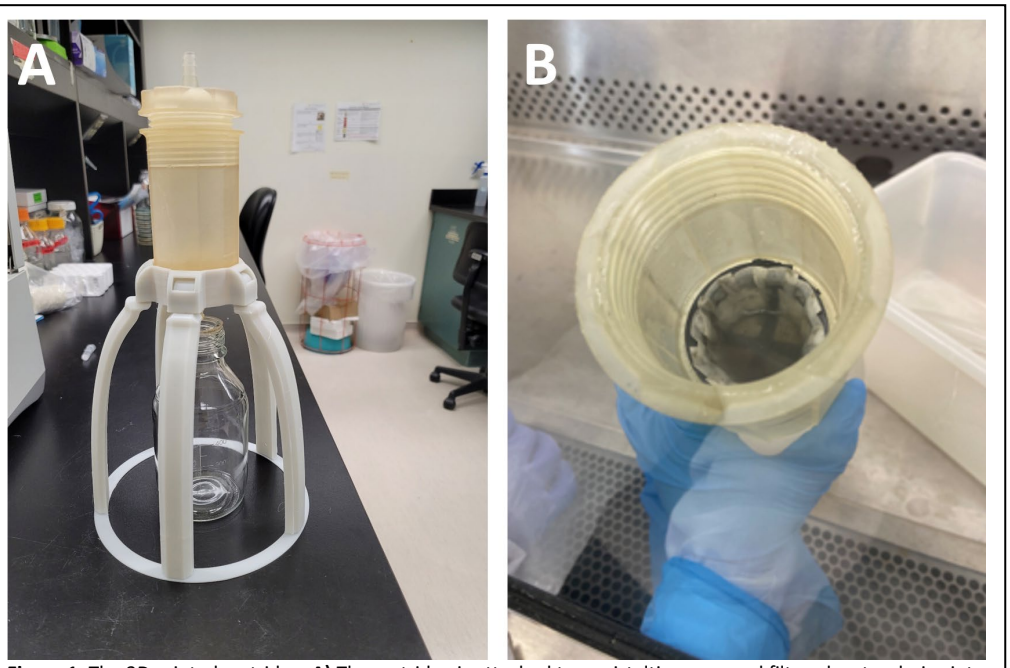

Figure 1. The 3D printed cartridge. **A)** The cartridge is attached t a peristaltic pump and filtered water drains into a bottle or flask. **B)** Inside the cartridge, a disposable K-Cup paper filter sits inside a reusable K-Cup filter, which acts as a holder. As water is filtered through the cup, bacteria bind to the surface and are captured.

Aim 2. Following validation of the cartridge using wastewater, experiments were conducted to evaluate the cartridge using tap water followed by integration with a diagnostic assay. Five litre samples of water were autoclaved in a 10 L jug. Overnight cultures of three *E. coli* O157:H7 isolates were diluted and individually added to the water samples to final concentrations of 10^1, 10^0 and 10^{-1} CFU/ml. water samples were filtered through the cartridge using a peristaltic pump, at a flow rate of 0.50 L/min (Figure 2).

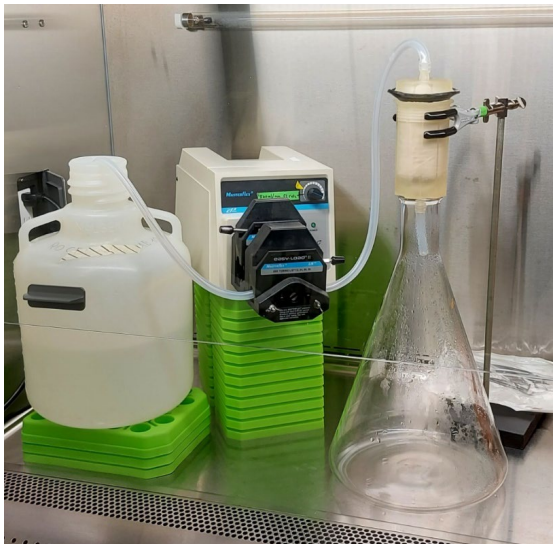

Figure 2. The irrigation water concentration setup.

Initial studies showed that the *E. coli* O157:H7 concentrations captured by the cartridge were too low for direct detection. Therefore, an enrichment step was needed to increase the *E. coli* O157:H7 concentrations to a detectable level. After filtering the water, the K-Cup paper filter was removed and placed in a flask containing 50 mL Tryptic Soy Broth (TSB), followed by incubation at 37°C for 8 and 24 hrs to allow bacterial growth. Following incubation, the broth culture was assessed for the presence of *E. coli* O157:H7 using the PhageDx *E. coli* O157:H7 Easy Phage assay (Figure 3).

The Easy Phage assay is based on the infection of target bacteria by bacteriophages and replication of the infecting bacteriophages within their specific

hosts. Bacteriophages demonstrate a high specificity for their bacterial host and are capable of replicating within their host quickly to high numbers. The genetically modified phages used in the Easy Phage assay express a luciferase enzyme during replication in bacteria. Following bacteriophage infection, the presence of the target bacteria is determined by incubating the infected bacteria (which are lysed to release the luciferase enzyme into solution) with a luciferase substrate and detecting emitted light in a handheld luminometer. An absence of detected light indicates that no target bacteria are present in that sample. An additional advantage of this system is that only viable bacteria cells are detected as bacteriophage only replicate in living cells.

To complete the Easy Phage assay, the sampling device (swab) was removed from the sample tube and dipped into the culture. The swab was placed back into the sample tube. The Snap Valve at the top of the sample tube was snapped, releasing the liquid reagent (containing the genetically modified bacteriophages) down the tube shaft where it interacted with the culture sample on the swab (and infected any *E. coli* O157:H7 cells that were present). The sample tube was incubated for 2 h at 37 °C. After incubation, the lysis buffer and luciferase reagent were added to the sample tube, and Relative light units were measured using the Hygiena EnSURE™ handheld luminometer (Figure 3B).

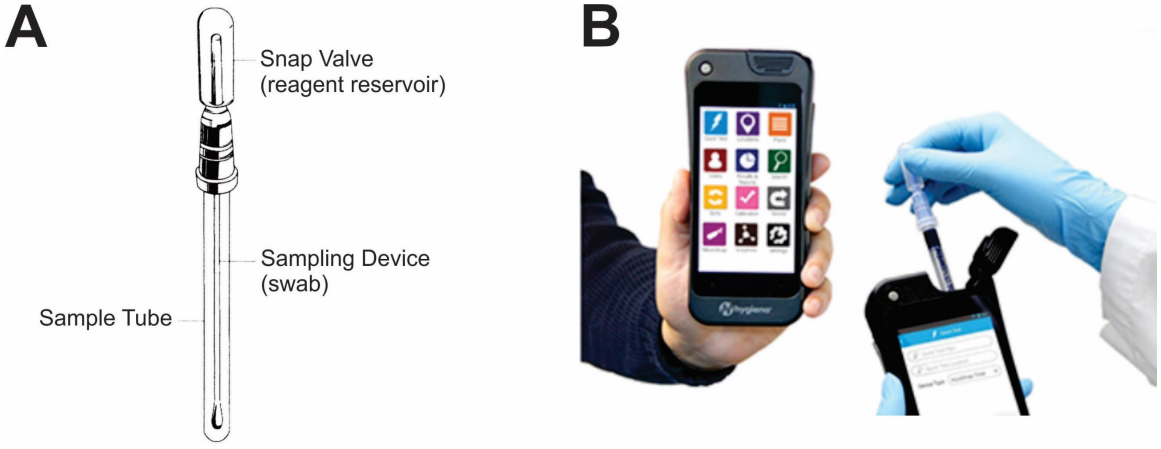

Figure 3. The PhageDx *E. coli* O157:H7 Easy Phage assay. **A)** The assay consists of a sample device consisting of a sample tube, a sample device (swab) and a reagent reservoir, which contains a luciferase reporter bacteriophage reagent. **B)** Following completion of the assay. The sample tube is placed into a handheld luminometer which reads the relative light units (RLUs) if any luciferase was produced during the assay.

iv. Results and discussion

Aim 1. Figure 4 shows the results of the evaluation of the 3D cartridge for its ability to concentrate foodborne bacterial pathogens from wastewater. A focus was placed on *Salmonella* spp., *Listeria monocytogenes* and *E. coli* O157:H7 because these pathogens have been commonly implicated in outbreaks associated with fresh produce, and in which irrigation water was the suspected vehicle (Gartley *et al*. 2022, Callejón *et al*. 2015). Untreated wastewater provided a convenient sample to evaluate the cartridge because the presence of foodborne pathogens in wastewater is well documented (Diemert *et al.* 2019, Hellmér *et al*. 2014.).

The results showed that the cartridge was capable of detecting all three bacterial pathogens at levels as low as between 10 and 100 CFU/ml (Figure 4).

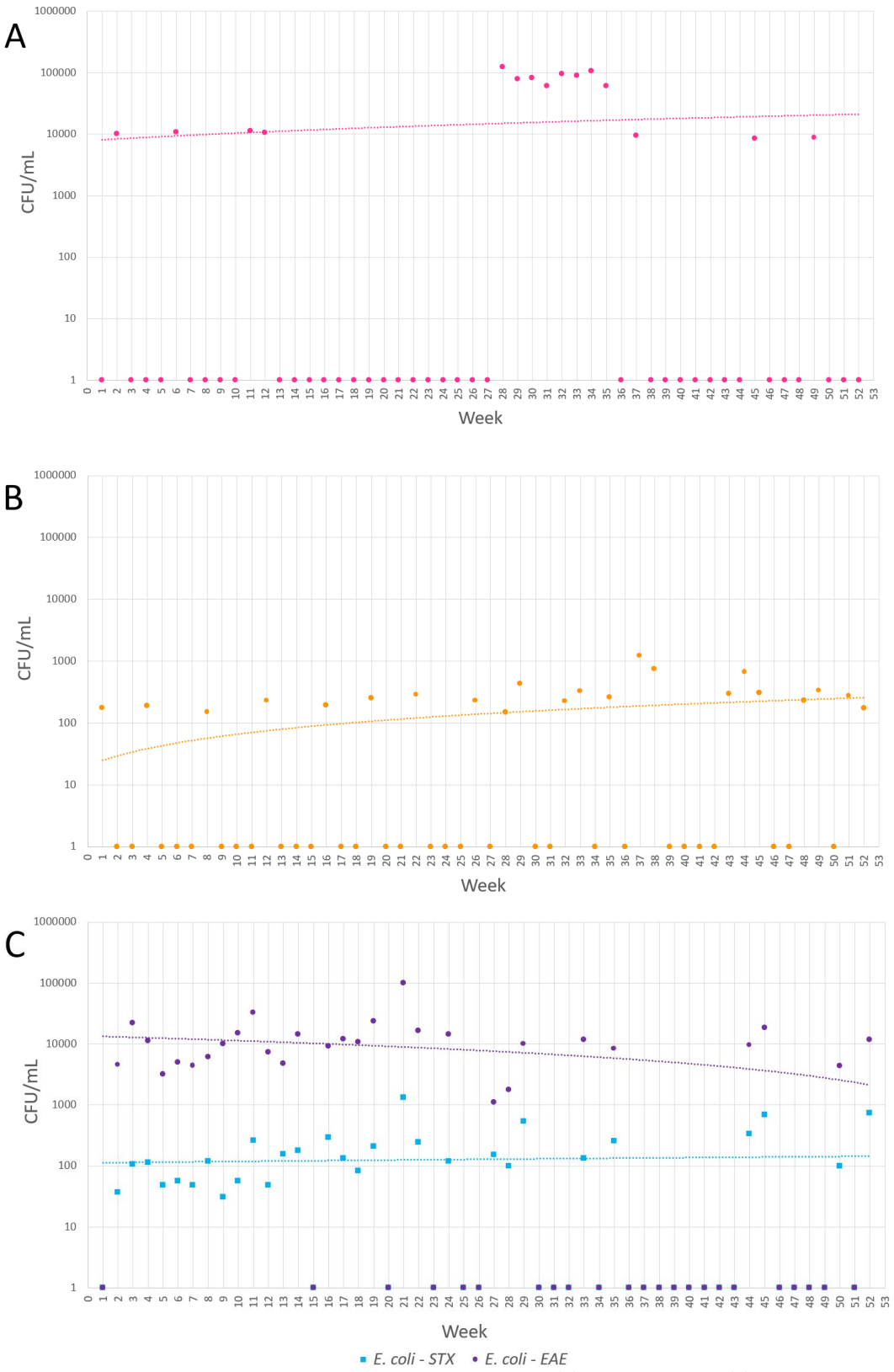

Figure 4. Wastewater levels of *Salmonella* spp. **(A)**, *Listeria monocytogenes* **(B)** and *E. coli* O157:H7 **(C)** following concentration with the 3D cartridge, and detection by RT-PCR. For *E. coli* O157:H7, two genetic markers were used including one for Shiga toxin (STX) and one for a cell attachment protein (EAE).

Aim 2.

Figure 5 shows the results of the irrigation water testing analysis. Water samples were individually spiked with three different strains of *E. coli* O157:H7 to three different concentrations (10^1, 10^0, 10^{-1} CFU/ml). A focus was placed on *E. coli* O157:H7 as compared to the other pathogens because since 2018, *E. coli* O157:H7 has been implicated in numerous outbreaks in which leafy greens have been the implicated vehicle (CFIA 2022). The results indicated that after 8 hours of enrichment, all samples were positive in the PhageDx *E. coli* O157:H7 Easy Phage assay. For example, after 8 hours, the 10^1 CFU/ml sample for all three *E. coli* O157:H7 strains (#122, #125, #129) produced an average reading of 20,000 RLUs, while the 10^0 CFU/ml and 10^{-1} CFU/ml samples produced average RLU readings of 13,719 and 1,573 respectively. Therefore, as few as 10^{-1} CFU/ml were detectable after 10 hours (8 hour enrichment and 2 hour assay). The difference in RLUs between the samples is reflected by the fact that, following 8 hours of enrichment, the concentrations of *E. coli* O157:H7 in the samples differed by approximately 1 log (initial 10^1 CFU/ml inoculum: final concentration after 8 hours enrichment 10^6 CFU/ml; initial 10^0 CFU/ml inoculum: final concentration after 8 hours enrichment 10^5 CFU/ml; initial 10^{-1} CFU/ml inoculum: final concentration after 8 hours enrichment 10^4 CFU/ml). After 24 hours of enrichment, all samples had RLU readings of 20,000, which is the upper limit of detection for the handheld luminometer.

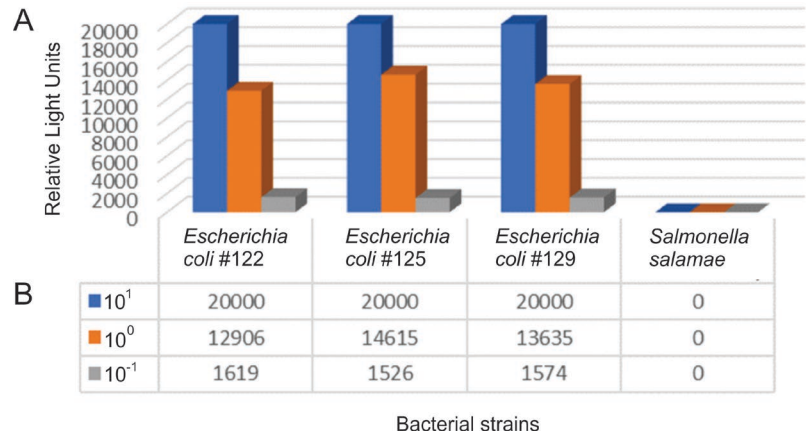

Figure 5. Detection of *E. coli* O157:H7 in spiked irrigation water. **A)** Three different isolates (#122, #125, and #129) were tested using the PhageDx *E. coli* O157:H7 Easy Phage assay, and each isolate produced a positive test result at all initial spiking concentrations (10^1, 10^0, 10^{-1}) after 8 hours of enrichment. In contrast, *Salmonella salamae* did not produce a positive test result. **B)** The Relative Light Unit readings for the different initial spiking concentrations after 8 hours of enrichment.

In this project, we combined a novel and quick concentration method using a 3D printed cartridge and disposable coffee cup filter with a new diagnostic assay based on the use of genetically modified bacteriophages, which upon infection of their target *(E. coli* O157:H7) cells, produced luminescence which was measured using a handheld luminometer. The luminometer was designed and is widely used in the food industry to measure ATP luminescence as a measure of hygiene, and was repurposed for this project.

The results of this study demonstrate that the rapid assay developed for *E. coli* O157:H7 detection in water exhibits promising potential for application with irrigation water samples. A goal of this study was to develop the assay for field-based detection of *E. coli* O157:H7 in irrigation water used for food crops. The assay consists of three components including a portable peristaltic pump, and plastic cartridge, and the PhageDx *E. coli* O157:H7 Easy Phage assay. The cartridge is produced from a resin that makes it easy to sterilize in boiling water, making it reusable. Therefore, for each new sample, only a new K-Cup paper filter and a new Easy Phage assay would be needed. In order to make the assay field ready, several modifications will need to be made. These included conducting the enrichment step directly in the cartridge, and integrating all Easy Phage diagnostic reagents into the sample device. Future work will address these two aspects.

v. Acknowledgements

In addition to acknowledging the funding of the Canadian Produce Marketing Association, we also acknowledge the support of Steve Erickson and Minh Nguyen at Labcorp, who provided the PhageDx *E. coli* O157:H7 Easy Phage assays and helpful comments.

vi. Supplemental Resources

This research was presented at the 2023 International Association for Food Protection Annual meeting, as follows:

C. Chiappe, H. M. Davidson, T. Bakker, and L. Goodridge. 2023. Development of a Rapid, Field-Based Assay for Detection of *Escherichia coli* O157:H7 in Irrigation Water. Abstract P2-228. A copy of the poster is attached to this report.

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Development of a Rapid, Field-Based Assay for Detection of *Escherichia coli* O157:H7 in Irrigation Water

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Abstract

The contamination of Irrigation water with *Escherichia coli* O157:H7 is of great concern since this microorganism can survive in leafy green vegetables. Traditional methods to detect *E. coli* O157:H7 can take several days or weeks. In this study, we developed a rapid, field-based assay for the detection of *E. coli* O157:H7 in irrigation water using the PhageDX *E. coli* O157:H7 assay. Our results indicate that after 8 H of enrichment it was possible to detect as few as 10^{-1} *E. coli* O157:H7 cells/ml.

Introduction

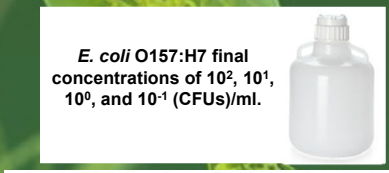
Contaminated irrigation water has been implicated in outbreaks of *E. coli* O157:H7 in which leafy greens was the identified vehicle. Foodborne illness outbreaks associated with pathogenic *E. coli* O157:H7 have been consistently reported worldwide, with a strong correlation to the rising consumption of fresh, ready-to-eat leafy greens [1]. The most common reservoir of *E. coli* O157:H7 is cattle [2] [3]. The pre-harvest contamination of field-grown vegetables by foodborne pathogens like *E. coli* O157:H7 is hypothesized to occur through cross-contamination from irrigation water contaminated with fecal waste or inadequately composted manure [4]. The conventional methods for detecting *E. coli* O157:H7 typically require a functioning laboratory, using testing procedures that are both time-consuming and labor-intensive [5]. In this study, we developed a rapid, field-based assay for the detection of *E. coli* O157:H7 in irrigation water using the PhageDX *E. coli* O157:H7 assay. The PhageDx™ *E. coli* O157:H7 Assay is based on the infection of *E. coli* O157:H7 by a genetically modified bacteriophage that subsequently replicates within its specific host and produces luminescence. This system offers the benefit of exclusively identifying viable bacterial cells, as bacteriophages can only replicate within living cells [6].

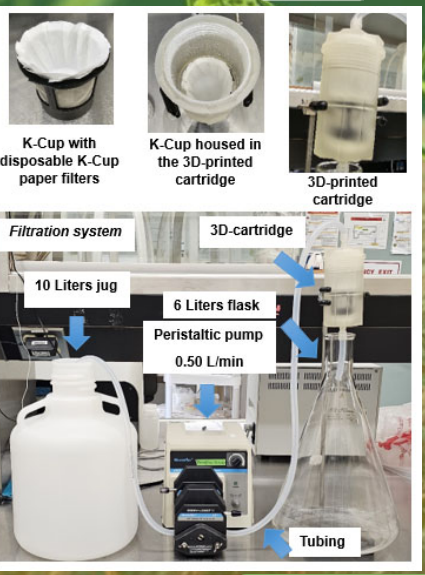
Significance

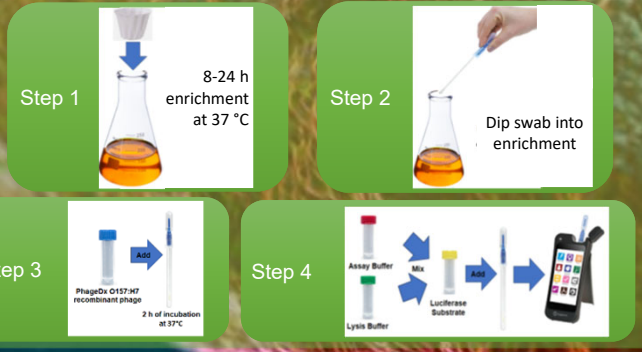
Current generic *E. coli* irrigation testing requirements have not proved effective at indicating the presence of foodborne pathogens in water that was subsequently implicated in leafy green foodborne outbreaks. This research could lead to a field-based assay for direct detection of *E. coli* O157:H7 in irrigation water within 10 hours.

Materials & Methods

***E. coli* O157:H7 final concentrations of 10^2, 10^1, 10^0, and 10^{-1} (CFUs)/ml.**

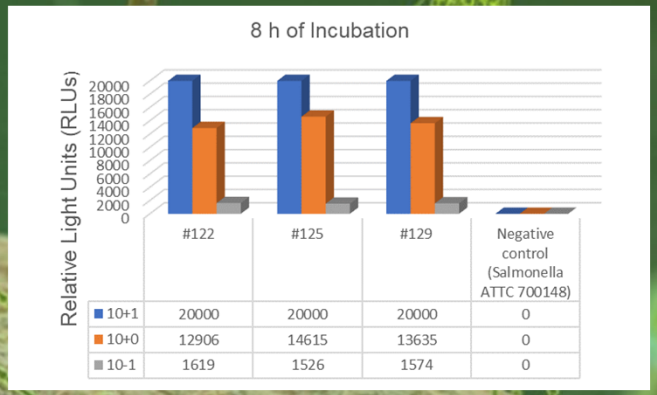






Results

All samples tested positive for *E. coli* O157:H7 after 8 and 24 hours of enrichment.



* The graph represents the average of two trials.

After 24 h of enrichment, all samples produced RLU readings of 20,000.

Conclusion

Based on the obtained results, we can assert that the rapid assay employed for *Escherichia coli* O157:H7 detection in water exhibits promising potential for application with irrigation water samples. Further investigations are planned to be conducted in subsequent studies.

Acknowledgement

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