Lab Report

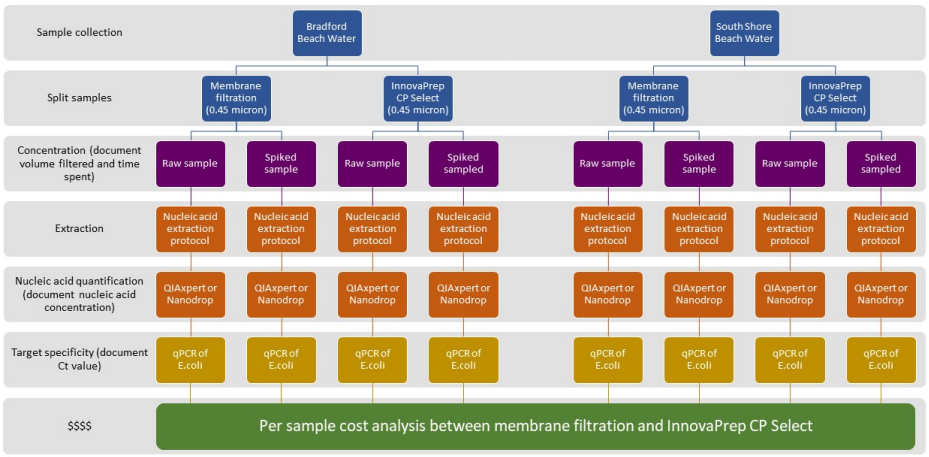
Denice Benitez

Comparing Beach Water Filtering Methods

Project Goal

The goal of this project was to compare two methods for processing beach water samples in the Milwaukee public health laboratory. The first analysis was done through traditional membrane filtration. The second analysis was done through a hollow fiber concentrating pipette tip made by Innova Prep. In order to evaluate their performance, we compared volume (mL), throughput (max volume/time), nucleic acid (DNA and RNA) quantity and quality, target specificity for *E. coli,* total bacterial 16S, and cost analysis. The most efficient and cost-effective method will be selected to filter beach samples. All tests were conducted by testing water from two different, regularly monitored Lake Michigan beaches: Bradford Beach and South Shore Beach.

Procedure

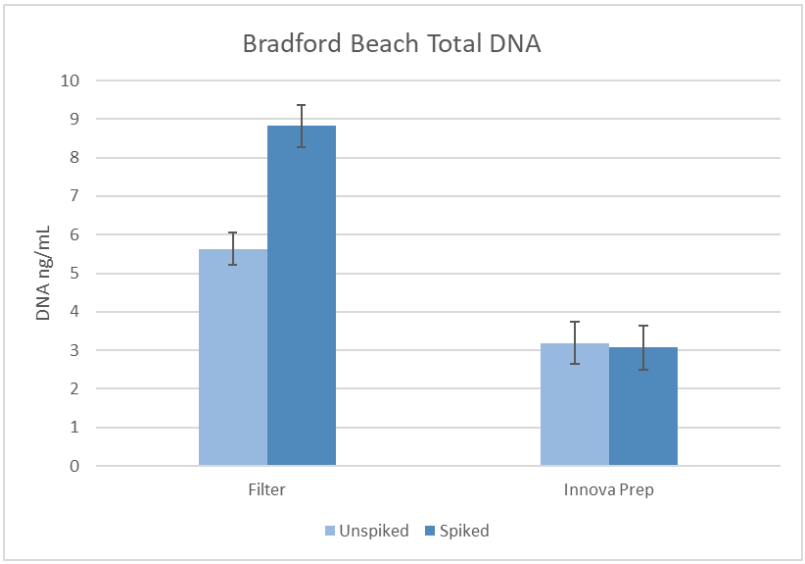


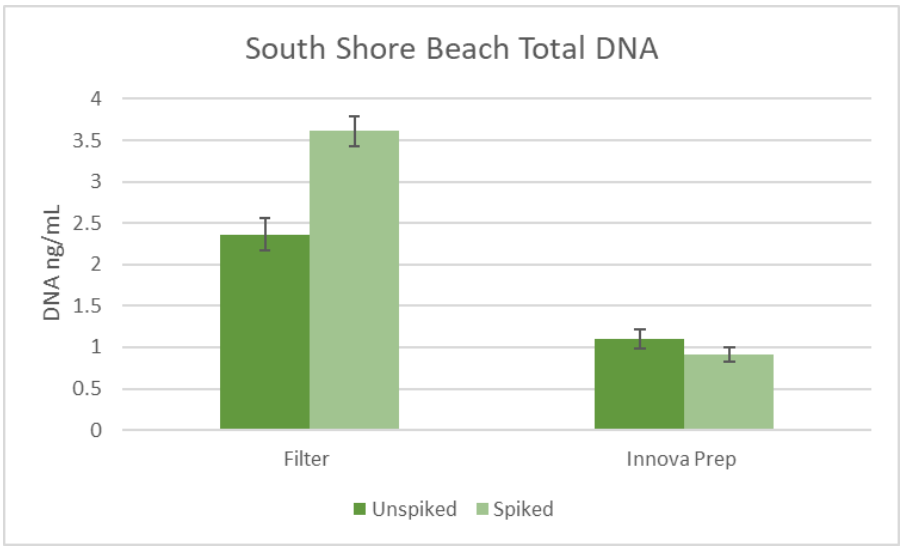
In total, 80 different beach water samples were collected. From those 80, 40 water samples were from Bradford Beach and 40 water samples were from South Shore Beach. Those 40 samples were then broken up by methods. Twenty samples were conducted by standard filtering and 20 were conducted using the Innova Prep. From those 20 samples, 10 were spiked with a known *E. coli* concentration and 10 were not. For the first experiments, all samples had the exact same volume filtered, 100 mL. According to the EPA, filtering for *E. coli* by membrane filtration must be done in 15 minutes when filtering begins. Once these water samples were filtered using the two methods, the extraction and analysis were identical. All 80 samples were extracted through the QIAcube (Qiagen) using the Qiagen Power Soil kit materials. Nucleic acids were then analyzed using a Qubit fluorometer (Thermo Fisher Scientific), which uses fluorescent dyes that emit signals when bound to the specific target molecules such as DNA. After that, all samples were analyzed through qPCR to target *E. coli* specifically.

After this initial round of experiments, for each method, an additional 10 samples were collected from Bradford Beach to determine a maximum volume that could be filtered in a given time and how much total DNA and bacteria was quantified in those samples.

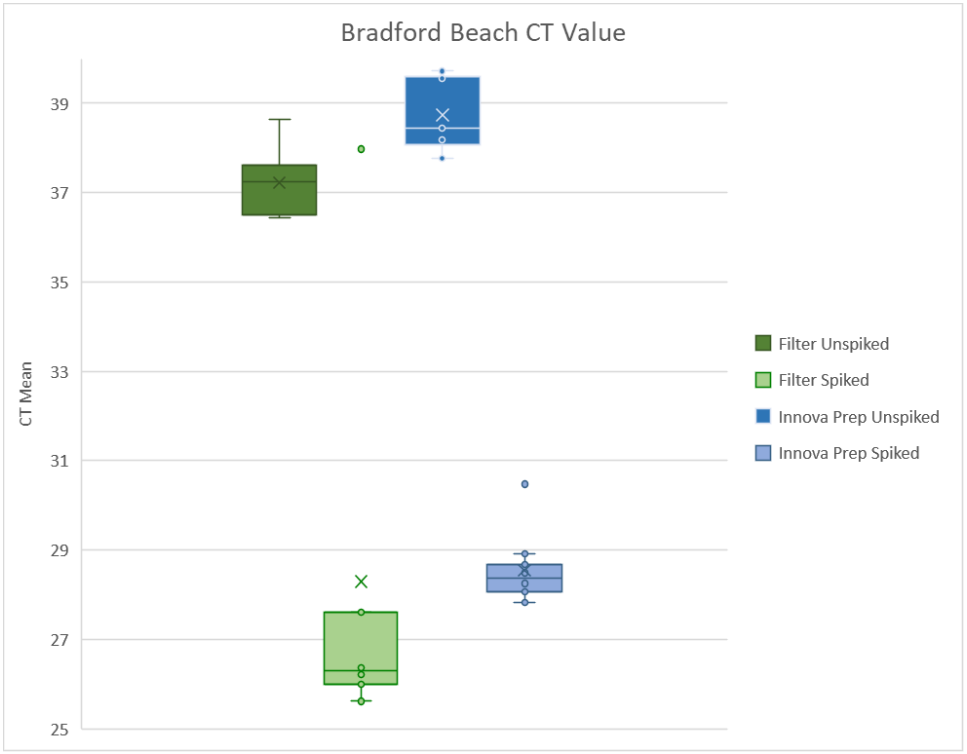
Results

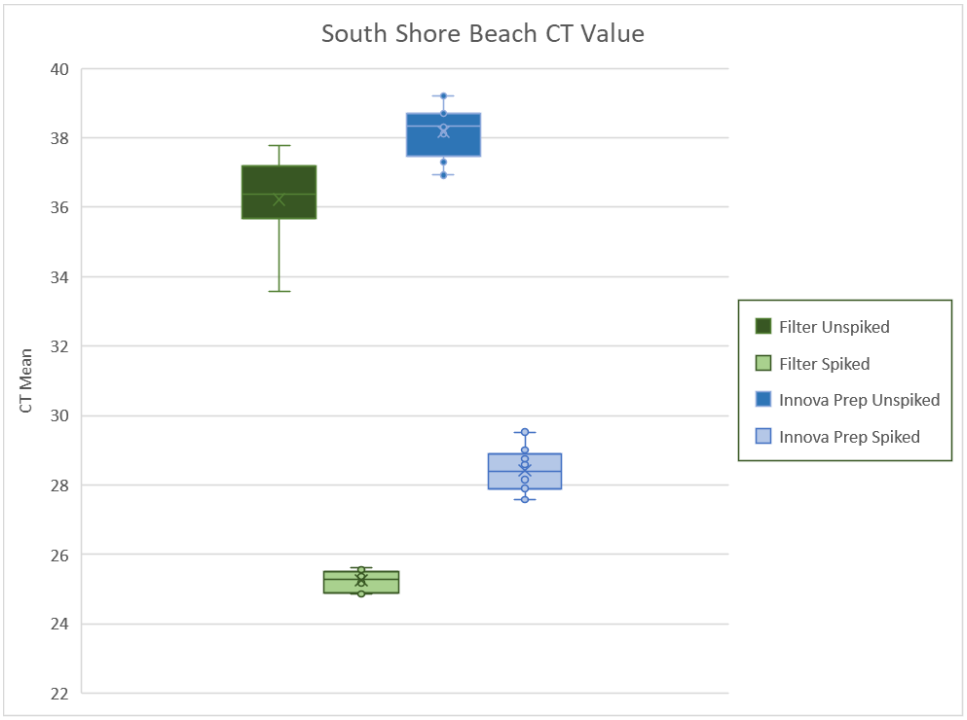
Results show that standard filtering 100 mL DNA comparison had more total DNA for both Bradford and South Shore Beach.



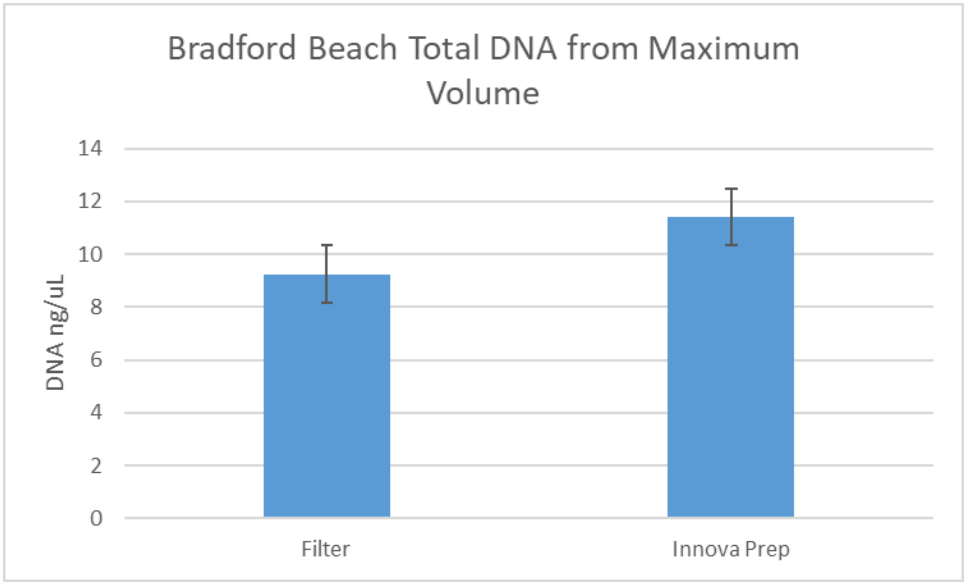


qPCR results show that the spiked samples for both methods have a lower average cycle threshold (CT), which means it had a higher level of *E. coli*. Comparing both beaches and methods, standard filtering had a lower CT value.

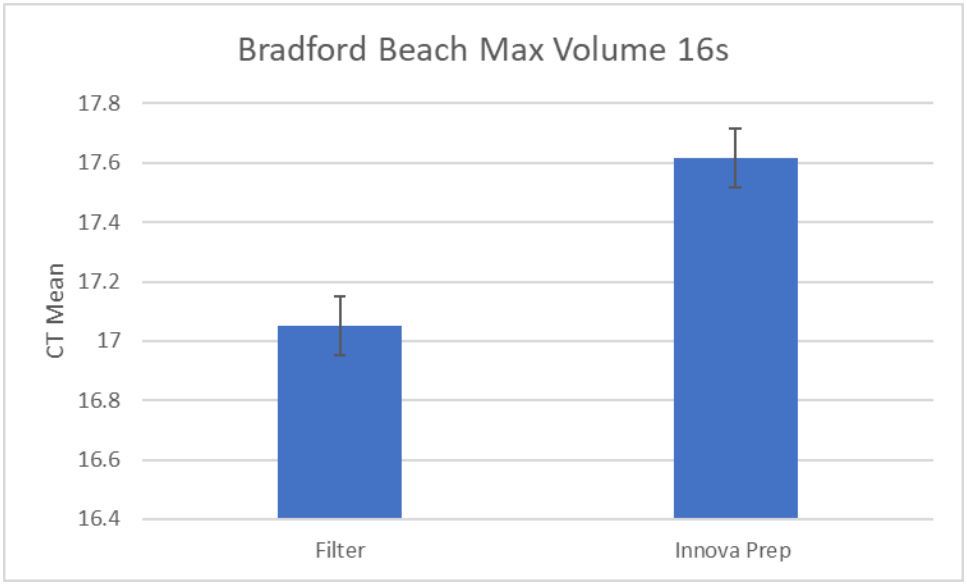




The Innova Prep had more total DNA compared to standard filtering when collecting maximum beach water sample from Bradford Beach.



After this discovery, the next steps were to perform a 16S qPCR to analyze total bacteria in the maximum volume samples from Bradford Beach. Although the Innova Prep had more total DNA, likely because of more volume, 16S qPCR shows that there were more bacteria found when performing standard filtering.



The cost analysis comparison shows that standard filtering is more cost effective compared to the Innova Prep. The Innova Prep requires ordering new filtering pipette tips and other consumables while standard filtering only requires new membrane. When calculating what it would cost for beach samples for summer of 2024, filtering would cost approximately$940.80. The InnovaPrep would cost approximately $4,189.18.

Conclusion

After analyzing the results, we have concluded that standard filtering is the most efficient and cost-effective method for collecting routine beach water samples for *E.coli* enumeration. This standard practice has shown to be effective with beach water samples. The InnovaPrep was believed to be more efficient when total volume of water collected was the goal. DNA concentrations were higher in these samples, target specificity using 16S was still better using the standard filtering method.

Both Bradford Beach and South Shore Beach showed that standard filtering collected more total DNA, when filtering equal volumes, compared to the Innova Prep. This shows that standard filtering is more effective when collecting total DNA.

This was also shown analyzing qPCR because the higher the CT value, the less sensitivity to the known *E. coli* samples are in the water beach samples. Standard filtering samples shows a lower CT value which means it was able to analyze the *E. coli* in the samples, while the InnovaPrep samples had a higher CT value. Overall, filtering had a lower CT value compared to InnovaPrep. This was shown when both methods were spiked with the known *E. coli* value and shows that filtering was more sensitive to *E. coli.* This was shown for both Bradford and South Shore beach.

The InnovaPrep is known to collect large volumes of water. This was put to the test when collecting beach water samples from Bradford Beach. With larger volumes, The InnovaPrep also filtered more total DNA compared to standard filtering. The InnovaPrep filtered an average of 420.6 ml compared to standard filtering that averaged 145.4 ml of beach water sample. When analyzing the total DNA using the Qubit, the InnovaPrep shows that it collected more DNA because of the large amounts of water it was able to filter within the 15 minutes.

When analyzing the 16S qPCR results, it was interesting to see that even though there was more total DNA read in the InnovaPrep samples, standard filtering showed that it collected more total bacteria. This can mean that there could have been other sorts of DNA collected in the sample such as plant-based DNA and not necessarily bacteria.