Is the water safe to drink?
The rapid test is the missing link!

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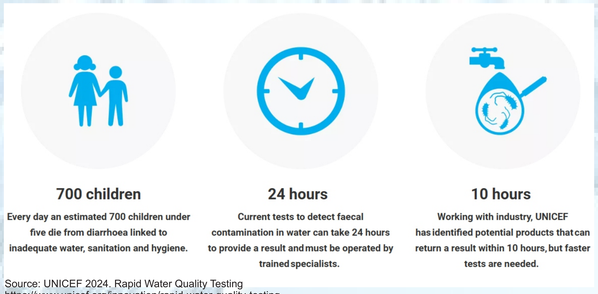
INTRODUCTION

- 2.2 billion people globally do not have access to clean, safe drinking water (UNICEF 2024).
- 1 million people die each year from scarce and contaminated water sources (WHO 2024)
- 255,500 children aged under 5 years dies each year (1 death every 2 minutes) (UNICEF 2024)
- Many more children miss school because the water they drink makes them sick
- Many people rely on drinking water that is contaminated with bacteria like *E. coli*, *Salmonella* and *Vibrio*
- Current testing approaches are time consuming, taking 24 hours to obtain a result
- The long test time, limits the ability to understand and communicate risks, launch community-led water quality monitoring programs, and integrate water testing into large-scale national household surveys
- Most water testing methods require complex equipment and need to be completed in a lab
- The United Nations International Children's Emergency Fund (UNICEF) has issued a global innovation project challenge to develop an easy-to-use, rapid detection method or portable kitt that can accurately identify fecal contamination in drinking water



Source: UNICEF 2024. Rapid Water Quality Testing
https://www.unicef.org/innovation/rapid-water-quality-testing

PURPOSE

- To develop a portable, field ready test to assess the safety of drinking water, addressing some of the UNICEF innovation project goals, while also addressing shortcomings of the UNICEF approach

HYPOTHESIS

- A major challenge of a field based water test is the need to concentrate the bacteria from the water instead of enrichment
- I hypothesized that the use of affinity capture beads will be the most effective method for concentrating microorganisms from water compared to polyethylene glycol and anionic exchange resin beads due to their specificity and potential for higher selectivity compared to PEG and anionic exchange resin beads.

VARIABLES

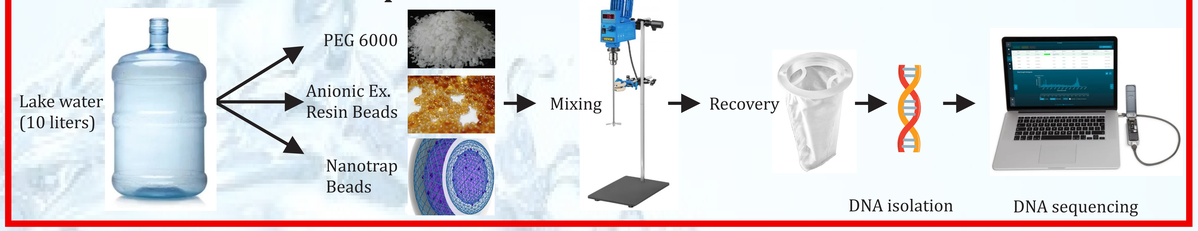
- Independent variable: the concentration method (Polyethylene Glycol, Anionic Exchange Resin Beads, Affinity Capture Beads)used to capture microorganisms from water samples
- Dependent variable: the efficiency and effectiveness of each concentration method in capturing microorganisms, measured by bacterial diversity determined by sequencing of the 16s rRNA gene
- Control variables: water volume (10 liters), water source (Lake Wilcox), Experimental conditions
- Positive control: filtered water sample
- Negative control: Sterilized (1 ml) sample of water

References

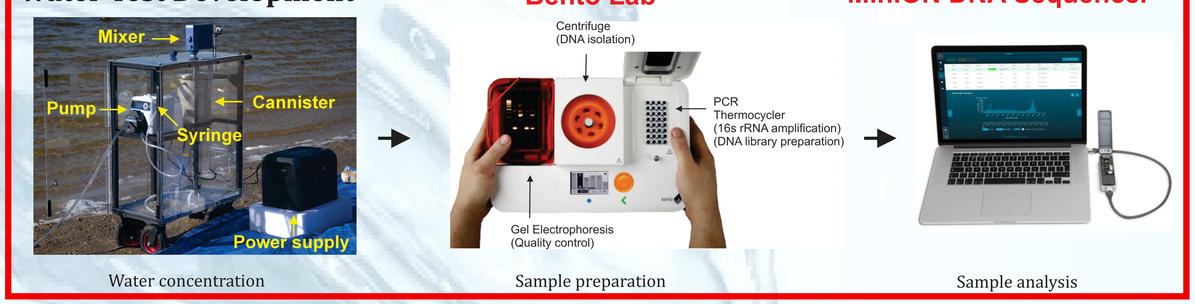
UNICEF 2024. Rapid Water Quality Testing. https://www.unicef.org/innovation/rapid-water-quality-testing
World Health Organization. 2023. Drinking-water. https://www.who.int/news-room/fact-sheets/detail/drinking-water

PROCEDURE

Bacterial Concentration Experiment



Water Test Development



RESULTS

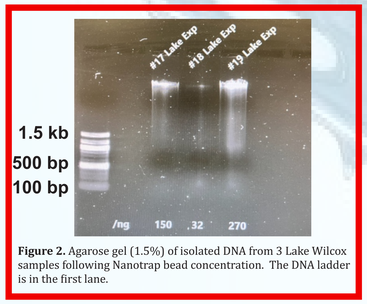


Figure 2. Agarose gel (1.5%) of isolated DNA from 3 Lake Wilcox samples following Nanotrap bead concentration. The DNA ladder is in the first lane.

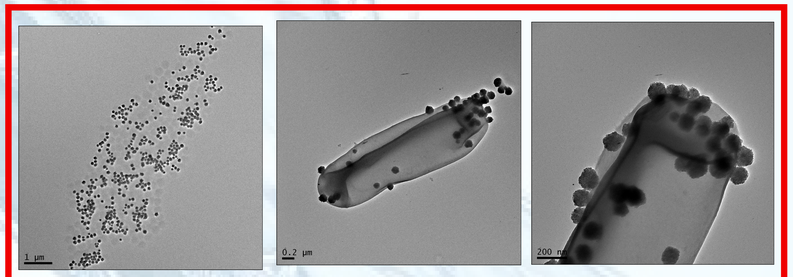


Figure 1. Transmission Electron Micrographs (TEMs) of Nanotrap beads attached to a bacterial cell.

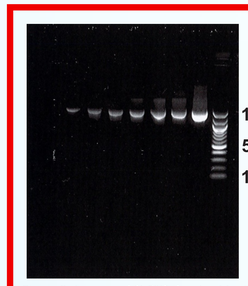


Figure 3. Agarose gel (1.5%) showing 16s rRNA amplicons from 2 different samples of water from Lake Wilcox. The positive control (*E. coli*) is in the 2nd to last lane. The DNA ladder is in the last lane.

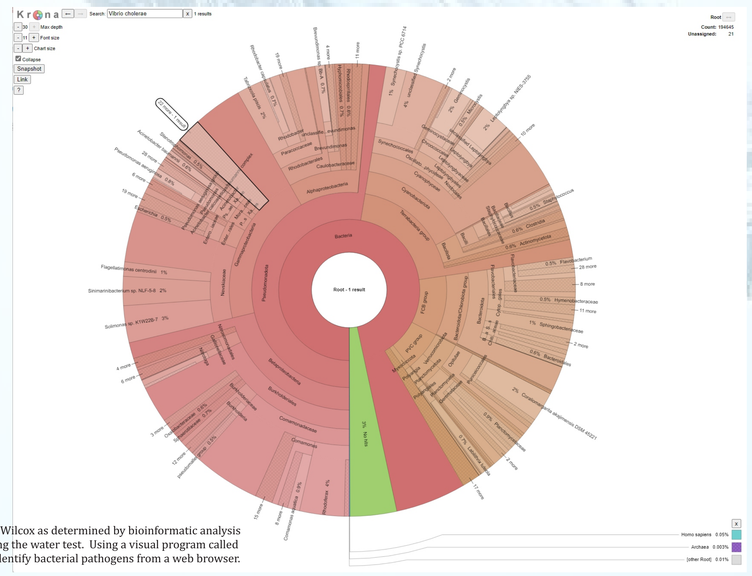


Figure 4. Bacterial diversity in Lake Wilcox as determined by bioinformatic analysis of 16s rRNA gene sequences following the water test. Using a visual program called Krona, the data can be searched to identify bacterial pathogens from a web browser.

Table 1. Results (DNA concentration, PCR and DNA sequencing) from the bacterial concentration experiment

Concentration Method	Average Recovered DNA Concentration (2 replicates)	16s rRNA gene PCR amplification	16s rRNA gene sequencing
Nanotrap Microbiome A Particles	21.5 ng/µl	Yes	Yes
Polyethylene glycol 600	13.0 ng/µl	No	N/A
Anionic Exchange Resin Beads	0.0 ng/µl	N/A	N/A

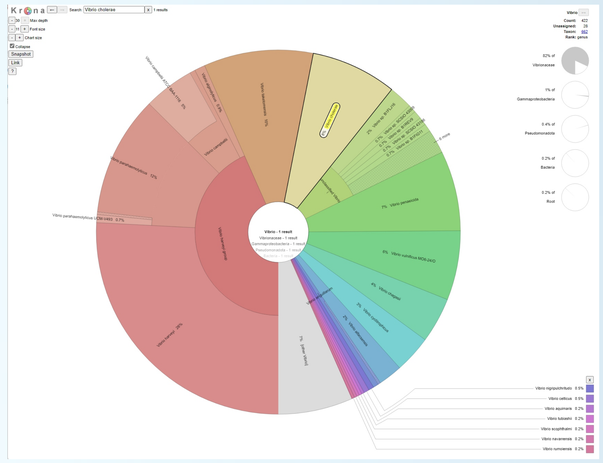


Figure 5. The Krona visualization data were searched for *Vibrio* species, and a number of pathogens were identified including, the waterborne pathogen *Vibrio cholerae*, and the foodborne pathogens *Vibrio parahaemolyticus* and *Vibrio vulnificus*. The water test was validated by culturing a Lake Wilcox sample in bacterial growth media, followed by isolation of *Vibrio cholerae* bacteria (see publication).

ANALYSIS

Bacterial Concentration Experiment

- The Nanotrap beads worked the best to concentrate the bacteria from the water
- PEG 6000 needs to be refrigerated and incubated for longer time to likely be effective
- Anionic exchange resin beads did not reliably capture bacteria likely due to variable and weak bacterial surface charge

Water Test Development

- Field testing of the water test indicated that ambient air temperate can affect the test results
- On cold days, PCR amplification and DNA sequencing can fail
- Placing the Bento Lab and MinION DNA sequencer in an insulated container should address this issue
- Hand warmers can also be used to ensure that the temperature inside the insulate container remains satisfactory

CONCLUSION

- A rapid and portable water test was developed that directly identifies the presence of bacterial pathogens in water in approximately 9 hours
- The test integrates large volume (10 l) water sampling, bacterial concentration, DNA isolation and amplification, DNA library preparation and DNA sequencing into a single assay
- The water test takes 9 hours to complete and costs $240/sample
- The test can be used by public health officials for routine surveillance of water sources that are suspected to be contaminated with bacteria
- **Future directions:** The water test will be field tested at additional water sources (lakes, rivers, drinking water sources)
- The water test will be validated against existing fecal indicator water tests