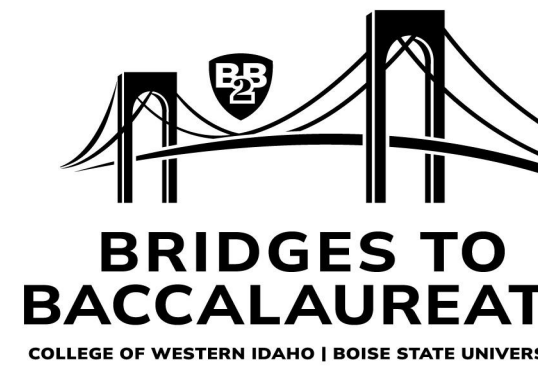


What's in the Water? Detecting Chlamydia and Gonorrhea in Treasure Valley Wastewater



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Abstract Wastewater-based epidemiology (WBE) gained prominence as a reliable method for predicting SARS-CoV-2 outbreaks and trends during the COVID-19 pandemic. We modified the technique to detect Chlamydia (*C. trachomatis*) and Gonorrhea (*N. gonorrhoeae*) in wastewater sourced from four wastewater facilities in the Treasure Valley (West, Lander, Meridian, and Nampa) using variations of two extraction methods, the AllPrep PowerViral DNA/RNA Mini Kit and paper filtration (0.22µm and 0.45µm). Both pathogens were detected in samples from both Boise, Idaho facilities (West and Lander) on three of the six days tested (*N. gonorrhoeae* 6/14/23, and *C. trachomatis* and *N. gonorrhoeae* 6/19/23 and 6/28/23). Neither pathogen was detected in samples from the Meridian and Nampa facilities. Pathogens were detected in 25% of all treatment facilities sampled. The 0.22µm filter paper produced positive results in 3 of 4 tests (75%), while the 0.45µm paper did not produce any positive results. Surprisingly, Nanotrap A, designed for viral detection, was more effective in detecting the bacteria than Nanotrap B, designed for bacterial detection (50% vs 33%).

Introduction

The Boise State University Wastewater Surveillance Lab (BSUWSL) began using WBE in June 2020 to quantify SARS-CoV-2 in Treasure Valley wastewater, and has been providing data for the CDC and Idaho H&W Laboratory Dashboards' (see QR code) From this data the BSUWSL modelled and predicted future COVID hospitalizations and deaths using machine learning. We have recently begun to expand the scope of WBE to measure other pathogens. In 2021, *C. trachomatis* and *N. gonorrhoeae* were the first and second most reported STIs in the United States with 1,644,416 and 710,151 cases, respectively (1). Incidence of both of these bacterial STIs are on the rise, and each carries the risk of reinfection and adverse effects on reproductive health without proper treatment. For these reasons, the BSUWSL is expanding testing to include both *C. trachomatis* and *N. gonorrhoeae*. In this pilot study we aimed to determine the best method for the detection of *C. trachomatis* and *N. gonorrhoeae* in Treasure Valley wastewater.



H&W Laboratory Dashboard

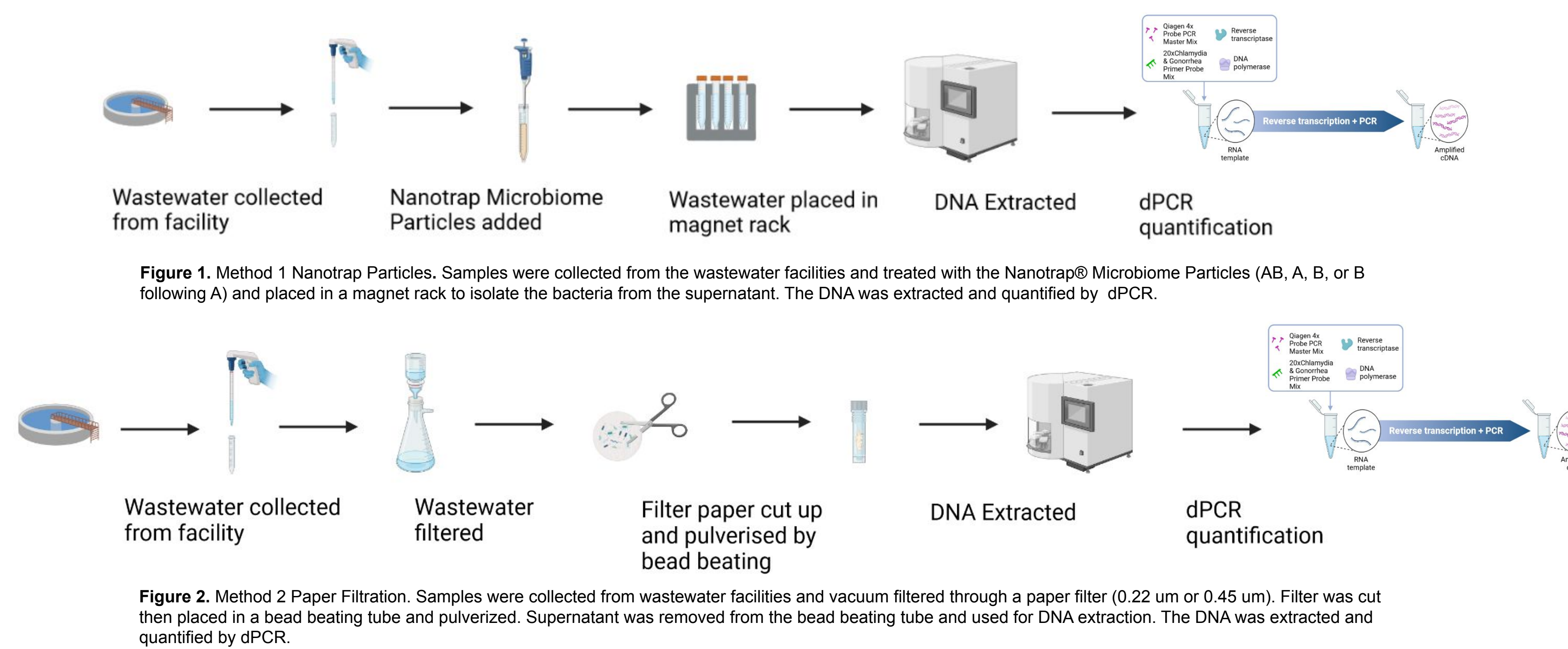
Objective

Develop a sensitive method to detect and quantify Chlamydia and Gonorrhea in Treasure Valley wastewater using digital PCR (dPCR).

References

- Centers for Disease Control and Prevention. (2023). *Impact of Covid-19 on STDs*. Centers for Disease Control and Prevention. <https://www.cdc.gov/std/statistics/2021/impact.htm>.
- Get Healthy Idaho. (2023). *Sexually Transmitted Diseases (STD) Reported Among Residents of Idaho*. Idaho Department of Health & Welfare. <https://www.gethealthy.dhw.idaho.gov/infectious-diseases-std-hiv-data>.

Methods



Results

Method 1: Nanotrap Particles Volume	Sample (mL)	Positive Control C. trachomatis (copies/mL)	Negative Control C. trachomatis (copies/mL)	Positive Control N. gonorrhoeae (copies/mL)	Negative Control N. gonorrhoeae (copies/mL)	Sample Date	Facility Location	C. trachomatis (copies/µL)	N. gonorrhoeae (copies/µL)
Nanotrap AB (150µL)	10	2611.4	0	5814.2	0	6/12/23	W	0	0
						6/12/23	L	0	0
						6/12/23	M	0	0
						6/14/23	N	0	0
		3.291	0	13.36	0	6/19/23	W	0.073	0
						6/19/23	W	0	0.085
Nanotrap A followed by B (150µL)	10	3.243	0	10.78	0	6/28/23	W	0	0
						6/28/23	L	0	0
						6/28/23	N	0	0
		***	0	0.053	0	6/23/23	N	0	0
						6/26/23	N	0	0
						6/28/23	W	0	0
Nanotrap A (150µL)	10	***	0	***	0	6/28/23	W	0	0
						6/28/23	L	0	0.074
Nanotrap B (150µL)	10	***	0	***	0	6/28/23	W	0	0
						6/28/23	L	0	0
Nanotrap B (300µL)	10	3.243	0	10.78	0	6/23/23	N	0	0
						6/26/23	N	0	0
						6/28/23	W	0.058	0.058
						6/28/23	L	0	0.071
Nanotrap B (450µL)	10	***	0	10.37	0	6/28/23	W	0	0
						6/28/23	L	0.056	0.056
Nanotrap B (525µL)	35	***	0	10.37	0	6/28/23	W	0	0
						6/28/23	L	0	0
Nanotrap B (525µL)	40	***	0	10.37	0	7/7/23	W	0	0
						7/7/23	L	0	0
						7/10/23	W	0	0

Table 1. Detection of pathogens using Nanotrap Microbiome Particles A and B Combined (pink), Nanotrap Microbiome Particle A followed by Nanotrap Microbiome Particle B (blue), Nanotrap Microbiome Particle A (green), and Nanotrap Microbiome Particle B (lavender, orange, gray, yellow, and burgundy). Particles A and B combined detected both pathogens in 6/19/23 West sample. Nanotrap Particle A followed by B produced no positive results. Nanotrap Particle A detected Gonorrhea (*N. gonorrhoeae*) 6/28/23 Lander. Both 300µL and 450µL of Nanotrap B particles in a 10mL sample detected both pathogens in 6/28/23 West and Lander samples at equal concentrations (0.058 and 0.056 copies/µL). Using 300µL of Nanotrap B particles yielded 0.071 copies/µL in the Lander sample.

Method 2: Filter Paper Size	Sample (mL)	Positive Control C. trachomatis (copies/mL)	Negative Control C. trachomatis (copies/mL)	Positive Control N. gonorrhoeae (copies/mL)	Negative Control N. gonorrhoeae (copies/mL)	Sample Date	Facility Location	C. trachomatis (copies/µL)	N. gonorrhoeae (copies/µL)
0.45 (µm)	40	3.291	0	13.36	0	6/14/23	W	0	0
						6/14/23	W	0	0
						6/14/23	W	0	0
						6/14/23	W	0.055	0
0.22 (µm)	40	3.291	0	13.36	0	6/28/23	W	0	0.053
		***	0	***	0	6/28/23	L	0.054	0

Table 2. Detection of pathogens using 0.22 and 0.45µm filter. Filter paper 0.45µm (yellow) did not produce any positive results. Chlamydia (*C. trachomatis*) was detected in 6/14/23 West and 6/28/23 Lander samples using the 0.22µm filter paper (pink) at 0.055 and 0.054 copies/µL extract. Gonorrhea (*N. gonorrhoeae*) was detected at West on 6/28/23 at 0.053 copies/µL extract.

Discussion/Conclusion

There was a 25% detection rate for *C. trachomatis* (5 positive partitions) and *N. gonorrhoeae* (6 positive partitions) in Treasure Valley wastewater samples. The method that resulted in the most positive readings was using a 10mL wastewater sample with pathogens adsorbed on a 0.22µm paper (3 positives). The 0.22µm paper filters gave a 75% detection rate making this the most sensitive method we tested for monitoring *C. trachomatis* and *N. gonorrhoeae*. The pilot study demonstrates that *C. trachomatis* and *N. gonorrhoeae* can be detected in Boise wastewater, and provides comparisons of several detection methods. This new test will provide health officials with a noninvasive measure of disease prevalence that doesn't rely on clinical reporting. Monitoring can help model and predict outbreaks, informing health priorities and policies. Using 0.22µm filter paper and bead beating provided the most sensitive results. This study also creates a framework for future detection of other bacterial and viral STIs.

Limitations

- Only ~500 cases of *C. trachomatis* and *N. gonorrhoeae* from Jan-March in the Treasure Valley (2)
- Each facility serves 50,000-150,000 people with flow rates of up to 20 Million gallons per day.
- Samples are stored at 4°C for three days before testing which could lower detection rates due to cell death and DNA degradation.

Future

- Optimize protocols for *C. trachomatis* and *N. gonorrhoeae* detection.
- Model outbreaks using machine learning correlating wastewater and clinical reports.
- Expand testing for other bacterial and viral STIs in wastewater.

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