Undergraduate Student Led COVID-19 Wastewater Surveillance on a university campus

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BACKGROUND:

Infections have been affecting and wiping out citizens and societies for centuries(LePan). Currently (2020-2021), the world is experiencing a global pandemic impacted by the novel coronavirus, SARS-CoV-2. Coronaviruses have been around for a significant amount of time and cause a respiratory infection that can transmit from person to person with a few severe cases. SARS-CoV-2, which causes COVID-19 as announced by the World Health Organization (WHO) in early February 2020, has proven deadly to humans (*Timeline*). It attacks the respiratory system, weakens the immune system, and can transmit rapidly while remaining asymptomatic. SARS-COV-2 can spread quickly with mild to no symptoms at all (CDC, "Coronavirus Disease 2019 (COVID-19)"). This ability of the SARS-CoV-2 virus means that most people do not go for testing until the symptoms are severe and apparent, prompting a virus outbreak that is delayed compared to the onset of symptoms. This feature of SARS-CoV-2 could delay effective control measures such as preventative quarantines and, when necessary – lockdowns.

Sewage or wastewater includes water from building usage (i.e., toilets, showers, sinks) that can contain human fecal waste, as well as water from non-household sources (rainwater and industrial use) (CDC, "National Wastewater Surveillance System") The World

Health Organization and the Center for Disease Control and Prevention have confirmed that the SARS- CoV-2 virus is found in gastrointestinal tracts and feces from an infected person. Before positive clinical tests, it can be detected in wastewater samples, making surveillance of wastewater for SARS-CoV-2 a potential early screening tool. If a sewage sample from a treatment plant is positive for SARS-CoV-2, samples could be collected from multiple community locations where possible to determine the most impacted area. Clinical testing in the affected area could help identify infected residents who may then be placed in quarantine, preventing further transmission. This was previously proven in 1989 when the Israeli Health Department set up a sewage surveillance system to detect Poliovirus (Brouwer et al.). Poliovirus is known to affect the human body's nervous system and eventually paralyzes the victim. Although Poliovirus works slower than the SARS-CoV-2 virus, it targets children and pregnant women. Human feces were collected through sewage trunk lines and checked for Poliovirus at the Central Virology Laboratory using this method. It took weeks to get the results, but the laboratory immediately informed the general public about the outbreak when receiving the results. Following this incident, the polio vaccine was produced. This process slowed down the virus transmission, stopping the pandemic, and resulted in zero paralysis cases.

More recently, in the context of the SARS-CoV-2 virus, some cities in the Netherlands adopted this early measure to detect the SARS-COV-2 spread. Amersfoort was the first to report a SARS-COV-2 case in the Netherlands, but SARS-CoV-2 RNA was detected in their wastewater six days before. After the eruption of the pandemic in China by the end of January 2020, the Netherlands started sewage surveillance in five cities in early February 2020. SARS-CoV-2 RNA was detected in early March 2020 in Amersfoort, even before the city's first confirmed case. By mid-March, there was an increase in SARS-CoV-2 RNA concentration in wastewater in all six wastewater treatment plants in the city. All five cities surveilling sewage reported data in which the confirmed case number and virus RNA concentration coincides (*COVID-19: Sewage Monitoring for Public Health - International Water Association*).

Since March 2020, the University has been actively fighting against the SARS-COV-2 pandemic and working on creating and maintaining a safe campus community. A "COVID-19" task force was formed and successfully ensured a safe return to campus for students who arrived in fall 2020. The task force also developed and enforced practices that kept positive SARS-COV-2 rates minimum throughout the fall semester. In September 2020, a Wastewater-Based Epidemiology (WBE) Team was developed as part of a university research center initiative to determine the use of wastewater surveillance for early detection of the SARS-CoV-2 virus and a noninvasive screening tool for SARS-COV-2. The initiative comprised an interdisciplinary team with undergraduate students in engineering, construction management, biochemistry, and communications working with faculty in these domains. The focus of this paper is the technical team's research questions. These included determining the best techniques and equipment and testing the efficacy of the equipment for on-campus wastewater sampling.

The idea of wastewater surveillance is to test wastewater at regular intervals to capture a representative sample that may contain the SARS-CoV-2 virus if it is released into wastewater from infected hosts. To collect wastewater, manholes around campuses are to be surveyed and located.

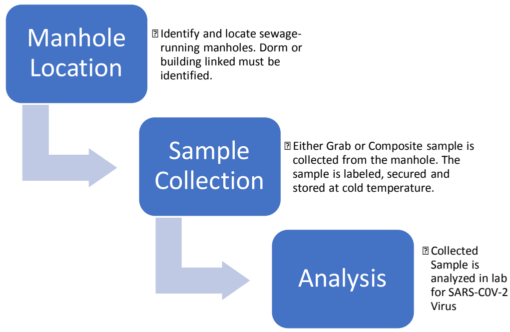

Figure 1Sampling Process

From the manholes, two types of samples can be obtained: grab samples and composite samples. Just like it sounds, a grab sample is a sample collected at one time from the source or manhole. In contrast, the composite sample is a collective sample from numbers of individual samples collected at a very regular time interval, usually every hour for 24-hours. For SARS-CoV- 2 virus detection purposes, the composite sample is preferred as the sample is collected over time is likely to be the most representative of wastes from the greatest number of residents(*How ASU, NAU and UA Are Testing Wastewater for COVID-19*). This sample was then analyzed by the scientific team to detect the presence of the SARS-CoV-2 virus. The two most prominent methods of testing the sample for the SARS-CoV-2 virus are:

RT PCR (Reverse-Transcription Polymerase Chain Reaction)

RT PCR is a scientific technique used in medical research for around 20-30 years to detect genetic information (Green et al.). In this method, the RNA (ribonucleic acid) of a virus is detected, and now it is being used in SARS-CoV-2 testing. The virus' RNA is converted into DNA (deoxyribonucleic acid) and copied multiple times using a PCR machine. RT-PCR captures and amplifies regions of the virus' genetic material and fluorescent markers to detect the presence of the virus by a series of temperature change processes. If the amount of fluorescence goes above a certain level, that confirms the virus' presence.

Loop-Mediated Isothermal Amplification (LAMP)

The Loop-Mediated Isothermal Amplification (LAMP) technique utilizes the same processing of RT-PCR but without variation of temperature. LAMP's process is conducted at a constant 60-65°C temperature. Like PCR, LAMP also converts RNA into DNA which can be copied into multiple forms. LAMP uses reagents to detect the presence of the virus. When the reaction mixture turns cloudy due to the production of a chemical called "magnesium pyrophosphate," it indicates the presence of the SARS-CoV-2 virus. Unlike RT-PCR, the LAMP method allows us to quickly diagnose the virus, as the cloudiness is visible to the naked eye (Green et al.).

Some other methods that can be used to detect SARS-CoV-2 are Lateral flow/Colloidal Good Immunochromatography and Enzyme-Linked Immunosorbent Assay (ELISA).

APPROACH AND METHODOLOGY:

The research methodology included comprehensive literature reviews, reviewing existing designs for grab and continuous sampling equipment and making improvements, as well as field and lab work. The following section describes the methodology used in this study:

A. Locating Campus Manholes

An extensive survey was started in fall 2020 in coordination with sophomore civil engineering students to determine the exact location and elevation of each campus manhole. Severe weather conditions prevented the completion of a fully successful survey. So, the team focused on campus site plans and used the help of the university's facility operations personnel, to shortlist key manholes that linked back to campus dormitories and that could be used in this study. The facilities operations staff also assisted in opening the selected manholes starting on October 21, 2020. At each manhole, a Vernier flow rate sensor was used to ensure that there was enough flow in any given 24-hour period for a composite sample that would be sufficient for the science team to analyze. The amount of wastewater we aimed for in each sample was 100 milliliters. These data were used to determine the final list of campus manholes that would be used for sample collection.

B. WBE Sample Collection Equipment

Two types of sample collection equipment were designed for this project: a grab sampling device and an autosampler.

1. Grab Sampling Device

The concept of grab sampling is straightforward: it comprises a long adjustable rod (15' long) with a collection reservoir attached to the end for manual sampling to scoop in the wastewater sample. This method of wastewater sampling is mainly used for one-time sample collection or sampling that does not occur often. The team ordered a long multi-purpose extension rod with various tool attachment options for an initial prototype and altered the design to fit our end goal of collecting a viable wastewater sample.

2. Autosampler

The 24-hour composite sample is a sample collected at a regular hourly interval over 24 hours. The amount of the composite sample collected depends on the manhole depth and the flow rate. The team modified a design developed by Syracuse university to build an automatic sampling unit (Kilaru et al.). The autosampler's main component is a submersible pump. The pump's motorized set of plastic rollers that roll on a rubber tube collect samples periodically over 24 hours. The autosampler kit consists of the components described below:

i. The Cooler: The cooler is set up with 48-quart fiberglass insulation. The insulation is cut into 5 long and short parts and wrapped in plastic wrap. They are placed in the cooler to cover each side. Once the insulation is in place, 12 cold packs are put in and surround the sample container to keep it cool

ii. Kit Bin: The Kit Bin (Figure 2) is a gray storage bin that holds all equipment needed for a field setup. It contains a 100 ft extension cord, a surge protector, three cord protectors, a timer outlet, the cooler, a biohazard sign, and a tarp. It also includes the smaller parts necessary for the autosamplers, such as the tubes, ferrules, and screws.

Figure 2 Complete KIT BIN

iii. The Pump Unit The most crucial piece of the sampler is a peristaltic pump. The pump pulls the wastewater through a set of rollers that "reel in" the water through its tube while also isolating it with a second tube inside the pump, preventing any spillage and contamination from occurring. The connection nut, ferrules, and weighted head are used in the field with the pump package. Accompanied with a 25ft plastic tubing cut to the depth of the manhole, these materials are needed for proper pumping from the wastewater line. The autosampler's pump unit is set up in a separate clear container. The clear plastic bin held the pump and had pre-cut holes for the tubes and electrical wire to power the machine

Figure 3. Pump in Action

and obtain the samples. The bin protected the pump from harsh weather conditions. (Figure 3)

iv. *Electrical Wiring* The electrical supply of the automatic sampling unit requires the buildings to be within 100' proximity of the manhole, as the design calls for a GFCI (ground-fault circuit interrupter) extension cord. This cord is then connected to a GFCI power strip, acting as a double fault protector. It can be connected to both an indoor and outdoor outlet. As the line is run towards the pump, cord protectors are placed over them to protect against pedestrians tripping over the line. The extra wiring would then sit inside a gray tote for storage and to declutter.

C. Sample Collection in the Field / Off-Campus

Two types of samples were collected for this project. A weekly sample from the local Wastewater Treatment Plant was taken each Wednesday from September 23rd, 2020, to November 5th, 2020, to provide a comprehensive picture of the community level of SARS-CoV-2 in wastewater system. Once the sample was received, it was brought to campus and immediately placed in the refrigerator for the science team to analyze. Simultaneously, the WBE team also collected on-campus samples during the same time frame to identify the campus level spread of SARS-CoV-2.

Off-campus samples were collected by the Wastewater Treatment Plant using a grab sampling technique and was handed over to WBE team for further analysis, whereas on-campus samples were collected using grab sampling and automatic sampler unit techniques.

D. WBE Sample Collection On-Campus

The technical team was responsible for collecting samples on-campus. This activity included opening a manhole, using sampling equipment, and sample collection. These activities are described as follows:

Figure 4. Students Opening Manhole

<u>*i. Opening a manhole:*</u> To open and operate the manholes, team members had to receive additional training and certifications including the "American Red Cross: Bloodborne Pathogens Training" and the "OSHA Confined Space Certification". The team also used personal protective equipment (PPE) in the field that included but was not limited to a full-length disposable gown, a disposable face mask, a face shield, and disposable lab gloves (Figure 4).

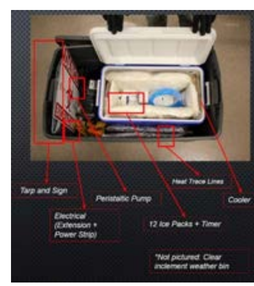

Figure 5. Complete Unit Description

<u>*ii. Installation of Sampler Unit*</u>: For grab samples, the sampling rod and a sampling container were brought to the site. The reservoir cup was manually dipped in the manhole to collect the sample. This method worked best in a two-person team where one dips the rod in the manhole for the sample, and the other holds the sampling container, both working together to guide and pour the wastewater in the container. For the 24-hour composite samples (Figure 5), the team set up the power source by running an extension cord to the nearest outlet and adding wire covers to high traffic areas. The manhole cover was opened from there, and the weighted head was lowered in the opposite direction of flow. The tubing sections were attached to the pump and pulled liquid into the reservoir when the pump was turned on. The pump was connected to the timer, which was set based on the manhole depth and flow rate.

<u>*iii. Sample Collection*</u>: The sampling procedure started with gathering materials in the lab and prepping the sample bottles. Each bot-

tle was set up in a temperature-controlled container with two covers: one with a drilled hole for the autosampler tubing and one sealed. The bottle was placed in an upright position in the cooler surrounded by ice packs to keep the sample cold. The ice packs in the container are kept cold by insulation wrapped in plastic wrap to keep the cold as previously described. Once the sample was collected, the tubing was removed from the sample bottle and the cap replaced. Finally, the sample bottle was sanitized and placed with the other samples in the lab's refrigerator. The clean-up procedure for manual and auto sampling involved sanitizing all parts involved in the sampling process with disinfecting wipes and washing them in a cleaning solution. The sample bottle was wiped down, labeled, and immediately taken to the environmental lab refrigerator for the storage unit. The sample was then ready for the science team to analyze.

a. Procedure for using the autosampler: The procedure for each sample collection day consists of the initial brief of where to go. On the first day, the team headed out to the manhole locations. Beforehand, the team suited up with personal protective equipment (PPE): disposable gown, gloves, face shields, and masks. The kits were then deployed to each site. Typically, two people ran the electrical: finding an outlet inside the building, connecting the surge protector, then the extension cord, and laying the wire down in a least-invasive manner. Trip-stops were then be lapped over the wiring to prevent individuals from tripping on cross wires. Next, the manhole cover was opened. To initiate sample collection, tubing connected with a heavier head was placed into the manhole, such that it opposed the flow direction to maximize the collection of inflow. The next step was to program the timer outlet for the 24-hour composite sampling.

Once the timer was programmed, and the pump was turned on, the manhole cover was returned to cover the opening, and a tarp was unfolded over the entire unit.

A biohazard sign was placed on top to warn potential onlookers of the danger. Waste wipes and any other PPE-type waste were collected and trashed into a red biohazard trash bag.

The next day, after the 24-hour composite sample was collected, the team typically broke into two groups: one group dismantled and field-sanitized. The second group transported the sample back into the laboratory to store it in the fridge. When returning, the second group transported the kit back to the lab while the first group walked to the following site and repeated the cycle.

Field sanitizing consisted of retrieving the inlet line from the manhole, wiping down the line with several sanitizer wipes. Once the inlet was above ground, a team member took off any clogging clutter and tossed it back down the manhole, then disinfected it. The inlet then sat inside a sanitizing bottle, and the pump was turned on, cleaning the inside for about 5-8 minutes. The outlet was taken from the collection bottle and pointed back at the manhole to drip inside. Once complete, the lines were wiped down once again, stored away, and the manhole was sealed shut.

RESULTS:

1. Local WWTP Sample Collection Results

The local Wastewater Treatment Plant (WWTP) was the initial control sample for the beginning of the wastewater surveillance. The initial sample was sent to two commercial labs, Biobot and Quadrant Analytics, and in-house analysis by the science team. In each case, the result was a non- detect for the SARS-CoV-2 virus.

2. Flowrate Results

Flowrates from manholes were tested between 0600 and 0700 with an assumption that we may encounter high flowrates as compared to other times, making sample collection easier. Additionally, each manhole was measured for its depth, and the width of the influent pipe was estimated. These key elements were crucial in determining the length of the tubing for the autosampler and for setting the timer. The results are shown in Table 1.

Table 1, Results of Manhole Flowrate

Time	**Location**	**Pipe Diameter (D)**	**Flow Rate Velocity (V)**	**Flow Rate (CFS) (D*V)**	**Remarks**
0645	Gerard Hall	8” (0.667’)	0.006 m/s (0.0197 ft/s)	0.0131 cfs	First time opened manhole.
					Manhole depth: 8’ deep
0640	Patterson Hall (Grass)	6” (0.5’)	Blocked	-	Flow-ways blocked. Manhole depth: 6’ deep
0635	U Building	8” (0.667’)	0.009 m/s (0.0295 ft/s)	0.0197 cfs	Manhole depth: 8’ deep
0610	South Hall	6” (0.5’)	<0.001 m/s Negligible	-	Small stream, not enough to catch. Manhole depth: 6’ deep
0620	Dalrymple Hall (Generator)	6” (0.5’)	None	-	Looked dry/nothing flowing. Manhole depth: 8’ deep
0625	Dalrymple Hall (Grass)	8” (0.667’)	<0.001 m/s Negligible	-	Small stream, not enough to catch. Manhole depth: 6’ deep

3. Grab Sample Results

Grab sampling was initially deployed to collect wastewater samples while the WBE team was developing the autosampler to collect a 24-hour composite sample. While a grab sample does not offer the representativeness of a 24-hour composite sample, the manual grab sampling method allows for the sample to be collected quickly, especially in manholes located in the roadway, and minimizes potential hazards from sampling equipment that has to stay longer (over a 24-hour period) and has minimal impact on traffic flow along a busy roadway.

4. Auto sampling Results

In the last two weeks of fall 2020 semester, the WBE team switched from grab sample collection to using autosampler sampling.. Two wastewater samples linked to three dormitories yielded positive results for the SARS-CoV-2 virus. Residents of these buildings were placed in quarantine. This was followed by clinical testing to determine who needed to be isolated, and to initiate contract tracing. This was right before Thanksgiving break when the campus was transitioning online for the remainder of the semester and the students prepared to leave campus. By using the WBE screening tool and isolating positive individuals, the efforts were successful in preventing a potential increase in transmission on campus as well as through student transit.

5. Affordability of the sampling equipment

The overall cost of the project is $8,203.85. The project's initial cost, which was for building one of the autosamplers and two manual samplers, was $911.56 and $133.55, respectively. With the inclusion of the seven others, to have a dedicated equipment kit for each manhole that was to be sampled, the total reached $7,158.85. Table 3 and Table 4 present the initial costing and final cost of building the autosamplers, respectively.

Table 2. Itemized costs of building one automatic sampler

Item	Quantity	Cost
Peristaltic Pump	1	$529.99
Extension Cord (3-way)	1	$99.00
Surge Protector	1	$40.00
Cord Protector	3	$72.00
Timer Outlet	1	$15.99
Flex Tubing	1	$48.50
Cold Packs	12	$12.00
Cooler (48qt)	1	$44.99
Clear Plastic Bin (21qt)	1	$5.98
Gray Storage Bin	1	$10.37
Soap	1	$5.24
Fiberglass Insulation Roll	1	$27.50
Total Cost		$911.56

Table 3. Itemized costs of building seven additional automatic samplers

Item	Quantity	Cost
Peristaltic Pump	7	$3,709.93
Extension Cord	7	$693.00
Surge Protector	7	$280.00
Cord Protector	21	$483.00
Timer	7	$111.93
Flex Tubing	7	$339.50
Cold Packs (12pk)	7	$294.00
Cooler	7	$314.93
Clear Plastic Bin	7	$41.86
Gray Storage Bin	7	$430.22
Cling Wrap	3	$34.44
Insulation	2	$55.00
Tarp	8	$159.92
Tubing Cutter	1	$15.47
Nashua Foil Tape	1	$13.99
1/4" Fittings	s3	$35.97
Pipe Insulate	2	$25.98
Channel Locks	1	$33.70
Heat Tracing	25'	$86.01
Total Cost		$7,158.85

Table 4. Itemized costs of building seven additional automatic samplers

Grab Sampling Design Itemized Cost		
Items	Quantity	Cost
Extension Rod (12′)	1	$39.99
Cotton Twine	1	$6.99
Dynarex Specimen Containers Sterile (4 oz)	1	$0.25
	Total:	$47.23

DISCUSSION AND LESSONS LEARNED

With the development, planning and experiments with autosampler, there have been many mishaps, mistakes, and otherwise unseen lessons in the field. Additionally, the technical team had to consider many potential problems that may have occurred. The lessons learned included in this discussion cover how the autosampler is typically set up. The lessons learned are derived from the practices, fieldwork, and trial and error from the sample collection duration.

1. Clogging: Clogging is one potential problem that can happen at any time when implementing autosamplers for testing. The end of the pump's inlet line, where the weighted head has a built-in filter, can become clogged with tissues and other solid waste flushed down the sewage line. A potential solution the technical team found was to cut the exact length of tubing to allow the inlet line to sit at the opening of the wastewater pipe, allowing waste to flow around the weighted head. Another method is to reverse the flow of the pump to push any debris out.

2. Pinching of the Line: Another expected issue is the inlet line being pinched by the manhole cover. The manholes are moved back from the opening after the inlet lines were placed to keep exposure to

a minimum. However, the objective is to provide ample room for it to pump without the manhole cover resting on the line. Over 24-hours, the manhole cover can shift and pinch the inlet line, stopping the flow. The easiest solution is to move the manhole cover further away to reduce the risk.

3. Overflow and Underflow: Estimating the collection timing is tricky, as it involves balancing between low timing and representative sampling. Setting the timer for the ON time for far too long may cause overflow from the container. There must be extensive trials to determine an optimum sample collection time at each manhole. The only way to circumvent this is to survey the field beforehand and estimate the number of people living in the dormitory building and the flow rates at each manhole at different times of the day.

4. Reversing Lines: A small but costly problem that can occur is mixing up the inlet and outlet lines—a simple fix of switching the lines allowed the test to continue. A good reminder for the peristaltic pump model the technical team used has the inlet on the bottom (from the sewer) and the outlet (to the container) on the top.

5. Pump Rollers: The pump can experience a significant problem: the rollers not compressing the rubber tubing inside, which can cause zero suction and zero flow. The solution rests with the roller assembly. Removing the rubber tubing and rotating the rollers to the right compresses the tubing and provides ample suction for pumping. Feeding the rubber tube back in from the bottom-up and sealing the cover fixed this issue.

6. Freezing Temperatures: Sewer water is still water at its base, and can still freeze, which becomes the biggest problem with the collection. As the entire apparatus is exposed, the lines going in and out are fully exposed to sub-zero temperatures. The only way to combat this would be to attach a heat tracing unit to keep the temperature over the freezing point. One method to solving the freezing issue is using

heat tape around the lines. This keeps fluid inside the lines above freezing. Another method is reversing the lines, like clearing clogs. Reversing the line will ensure that there is no water in the lines for a long period of time.

CONCLUSIONS AND RECOMMENDATIONS

1. WBE Sample Collection with AS

The WBE technical team has reached the general conclusion that the WBE project can be followed up in the 2021 fall semester for SARS-CoV-2 surveillance. The eight autosamplers have been tested, serviced, and ready for the next project for the incoming semester. All equipment is fully functional; the team created walkthroughs for setup and breakdown, and the cold weather attachments have been assembled.

Due to the nature of the project, the team has run into many complications throughout the year. Some of the problems were within the team's control, but the others were out of our hands. The cold weather attachments should be further investigated and tested, as time was a very limiting factor for the WBE technical team. We also highly recommend that an extra sets of tools and parts should be included with every kit, especially during autosampler setup sessions. Doing so reduces the amount of stress running around campus, and overall, it makes things go smoother even when minor inconveniences present themselves.

2. Use of Tampons

Northern Arizona University is using tampons in replacement of autosamplers due to the high price tag on them(Brizee). The procedure is as follows: tightly attach a tampon to the end of the fishing line and lower it down into the manhole and leave it in there for 24-hours. During that time, the tampon traps a continuous flow of the sample. Once removed, it is transported in a cooler to preserve the sample before getting to the lab.

Next year, it is highly recommended to try the Moore Swab method, as it also gathers a 24-hour composite sample. The Moore Swab is surveillance method in which a absorbing pad or gauze pad is tied up with a string and suspended in flowing wastewater(Sikorski and Levine). This method is intended to trap microorganisms over 24-hour period. The team also believes that both the auto sampling and the Moore Swab methods can bring results. A comparison on cost, effectiveness, and result on both methods can be of great value to literature.

3. Future Work

Future possibilities for the WBE project may expand beyond testing for SARS-CoV-2. The virus testing is but a steppingstone. Wastewater surveillance can extend to tracking other trends in , public health hazards.

Acknowledgments

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