Water Quality Monitoring for Diarrheal Pathogens (WQMDP), Fall 2017

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Abstract

AguaClara plants effectively remove turbidity and fecal indicator bacteria (FIB) from drinking water sources in Honduras. However, no study has documented the plants' ability to remove specific diarrheal pathogen species. This study identified best practices for collecting and shipping pathogen DNA from sampling locations in Honduras back to Cornell University. A literature review identified options for filtration systems, chemical DNA preservation solutions, and shipping protocols. Tests of the clogging behavior of 5 µm and 0.1 µm pore size membrane filters demonstrated that this filtration method's 60 h projected run time for a 10 L sample outweighs its gains in simplicity.

Introduction

AguaClara water treatment plants supply drinking water to approximately 50,000 people in Honduras and India (Cornell University AguaClara, 2017). Until recently, however, the only data collected on how effectively AguaClara plants operated in the field were turbidity measurements. This convention matched the convention used by AguaClara design teams in Ithaca, NY, who gauge plant performance based on the turbidity of the processed water. Turbidity is easy to measure and understand: even a cursory visual inspection can tell an observer a lot about a water sample's turbidity. However, because turbidity measurements only describe how much suspended material there is in a sample and not what makes up the suspended material, turbidity is at best a proxy measure for the potential of a water sample to cause disease (potability). An improvement over turbidity measurement is the measurement of fecal indicator bacteria (FIB), which are used to define important drinking water standards in the United States (U.S. Environmental Protection Agency, 2017). These bacteria originate in the guts of humans and animals, and, in theory, are at least as persistent in the environment as disease-causing pathogens. Work performed by Yolanda Brooks at the Richardson Lab in 2016 quantified turbidity and *E. Coli* removal by AguaClara plants in Honduras using portable turbidimeters and compartment bag tests, respectively. That work showed that AguaClara plants effectively remove turbidity and *E. Coli* from drinking water (Brooks et al., 2017a).

Even though metrics such as turbidity and FIB concentrations are useful for quickly determining the potability of a water sample, neither method directly measures the concentration of disease-causing bacteria, protists, and viruses in water. AguaClara would benefit from direct measurement of pathogens because the results would either confirm the team's belief that its plants are doing a good job of providing safe water, or the results would suggest important areas for improvement. To achieve direct measurement, the Richardson Lab has pioneered the use of an OpenArray qPCR analysis protocol that will detect not only species-specific FIB from humans, chickens, and cattle, but also the pathogens *E. Coli*, *Salmonella* spp., Hepatitis A, norovirus, *Giardia lamblia*, and *Cryptospordium parvum*, among others. Unfortunately, this protocol requires large water samples, on the order of 10 L in volume, to achieve low enough limits of detection to measure the concentrations of individual pathogen species (Brooks et al., 2017b). Additionally, the protocol requires equipment unavailable in Honduras, so samples must be concentrated down to volumes of less than 200 ml for shipment back to Cornell University before the DNA molecules degrade (Monroe Weber-Shirk and Ruth Richardson, 2017). This study first examines the sample collection process from Honduran water source all the way to air shipment by means of a literature review, and second seeks to develop a simpler method of large-sample filtration than those found in the literature.

Literature Review

A selective literature review was conducted to understand current techniques and technologies for environmental DNA (eDNA) collection and transportation. Three questions based on conversations with Professors Ruth Richardson and Monroe Weber-shirk, focused the search (Monroe Weber-Shirk and Ruth Richardson, 2017). First, how do scientists collect aquatic eDNA samples? Second, how do scientists preserve DNA when they don't have access to laboratory facilities such as refrigeration or cryo-storage? Third, what regulations apply to the shipment of potentially infectious biological samples by air?

The first focusing question was, "How do scientists collect aquatic eDNA samples in the field?" The most common methods to collect eDNA is with pump and filter mechanisms. The first example of this paradigm was Smith-Root Inc.'s *ANDe* eDNA collection system. The system takes the form of a backpack with a long hose arm for collecting water far out into a body of water, and it retails for $5975. Although this water analysis tool is fairly expensive, it's also portable and facilitates the analysis of water in remote areas greatly, so it is an option worth considering or comparing the final system to. In addition to this, the Smith-Root *biomeme* qPCR machine facilitates the rapid, on-site analysis of the eDNA collected (Smith-Root, Inc., 2017). Even though an option as expensive as Smith-Root might not be feasible, looking at their process could be beneficial towards the goal of searching for diarrheal particles in wastewater. A second example of the pump and filter paradigm came from the USGS Environmental DNA Sampling Protocol. The protocol describes three possible methods for extracting eDNA from the water. Each of the three methods uses the same open-topped membrane filter system. However, the three protocols differ on the type of pump used to drive the sample water through the filter. Where access to electricity is easy, the protocol recommends using a peristaltic lab pump. Where there is no direct electricity access, but weight is not a concern, the protocol recommends using a cordless drill to run peristaltic pump heads. Finally, where no electricity is available, and weight is a concern, the protocol recommends a hand vacuum pump (Laramie et al., 2015). Professor Ruth Richardson of Cornell University's Civil and Environmental Engineering department uses protocols similar to the first two USGS protocols, with peristaltic lab pumps and cordless drills, respectively. However, for the filter medium, her lab has used both membrane filters and dialysis cartridge ultra-filters. Please see the **Previous Work** section of this document for a more complete description of the Richardson Lab's filtration protocol.

The information gathered to answer the first focusing question prompted several important sub-questions. In particular, what is the ideal filter pore size for the collection of eDNA particles? Professor Richardson recommended the use of two filters in series, the first having pores of 3 micrometers and the second having pores of 0.1 micrometers (Monroe Weber-Shirk and Ruth Richardson, 2017). The USGS eDNA collection protocol, on the other hand, recommends a single filter with a pore size between 0.45 micrometers and 3 micrometers, depending on the volume of water desired to be pumped before clogging (Laramie et al., 2015). Turner et al. investigated the size distribution total suspended particle matter (SPM) and total DNA containing particles (total eDNA) by using a series of filters of decreasing pore size (See Figure 1). Their filter series had filters with 180, 100, 60, 20, 10, 1.0, and 0.2 micrometer pore sizes. Most of the SPM was particles larger than 100 micrometers in size. Of all the SPM collected, only 0.1 percent of it was eDNA, and most of the eDNA was found in particles less than 0.2 micrometers in size. Turner et al. also found that filter clogging at the 0.2 micrometer pore size allowed for less than 250 milliliters of 1.0 micrometer filtrate to pass through, so their trials only used 250 milliliters of sample water (Turner et al., 2014). These results suggest three things to this research team. First, just as Professor Richardson originally suggested, the primary problem with isolating eDNA from aquatic samples will be clogging of the necessarily small-pore-sized filters. Second, chemical precipitation of DNA, similar to Turner et al.'s procedure, might be a good way to recover the large portions of DNA that pass through even the 0.2 micrometer filter. Third, it might be possible to accomplish AguaClara's pathogen identification goals with less than the 10 liters of sample water that Professor Richardson suggests, as Turner et al. used only 250 milliliters to find carp eDNA.

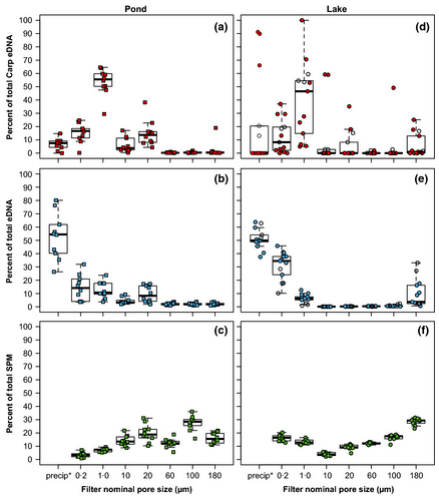

Figure 1: Per cent of total Carp eDNA, total eDNA and SPM in each particle size fraction per water sample from the pond (a, b, c, respectively) and lake (d, e, f, respectively). *The final fractionation step was DNA precipitation using the 0·2 micrometer filtrate, but fractionation for SPM measurement did not include this step (see 'Materials and methods'). Filled symbols are winter; open symbols are spring (only the lake was sampled in spring; SPM was not measured in spring). Data points are horizontally 'jittered' within each size fraction to reduce visual overlap. Boxplots include the spring data from the lake in (d) and (e).(Turner et al., 2014)

Another important sub-question that arose during the literature review was, "How do researchers pump water through their filter systems, especially when they have little access to electricity?" Beyond the three methods suggested in the USGS Environmental DNA Sampling Protocol (hand vacuum pump, cordless drill with peristaltic pump heads, and peristaltic lab pump), Professor Richardson also suggested the use of a hand-powered bicycle pump reconfigured so that it pulls a vacuum instead of pumping a vessel up to high pressure. Such a pump would be much more powerful than the hand-vacuum pump, but could still operate without any electricity (Monroe Weber-Shirk and Ruth Richardson, 2017).

The second focusing question was, "How do scientists preserve DNA when they don't have access to laboratory facilities such as refrigeration or cryo-storage?". Several chemical preservation methods were found which allow samples to be stored at ambient temperature over time scales of weeks or even months. The techniques were primarily designed for field biologists using eDNA techniques in locations that can only be accessed on foot, which limits the ability to refrigerate or cryo-store samples. These preservation methods fell into two broad categories: chemically treated cards that DNA samples are dried onto and liquid chemical solutions that samples are placed into. The purpose of both methods is to inactivate DNases, proteins that break down DNA. Overall, liquid chemical solutions performed much better than chemically treated cards, so this literature review's focus narrowed to selecting the best solution to use for this study. Ambient temperature preservation solution (hereafter "ATPSs") were evaluated by how long they could preserve DNA samples at high quality, how expensive they were on a per-volume basis, and how toxic they were to humans. Better ATPSs had longer preservation times, cost less, and were less toxic than worse ATPSs. Gray et al. paper was particularly useful in comparing ATPSs, because it tested several types against each other (Gray et al., 2013). Unfortunately, the samples used in the study were taken from coral reefs by swabbing the surfaces of coral polyps. Samples of from coral polyps might differ significantly from the highly turbid aquatic samples expected in Honduras, so other studies with more similar samples were also considered. De Ley et al. discusses the use of ATPSs on bulk soil samples to collect nematode DNA (De Ley et al., 2006). Nechvatal et al. used ATPSs similar to those in Gray et al. to preserve human fecal samples for shipment across the U.S. (Nechvatal et al., 2008). In all three studies, ATPSs preserved eDNA well at ambient temperatures for later analysis in the lab. Among the ATPSs tested, the most attractive for AguaClara's purposes is DESS. This solution's per-volume cost is one-tenth that of the next cheapest solution, it is safe to ship by air, and it preserves DNA over time scales of months. Also, consistent with AguaClara's goals to use open-source technology, DESS non-proprietary, unlike every other ATPS encountered in this literature review (Gray et al., 2013).

The third focusing question was, "What regulations apply to the shipment of potentially infectious biological samples by air?" According to the guidelines compiled by U.S Department of Health and Human Services, once a substance is found to be "infectious" there are steps to be followed to protect

the public, workers, property, and the environment from contamination during shipment. For this purpose, the Department provides guidelines on UN Standard Triple Packaging for infectious microbial or biomedical samples. Since samples from this study will be treated with DESS before shipment, and therefore be nonviable, they fit into Category B outlined in the handbook. Category B is defined as an infectious substance which does not cause permanent disability or life-threatening or fatal disease to humans or animals when exposure to it occurs (Chosewood and Wilson, 2009). See Figure 2 for an image of the UN Standard Triple Packaging method for Category B infectious substances.

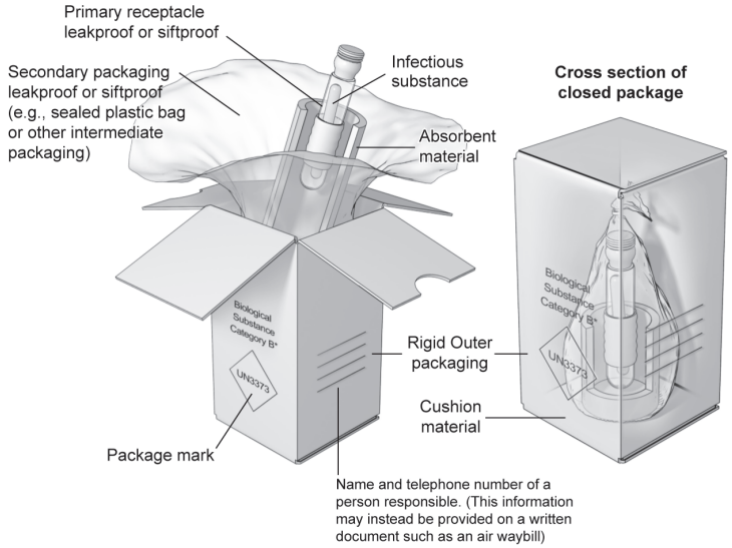

Figure 2: Triple Packaging for Category B Substances.(Chosewood and Wilson, 2009)

Previous Work

Professor Richardson's lab, while pioneering the OpenArray qPCR tests for specific pathogen species in water, also tested protocols for isolating pathogenic eDNA from large volume water samples. Their work, based on the work of Francy et al., uses ultra-filters designed for medical dialysis procedures to decrease sample volumes from 10 L to volumes small enough for centrifugation (See Figure 3) (Brooks et al., 2017b). Two filtration protocol options for ultra-filtration systems are detailed by Francy et al (Francy et al., 2013). The Richardson Lab uses the recycling, tangential flow method, which pumps water through the ultra-filtration cartridge, where pure water flows out at one outlet and more concentrated sample flows out at the other outlet into a retentate bottle. As the retentate bottle fills with a concentrated sample, some of the retentate can be recycled back into the ultra-filter cartridge for another round of concentration. Once the sample carboy is empty and the retentate bottle is as concentrated as possible, the retentate is taken out of the ultra-filtration apparatus and filtered through very small pore-size membrane filters. The material captured on the membrane filters is then re-suspended into small volumes of the solution so that it can be centrifuged into a pellet. The pellet is then resuspended in another solution, and the OpenArray qPCR tests are performed (Sausele and Richardson, 2017).

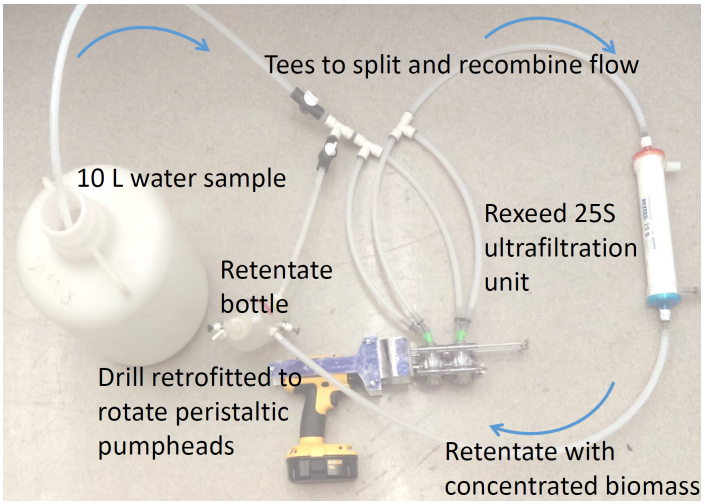

Figure 3: Richardson Lab Ultrafiltration Process.(Brooks et al., 2017b)

This study aims to test whether the ultra-filtration cartridge step could be skipped altogether, simplifying the process of collecting pathogen eDNA samples considerably. Instead of ultra-filtration, a series of successively smaller pore-size membrane filters would be used to collect the eDNA found in suspended particles in the water sample. This method would decrease the amount of tubing required for the filtration apparatus, and would also decrease the size of the material that would need to be shipped back from Honduras. Under the current ultra-filtration system, the whole ultra-filter cartridge and the retentate must be shipped back to Ithaca, NY. With the membrane filtration-only system, the filter membranes could be stored in small vials for shipment (Monroe Weber-Shirk and Ruth Richardson, 2017).

Methods

To determine the validity and efficiency of our process, the filtration system was equipped with two 200 kPa pressure sensors to determine when the system was functional. The pressure differential between pre-filter and post-filter sensors is a useful measure for clogging because, within a certain flow regime, as filter permeability decreases because of clogging, the pressure difference across the membrane must increase to keep up with the constant flow rate set by the peristaltic pump (See Equation 1).

$$N_{Ax} = \frac{\rho_A \kappa}{\mu} \frac{dp}{dx} \tag{1}$$

Where N_{Ax} is the mass flux through the membrane in the x direction, ρ is the density of the filtered fluid, κ is the Darcy Law permeability of the filter medium, μ is the fluid viscosity, and $\frac{dp}{dx}$ is the differential of pressure with respect to x. This equation assumes one-dimensional flow in the x direction, which is normal to the filter surface, and that backward diffusion through the membrane is negligible (Sagle and Freeman, 2004).

ProCoDA and EXCEL were used to track and analyze the amount of water that could run through the system before the filter became clogged. Several tests were conducted to guarantee that this process was viable. The first, a membrane-clogging pressure test, was designed to give a better understanding of how quickly the membrane filters clog when water of varying turbidities are passed through them. The second, a flow test, was conducted to measure how much water can be run through the system as the quantity and location of filters is varied. The results of these tests guided future designs and modifications of the overall process. Please see the **Manual** section for the finalized methods.

Membrane-Clogging Pressure Test

Experimental Apparatus

For the initial apparatus, the 10 L tank was placed on an elevated location so that gravity assisted the water flow. Quarter inch tubing was used for all the connections, and the pump tubing used for the peristaltic pumps was size 16. A valve was located right after the peristaltic pump to minimize water

loss. A 200 kPa pressure sensor was placed after the valve to measure the inlet pressure and see how the pressure at this location varied with clogging. After the pressure sensor, the filter housings were installed with the 5 µm and then 0.1 µm pore-size filter membranes. These membrane sizes were chosen based on the recommendations of Professor Richardson. After each filter housing, a pressure sensor was placed to determine the outlet pressure of when the apparatus clogs, which should be at about vacuum pressure. The water that went through the system was collected in the water outlet bucket. When placing the filter membranes, it was ensured that the membrane lay flat within the filter housings, so that water would not leak around the edge of the filter membranes. The clamps were placed on the tubing connections around the peristaltic pump to make sure none of the barbed fittings failed due to the pressure increase.

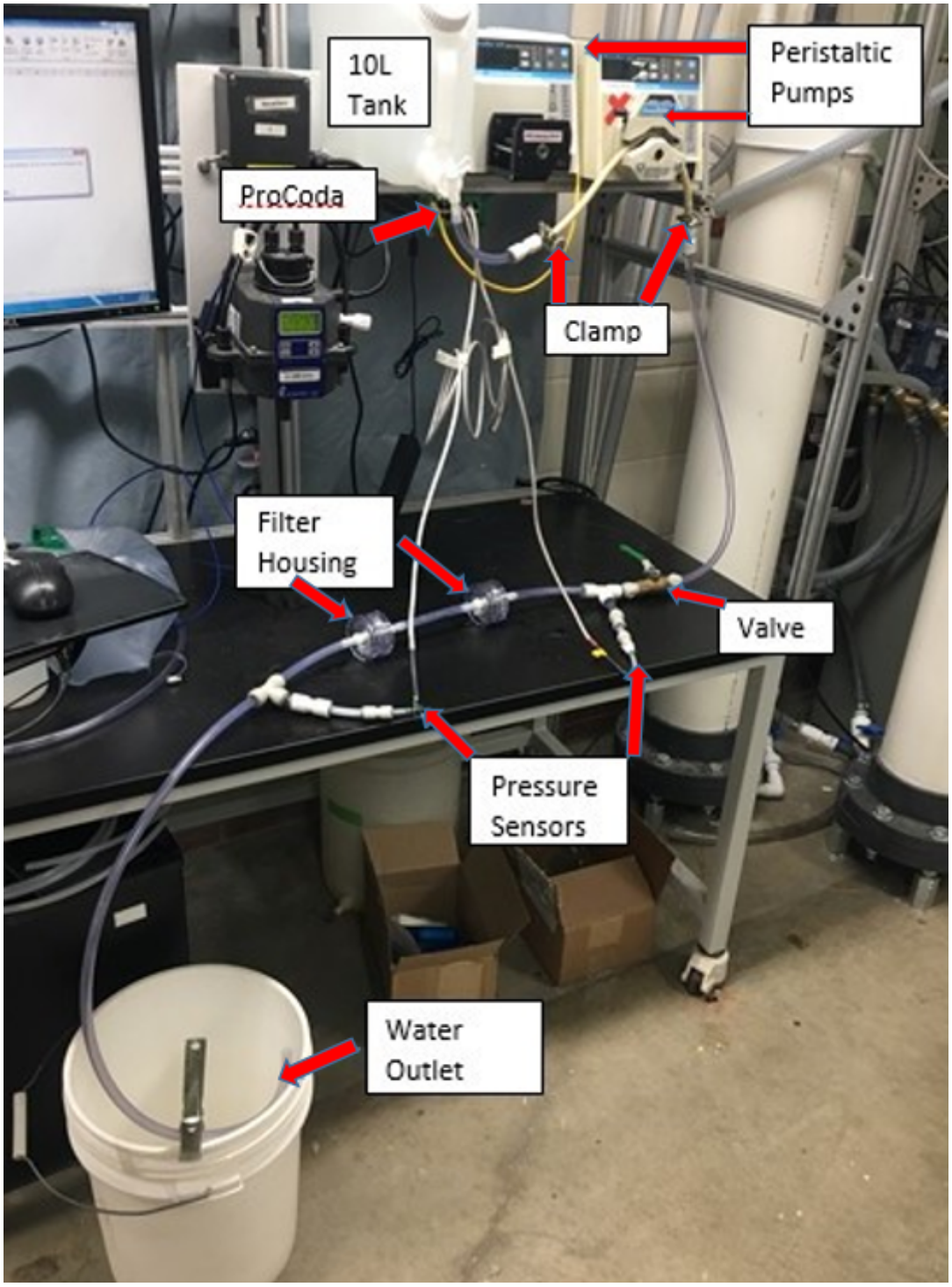

Figure 4: Lab Set-up

The final apparatus was modified to account for clogging time and changes in flow rate. The water tanks were placed at any arbitrary level due to the realization that gravity had a negligible effect on the pressure driven flow. Two peristaltic pumps were set up, one for priming and one to run the process. Quarter inch tubings were used for all the connections and the pump tubing used for the peristaltic pumps was size 16. Two 200 kPa pressure sensors were placed again on either side of the filter housing but this time only one filter housing was placed at a time. A large container was used for effluent water for the 5 µm runs and a graduated cylinder was used to contain the effluent in the 0.1 µm runs. An electronic balance was placed under the container and connected to ProCoDA to determine how flow rate varied with time.

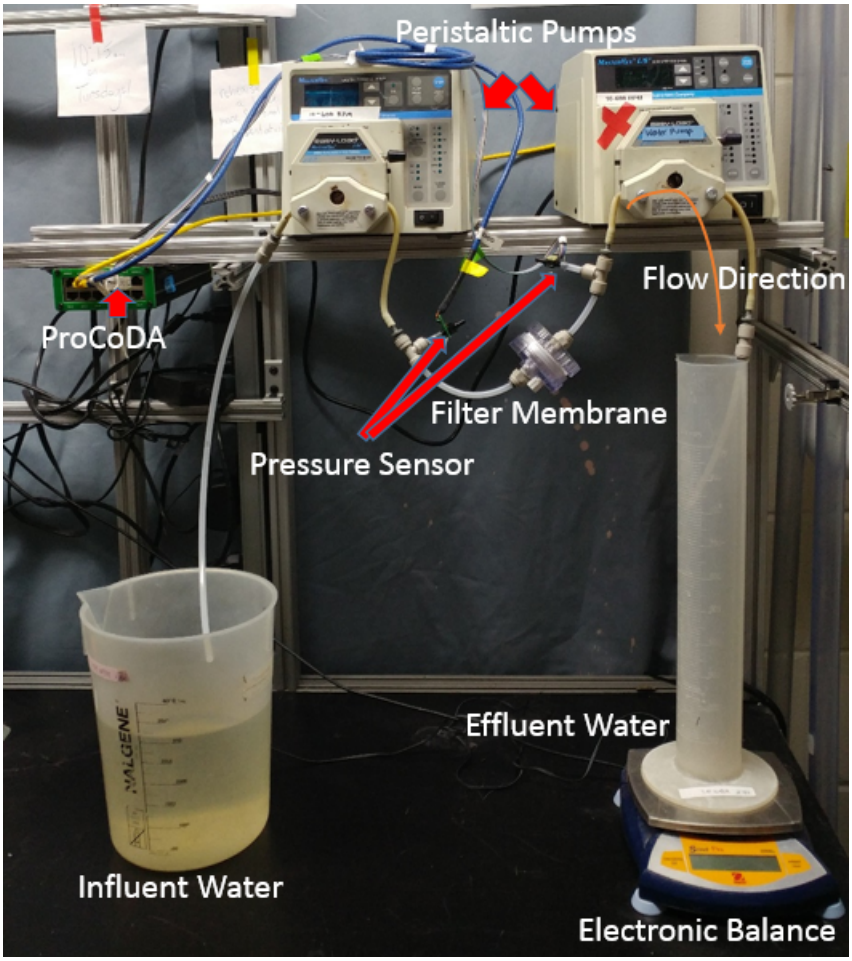

Figure 5: Final Lab Set-up

The aforementioned apparatus was constructed with size 16 tubing, two filter housings, two clamps, a valve, a peristaltic pump, and two 200 kPa pressure sensors. These materials were adjusted for the final apparatus by only including one filter housing, one clamp, quarter-inch tubing, and a balance to measure flow rate. During construction, several complications were encountered such as the build up in pressure from filter clogging, the difficulty in keeping the tubing attached, and the fact that water could stop flowing through the filter. Some adjustments that were made to the apparatus to guarantee its functionality were clamping down every piece of loose tubing, and priming the filter housing with water to ensure proper flow without air bubbles. It was also required to frequently swap out the filter membranes depending on how turbid the water was to make sure water continued to flow. This was checked using the mass balance to study the change in flow with time. These modifications are reflected in the change from the initial setup in Figure 3 to the final setup in Figure 4.

ProCoDA

At the beginning of the semester, ProCoDA was used at a minimal level, just to log the pressure data over time and run pumps. In the previous section, there are two pumps in the photo of the experimental

setup. However, only one of the pumps was connected to the ProCoDA because the other one was used to prime the filter.

There were different needs for pressure monitoring. Initially, when the influent water was being pushed through the membrane, the pressures before and after the filter were monitored to make sure the pressure increase did not detach the tubing. After changing the pump direction to pull flow through the filter membrane, the pressures were monitored to determine if the membrane was clogged. From several control experiments with filter membranes, the team found out that the post-filter pressure went below -94 kPa when the membrane was clogged.

In an effort to automate the experiments, a pressure threshold set point was created for a new rule called "Stop Running Auto." The rule stops the pump when the post-pressure sensor data exceeds the "pressure threshold" set point. This set point was set to -94 kPa unless the experiment was aiming to observe the pressure behaviors near the failure modes.

In addition to pressure measurement, an Ohaus Scout Pro electronic mass balance was added to measure and record the instantaneous mass of the collected effluent flow. The measured mass was used to calculate the rate of change in the flow rate through the membrane, to assess the performance of the membrane for specific turbidities.

Detailed descriptions of how to add the above devices, set points, and rule are located on the **Manual** section.

Procedure

To perform a filter membrane clogging test, the team used a peristaltic pump to pull water from a feed water carboy through a filter housing to a waste bucket. On the upstream side of the filter housing, where water would pass before reaching the filter, and on the downstream side of the filter housing, where water would pass after going through the filter, 200 kPa pressure sensors were mounted to track the pressure differential across the filter membrane as demonstrated in Figure 5.

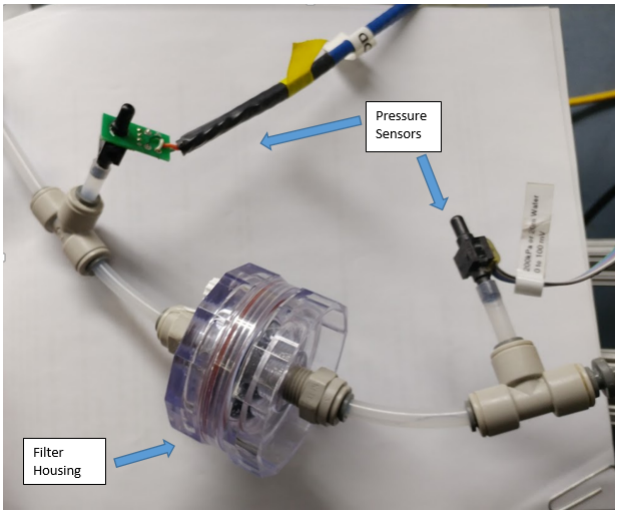

Figure 6: Pressure Sensor Setup

Higher pressure differentials would signal a filter was inhibiting water passage, either because of smaller pore sizes or because of clogging. The pressure sensors read pressure data as negative values when the pressure in the tubing was less than atmospheric pressure and as positive values when the pressure in the tubing was more than atmospheric pressure. The pressure and effluent flow were both monitored during each trial to determine when the filter had clogged. Although the team aimed to run each trial until flow through the filter stopped, on some occasions the trials were stopped earlier due to scheduling constraints(e.g. classes) or apparatus malfunctions.

Results and Analysis

Filter Membrane Clogging Test

Prior to collection of pathogen samples from raw surface water, the team conducted several control runs to build baseline data regarding clogging pressure, filtration rate, flow rate and running time of each size of membrane.

The first set of filter membrane clogging tests used 5 µm filter membranes. The second set of filter membrane clogging tests used 0.1 µm filter membranes. The tests were iterated using tap water as influent, to find representative data.

Flow Rate Estimation

The goal of each trial was to filter 10 L of water. Using size 16 pump tubing with the peristaltic pump, the expected flow rate was 0.01333 mL/s per each increment of rpm. For example, if the pump was running at 500 rpm, the expected flow rate of the effluent is

$$(0.01333mL/s - rpm) * (500rpm) = 6.67mL/s \tag{2}$$

Once the optimal pump speed, at which the raw water can be filtered continuously without experiencing extreme pressures, is discovered empirically, the effluent flow rate was measured manually and repeatedly. It was done by dividing the volume of collected effluent with the collection time. For example, average flow rate in trial 1 can be calculated as shown below:

$$(4L)/(575s) = 6.96mL/s \tag{3}$$

Control experiment on 5 µm filter membrane

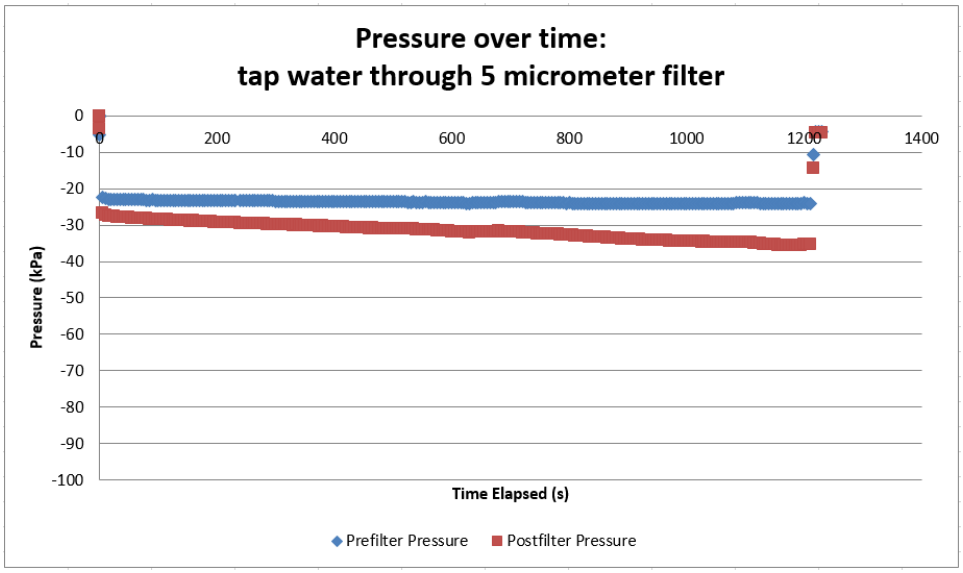

Figure 7: Pressure difference across the filter increases linearly when total 10 L of tap water is filtered through 5 µm membrane at pump speed of 500 rpm. The total run time was approximately 1200 s.

Among the results of five identical trials that executed the same procedures, the graph of this trial shows the behavior of the pressure associated with the flow through the 5 µm pores, most accurately. Measurements of the flow rate were 7.4 ml s^{-1}, 8 ml s^{-1}, and 7.79 ml s^{-1} at 680 s, 760 s, and 850 s, respectively. This graph supports the initial hypothesis that the filters would clog gradually. It seems that at low turbidities, the 5 µm pore-size clogged at a visible, linear rate.

Control experiment on 0.1 µm filter membrane

In this trial, the pump speed was constant in 100 rpm. The 0.1 µm pore-size filter did not clog and steadily yielded about 337 ml over 2000 s.

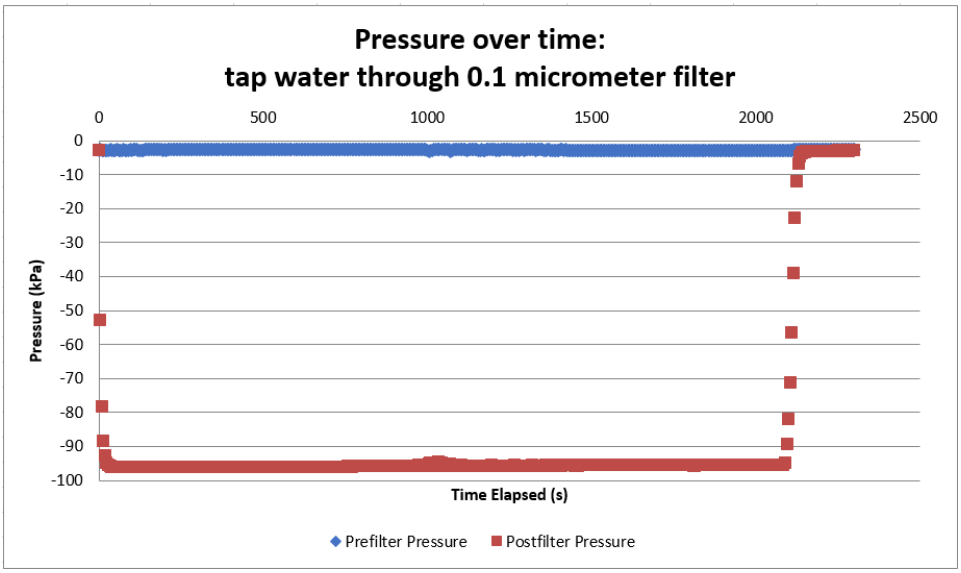

Figure 8: When the tap water is filtered through 0.1 µm membrane at pump speed of 100 rpm, the pressure fluctuates around -94 kPa. Over total run time of 2000 s, 337 ml effluent water was collected.

The 0.1 µm pore-size filter tests had very different pressure profiles than the 5 µm tests. The team had hypothesized that the 0.1 µm pore-size filters would clog more quickly than the 5 µm pore-size filters. This hypothesis proved to be true in the extreme. The upstream pressure stays at its original state, slightly negative, for the duration of the trial, while the downstream pressure precipitously drops to around −94 kPa in the first few seconds of the trial and stays at about that pressure until the pump is turned off (See Figure 8).

At these extremely low downstream pressures, some water does come through the membrane, but the volume is extremely small compared to the volumes filtered through the 5 µm pore-size filters. These volumes filtered are consistent with the 250 ml of volume pumped until clogging for a 0.2 µm pore-size membrane filter given in Turner et al. (Turner et al., 2014).

Series Filter Control Test

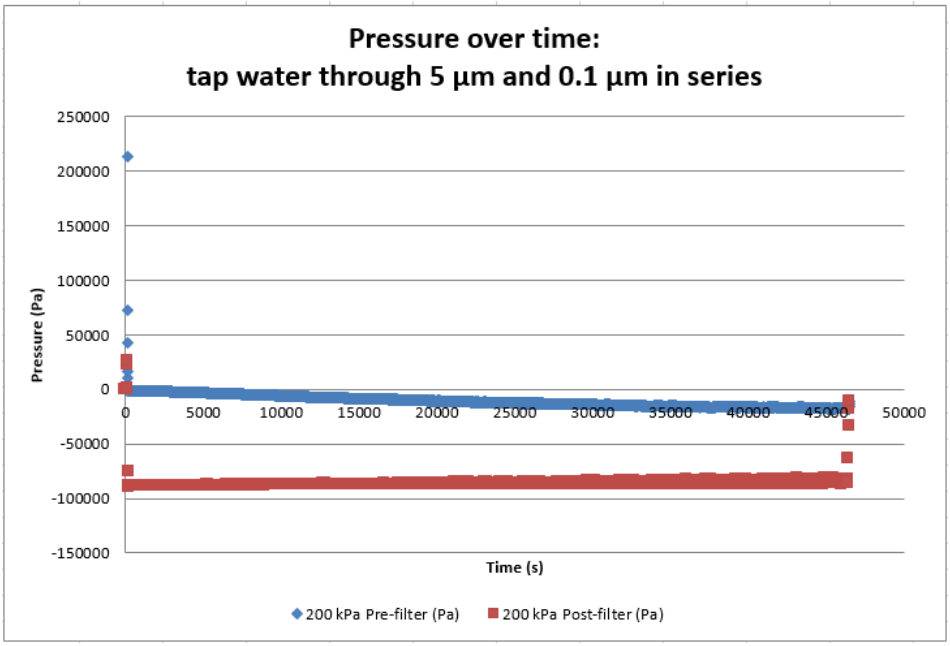

Figure 9: When the tap water is filtered through 5 µm and 0.1 µm membrane in series at 300 rpm, the pressure fluctuates around -84 kPa. Over total run time of 46 500 s, or 775 min, the record of total amount of effluent water was collected was lost.

The series filter test had a 5 µm pore-size filter followed by a 0.1 µm filter, to build baseline performance data for filters in series. To assess maximum capacity, tap water was used for influent. The team hypothesized that 0.1 µm pore-size filters would perform better in this setup. Because the influent water for the membrane was tap water filtered by 5 µm, the team anticipated to have less clogging in 0.1 µm membrane. As shown in Figure 9, the pressure increases slowly. Steady and slow effluent flow was observed during the experiment.

Series Filter Creek Water Test

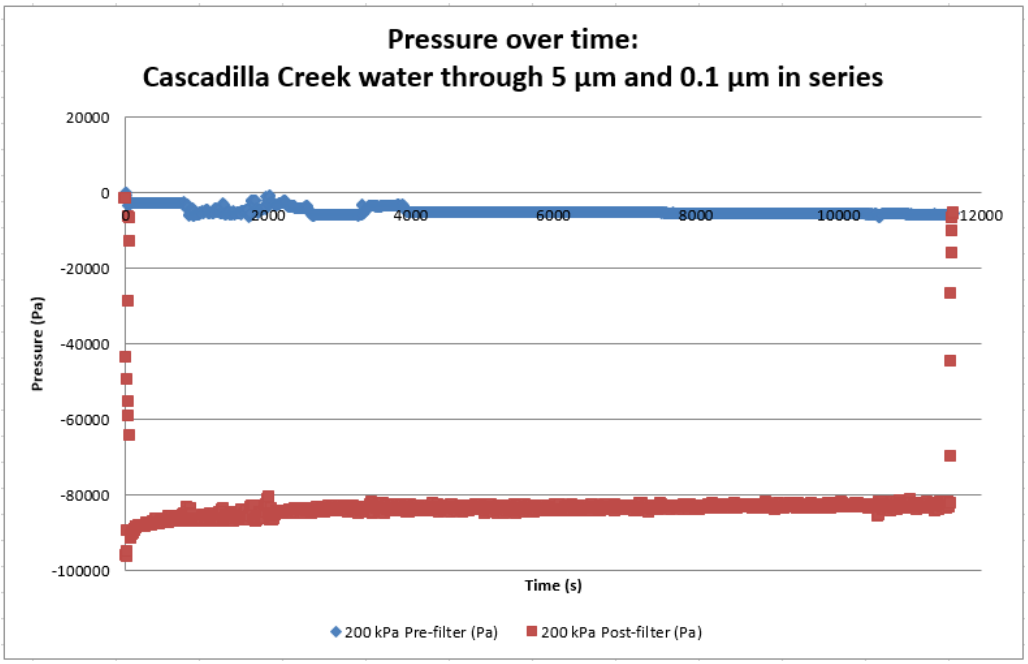

Figure 10: When the creek water was filtered through 5 µm and 0.1 µm membrane in series at pump speed of 300 rpm, the pressure fluctuated at -84 kPa. Over total run time of 11 600 s, or 193 min, 340 ml effluent water was collected.

10 L sample of Cascadilla Creek water was taken and stored in a refrigerator. The turbidity of the water was 1.35 NTU. Having the identical setup with the previous experiment, the change in pressure during the experiment seem similar as it is shown in Figure 10. Again, steady and slow flow was observed throughout the test.

While it had been originally hoped that an OpenArray qPCR analysis could be run using the material collected on the filters during this test, it was discovered upon discussion with Professor Richardson and Desiree Sausele of Professer Richardson's lab that all the tubing and filter housings would need to be sterilized beforehand for the qPCR analysis to be useful (Sausele and Richardson, 2017). The WQMDP team decided that it was not ready to learn to sterilize equipment in a short time frame, and determined that even if the team learned to sterilize equipment, the risk of the team making an error was too high for the learning to be worthwhile. See the **Multi-Membrane Volume Throughput Test** and **Conclusion** sections for further discussion of contamination risks.

Multi-Membrane Volume Throughput Test

Only one multi-membrane volume throughput test was performed by the WQMDP team. However, since so many filter membranes were used to perform the test, the results can be viewed as predictive of future behavior. The test was performed using a 10 L sample of 12.12 NTU water from Fall Creek in Ithaca, NY. See Table 1 and Figure 11 for exact numbers and illustrations of the experiment, respectively.

Table 1: Multi-Membrane Volume Throughput Test Results Summary

Filter Type (Pore Size)	5 µm	0.1 µm
Filters Used (Count)	8	5
Total Water Volume Filtered (ml)	3776.4	518.7
Membrane Average Water Volume Filtered (ml)	472.05	103.74
Membrane Avg. Time Until Clogging (including changeover) (min)	9.5	35.6

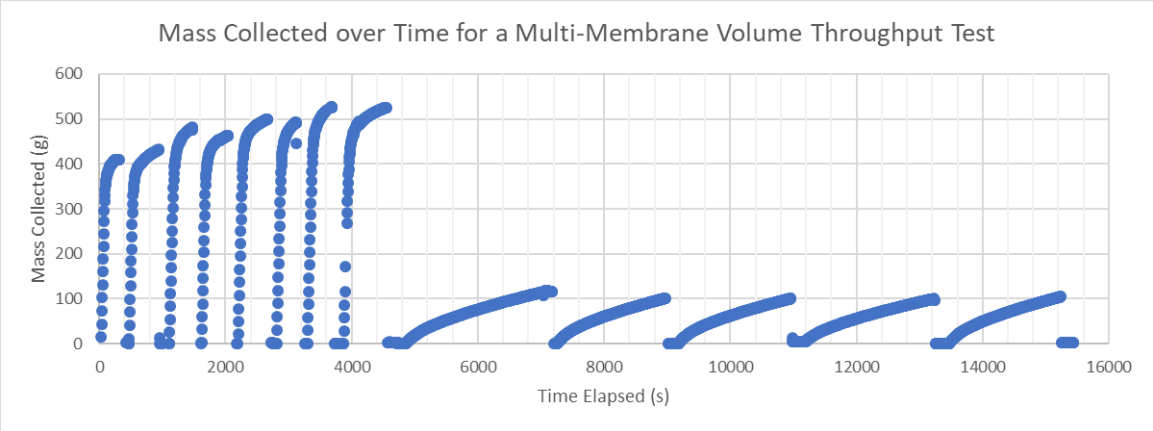

Figure 11: The filtrate mass of water collected from each filter membrane. When each filter membrane was changed out for the next membrane, the graduated cylinder containing the filtrate was emptied into a storage container so that mass for the new filter would begin again at zero.

Assuming that each filter's behavior is independent of the behavior of other filters, the number of filters of each type required to filter 10 L of 12.12 NTU water was estimated by simply dividing 10 L by the average volume of water processed by each type of filter. This yielded a projection of required filter count for each type of filter membrane. Multiplying these filter counts by the average time required to filter for each filter membrane and summing together the result for both filter types yielded a projected process time. See Table 2 for numerical results. Note the extremely high cost in both time and money to filter a single 10 L sample of water. Beyond time and money costs, the extremely large number of filters and filter changeovers required for membrane filtration also increases risk of contamination. Every time a filter is changed out, the operator runs the risk of contaminating the filter with bacteria from the environment. Even worse, the operator also runs the risk of being exposed to high concentrations of infectious pathogens (Richardson, 2017).

The Ultrafiltration Total column contains estimates of performance for the Richardson Lab's ultrafiltration system (Sausele and Richardson, 2017). See the **Conclusions** section for discussion and comparison of the membrane and ultrafiltration processes.

Table 2: Predicted characteristics of the membrane filtration procedure for 10L of 12.12 NTU sample water

Filter Type (Pore Size)	5 µm	0.1 µm	Membrane Total	Ultrafiltration Total
Filters Used (Count)	21	96	118	5
Total Filtering Time (hrs)	3.3	57.2	60.6	1-3
Total Filter Membrane Cost ($)	24.15	192.00	217.15	20.00

This total time estimate for filtering 10 L of water may exhibit a slight upward bias because the stopping criterion for each filter membrane was not optimized. All filters exhibited expected clogging behavior, with high volume flow rates at the beginning of their run which decreased over time down to an asymptote near zero. This trend means that shorter run times for each filter membrane increase the time-average flow rate, but also increase the number of membranes required to filter the same target volume of water. The WQMDP team, in its single run of the multi-membrane volume throughput test, erred on the side of using fewer filters but decreasing the time-average flow rate. This suggests that lower 10 L sample filtration times could be achieved by slightly increasing the number of filters used. The available gains from this optimization would probably be small, however, because most of the time gained from using more filters would come from the 5 µm filtration part of the process. The 5 µm filtrations have very high initial flow rates of about $6\,\mathrm{ml\,s^{-1}}$ which fall of sharply to flow rates of about $0.1\,\mathrm{ml\,s^{-1}}$ (See Figure 12). In contrast, 0.1 µm pore-size filters have very little change in flow rate between initial unclogged and limiting clogged states, which have flow rates of about $0.15\,\mathrm{ml\,s^{-1}}$ and $0.05\,\mathrm{ml\,s^{-1}}$, respectively (See Figure 13).

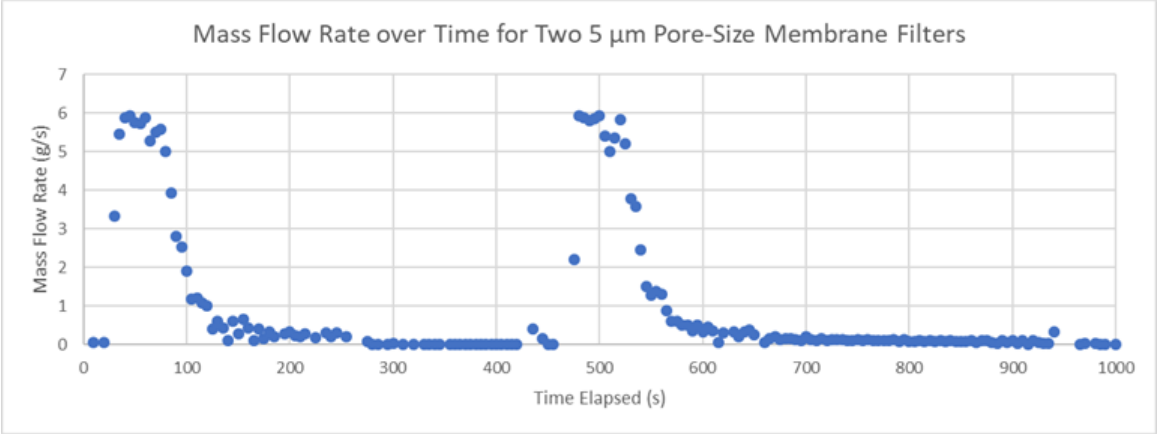

Figure 12: The decay of mass flow rate over time due to clogging in two 5 µm pore-size filter membranes. Taken from the multi-membrane volume throughput test, so times elapsed refer to the much longer full trial. Note that the range of mass flows shown here is much wider than the range of mass flows shown in Figure 13

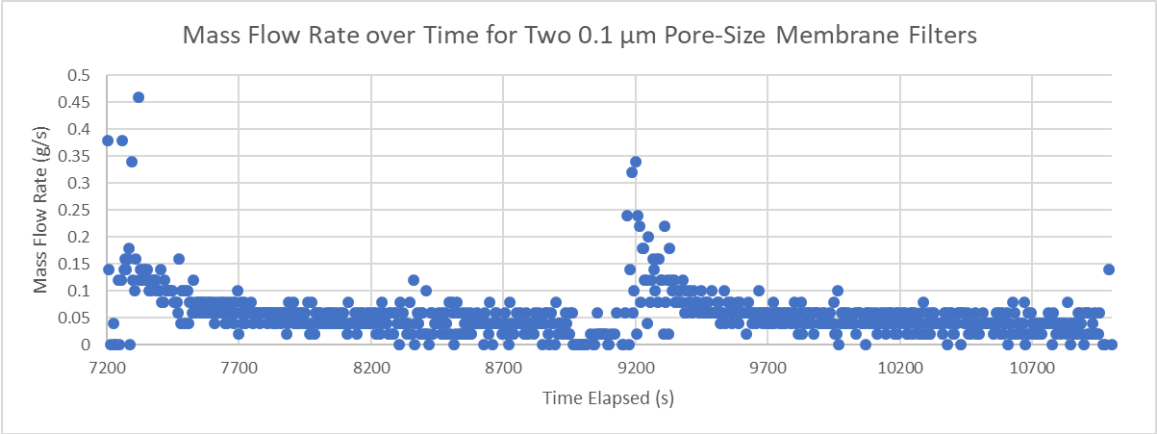

Figure 13: The decay of mass flow rate over time due to clogging in two 0.1 µm pore-size filter membranes. Taken from the multi-membrane volume throughput test, so times elapsed refer to the much longer full trial. Note that the range of mass flows shown here is much narrower than the range of mass flows shown in Figure 12

This observation means that gains from optimization would mostly affect the small 3.3 h contribution from 5 µm pore-size filters and not change the much larger 57.2 h contribution from 0.1 µm pore-size filters, leaving the total process time of about 60 h almost unchanged.

Conclusions

Based on the literature review and all the tests run this semester, the WQMDP team concluded that membrane filtration is not a preferred option for pathogen sampling in Honduras because of time constraints, cost constraints, and contamination risks. As discussed in the **Results and Analysis** section, the projected time required to filter 10 L of water using the membrane filtration system is about 60 h of continuous processing. This length of time is not feasible for practical use in Honduras, especially when compared to the 1 h to 3 h processing time of the Richardson Lab's ultra-filtration system. With regard to cost constraints, 0.1 µm pore-size membranes, 5 µm pore-size membranes, and ultra-filtration cartridges cost \$2, \$1.15, and \$10 each, respectively . Given the estimated numbers of membrane filters required to filter 10 L of water, the membrane filtration process is projected to cost \$217.15 per run. In contrast, the Richardson Lab's ultra-filtration process, which uses 1 ultra-filtration cartridge and 10 0.1 µm pore-size filters, would cost only \$20.00 per run (See Table 2)(Richardson, 2017). With regard

to contamination, since the membrane filtration process requires a projected total of 118 membranes to process 10 L of water, the filter housing would have to be opened more than 118 times for each 10 L run. Even a very careful operator would be at risk for contaminating the apparatus or themselves on one of the 118 filter membrane changeovers. The ultra-filtration process, on the other hand, requires only 6 total set-ups, one for the ultra-filtration cartridge and five for membrane filters, which means a much lower chance of contamination.

These drawbacks suggest that membrane filters are not a viable substitute for the dialysis cartridge. This membrane filtration method is still planned to be conducted in Honduras in this January. Although the developed protocols and manuals will not be used for pathogen monitoring, the polished manual and ProCoDA methods files are now available for future use for other AguaClara sub-teams. Additionally, the WQMDP team's literature review findings about DNA preservation chemicals and shipping methods may serve as a starting point for AguaClara sub-teams working on pathogen sampling campaigns in the future.

Future Work

Since this semester's WQMDP team concluded that pathogen collection using membrane filtration is less feasible than ultra-filtration collection methods, the team will explore other options for water quality monitoring for diarrheal pathogens next semester. One idea is to improve current technologies, including the compartment bag test and ultra-filtration. In the literature review section, compartment bag test (CBT) was mentioned as a simple way to detect FIB in the field. It would be valuable to have a CBT kit and training manual prepared in advance for future sampling campaigns that will happen at prospective AguaClara plant sites in Honduras and other countries. For ultra-filtration, Professor Richardson has highlighted the need for a more concise and intuitive written manual which the WQMDP team could help to write. These step-by-step instructions and tutorial videos would help future teams to take field measurements. Also, Professor Richardson expressed that the results from tangential methods of ultra-filtration, which collect biomass in a retentate bottle have not been satisfactory, and would now like to shift to dead-end methods, which collect biomass in the filter cartridge itself (Francy et al., 2013). Next semester's WQMDP team could work on developing procedures for dead-end ultra-filtration.

Regardless of which filtration technology is selected, another option for future work is testing different methods of pumping water through the filters. As discussed in the **Literature Review** section, using peristaltic lab pumps in the field is not always possible, so alternative methods may be important to explore, especially if AguaClara makes pathogen detection a normal part of plant quality control. Of particular interest are retrofitted bike pump and hand drill peristaltic pump options. However, these pumping methods might be of less interest to AguaClara as a whole than the aforementioned work on compartment bag test and ultra-filtration procedures. The collaboration with Zamorano University in Honduras reduces the need for electricity-free sample processing methods, because the university has pumps and centrifuges available on their campus in Tegucigalpa.

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Semester Schedule

Task Map

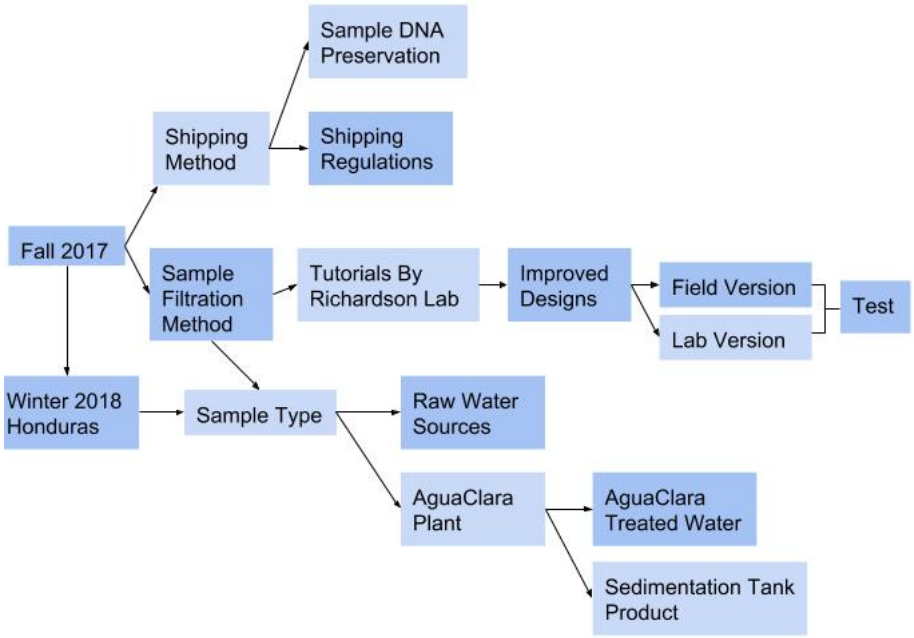

Figure 14: Task Map Fall 2017

Task List

1. ✓Check domestic/international shipping regulations (9/22/2017) - Ji Young. Learn if there are specific regulations for bringing in water samples back to the lab
   (a) Toxic chemicals
   (b) Pathogens/Raw water
   (c) Quantity of liquid and ice
2. ✓Explore sample DNA preservation methods (9/12/2017) - Fletcher. Research if there are ways to kill the pathogens safely to bring back.
   (a) Freeze-dry and autoclave
   (b) Other DNA preservation technologies
3. ✓No-shipping or heavy pre-processing option (9/15/2017) - Steven. Do as much processing and analyzing as possible at Zamorano University. Reach out to Zamorano to explore possibility of doing as much analysis as possible there.
   (a) Erika Alejandra Tenorio Moncada ¡etenorio@zamorano.edu¿
   (b) What to send Zamorano team : protocol or prototype of our setup or specific equipment
   (c) Existing facilities and equipments at Zamorano
   (d) Collaborative work specifics: Sharing technology and funding issues
   (e) Shipping method and cost within Honduras
4. ✓Explore filtration options (9/22/2017) - All. With help with Richardson Lab, find out which experimental setup the team is going to work on. Candidates are pre-filtration, multi-stage filtration, and ultra-filtration.

(a) Tutorial by Richardson Lab (Orville)

(b) Read about membrane filtration protocol used in Richardson Lab

(c) Check on available experimental devices, such as filter housings and filter membranes. If in need, order them.

5. ✓Tackle clogging issue (11/01/2017) - All. There will be clogging issue with filtration.

   (a) Pre-filtration or Multi-stage filtration

   (b) If filter housing needs to be replaced to another size, develop a python file for related calculation.

   (c) If there is no filter housing of capacity for our experiment, explore 3D-printing option.

   (d) Settling of sample water before filtration.

6. Develop sampling protocol (11/20/2017) - All. Visit local surface water sources, such as buttermilk falls, cayuga river, fall creek, and cascadilla creek, and create a detailed protocol for taking 10L raw water sample.

7. ✓Develop field version of filtration protocol - Fletcher. Find a way to replace a peristaltic pump with a hand drill or a bike pump and to run filtration without proCoDA.

8. ✓Write a ProCoDA file for sample filtration (11/30/2017) - Jiyoung. Write a ProCoDA file - set points and rules- for sample filtration.

   (a) Set pressure threshold to prevent explosion of the experimental setup. The pump has to stop when the pre-filter pressure reaches the threshold

   (b) Find optimal pump speed or make the system to find and adjust to the optimal pump speed.

   (c) Monitor effluent turbidity to check if the filter(s) fail.

   (d) Organize the logs in excel files.

9. Train Clare O'Connor who is going on the Honduras trip in January how to process the sample and pack the sample for the flight travel (12/13/2017) - Steven.

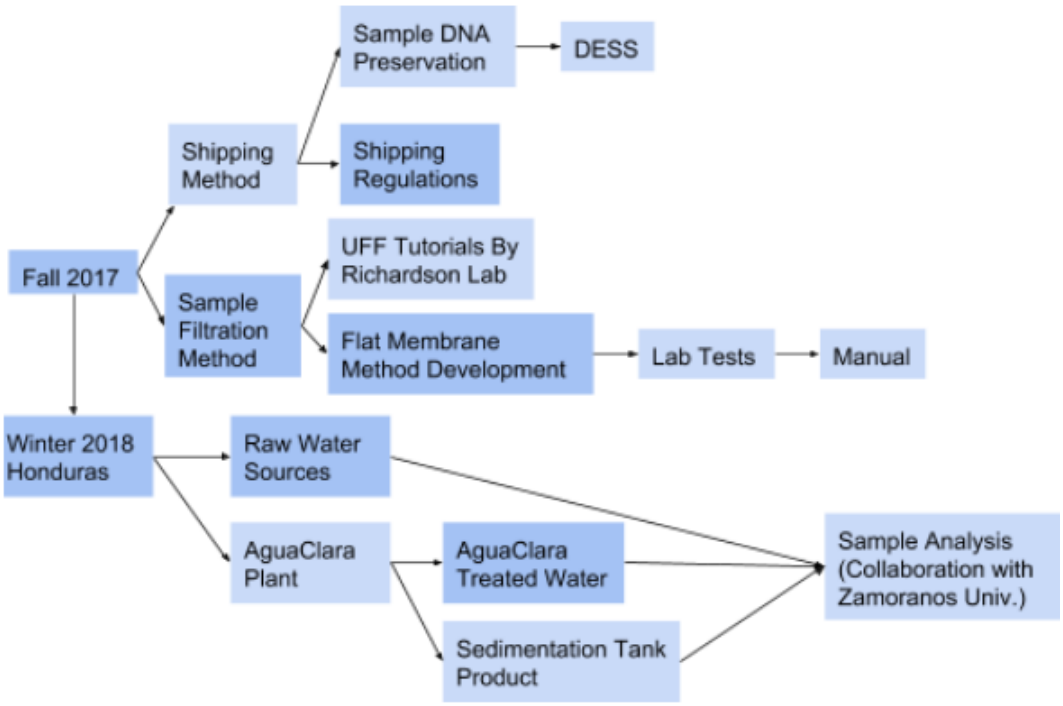

Figure 15: Revised Task Map Fall 2017

Report Proofreader: Ji Young Kim

Manual

Experimental Methods

Filter Membrane Clogging Test

For membrane clogging pressure over time trials with a single filter housing and membrane, given that the apparatus is already set up, repeat the following for every trial:

1. Remove the new 47 mm diameter filter membrane from its packaging. Make sure to take the membrane itself, which should look like and have the texture of a sheet of plastic, rather than the packaging material, which should look like and have the texture of a slightly blue sheet of paper. For these trials, the 0.1 µm pore-size filters used were Millipore Isopore VCTP membrane filters, and the 5 µm pore-size filters used were Poretics PCTE membrane filters.
2. Twist open the filter housing and insert the new filter membrane by placing it on the inner face of the downstream side of the PALL Life Sciences 47mm in-line filter housing. Make sure that the filter membrane is centered on the black mesh, then place the o-ring on top of the filter membrane. The o-ring should fit snugly into the downstream side of the filter housing. Then screw the upstream and downstream sides of the filter housing together.
3. Prime the filter housing by the following steps:
    - Remove the air plug from the upstream side of the filter housing
    - Run the peristaltic pump on the upstream side of the filter housing until all the air is pushed out of the upstream tubing and the upstream side of the filter housing
    - Replace the air plug on the upstream side of the filter housing and shut off the upstream peristaltic pump
4. Disengage the upstream peristaltic pump from the upstream tubing, and engage the downstream peristaltic pump with the downstream tubing.
5. Turn on the ProCoDA logging feature, and let it record the initial pressures for a few seconds
6. Begin running the downstream peristaltic pump, it should continue running for the remainder of the trial
7. Once the trial is complete, shut off the downstream peristaltic pump
8. After waiting a few seconds for the pressure to return to equilibrium, shut off the ProCoDA logging feature

For membrane clogging pressure over time trials with two filter housings and membranes, use the same procedure as for trials with a single filter housing and membrane, except for the following changes:

- Select the upstream and downstream filter membranes one at a time and place each in their respective filter housings, not at the same time. The membrane with the larger pore size would go before the one with the smaller pore size. This process avoids confusion, because filter membranes of all pore sizes look remarkably similar.
- Prime both filter housings using the priming method detailed in the single filter housing procedure, as follows. This avoids pumping water through the filter membranes until both filter housings are in place:
    - Prime the downstream filter using the single filter housing priming procedure, then cap the primed filter housing and tubing with the first filter housing.
    - Prime the upstream filter housing using the single filter housing priming procedure.

Multi-Membrane Volume Throughput Test

For membrane clogging pressure over time trials with a single filter housing and multiple membranes. This procedure is suitable for influent water with turbidity greater than 5 NTU:

1. Remove the new 47 mm diameter 5 µm pore size filter membrane from its packaging. For these trials, the 0.1 µm pore-size filters used were Millipore Isopore VCTP membrane filters, and the 5 µm pore-size filters used were Poretics PCTE membrane filters.
2. Twist open the filter housing and insert the new filter membrane by placing it on the inner face of the downstream side of the PALL Life Sciences 47mm in-line filter housing. Make sure that the filter membrane is centered on the black mesh, then place the o-ring on top of the filter membrane. The o-ring should fit snugly into the downstream side of the filter housing. Then screw the upstream and downstream sides of the filter housing together.
3. Prime the filter housing by
    - Removing the air plug from the upstream side of the filter housing
    - Running the peristaltic pump on the upstream side of the filter housing until all the air is pushed out of the upstream tubing and the upstream side of the filter housing
    - Replacing the air plug on the upstream side of the filter housing and shutting off the upstream peristaltic pump
4. Disengage the upstream peristaltic pump from the upstream tubing, and engage the downstream peristaltic pump with the downstream tubing.
5. Turn on the ProCoDA logging feature, and let it record the initial pressures for a few seconds
6. Begin running the downstream peristaltic pump, it should continue running for the remainder of the trial
7. Once the filter clogs, shut off the downstream peristaltic pump
8. Twist open the filter housing and replace the membrane with a new one with same pore size.
9. Repeat previous steps until filtering all of the prepared influent water. Collect the filtered water in a clean container
10. After a final run using 5 µm membrane, begin filtering the collected filtered water using 0.1 µm membrane.
11. Repeat the process as long as the time permits, or the influent water batch runs out.
12. Once the trial is complete, shut off the downstream peristaltic pump
13. After waiting a few seconds for the pressure to return to equilibrium, shut off the ProCoDA logging feature

Experimental Checklist

- Pump direction - Pushing water against the membrane is not a good idea. Always pull water through the membrane from the effluent side.
- Filter Membrane - Carefully read the packaging and check which circular piece of material is membrane and which is spacing paper.
- Pressure Sensors - Check which port has which pressure sensor. Make sure the location of the pressure sensor lines up with the label on ProCoDA. For example, check if a post-filter pressure sensor on ProCoDA is actually located after the filter housing. Also, check whether the sensors are reading realistic values prior to logging the experiment.

ProCoDA Method File

The WQMDP Fa17 method file can be retrieved at the WQMDP team page on AguaClara wiki. Descriptions of several components are shown below:

States

There are three states define in this method file:

- OFF - Resting state of ProCoDA. All sensors, relays, and pumps are turned off.
- Run Pump - Starting the experiment at manual state. All sensors, electronic balance, and pump are turned on.

Once the method file is successfully loaded, look for process operation window as shown below:

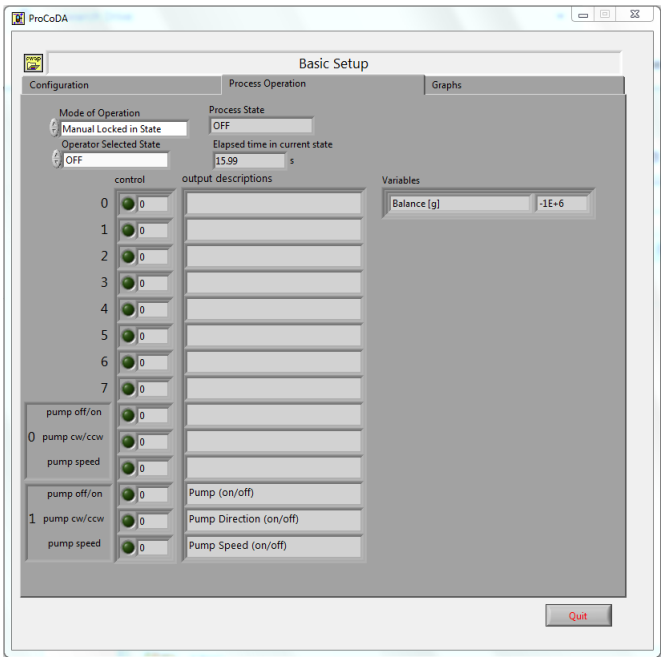

Figure 16: Process Operation Window

When setting up the pump, go to 'edit rule' tab, and modify outputs of the state, 'Run Pump', to fit the need of the experiment.

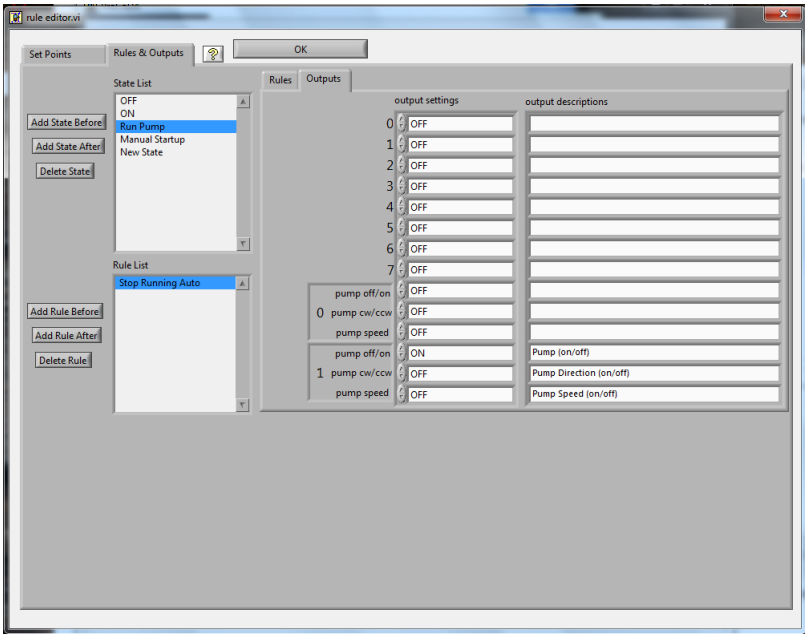

Figure 17: Run Pump Output

In this semester, a rule named **'Stop Running Auto'** was embedded in 'Run Pump' state. As shown in Figure 18, the rule turn the current state 'Run Pump' to 'OFF' when the '200 kPa Post-Filter' pressure sensor reading is less or equal to 'Pressure Threshold' set point value.

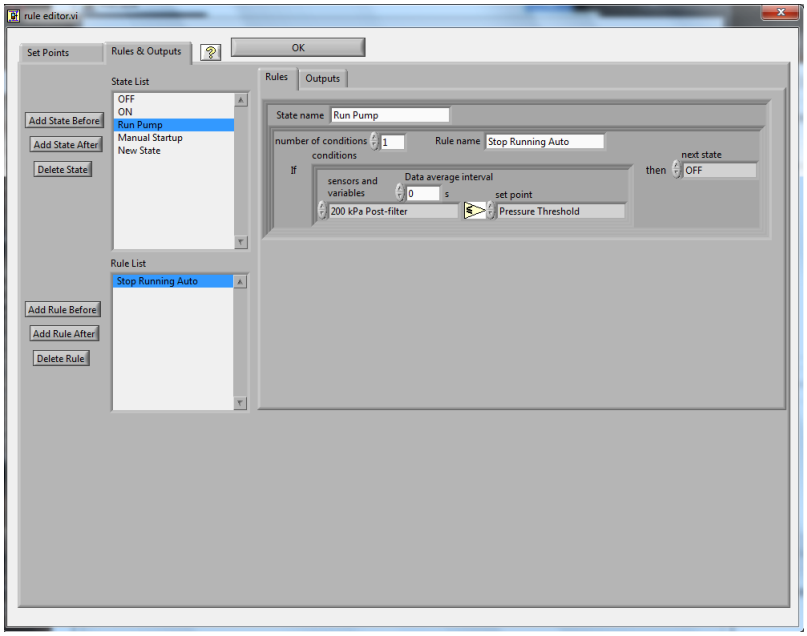

Figure 18: Rule: Stop Running Auto

Set Points

Below are the set points used in WQMDP FA17 method file and how each was calculated.

- Pressure Threshold - This set point is used for the rule "Stop Running Auto". When the input from the post-filter pressure sensor reaches a value below this set point value, all sensors, electronic balance, and pump are turned off.

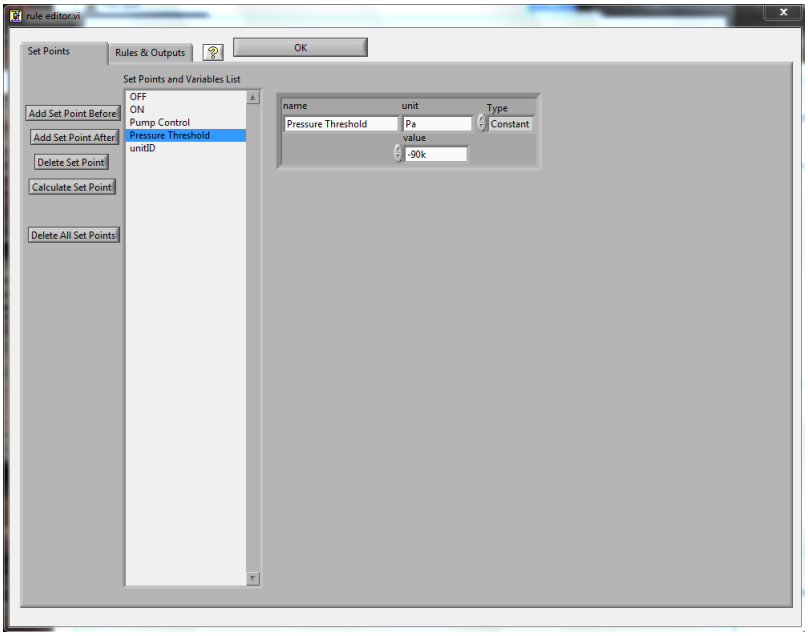

Figure 19: Set Point: Pressure Threshold

To get utilize the set point and the rule, make sure to have pressure sensors connected to ProCoDA. Click on 'Calibrator' or 'Volts' button, and add sensors. Match with correct port number, and name the sensors with correct names. The sensors can be calibrated on this window.

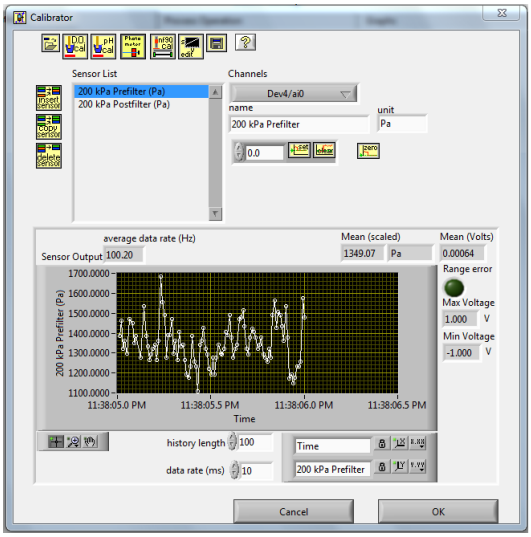

Figure 20: Pressure Sensor Calibration

- Balance - This set point is used for calculating flow rate. A Ohaus Scout Pro electronic balance connected to ProCoDA, gives the input.

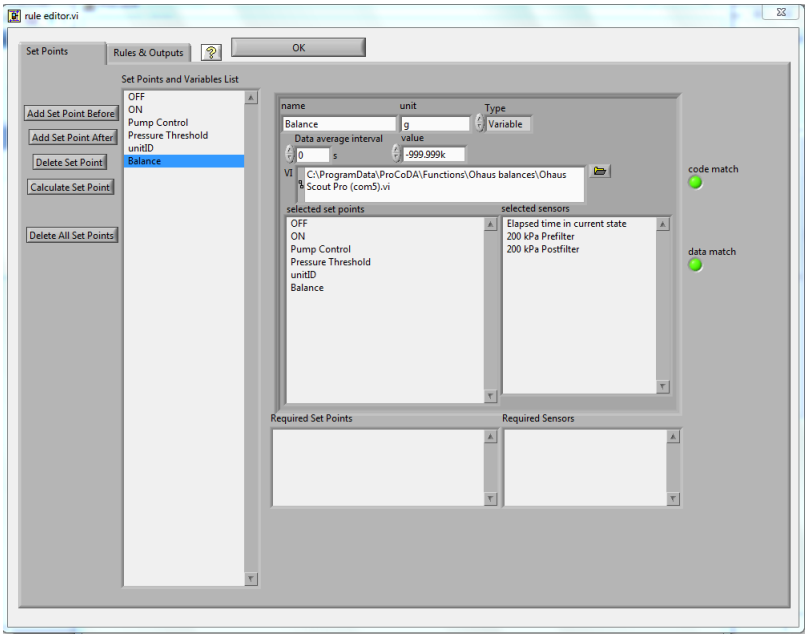

Figure 21: Set Point: Balance

Click on button with the folder diagram to find the method file for the balance. Make sure the file has the correct model name. Try all the com numbers (com1 to com6), or go to windows setting to find at which USB port is connected with the balance.

Special Components

Detailed Product Names for Experimental Equipment

- Isopore TM Membrane Filters, Filter Type: 0.1 um VCTP, REF no: VCTP04700, LOT no: R7EA97095
- Osmonic INC, Poretics, Polycarbonate, Black, 5.0 Micorn, 47 mm, Catalog No . K50BP04700
- PALL Life Sciences, 47 mm In-Line Filter Holder