

Identification of a Testing Method for BLUElab Designed Filtration Systems

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EXECUTIVE SUMMARY

This report contains a detailed investigation of water testing methods for purification systems designed by BLUElab. BLUElab is a student-run organization at the University of Michigan dedicated to improving the quality of life for people around the world using engineering. They have designed a number of water purification systems for residents of developing countries. Although these systems can be shown to work when constructed in the United States, a number of factors can lead to failures of the purification processes when built onsite. These factors include less than ideal materials and large variations in skilled labor in these nations. A test is therefore required to ensure that those who rely on these purification systems are safe. This test is especially important when the filtration process must be used to purify water for visitors to the developing country.

For our test to be useful for BLUElab, it must meet the following criteria. First, our design should be able to test for fecal coliforms, the main contaminant found in the water of many developing countries. Second, the test should also be portable so it can be performed on location, and be moved to various other locations for testing. Third, students, teachers, and BLUElab members should also be able to perform this test on their own with limited training. Finally, this test should be able to be completed within a day and be accurate enough to the point that if a low bacteria count is given, the water is safe to drink. This is to give those testing the samples onsite sufficient time to procure new water if the current supply is contaminated.

We investigated two main testing methods. Heterotrophic plate count tests the number of bacteria in a water sample by collecting bacteria onto a surface and allowing them to grow over a long period of time. The bacteria form colonies. By counting these colonies the total bacteria concentration can be estimated. This method is accurate, but not practical for onsite testing in a developing country because it requires at least two days to obtain results.

ATP Bioluminescence is a quick and accurate process to test the bacteria count in a sample of water. The process consists of placing the water into a container called a Filtravette™. A number of solutions are applied to lyse various cells and leave only bacterial ATP in the sample water. Another solution is then added to cause the bioluminescence and the sample is then placed in a micro luminometer to read the amount of bacteria.

We recommend the use of ATP Bioluminescence. To verify that this test met our design criteria we conducted a number of prototype tests. These experiments confirmed the accuracy, speed, and ease of bioluminescence as a testing method. These tests also helped to verify the robust purification system that we recommend. Bioluminescence should be implemented by BLUElab for use throughout the world to test purification systems. This testing method should also help BLUElab find the best water sources in areas of high contamination.

A few challenges remain before ATP Bioluminescence can be implemented by BLUElab. Current micro luminometers require a small amount of electricity to function. Electricity is not available in most developing countries. We recommend that battery packs be used to power the micro luminometers, or that a future group designs a small solar-cell recharge system to power the device.

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1 INTRODUCTION

When devising a method for purifying water, a procedure for testing the water is necessary to ensure drinkability. Methods of testing for bacteria in water can be used through out the world for finding suitable sources of drinking water, preventing biological warfare, and testing water purification systems. The best testing methods are quick, accurate, and able to be conducted in the field. Field-testing can greatly improve the quality of drinking water in third-world countries. We have found the testing method of ATP Bioluminescence using a microluminometer to be the best method for testing both contaminated and purified water.

BLUElab, a University of Michigan student organization, is involved with many water purification projects through out the world. They have done worked with water purification in Hagley Gap, Jamaica, and in Malawi, Africa. Many various methods of water purification have been proposed. We recommend the use of a clay pot filter to remove turbidity and bacteria, followed by boiling the water in a solar oven for added robustness. This system ensures that water will be drinkable upon both visual inspection and bacteria count tests.

This report presents our inspection, through both literature review and prototype testing of which system is best for testing bacteria count in water. A thorough recommendation of a robust purification process will also be presented in this report.

2 BACKGROUND

The following section gives background on BLUElab and why water purification is needed.

2.1 BLUElab

BLUElab is a University of Michigan organization dedicated to improving the quality of life for people throughout the world by coming up with solutions to developmental problems. They work on a variety of projects from the Hagley Gap Bridge in Jamaica to outreach projects in Detroit. BLUElab is working together with Leapfrog Partners to help create a water filter and solar oven for orphan care centers in Malawi (BLUElab, 2008).

2.2 Need for water test

The conditions that make a water contamination testing method necessary are described below.

2.2.1 Prevention of biological warfare

Methods of testing water for bacteria are needed through out the world. In an interview, Dr. Deininger of the School of Public Health told us that a large motivation behind developing methods of testing water for bacteria is to prevent biological warfare. If an almost real-time

testing method is in place at water treatment plants, harmful contaminants can be detected before the water is consumed.

2.2.2 Testing purification systems

Many proposed water purification systems that BLUElab deals with are designed to work with materials that may not be available in the country where the system will be used. Some proposed methods of water purification use Moringa Tree seeds, clay pot filters, boiling, and bio-sand filters. These processes are described in-depth in Appendices E, F, G, and H. Once filters are actually set up in the country where they will be used, their effectiveness must be tested. If the filters fail, the health of many people may be at stake. A reliable testing method is needed so that relevant, accurate results can ensure drinkability of water in many different applications throughout the world.

3 DESIGN CRITERIA

We compiled the following criteria based on our literature review and interviews.

3.1 Test should work for fecal coliform and other water born pathogens

A useful water contamination test should be able to measure the amounts of fecal coliform and water born pathogens in a sample of water (Appendix Q). A quantitative reading of the number of harmful contaminants must be given by the test so a determination can be made about whether water is safe to drink or not.

3.2 Test should be portable

A water test that can be performed in the field is most useful. For this to be possible, the testing unit should be portable. Portability is enhanced by small size and low weight. The unit should also be able to be powered by batteries, generate its own power, or not require electricity. This increases the ability for the testing unit to be used in rural areas.

3.3 Test should be able to be administered by U of M students in BLUElab

BLUElab is made up of undergraduate and graduate students. Therefore, the water test should only require a minimal amount training so that people who are not professionals in the area of water contamination can use it. A simple user interface allows for the test to be easily used throughout the world and by many different people (BLUElab, 2008).

3.4 Test should be accurate

A good water contamination test should give accurate results. The health of many people may rely on the results of the test. Accurate measures of bacteria count are required to determine

plate at 35 degrees Celsius and let it incubate for 48 hours. The second method is to store the plate between 20 and 28 degrees Celsius and let the bacteria grow for five to seven days. This method generally produces small, compact colonies that are very easy to count. On the other hand, in this method the bacteria grow underwater, causing a slow growth and problems transferring the colonies. Also, introducing agar at the high heats needed for this method may cause heat shock in the bacteria, thus possibly marring the results (Canadian Drinking Water Quality, 2006).

4.1.3 Spread Plate Method

The Spread Plate Method uses the same incubation process as the Pour Plate Method; otherwise, two methods are very different. The Spread Plate Method uses a solid agar, thus removing the problem of heat shock. Because this method uses a solid agar, the sample water must be absorbed into the agar, therefore samples must be between .1 and .5 mL. The colonies here are also easily transferrable and their colony morphology, or form, is easily distinguishable (Canadian Drinking Water Quality, 2006).

4.1.4 Membrane Filtration Method

Membrane Filtration has its advantages and disadvantages. For this process, water is run through a filter with 0.45- μ m pores. The bacteria get trapped within this filter and the filter is then placed onto the culture media. This also removes the problem of heat shock, but since the colonies need to grow in the small filter pores, the viewing surface is much smaller. The individual colonies are, therefore, harder to count. In this method it is also possible for colonies to get damaged from too much pressure from the filtration, but this method allows for large amounts of water to be examined at once (Canadian Drinking Water Quality, 2006).

4.2 TESTING FOR BACTERIA USING ADENOSINE TRIPHOSPHATE BIOLUMINESCENCE

ATP Bioluminescence is a method of testing for the amount of bacteria in water, and will be elaborated upon in this section.

4.2.1 Finding our Testing Method

We initially contacted the Environmental and Water Resources Engineering (EWRE) department and emailed and called various people. We contacted Dr. Rolf Deininger, a retired professor from the University Of Michigan, School Of Public Health. Dr. Deininger, along with JiYoung Lee, Assistant Professor of Bioengineering, Ohio State University, developed a method of testing for bacteria with ATP bioluminescence. He helped us obtain contaminated water to test and supplied us with a testing kit.

4.2.2 Background of testing method

ATP bioluminescence is a process that finds the bacteria population in a sample of water within minutes and can be performed almost anywhere. This method of testing is a great improvement over other methods that use an ATP assay. Other methods using an ATP assay require more than one hour, over one liter of water, and have a high sensitivity of 100,000 cells. Sensitivity is the minimum amount of cells that need to be in the sample for the test to detect them. The new method of bioluminescence is over 100 times more sensitive and much more efficient than any other method currently in use (Deininger, 2005).

4.2.3 Microluminometer and bacteria count

A microluminometer is a small, portable device that records the light emission from the ATP of living samples over a 10-second interval and integrates the light impulses. The result is output as relative light unit per milliliter (RLU/ml), which gives an accurate count of bacterial ATP in the sampled water. The amount of ATP is directly proportional to the number of bacteria; therefore, the number output is the amount of bacteria in the sample (Deininger, 2005).

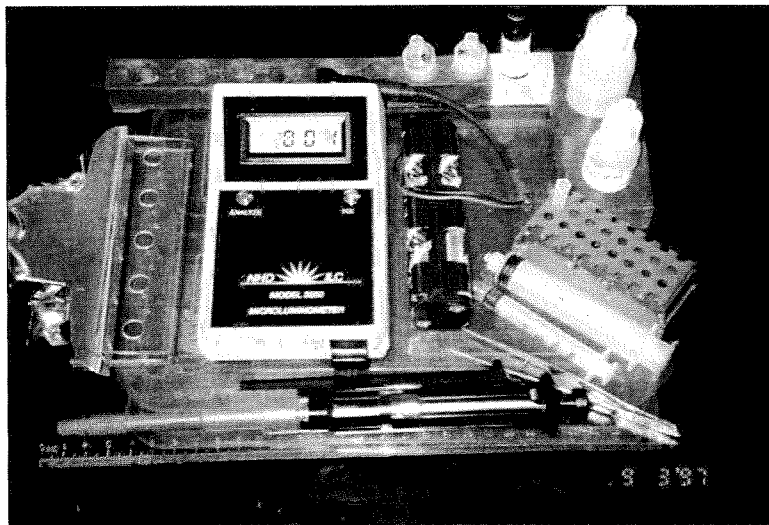


Figure 1: Picture of the testing kit courtesy of Dr. Deininger

4.2.4 SRA, BRA, and Luciferin-Luciferase Application

A somatic cell releasing agent (SRA) is mixed into the sample of water and lyses all non-bacterial cells and releases their ATP. Air pressure is then used to remove the non-bacterial ATP so only bacteria remains in the Filtravette™, which is a combination of a cuvette and a filter with a pore size of 0.45 microns. The bacterial ATP is still inside the bacteria itself, so the Filtravette™ is placed into the microluminometer and the bacterial cell-releasing agent (BRA) is added to lyse the bacterial cells and release their ATP. Therefore, only bacterial ATP is left in

6

the Filtravette™. Next, 50 µL of luciferin-luciferase is mixed into the sample water that is in the Filtravette™, and the drawer of the microluminometer is closed, which starts the test (Deininger, 2001).

4.2.5 Luciferin-luciferase

Luciferin-luciferase is a combination of two chemicals that react and cause bioluminescence. Luciferin is a solution that sensitizes, or induces the condition of hypersensitivity, when UV light in the range of 310–390 nm is applied. With histidine, an amino acid, or dithiothreitol, a redox reagent, as a substrate, a type II photo-oxidation occurs. Then an ATP-driven luciferin-luciferase reaction occurs (Steveninck, Boegheim, Dubbelman, 1986). In this reaction, luciferase uses the ATP of a living sample to activate the luciferin, and the resulting product combines with oxygen on the molecular level. This now becomes an excited-state of the luciferin-luciferase called oxyluciferin. When oxyluciferin relaxes back to its ground state, it releases energy in the form of light, resulting in bioluminescence (Pepling, 2008).

5 Selection of Method

This section compares ATP Bioluminescence and Heterotrophic Plate Count on the basis of our criteria established in Section 3.

5.1 Method should work on fecal coliforms

Many pathogens that are tested for with the HPC test are not in the coliform group. A high HPC has actually been shown to hinder the determination of coliforms in tests. The ATP test is less accurate but it was built to be a quick assessment of possible contamination of the water and tests for all types of bacteria. Both of the tests somewhat meet this criterion, and both would be applicable tests for the water in Malawi.

5.2 Method should be implementable on-site

The HPC test needs to be performed under strict temperature requirements, which would be very hard to maintain in Malawi considering there isn't electricity in the areas under question. Therefore, this test would not be able to be performed onsite.

ATP Bioluminescence can be run off of a small tray almost anywhere. The only requirement to run it is that it needs a power source for the short time it takes the microluminometer to count the bacteria. This could be accomplished by a small generator or a solar panel.

5.3 Method should be able to be used with training

A standard method of testing has been developed and agreed upon, but varying test conditions lead to different results. Testing temperature can range from 20 to 40 degrees Celsius and the

6 PROTOTYPE TESTING PROTOCOL

We conducted three prototype tests over a period of two days. These tests accomplished two main goals. They confirmed the effectiveness of both boiling and bio-sand filtration as methods for water purification. Additionally, they showed that two undergraduate engineers could successfully determine the bacterial concentration of multiple water samples using bioluminescence.

6.1 Ann Arbor Waste Water Plant: 1st Boiling Test

Our first prototype test used water from the Ann Arbor Waste Water Treatment Plant to simulate the water found in developing countries.

6.1.1 Method for Obtaining Water

To obtain our sample of contaminated water, Dr. Deininger set up an appointment for us to visit the Ann Arbor Waste Water Plant. We took samples of the water from the secondary effluent, which is after the point where all the sludge has been removed, but most of the bacteria still remain. This water was recommended to us by numerous individuals within the School of Public health to serve as a model for drinking water found in developing nations.

6.1.2 1st Test

Our original intent for the first test was to demonstrate the effectiveness of boiling as a method for purifying water contaminated with fecal matter. Therefore, we devised the following experimental procedure.

- 1.) Obtain materials
 - a. Styrofoam cups
 - b. Standard pot (See Appendix J.2) and stove
 - c. Microluminometer, pipette tips, necessary solutions
 - d. Timer and Notebook
 - e. Contaminated water
- 2.) Prepare Test equipment
 - a. Preheat the stove to simulate the cooking surface inside a solar oven
 - b. Label Styrofoam cups 0-10
 - c. Plug in microluminometer
 - d. Pour water into pot
- 3.) Purify sample
 - a. Place pot on stovetop and start timer
 - b. Remove sample from surface of boiling water at 1 minute intervals
 - c. Allow to cool

- 4.) Test sample
 - a. Place fresh Filtravette™ into microluminometer
 - b. Obtain new tip for pipette
 - c. Pipette 200 μL of water from cup into Filtravette™
 - d. Pipette 50 μL of Luciferin Luciferase into Filtravette™
 - e. Stir contents of Filtravette™ using micropipette
 - f. Push Filtravette™ into microluminometer
 - g. Record output
 - h. Repeat for all other samples

- 5.) Establish baseline
 - a. Test a sample to known purity to use as a basis for comparison

6.1.3 Results

The results from our first boiling test can be seen below. A complete table of the experimental data for this test can be seen in Appendix P. To provide a basis for comparison a value of 500 RLU/mL is the maximum allowable bacteria count in the US. Additionally, Ann Arbor drinking water measured 115 RLU/mL.

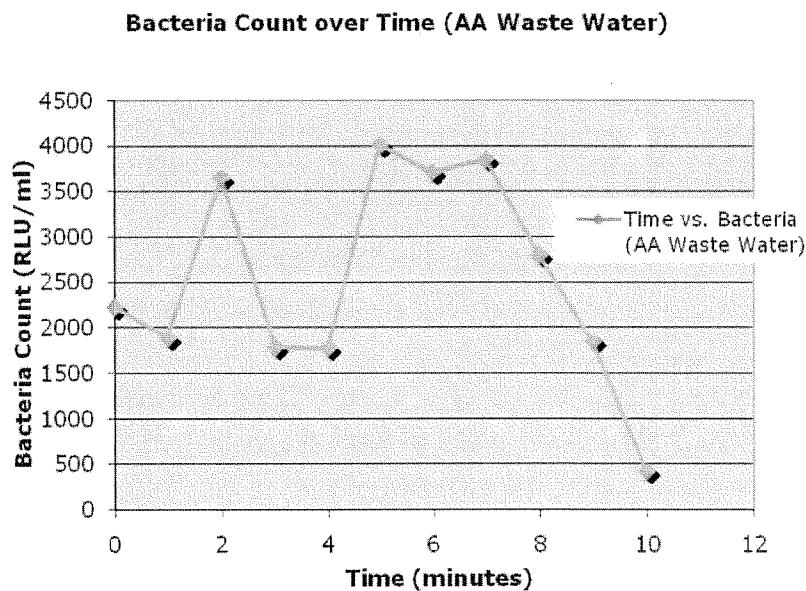


Figure 2: Results from 1st boiling test

For the samples from the first five minutes of heating, we noticed that the count of bacteria did not follow any pattern. From the sample at time of two minutes, we measured the bacteria count to be 3,650 bacteria per milliliter, which was much higher than the initial count of bacteria in the raw water. At time of three minutes, however, the water had 1,785 bacteria per milliliter, which

was almost 2,000 less than test one minute before. When the water began to boil, which was around five minutes after heating started, the bacteria count began to steadily decrease. The count went from a maximum of 4,010 bacteria per milliliter at five minutes to 420 bacteria per milliliter at ten minutes.

6.1.4 Lessons Learned from 1st boiling test

Bacteria Settling in Contaminated Water

We realized following our first test that we had failed to include a few critical steps in our procedure. Most importantly, we had forgotten to shake our water sample before boiling. Shaking the contaminated water ensures that the bacteria is evenly distributed throughout because as the water sits the bacteria settles towards the bottom of the water. Shaking the sample gives a somewhat more accurate account of the actual amount of bacteria in the source.

Convection

Another possible source of error for our results may be convection. Convection causes the bacteria to circulate, and could unevenly spread the bacteria, which may have resulted in the major fluctuations of our samples. It is also possible that as time goes on, the bacteria has evened out in the sample because of the convection, seen in the steady decrease of bacteria as the water starts to actually boil. To counter this error, stirring the water while it is being boiled in the pot would most likely make the results more accurate.

6.2 2nd Boiling Test and Bio-sand Water from Team Enigma

This section describes the second boiling test, during which water from Team Enigma's bio-sand filter was tested.

6.2.1 Method for Obtaining Water

For our second test and third prototype test we collaborated with Team Enigma, a team from our section constructing a prototype of a past bio-sand Filter design. For more information on bio-sand filters and Team Enigma see Appendix E. They provided us with approximately one gallon of water from the Huron River. Huron river water was also widely recommended by our contacts within the School of Public health for its high bacteria count. More importantly, they gave us a sample of Huron River water that had been filtered that day using their bio-sand filter. This allowed us to quantitatively compare our experimentally measured effectiveness for their filter to the effectiveness of similar bio-sand filters found in peer-reviewed literature.

6.2.2 Test

We used almost the same testing method outlined in Section 6.1.2 for our second boiling test; however, this time we were careful to stir the water samples throughout the boiling and testing process. Testing of the bio-sand filtered water required only steps 1 and 4 in the procedure.

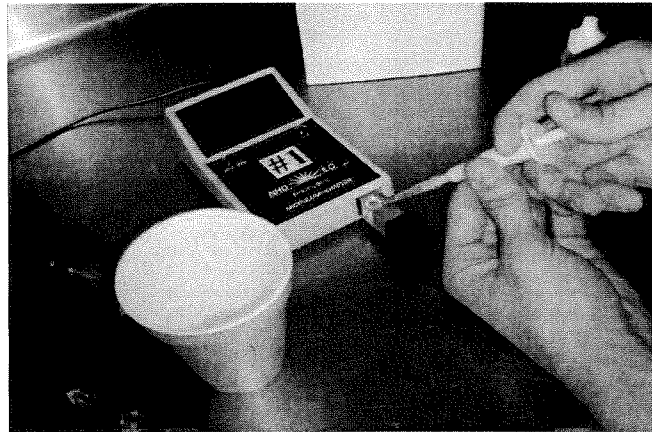


Figure 3: Testing of a purified sample

6.2.3 Results

The results from our second boiling test can be seen below. A complete table of the experimental data for this test can be seen in Appendix P. To provide a basis for comparison a value of 500 RLU/mL is the maximum allowable bacteria count in the US. Additionally, Ann Arbor drinking water measured 115 RLU/mL.

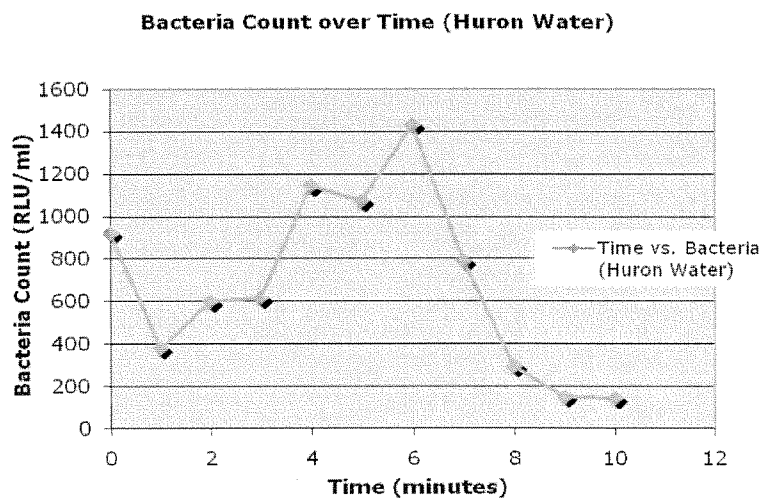


Figure 4: Results from the Second Boiling Test

Second Boiling Test Results

For our second test, in which we tested the water straight from the Huron River, we shook up our sample water before testing evenly spread the bacteria. We still ended up receiving strange results but the deviation from the raw water was not as high. From this data we determined that shaking the water helps improve the accuracy of the data, but other errors must be present.

Additionally, during the second boiling test we obtained a bacteria count of 144 RLU/mL after ten minutes. This was impressive because it approached value of 115 RLU/mL seen in water purified professionally by the Ann Arbor Waste Water Treatment plant.

Results for Team Enigma

Our most significant results came from our testing of the filtered water given to us by Team Enigma. Before filtration, the Huron River water registered 921 RLU/mL. However, after traveling through the bio-sand of Team Enigma's prototype only 244 RLU/mL remained. This is less than half of the maximum concentration allowed by the United States.

6.3 Prototype Conclusion

From this data we have determined that if the water is boiled in a solar oven that meets the specifications given by Mt. Hillox's design, the contaminated water in Malawi can be made safe for the volunteer teachers to drink. We also concluded that ATP bioluminescence is a very accurate, quick and efficient way to obtain the bacteria count of water and can be very valuable to improving the quality of life in Malawi and around the world.

7 USE IN MALAWI

Malawi is a developing country in Southern Africa facing problems from many different causes such as disease, poverty and lack of resources (Appendix A).

7.1 Importance of Water Purification

The AIDS epidemic has plagued much of Africa. In Malawi this epidemic kills thousands of people each year, has lowered the average age to 16 years old, and has decreased the average life expectancy to 42 years. (CIA, 2008) Currently, Malawi has a population of about 13.5 million people (CIA The World Fact Book). Of this 13.5 million, approximately 1 million are orphans. This high number of orphans is the result of the AIDS epidemic in Malawi. Many of the adults who are killed by this disease leave behind their children, causing the number of orphans in Malawi to increase rapidly. It is estimated that nearly 500,000 (Mt. Hillox Final Report, 2007) of the 1 million orphans in Malawi come from families whose parents have died from AIDS.

Due to the high number of orphans, orphan care centers are being established. These care centers provide education and food for the orphans during the day. The orphan care centers are not orphanages since the children usually live with extended family; however, many families rely on the services provided by the orphan care centers.

The water in Malawi is very poor in quality and contains many different types of bacteria and protozoa. The main contaminant of the water is human fecal matter. The large amount of contamination makes drinking the water dangerous for those who are not from the area. Much of

the drinking water in Malawi also has a cloudy appearance, known as turbidity. Since the volunteer teachers at the orphan care centers are not from Malawi, they are not immune to the contaminated drinking water. If they drink the water they could become very ill. Since the water contamination causes this problem, a water purification system is needed to protect the health of the teachers. Although the locals are immune to the water, they could also benefit from clean drinking water, if the contaminated water can be purified in large enough volumes. (Science Direct, 2007)

7.2 Method of Purification

To filter the contaminated water in Malawi, we decided to use a clay turbidity filter and a solar oven in sequence (Appendix B).

7.2.1 Turbidity Filter and Boiling

A clay filter (Appendix G) should be used to remove turbidity from the water before it is boiled. Water in Malawi is highly contaminated with bacteria from fecal matter, but it also contains other material such as dirt. The boiling process alone would make the water containing dirt safe to drink, but the appearance and visual debris would be undesirable for the teachers. By removing the turbidity from the water, the teachers can feel more comfortable drinking our water and therefore will not risk dehydration. We recommend the use of a porous colloidal clay filter, which is available in the region through Potters for Peace. If this filter cannot be obtained, any turbidity filter can be used because of the robustness of our process.

Some bacteria will still remain in the water after the turbidity has been filtered out. Boiling this contaminated water will kill the remaining bacteria. Temperature is fatal to any bacteria even if they are too small to be removed by a conventional filter. The filtered and boiled water is suitable for drinking by the teachers at the orphan care centers. This clean water can be used immediately or stored for later use.

7.2.2 Robustness

Our purification process is robust due to the redundancy in removing bacteria. The porous colloidal clay filter removes many bacteria, but the boiling process ensures the drinkability of the water.

8 CONCLUSION

Through literature review and several prototype tests, we have proven bioluminescence with a micro luminometer to be the best method for testing water contamination. This process is fast and accurate. It also requires very little training and can be conducted in the field. BLUElab can use this testing system throughout the world as they work on projects that concern water contamination and purification.

BLUElab can specifically apply the bioluminescence testing process in Malawi, Africa. The drinking water in Malawi is unsafe for visitors and must be purified. BLUElab can use bioluminescence to gather the information necessary to analyze which filtration process is best for purifying the drinking water for volunteer teachers in Malawi. We have specifically recommended a robust process of filtration, which first uses a clay filter to remove turbidity and then kill all bacteria by boiling the water in a solar oven. We have used bioluminescence to prove that this process is effective, and we recommend that further work be done to implement the systems we have used for purification and contamination testing.

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