

Entry into the Stockholm Junior Water Prize 2011

Development and Evaluation of a Microfluidic Co-Flow
Device to Determine Water-Quality

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I. Abstract: It was hypothesized that by combining co-flow microfluidic devices, cell-phones, and the bacteria growth chemical Colilert-18, a novel way of determining water qualities may be found. The device allows mixing of Colilert-18, a chemical that becomes yellow in the presence of coliform bacteria and a water sample in a single channel. This channel is photographed and analyzed by a cell-phone. A statistically significant positive correlation between bacteria concentration (coliform and E. coli) and yellow pixel intensity was found. A formula can be derived to assess bacteria concentration based on the distance between the initial mixing and the complete mixing points in the channel.

The device was tested in two ways, in the laboratory setting with known quantities of bacteria and in the field setting with unknown concentrations of bacteria. Additionally, mathematical and computer modeling of experimental tests successfully predicted the device’s performance. In comparison the new device is faster, more mobile, and the results are as accurate as the standard bacteria tests.

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III. Key Words:

Potability	Co-flow
Microfluidic	Water Quality
E. coli and Coliform Bacteria	Colilert-18
Cell-phone	

IV. Abbreviations and Acronyms:

COMSOL
AutoCAD
MATLAB

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VI. Biography: Alison Bick, 17, of Short Hills, sought a low-cost, portable and publicly accessible method for testing water potability. Concerned by the threat of contaminated drinking water in her community, Alison worked for over four years researching and developing several devices to accurately test water for inorganic materials and harmful bacteria. Alison attends Millburn High School, where she competes on the varsity fencing, track and cross country teams. She is a certified sailing instructor.

1. Introduction: Availability of water is perhaps the most essential factor in determining where humans can live, grow food, and develop industry. The United Nations reports that nearly 5,000 children die each day due to a lack of clean water [1]. Additionally, with the rise of terrorism, attempts to contaminate municipal drinking water supplies are more likely than ever. Public water treatment facilities are only effective against some infectious replicating agents, as few biotoxins are inactivated by chlorination [2]. Even the popular press has expressed concerns about the availability of water and water quality tests, as evidenced by a recent series of New York Times articles [3]. Natural disasters also give rise to the need for a system and method of water quality testing that is easy to use, inexpensive, yields quick and accurate results, and is available to the public. In Third World countries, such a device could save millions of lives and billions of dollars [4]. Cholera, which is caused by the bacteria *Vibrio cholerae*, and *E. coli*, are highly correlated to poor water quality [5]. The devices described in this paper are immediately useful in Haiti or other disaster areas for preventing death and

reducing suffering. In First World countries, this device could enable people to self-assess water quality in the event of a natural disaster or terrorist attack.

Therefore, there is an urgent need for a faster, simpler, low-cost, real-time device suitable for water testing throughout the world for reasons of health and national security. Standard water quality tests include the following: Mardel ® Test Strips (Sergeant’s Pet Care Products, Inc., Omaha, NE) which tests for pH, ammonia, and alkalinity; Vernier LabPro® (Venier Software & Technology LLC, Beaverton, OR) with appropriate probes which tests for dissolved oxygen; and Colilert-18 (Idexx Labs, Westbrook, MA) fifteen test tube test, which tests for coliform bacteria and E. coli. Commercially available tests for water quality are often subject to human errors, chemical reaction failures, and mechanical calibration failures [6]. Additionally, most water quality testing equipment is unavailable to the general public due to its technical operation and high cost. In many situations, these shortcomings result in unnecessarily high costs, slow results, and inaccurate information.

In addition to the standard laboratory tests noted above, water quality has been measured in more creative ways. Aerial photography has been used to determine water pollution in catfish ponds [6]. Luminescence may determine dissolved oxygen levels in surface water [7]. Lastly, pattern recognition is used for water quality assessment [8]. The present work uses a combination of these three elements: photography, luminescence, and pattern recognition, as well as microfluidic co-flow technology, to create two distinct devices to determine alkalinity, ammonia, dissolved oxygen, turbidity, and pH water qualities, and coliform bacteria and E. coli bacteria concentrations.

Initial Detection Devices: From 2007 to 2009, work was carried out to combine photography, luminescence, and pattern recognition in order to determine water quality. As a result, a series of water quality assessment devices were developed which have overcome many of the aforementioned shortcomings. In particular, the initial device was comprised of a computer, camera and shoe box whose interior was painted with fluorescent and phosphorescent paint (Device A). Subsequently, a cell-phone

was used to replace the computer and camera (Device B). Figure 1 shows images of Device A and Device B. These efforts produced devices with sufficient ease of use



and range for the general public, but lacked sufficient sensitivity with respect to bacterial concentrations. Devices A and B could only accurately detect inorganic water qualities and required custom made elements, such as the fluorescent and phosphorescent painted shoe box. The two devices could not determine if water was potable.

Background - Developing a Microfluidic Approach: While testing water samples using standard coliform and E. coli tests to confirm the cell-phone device results, it was observed that when the chemical Colilert-18 is mixed with a water sample containing high levels of bacteria a momentary yellow color change occurred and then the water turned clear again. A similar momentary color change was observed at low bacteria concentrations. The higher the bacteria concentration, the darker the yellow color.

Device B demonstrated that cell-phones can effectively capture images and analyze their colors. This indicated that Device B could be useful to quantify the yellow intensity and relate it to concentration. Additionally, similar custom programs that were written for Device B could be used to analyze the water sample with the added chemical.

The color change event was short lived; after approximately five seconds the water would revert to its original color. A new device was needed to either slow this event or allow the event to occur over and over again in a serial manner in the same location so as to allow more observation time. It was hypothesized that a microfluidic co-flow device should allow such an event to occur.

Microfluidic devices are instruments that use very small amounts of fluid, only one or two drops, to perform certain laboratory tests. Co-flow refers to two streams that are brought into contact and then flow side by side down a channel allowing mixing.

Just as a strobe light or stroboscopic lamp is used to "stop" the motion of rotating and other repetitively operating machinery to allow scientific measurement, a microfluidic co-flow device can also "stop" the color change resulting from the interaction of bacteria and Colilert-18 which is flowing along a channel. This will allow for sufficient time to make experimental measurements.

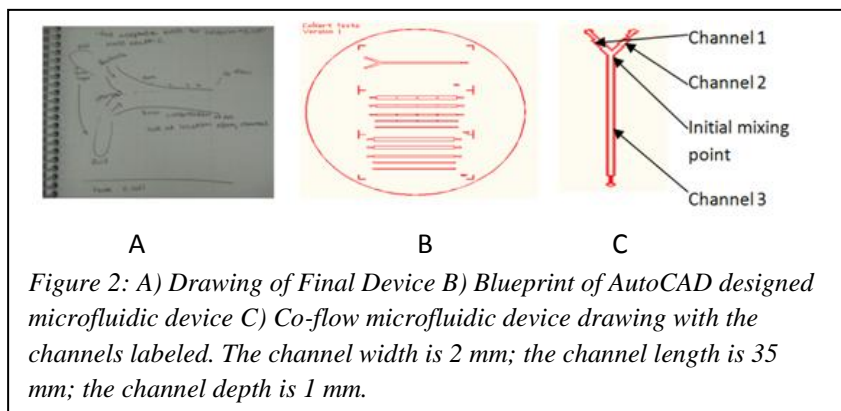
In particular, the strobe light uses regular flashes of light to deceive the human eye into seeing "frozen" motion. The microfluidic co-flow device lines up a set of continuously changing droplets of bacteria and Colilert-18 which deceives the human eye into seeing each stage of color change resulting from the interaction of bacteria and Colilert-18 frozen along the co-flow device channel.

It is known that microfluidic devices have shown the potential for bacteria analysis [10][11][12]. Consequently, it was hypothesized that by combining microfluidic devices with cell-phones and Colilert-18, a novel way of determining water qualities may be found.

It was also hypothesized that the microfluidic co-flow device would offer a faster, more flexible and less expensive method for detecting certain chemical reactions which produce momentary color change and determining the concentration of certain material suspended in fluids which exhibit color change. The use of the co-flow microfluidic device for determining water quality is just one of many possible applications.

Many chemical reactions and fluid mixing events produce temporary color changes. Every compound absorbs a characteristic set of colors of light. This absorption spectrum is a chemical fingerprint for detecting the presence of that compound. When the compound is altered in a chemical reaction, the chemical fingerprint will change and the color of the reacting mixture may change as the reaction progresses.

2. Procedure/Materials and Methods: The device consists of a cell-phone, a microfluidic device and the chemical, Colilert-18. The microfluidic device is composed of a glass microscope slide and polydimethylsiloxane (PDMS (Dow Corning Corporation, Midland, MI)). To design the co-flow microfluidic device, first the general characteristics of the device were identified and modeled using the computer program COMSOL Multiphysics Simulation Software (COMSOL, Inc., Burlington, MA).



These characteristics include the number of channels, the channels relative positions, and the approximate channel dimensions. Figure 2A shows the drawing which was the basis for the final device. Next, the drawing was used as a guideline for the program

AutoCAD (Autodesk, Inc. San Rafael, CA) to create an electronic blueprint suitable for use by the mask manufacturer (Stanford Microfluidics Foundry, Stanford, CA). The mask is made of a silicon wafer and used to produce an element of the microfluidic co-flow device, in accordance with the description above. Figure 2B shows the blueprint of the device generated by the AutoCAD program.

To create the device, the following procedure was used as illustrated by Figure 3. First, a convex pattern of the channels is etched into a silicon wafer, using standard methods [12], to create the mask.

Then 100 mL PDMS is mixed in a 10 to 1 ratio of PDMS base to PDMS curing agent. Next, the PDMS

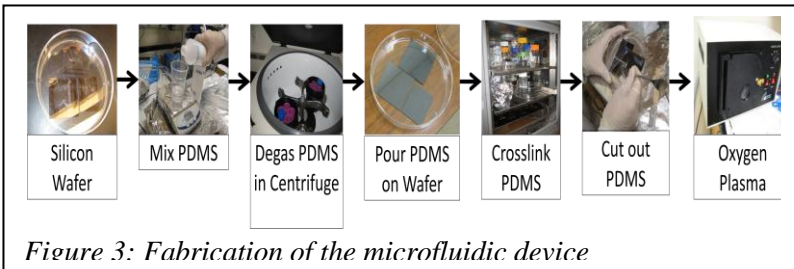


Figure 3: Fabrication of the microfluidic device

is degassed in the centrifuge for 60 minutes at 500 rpm. Then, the PDMS is poured onto the mask and placed in an oven at 450 ° F for three hours,

solidifying the PDMS. The PDMS is

taken out of the oven and, using a scalpel, the solidified PDMS is removed from the mask. The PDMS piece is put into the oxygen plasma machine (ValTEC Vacum Plasma Treating System, Lunderskov, Denmark) along with a glass microscope slide; this machine oxidizes both surfaces so they can be combined to create sealed channels. Finally, the concave pattern on the PDMS is combined with the flat surface of the glass slide, to make a channel. The channel is hydrophilic because of the plasma process which adds oxygen to the hydrophobic PDMS. The excess oxygen ions attract the water which makes it easier to pump water through the channels.

To use the device, one mixes 100 mL of distilled water with 1 packet of Colilert-18. Then, this solution is poured into one of the two channels of the microfluidic co-flow device while water that is to be tested is poured into the other channel. The liquids diffuse along the third channel which is formed when the two combined channels meet. Figure 2C is a drawing of the co-flow microfluidic device with the channels labeled. Once the third channel is full, a cell-phone containing a custom C sharp mobile phone application, developed as part of this project, is activated. This application acquires the image of this channel from a point directly over the center of the central channel and at a height of three to five inches above the plane of the device. The image is sent to a subroutine which analyses the channel and determines what point along the channel has the darkest color and correlates this with bacteria and E. coli concentrations. The subroutine is illustrated in an algorithm schematic below (Figure 4).

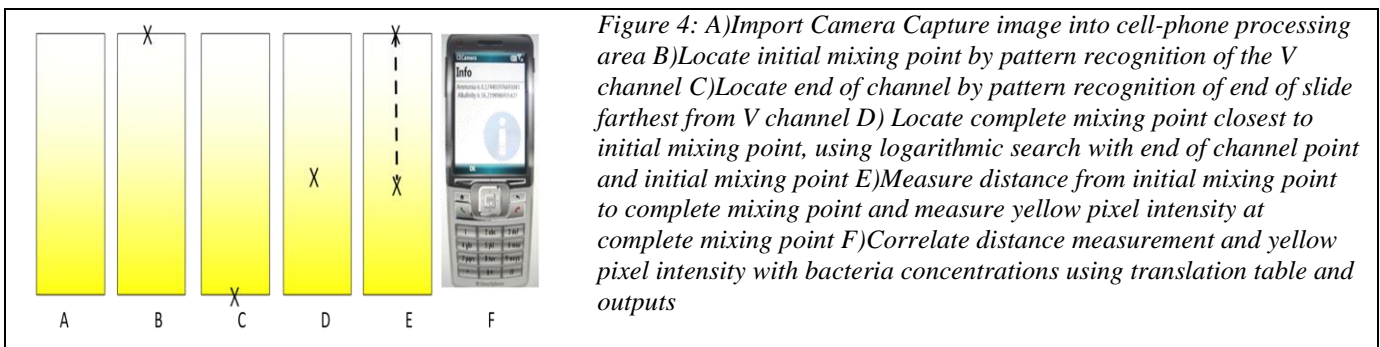
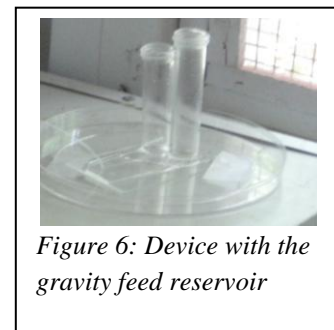
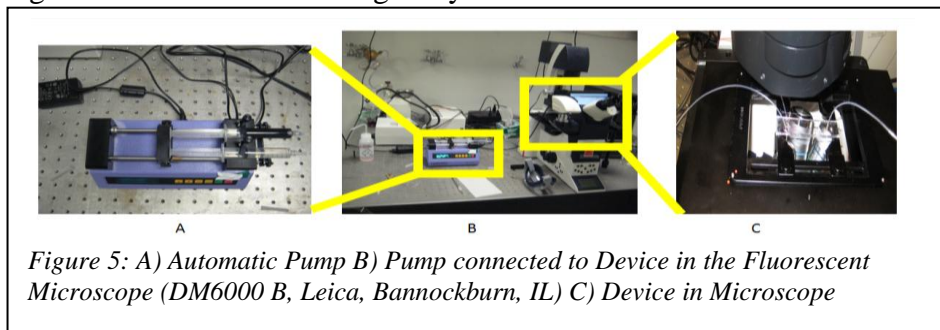
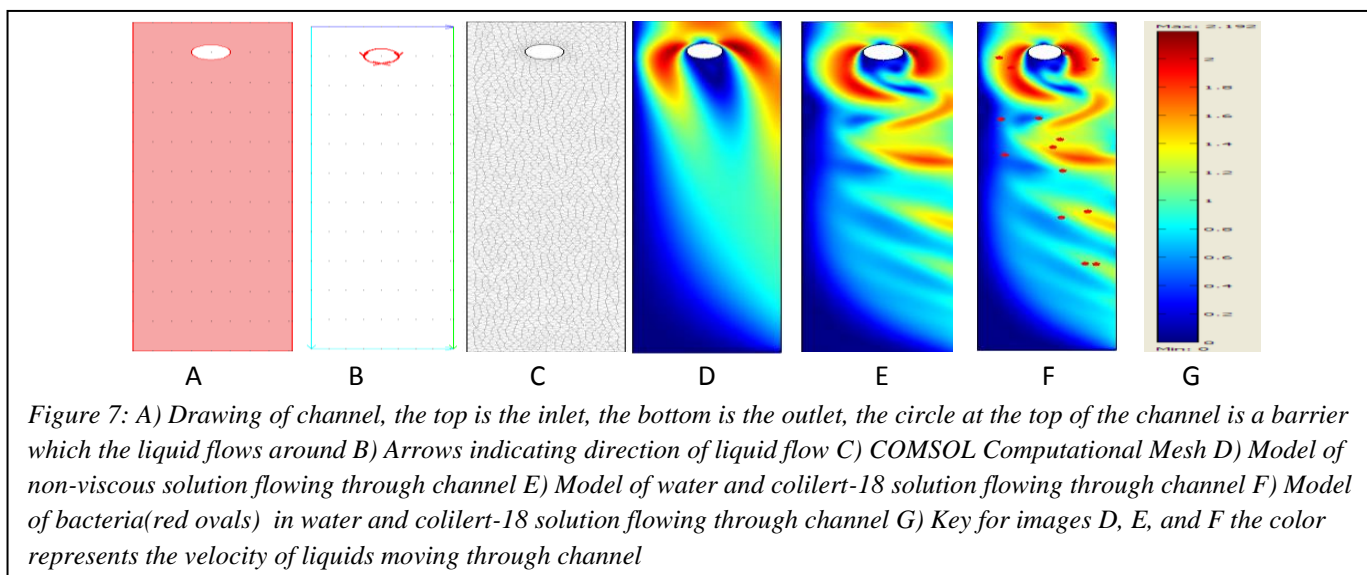


Figure 4: A) Import Camera Capture image into cell-phone processing area B) Locate initial mixing point by pattern recognition of the V channel C) Locate end of channel by pattern recognition of end of slide farthest from V channel D) Locate complete mixing point closest to initial mixing point, using logarithmic search with end of channel point and initial mixing point E) Measure distance from initial mixing point to complete mixing point and measure yellow pixel intensity at complete mixing point F) Correlate distance measurement and yellow pixel intensity with bacteria concentrations using translation table and outputs

Finally, the device was tested using known samples of *E. coli* bacteria, a type of coliform bacteria, in the laboratory setting. Automatic electric pumps (Harvard Apparatus, Harvard, MA) were used to input the solutions into the two channels. Figure 5 shows the automatic pump. After that, the device was tested in a field setting with an unknown amount of bacteria in pond water. For these tests, gravity feed reservoirs were built due to the lack of access to automatic pumps in the field setting. Figure 6 is a device with the gravity feed reservoir.



3. Results: This research resulted in ten significant results. First, several co-flow devices were conceived and designed. Using mathematical analysis and the computer modeling program COMSOL,



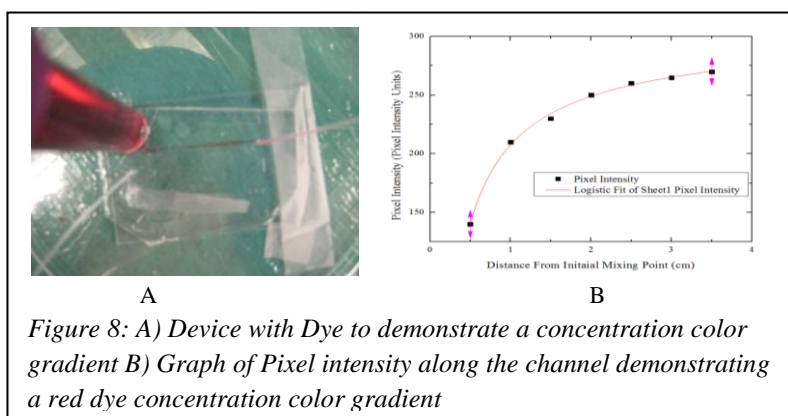
the device designs were simulated and optimized. It was designed so that the device could have 1) channels that were a sufficient size so bacteria could flow through them, 2) channel width that could be photographed with a cell-phone, 3) channels that were short enough so that the entire time to create a concentration color gradient only took five seconds, 4) channels that were long enough to allow sufficient mixing, and 5) channels that were thin enough so that PDMS on top of the channel would not

collapse into the channel. Rather than trial and error, mathematical techniques were used to satisfy these criteria. In particular, the Hagen-Poiseuille equation was used: $P = \frac{8Q\mu L}{\pi R^4}$, where ΔP is the pressure drop, Q is the volumetric flow rate, μ is the viscosity, L is the channel length, and R is the channel radius.

Having narrowed the possibilities, COMSOL was then used to model the possible solutions and determine the optimum one. Using the program, the ideal device would be 2 mm wide, 6 cm long, and 1 mm high. Figure 7 shows models that were generated by the COMSOL program using specifications mentioned above.

The co-flow device that was made could create a constant color gradient. This finding was demonstrated using Shaw's Food Coloring (97% water, 2.9% propylene glycol, 1% Yellow 5, 0.1 % Propylparaben, Shaw's Scan Code 45674 514). Figure 8A shows a constant color gradient created with

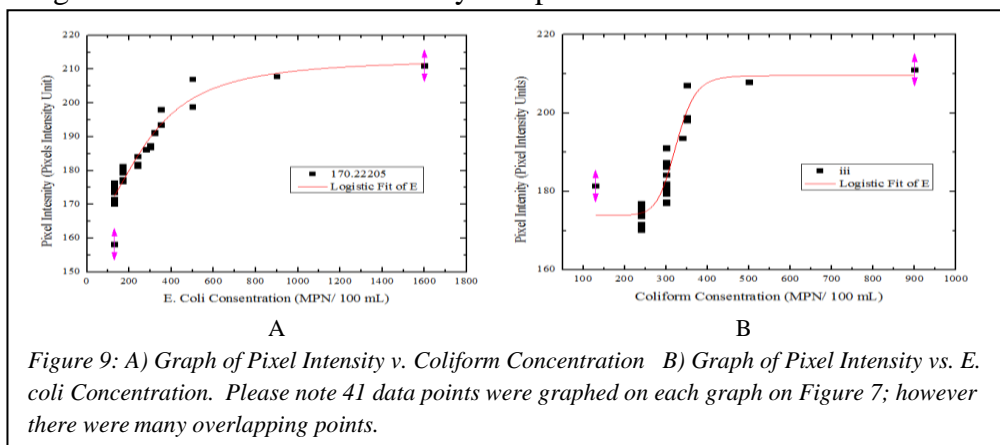
food coloring and water. Figure 8B



graphs the mixing along the channel. The co-flow device was of sufficient size so coliform and E. coli bacteria could pass through the channels. This was tested by running 10 micron beads (Microbeads Spheromers® CA10) through the

channel and observing them under the microscope. This test confirmed that bacteria sized objects would mix and flow through the channel.

There is a statistically significant positive correlation between bacteria concentration (coliform and E. coli) and yellow pixel intensity. This was determined by taking a picture of the mixing channel, using MATLAB to find the intensity of a point on the third channel that is 3 cm from the initial mixing

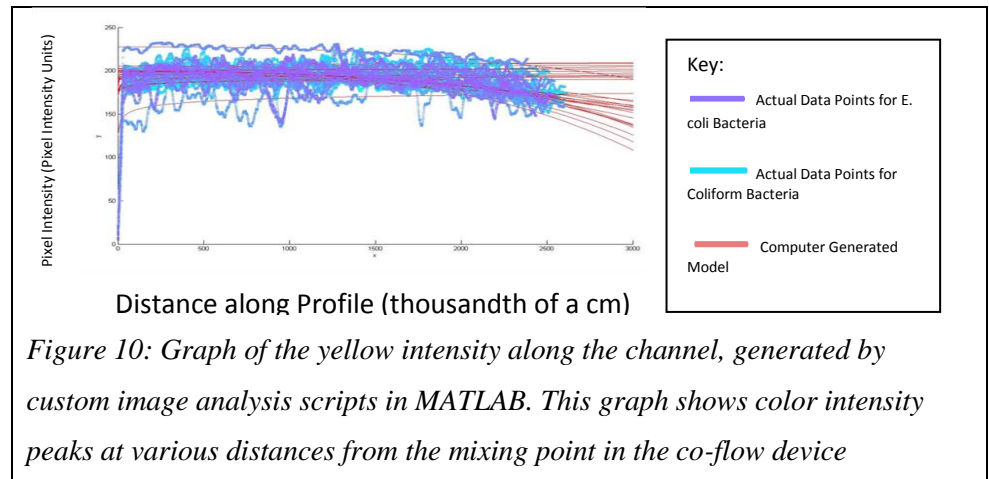


point and graphing that pixel intensity against the bacteria concentration determined by traditional means.

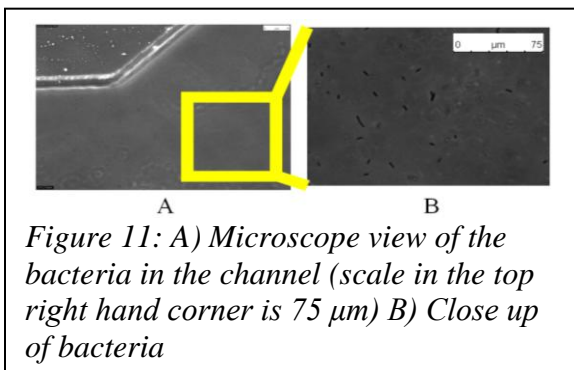
Figure 9A and 9B graph these trends for coliform bacteria and E. coli bacteria concentrations respectively. The best fit line for both coliform bacteria and E. coli bacteria data points is a logarithmic shape. The R² value for Figure 9A is 0.7139. The R² value for Figure 9B is 0.8852. R² values in this instance means that from 71 percent to nearly 89 percent of the variance of the dependent variable was explained, which suggests the device is useful.

It was found that the higher the bacteria concentration in the sample, the closer the total mixing point is to the initial mixing point. In particular, for 160 MPN/ 100 ML, the total mixing point was approximately 1 cm along the channel whereas for 1 MPN/ 100 ML the total mixing point was approximately 3.5 cm along the channel. This result was for coliform bacteria, results for other bacteria may vary.

A formula can be derived to assess bacteria concentration based on the distance between the initial mixing point and the complete mixing point. The relationship appears to be a concave line with a positive slope (see Figure 10).



Additional tests were done on the devices using gravity feed reservoirs (See Figure 5), instead of automatic pumps (see Figure 4). The gravity feed reservoirs were designed to have the same flow rate as the pumps, which was between .0035 mL/ minute and .0018 mL/ minute. Both the gravity feed reservoirs and the automatic pumps created the same stable concentration gradients, as predicted using Bernoulli's equation, $p + \frac{1}{2}\rho V^2 + \rho gh = \text{constant}$, where p is pressure, ρ is the density, V is the velocity, h is elevation, and g is the gravitational acceleration.



The device was tested in two ways. First, the device was used to predict samples with known concentrations of bacteria in the laboratory setting. Figure 11 shows a microscopic view of the bacteria in the channel. The device was able to accurately determine the

coliform bacteria concentration from the known samples with a 99.8% confidence level. The device was able to accurately determine the E. coli bacteria concentration from the known samples with a 95.0% confidence level. A chi-squared test was used to analyze the results because the measurement of

Water Quality	Degrees of Freedom	χ^2 Value	Probability
Coliform Bacteria	40	69.70	.998
E. coli Bacteria	40	5.76	.950

Table 1: Table of χ^2 Calculations for data collected in the lab with known quantities of bacteria

variance was of interest. Table 1 shows the chi-squared calculations for data collected in the laboratory.

Upon demonstrating that

the device worked on known concentrations of bacteria, field tests were conducted with unknown concentrations of bacteria. In particular the device was used to predict bacteria concentrations from a pond in Cape Cod, MA, and those predictions were compared with the results from the same pond water assessed using traditional bacteria tests, specifically Colilert-18. The field test included testing multiple samples of pond water for 43 days during a period of six weeks during July and August 2010 using the industry standard chemical, Colilert-18, to test for coliform bacteria and E. coli. Colilert-18 is a chemical that is mixed with water that is to be tested for coliform bacteria and E. coli. If the solution turns yellow after 18 hours of incubation, the test indicates there is a presence of coliform bacteria and E. coli in the solution. If the solution is diluted with distilled water in the ratio of 1:0, 1:10, and 1:100 then incubated for 18 hours, the bacteria count can be determined.

For coliform bacteria the formula that was created had a 97.5 % confidence level. For E. coli

Water Quality	Degrees of Freedom	χ^2 Value	Probability
Coliform Bacteria	40	59.43	.975
E. coli Bacteria	40	51.81	.900

Table 2: Table of χ^2 Calculations for Data collected in the Field. It compares the results from the microfluidic device with the results of the traditional Colilert-18 test analyzing the same pond water samples.

bacteria, the formula that was created had a 90.0% confidence level. These results were based on the results from the field test. A chi-squared test was used to analyze the results, because the measurement of variance was also of

interest here. Table 2 shows the chi-squared calculations for data collected in the field.

The co-flow microfluidic devices were found to have the same sensitivity as the chemical Colilert-18 procedure. This finding is significant because Colilert-18 has been approved by the EPA for use in determining water potability [14]. The Colilert-18 upper limit of enumeration is 2,419 organisms for both coliform and E. coli, and the lower limit is 0 organisms for both [15].

It was found that the cost of standard water quality tests were at least \$20 per test where as the cost of the co-flow test is less than 20 cents per test. Thus the saving is more than two orders of magnitude. Table 3 shows the cost comparison between the two devices. Water tests and water test laboratory fees will vary depending on the lab chosen. Typical water test fees include a base collection

Bacteria Test	Co-Flow Costs	Lab Testing Cost
Total Coliform	\$0.08	\$20.00
Fecal Bacteria/ E. coli	\$0.08	\$20.00
Other Bacteria	\$0.20	\$50.00

Table 3: Cost Comparison Chart between Co-Flow Device and Lab Testing

fee plus the actual lab costs for the specific water tests performed. The cost below does not include any markup on the lab costs, such as profit or overhead [16]. The co-

flow device costs include a mask manufactured specifically for this study[17], material for making the device (PDMS, PDMS curing agent) and bacteria reaction agent (such as Colilert-18).

4. Discussion:

The results of the co-flow devices failed to disprove the hypothesis that a novel way of determining water quality may be found using microfluidic devices, cell-phones, and Colilert-18. The results included investigations concerning mathematical and computer modeling, experimental tests in the laboratory and field settings, and statistical analysis.

The co-flow device design is consistent with prior research and mathematical findings. In particular, the device design allowed a flow of between .0035 mL/ minute and .0018 mL/ minute. This information is important because in the event of a need for a change in rate due to bacteria change or a change in chemical, instead of Colilert-18, a new device can be designed quickly and inexpensively by using computer modeling or mathematic formulas.

A color gradient was formed in the co-flow devices tested. While this result is consistent with prior research which indicates that color concentration gradients can occur in co-flow devices [18], there is no research which indicates that Colilert-18 could form a color concentration gradient. This suggests that other bacteria growth media and identification mixtures might also form color concentration gradients suitable for use in water quality testing. An example of a mixture to be tested is brilliant green lactose bile broth [19].

It was found that the time for complete mixing to occur and the concentration of yellow observed are statistically positively correlated. This is true with a 99% confidence level. This indicates the higher the concentration of the bacteria, the less time it takes to achieve complete mixing and hence the maximum concentration of yellow color.

The higher the bacteria concentration in the sample, the closer to the initial mixing point the total mixing will occur and, hence, the maximum concentration of yellow color. This is significant because the higher the bacteria concentration, the shorter the microfluidic device channel needs to be. Thus the accuracy of the co-flow microfluidic device may depend upon the channel length. One must also conclude that the longer the channel the more accurate the device is for measuring low bacteria concentrations.

The high confidence level for the coliform and E. coli formulas are beneficial to the scientific community. This is because there is a new statistically significant way of determining water quality, at least for coliform bacteria. Additionally, this new method takes only a few seconds instead of 18 hours. Additionally, the accuracy of the co-flow microfluidic device is superior to the cell-phone mirror device. This satisfies the design objective for this independent research.

The results show that the use of gravity feed reservoirs and the automatic pumps are identical. However, the gravity feed reservoirs, unlike the automatic pumps allow testing in locations that do not have access to sufficient laboratory equipment. Additionally, the gravity feed reservoirs are a fraction of the cost of the laboratory pumps, and do not require setup time, calibration, and maintenance. They are easier to use and easier to transport.

When colored demarcations are added to the device, its use is improved outside the laboratory. Use of demarcations simplifies the process to determine if water is potable. With a single line device, the general public can simply determine if water is safe to drink (less than 1 MPN/ 100 mL). The double line device can be used to determine the level of bacteria in the water. Specifically, the two line device will help determine if water is dangerous, questionable, or safe to drink.

	Mobility (Based Equipment weight)	Accuracy (Quantifiable results)	Speed (Time to determine result)	Ease of determining results (Steps required to determine result)	Cost (Dollars)	Key + Benefit -Limitation = Same *** Each + or - represents an order of magnitude
Co-flow device with automatic pump	=	=	+++	+	=	
Co-flow device with gravity feed reservoirs	++	=	+++	+	+	
Single demarcation device	++	-	+++	++	+	
Double demarcation device	++	-	+++	++	+	

Table 3: Devices in comparison to colilert-18, traditional procedure

In comparison to the Colilert-18 bacteria test procedure, the new device offers one or more advantages and a few disadvantages in areas including mobility, accuracy, speed, ease of use, and cost. Table 3 details the advantages of each device in comparison to the traditional Colilert-18 tests.

5. Conclusions:

In the future, the co-flow devices will be tested at different laboratory locations and different field locations. The Red Cross (Millburn, NJ Chapter) is preparing to test the co-flow devices with non-laboratory users. Also, the use of staining and growth material other than Colilert-18 is being evaluated for use in the co-flow devices, to determine bacteria concentrations and additional water qualities. In particular, brilliant green lactose bile broth is among the chemicals to be tested [19].

Additionally, the potential use for microfluidic co-flow devices as a low cost, accurate, easy to use testing device is wide-ranging. The microfluidic co-flow device has the potential to be a food safety device or a product quality control device. The food industry uses ammonia to test the edibility of mushrooms. A drop of ammonia combined with a few mushroom spores in a microfluidic co-flow device will detect a change that identifies whether the mushroom is edible. The chemical industry uses sodium hydroxide solution to test for the presence of the pollutant phenolphthalein in some products. A drop of sodium hydroxide solution combined with a few drops of the test solution in a microfluidic co-flow device will detect the pink changes which not only identifies the pollutant but also the distance from the mixing point will determine the pollutant's concentration.

By combining microfluidics, cell-phones, and Colilert-18, a novel way of determining several water qualities has been found. The research indicates that two new water quality devices may be useful. For laboratory use, the co-flow device with the automatic pumps and cell-phone can be used to quickly and accurately determine coliform and E. coli concentrations in water. For non-laboratory use, the co-flow device with gravity feed reservoirs and demarcations can quickly and accurately determine if water is potable. These results may prove to be useful to those in need of rapid water quality assessment, which would certainly be useful to people experiencing the results of natural disasters or those dealing with the aftermath of man-made hostilities.

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