Point-of-Care Medical Tests Devices and their Value as Educational Projects for Engineering Students

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Abstract

Point of Care Medical Diagnostics devices are portable microfluidics-based systems that test for infectious diseases in clinical specimens such as blood. They provide fast, easily-interpreted test results in a low-cost format and do not require highly skilled operators. This technology is expected to play a prominent role in future healthcare, especially in resource-limited regions of the developing world.

We believe point of care diagnostics devices are excellent topics for student projects. The projects involve microfluidics, cell phone applications, image processing, optics, CAD/CAM and rapid prototyping, microcontrollers, sensors, C programming, instrumentation and process control, product development; and give engineering students exposure to important medical and biotechnology applications. For example, students have designed, constructed, and validated portable devices to test for viruses in blood samples, using a device that integrates a cellphone as a detector, controller, and communications port. This work provides ample opportunities for students to integrate their foundational knowledge and skills in an increasingly important enabling technology for sustainable healthcare.

Background

Point-of-care (POC) medical diagnostics refers to medical tests that can be performed outside of hospitals and centralized laboratories, such as doctors and dentists offices, day care centers and schools, neighborhood clinics, ships at sea, and even at home. POC medical diagnostics devices, or more generally, point of care tests (POCT) are likely to play an important role in future healthcare, especially in the developing world and other resource-limited settings where healthcare infrastructure is lacking. According to recent market surveys, the POC diagnostics global market is expected to reach $16.5 billion in 2016 [MONEGAIN 2014]. Applications include tests for infectious disease, glucose monitoring, blood chemistry, pregnancy and fertility, cardiac markers, cholesterol, drugs and alcohol, hemoglobin, tumor markers, and urine chemistry.

POC devices are typically comprised of a credit-card sized plastic single-use (disposable) cassette that hosts a microfluidic network of channels, conduits, filters, reaction and reagent chambers to process clinical specimens such as blood, saliva, urine, or environmental samples such as drinking water, food, air. The cassette or 'chip' is mated with a small, portable instrument that provides the cassette with controlled heating, fluidic actuation and flow control, and detection capabilities. Most commonly, the test result is determined by measuring an optical signal such as fluorescence. Ideally, the system is self-contained, can be operated by non-technical users, costs about $10 per test, and provides an easily-interpreted clinically-relevant test result in a time frame of one hour or less.

From the perspective of engineering education, POC technology offers many opportunities and vehicles for interdisciplinary, capstone projects. These systems involve the integration of prototyping methods, optics, electronics and microcontrollers, fluid mechanics, and temperature control. Further, they offer a gateway to biomedical areas for engineering students. The basis of these devices involve fairly straightforward concepts in microbiology, immunoassays, and molecular diagnostics. Engineering students can be taught the principles of detecting 'biomarkers' of disease or infection (i.e., pathogen-specific proteins, nucleic acids, and metabolites), as routinely practiced in medicine, agriculture, public health and hygiene, homeland security, and food safety. The chemistry (materials, methods, and protocols) are well-established. The objective is to translate technologies routinely used in laboratories to portable microfluidic systems. The students adapt a commercial test kit for laboratory use that typically relies on benchtop instruments such as controlled water baths for incubating samples, centrifuges, thermal cycler instruments, electrophoresis equipment, and fluorometers or spectrophotometers. A basic aim then is to remove the need for these instruments by using a microfluidics format.

Here, we describe a POC viral load test (i.e., measurent of the number of virus particles in a specified volume of blood) directed toward a convenient means to monitor HIV in patients undergoing anti-viral drug therapy. In brief, viral load needs to be measured every three to four months in order to verify the drug treatments that suppress the virus are still effective. These tests as currently performed in centralized laboratories and cost on the order of $100 per test. This is prohibitive in developing countries. **Table 1** compares conventional viral load tests as performed in developed countries in centralized medical labs to what is needed in the developing world. The objectives are challenging but

realistic. **Table 1** compares the features of current tests with those needed for POC tests in the developing world.

We should emphasize that it is not necessary for students to work with hazardous materials or medical samples in such a project. The commercial kits include benign positive control samples that simulate test material for purposes of such development work, but are completely safe. These materials are commonly used in high school and college student laboratories, and are available from a number of commercial suppliers.

This project offered Engineering Technology Senior Design students with a product development exercise for a highly useful device that can provide appropriate, sustainable healthcare to the developing world. The work reported here was the product of a three-student team over the three terms of their Senior Year. The basic microfluidic chip, design and protocol for application to viral load testing was the work of Dr. Chagchun Liu of the University of Pennsylvania. Dr. Liu served as a co-advisor for the project.

Table 1: Comparison of Current Viral Load Tests and What is Needed for the Developing World

	Now in Clinical Labs	Needed for the Developing World
Sample	1 milliliter of venous blood	0.1 ml of blood from finger prick
Test time	> 8 hours	about 1 hour
Instruments	>$100,000	< $1000
Operator skill level	advanced training in molecular biology lab techniques	1-2 days training
Test Environment	well-equipped lab with hoods, HVAC	anywhere
Power	mains electricity	battery
Reagent and sample	'cold chain' refrigeration	room-temperature storage

Figure 1 shows the steps needed to perform a viral load (or bacteria count) test. A sample, such as blood, urine, or a food sample is mixed with detergent and lysing agents. The lysed sample is then filtered through a silica glass fiber or cellulose filter to bind the nucleic acids in the sample. The nucleic acids are then eluted into a small chamber where they are subjected to pathogen specific enzymatic amplification which increases the DNA or RNA sequences of the virus or bacterial pathogen. We use polymerase chain reaction (PCR) or loop mediated amplification (LAMP) for amplifying the DNA. PCR requires precise, rapid thermal cycling, which makes for an interesting PID control problem with the Arduino microcontroller. LAMP requires constant temperature incubation (~65 C°), but the reagents are more expensive. A dye (SYTO Green® or EVO Green®) is included in the reaction that binds to DNA and fluoresces green when excited with blue light (e.g., from an LED). Thus, green fluorescence is an indicator of a positive test result (i.e., presence of a virus). The amplification reagents are pre-stored in the form of freeze dried pellets that are commercially available.

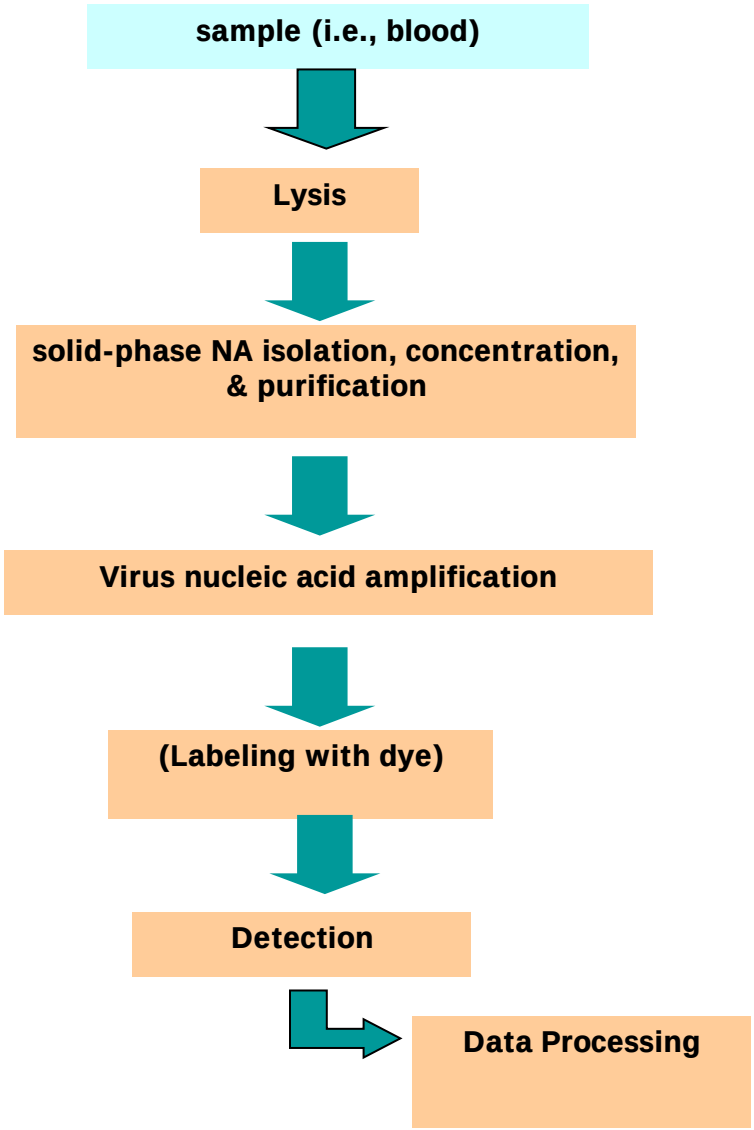

Figure 2: Process for testing for a virus in a clinical specimen.

Some examples of microfluidic cassettes ('chips') made by students are shown in **Figure 2**. The students designed the chips with SolidWorks® CAD software. The chips were made by CO2 laser cutting (Universal Laser Systems, 30 W). Sheets of acrylic plastic (0.1 to 1 mm thick) were patterned with microfluidic circuits and then bonded with adhesives or double-sided tape as a laminated structure. A 3-mm diameter silica glass fiber (Whatman GF) or cellulose (Whatman FTA) disc was embedded in the chip as a nucleic acid binding membrane. Details of the chip and fabrication process are available in XXXX [2014].

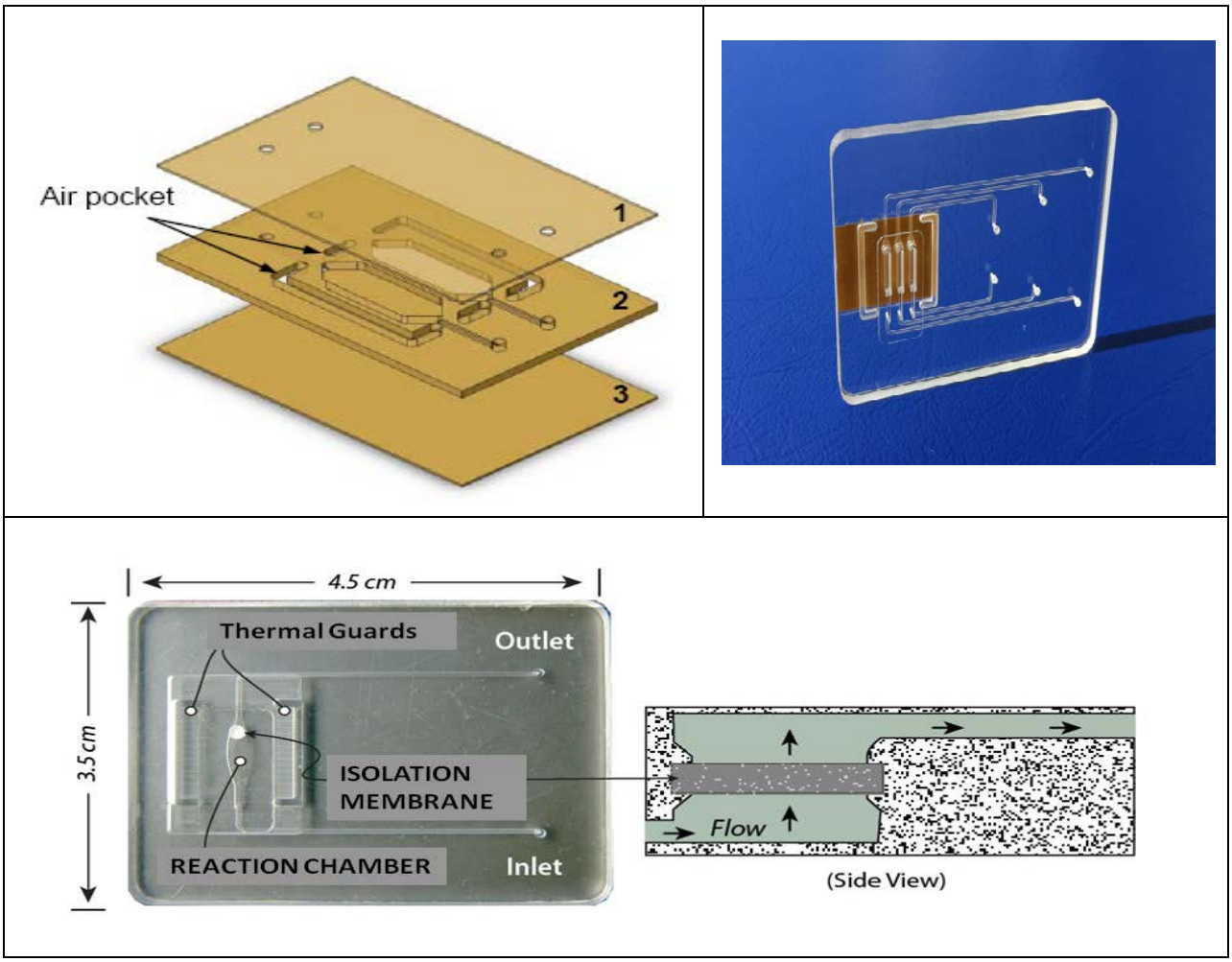

Figure 2: Examples of microfluidic chips for POC diagnostics.

The chip is used on an instrumented platform. **Figure 3** shows the various components of the platform: thermoelectric heater/cooler element, Arduino® Microcontroller, H-bridge, DC/DC converter to power the thermoelectric, a thermocouple for temperature measurement, and a blue LED to excite the fluorescent dye in the chip and a detector. The device can be battery powered. In this project, the students used a cellphone camera with an appropriate optical filter for detection of fluorescence. The schematic represents the "breadboard" stage of development, where students could explore and validate various subsystems and components. The Samsung Galaxy® cellphone and Arduino® communicated through Bluetooth® and the cellphone was programmed with Android®. **Figure 4** shows the temperature control scheme. At this stage of development, the chip was loaded with sample and reagents by manual pipetting. Future versions will automate the fluid actuation and flow control.

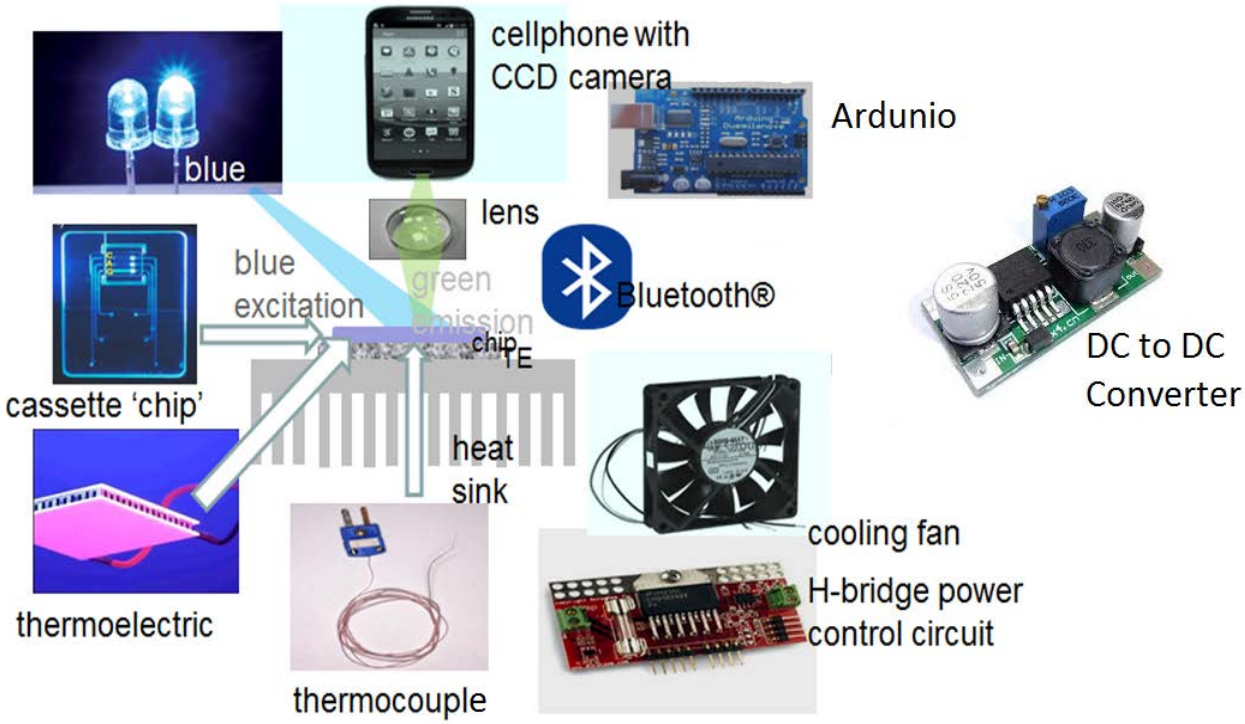

Figure 3: Components of instrumented stage to operate microfluidic diagnostics chip.

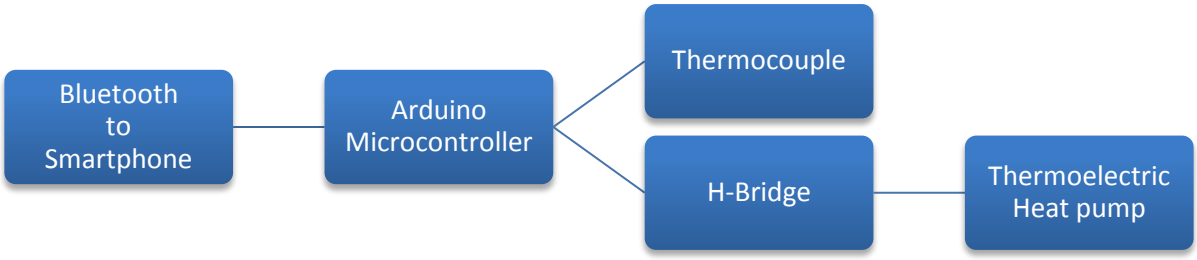

Figure 4: Temperature control scheme for POC system using Arduino Microcontoller, thermocouple, H-Bridge, and thermoelectric element (heat-up).

A packaged instrument, i.e., housing for the instrumented platform shown in **Figure 3,** was designed in SolidWorks® and made on a 3-D printer in ABS plastic— see **Figure 5**. The smartphone could be mounted on the package to serve as a communication port and detector. The image capture capability of the phone (centered over and focused on the amplification chamber of the chip) provided color image data for red, green, and blue pixels, thus assessing the amount of green fluorescence due to the blue LED excitation. **Figure 6** shows the green fluorescence of the reaction chamber for a positive control. This was digitized and converted to a fluorescence intensity by Android software on the Smartphone. Fluorescence above a specified threshold intensity was deemed a positive test result. It is also possible to quantitate the amount of pathogen DNA based on the fluorescence intensity. Parallel chambers could be used as controls or for calibration, or for multiplex testing for different pathogens.

The integration of a Smartphone with POC diagnostics provides a host of capabilities based on telemedicine, GPS tracking of epidemics, more personalized medical care. Smartphones are widely available in the developing world, even in places without electricity. This approach allows leverage of already disseminated technology to healthcare.

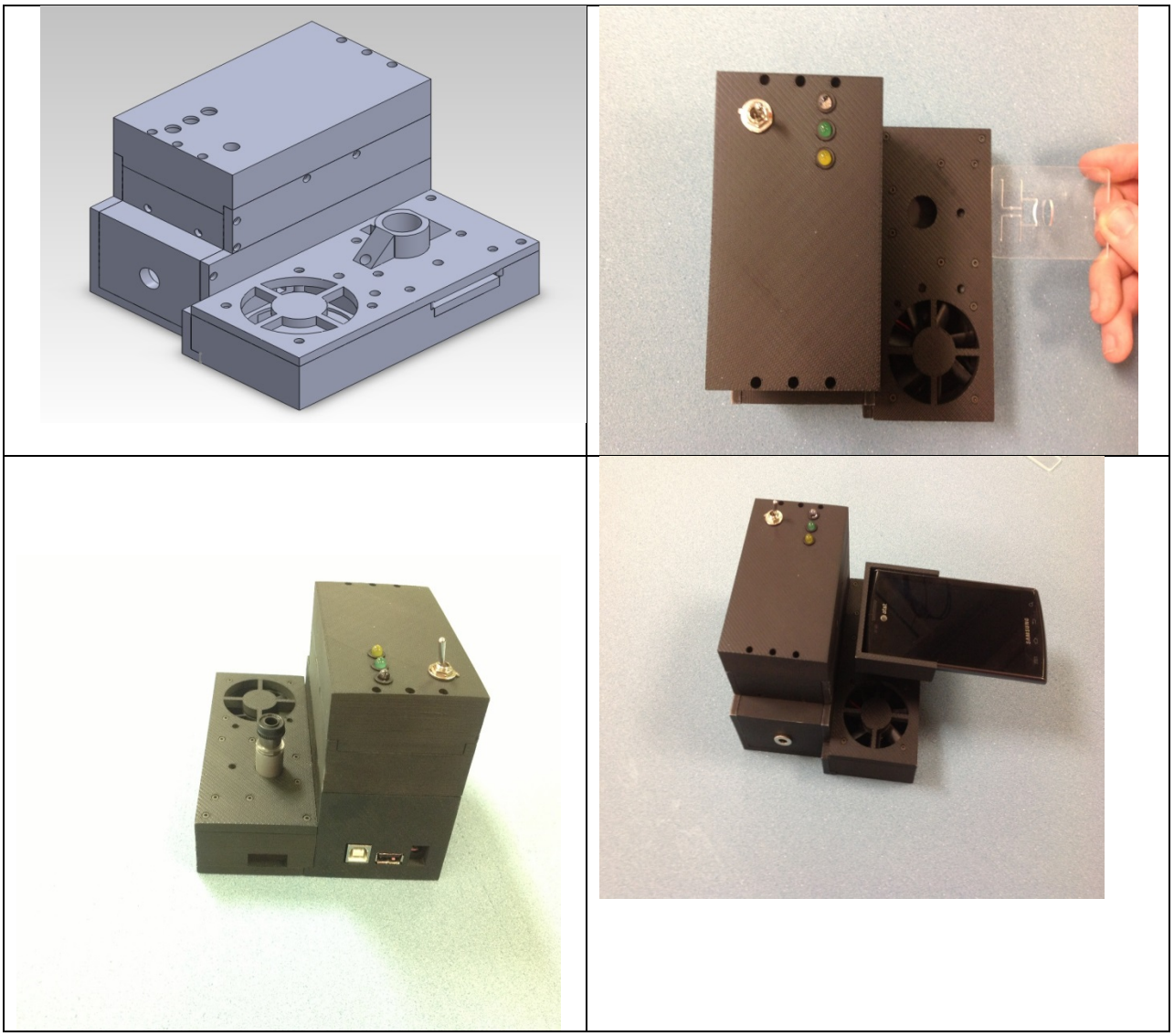

Figure 5: Rapid Prototyped Test system. Upper left: SolidWorks rendition, Upper right: insertion of microfluidic chips, Lower left: side view, lower right: Smartphone mounted on system.

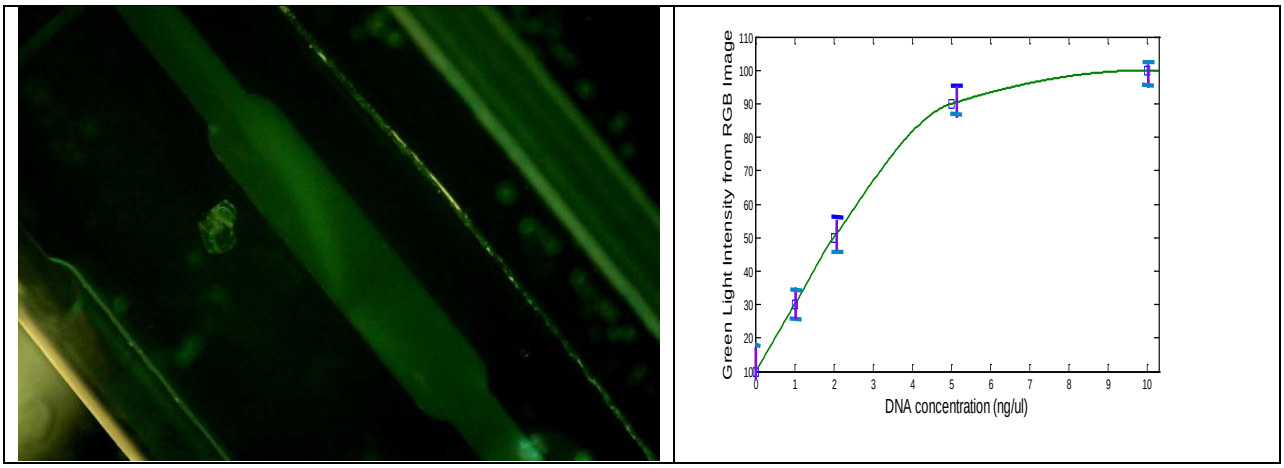

Figure 6: Flourescence image showing showing successful amplification of DNA sequence in microfluidic chip (left), and calibration curve for green light intensity vs. DNA concentration (right).

Conclusion and Discussion: POC tests offer many interesting topics for capstone Senior Design courses. We report a project where students could make a viable demonstration device for an increasingly important healthcare technology. This project offered small groups of students opportunities to utilize and integrate microfluidics, rapid prototyping, optics, sensors, microcontrollers, biotechnology and medical diagnostics. This paper reports the suitability of POC devices and systems as Senior Design projects and can serve to stimulate similar work at other schools. POC projects also promote more interaction and collaboration between Engineering and Biology departments, which will be of increasing importance in coming years. Many of the facilities (3D printers and CO2 laser cutters, as well as CNC machining and support for microcontrollers and other instruments) will be typically available at educational institutions for which this type of work is targeted. A Bill of Goods for the project is shown in the Appendix. The total cost of basic materials was only a few hundred dollars.

A working prototype of a portable Lab-on-a-chip analysis device was produced over the course of this project. While still in its early stages this project has set the groundwork for the further development of this technology which can provide better healthcare to resource-limited areas of the world.

This and related technologies have the potential for considerable societal impacts. The short lead time and portability of it would make identification and quantification of diseases much quicker, cheaper, and more effective. The incorporation of a smart phone into the device would make sending data about the spread of disease much quicker. This would lead to the increase in ability to track a pathogen and take steps to contain outbreaks before they spread to a large proportion of a population. A device such as this has the potential to substantially change how infectious diseases are screened, diagnosed and treated in the developing world.

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Appendix: Bill of Materials for Project

Circuitry				
Part Name:	**Description:**	**distributer:**	**Qty:**	**Price ($):**
Arduino Mega ADK	Microcontroller	Amazon	1	61.95
H-Bridge Shield	Motor Controller	b2cqshop	1	21.00
MAX31855 Thermocouple	Thermocouple	Sparkfun	1	17.50
Buck DC-DC Converter	3 - 40V to 1.5 - 35V	Amazon	1	4.52
Thermoelectric Heater	Peltier Heater/Cooler	LairdTech	1	20.00
RN41 Bluetooth Breakout	Bluetooth module	Sparkfun	1	59.95
SN74HC32N Chip	Quad 2-input OR Gate	Digikey	1	0.33
TIP31C transistor	Power Transistor	Digikey	1	0.54
High Intensity Blue LEDs	5mm Blue 2600 MCD LE	Radioshack	2	9.98
Green LED	5mm Green LED	Radioshack	1	1.99
Yellow LED	5mm Yellow LED	Radioshack	1	1.99
Red LED	%mm Red LED	Radioshack	1	1.99
1K Resistor	1k 1/2 Watt Resistor	Radioshack	5	1.49
Misc parts		Radioshack		20.00

Table A1: Costs of heater circuit components

Casing (3D Printer)	Model/Support Material ($/in^3) = 5			
Part Name:	**Model material (in^3):**	**Support material (in^3):**	**Total Material:**	**Price ($):**
3 x Printing Tray	N/A	N/A	N/A	15.75
Heater Base	7.51	0.89	8.40	42.00
Heater Bottom Tray Section	0.46	0.10	0.56	2.80
Heater Lid	0.10	0.42	0.52	2.60
Bottom Circuitry Holder	6.67	1.92	8.59	42.95
Top Circuitry Holder	2.68	0.55	3.23	16.15
Circuitry Side	1.29	0.36	1.65	8.25
Circuitry Lid	4.68	0.46	5.14	25.70
Cell Phone Holder	1.07	1.13	2.20	11.00

Table A2: Costs of heater Casing