Design of an HIV Treatment Failure Detection System

Engineering World Health
Design Project Competition

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University of California San Diego Chapter
Department of Bioengineering

1. Problem definition

The impact of HIV drug resistance (DR) following the initiation of first-line antiretroviral therapy (ART) is an emerging issue of critical importance to the success of HIV treatment in developing countries. Current antiretroviral treatment involves regular viral load testing, which costs roughly $80,000 for equipment and $65 per test. Although this is affordable in countries such as the US and Western European nations, patients in limited resource settings including Mozambique cannot afford such testing. Instead, hematological, immunological and clinical parameters are monitored, which are not nearly as effective as viral load testing, which increases the likelihood of developing DR. Furthermore, in resource limited settings, lab technicians may not be as highly trained or as available, lab equipment may not be of ideal quality, and necessary precautions may not be taken during sample preparation and analysis

Our device will:

1. *Increase access to effective anti-retroviral treatment failure testing by developing a cheaper alternative to current methods. To increase affordability of the viral load test, we will develop and validate an in-house prototype for under $2000 capable of performing assays used to screen for treatment failure for under $5 per patient. In reducing the price point of this testing procedure, we hope to make this technology affordable enough to be distributed to clinics in low-resource settings.*

2. *Streamline the entire process to minimize human error and be operable by minimally trained individuals with limited access to equipment. It is important to minimize human error and possible contamination by streamlining the testing process. Our device will be limited to one specific assay, and will achieve design usability by consolidating RNA extraction, PCR, and gel electrophoresis and minimizing human input.*

3. *Utilize efficient pooling techniques to increase efficiency of macro detection. The assay described in this design gives either a positive or negative result, depending on the presence or absence of viral RNA in blood. By pooling the blood samples of five patients together, the number of tests required is significantly reduced, as the majority of patients (approximately 95%) test negative.*

Our design aims to validate this method in clinical settings in the developing world through a partnership between the UCSD Stein Clinical Research and Eduardo Mondlane University in Mozambique. Our intention is to develop this technology to the degree of accuracy currently achieved by manually performed pooling standards. Based on an estimation of clinical need, our target goal is to provide the means for the Eduardo Mondlane HIV ward to be able to process 25 pooled samples per day, mapping to 125 patients.

2. Impact in developing world

In areas with abundant resources, patients on first-line therapy are monitored through regular viral load testing and DR genotyping is done on patients identified with failure. However, these techniques are expensive and require sophisticated laboratory facilities and technical expertise that are not available in most resource-limited settings.

To address this need, we plan to develop a rapid, efficient and cost-effective device to monitor for detectable HIV in patients on ART that minimizes user operation and allows for subsequent drug resistance testing. Our device will utilize pooling techniques to rapidly evaluate ART effectiveness for multiple patients.

Our testing method employs the use of a novel pooled-sample assay which has been shown to be an effective way of minimizing the cost per patient for HIV treatment analysis in resource limited settings.[1] The use of qualitative instead of quantitative polymerase chain reaction (PCR) testing dramatically reduces costs while still being able to detect the presence of HIV in human blood. Lastly, by designing a semi-automated device, the time technicians spend on performing this assay will be reduced, along with the potential for contamination due to operator error.

The device will consist of three primary components: an HIV RNA extraction apparatus, a PCR thermocycler, and a gel electrophoresis chamber. Pooled patient blood plasma specimens are introduced via tubing into a chamber and valve system. The RNA will be bound to a mesh, and a pressurized pump will induce fluid flow of reagents over the RNA. In this manner, the RNA will be purified with limited user input and without the use of a rotational centrifuge. A valve system will regulate the flow of reagents through the system and direct the purified RNA product to the thermocycler component of the device, where HIV RNA will be reverse transcribed and amplified via standard PCR. This project will incorporate inexpensive heat-modulation system designs that will deliver the most basic functionality required by the PCR assay. Amplified DNA from the HIV RNA will be transferred via a small tube for agarose gel electrophoresis. The presence of a band in the gel will indicate the binding of primers to the viral RNA, and therefore a positive result. If an amplified product is found, it can be sequenced to determine if DR is present. Clinically, a positive result would mean that the patient would need to be switched to a new regimen of drugs.

By significantly reducing technological costs and the need for trained technicians, this platform would enable the implementation of virologic monitoring in resource-limited settings. We anticipate a maximum production cost of $2,000, a price that will facilitate widespread use.

3. Technical Specifications

The three primary components of our device have been given the following overarching design specifications:

- The device should be simple enough to be used and modified in settings with minimal technical-expertise
- The device should be able to run at least 25 times a day, for a total of 125 samples processed.
- The device should have a target cost of $2,000 or less

During the RNA extraction stage, the human blood serum will be converted into a purified sample of HIV RNA. The RNA extraction device shall:

- Accept 5 pooled samples of blood serum of 5 patients each in addition to one control.
- Be compatible with reagents in the "High Pure Viral RNA Kit" by Roche (Cat no. 11 858 882 001) or other similar extraction kits. (Appendix Protocol 1)
- Limit technician handling of reagents with an accurate method of fluid measuring using a time based flow metering system accurate to within 5%.
- Drive 500 uL inhibitor removal buffer, 450 μL wash buffer (2x), and 50 μL elution buffers sequentially over an RNA-adherent glass mesh
- Dispense a minimum volume of 12.5 μL over five samples

During the thermocycler stage, viral RNA is converted to cDNA and amplified via polymerase chain reaction. The thermocycler shall:

- Rapidly heat 50 μL samples uniformly at a ramp rate of at least 1°C per second
- Cycle through temperatures of 95 degrees, 50 degrees, and 72 degrees Celsius necessary to catalyze the denaturation, annealing, and elongation reactions (Appendix Protocol 2)
- Be extensible and able to load and perform multiple protocols without use of a computer
- Be able to hold the samples at a temperature of 4 degrees Celsius to preserve the samples after the protocol is complete
- Keep the sample intact by limiting evaporation and condensation on the sample lid

The gel electrophoresis stage determines if HIV RNA has been amplified and is present, indicating the likelihood ART failure and DR. The gel electrophoresis stage shall:

- Use a cost-effective 100V power supply to run amplified cDNA on a 2% agarose gel stained with ethidium bromide alongside a standard DNA length ladder
- Be designed using banana plugs and test lead wire to create a safe electrical connection
- Be made from acrylic of varying thickness, between .25 and .5 inches.
- Be able to accurately resolve an HIV band in patients with diagnosed ART failure

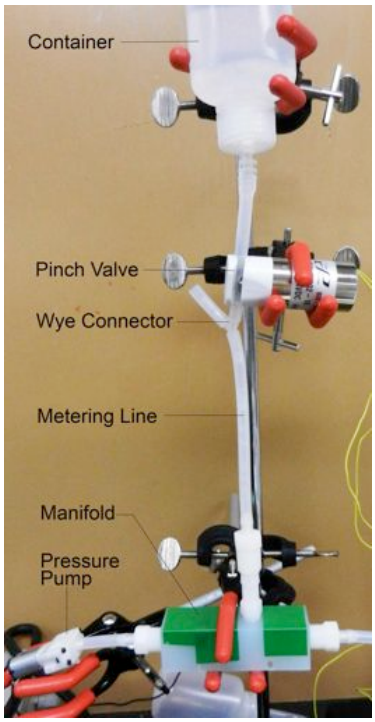
In order for the system to be clinically relevant, it must detect viral load levels at 1000 copies/mL. At this level it would be clear that ART failure had occurred for a patient in the pool. Deconvolution of the pool would then be required to identify the specific patient that had the treatment failure. Based on published rates of ART failure, the vast majority if pools would be negative and therefore not require deconvolution, which results in considerable efficiency and the need for fewer assays being performed.

4. Prototype Implementation

RNA Extraction:

In an attempt to keep the device as simple and robust as possible, several design options were considered before settling upon a configuration that replaced rotational platforms and a pressure actuator with a gravity-based system using a deformable bag to control pressure. (Appendix Figure 7) As seen in Fig. 1, containers holding reagents are open to the atmosphere to prevent back pressure in the system. An air permeable, hydrophobic filter covers each container to prevent reagent contamination. Silicone tubing (ID: 1/8'', OD: ¼'') connects the containers to a normally closed pinch valve, which acts as a timed metering device. An Arduino chip controls the power to the pinch valve and will be programmed to open and close the pinch valve to allow specific amounts of reagent to flow through the system. A barbed wye connector, also protected from contamination by a filter, is attached directly below the valve in order to keep the remainder of the system open to atmospheric pressure. The reagents flow down the silicone tube into a rectangular manifold. A pressure pump attached to the rectangular manifold forces fluid into a radial manifold, which splits the fluid equally between five samples. As the reagent fluids are forced over the samples, the RNA bound to the glass mesh of each sample tube is purified, and finally extracted with the elution buffer. This system is built to be both robust and minimize electrical components. By doing this, the cost of the device is significantly reduced, and the need for technical expertise in maintenance is reduced.

Figure 1. RNA Purification Device



PCR Thermocycler:

A thermoelectric chip (TEC) is used to heat and cool the samples. These devices are measured in delta T and W values, which are then graphed to show the efficiency at certain temperatures. The chip functions as a heat pump by using electrical current to create a large temperature gradient and direct heat flow. Thus, the delta T value refers to the amount of temperature difference that the chip can generate between its two sides. The W values refer to wattage, which relates to the actual amount of heat that the chip can move. Peltiers are a cost-effective solution compared to more conventional heating methods, as they are relatively inexpensive and are cheap from a space standpoint, thus minimizing external containment material. Connecting a motor driver, capable of switching at least 6A/12V, allows the same thermoelectric chip to switch between heating and cooling. This driver is controlled by an AVR microcontroller, which receives data from a thermistor circuit to regulate the temperature according to protocol (Figure 4). The use of the microcontroller allows the use of the device without a computer, as a pre-loaded algorithm can be run for a specific thermocycling protocol. Currently, the thermocycler runs based on a PID (proportional, integrative, derivative) algorithm, which uses PWM (pulse-width modulation) to provide varying amounts of voltage to the thermoelectric (Peltier) chip, to modulate the

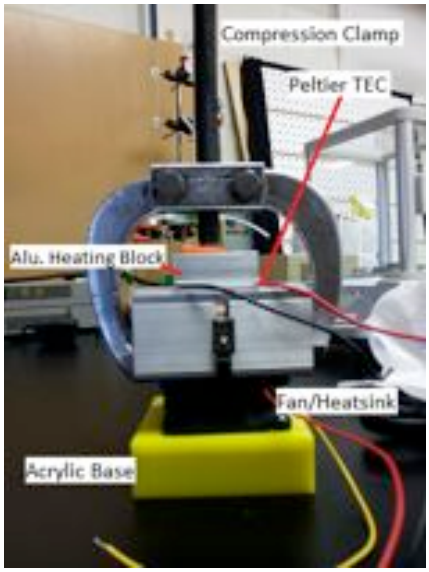


Figure 2. PCR Thermocycler

temperature. PID uses several gain values to adjust the output based upon input from a loop source, and thus provides closed-loop control to hold temperatures at a certain value (Figure 8). In the event that a change in protocol is required, a computer would only be needed to re-load updated code to the microcontroller. The thermoelectric chip lies below an aluminum heating block with 6 step drilled holes for each 50uL PCR tube. Care was taken to limit the thermal mass of the heating block in order to induce rapid heat transfer. In addition, the heating block setup lies on top of a cooling fan to maintain adequate temperature differential and maximize the heat transfer by the Peltier chip. The entire setup lies on top of an acrylic stand to allow for air clearance underneath the device. A small LCD screen is expected to appear in the final iteration of the design, enabling the user to have greater control over the device through diagnostic feedback, including on/off status, current temperature, current stage in the protocol, and current protocol. In future iterations, an interrupt switching mechanism to power the device is also expected to be included.

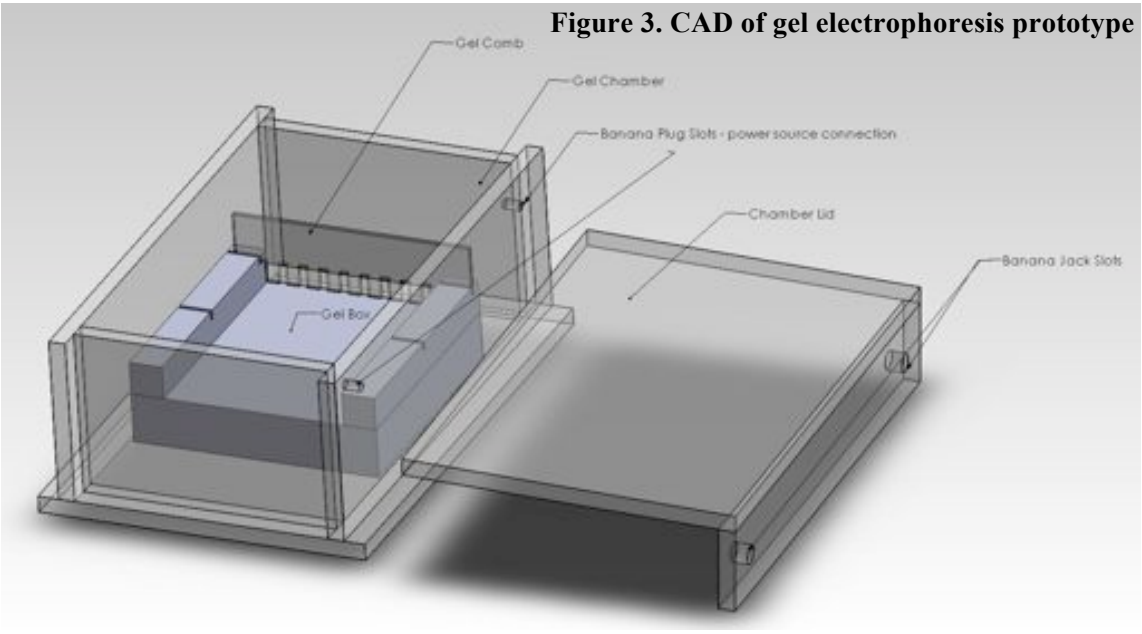
Gel Electrophoresis:

A gel box is built utilizing ¼" acrylic, which was chosen for its low cost and ease of use (Figure 11). The acrylic is cut using a laser cutter and is assembled using water-proof epoxy. Holes are drilled in the acrylic, allowing for the placement of two banana plugs for the power supply. These plugs are each connected to a wire that runs horizontally along the top or bottom of the box. The banana plugs will plug into the lid of the box, which will connect the box to a power supply. To minimize the cost of the power supply, used power supplies have been explored, as well as potentially building a power supply. One concern is adapting the power supply to be compatible with Mozambique, since in the United States, outlet voltage is 120V, while in Mozambique it is 220V. However, this concern should be relatively easy to address with the use of a power converter.

Once fully constructed, the box was tested for electrical conductance. Connected in series, an ammeter will indicate the current through the circuit in the box. If this proves successful, a gel made from 1% agarose is run to ensure the box's functionality. The gel is placed in a buffer of TAE, which is a combination of Tris base, acetic acid and EDTA that contains ions to help conduct the current, as well as maintains the proper pH. After the gel is loaded with a sample dye, the box is connected to the power supply, which is set at 100 V for 20 minutes, to close the circuit and provide a current. The sample dye should travel down the gel but remain in the gel after 20 minutes if the box is working properly.

Ultimately, ethidium bromide will be used to stain the DNA so it is visible under UV light. Since ethidium bromide is carcinogenic, proper disposal will be necessary. Current standards for biohazardous waste disposal involves incarnating and burying materials in order to prevent contamination. Our goal is to work closely with hospitals where our technology will be deployed in order to insure the proper disposable of all hazardous materials.

Figure 3. CAD of gel electrophoresis prototype



5. Proof of Performance

RNA Extraction:

The main function of this portion of the device is to measure and force fluid over a sample of RNA. The RNA is bound to a glass mesh inside of a spin column. In order to ensure that the correct amount of each reagent is being used to purify the RNA, flow rate testing was done. The amount of liquid in the container was measured using an analytical mass balance (accurate to the hundredth of a gram). This liquid was then metered through the flow line and timed using a stopwatch. The amount of liquid that flowed through the system was then massed again, and the volume was calculated. In this manner a volume per time graph was formed and the trend for the flow rate was observed. The flow rate through the spin column was approximately 3.55 μL/s. Assuming uniform viscosity for all reagents, the time required to force each reagent through the mesh would be 141.18 seconds for the inhibitor removal buffer, 127.07 seconds for the wash buffer, and 14.11 seconds for the elution buffer. This means the run time for a single sample would be less than five minutes, which is a significant improvement over current methods that require time for centrifugation and pipetting.

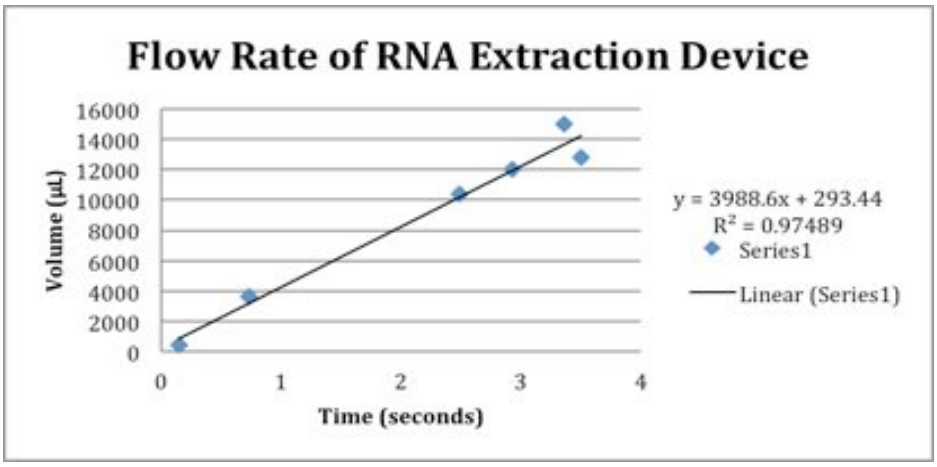


Figure 4. Flow rate through RNA extraction system was determined to be relatively linear up to 16 mL

In testing the flow rate of our system, the average flow rate of our system proved to be 3,905 μL with a standard deviation of 327 μL. Given that our pinch valve has a delay of .035 milliseconds, the resolution of the fluid measurement system is 136 μL with a standard deviation of 11.4 μL. The current system, therefore, can meter 250 μL with a standard deviation of 21 μL (see Table 1). Although the specifications for the amount of fluid needed for five samples is met, the standard deviation shows that the system is not sensitive enough to measure within a 5% accuracy. We wish to address this issue by using thinner tubing and reducing the variable pressure in the system. Our next design iteration will have a deformable container which will remove the variation due to a container open to atmospheric pressure with a changing fluid height.

These results prove that our current system is capable of measuring fluid. We were able to demonstrate the ability to rapidly force fluid over the bound proteins via our pressure pump. Our design passes the current proof of concept test, and we intend to address issues of sensitivity in the next design iteration.

Thermocycler:

The thermocycler device prototype was able to successfully run up to a temperature of 93 degrees while at the same time cooling to 3 degrees, with an average ramp rate of 0.3 degrees per second throughout the temperature cycle. As such, the temperatures are within the ranges of the protocol, but the ramp rate may not be fast enough to ensure the protocol runs at optimum time frequency. This was solved by improving

the quality and power of the Peltier thermoelectric element used. Thus, in the next iteration of the device we plan to increase the power from 138W to 196W, which is expected to achieve a marked improvement in ramp rate, with an expected increase of ramp rate over 0.6 degrees per second.

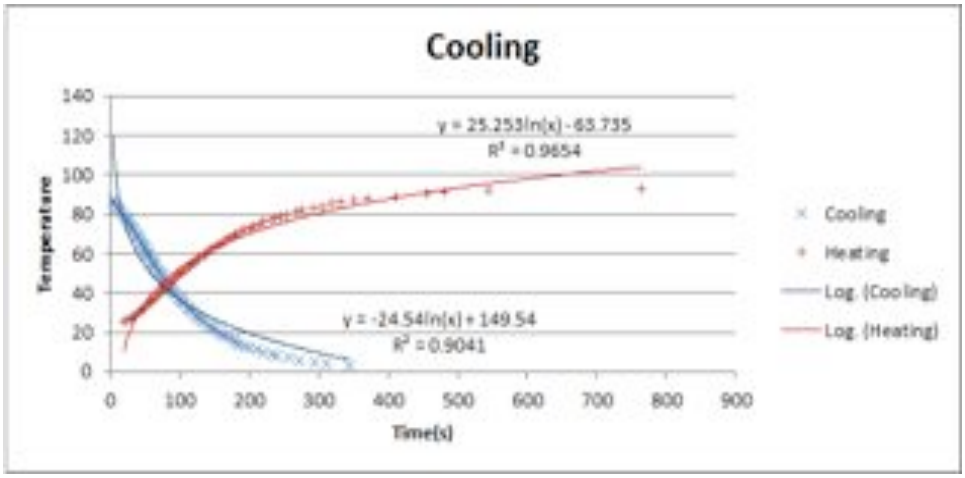


Figure 5. Initial Heating and cooling time curves of PCR thermocycler device using 138W TEC. Curves were roughly logarithmic, indicating that further optimization is necessary to rapidly reach 95°C

Figure 5 indicates that the heating curve slows down considerably as the final temperatures are reached, which is as expected as the temperature difference approaches the maximum temperature difference supported by the thermoelectric chip. This can be optimized by finding a chip with greater power, which would be able to pump more heat and generate its maximum temperature difference more rapidly. We found that a heat-sink and fan were crucial to Peltier operation as they enabled more heat to be pumped from the source to the sink. A Peltier thermoelectric chip was found to "saturate" without the use of these elements and thus would stagnate at a certain temperature without heat being removed from the other side. The LCD that was chosen for the system was a 16x2 LCD display, as such a display is large enough to provide a great deal of information, but still inexpensive enough so as not to add considerably to the project.

Gel Electrophoresis:

Once the gel box was completed, testing ensued. First, a 1% solution of agarose and buffer was poured into the gel carrier and was refrigerated for 30 minutes to allow it to set. A 10-teeth comb was set in the gel before congealing to make the wells for the loading sample. The box was tested for leaking and any leaks were patched with additional epoxy glue. Next, gel was placed into the box and the box was filled with enough buffer to cover the top of the gel and the copper wires. Two samples of a New England BioLabs DNA ladder (PhiX174 DNA-HaeIII Digest, Cat N3026S) were loaded into the gel along with a third sample of only a loading dye (Cat B70215) as a control, shown in Figure 5. 100 V were run through the gel for approximately a hour, which yielded the results seen in Figure 13. This picture shows the box can successfully propagate a DNA sample through the gel, as can be seen by the separation of the bands by base pairs. This can be compared to the base pair separation of the ladder shown by BioLabs (Figure 12).

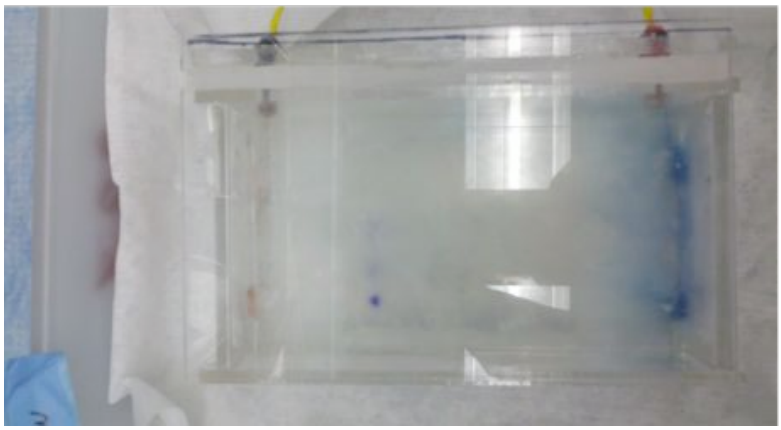


Figure 6. Final Constructor Gel Electrophoresis Chamber stained with Ethidium Bromide

6. Business Plan

6.1. Manufacturing

6.1.A. Short-term

In the short-term we are implementing an ad-hoc manufacturing scheme. Devices are being built in house specifically for our Mozambique clients.

6.1.B. Long-term

One of our key aims is to move manufacturing of this device into the developing world. Maintaining high-end technologies is difficult in resource-limited settings, and lack of maintenance can often lead to disuse of technologies. Our design was specifically created for simple manufacturing and maintenance; this is vital to having a system that can be maintained locally and that doesn't need highly technical equipment to repair or update. Eventually, we aim to make our technology open source and to provide blueprints for construction to developing nations, so that manufacturing can be done in limited-resource settings.

6.1.C Costs

Currently in Mozambique, most patient care is paid for through the President's Emergency Plan for AIDS Relief (PEPFAR), an initiative that promotes sustainable programs of AIDS relief in developing countries. PEPFAR funds the training of clinicians, and is responsible for the majority of patient care, although there are some patients who pay for their own care. PEPFAR is not screening for drug resistance in HIV, and is instead relying on CD4 counts alone to confirm HIV suppression, even though studies have shown that drug resistance screening is significantly more effective than CD4 counts. We have simplified our design significantly so that PEPFAR and other funding agencies are able to afford ART failure and subsequent drug-resistance screening with our device. In the long-term, we hope that this significant reduction in price will lead to limited-resource countries being able to afford screening technologies with their own budgets.

Through design simplification we seek to reduce equipment costs substantially: The RNA extraction component of our device will cost approximately $467; The PCR component part will cost approximately $172; the gel component will cost approximately $25. These costs are detailed in the appendix.

6.2. Distribution

6.2.A. Target Market

Our initial target market is Mozambique. The device is currently being made region specific for hospitals in the area. Grants have also been submitted to build the device for regions in India, and we plan to make the device region-free after the initial prototype release.

6.2.A.1. Customers

Clinical labs in developing nations are our clients. Our initial major client is the Central Hospital of Maputo located in Maputo, Mozambique. This hospital and universities associated with it are funded by PEPFAR and other major grants. Other grants from associations such as the Gates foundation, the Clinton foundation, the CDC, or the NIH, fund AIDS relief and associated technologies; these grants will fund our technology in developing nations. Eventually, when the

technology is inexpensive enough, we expect Mozambique and other impoverished nations to be able to purchase the technology directly.

6.2.B. Costs

Bringing technology to Mozambique and other developing countries is expensive if shipping a completed device. We expect costs to be approximately $500 to ship and set-up our initial prototype. In the long-term, we envision that distribution of the device will be done locally and costs will be at a minimum. Although the development of this technology greatly decreases the current costs of technology, our device will not generate revenue but will just cover its costs in sales.

6.2.C. Logistics

We will work through our funders to distribute this technology to clinics in the developing world. In addition, UCSD has developed a program that sends medical clinicians to Mozambique to train the indigenous clinicians and to work with the technology there; we will initially be able to setup our prototype using this resource.

6.3. Funding

This platform needs to be validated in local settings to prove its efficacy, which will require startup funding. It will require funding for the roughly 2-5 years of clinical trials before it can be adopted for widespread use. Our funders, whether it is an NGO or other non-profit organization, may need to fund these developing stages of our device. In the future, our aim is for the technology to be self-sustainable and affordable so that the developing countries will be able to pay for it themselves. But preliminary testing and clinical trials will require outside support. Given the potential our design has on the developing world, we are currently looking into funding from a number of NGOS such as the Gates Foundation, Clinton Foundation, CDC, and NIH.

6.4. Novelty

6.4.A. Patentability

The prototypes developed by EWH at UCSD do not infringe upon any known patents. The use of RNA extraction, PCR, and gel electrophoresis reagents and protocols made available publicly does not come under any patent. The use of pooling methods for HIV treatment failure detection in resource-limited settings has been filed by advisors Dr. David Smith and Dr. Winston M. Tilghman with the UCSD Tech Transfer Office per university policy. Disclosure has been filed so that UCSD may hold the rights to this patent for a one-year period. While the assay used to run the diagnostic is being protected by UCSD, the actual technology being developed by EWH at UCSD remain open source, thereby giving EWH at UCSD the Freedom to Operate.

6.4.B. IP Rights

The components used in this design prototype are readily available and do not require the use of proprietary in order to be completely manufactured. The consumables used to test patients using the device may be purchased from different companies with comparable results.

6.4.C. Regulatory Considerations:

The continued development of an HIV treatment failure detection system will require all

necessary safety testing pre-cautions for testing of HIV-positive patients. When the time comes, the device will be validated under UL and CE standards to ensure the safety of the components that may not have already been certified. Should the device require approval for testing in the U.S. before deployment in the resource limited settings, the leaders of this project will work closely to seek approval from the IRB and file an IDE with the FDA. For continued work in Mozambique, the device will be designed to closely follow all other biomedical device regulations set forth by the WHO.

6.5. Impact

This technology will eventually create a global positive feedback cycle in terms of economic output and quality of health. The manufacturing and distribution of this device will generate jobs and increase local employment while allowing the purchasers, namely hospitals, to improve quality of care. The HIV positive patients who will benefit from this device will have a better quality of life, and therefore be able to contribute to the overall economy and be more productive members of society- similar to the initial roll out of ART. Additionally, effective HIV treatment, monitored using virologic methods, will greatly help to prevent the spread of the disease, thereby reducing the number of infected individuals.[2]

While initial testing and validation efforts will require a certain amount of resources, we would like this technology to eventually be self-sustainable. Having shown both proof of concept and a working prototype, we hope to have a field-ready device by the end of 2012 that will be delivered to Mozambique by mid-2013. The potential impact of this device is widespread, and requires only an initial investment and design in order to make it sustainable in limited resource settings.

Citations

[1] Smith, David M. "The Use of Pooled Viral Load Testing to Identify Antiretroviral Treatment Failure." *AIDS* 23 (2009). *Viral Path*. UCSD. Web. <http://viralpath.ucsd.edu/Papersforweb40-53/Davey_paper52.pdf>.

[2] Smith, David M. "Running with Scissors: Using Antiretroviral Therapy without Monitoring Viral Load." *Division of Infectious Diseases* 46 (2008). UCSD. Web. <http://viralpath.ucsd.edu/Papersforweb40-53/Davey_paper40.pdf>.

Appendix

Table 1: Raw Flow Rate Data for RNA Extraction

Trial	Mass H20 (g)	Volume (ml)	Flow Rate* (ml/s)	ΔHeight of Water (cm)
1	8.953	8.937520263	4.905334941	0.47498
2	8.891	8.875627461	4.871365237	0.635
3	7.929	7.915290759	4.344286915	0.3175
4	7.899	7.885342629	4.327849961	0.3175
5	8.064	8.050057344	4.418253207	0.47752
6	8.118	8.103963978	4.447839724	0.3175
7	7.991	7.977183561	4.37825662	0.3175
8	7.563	7.549923573	4.143756077	0.635
9	6.976	6.963938496	3.822139679	0.3175
10	7.265	7.252438815	3.980482335	0.3175
		AVG	4.36395647	0.41275
		STDEV	0.341815409	0.12700127

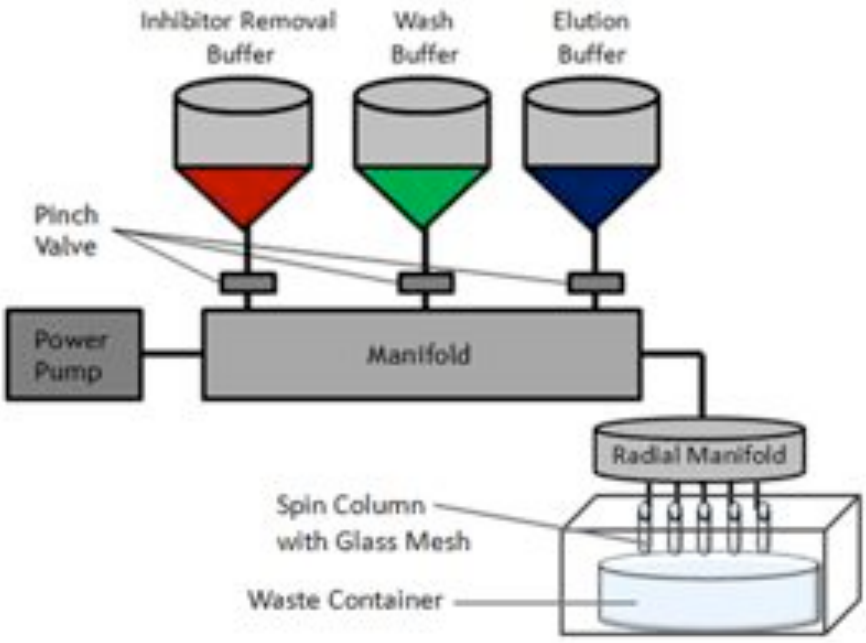


Figure 7. Final Design Schematic for RNA extraction device using gravity-based flow and a radial manifold

RNA Extraction Cost to Build:

Item	Vendor	Bulk Price	Amount Used	Cost
1. Polythylene Container (125 mL)	Cole Parmer	$24.50 (6 Pack)	3	$12.25
2. Barbed Wye Connector	Cole Parmer	$6.55 (25 Pack)	1	$00.26
3. Pinch valve	Cole Parmer	$113.00	3	$339.00
4. Pipe Adaptor (⅛’ x ⅜’)	Cole Parmer	$10.50 (10 pack)	3	$3.15
5. Straight Tube Connector (1/16’’)	Cole Parmer	$9.55 (10 pack)	6	$5.70
6. Platinum-cured Silicone tubing (⅛’ ID x ¼’ OD)	Cole Parmer	$40.00 (25 ft)	2 ft	$3.20
7. Pressure Pump (0.4 LPM 5.9" Hg/6.8 psi, 3/6 VDC)	Cole Parmer	$27.00	1	$27.00
8. Polypropylene manifold; 4 outlets; 1/4" inlet NPT(F)	Cole Parmer	$26.00	1	$26.00
9. Male Pipe Adapter Nylon, 1/4" x 1/8", 10 PACK	Cole Parmer	$10.00 (10 Pack)	2	$2.00
10. Six-Way Radial Manifold	Aquatic EcoSystems Inc	$49.23	1	$49.23
Total				$467.79

Thermocylcer Cost to Build:

Item	Vendor	Bulk Price	Amount Used	Cost
1. Arduino Microcontroller (AT-Mega328P)	Arduino	$17.00	1	$17.00
2. Pololu Motor Driver (VNH5019)	Pololu	$20.97 ea (if buying 100)	1	$20.97
3. Peltier Thermoelectric Chip (TEC-1-12709)	Aliexpress	$5.68	1	$5.68
4. Heatsink /Fan	Thermaltake	$14.98	1	$14.98
5. Acrylic (36”x30”)	Home Depot	$21.98	1	$21.98

6. Power Supply (12V/12A)	Digi-Key	$73.00	1	$73.00
7. Aluminum Block (12”x3/16”x2”)	Metals Depot	$23.95	1/2	$11.97
8. Thermistor (10k ohm, NTC)	Digi-Key	$0.39	1	$0.39
9. Power Supply (5V/1A)	Sparkfun	$5.95	1	$5.95
Total				$171.92

Gel Electrophoresis Cost to Build:

Item	Vendor	Bulk Price	Amount Used	Cost
1. Acrylic	RidOut Plastics	$100	1/5	$20
2. Heat Shrink Tubing (1/2”)	RadioShack	$7.50	1/10	$0.75
3. Banana Plugs	RadioShack	$15	1/10	$1.50
4. Banana Jacks	RadioShack	$15	1/10	$1.50
5 Copper Wire (180 ft, 22 gauge)	RadioShack	$15	1/20	$0.75
Total				$24.50

Figure 8. Temperature Control Feedback Circuit Schematic

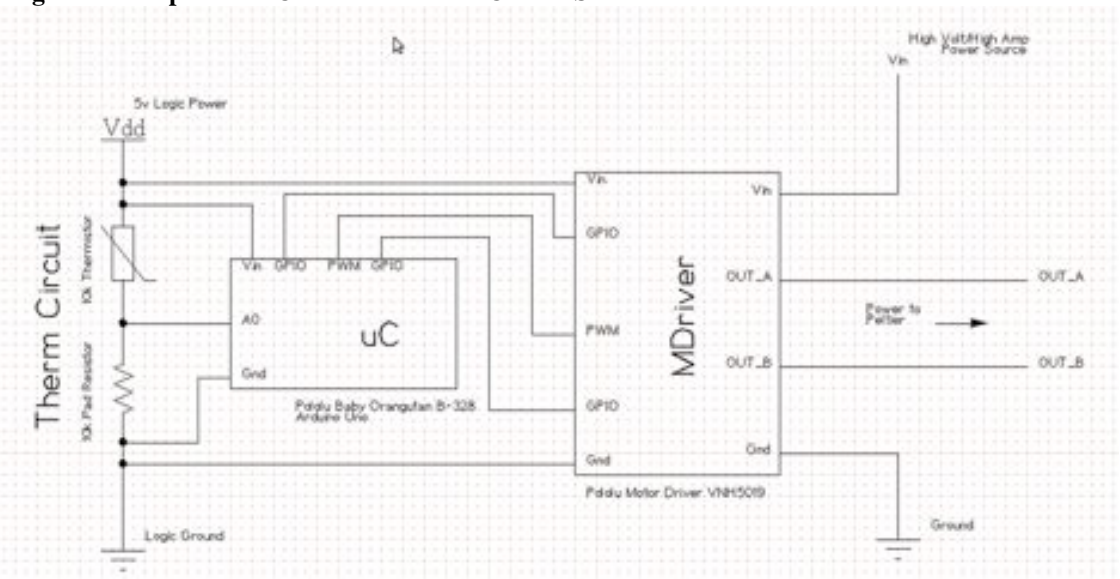


Figure 9. Heating Curve for 196W Peltier Chip Prototype with 50 uL sample

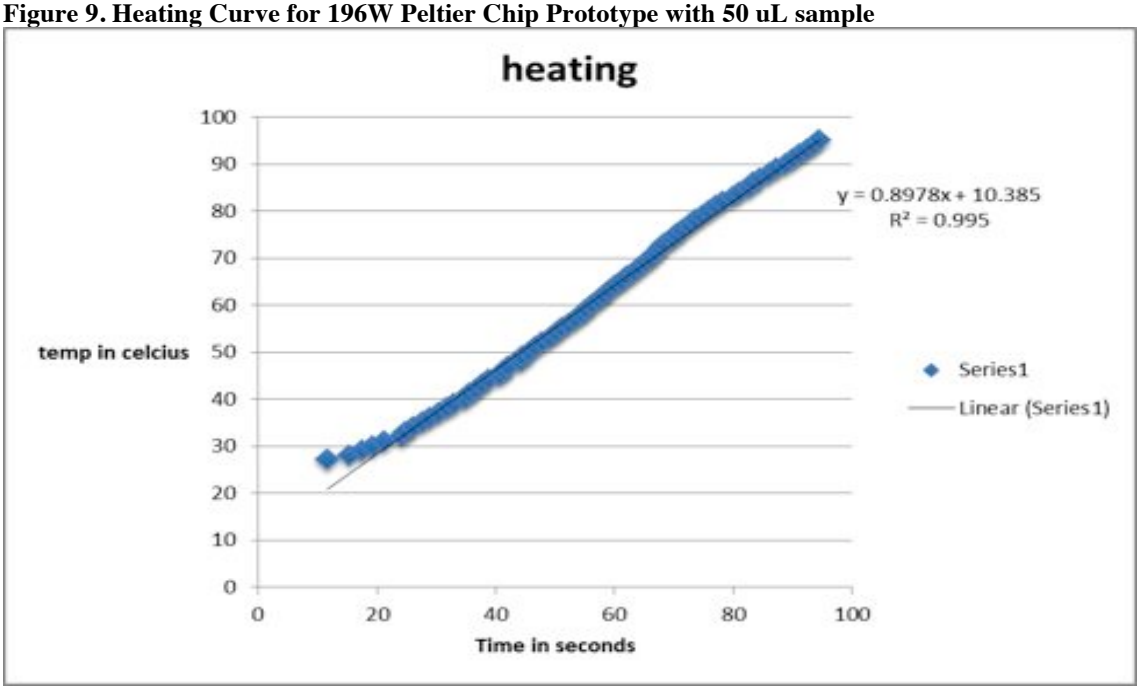


Figure 10. PCR Thermocycler Protocol

Masterbook # **Nested PCR Using alternate RT (pol) Primers** **Date**

Purpose: Amplify the RT region of HIV-1. This is a nested (hemi-nested) protocol for samples with very low viral loads. Also, it provides troubleshooting solutions if smears or multiple nonspecific bands are observed.
*Protocol may be used for modified 5RT/3RT primers.

Procedure:

(1) First Round with CI-Pol1 and 3RT

	1	14
Nuclease-free water	31.5	441
10X PCR Buffer + $MgCl_2$	5	70
10 mM dNTPs	1	14
CI-Pol1(20 pmol/µl)	1	14
3RT(20 pmol/µl)	1	14
Taq Polymerase	0.5	7
TOTAL VOLUME =	**40**	

To this mix, add 10µl of extracted DNA or cDNA.

TOTAL REACTION VOLUME = 50.0µl

Cycling Conditions: Richy 2 #25

95°C	2 min	
95°C	30 sec	> 35 cycles
50°C	30 sec	
72°C	1 min	
72°C	10 min	
4°C	Hold	

(2) Second Round with Inner Primers 5RT and 3RT

	1	14
Nuclease-free water	36.5	511
10X PCR Buffer + $MgCl_2$	5	70
10 mM dNTPs	1	14
5RT (20 pmol/µl)	1	14
3RT(20 pmol/µl)	1	14
Taq Polymerase	0.5	7
TOTAL VOLUME =	**45**	

To this mix, add 5µl of 1st Round Product.
If nonspecific binding occurs, reduce volume of 1st Round Product, starting at 2.5µl. Reduce to 1µl if needed. Fill to 5µl with molecular grade water.

TOTAL REACTION VOLUME = 50.0µl

Cycling parameters same as in first round.

(3) Clean-up of PCR Products

PCR products that will be used as sequencing templates have to go through a clean-up process to remove any excess dNTPs and primers. Use Qiagen's QIAquick PCR Clean-up Kit according to manufacturer's protocol.

Reviewed by Caroline Ignacio July 2010

Figure 11: CAD Schematic for Gel Electrophoresis Box

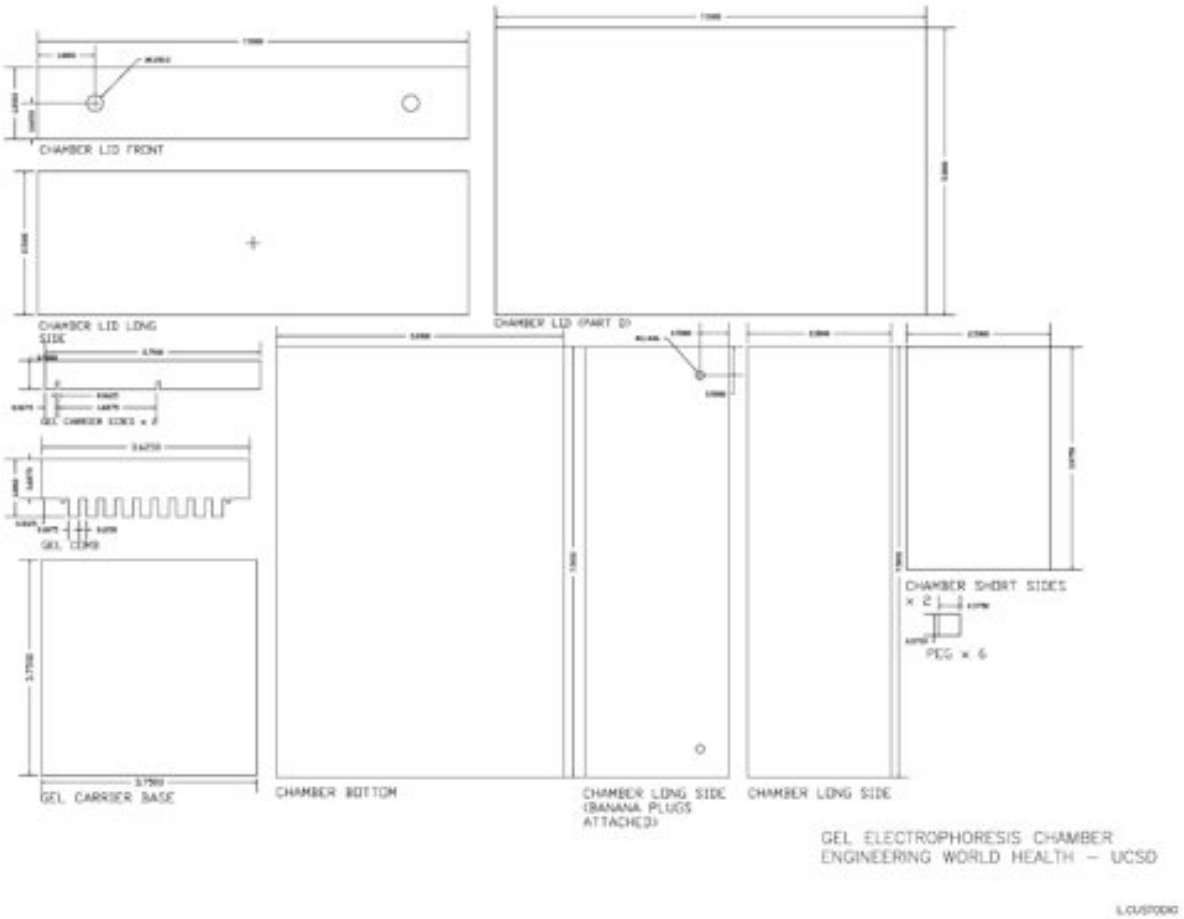


Figure 12: Ideal ladder used for comparison in gel run

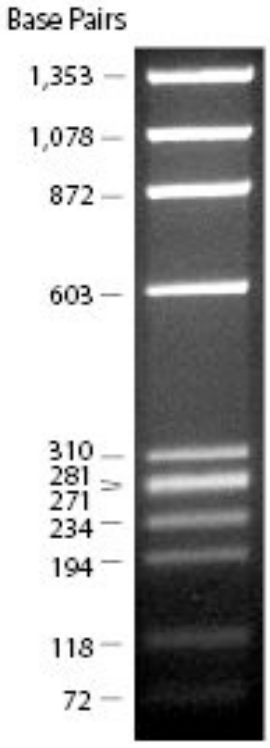


Figure 13: Sample DNA run through gel to test efficacy of gel