A Platform for the Detection of Antiretroviral Therapy Failure for HIV Positive Patients in Mozambique, Africa

Gillie Agmon; Magda Holda; Vyshaali Jagadeesan; Kevin Le; Mallika Malleswaran; Tammy Soo-Hoo; David Smith, MD
Department of Bioengineering, Jacobs School of Engineering, University of California San Diego

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Introduction

Currently 34 million people worldwide are living with HIV and AIDS and over two thirds of those people live in sub-Saharan Africa. The president's Emergency Plan for AIDS Relief (PEPFAR) has increased the access to antiretroviral therapy. But detection of HIV drug resistance following first-line antiretroviral therapy is a critical issue in HIV treatment in developing nations.

A patient is drug resistant when their medication is no longer effective in suppressing the virus. To ensure the efficacy of the medication and detect drug resistance Viral Load tests must be performed every 3-6 months.

Current Viral load test cost roughly $80,000 in equipment and $65 per test. This cost constraint is not feasible in resource-limited settings. Furthermore, the procedure is time intensive, requires precision lab techniques, and highly trained lab technicians.

Our group is currently collaborating with Eduardo Mondlane University Hospital in Mozambique to design a cost effective set of devices, to automate the viral load testing, this will allow for the qualitative determination of drug resistance.

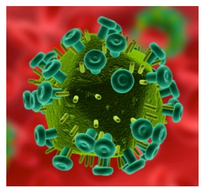
Eduardo Mondlane Hospital

Aims

1) **Increase access to effective anti-retroviral treatment failure testing by developing a cheaper alternative to current methods. We hope to sufficiently lower the price point of this testing procedure to make distribution of this technology to clinics affordable in low-resource settings.**

2) **Streamline the entire process to make the technology operable by minimally trained individuals, and to minimize human involvement. Streamlining the testing process is important to minimize human error, minimize possible contamination, and facilitate widespread application of anti-retroviral treatment failure testing.**

3) **Utilize sample pooling techniques to increase efficiency of detection. By pooling the blood samples of five patients together, the number of tests required can be reduced by 30-80%, as the majority of patients test negative.**



HIV Virus

Our design aims to validate this detection method in clinical settings in the developing world. We hope to make this technology as accurate as current manually-performed pooling standards. Our target goal is to be able to process 25 samples per day, mapping to ~50 patients per day.

Methods

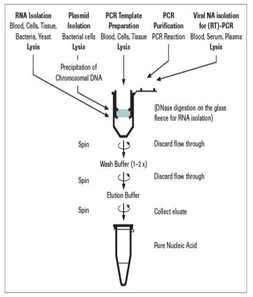
In order to increase affordability of HIV drug resistance detection, we are developing and validating an in-house prototype for under $2000 that can qualitatively confirm HIV drug resistance in patients.

Our device will be limited to one specific assay, and will achieve design usability by consolidating RNA extraction, PCR, and gel electrophoresis, and by minimizing human input. The assay described in this design gives either a positive or negative result, depending on the presence or absence of viral RNA in blood, which is used to determine anti-retroviral treatment failure.

Step	Assay Description
1.	**Separate blood plasma from patient blood sample**
2.	**Extract RNA from virus in patient's blood plasma**
3.	**Reverse transcribe cDNA from extracted viral RNA**
4.	**Use PCR to amplify viral DNA sequence**
5.	**Increase quality of PCR amplicons using clean up kits**
6.	**Visually verify amplicons using 1% agarose gel**

RNA Extraction Device

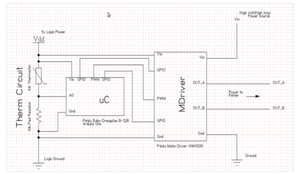
The RNA extraction device automates the precise measurement and delivery of reagents for the purification process. Samples of blood serum and binding buffers are forced through a glass mesh, this binds RNA to the mesh. Wash reagents are forced through the mesh to lyse and remove all other material. This device uses an arduino-controlled vacuum pump to replace precision pipetting and a vacuum manifold instead of a micro-centrifuge.



Traditional RNA buffer extraction method

PCR Thermocycler Prototype

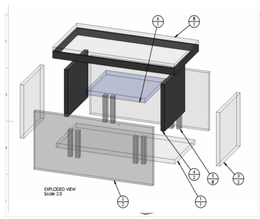
The purified RNA is turned into cDNA via reverse transcriptase. The thermocycler runs PCR by rapidly cycling through enzyme-specific temperature ranges. Each temperature activates a different set of primers that select for the gene of the envelope region of the HIV virus. If the virus is present, then its DNA sequence is amplified.



Thermocycler Control Circuit Design

Gel Electrophoresis

The DNA is tagged with ethidium bromide and the samples are run through an electrophoresis gel. The presence of a viral band indicates a significant viral load level, and therefore drug resistance in the patient.

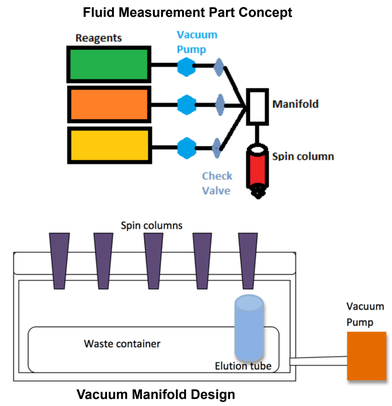


Gel Chamber Design Concept

Design Results

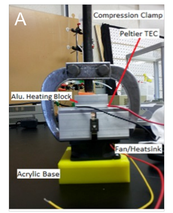
RNA Extraction

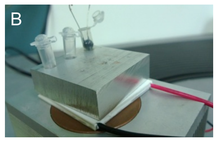
- Consistent flow during rinse cycles
- Capable of delivering minimum required volume (50 μL)
- Arduino-control pump delivers precise buffer volumes
- Ardunio controlled vacuum manifold forces reagents through the mesh



Vacuum Manifold Design

PCR Thermocycler



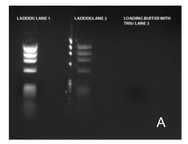


A. Part Diagram of first prototype
B. PCR Samples with thermistor to detect temperature

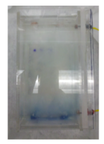
- Arduino-control cycles temperatures of samples in PCR tube according to PCR protocol from 75 °C to 90 °C to 50 °C
- Powered using 150W Peltier TEC with 12V/12A supply
- Preliminary Arduino-controlled LCD interface for real time temperature display

Gel Electrophoresis

- Fabricated second low-cost acrylic chamber box prototype
- Able to successfully resolve distinct DNA ladder bands



A. DNA Ladder Results



B. Gel Box Prototype

Conclusion

Our first round of prototyping has produced promising results. The next phase of development will work towards reproducing the complete assay and benchmarking reliability against gold standard first-world lab equipment.

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