A Platform for the Detection of HIV through Viral Load Testing in Tijuana, Mexico

The Open Viral Load - Global TIES Team

Business Lead: Kirk Hutchison - **Technical Lead:** Hayley Chong

Biochemistry Team: Allison Duchnak, Kushagra Mathur, Uma Mahto, Chris Squiers, Taylor Williams, Martin Hartel, Ron Rubio, Elodie Sandraz, Connie Gean, Neha, Chhugani, Andrea Kim, Brianna Parish, Megan Lo, Bella Iriarte, Steven Windas, Saumya Bhatia

Devices Team: Alex, Smith, Christopher Liu, Brian Nguyen, Smita Sahay, Timothy Han, Phuong Ho, Ori Gilad, Lucas Petit, Kanan Saito, Sean Ghaderi, Xinyu Wu, Ben Chafik, Bahari Hasjim, Amitoj Setia

Introduction

Currently **37 million people worldwide are living with HIV** 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 initial HIV infection**, as well as drug resistance following first-line antiretroviral therapy is a critical issue in HIV treatment in developing nations.

Current Viral Load tests cost roughly $10,000 in equipment and $15 per test. This cost constraint is not feasible in resource-limited settings. Furthermore, the procedure is time intensive and involves precise methods carried out by highly trained lab technicians.

Our client, **Albergue Las Memorias in Tijuana**, aims to provide an affordable, accurate, and effective method for diagnosing HIV.

Together, the OVL team and Las Memorias clinic have been making strides in designing a **cost effective device** that will **automate** viral load testing and enable **prompt diagnosis** of HIV and other diseases.

Overall Aims

1) Devise an open source system to sufficiently **lower the price point** of the testing procedure and make its distribution to clinics affordable and widespread in low resource settings.
2) Engineer an **automated and simple** device protocol. Simplicity and automation will minimize the chance of human contamination and allow for operation by unskilled laborers.
3) Create an **effective and rapid detection** method for HIV and other infectious viral diseases in low-resource areas. This is achieved through imaging the fluorescent results of digital PCR for the viral load, which efficiently and precisely diagnoses positive patients.

Software Team

Aim:

To develop a computer vision program using neural networks to image and quantify the HIV RNA.

Design

Neural networks are a method used to train a computer program to recognize objects. For example, if the program is shown 1,000 images of boxes or channels on a microfluidic chip, then eventually it will be able to recognize a shape as either being a box/channel or not being a box/channel.

We can use this method to identify boxes/channels in an image, and further, to determine if the boxes or channels are fluorescing, which will allow us to measure the presence of HIV RNA after PCR.

Biochemistry Team

Aim:

1. To effectively bind viral HIV RNA strands with a low-cost, customizable RNA Extraction protocol.
2. To design and manufacture a cost effective and low auto-fluorescent microwell chip to efficiently contain extracted RNA strands and PCR ingredients during the PCR process and during fluorescent microscopy analysis.

RNA Extraction Design

The design by the RNA Team is innovative in that the protocol uses magnetic nanobeads to bind RNA rather than silica gels. The magnetic nanobeads are customizable, making them a powerful tool for processes requiring customization. In addition, in efforts to reduce costs of RNA extraction, the team is working on a tube enclosure (Fig. 2) for the extraction to take place, sequentially preloaded with the required reagents as outlined in Fig. 1. A magnet is used to slowly guide the microbeads through the tube, which eliminates the need for expensive micropipettes. Finally, an automated version of this process is in the making, such that all reagents are preloaded, and the magnet is moved by a motor.

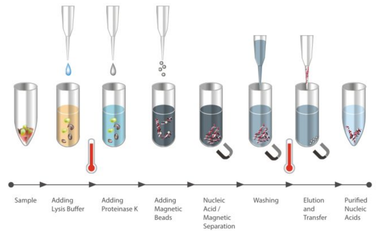

Fig. 1: RNA Extraction with Magnetic NanoBead Technology

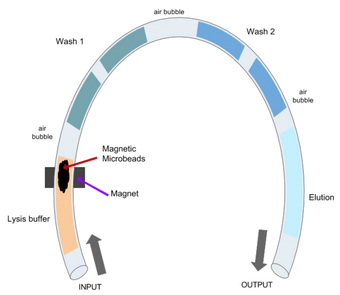

Fig. 2: Microchannel Design for Reagent Delivery

Serial Dilution Chip - Vessel for Amplifying and Imaging RNA

The team is currently designing a chip that will load the elute from the RNA extraction and perform a serial dilution of the RNA. Compared to a standard dilution array, depending on how many diluted wells fluoresce will indicate either: no HIV RNA, low levels, medium levels, or high levels, corresponding to various ranges of viral load. The team is still heavily in the design phase of this process and any initial designs are preliminary.

We have two designs we are pursuing: one involves “etching” a design into an acrylic piece to create microfluidic channels (Fig. 3). The other involves a series of wells that range from shallow to deep, into which the extracted RNA is “slipped” onto, following a design published called a Slip Chip (Fig. 4).

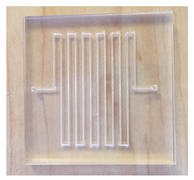

Fig. 3. Acrylic chip with channels etched in.

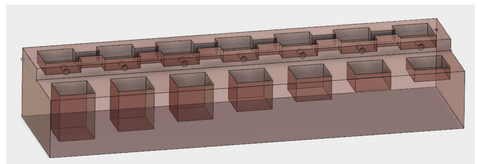

Fig. 4. Top layer contains RNA, dispersed via a channel. Bottom piece has wells with different amounts of water.

Hardware Team

Aim:

1. To create an open-source, low cost, portable, and reliable thermal cycler for use in RNA/DNA amplification.
2. To create an open-source, low-cost hardware and software integrated system that can effectively visualize and quantify the HIV RNA count on a chip.

Thermal Cycler Design

The current thermal cycler design, currently in its fourth prototype, is composed of a ceramic heating element and a fan controlled by an Arduino. The Arduino microcontroller allows the device to cycle between a high and low temperature, required for DNA amplification, and takes frequent temperature measurements from a thermistor to ensure accuracy of temperature. The team is currently working on a more user friendly design.

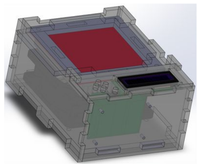

Fig. 5. Thermal Cycler Prototype 3.

Imaging Design

The genetic material quantification design, or the imaging device, illuminates the chip with blue LEDs, and an image of the chip is taken with a monochrome CMOS camera through a green filter. This image is sent to a computer for image analysis. The imager is essentially a very low cost version of a fluorescence microscope.

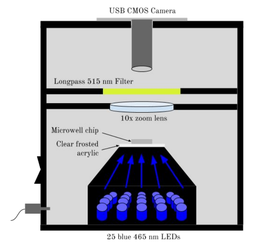

Fig. 6. Imaging Device

Client: Albergue Las Memorias

Tijuana, Mexico

The team is partnering with the Albergue las Memorias clinic for development and clinical trials. In addition, the team plans to partner with students at a local university to establish a network for production and distribution in Mexico.

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