I. Background and Objectives

Increased access to HIV drug resistance testing in resource limited settings is needed to improve treatment monitoring and patient care.

Dried blood spots (DBS) provide an alternative to gold-standard plasma-based genotyping, reducing the need for cold chain transport and skilled laboratory operations. DBS limitations include requirement for training, a relatively long drying process and limited sensitivity.

HemaSpot is a novel dried blood storage device which combines an absorbent paper to hold samples inside a plastic cartridge (Figure 1). Compared to DBS, HemaSpots store slightly larger volumes (80-100µL) and include an internal desiccant and storage system greatly facilitating drying and shipping.

In this study we, for the first time, evaluated the efficacy of HemaSpots for HIV drug resistance testing. We investigated its feasibility in resource rich and resource limited settings, using fresh and frozen blood samples, respectively.

II. Methods

United States (US) Study

A total of 30 fresh blood HemaSpots were prepared in the US from two antiretroviral treatment (ART) naïve patients. Pooled plasma samples were prepared in parallel. For each patient, blood samples were diluted to five concentrations and samples of each concentration were stored for 24 hours, 2 weeks or 4 weeks.

Kenya Study

Fifty-four frozen blood HemaSpots and pooled plasma samples were prepared from HIV-infected patients taking 1st line ART in Eldoret, Kenya (Figure 2) as part of a study at the Academic Model Providing Access to Healthcare (AMPATH) and the Moi Teaching and Referral Hospital (MTRH). AMPATH is largest HIV/AIDS program, MTRH manages 27,800 of AMPATH’s 140,000 HIV-infected patients, 68% of which (19,935) have started ART.

HemaSpot Preparation and Data Analyses

Samples were prepared in the US and Kenya by absorbing 100µL of whole blood onto each HemaSpot. Upon full absorption of the blood by the filter the cartridge was closed, and kept in room temperature for drying or refrigeration or for shipping, according to study design.

Predicators of sequence amplification success (yes/no) were examined for both studies, using methods for correlated outcomes. For the Kenya study, predictors included log VL, CD4 count and the amount of time between study enrollment and sample preparation in Kenya.

Bioinformatic analyses were done with the Sequence Quality Assessment Tool (SQUAT), Molecular Evolutionary Genetics Analysis (MEGA) and Stanford HIV Sequence Database tools. Drug resistance mutation concordance was assessed for all plasma HemaSpots. Mixtures were considered mutations and not counted towards discordance.

To estimate the potential effect of plasma HemaSpot discordant drug resistance mutations, predicted susceptibilities were determined for each ART drug using Stanford Database tools. For this evaluation, a “clinically-relevant discordance” was defined as high or intermediate predicted resistance to at least one ART drug in only one analyzer.

Figure 2: AMPATH Clinical Sites in Western Kenya

IV. Results

US Study

Patient 1 was a 28 year old male with CD4 count <20 cells/µL (CD4% < 5); and Patient 2 was a 22 year old male with CD4 count 300-350 cells/µL (CD4% 10-15).

Both patients had a viral load (VL) of 100,000-150,000 copies/mL.

HemaSpot genotyping was successful in 67% (20/30), at a VL range of 1,000-150,000 copies/mL (Table 1). Of unsuccessfully genotyped HemaSpots, 5/10 (50%) had VL<5,000 copies/mL (genotyping success of 78% above this VL), and 6/10 (80%) had VL>5,000 copies/mL (genotyping success of 81% above this VL).

The odds of successful HemaSpot genotyping were associated with higher VL (OR=23.0 per 1 log unit higher VL, CI=1.98 to 270.1, p<0.05) and shorter storage times at both 2 weeks (OR=0.01 CI=0 to 0.79, p<0.04) and 4 weeks (OR=0.03 CI=0 to 1.40, p<0.08) compared with 24 hours.

HemaSpot and plasma paired sequences clustered well phylogenetically with high (>98%) bootstrap values (Figure 3).

Two drug resistance mutation mixtures were detected in two HemaSpots, neither of which was detected in plasma.

Mean HemaSpot plasma nuclear acid genetic distances were 1.3% in protease and 1.4% in reverse transcriptase.

Kenya Study

Demographic, laboratory and study data of participants are shown in Table 2.

Genotyping was successful in 35% (19/54) of HemaSpots, prepared with frozen blood after median storage of 141 days (range 31-246) for 100,000-150,000 copies/mL of paired plasma samples (p=0.05). Of unsuccessfully genotyped HemaSpots, 22/35 (63%) had VL<5,000 copies/mL (genotyping success of 58% above this VL) and 27/35 (77%) had VL>5,000 copies/mL (genotyping success of 68% above this VL).

In unfavorable analyses, HemaSpot genotyping was more successful for samples with higher VL (p<0.001) and lower CD4 count (p=0.002) (Figure 4). In multivariate analyses, the odds of successful HemaSpot genotyping were associated with higher VL (OR=0.02 per 1 log unit higher VL, CI=0.32 to 1.24, p=0.001) and shorter storage times at 2 weeks (OR=1.5 for time <6 months versus >6 months, 95% CI=1.01 to 2.28, p=0.04) and 4 weeks (OR=0.03 CI=0 to 1.40, p<0.08) compared with 24 hours.

HemaSpot and plasma paired sequences clustered well phylogenetically with high (>95%) bootstrap values (Figure 5).

Full drug resistance mutation concordance was seen in 10/19 (53%) patients (Table 3). Only 4/19 (21%) had more than one discordant mutation between analyte pairs.

90 mutations found in 19 plasma sequences, 77/90 (85%) were seen in paired HemaSpots. Of 86 mutations found in 19 HemaSpot sequences, 74/86 (85%) were seen in paired plasma sequences. Mutation detection did not differ significantly between plasma and HemaSpot sequences.

Of the 19 patients for whom HemaSpot/plasma paired sequences were available, 12 (63%) had complete concordance in their predicted susceptibility to all drugs.

The clinical impact of discordant resistance was low (3/19 (16%) with high or intermediate resistance to at least one drug in one analyzer, see Table 3).

Mean HemaSpot plasma nucleic acid genetic distances were 1.1% in protease and 1.1% in reverse transcriptase.

IV. Summary and Conclusions

We demonstrate for the first time successful HIV genotyping from HemaSpot, a novel dried blood storage device.

Good quality sequences were obtained from HemaSpot samples in the US and Kenya with a wide range of VL, using both fresh (HemaSpot) and fresh blood with prolonged storage at ambient temperatures (US).

Genotyping success rates were better with higher VLs and shorter storage times in both studies. Observed differences in genotyping success rates by use of fresh vs. frozen blood should be further evaluated.

HemaSpot/plasma sequence comparison demonstrated identical subtype determination, comparable drug resistance mutation profiles and predicted drug susceptibilities, and high overall sequence concordance.

HemaSpot samples preparation, drying, storage and shipping procedures offered logistical improvements compared to DBS and plasma.

Table 1: 1 Viral Load and Genotyping Success of Fresh Blood Samples.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Plasma Successful</th>
<th>HemaSpot Successful</th>
<th>Successful Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Load (VL&lt;5,000)</td>
<td>Yes</td>
<td>Yes</td>
<td>87%</td>
</tr>
<tr>
<td>High Load (VL&gt;5,000)</td>
<td>No</td>
<td>Yes</td>
<td>68%</td>
</tr>
</tbody>
</table>

Figure 3: Maximum Likelihood Tree of Plasma and HemaSpot Sequences

Table 2: Demographic, Laboratory and Study Data According to Genotype Success

<table>
<thead>
<tr>
<th>Analizer</th>
<th>Successful Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Female Gender</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
</tr>
<tr>
<td>Successful Plasma Samples</td>
<td></td>
</tr>
<tr>
<td>Successful HemaSpot Samples</td>
<td></td>
</tr>
<tr>
<td>Successful Rate (VL&lt;5,000)</td>
<td></td>
</tr>
<tr>
<td>Successful Rate (VL&gt;5,000)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Drug Resistance Mutations in HemaSpot and Plasma Sequence Pairs from the Kenya Study According to Analyte Discordance

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Successful Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
</tr>
<tr>
<td>HemaSpot</td>
<td></td>
</tr>
<tr>
<td>Successful Rate (VL&lt;5,000)</td>
<td></td>
</tr>
<tr>
<td>Successful Rate (VL&gt;5,000)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4: Genotype Success of Kenya Samples at Different VLs by Analyte

Figure 5: Maximum Likelihood Tree of Plasma and HemaSpot Sequences

Table 4: Maximum Likelihood Tree of Plasma and HemaSpot Sequences