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HemaSpot, a Novel Blood Storage Device for HIV-1 Drug Resistance Testing

K. Brooks,[a] A. DeLong,[b] M. Balamane,[a] L. Schreier,[a] M. Orido,[c] M. Chepkenja,[c] E. Kemboi,[c] M. D'Antuono,[a] P. A. Chan,[a] W. Emonyi,[c,d] L. Diero,[c,d] M. Coetzer,[a] R. Kantor[a]

Division of Infectious Diseases, Alpert Medical School, Brown University, Providence, Rhode Island, USA[a]; Center for Statistical Sciences, School of Public Health, Brown University, Providence, Rhode Island, USA[b]; Academic Model Providing Access to Healthcare (AMPATH), Eldoret, Kenya[c]; Department of Medicine, School of Medicine, College of Health Sciences, Moi University, Eldoret, Kenya[d]

HemaSpot, a novel dried-blood storage filter device, was used for HIV-1 *pol* resistance testing in 30 fresh United States blood samples and 54 previously frozen Kenyan blood samples. Genotyping succeeded in 79% and 58% of samples, respectively, improved with shorter storage and higher viral load, and had good (86%) resistance mutation concordance to plasma.

Drug resistance is a major challenge to sustained treatment success, particularly in resource-limited settings (RLS) with few antiretroviral therapy (ART) regimens and limited access to virologic monitoring and drug resistance testing (DRT) (1, 2). Availability of these tests is restricted mostly due to cost and expertise.

HemaSpot is a novel dried-blood storage device that combines an absorbent paper to hold samples and a desiccant to maintain dryness, within a plastic cartridge (Spot On Sciences, Austin, TX). To date, HemaSpots had only been applied to *Leishmania* diagnosis via antibody detection in dogs, showing high sensitivity and specificity (3). We examine the potential of HemaSpot use for HIV-1 DRT.

HemaSpot DRT was evaluated using fresh blood in the United States study (30 samples from two patients with various storage times and viral load [VL] dilutions) and previously frozen blood in the Kenya study (54 samples from patients failing first-line ART). Sequences were compared to those derived from plasma. Further study design and laboratory and data analysis methods are given in the supplemental material.

In the U.S. study, genotyping was successful in 67% (20/30) of HemaSpots at all tested time points, with detectable VL (range, 1,000 to 100,000 copies/ml) (Table 1), 79% with a VL of >1,000 copies/ml, and 83% with a VL of >5,000 copies/ml (see Fig. S1 in the supplemental material). Odds of successful genotyping were 23.1 times higher for each 1-log-unit-higher VL (confidence interval [CI], 1.98 to 270.1; $P = 0.01$). Genotyping success was significantly lower at 2 weeks (50%) of storage compared to 24 h (90%; odds ratio [OR], 0.01; CI, 0.00 to 0.78; $P = 0.04$) and marginally significantly lower at 4 weeks (60%; OR, 0.03; CI, 0.00 to 1.40; $P = 0.075$), with very small odds ratios.

In the Kenya study (Table 2), genotyping was successful in 35% (19/54) of HemaSpots with detectable VL (range, 110 to 1,175,462 copies/ml) and in 65% (35/54) from paired plasma samples (VL range, 41 to 1,175,462 copies/ml), including all 19 for which HemaSpot genotyping was successful. Using a cutoff VL of >1,000 copies/ml, genotyping was successful in 58% of HemaSpots (89% in plasma), and for a VL of >5,000 copies/ml, genotyping was successful in 68% (94% in plasma).

Plasma samples had 7.29 times the odds of successful amplification (95% CI, 2.68 to 19.83; $P < 0.001$) compared to HemaSpots. Additionally, successful amplification was related to higher VL (OR, 4.50 per 1-log-unit-higher $\log_{10}$ VL; CI, 2.32 to 8.70; $P < 0.001$) and shorter storage time (OR, 0.11 for time of >8 months versus ≤8 months; 95% CI, 0.03 to 0.41; $P = 0.001$). The interaction between analyte type and VL was insignificant, indicating the relationship between VL and amplification success did not significantly differ between HemaSpot and plasma.

In the U.S. study, no resistance mutations were detected in plasma, but two mutation mixtures of wild-type and mutated viral populations were detected in HemaSpots from patient 1 (T215AT at week 2 and V75VL at week 4).

Of 19 patients with HemaSpot and plasma sequences in the Kenya study, 17 (89%) had a total of 90 *pol* resistance mutations in plasma. Of those, 77/90 (86%) in 16/19 (84%) patients were detected in HemaSpots. Of 13 mutations (in seven patients) not detected in HemaSpots, four (31%) were mixtures. Nine additional mutations (in five patients) were detected in HemaSpots, not in plasma (3/9 [33%] mixtures) (see Table S1 in the supplemental material).

HemaSpot sequences were of good quality, and HemaSpot-plasma paired sequences formed expected phylogenetic clusters with high (>95%) bootstraps. Mean plasma-HemaSpot nucleic acid percentages of discordance for protease and reverse transcriptase sequence pairs were 1.7% (range, 0 to 3.4%) and 1.8% (range, 0 to 3.9%) in the U.S. study and 1.0% (range, 0 to 3.7%) and 0.97% (range, 0 to 2.9%) in the Kenya study (see Table S2 in the supplemental material). This high concordance was only slightly lower than plasma-plasma intrapatient sequence pairs ob-

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Address correspondence to R. Kantor, rkantor@brown.edu.

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TABLE 1 Viral load and HemaSpot genotyping success of fresh blood samples in the U.S. study

Viral load (copies/ml)[a]	Time point	Genotyping success
Patient 1		
~100,000	24 h	Yes
	2 wk	Yes
	4 wk	Yes
~50,000	24 h	Yes
	2 wk	Yes
	4 wk	Yes
~20,000	24 h	Yes
	2 wk	Yes
	4 wk	Yes
~10,000	24 h	Yes
	2 wk	No
	4 wk	Yes
~1,000	24 h	No
	2 wk	No
	4 wk	No
Patient 2		
~100,000	24 h	Yes
	2 wk	Yes
	4 wk	Yes
~50,000	24 h	Yes
	2 wk	No
	4 wk	Yes
~20,000	24 h	Yes
	2 wk	No
	4 wk	No
~10,000	24 h	Yes
	2 wk	Yes
	4 wk	No
~1,000	24 h	Yes
	2 wk	No
	4 wk	No

[a] Real VL values are rounded to protect patient confidentiality.

tained within 7 days of each other at the Immunology Center (data not shown).

Full concordance of resistance mutations was seen in 53% of patients (12/19) (see Table S1 in the supplemental material). Discordance was not explained by differential mutation detection between analytes (mutations in plasma only 1.05 times the number of mutations in HemaSpots by Poisson regression; rate ratio [RR] CI, 0.92 to 1.20; $P = 0.50$). Only three (16%) patients (shown by asterisks in Table S1) had clinically relevant differences between analytes, defined as high or intermediate resistance levels to ≥1 antiretroviral drug in one analyte type but not the other, based on Stanford Database tools (hivdb.stanford.edu).

Similarly to dried blood spots (DBS), HemaSpots are applicable to DRT in settings where conventional frozen plasma use is limited (4). HemaSpots are advantageous in their limited requirements for sample preparation, technical skills, training, drying, storing, and shipping. They contain integrated desiccants, the option to splice into 10 separable segments for multiple testing, and the ability to store large volumes (80 to 100 μl versus 50 to 75 μl per spot in DBS), with even higher volumes (200 μl) in development (HemaSpot-DS; personal communication, Spot On Sciences). The unique, nonconventional, use of frozen blood on a filter-based analyte is a deviation from the HemaSpot instructions for use but is an additional benefit, which can further conserve resources.

HIV-1 successful DBS amplification rates in RLS for a VL of >1,000 copies/ml vary and range from 42% to 96% (average, 64%) (5–9). Genotyping success with HemaSpots at a VL of >1,000 copies/ml was within this range, with higher success in higher VLs. Lower amplification success in HemaSpots versus plasma and in frozen versus fresh blood may be associated with lower sample volumes, nucleic acid degradation due to storage conditions, and technical extraction difficulties (4, 10, 11).

High sequence and resistance mutation concordance data are similar to those reported on DBS-plasma sequences (4, 8, 12). Such small discordances, which may be unavoidable, might be related to PCR, storage time and RNA degradation, VL, and exclusive existence of proviral DNA in filter-based analytes (11, 13).

Further exploration of DRT with HemaSpots is needed. In this initial assessment, for instance, rotation of HemaSpot filter papers in lysis tubes promoted their disintegration and complicated their removal from the buffer. Modification to only gentle manual agitation in the buffer improved outcomes significantly. Other possible improvements include modifications to RNA extraction, working on filter paper stability, and using >1 HemaSpot per patient to increase sample input volume and amplification sensitivity (6, 14).

The main study limitations include its preliminary nature and small sample size. Additionally, time from HemaSpot preparation to extraction in Kenya was not varied, and factors that can affect sample degradation, such as sample mishandling, repetitive and lengthy freeze-thawing, and prolonged exposure to higher temperatures, were not completely controlled.

In summary, this is the first demonstration of successful HIV-1 (subtypes A, B, C, and D) *pol* genotyping from HemaSpot, a novel dried-blood storage device, using fresh and frozen blood samples in diverse U.S. and Kenya settings. High HemaSpot-plasma sequence and resistance mutation detection concordance were demonstrated. Genotyping success rates varied by storage time and VL, and HemaSpot sample preparation and shipping procedures offered logistical improvements over plasma and DBS in terms of ease of operation and transport. Although further larger studies are required and improvements to sensitivity are needed, HemaSpot is a promising technology to be evaluated for the increasingly utilized HIV-1 DRT in RLS.

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TABLE 2 Demographic, laboratory and subtype data in the Kenya study according to genotyping success

Parameter	Result for: Total HemaSpots prepared ($n = 54$)	Unsuccessful plasma and HemaSpot genotypes ($n = 19$)	Successful plasma and HemaSpot genotypes ($n = 19$)	Successful plasma but unsuccessful HemaSpot genotypes ($n = 16$)	P value[a]
Age, yr (range)[b]	38 (23–82)	39 (28–64)	37 (27–82)	36 (23–67)	0.802*
Female, no./total (%)	32/54 (59)	11/19 (58)	11/19 (58)	10/16 (62)	1.000*
CD4 count, cells/μl (range)[b]	216 (5–869)	250 (49–759)	74 (5–423)	258 (13–869)	0.002*
CD4, % (range)[b]	14 (1–48)	17 (7–48)	9 (1–28)	15 (2–39)	0.016*
Viral load; copies/ml (range)[b]	3,671 (41–1,175,462)	107 (46–35,551)	58,286 (110–1,175,462)	4,404 (41–150,649)	<.001*
Time between collection and HemaSpot preparation, mo (range)[b]	4.6 (1.2–12.0)	4.2 (1.3–12.0)	4.5 (1.2–9.1)	4.7 (1.3–8.6)	0.77
HIV-1 subtype, no./total (%)					1.000**
A	NA[c]	NA	11/19 (58)	11/16 (68)	
C	NA	NA	3/19 (16)	2/16 (13)	
D	NA	NA	3/19 (16)	2/16 (13)	
AD recombinants, no./total (%)	NA	NA	2/19 (11)	1/16 (6)	

[a] *, P values from comparison of the 19 samples with successful HemaSpot genotypes with the 35 samples without HemaSpot genotypes; **, P value from comparison of the 19 samples with successful HemaSpot genotypes with the 16 samples with successful plasma genotypes but no HemaSpot genotype.
[b] Values are presented as median (range) for continuous variables.
[c] NA, not available.

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