Published in final edited form as:

AIDS. 2017 May 15; 31(8): 1191–1193. doi:10.1097/QAD.0000000000001477.

Detection of HIV RNA in Dried Blood Spots and Oral Fluids

Joanne D. Stekler^{1,2}, George Ure¹, Joan Dragavon³, Ming Chang³, and Robert W. Coombs^{1,3}

¹Department of Medicine, University of Washington, Seattle, WA

²Department of Epidemiology, University of Washington, Seattle, WA

³Department of Laboratory Medicine, University of Washington, Seattle, WA

Abstract

We evaluated detection of HIV-1 RNA from dried blood spots (DBS) and oral fluid specimens. Between February 2010 and August 2014, HIV-1 was newly diagnosed in eight (2.6%) subjects who had median blood HIV-1 RNA of 61,500 copies/mL (IQR 7500-146,000). RNA was detected in 7 (87.5%) DBS and 3 (37.5%) oral fluid swabs but was not detected in either specimen from one participant. DBS may be a reasonable specimen collection method to detect acute infection.

Keywords

HIV testing; rapid HIV test; oral fluids

Background

There has been increasing appreciation for the need to employ HIV-1 tests able to detect acute infection during the antibody-negative “window period” [1]. Beginning in 2001, several health departments created pooled nucleic acid amplification testing (NAAT) programs to increase acute infection case-finding [2-5]. However, there are barriers to widespread implementation of HIV-1 NAAT, including the fact that high risk persons prefer less invasive specimen collection methods over venipuncture [6]. In developing countries, needs for venipuncture, sample storage, and advanced technology are other potential barriers.

In 2010, we began a prospective, cross-sectional study to compare the ability of point-of-care (POC) and laboratory-based HIV-1 tests to detect acute and early infection in real-time [6-8]. This report describes findings from a pilot substudy to evaluate the performance of NAAT on non-venipuncture specimens that could be collected at home or other settings without immediate access to NAAT.

Study Design

Population

Men and transgender persons reporting sex with men in the prior year were recruited when seeking HIV testing at the Public Health – Seattle & King County Sexually Transmitted Disease Clinic. The University of Washington Institutional Review Board approved this study, and participants gave verbal consent. An additional \$20 was given for substudy participation. Persons testing HIV-negative could participate quarterly.

Specimen Collection and Testing

HIV screening procedures are described elsewhere [6-8]. Oral fluid was collected using a flocked swab (Copan Diagnostics) rubbed twice in the buccal sulcus between the top and bottom teeth and gums and inserted into a 2-mL cryovial. Swabs were transported within 60 minutes of collection to the UW Retrovirology Laboratory and immediately placed on dry ice until they could be stored at -80° C. Based on our preliminary work (data not shown), HIV-1 NAAT had lower yield when performed on saliva compared to oral mucosal surface fluid collected as described here.

Dried blood spots (DBS) were collected through fingerstick whole blood specimens into a 50µL microcollection pipette and dispensed onto a DBS card (Whatman 903 protein saver card; GE Healthcare). DBS were allowed to dry undisturbed for 24-72 hours at ambient temperature and were placed into a foil packet with desiccant pouch and humidity indicator card (Humonitor MS20003-2; Multisorb Technologies Inc.).

Frozen specimens from all HIV-infected participants, including antibody-positive and antibody-negative persons, were selected for batch testing after enrollment was complete. An equal number of specimens from HIV-negative participants were tested to blind laboratory staff to HIV status.

One DBS was excised using a laser cutter [9] and soaked in 2.5 mL lysis buffer (bioMerieux) with agitation for two hours. The lysate then was quantified using the Abbott RealTime HIV-1 assay and the result was corrected for a dilution factor of 50 to give an estimated HIV-1 RNA copies/mL of blood [10].

Data Analysis

We conducted chart reviews for participants with discordant test results to confirm HIV-1 infection. Participants with reactive EIA and positive Western blot or detectable HIV-1 RNA were considered to have confirmed infection. Analyses were descriptive, focusing on mean, median, and range of HIV-1 RNA levels detected in blood, DBS, and oral fluid swabs of persons with acute and established infection.

Results

Between February 22, 2010 and August 1, 2014, there were 3438 study visits through the main study; 3407 visits were by men, 24 visits were by transgender women, and seven visits were by transgender men [8]. Table 1 shows demographic and HIV test results of the eight

(2.6%) participants newly diagnosed with HIV-1 infection among 308 substudy visits. Two had acute HIV-1 infection (EIA-negative), two had discordant POC test results, and four tested positive on all HIV tests performed. These subjects had a median blood plasma or serum HIV-1 RNA of 61,500 copies/mL (IQR 7500-146,000, range 6000-347,000); HIV-1 RNA was detected in 7 (87.5%, 95% CI 47.3-99.7%) DBS and 3 (37.5%, 95% CI 8.5-75.5%) oral fluid swabs. HIV-1 RNA was not detected in either DBS or oral fluid from one participant with established infection. All HIV-negative specimens tested negative [data not shown].

Discussion

These data suggest that HIV-1 NAAT performed on DBS may be a relatively accurate substitute for performance on blood specimens but do not support the use of oral fluids. This observation, in conjunction with our prior work which showed that HIV antibody testing performed on oral fluids is significantly less accurate than testing on fingerstick whole blood [7, 11], suggests that oral fluid specimens should not be prioritized in the development of new testing technologies.

Prior studies of oral fluids and saliva have shown greater yield [12-16]. HIV-1 RNA levels in oral fluids are 10- to 100-fold lower than in blood plasma [12, 13, 16] and may be less than 10,000 copies/mL, even during acute HIV-1 infection [12]. However, in one study, HIV-1 RNA was detected in saliva of three untreated subjects who had undetectable blood plasma HIV RNA levels [16].

Our study had several limitations that were primarily due to small numbers. We recruited persons not previously diagnosed with HIV-1 infection in order to enroll individuals naïve to antiretroviral therapy and identify persons with acute infection. It was particularly challenging to recruit persons with established infection who had just received a new HIV-1 diagnosis through the main study.

Our results suggest that research into HIV testing using DBS should be advanced. Home self-testing for HIV has potential to reach HIV-positive persons who would not otherwise test for HIV. However, self-testing will reduce access to sensitive tests and could result in increased HIV prevalence due to window period testing [17] unless NAAT can be tailored to home specimen collection. Of the 116 men randomized to self-testing in our iTest Study [18], 34 (29%) accepted DBS back-up testing. During the 15-month study period, we received 41 DBS from 17 participants, and all test results were negative [unpublished data]. Pooling DBS eluates could reduce cost and time [19]; however pooling would have resulted in our failure to detect two participants with DBS HIV-1 RNA levels below the level of quantitation. Future work should continue to expand HIV testing and testing options for high-risk populations.

Acknowledgments

We would like to thank everyone who participated in this project.

This study was supported by NIH R01 MH-83630, U01 AI-38858, UM1 AI-068618, UM1 AI-106701, and P30 AI-27757. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Alere provided Determine™ HIV 1/2 Ag/Ab Combo tests and controls throughout the duration of the project.

Sources of support: NIH R01 MH-83630, U01 AI-38858, UM1 AI-068618, and P30 AI-27757 Alere provided Determine™ HIV 1/2 Ag/Ab Combo tests and controls for investigational use.

References

1. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS*. 2003; 17:1871–1879. [PubMed: 12960819]
2. Stekler J, Swenson PD, Wood RW, Handsfield HH, Golden MR. Targeted screening for primary HIV infection through pooled HIV-RNA testing in men who have sex with men. *AIDS*. 2005; 19:1323–1325. [PubMed: 16052089]
3. Pilcher CD, Fiscus SA, Nguyen TQ, Foust E, Wolf L, Williams D, et al. Detection of acute infections during HIV testing in North Carolina. *N Engl J Med*. 2005; 352:1873–1883. [PubMed: 15872202]
4. Klausner JD, Grant RM, Kent CK. Detection of acute HIV infections. *N Engl J Med*. 2005; 353:631–633. author reply 631–633.
5. Priddy FH, Pilcher CD, Moore RH, Tambe P, Park MN, Fiscus SA, et al. Detection of acute HIV infections in an urban HIV counseling and testing population in the United States. *J Acquir Immune Defic Syndr*. 2007; 44:196–202. [PubMed: 17312561]
6. O'Neal JD, Golden MR, Branson BM, Stekler JD. HIV nucleic acid amplification testing versus rapid testing: it is worth the wait. Testing preferences of men who have sex with men. *J Acquir Immune Defic Syndr*. 2012; 60:e117–120. [PubMed: 22772351]
7. Stekler JD, O'Neal JD, Lane A, Swanson F, Maenza J, Stevens CE, et al. Relative accuracy of serum, whole blood, and oral fluid HIV tests among Seattle men who have sex with men. *J Clin Virol*. 2013; 58 Suppl 1:e119–122. [PubMed: 24342471]
8. Stekler JD, Ure G, O'Neal JD, Lane A, Swanson F, Maenza J, et al. Performance of Determine Combo and other point-of-care HIV tests among Seattle MSM. *J Clin Virol*. 2016; 76:8–13. [PubMed: 26774543]
9. Murphy SC, Daza G, Chang M, Coombs R. Laser cutting eliminates nucleic acid cross-contamination in dried-blood-spot processing. *J Clin Microbiol*. 2012; 50:4128–4130. [PubMed: 23052309]
10. Chang, M., Dragavon, J., Hart, S., Selle, A., Murphy, S., Michaels, S., et al. Thirtieth Annual Clinical Virology Symposium. Daytona Beach, Florida. In: 2014. Application of a 4th Generation HIV Diagnostic Testing Algorithm Using Finger-Prick Dried Blood Spot Cards.
11. Stekler JD, Swenson PD, Coombs RW, Dragavon J, Thomas KK, Brennan CA, et al. HIV testing in a high-incidence population: is antibody testing alone good enough? *Clin Infect Dis*. 2009; 49:444–453. [PubMed: 19538088]
12. Pilcher CD, Shugars DC, Fiscus SA, Miller WC, Menezes P, Giner J, et al. HIV in body fluids during primary HIV infection: implications for pathogenesis, treatment and public health. *AIDS*. 2001; 15:837–845. [PubMed: 11399956]
13. Liuzzi G, Chirianni A, Clementi M, Bagnarelli P, Valenza A, Cataldo PT, et al. Analysis of HIV-1 load in blood, semen and saliva: evidence for different viral compartments in a cross-sectional and longitudinal study. *AIDS*. 1996; 10:F51–56. [PubMed: 8970677]
14. Nugent, C., Kolk, D., Giachetti, C., Busch, M., Hecht, F. Detection of HIV-1 in saliva using the APTIMA HIV-1 RNA Qualitative Assay. 14th Conference on Retroviruses and Opportunistic Infections; Los Angeles, CA. February 25–28, 2007; abstract # 679
15. Zuckerman RA, Whittington WL, Celum CL, Collis T, Lucchetti A, Sanchez JL, et al. Factors associated with oropharyngeal human immunodeficiency virus shedding. *J Infect Dis*. 2003; 188:142–145. [PubMed: 12825183]

16. Balamane M, Winters MA, Dalai SC, Freeman AH, Traves MW, Israelski DM, et al. Detection of HIV-1 in Saliva: Implications for Case-Identification, Clinical Monitoring and Surveillance for Drug Resistance. *Open Virol J.* 2010; 4:88–93. [PubMed: 21673840]
17. Katz D, Cassels S, Stekler J. Replacing clinic-based tests with home-use tests may increase HIV prevalence among Seattle men who have sex with men: evidence from a mathematical model. *Sex Transm Dis.* 2013 in press.
18. Katz DA, Golden MR, Stekler JD. Use of a home-use test to diagnose HIV infection in a sex partner: a case report. *BMC Res Notes.* 2012; 5:440. [PubMed: 22894746]
19. Sarge-Njie R, Schim Van Der Loeff M, Ceesay S, Cubitt D, Sabally S, Corrah T, et al. Evaluation of the dried blood spot filter paper technology and five testing strategies of HIV-1 and HIV-2 infections in West Africa. *Scand J Infect Dis.* 2006; 38:1050–1056. [PubMed: 17148076]

Table 1
Clinical and Research HIV Test Results for 8 HIV-Infected Study Participants

Age	Race	OraQuick	OraQuick	FS	Uni-Gold	INSTI	Determine	Ag	Ab	3rd gen	WB	WB Bands	Blood RNA	DBS RNA copies/mL	OF RNA
		OF								EIA					
1	25	Black	—	—	ND	—	—	—	—	—	ND		8000s	<2000	Not detected
2	34	White	—	—	—	ND	—	—	—	—	ND		25,000s	71,000	13,000
3	22	Mixed	—	+	ND	+	—	+	+	+	+	18, 24, 31, 41, 51/55, 65, 120, 160	7000s	<2000	Not detected
4	31	White	—	—	ND	+	—	+	+	+	+	24, 51, 55, 160	347,000s	634,000	5000
5	33	Black	+	+	+	ND	—	+	+	+	+	18, 24, 41, 55, 120	167,000p	64,000	Not detected
6	55	White	+	+	+	ND	—	+	+	+	+	18, 24, 31, 41, 51/55, 65, 120, 160	125,000p	5000	Not detected
7	21	White	+	+	+	ND	—	+	+	+	+	18, 31, 41, 55, 65, 120, 160	98,000p	30,000	1000
8	25	Asian	+	+	ND	+	—	+	+	+	+	18, 24, 31, 40, 51/55, 65, 120, 160	6000p	Not detected	Not detected

DBS: Dried blood spot; OF: oral fluids; FS: fingerstick; Ag: antigen; Ab: antibody; EIA: enzyme immunoassay; WB: Western Blot; ND: not done;