JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2004, p. 3847–3849 Vol. 42, No. 8
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.8.3847–3849.2004

Persistence of Human Immunodeficiency Virus Type 1 Subtype B DNA in Dried-Blood Samples on FTA Filter Paper

Chung-Chen Li,[1,2] Ingrid A. Beck,[1] Kristy D. Seidel,[3] and Lisa M. Frenkel[1,4]*

Departments of Pediatrics[1] and Laboratory Medicine,[4] University of Washington, and Department of Biostatistics, Children's Hospital and Regional Medical Center,[3] Seattle, Washington, and Department of Pediatrics, Chang-Gang Children's Hospital, Kaohsiung, Taiwan[2]

Received 22 September 2003/Returned for modification 31 January 2004/Accepted 4 May 2004

The stability of human immunodeficiency virus type 1 (HIV-1) DNA in whole blood collected on filter paper (FTA Card) was evaluated. After >4 years of storage at room temperature in the dark our qualitative assay detected virus at a rate similar to that of our initial test (58 of 60, 97%; $P = 0.16$), suggesting long-term HIV-1 DNA stability.

Passively transferred maternal antibodies persist in infants for up to 18 months after birth, which renders antibody screening ineffective for the early diagnosis of human immunodeficiency virus type 1 (HIV-1) infection in infants (13). Simple, sensitive, specific, and inexpensive assays that detect the virus are needed for optimal management of HIV-1-exposed infants. DNA PCR has been useful in diagnosing HIV-1 infection in young infants (12). Generally these tests are performed on peripheral blood mononuclear cells (PBMC) that have been separated from whole blood by density gradient centrifugation; however, several groups have published methods using whole blood collected on filter paper (3, 5, 10).

Use of filter paper is an attractive alternative to collection of blood in tubes; less blood is needed, immediate processing is not required, the paper can be stored at room temperature, and once dried, the blood samples are not infectious. In addition, filter paper methods require less equipment and less technical expertise and are less expensive. Thus, combined with the DNA PCR, blood collected on filter paper provides a simple approach for HIV-1 diagnosis in infants (5, 7, 9, 10).

To further simplify detection of HIV-1 in whole blood on filter paper, we previously developed an assay using filter paper that lyses cells and binds DNA (FTA Card; Whatman, Inc., Clifton, N.J.), eliminating the need to elute and extract DNA from the filter paper (1). Our assay proved sensitive and specific among HIV-1-exposed infants in Lima, Peru, and children followed in our Seattle HIV Clinic who were deemed HIV-1 infected on the basis of positive enzyme immunoassay and Western blot assay results after 18 months of age. After these specimens were tested, they were stored in a sealed plastic bag inside a laboratory cabinet (no direct light) at room temperature (22 to 24°C) for >4 years in Seattle, Wash.

Knowledge concerning the stability of HIV-1 DNA and RNA in dried-blood samples is required to gauge the validity of both qualitative and quantitative assays when specimen transport is not expeditious or testing is delayed, such as with batched assays used in some clinical studies. While HIV-1 RNA appears stable in dried blood and plasma collected on 903 (Guthrie Card) filter paper (Schleicher & Schuell, Inc., Keene, N.H.) stored at room temperature for 2 weeks to 13 months (2, 4, 11), the stability of HIV-1 DNA in dried blood has been examined only up to 15 weeks (3). To evaluate HIV-1 DNA persistence on FTA Cards, all HIV-1-positive samples from our Seattle clinic collected 4.2 or more years ago (1) were retested.

The repeat assay for HIV-1 DNA in the whole blood collected on FTA Cards was done as previously described (1). Briefly, two 3-mm-diameter disks were punched from the blood spot of each filter paper. The disks were then transferred to separate 0.2-ml PCR tubes, washed in the PCR tube three times with 200 μl of FTA Purification Reagent (Whatman, Inc.) and twice with Tris-EDTA buffer, and then air dried in the same tube. The filter paper disks were subsequently subjected to nested PCR amplification targeting the HIV-1 *pol* gene, with primers RT1 and RT2 as the outer primers and RT3 and RT4 as the inner primers (8). An additional punch of filter paper from each specimen was used to control for the presence of PCR inhibitors by amplification of the human β-globin gene, with primers KM38 and Fluo-GH120 (6) and the same PCR reagents and cycling conditions used to amplify HIV-1 *pol*. Amplicons of 665 bp, containing most of the gene encoding HIV-1 reverse transcriptase, and 350 bp, containing the human β-globin gene, were visualized by ethidium bromide staining in a 1.5% agarose gel. A sample was designated positive if *pol* was amplified in one or both of the duplicate PCRs, negative if *pol* was not amplified in either duplicate but the β-globin gene amplified, and indeterminate when the PCR was inhibited and neither *pol* nor the β-globin gene was amplified. The rate of positive tests and the frequency of positive PCRs in 1998 and those in 2002 and 2003 were compared by McNemar's test and the marginal homogeneity test, respectively.

A comparison of the 1998 and the 2002 and 2003 testing results is shown in Table 1. Sixty subjects tested positive when assayed in 1998, and 58 (97%) of these tested positive for HIV-1 DNA by PCR in 2002 and 2003 (P = not significant). The specimens were free of PCR inhibitors, as β-globin was amplified from all of the specimens at both time points.

When tested in 1998, 50 subjects had a positive *pol* PCR

* Corresponding author. Mailing address: 307 Westlake Ave. N, Suite 300, Seattle, WA 98109. Phone: (206) 987-5140. Fax: (206) 987-3890. E-mail: lfrenkel@u.washington.edu.

TABLE 1. Evaluation of the persistence of HIV-1 DNA in whole blood collected on FTA filter paper by comparison of qualitative nested PCR results of HIV-1 *pol* in specimens collected in 1998 and assayed in 1998 and again in 2002 and 2003

PCR result in 1998	No. of PCR results in 2002 and 2003	
	Positive	Negative
Positive	58[a]	2
Negative	0	0

[a] $P = 0.16$ by McNemar's test.

assay result in both of the duplicate filter paper disks and 10 subjects had the virus amplified in only one of the duplicates (Table 2). In 2002 and 2003, both duplicates were amplified in only 31 of the 50 specimens that had both duplicates positive in 1998. The 10 subjects with one positive duplicate in 1998 tested similarly in 2002 and 2003; both duplicates were amplified in two, one of the duplicates was positive in seven, and both duplicates were PCR negative in the specimen from one subject. Those with persistent dual-positive tests (both the first and second tests with both duplicates PCR reactive) had a higher number of HIV-1 RNA copies per ml of plasma than those with two reactive tests in 1998 and one reactive test in 2002 and 2003 (RNA copy number, 78,099 ± 144,391 versus 7,077 ± 17,958 copies per ml; $P < 0.05$ by Student t test and $P = 0.18$ by Wilcoxon test). Overall, there was a change in the distribution of the three possible PCR outcomes for a specimen when the recent test results were compared to those from 1998 ($P = 0.0007$ by marginal homogeneity test), suggesting that there was a slight decay in the HIV-1 DNA. The decay in HIV-1 DNA appeared minimal, as nearly all (97%) of the specimens were defined as positive in the second test conducted 4.2 or more years after the first assay.

We estimate that ~25,000 PBMC were present in each 3-mm disk punched from blood-saturated filter paper. Assuming reliable amplification of a single copy of HIV-1, application of our assay to one 3-mm-diameter filter paper punch would detect infection when the viral load is ≥40 copies of HIV-1 per 10^6 PBMC (1). Given that untreated infants and children may have viral DNA levels as low as several hundred copies per 10^6 PBMC (14), we recommend that a minimum of two 3-mm disks be evaluated. In the specimens we studied, the distribution of HIV-1 did not appear to be uniform in the filter paper, as two specimens with one of two duplicates positive in 1998 tested positive in both duplicates in 2002 and 2003. It is worth noting that, given that relatively few cells are in each 3-mm disk, the reproducibility of the assay will diminish when the HIV-1 DNA copy number is low, such as when blood is diluted with serous fluid by vigorous squeezing of the infant's heel.

TABLE 2. Frequency of positive PCR results when specimens were amplified in duplicate in 1998 and again in 2002 and 2003 to evaluate decay of HIV-1 DNA in whole blood collected on FTA filter paper and stored at room temperature[a]

Results of duplicate testing in 1998	No. of samples with following duplicate testing results in 2002 and 2003:			Total no. of samples
	+/+	+/−	−/−	
+/+	31	18	1	50
+/−	2	7	1	10
−/−	0	0	0	0
Total	33	25	2	60

[a] Two 3-mm-diameter punches of filter paper from each specimen were tested Symbols: +/+, positive in both duplicates; +/−, positive in one of the duplicates; −/−, negative in both duplicates. Distribution of positive (+) PCR amplification differed significantly over time ($P = 0.0007$ by marginal homogeneity [Stuart-Maxwell] test).

Both RNA and DNA PCRs have been used to diagnose HIV-1 infection in young children. HIV-1 RNA appeared stable in plasma dried on 903 filter paper following storage for 2 weeks at 20°C (4) and in a separate study for up to 1 year when stored at room temperature or at −70°C (2). However, as discussed above collection of whole blood from infants is more convenient than collection and processing of plasma. HIV-1 RNA quantified from whole blood dried on 903 filter paper stored at −70°C for up to 13 months had values similar to those of plasma and whole blood collected in test tubes and stored in lysis buffer (11) and for up to a year when stored at room temperature or at −70°C (2).

The stability of HIV-1 DNA has not been evaluated extensively. HIV-1 DNA was detected on 903 filter paper after 15 weeks of storage at 22°C (3) but was not evaluated after additional time had passed. Data from our study indicate that HIV-1 DNA can persist in filter paper for extended periods of time.

In conclusion, our study demonstrated that HIV-1 DNA in whole blood stored on FTA Card filter paper at room temperature for 4.2 or more years decayed slightly. This decay, however, was minimal, such that 97% of the specimens retained sufficient viral DNA to test positive in our qualitative assay. These data are relevant to patient care and clinical trials and suggest that whole blood stored on FTA filter paper would provide a simple and economical method for collection and storage of specimens for later testing.

REFERENCES

1. **Beck, I. A., K. D. Drennan, A. J. Melvin, K. M. Mohan, A. M. Herz, J. Alarcón, J. Piscoya, C. Velázquez, and L. M. Frenkel.** 2001. Simple, sensitive, and specific detection of human immunodeficiency virus type 1 subtype B DNA in dried blood samples for diagnosis in infants in the field. J. Clin. Microbiol. **39:**29–33.
2. **Brambilla, D., C. Jennings, G. Aldrovandi, J. Bremer, A. M. Comeau, S. A. Cassol, R. Dickover, J. B. Jackson, J. Pitt, J. L. Sullivan, A. Butcher, L. Grosso, P. Reichelderfer, and S. A. Fiscus.** 2003. Multicenter evaluation of use of dried blood and plasma spot specimens in quantitative assays for human immunodeficiency virus RNA: measurement, precision, and RNA stability. J. Clin. Microbiol. **41:**1888–1893.
3. **Cassol, S., T. Salas, M. Gill, M. Montpetit, J. Rudnik, C. Sy, and M. O'Shaughnessy.** 1992. Stability of dried blood spot specimens for detection of human immunodeficiency virus DNA by polymerase chain reaction. J. Clin. Microbiol. **30:**3039–3042.
4. **Cassol, S., M. J. Gill, R. Pilon, M. Cormier, R. F. Voigt, B. Willoughby, and J. Forbes.** 1997. Quantification of human immunodeficiency virus type 1 RNA from dried plasma spots collected on filter paper. J. Clin. Microbiol. **35:**2795–2801.
5. **Comeau, A. M., J. Pitt, G. V. Hillyer, S. Landesman, J. Bremer, B. Chang, J. Lew, J. Moye, G. F. Grady, and K. MacIntosh.** 1996. Early detection of human immunodeficiency virus on dried blood spot specimens: sensitivity across serial specimens. J. Pediatr. **129:**111–118.
6. **Coutlée, F., Y. He, P. Saint-Antoine, C. Olivier, and A. Kessous.** 1995. Coamplification of HIV type 1 and β-globin gene DNA sequences in a nonisotopic polymerase chain reaction assay to control for amplification efficiency. AIDS Res. Hum. Retrovir. **11:**363–371.
7. **DeVange Panteleeff, D., G. John, R. Nduati, D. Mbori-Ngacha, B. Richardson, J. Kreiss, and J. Overbaugh.** 1999. Rapid method for screening dried blood samples on filter paper for human immunodeficiency virus type 1 DNA. J. Clin. Microbiol. **37:**350–353.
8. **Edelstein, R., D. Nickerson, V. Tobe, L. Manns-Arcuino, and L. M. Frenkel.**

1998. Oligonucleotide ligation assay for detecting mutations in the human immunodeficiency virus type 1 *pol* gene that are associated with resistance to zidovudine, didanosine, and lamivudine. J. Clin. Microbiol. **36:**569–572.
9. **Kunisada, T., S. Ando, K. Saito, Y. Eshita, W. Roder, M. Kruse, W. E. G. Muller, and H. Ushijima.** 1994. Detection of human immunodeficiency virus-1 nucleic acids on inactivated filter paper disks by polymerase chain reaction and microtiter plate assay. Microbiol. Immunol. **38:**649–654.
10. **Nyambi, P. N., K. Fransen, H. De Beenhouwer, E. N. Chomba, M. Temmerman, J. O. Ndinya-Achola, P. Piot, and G. van der Groen.** 1994. Detection of human immunodeficiency virus type 1 (HIV-1) in heel prick blood on filter paper from children born to HIV-1-seropositive mothers. J. Clin. Microbiol. **32:**2858–2860.
11. **O'Shea, S., J. Mullen, K. Corbett, I. Chrystie, M. L. Newell, and J. E. Banatvala.** 1999. Use of dried whole blood spots for quantitation of HIV-1 RNA. AIDS **13:**630–631.
12. **Owens, D. K., M. Holodniy, T. McDonald, J. Scott, and S. Sonnad.** 1996. A meta-analytic evaluation of the polymerase reaction for the diagnosis of HIV infection in infants. JAMA **275:**1342–1348.
13. **Rakusan, T. A., R. H. Parrott, and J. L. Sever.** 1991. Limitations in the laboratory diagnosis of vertically acquired HIV infection. J. Acquir. Immune Defic. Syndr. **4:**116–121.
14. **Tetali, S., E. Abrams, S. Bakshi, M. Paul, N. Oyaizu, and S. Pahwa.** 1996. Virus load as a marker of disease progression in HIV-infected children. AIDS Res. Hum. Retrovir. **12:**669–675.