JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2008, p. 1104–1105 Vol. 46, No. 3
0095-1137/08/$08.00+0 doi:10.1128/JCM.02259-07

Comparison of Results Obtained with Amplicor HIV-1 DNA PCR Test Version 1.5 Using 100 versus 500 Microliters of Whole Blood▽

Estelle Piwowar-Manning,[1,2] Lebah Lugalia,[1,2] Bosco Kafufu,[2] and J. Brooks Jackson[1,2]*

Department of Pathology, Johns Hopkins Medical Institutions, 600 North Wolfe Street, Baltimore, Maryland 21287,[1] *and Makerere University-Johns Hopkins University Research Collaboration Laboratory, Old Mulago Hill Road, Kampala, Uganda*[2]

Received 21 November 2007/Returned for modification 19 December 2007/Accepted 4 January 2008

The Amplicor HIV-1 DNA PCR assay (Roche Diagnostics, Branchburg, NJ) requires 500 μl of whole blood for a diagnosis of human immunodeficiency virus type 1 (HIV-1) infection, and this amount is often difficult to obtain from infants. A comparison was performed using 100 and 500 μl of whole blood from infants less than 18 months of age. The concordance rate for HIV DNA PCR-negative and -positive samples was 100% for the two different volumes.

The Amplicor HIV-1 DNA PCR assay (Roche Diagnostics, Branchburg, NJ) has been used in clinical trials and in clinical practice for the diagnosis of human immunodeficiency virus (HIV) infection in infants less than 18 months of age who were born to HIV-infected mothers (1). In May 2005, Roche Diagnostics replaced the version 1.0 Amplicor HIV-1 DNA PCR assay with the version 1.5 assay, which has been shown to have excellent sensitivity and specificity in testing adult venous blood samples and infant dried blood spots (2, 3). Unlike the version 1.0 package insert, the manufacturer's version 1.5 package insert does not address the issue of testing infant samples with respect to the volume of blood that can be used (Amplicor HIV-1 DNA test version 1.5 package insert; Roche Molecular Systems, Inc., Branchburg, NJ).

The Roche Amplicor HIV-1 DNA test version 1.0 required a 100-μl blood volume from infants less than 18 months of age and 500 μl from infants greater than 18 months of age. However, according to the manufacturer's new version 1.5 package insert, 500 μl of blood is required regardless of age. As it is often difficult to obtain 500 μl of blood or more from infants who may require multiple tests on a small volume of blood, the objective of this study was to validate the use of 100 μl of blood for infants under 18 months of age in the version 1.5 assay by comparing the concordance rates of positivity and negativity for the two different blood volumes.

In this study, blood samples were obtained from 38 babies previously determined by PCR (using 500 μl of whole blood) to be positive for HIV type 1 (HIV-1) DNA and 16 HIV-1 DNA PCR-negative babies aged 6 weeks to 18 months who visited the clinics at Mulago Hospital, Kampala, Uganda, and whose mothers or guardians gave consent for HIV DNA testing. Approximately 1 ml of blood from each baby was collected in EDTA anticoagulant and transported to the Makerere University-Johns Hopkins University Uganda Core Laboratory. The processing technician at the core laboratory made one or more cell pellets from 100- and 500-μl aliquots of each blood sample (Table 1). Three milliliters of specimen wash buffer was used to prepare and wash each cell pellet from 100 or 500 μl of whole blood. The Amplicor HIV-1 DNA PCR test version 1.5 was run on the two pellets from each specimen according to the manufacturer's instructions. Kit controls and coded control pellets were used for the validation of each assay run. The technologist running the assay was blinded as to the HIV status of each infant.

The concordance rate was 100% for the 16 HIV DNA PCR-negative samples and 100% for the 38 HIV DNA PCR-positive samples (Table 1). The optical density (OD) readings for the tested pellets made from 100- and 500-μl aliquots of samples from 54 different infants were not significantly different (Table 1). The mean OD of the HIV-1 DNA-positive samples made from 100 μl of whole blood was 3.91, compared with a mean OD of 3.96 for those made from 500 μl. The mean OD of the HIV-1 DNA-negative samples made from 100 μl of whole blood was 0.062, compared with a mean OD of 0.056 for those made from 500 μl.

Our comparison revealed no significant difference in the HIV-1 DNA PCR test results obtained using 100- and 500-μl blood volumes in the cell pellet preparation for the Roche Amplicor HIV-1 DNA PCR test version 1.5. A limitation of our study is that blood samples from infants less than 6 weeks of age, when HIV DNA levels may be lower, although lymphocyte counts are typically higher in very young infants, were not available for testing. Nevertheless, because of the difficulties encountered during venipuncture in infants less than 18 months old and the need to minimize the risk of anemia, our data indicate that a volume of 100 μl of blood can be used in the cell pellet preparation for the Amplicor HIV-1 DNA PCR test version 1.5, at least for infants greater than 8 weeks of age.

* Corresponding author. Mailing address: Department of Pathology, Carnegie 415, 600 North Wolfe St., Johns Hopkins Medical Institutions, Baltimore, MD 21287. Phone: (410) 614-4966. Fax: (410) 614-2907. E-mail: bjackso@jhmi.edu.

▽ Published ahead of print on 16 January 2008.

TABLE 1. HIV-1 DNA PCR results for testing of 100- and 500-μl samples of whole blood from infants 6 weeks to 18 months of age born to HIV-infected mothers

Sample identification no.	infant (mo)	100-μl blood sample OD reading:		Interpretation	500-μl blood sample OD reading:		Interpretation
		1	2		1	2	
1	12	0.064	0.064	Negative	0.072	0.074	Negative
2	12	0.057	Not done	Negative	0.097	0.123	Negative
3	2	0.056	Not done	Negative	0.049	0.045	Negative
4	2	0.058	Not done	Negative	0.062	0.085	Negative
5	1.75	0.066	Not done	Negative	0.041	0.042	Negative
6	1.5	0.057	Not done	Negative	0.037	0.056	Negative
7	2.75	0.058	Not done	Negative	0.038	0.047	Negative
8	3.5	0.061	Not done	Negative	0.045	0.046	Negative
9	1.5	0.065	Not done	Negative	0.051	0.041	Negative
10	2	0.060	Not done	Negative	0.044	0.085	Negative
11	3	0.067	Not done	Negative	0.076	0.075	Negative
12	5	0.059	Not done	Negative	0.030	0.068	Negative
13	5	0.063	Not done	Negative	0.056	0.045	Negative
14	2	0.064	Not done	Negative	0.044	0.045	Negative
15	1.5	0.071	Not done	Negative	0.042	0.04	Negative
16	1.5	0.065	Not done	Negative	0.042	0.043	Negative
17	12	3.891	4.000	Positive	3.957	4.000	Positive
18	12	3.956	3.955	Positive	3.955	3.954	Positive
19	7	3.800	3.909	Positive	4.000	3.975	Positive
20	18	3.037	3.522	Positive	3.849	3.71	Positive
21	16	3.905	3.905	Positive	3.954	4.000	Positive
22	9	3.953	4.000	Positive	3.952	4.000	Positive
23	8	3.951	3.951	Positive	3.915	4.000	Positive
24	12	3.993	3.992	Positive	3.992	3.991	Positive
25	4	3.959	4.000	Positive	3.891	3.89	Positive
26	13	3.890	3.956	Positive	3.956	4.000	Positive
27	13	4.000	4.000	Positive	3.976	3.909	Positive
28	2	3.908	3.908	Positive	3.974	3.974	Positive
29	5	3.976	3.975	Positive	3.908	3.974	Positive
30	12	3.973	3.973	Positive	3.956	3.955	Positive
31	6	3.889	3.954	Positive	3.887	3.953	Positive
32	9	3.953	3.885	Positive	3.995	4.000	Positive
33	5	3.915	3.847	Positive	3.914	3.913	Positive
34	18	3.957	3.890	Positive	4.000	4.000	Positive
35	6	3.801	3.977	Positive	3.909	3.851	Positive
36	18	3.851	3.523	Positive	3.850	3.849	Positive
37	12	3.908	Not done	Positive	4.000	3.956	Positive
38	2	3.831	Not done	Positive	3.956	4.000	Positive
39	9	3.849	Not done	Positive	4.000	3.954	Positive
40	9	3.954	Not done	Positive	4.000	4.000	Positive
41	12	3.953	Not done	Positive	3.975	4.000	Positive
42	6	3.974	Not done	Positive	4.000	3.974	Positive
43	10	4.000	Not done	Positive	3.972	4.000	Positive
44	6	4.000	Not done	Positive	4.000	4.000	Positive
45	12	4.000	Not done	Positive	4.000	3.953	Positive
46	18	3.993	Not done	Positive	4.000	3.952	Positive
47	4	4.000	Not done	Positive	4.000	4.000	Positive
48	2	3.913	Not done	Positive	3.994	3.993	Positive
49	4	3.849	Not done	Positive	3.993	3.992	Positive
50	7	3.845	Not done	Positive	4.000	3.991	Positive
51	16	3.992	Not done	Positive	4.000	3.952	Positive
52	4	4.000	Not done	Positive	3.995	3.994	Positive
53	5	4.000	Not done	Positive	4.000	4.000	Positive
54	12	3.974	Not done	Positive	3.993	3.992	Positive

No sponsored support was received for this project. J. Brooks Jackson has received honoraria in the past year from Roche Diagnostics for two scientific presentations he gave at Roche-sponsored scientific meetings, but not on this topic.

REFERENCES

1. **Creek, T. L., G. G. Sherman, J. Nkengasong, L. Lu, T. Finkbeiner, M. G. Fowler, E. Rivadeneira, and N. Shaffer.** 2007. Infant human immunodeficiency virus diagnosis in resource-limited settings: issues, technologies, and country experiences. Am. J. Obstet. Gynecol. **197:**S64–S71.
2. **Germer, J. J., T. M. Gerads, J. N. Mandrekar, P. S. Mitchell, and J. D. C. Yao.** 2006. Detection of HIV-1 DNA proviral DNA with the AMPLICOR HIV-1 DNA test, version 1.5, following sample processing by the MagNa Pure LC instrument. J. Clin. Virol. **37:**195–198.
3. **Patton, J. C., E. Akkers, A. H. Coovadia, T. M. Meyers, W. S. Stevens, and G. S. Sherman.** 2007. Evaluation of dried whole blood spots obtained by heel or finger stick as an alternative to venous blood for diagnosis of human immunodeficiency virus type 1 infection in vertically exposed infants in the routine diagnostic laboratory. Clin.Vaccine Immunol. **14:**201–203.