

Detection of HIV RNA in Whole Blood Using Procleix® Discriminatory HIV-1(dHIV-1) Assay

W. Andrews, P. Yan, C. Harrington, B. Phelps, T. Elbeik*, E. Fiebig*, and V. Ng*

Blood Testing Division, Chiron Corporation, Emeryville, California 94608

and *Dept. of Laboratory Medicine, University of California, San Francisco and Clinical Laboratories, San Francisco General Hospital, San Francisco, CA

BACKGROUND

- Currently NAT is performed only on serum or plasma.
- In TMA NAT assays the use of whole blood produces negative internal control and analyte results. This phenomenon has been seen in the field with heavily hemolyzed samples.
- Report in the literature that HIV-1 virus may be found on red blood cells (RBC) of plasma negatives (Hess et al, Lancet 359:2230-34 (2002)). 22/23 individuals that were BDL (<20 copies/ml) were found to have RBC-bound virus at some level, the highest being 83,000 copies/ml.

OBJECTIVES

- Develop protocol to use whole blood in the Procleix® HIV-1/HCV assay.
- Confirm the findings of Hess et al.

METHODS and RESULTS

Titration of whole blood samples in the Procleix® HIV-1/HCV assay

SAMPLE	Internal Control		Analyte		
	RLU	Result	RLU	S/CO	Result
Whole blood dilutions + 20 copies HIV					
1:2	852	Invalid	6,679	0.19	Invalid
1:2	847	Invalid	8,307	0.24	Invalid
1:4	197,058	Valid	458,547	13.01	Reactive
1:4	157,805	Valid	102,295	2.90	Reactive
1:8	170,174	Valid	411,573	11.68	Reactive
1:8	165,560	Valid	242,767	6.89	Reactive
serum control	149,690	Valid	552,108	15.66	Reactive
serum control	143,996	Valid	886,524	25.15	Reactive
Whole blood dilutions - no virus added					
1:2	1,007	Invalid	8,017	0.23	Invalid
1:2	555	Invalid	5,768	0.16	Invalid
1:4	180,397	Valid	7,191	0.20	Nonreactive
1:4	171,893	Valid	4,589	0.13	Nonreactive
1:8	167,618	Valid	5,893	0.17	Nonreactive
1:8	183,520	Valid	4,630	0.13	Nonreactive
serum control	157,320	Valid	4,709	0.13	Nonreactive
serum control	156,210	Valid	5,163	0.15	Nonreactive

At 1:2 dilution of whole blood (with negative sera) invalid results are obtained on the Procleix® HIV-1/HCV assay due to failure of the internal control to amplify. At whole blood dilutions of 1:4 and 1:8 valid internal controls are obtained.

METHODS and RESULTS, cont

Testing of HIV positive whole blood samples with detectable or undetectable viral load

- To test the findings of Hess et al, we assayed two samples- one sample that was BDL (<50 copies/ml) on an HIV viral load assay and one sample that had a detectable HIV viral load.
- Procleix® dHIV-1 assay testing of the HIV sample with no detectable virus in plasma was positive on the 1:10 dilution of whole blood while the plasma was negative when tested neat.
- Procleix® dHIV-1 TMA testing of the HIV sample with detectable viral load (15,539 copies/ml) was positive up to the 1:1000 dilutions of both whole blood and plasma.

Testing of HIV positive whole blood samples with detectable or undetectable viral load on Procleix® dHIV-1 assay

SAMPLE	Internal control		Analyte		
	RLU	Result	RLU	S/CO	Result
ProMedDx #10292439; HIV positive, viral load < 50					
Whole Blood					
neat	630	Invalid	7,388	0.18	Invalid
neat	0	Invalid	125,690	3.03	Invalid
1:10	156,303	Valid	346,653	8.36	Reactive
1:10	184,408	Valid	640,359	15.45	Reactive
1:100	180,726	Valid	63,821	1.54	Reactive
1:100	187,991	Valid	10,683	0.26	NonReactive
1:1000	195,792	Valid	9,643	0.23	NonReactive
1:1000	192,583	Valid	9,037	0.22	NonReactive
Plasma					
neat	178,272	Valid	8,258	0.20	NonReactive
neat	183,600	Valid	5,924	0.14	NonReactive
1:10	163,257	Valid	6,668	0.16	NonReactive
1:10	173,317	Valid	10,411	0.25	NonReactive
1:100	184,269	Valid	8,582	0.21	NonReactive
1:100	183,148	Valid	12,599	0.30	NonReactive
1:1000	202,217	Valid	10,798	0.26	NonReactive
1:1000	194,465	Valid	8,454	0.20	NonReactive
ProMedDx #10292438; HIV positive, viral load 15,539					
Whole Blood					
neat	317	Invalid	7,906	0.19	Invalid
neat	821	Invalid	6,996	0.17	Invalid
1:10	145,456	Valid	489,717	11.81	Reactive
1:10	161,233	Valid	487,701	11.76	Reactive
1:100	171,580	Valid	505,762	12.20	Reactive
1:100	166,744	Valid	462,542	11.16	Reactive
1:1000	180,129	Valid	434,735	10.49	Reactive
1:1000	206,000	Valid	472,362	11.39	Reactive
1:100K	188,325	Valid	11,986	0.29	NonReactive
1:100K	200,845	Valid	8,417	0.20	NonReactive
1:100K	192,378	Valid	14,873	0.36	NonReactive
1:100K	192,207	Valid	10,678	0.26	NonReactive
Plasma					
neat	168,480	Valid	512,978	12.37	Reactive
neat	162,009	Valid	521,633	12.58	Reactive
1:10	167,630	Valid	479,361	11.56	Reactive
1:10	190,484	Valid	461,864	11.14	Reactive
1:100	198,239	Valid	502,517	12.12	Reactive
1:100	192,536	Valid	498,503	12.02	Reactive
1:1000	202,927	Valid	472,517	11.40	Reactive
1:1000	197,359	Valid	455,419	10.99	Reactive
1:100K	190,688	Valid	11,296	0.27	NonReactive
1:100K	198,410	Valid	405,715	9.79	Reactive
1:100K	190,340	Valid	7,122	0.17	NonReactive
1:100K	186,527	Valid	11,380	0.27	NonReactive

Whole blood TMA – SFGH study

- Set up a prospective study with Tarek Elbeik at SF General Hospital using HIV patient samples. These patients were not necessarily on HIV drug therapy.
- Each patient plasma was tested at SFGH on the Bayer Versant® HIV-1 RNA 3.0 Assay (bdDNA) to establish an HIV viral load.
- One tube of EDTA plasma was collected for analysis with the Procleix® dHIV-1 assay.
- For each sample both the whole blood and the plasma were tested on the Procleix® dHIV-1 assay, each at a 1:5 dilution into negative human serum.

METHODS and RESULTS, cont

Whole blood TMA – SFGH samples

Plasma testing- results

- 16 of 27 plasma samples that were BDL on the Bayer Versant® HIV-1 3.0 assay (< 75 copies/ml) were positive on the Procleix® dHIV-1 assay using plasma at a 1:5 dilution. 11 of 27 were negative on the Procleix® dHIV-1 assay.
- One plasma specimen was positive on the Bayer Versant® HIV-1 RNA 3.0 assay, @ 97 copies/ml, but was repeatedly negative on the Procleix® dHIV-1 assay. This sample (#143) was one of those found to be positive with whole blood and negative with plasma on the Procleix® dHIV-1 assay.

Testing of Plasma samples:

Procleix® dHIV-1 TMA vs Bayer Versant® HIV-1 RNA 3.0

Procleix® dHIV	Bayer Versant® HIV-1 3.0	
	Positive	Negative (BDL)
Positive	37	16
Negative	1	11

Whole blood testing - results

- 9 specimens had discrepant whole blood and plasma results on the Procleix® dHIV-1 assay. One specimen resolved on retesting to positive on both whole blood and plasma.
- The 8 remaining specimens were positive with a 1:5 dilution of whole blood and negative with a 1:5 dilution of plasma.
- 7 of the 8 show lower than maximum S/CO on the Procleix® dHIV-1 assay, indicating low RNA copy number. The Procleix® dHIV-1 assay, however, is not a quantitative assay, so no specific viral load numbers could be assigned.

METHODS and RESULTS, cont

Whole blood vs plasma (all 1:5 dilutions) Procleix®dHIV-1 testing

First pass samples only (reruns not included)		
Whole Blood (1:5)	Plasma (1:5)	
	Positive	Negative
Positive	51	8
Negative	1	3

Discrepant samples (9) whole blood vs plasma (all 1:5 dilutions) Procleix® dHIV-1 testing

Sample	Whole blood (1:5)		plasma (1:5)	
	S/CO	Result	S/CO	Result
116	13.77	Reactive	0.10	NonReactive
119	23.24	Reactive	0.08	NonReactive
120	3.23	Reactive	0.11	NonReactive
143	7.98	Reactive	0.10	NonReactive
150	14.52	Reactive	0.20	NonReactive
158	18.57	Reactive	0.18	NonReactive
159	1.75	Reactive	0.08	NonReactive
167	1.25	Reactive	0.11	NonReactive
169	0.12	NonReactive	5.50	Reactive
	9.96	Reactive	2.85	Reactive

SUMMARY AND CONCLUSIONS

- We demonstrate a method by which whole blood, diluted 1:5 into negative human serum, can be tested on Procleix® HIV-1/HCV assay with little or no loss of sensitivity to HIV-1 (except due to the 1:5 dilution).
- We confirm the overall findings of Hess et al that there are samples from HIV patients which are BDL in plasma but are RNA positive when whole blood is tested.
 - The viral levels in most of these samples are probably low since the S/CO values in the Procleix® dHIV-1 assay are below maximum (<20)