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Significant Closure of the Human Immunodeficiency Virus Type 1 and Hepatitis C Virus Preseroconversion Detection Windows with a Transcription-Mediated-Amplification-Driven Assay

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While the present generation of serology-based assays has significantly decreased the number of human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) infections acquired by transfusion, the possibility of infected donations escaping detection still exists. The average seronegative viremic window duration during which immunological assays are unable to detect the virus is estimated to be between 16 and 22 days for HIV-1 and approximately 70 days for HCV. Significant reduction of detection window duration was demonstrated using a nucleic acid amplification assay, the Procleix HIV-1/HCV Assay, which utilizes transcription-mediated amplification technology to simultaneously detect HIV-1 and HCV RNAs. For 26 commercially available HIV-1 seroconversion panels tested, specimens were reactive in the HIV-1/HCV assay at the same time as or earlier than in serological assays. Overall, the HIV-1/HCV assay was able to reduce the detection window duration by an average of 14 days and 6 days compared to tests relying on recognition of HIV-1 antibody and p24 antigen, respectively. For 24 commercially available HCV seroconversion panels tested, the specimens were reactive in the HIV-1/HCV assay at an earlier blood sampling date than in serological assays, reducing the detection window duration by an average of 26 days. Similar results were obtained in testing the HIV-1 and HCV seroconversion panels in the virus-specific HIV-1- and HCV-discriminatory assays, respectively. In conclusion, the HIV-1/HCV assay and corresponding discriminatory assays significantly reduced detection window durations compared to immunoassays.

Blood centers in the United States and around the world utilize multiple approaches to ensure that blood supplies are as safe as possible. The number of transfusion-transmitted infections with human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) has dropped dramatically since the introduction of serological assays in 1985. This screening has largely been carried out with methodologies relying on the detection of HIV-1 p24 antigen (Ag) and antibodies (Abs) to HCV or HIV-1 Ags along with confirmatory enzyme-linked immunosorbent assays and recombinant immunoblot assays (RIBA) (4, 15). However, a major concern is that the immunological methods, although improving in sensitivity since their inception, cannot detect infection prior to seroconversion. The period of time between HIV-1 infection and Ab seroconversion is usually approximately 22 days in duration but is thought in some cases to last as long as 6 months (1, 5). Likewise, the period of time between acute infection with HCV and the appearance of Abs is approximately 70 days, and the occurrence of false negative results in rare patients with chronic, Ab-negative infection further complicates accurate detection of HCV (1). Therefore, immunological testing is unable to prevent the transmission of HIV-1 and/or HCV from a blood donor to a recipient if the donation is tested during the preseroconversion infectious window period.

Nucleic acid amplification testing (NAT) allows for direct viral detection of HIV-1 and HCV and is predicted to reduce the window period for identification of these viruses (2, 5, 6). The HIV-1/HCV assay, a nucleic acid test developed at Gen-Probe Incorporated for blood bank applications and marketed as the Procleix HIV-1/HCV Assay, detects both HIV-1 and HCV RNAs (reference 11 and C. Giachetti et al., submitted for publication). The HIV-1/HCV assay utilizes three proprietary technologies: (i) target capture-based sample preparation, (ii) transcription-mediated amplification (TMA), and (iii) a hybridization protection assay, all carried out within the same tube. If a positive result is obtained in the HIV-1/HCV assay, the source of the reactivity can be determined to be due to infection with HIV-1, HCV, or both by discriminatory assays. The discriminatory assays differ from the HIV-1/HCV assay in that a probe reagent is used that contains probes specific for only one of the viruses. The HIV-1/HCV assay and the discriminatory assays are able to detect 10 to 13 copies of HIV-1/ml with 95% detection rates and can detect HCV to 30 copies/ml with 95% sensitivity (Giachetti et al., submitted), theoretically allowing for detection of the virus early after infection.

For this report, HIV-1/HCV assay results from tests of 26 HIV-1 seroconversion panels and 24 HCV seroconversion panels were compared to detection carried out with multiple serological tests. Significant reduction by NAT of the window period for detection in comparison to the period required by the immunoassays was observed, further reducing the risk of

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HIV-1 and HCV infection by blood transfusion. The results obtained by the HIV-1/HCV assay, the HIV-1 discriminatory assay, and the HCV discriminatory assay support NAT of all blood donations and replacement of the HIV-1 p24 Ag test for blood and plasma screening.

MATERIALS AND METHODS

Specimens. (i) HIV-1 seroconversion panels. Five HIV-1 seroconversion panels were obtained from BioClinical Partners, Inc. (BCP; Franklin, Mass.): panels 6240, 6243, 6244, 6245, and 6248. Seven HIV-1 seroconversion panels were from North American Biologicals, Inc. (NABI; Miami, Fla.): panels SV-0211, SV-0281, SV-0321, SV-0331, SV-0341, SV-0361, and SV-0371. Fourteen HIV-1 seroconversion panels were acquired from Boston Biomedica, Inc. (BBI; West Bridgewater, Mass.): panels PRB916, PRB923, PRB926, PRB929, PRB931, PRB932, PRB943, PRB944, PRB945, PRB946, PRB947, PRB948, PRB949, and PRB950. The frequency of sampling varied in the seroconversion panels. Frozen panels were typically thawed at room temperature and spun at 1,000 × *g* for 10 min to eliminate particulates before testing. Each vendor tested the seroconversion panels by using several U.S. Food and Drug Administration-licensed test kits, including Abbott HIVAG-1 Monoclonal and Abbott HIVAB HIV-1/HIV-2 (Abbott, Abbott Park, Ill.), Coulter HIV-1 p24Ag (Coulter, Fullerton, Calif.), and Genetic Systems HIV-1/HIV-2 (Bio-Rad, Hercules, Calif.). Results for serological and alternative NAT were derived from the package inserts provided with each panel. Qualitative and/or quantitative HIV-1 RNA detection by PCR was carried out by the vendors of the seroconversion panels by using the Amplicor HIV-1 monitor kit (Roche Diagnostics, Branchburg, N.J.) or by the National Genetics Institute (Los Angeles, Calif.).

(ii) HCV seroconversion panels. Eight HCV seroconversion panels were obtained from BCP (panels 6211, 6212, 6213, 6214, 6215, 6225, 6226, and 6228). Eight HCV seroconversion panels were from NABI (panels SC-0010, SC-0030, SC-0040, SC-0060, SC-0070, SC-0080, SC-0090, and SC-0100). Eight HCV seroconversion panels were acquired from BBI (panels PHV901, PHV904, PHV907, PHV908, PHV913, PHV914, PHV915, and PHV916). The frequencies of sampling differed among the seroconversion panels. Panel members were typically thawed and spun at 1,000 × *g* for 10 min to eliminate particulates prior to testing. Each vendor tested the seroconversion panels with several commercially available kits, such as Abbott HCV EIA 2.0, Abbott HCV EIA 3.0, Abbott Prism Anti-HCV, Abbott HCV EIA 2nd Generation (Abbott), Ortho HCV EIA 3.0, Ortho/Chiron RIBA HCV 2.0 SIA, Ortho/Chiron RIBA HCV 3.0 SIA, and ROCHE Cobas Core Anti-HCV EIA (Roche Diagnostics). Results for serological and alternative NAT were taken from the package inserts provided with each panel. Qualitative and/or quantitative HCV RNA detection by PCR was carried out under the direction of the vendors of the seroconversion panels by using the HCV RNA Roche Amplicor PCR kit (Roche Diagnostics) or was done by the National Genetics Institute.

HIV-1/HCV assay. The HIV-1/HCV assay and the corresponding HIV-1 and HCV discriminatory assays have been described in detail (Giachetti et al., submitted) and are summarized briefly as follows. During sample preparation, plasma is treated with detergent to solubilize the viral envelope, denature proteins, and release viral genomic RNA. Chimeric capture oligonucleotides, having a 5′ sequence complementary to highly conserved regions of HIV-1 or HCV, hybridize to any HIV-1 or HCV target present in the test specimen. The capture oligonucleotides also have 3′ deoxyribosyladenine sequences that are complementary to deoxyribosylthymine sequences attached to the magnetic microparticles. The hybridized target is captured on the magnetic microparticles, which are separated from the plasma in a magnetic field. Further wash steps ensure removal of extraneous plasma components.

The target is amplified by TMA, which relies on two enzymes, Moloney murine leukemia virus reverse transcriptase and T7 RNA polymerase. The reverse transcriptase generates a DNA copy of the target RNA sequence that contains a promoter sequence for the T7 RNA polymerase. The T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template that are then cycled back through the same process to achieve exponential amplification of the target. The HIV-1/HCV assay uses this TMA-based method to amplify specific regions of HIV-1 and HCV RNAs.

The hybridization protection assay detects the RNA product by using single-stranded nucleic acid probes with chemiluminescent acridinium ester labels (3, 10). The probes hybridize specifically to the targeted amplicon, and hybridized probe is specifically detected after a selection step inactivates the chemiluminescent labels on unhybridized probes. The chemiluminescent signal is measured in a luminometer and is reported in relative light units (RLU).

An internal control (IC), which controls for the specimen processing, amplification, and detection steps, is added along with the Target Capture Reagent to each sample tested. The IC signal in each reaction can be discriminated from the HIV-1 and/or HCV signal by the differential kinetics of light emission from probes with different acridinium ester labels (12). The IC amplicon is detected by using a probe with rapid emission of light, while the amplicons specific to HIV-1 and HCV are detected by using probes with slower kinetics of light emission. The Dual Kinetic Assay differentiates between these two signals (12).

Specimens found to be reactive in the HIV-1/HCV assay are run in the HIV-1 and HCV discriminatory assays to determine if they are reactive for HIV-1 or HCV or both. The discriminatory assays use the same three steps as the HIV-1/HCV assay, and the same protocol is followed except that HIV-1- or HCV-specific probe reagent is used in place of the HIV-1/HCV assay probe reagent.

Three negative calibrators, three HIV-1-positive calibrators, and three HCV-positive calibrators were positioned at the beginning of each TMA assay run, and the results obtained from these calibrators were used to determine the validity of the run and to establish the assay cutoffs as described below. The negative calibrator was defibrinated normal human plasma, the HIV-1 positive calibrator consisted of heat-inactivated HIV-1-positive plasma in defibrinated normal human plasma, and the HCV positive calibrator consisted of heat-inactivated HCV-positive plasma in defibrinated normal human plasma.

Formula-derived assay cutoff values. Each assay generated two results, one for the analyte (HIV-1 and/or HCV) and one for the IC. Formulas were established to allow for calculation of a floating analyte cutoff in each assay that falls within the range of relative light unit values indicated by receiver operating characteristic (ROC) analysis to achieve ≥99.5% sensitivity and specificity. By using a floating cutoff rather than a set value, day-to-day variations due to reagents, operators, and instruments are normalized. The formulas add a portion of the HIV-1 positive calibrator analyte signal and/or the HCV positive calibrator analyte signal to the negative calibrator analyte signal for the three assays. When a sample produced an analyte/analyte signal/cutoff (S/CO) ratio ≥1, it was considered reactive for the target RNA.

An IC cutoff was also established for each individual assay run, with the cutoff calculated to diminish the number of invalid results while at the same time minimizing the occurrence of false negative results. A cutoff calculated as 50% of the negative calibrator IC signal was found to meet these conditions for the HIV-1/HCV assay, the HIV-1 discriminatory assay, and the HCV discriminatory assay. In HIV-1- and HCV-negative samples, an IC S/CO ratio of ≥1 validates the reaction and an IC S/CO ratio of <1.0 indicates an invalid reaction. In samples with a positive analyte signal, the IC is not used to validate the reaction.

RESULTS

HIV-1 seroconversion panel testing. The results from testing 26 seroconversion panels in the HIV-1/HCV assay were compared to the results supplied by the vendors for several commercially available Ab and Ag tests. The numbers described below from Ab testing reflect those obtained in the Abbott HIV-1/-2 assay, and the Ag values were attained in either the Abbott or Coulter p24 Ag assay. Furthermore, either qualitative or quantitative PCR data were available for each panel member and the date of the earliest donation detected by this methodology was compared to that observed with the test assay.

The lengths of time from the initial donation until the first donation positive for HIV-1 in the seroconversion panels from BCP, NABI, and BBI by using HIV Ab, HIV Ag, PCR, and HIV-1/HCV assay testing are shown in Table 1. As expected, the times at which positive results were observed differed according to the test employed. In all BCP panels, the HIV-1/HCV assay indicated a reactive result sooner than the Ab and Ag tests and at the same time as, or sooner than, the PCR testing. The results obtained with the nine seroconversion panels from NABI were similar to the results with the BCP panels, as the donations were reactive by the HIV-1/HCV assay sooner than by Ab tests in all cases. Likewise, the HIV-1/HCV assay shortened the detection window duration compared to Ag

TABLE 1. Comparison of the abilities of HIV-1 Ab testing, HIV-1 Ag testing, PCR, the HIV-1/HCV assay, and the HIV-1 discriminatory assay to detect HIV-1 in seroconversion panels

Seroconversion panel	Vendor	Day with the first positive result by:[a]				
		HIV-1 Ab[b]	HIV-1 Ag[b]	PCR[b]	HIV-1/HCV assay	HIV-1 discriminatory assay
6240	BCP	28	23	16	16	16
6243	BCP	31	24	17	17	17
6244	BCP	33	28	25	22	22
6245	BCP	74	67	61	61	61
6248	BCP	25	18	14	11	11
SV-0211	NABI	14	1	1	1	1
SV-0281	NABI	34	8	6	6	6
SV-0321	NABI	12	8	1	1	1
SV-0331	NABI	20	13	6[d]	6	6
SV-0341	NABI	21	10	7[d]	7	1
SV-0361	NABI	16	9	1	1	1
SV-0371	NABI	22	17	17[d]	17	17
PRB916	BBI	30	15	9	0	9
PRB923	BBI	47	37	35	30	30
PRB926	BBI	27	7	2	0	0
PRB929	BBI	25	14	14	14	14
PRB931	BBI	28	15	15	9	9
PRB932	BBI	27	27	27	13	13
PRB943	BBI	14	7	5	5	5
PRB944	BBI	14	2	0	0	0
PRB945	BBI	13	13	0	0	0
PRB946	BBI	>11[c]	7	4	4	0
PRB947	BBI	9	9	0	0	0
PRB948	BBI	>23[c]	23	20	20	20
PRB949	BBI	>20[c]	18	6	6	6
PRB950	BBI	28	18	18	0	0

[a] The first donation date is designated 0 by BBI and BCP and 1 by NABI.
[b] HIV-1 Ab, HIV-1 Ag, and PCR results were taken from the package insert provided with each panel as described in Materials and Methods.
[c] Day of antibody detection is after that indicated, as these specimens did not seroconvert by this final donation date.
[d] Prior blood sampling was below the limit of quantitation.

testing, with the exception of samples SV-0211 and SV-0371, which were detected by both Ag and NAT on the same donation date. All samples of these panels that were reactive by qualitative PCR were also reactive in the HIV-1/HCV assay. The HIV-1/HCV assay also detected HIV-1 earlier than the serological tests in all panels from BBI and detected all samples reactive by PCR. The HIV-1/HCV assay detected HIV-1 from 2 to 18 days earlier than qualitative PCR in five of these panels (PRB916, 9 days earlier; PRB926, 2 days; PRB931, 6 days; PRB932, 14 days; PRB950, 18 days).

Overall, the HIV-1/HCV assay detected infection an average of 14.0 days earlier than Ab testing and an average of 6.2 days before the p24 Ag test (Table 2). The result for days of window duration closure with respect to Ab testing is an underestimation, as three samples, PRB946, PRB948, and PRB949, did not become HIV Ab positive within the donation dates tested. HIV-1 seroconversion panel PRB916 was coinfected with HCV; thus, the values obtained with this sample were not used in the average window duration reduction calculation for the HIV-1/HCV assay in order to ensure that closure for HIV-1 detection was specifically obtained.

When a positive result is obtained by the HIV-1/HCV assay in a blood bank setting, the sample must be retested to determine whether HIV-1 or HCV or both are present. Therefore, to test the detection window duration of the corresponding discriminatory assay, the seroconversion panels were also tested by the HIV-1 discriminatory assay. Results with these panels were identical to those observed with the HIV-1/HCV assay with the exception of three panels (Table 1). SV-0341 and PRB946 had a positive result in an earlier donation sample when tested in the HIV-1 discriminatory assay. In contrast, PRB916 was detected in a donation later in the discriminatory assay than in the HIV-1/HCV assay. Overall, the closure of the window duration with testing carried out with the discriminatory assay compared to serological testing was similar to that for the same specimens tested by the HIV-1/HCV assay (Table 2). The detection window duration was reduced with HIV-1 discriminatory assay testing by an average of 14.6 and 6.6 days compared to testing with Ab and Ag detection assays, respectively.

HCV seroconversion panel testing. The results from testing 24 HCV seroconversion panels by HCV Ab testing or by the HIV-1/HCV assay were compared with respect to the blood sampling date at which HCV was detected. The Ab test results, which were supplied by the seroconversion panel vendor, were obtained using the Abbott HCV EIA 2.0 or the Ortho HCV EIA 3.0 test. In addition, qualitative or quantitative PCR was carried out as an alternative NAT and the donation date associated with a positive result was compared to the donation dates for the samples positive by the other tests.

The abilities of the HCV Ab tests, PCR, HIV-1/HCV assay, and the HCV discriminatory assay to detect the presence of

TABLE 2. Detection window duration closure by the HIV-1/HCV assay and HIV-1 discriminatory assay as compared to immunological assays

Seroconversion panel	HIV-1/HCV assay closure of detection window (days) compared to:		HIV-1 discriminatory assay closure of detection window (days) compared to:	
	HIV-1 Ab	HIV-1 Ag	HIV-1 Ab	HIV-1 Ag
6240	12	7	12	7
6243	14	7	14	7
6244	11	6	11	6
6245	13	6	13	6
6248	14	7	14	7
SV-0211	13	0	13	0
SV-0281	28	2	28	2
SV-0321	11	7	11	7
SV-0331	14	7	14	7
SV-0341	14	3	20	9
SV-0361	15	8	15	8
SV-0371	5	0	5	0
PRB916[b]	30	15	21	6
PRB923	17	7	17	7
PRB926	27	7	27	7
PRB929	11	0	11	0
PRB931	19	6	19	6
PRB932	14	14	14	14
PRB943	9	2	9	2
PRB944	14	2	14	2
PRB945	13	13	13	13
PRB946	>7[a]	3	>11[a]	7
PRB947	9	9	9	9
PRB948	>3[a]	3	>3[a]	3
PRB949	>14[a]	12	>14[a]	12
PRB950	28	18	28	18
Average	14.0	6.2	14.6	6.6
SD[c]	6.3	4.5	6.2	4.4

[a] Panel never became HIV-1 antibody positive.
[b] Panel was coinfected with HCV and was not included in calculation of average for the HIV-1/HCV Assay.
[c] SD, standard deviation.

HCV infection in the seroconversion panels from BCP, NABI, and BBI are listed in Table 3. As with the HIV-1 seroconversion panels described above, the various assays detected the presence of HCV at different donation dates. In the panels from BCP, NAT by the HIV-1/HCV assay detected HCV infection at an earlier date than the serological tests. In addition, the HIV-1/HCV assay demonstrated a positive result on the same date as, or earlier than, the alternative NAT method, PCR. Similar results were obtained with the NABI panels, although the viral status of four panel members (SC-0070, SC-0080, SC-0090, and SC-0100) was not assessed by PCR. Finally, the results with the BBI panels indicated a positive result with the first donation sample (day 0) by both PCR and HIV-1/HCV testing, which was earlier than Ab detection in all cases. Overall, the detection window duration was reduced by an average of 25.9 days with the HIV-1/HCV assay compared to Ab testing.

To test the detection window duration of the corresponding discriminatory assay, these seroconversion panels were also tested in the HCV discriminatory assay. Comparison of the positivity date with this assay compared to Ab and PCR testing is presented in Table 3. The HCV discriminatory assay indicated a positive result earlier than the HCV Ab tests with all BCP panels tested. The results coincided with those obtained by PCR testing. In addition, all NABI panels tested were confirmed positive for HCV at an earlier date than were the HCV Ab tests and were found to be positive for the same donation as the PCR tests (the viral status of four panel members [SC-0070, SC-0080, SC-0090, and SC-0100] was not assessed by PCR). The samples from donations first indicating positivity were identical for panels tested by both the HIV-1/HCV assay and the HCV discriminatory assay, with the exception of panel 6213, whose positivity was first detected in the discriminatory assay in the donation provided three days later. Overall analysis of the results of the 16 panels demonstrated that the HCV discriminatory assay reduced the detection window duration by an average of 25.8 days (Table 3).

DISCUSSION

Detection of HIV-1 virus. For HIV-1, it is estimated that 1.48 infectious units per million donations, or 18 infectious units per year, go undetected by the present Ab assays due to donations provided during the window period between infection and seroconversion (5, 6). Fortunately, the window period for HIV-1 detection of approximately 16 to 22 days is relatively short compared to other viruses such as HCV, which has a window period of approximately 70 days (2). The abbreviated window period observed with HIV-1 infection is attributed to the rapid virus doubling time, estimated at slightly less than 1 day (5). In addition, the sensitivity of the present fourth-generation immunoassays has also helped minimize the seroconversion detection window (4, 8, 15). Current estimates suggest that NAT should be able to reduce the window period further, by approximately 7 to 15 days compared to tests based on Ab and/or Ag detection (2, 8, 9). The rapid doubling time for HIV-1 suggests that the interval between infection and the appearance of HIV-1 RNA, also known as the eclipse period, may be as short as 1 day (2).

In each of the 26 HIV-1 panels tested in this study, the TMA-based assays were able to detect HIV-1 RNA on the same date as, or prior to, the appearance of detectable HIV-1 Ab in the samples. From examination of all seroconversion panels together, the HIV-1/HCV assay and HIV-1 discriminatory assay were able to reduce the detection window duration for HIV-1 by about 14 to 15 days compared to presently licensed Ab detection techniques. Three of the panels (Table 1) further emphasize the importance of NAT screening of blood donations, as the subjects did not seroconvert within the time period of the seroconversion panel blood samplings.

The HIV-1 p24 Ag test was introduced in 1996 to help minimize the detection window duration period, and as of December 2000, only 6 p24 Ag-positive donors who were Ab-negative were identified in the more than 24 million donations screened (13). HIV-1 RNA appears before, or simultaneously with, p24 Ag and remains long after p24 Ag has disappeared. Thus, there have been suggestions that NAT could effectively replace the p24 Ag test. In the experiments described here, the window duration of detection was effectively decreased by an average of 6 to 7 days compared to present methods that detect HIV-1 Ag. There were no instances in which Ag was detected in a specimen without concurrent detection of HIV-1 RNA.

TABLE 3. Comparison of the abilities of HCV Ab testing, PCR, the HIV-1/HCV assay, and the HCV discriminatory assay to detect HCV in seroconversion panels

Seroconversion panel	Vendor	Day with the first positive result with:[a]				Closure of detection window (days) by HIV-1/HCV assay	Closure of detection window by the HCV discriminatory assay
		HCV Ab[b]	PCR[b]	HIV-1/HCV assay	HCV discriminatory assay		
6211	BCP	186	140	140	140	46	46
6212	BCP	23	0	0	0	23	23
6213	BCP	43	11	8	11	35	32
6214	BCP	32	0	0	0	32	32
6215	BCP	20	0	0	0	20	20
6225	BCP	78	39	39	39	39	39
6226	BCP	39	0	0	0	39	39
6228	BCP	31	0	0	0	31	31
SC-0010	NABI	5	1	1	1	4	4
SC-0030	NABI	45	1	1	1	44	44
SC-0040	NABI	8	1	1	1	7	7
SC-0060	NABI	6	1	1	1	5	5
SC-0070	NABI	72	NT	12	12	60	60
SC-0080	NABI	45	NT	1	1	44	44
SC-0090	NABI	53	NT	11	11	42	42
SC-0100	NABI	8	NT	1	1	7	7
PHV901	BBI	104	72	72	72	32	32
PHV904	BBI	14	0	0	0	14	14
PHV907	BBI	18	0	0	0	18	18
PHV908	BBI	19	0	0	0	19	19
PHV913	BBI	7	0	0	0	7	7
PHV914	BBI	19	0	0	0	19	19
PHV915	BBI	12	0	0	0	12	12
PHV916	BBI	23	0	0	0	23	23
Average						25.9	25.8
SD[c]						15.5	15.5

[a] The first donation date is designated 0 by BBI and BCP and 1 by NABI.
[b] HCV Ab and PCR results were taken from the package insert provided with each panel as described in Materials and Methods. NT, not tested.
[c] SD, standard deviation.

The HIV-1/HCV assay is presently being used in blood banks in the United States under an investigational new drug application. Currently, donations are tested in pools of 16 to increase throughput. Only when a pool is found to be reactive are the donations making up the pool tested individually. Although diluted 16-fold, 3 HIV-1 NAT-reactive, p24 Ag-negative, and Ab-negative donations were identified among the 11 million samples screened (13). A clinical trial carried out between August and October 2000 investigated the detection of HIV-1 in seroconversion panels tested in the 16-pool format. Even in these diluted samples, the HIV-1 seroconversion panels were found reactive an average of 10 and 3 days earlier than by Ab and Ag detection, respectively (16). These clinical data, together with the seroconversion panel results described in this paper, suggest that consideration should be given to replacing the p24Ag assay with NAT.

Overall, the HIV-1/HCV assay and HIV-1 discriminatory assay reduced the HIV-1 detection window duration by 7 to 15 days. The assays detected RNA in the same donation, or an earlier donation, as that detected by PCR. The 95% detection sensitivity of HIV-1 type B (down to approximately 10 to 13 copies/ml), as well as the ability of the assay to detect all subtypes of HIV-1, make these assays attractive for blood bank screening (reference 7 and Giachetti et al., submitted).

Detection of HCV virus. In the 24 panels tested, the TMA-driven assays were able to detect HCV RNA prior to the appearance of recognizable HCV Ab in the samples. From examination of all seroconversion panels together, the HIV-1/HCV assay was able to reduce the detection window duration for HCV by an average of approximately 26 days compared to presently licensed techniques that rely on Ab recognition. The maximum window duration reduction of 60 days was observed with panel SC-0070; however, intermediate donations between specimens that were positive by the HIV-1/HCV assay and those reactive by HIV-1 Ab testing were not available for this panel. The window duration of detection was decreased by an average of about 26 days when the HCV discriminatory assay was carried out with the panels. The result for reduction in the detection window duration provided by the assays may be an underestimation, as 18 of the 24 panels tested gave a reactive result in the sample from the first blood sampling date provided by the vendor (Table 3). Furthermore, there is evidence from tests involving intravenous drug abusers that HCV seroconversion could take as long as several years, during which time the only indicator of the presence of the virus is very low-level viremia (1). In these cases, NAT would likely decrease the detection window duration by months or years rather than days.

The decrease in the detection window duration with the HIV-1/HCV assay of at least 30 days was expected, due to the extremely rapid doubling time of HCV, estimated at <1 day (5). Although seroconversion may not occur for an average of 41 days postinfection, the number of RNA copies during this infectious stage averages 100,000 per ml. This level of RNA would readily be detected by NAT techniques, even when pooled samples are tested (reference 5 and Giachetti et al., submitted).

Serological tests depend upon the patient's immune response, but assays like the HIV-1/HCV assay directly detect viral RNA. As such, NAT provides better information about the dynamics of progression of HCV infection (14). For instance, in addition to reducing detection window periods, testing with the HIV-1/HCV assay has the ability to identify HCV-infected individuals who are immunosilent. There have been several cases of patients who have chronic HCV infection without detectable Abs; these patients do not seroconvert but remain HCV RNA positive (1). These patients may be unable to develop a lasting immune response to the HCV Ags used in the licensed HCV serological tests.

It is estimated that 9.70 HCV-infected units per million donations, or 116 infectious units per year, go undetected by the present Ab assays. With implementation of HCV NAT, such as by the HIV-1/HCV assay and HCV discriminatory assay, the estimated risk is reduced to 2.72 infectious units per million donations, or 32 infectious units per year (6). The HIV-1/HCV assay is presently used under an investigative new drug application and has been used to identify specimens containing HCV RNA that would have gone undetected by assays relying on detection of Abs to HCV. Forty-three such yield specimens were found overall among 11.0 million units (1:256,000) tested by the American Red Cross, America's Blood Centers, and America's Independent Blood Centers (13). In addition, in a recently completed clinical trial the HIV-1/HCV assay reduced by 25 days the detection window duration for HCV seroconversion panels diluted 1:16 (16). In conclusion, testing with the HIV-1/HCV assay will provide sensitive detection of HCV RNA in the blood supply, as well as assisting in diagnosing, treating, and monitoring HCV infection.

Conclusions. For the blood-banking industry to achieve the goal of a zero-risk blood supply, the few donations containing HIV-1 and/or HCV that escape detection using immunoassays must be detected. NAT using the HIV-1/HCV assay clearly will increase the detection rate of HIV-1 and/or HCV in donations that are immunosilent and help prevent these specimens from entering the blood supply.

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ADDENDUM IN PROOF

As of 8 November 2001, 7 HIV-1 NAT-reactive p24 Ag-negative donations and 88 HCV Ab-negative donations had been detected by the HIV-1/HCV assay in clinical trials.

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