Relative sensitivities of licensed nucleic acid amplification tests for detection of viremia in early human immunodeficiency virus and hepatitis C virus infection

Michael P. Busch, Simone A. Glynn, David J. Wright, Dale Hirschkorn, Megan E. Laycock, Joan McAuley, Yongling Tu, Cristina Giachetti, James Gallarda, John Heitman, and Steven H. Kleinman for the National Heart, Lung, and Blood Institute Nucleic Acid Test Study Group

BACKGROUND: Screening donors for human immunodeficiency virus (HIV) and hepatitis C virus (HCV) RNA is primarily performed on minipools (MPs) with one of two commercial nucleic acid amplification tests (NAT; Roche Molecular Systems; or Gen-Probe/Chiron). We compared these assays with respect to detection of RNA in early HIV and HCV infection.

STUDY DESIGN AND METHODS: Twelve HIV plasma donor panels (116 serial samples) and 12 HCV panels (180 serial samples) were selected to optimally represent early viremia. Initial testing was performed in singlicate or triplicate on separately coded aliquots, both neat and at dilutions corresponding to MP screening (1:16 for Gen-Probe; 1:24 for Roche); 20 additional replicates were performed when discordant results were observed. Odds ratios (ORs) comparing detection of RNA by different assays were derived with logistic regression models. Differences in window-period closure and yields of assays in MP or individual-donation (ID) format were estimated.

RESULTS: Differences in detection rates between Roche and Gen-Probe NAT assays were small and only observed with samples with very-low-level viremia. ORs for detecting RNA by the Gen-Probe versus the Roche assay were significant for HIV if conducted on MPs (1.8; 95% confidence interval [CI], 1.3-2.5) but not neat (1.0; 95% CI, 0.72-1.4). Odds of detecting HCV RNA were higher if the Gen-Probe assay was conducted either neat (2.3; 95% CI, 1.6-3.2) or on MPs (4.0; 95% CI, 2.8-5.8). These differences translated to <1 day window-period closure and ≤1 infection per 20 million donations. In contrast, comparisons of ID versus MP assays were highly significant for both viruses (ORs for ID vs. MP ranged from 45.3 to 93.4), with projected yields of one to two additional infections per 10 million donations.

CONCLUSIONS: Differences in sensitivities of licensed NAT assays for HIV and HCV are very small and clinically insignificant, particularly when compared to differences of MP versus ID NAT screening.

Nucleic acid amplification tests (NAT) for detection of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) RNA in blood donations were widely implemented by US blood banks in 1999 under FDA investigational new drug protocols.[1,2] NAT assays from two manufacturers were subsequently licensed by the FDA and are currently used to screen all blood donations in the US as well as donations in numerous other countries: the COBAS AmpliScreen HIV-1 and HCV assays, based on polymerase chain reaction (PCR; Roche Molecular Systems, Pleasanton, CA) and the Procleix HIV-1 and HCV assay and corresponding dis-

ABBREVIATIONS: BSRI = Blood Systems Research Institute; d = discriminatory; ID(s) = individual donation(s); MP(s) = minipool(s); NGI = National Genetics Institute; TMA = transcription-mediated amplification.

From the Blood Systems Research Institute, San Francisco, California; the Department of Laboratory Medicine, University of California, San Francisco, California; Westat, Rockville, Maryland; Blood Systems Laboratory, Tempe, Arizona; Gen-Probe Inc., San Diego, California; Roche Molecular Systems, Pleasanton, California; and the University of British Columbia, Vancouver, British Columbia, Canada.

Address reprint requests to: Michael P. Busch, MD, PhD, Blood Systems Research Institute, 270 Masonic Avenue, San Francisco, CA 94118; e-mail: mpbusch@itsa.ucsf.edu.

This work was supported in part by the National Heart, Lung, and Blood Institute Contracts N01-HB-97077 (superseded by N01-HB-47114), -97078, -97079, -97080, -97081, and -97082 through the Retrovirus Epidemiology Donor Study. Data on plasma donor panels were provided by Alpha Therapeutic Corporation and National Genetics Institute (NGI) and testing reagents by the manufacturers of the NAT assay systems (Roche Molecular Systems and Gen-Probe Inc.).

Received for publication March 20, 2005; revision received May 5, 2005, and accepted May 9, 2005.

doi: 10.1111/j.1537-2995.2005.00649.x

TRANSFUSION 2005;45:1853-1863.

criminatory assays, based on transcription-mediated amplification (TMA; developed and manufactured by Gen-Probe Inc., San Diego, CA; and distributed by Chiron Corporation, Emeryville, CA).[3,4] Screening is primarily performed with minipools (MPs) of 16 donation samples with the Procleix TMA system and 24 donation samples with the AmpliScreen PCR system, although some US blood banks perform individual-donation (ID) NAT screening and, in other countries, alternative MP sizes, ranging from 4 to 96 donation samples, are routinely employed.

Although studies have documented the analytical sensitivity (based on probit analysis of dilutions of international standards and reference panels)[3-7] and summarized the clinical yields and performance characteristics for the two US-licensed NAT assays,[1,8-10] no study has directly compared these assays with respect to their relative capacity to detect RNA in early HIV and HCV infection in the context of MP- and ID-NAT screening. These data are important to reassure users of these assay systems that the licensed tests have comparable clinical sensitivity for detecting early HIV or HCV infections when employed in MPs. Head-to-head comparison data would also be useful to support pool resolution and supplemental testing algorithms, including use of HIV-1 and HCV discriminatory assays to resolve the source of viremia in multiplex reactive samples detected with the Gen-Probe system and "cross-supplemental" use of Gen-Probe and Roche assays to confirm viremia in donation samples that screened reactive by the alternate manufacturers' system. Finally, data directly comparing RNA detection when the assays are conducted individually (i.e., neat) or in MPs would further inform the policy debate over the merit of moving from MP-NAT to ID-NAT.

This study was designed to address each of these issues by directly comparing the detection rates of US-licensed NAT screening assays on serial donation samples from selected HIV-1- and HCV-infected plasma donor panels. The NAT assays were performed in parallel in multiple replicates on both neat and diluted panel members to mimic ID- and MP-NAT screening. Rigorous retesting of all samples with discordant results allowed assessment of differences in the odds of detecting RNA between manufacturers' assays and, for each manufacturer, between MP and ID screening contexts. The data were also analyzed to estimate window-period differences and extrapolate the clinical impact of these differences with respect to interdiction of viremic donations in the US volunteer blood donor setting.

MATERIALS AND METHODS

Construction of the HIV-1- and HCV-positive plasma donor panels

Plasma donations (600-800 mL) given in 1996 through 1999 by source plasma donors were routinely collected at donor centers operated by Alpha Therapeutic Corporation (Los Angeles, CA) at approximately twice-weekly intervals. The collected plasma was frozen within 8 hours of collection and routinely stored frozen at not greater than –20°C for at least 60 days (quarantine period). Samples of plasma from each donation were submitted for infectious disease serologic screening and for HIV and HCV RNA testing in pools of 512 with sensitive HIV-1 and HCV reverse transcription–PCR (RT-PCR) assays (National Genetics Institute [NGI], Los Angeles, CA). HIV-1 and HCV infections were confirmed by follow-up individual-donor RNA testing and antibody seroconversion. HIV-1- and HCV-positive donors were notified, counseled, and permanently deferred. Frozen plasma donations from cases with confirmed viremia and/or seroconversion were retrieved from quarantine to construct 44 HIV-1 and 55 HCV anonymized panels composed of sequentially drawn plasma samples. The study protocol was approved by the UCSF Committee for Human Research Institutional Review Board.

Original RNA testing conducted on candidate HIV and HCV panels

In a previous study, we had quantified the viral RNA levels of the 145 serial plasma donations comprising the 44 HIV-1 panels.[11] Samples were tested by NGI with their proprietary SuperQuant HIV-1 RT-PCR assay, which quantifies HIV-1 RNA between 100 and 5×10^6 copies per mL.[12] These quantitative data were used to derive a doubling time for HIV RNA during the ramp-up phase of viremia, when RNA levels exponentially increase, of 20.5 hours (95% confidence interval [CI], 18.2-23.4 hr).[11] Nonquantifiable samples were further tested in 5 to 10 replicates with the UltraQual HIV-1 RT-PCR assay (NGI) with 50 and 95 percent detection limits of 1.4 and 5 HIV copies per mL, respectively.[13]

For the 55 HCV panels consisting of 629 serial samples, RNA levels were quantified at the Blood Systems Research Institute (BSRI, San Francisco, CA) with the COBAS AMPLICOR HCV MONITOR test, Version 2.0 (Roche Molecular Systems, Pleasanton, CA) with detection limit of 600 IU per mL or 1620 copies per mL (conversion factor, 2.7; see package insert).[14,15] The quantitative RNA data were used to derive a 10.8-hour (95% CI, 9.9-12.0) estimate for the RNA doubling time during the ramp-up phase of acute HCV viremia.[14] For all samples in which RNA was not quantifiable, neat testing in four replicates was conducted at Gen-Probe Inc. with the Procleix discriminatory (d)-HCV TMA assay.[6,10]

Selection of panels for inclusion in the study

To optimally represent the early phase of acute viremia, we chose a subset of 12 HIV-1 and 12 HCV plasma sero-

conversion panels for inclusion in this study. Panel selection was based on the following criteria: 1) the panel included one or more HIV-1 and HCV RNA–positive, antibody-negative samples preceded by at least two collections with RNA-negative results; 2) serial units were available for at least 2 weeks before samples were quantifiable by the SuperQuant HIV-1 RT-PCR or by the COBAS AMPLICOR HCV MONITOR Test, Version 2.0; and 3) there were short time intervals between serial units (usually <7 days). The 12 selected HIV and HCV panels contained a total of 116 and 180 samples, respectively.

Processing and further testing of the selected HIV and HCV panels

All aliquots, replicates, and dilutions were prepared by the central laboratory at BSRI with procedures and storage conditions in accordance with licensed package insert specifications. Dilutions were prepared with a pooled plasma-derived base matrix (Acrometix, Benicia, CA) consistent with pool sizes used for MP-NAT screening in the United States (1:16 for Gen-Probe; 1:24 for Roche). Testing with the COBAS AmpliScreen test with the multiprep specimen processing procedure (high-speed centrifugation of 1.0 mL of sample) was performed at BSRI. The multiprep procedure was also used to process neat samples to assess the potential optimal sensitivity of ID-NAT screening with the Roche system. (Normally the COBAS AmpliScreen test is performed with the standard sample processing procedure [extraction of RNA from 200 µL of sample with no centrifugation] for ID samples during the process of resolution of reactive MPs.) Gen-Probe Procleix TMA testing was performed at Blood Systems Laboratory (Tempe, AZ) with the same instruments and procedures employed for routine donor NAT screening.

Testing was initiated only after training and certification of proficiency with manufacturer-supplied proficiency panels by the respective manufacturers and was performed according to manufacturers' instructions as stipulated in licensed package inserts, except that the COBAS AmpliScreen test was performed with the multiprep procedure on neat samples, as noted above.[3,4] Proficiency panels were reperformed during and after completion of testing study samples and yielded appropriate results in all instances.

Figures 1 and 2, respectively, show the HIV and HCV panel testing algorithms. In all, approximately 3900 aliquots were tested. Samples containing fewer than 5000 copies per mL were tested in triplicate, whereas samples with 5000 copies per mL were tested in singlicate. These initial test results were compiled in a SAS data set[16] by the coordinating center for the study (Westat, Inc., Rockville, MD) and analyzed to identify samples that would need additional testing because they had discordant singlicate or triplicate results. The types of discordancies that triggered additional 20-replicate testing by TMA (multiplex or discriminatory) or PCR included discordancy in results between multiplex TMA and PCR assays; multiplex and discriminatory TMA assays conducted neat; assays conducted neat and on MP; and the NGI UltraQual HIV-1 RT-PCR or the d-HCV TMA assay originally done on the nonquantifiable samples from the 44 HIV and 55 HCV panels and the TMA and PCR assays conducted in one to three replicates on the subset of 12 HIV and 12 HCV panels. The maximum number of *additional* replicates done for a particular assay at the dilution that was discordant was 20 (if sample volume allowed). If a sample fell into two categories that would trigger additional testing, the given set of 20 replicates was only performed once. Hence, for any given assay conducted either neat or diluted, the final number of replicate results available on each sample varied from one (for those samples with 5000 copies/mL and no discordant results) to 23 (for samples with <5000 copies/mL and discordant results).

Statistical analysis

We were interested in comparing the performance of the Gen-Probe TMA and the Roche PCR test in the situation most often encountered in blood donor screening in the United States: that is, 1:16 MP Gen-Probe multiplex TMA compared to 1:24 MP Roche PCR. We also compared results from the multiplex TMA to those of the PCR assays (with the multiprep sample preparation procedure) when both assays were conducted neat to compare the two manufacturers' assays for ID-NAT screening. To complete our analysis, we also evaluated whether there was a significant loss in sensitivity when screening was conducted on diluted samples (MPs) compared to neat samples with each manufacturer's screening assay and compared the multiplex and discriminatory TMA assays' ability in identifying viremic samples.

Evaluations were conducted separately for HIV and HCV and consisted of descriptive tabulations and characterization of discrepancies occurring between assays. Analytically, we conducted logistic regression models to derive odds ratios (ORs) that compared the odds of detecting RNA by one assay to the odds of detecting RNA by another assay. These models only included data on samples with nonconcordant test results, because concordant samples (samples that have all negative or all positive results) are statistically noninformative. These models assumed that the variability of each assay was equivalent and essentially evaluated whether detection limits were different between any two assays. For example, the model estimated the odds of having a qualitative positive multiplex TMA versus the odds of having a qualitative positive PCR when both tests are conducted neat, assuming that the sensitivity curves of both assays were parallel (i.e., the 50% sensitivity levels may differ; hence,

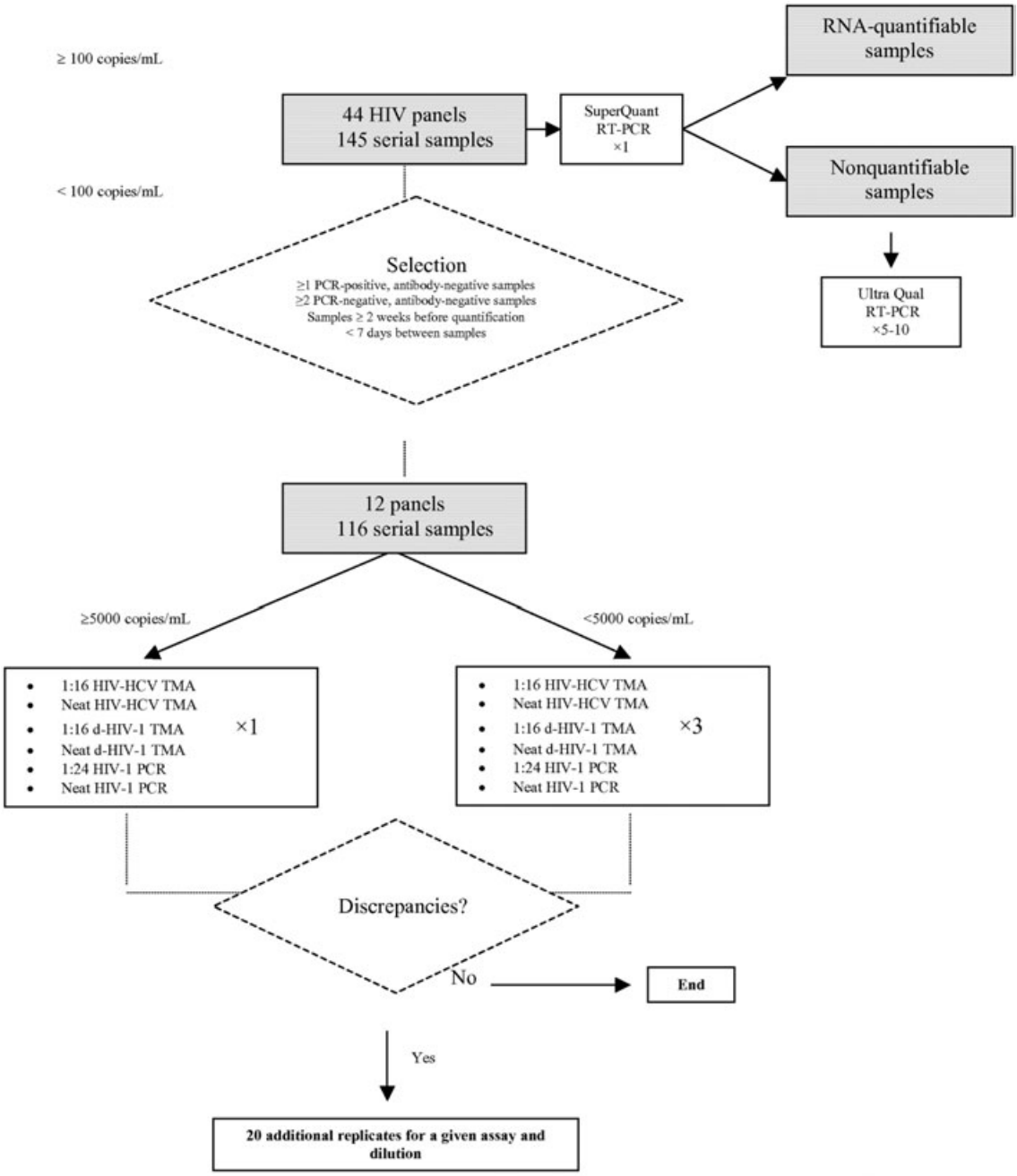

Fig. 1. HIV panel testing algorithm.

curves may be shifted right or left of one another, but otherwise the curves have the same shape). The logistic regression models included terms for estimating each test effect (e.g., multiplex TMA vs. PCR, multiplex vs. discriminatory TMA), a MP size effect, and the sample effect (a nuisance variable identifying the subject and sample date).

To calculate the differential window period between any two assays, we also used the logistic regression models to estimate the difference in viral loads needed to achieve a common sensitivity level, as derived from the difference in the assays' sensitivity curves (i.e., the shift—right or left between the two sensitivity curves).[17,18] The differential window period between two assays was then estimated by dividing the difference in viral loads by an estimate of the rate at which viral load increases during ramp-up (a function of the doubling time). Doubling times were previously estimated to be 20.5 hours for HIV (95% CI, 18.2-23.4 hr) and 10.8 hours for HCV-1 (95% CI, 9.9-12.0 hr) .[11,14] The differential win-

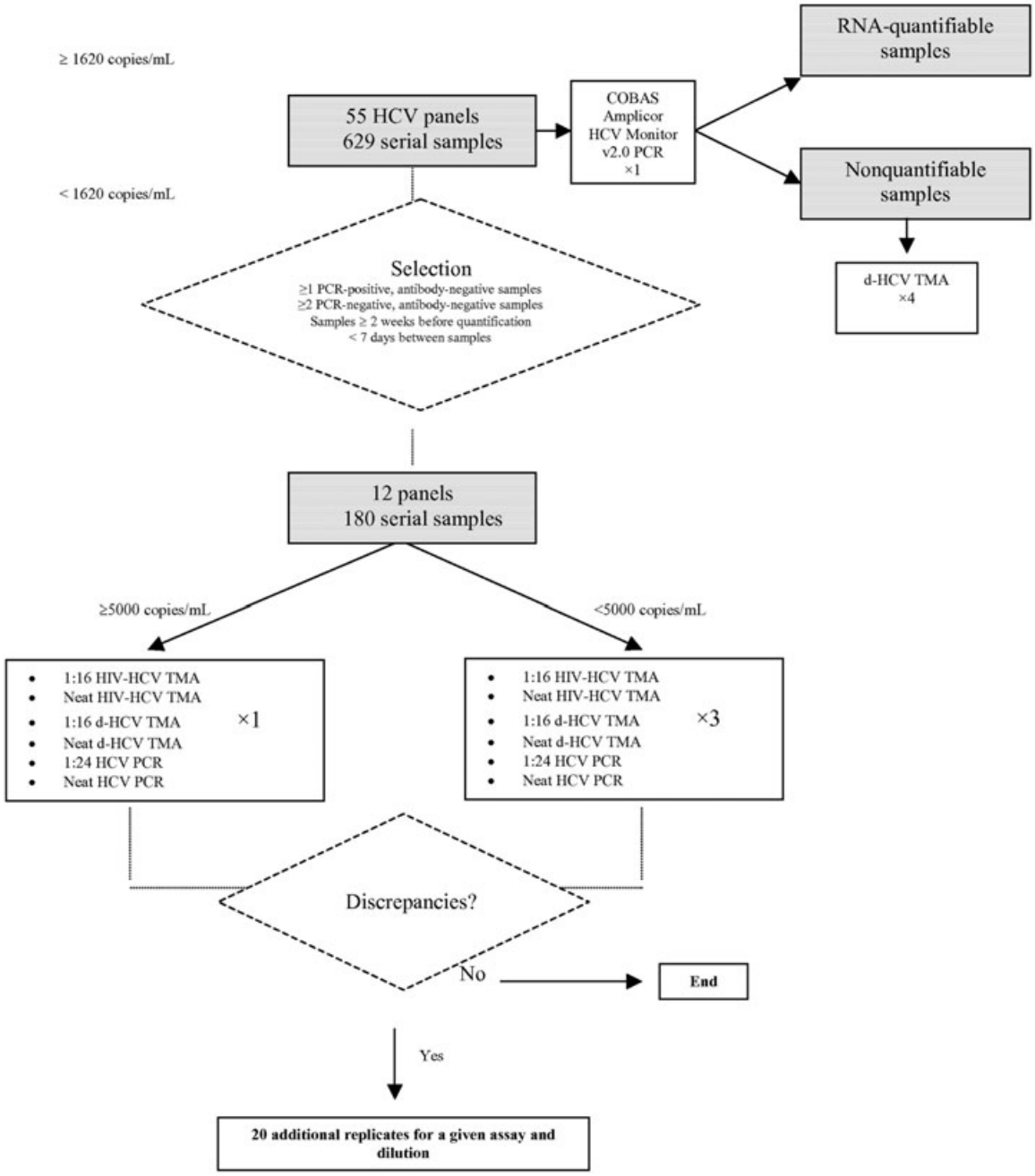

Fig. 2. HCV panel testing algorithm.

dow-period estimates were combined with estimates of HIV and HCV incidence in volunteer whole-blood donors (HIV-1, 2.16, and HCV, 2.80 per 100,000 person-years) to estimate the differential yield between two assays.[18,19] Wild-type 95 percent CIs for the differential window periods and differential yields were determined with Taylor series approximations of the standard errors of the differential window periods and differential yields.[20]

RESULTS

HIV panels

Test data were available for 113 HIV panel members (insufficient volume precluded testing of three samples). A little more than half of the samples (n = 59) had quantifiable RNA levels of 100 copies per mL. There was complete agreement between all assays for 77 percent of samples; that is, all replicate results (1-23) were either all

negative or all positive. There were 26 samples for which the results were not in complete agreement. All occurred when RNA levels were very low (n = 8) or nonquantifiable (n = 18). RNA levels in the eight quantifiable samples with discrepancies varied from 100 to 340 copies per mL.

Samples with discrepancies between 1:16 MP HIV-HCV TMA and 1:24 MP HIV PCR and between neat HIV-HCV TMA and neat HIV PCR are shown in Table 1 (samples with other types of discrepancies are not shown). As seen in Table 1A, there were 10 samples (of 113 evaluated) with discrepancies between these 2 assays when conducted in MPs. A similar number of discrepant samples was observed when testing was conducted neat (Table 1B). Differences in detection rates between the two assays appeared slight. As shown in Table 3, the logistic model confirmed a lack of a significant difference in sensitivity when the assays were conducted neat (OR, 1.0; 95% CI, 0.72-1.4) but detected a nearly twofold increase in the odds of having a positive replicate if using multiplex TMA relative to HIV-1 PCR when these assays were conducted in MPs (OR, 1.8; 95% CI, 1.3-2.5).

We also compared detection rates for the multiplex and discriminatory HIV-1 TMA assays. There were 15 samples (all nonquantifiable) with nonmatching results when testing was conducted neat; and 12 nonmatching samples, half being nonquantifiable, when testing was done diluted (data not shown). The sensitivity of the two tests was not significantly different (OR, 1.2; 95% CI, 0.72-2.1).

Finally, as expected, an assay conducted on an undiluted sample was much more likely to detect RNA (if present) than if conducted on a diluted sample (ORs, 45 and 79 for the neat vs. 1:16 and 1:24 MP comparisons, respectively).

HCV panels

Fifty-five percent (n = 100) of the 180 HCV panel samples were nonquantifiable (<1620 copies/mL). All assays were in complete agreement for 83 percent of the samples. Of 31 samples that were not in complete agreement, only one had quantifiable RNA (3600 copies/mL).

Discrepancies in the number of positive replicates detected by multiplex TMA and PCR conducted in MPs or neat were assessed separately. As shown in Table 2, six (one of them having 3600 copies/mL) and 16 (all with <1620 copies/mL) samples had slightly different numbers of positive replicates detected by each of these two assays when conducted in MPs (Table 2A) or neat (Table 2B), respectively. At these low viral concentrations, multiplex TMA appeared in both cases to have higher sensitivity than PCR (OR 4.0, 95% CI 2.8-5.8 for the MP comparison; and OR 2.3, 95% CI 1.6-3.2 for the neat comparison).

Similar to HIV, we did not observe any significant difference in sensitivity between multiplex and discriminatory TMA assays (OR, 1.2; 95% CI, 0.76-1.8); there were 25 samples with minor discrepancies between results when

TABLE 1. Replicate results on samples from HIV panels with discrepancies between HIV-HCV TMA and HIV PCR

		Number reactive/number of replicates					
Sample	RNA copies/mL	HIV-HCV TMA 1:16	HIV PCR 1:24	HIV-HCV TMA neat	HIV PCR neat	d-HIV TMA neat	d-HIV TMA 1:16
		A. Discrepancy between 1:16 MP HIV-HCV TMA and 1:24 MP HIV PCR					
1	<100	5/23	4/23	23/23	3/3	2/3	0/3
2	<100	6/23	7/22	3/3	3/3	3/3	0/3
3	<100	12/23	7/23	23/23	3/3	3/3	1/3
4	<100	0/23	2/22	5/23	12/23	1/3	0/3
5	100	22/23	18/23	3/3	3/3	3/3	2/3
6	100	9/23	8/23	3/3	3/3	3/3	2/3
7	100	9/23	3/22	3/3	3/3	3/3	2/3
8	200	7/23	8/21	3/3	3/3	3/3	0/3
9	300	16/23	9/23	3/3	3/3	3/3	1/3
10	340	18/23	22/22	3/3	3/3	19/19	3/3
		B. Discrepancy between neat HIV-HCV TMA and neat HIV PCR					
1	<100	0/3	0/3	12/23	4/23	1/3	0/3
2	<100	0/23	0/3	1/23	0/3	1/21	1/23
3	<100	0/3	0/3	2/23	3/22	1/21	0/3
4	<100	0/3	0/3	3/23	2/23	0/3	0/3
5	<100	1/23	1/23	2/23	1/23	0/3	0/3
6	<100	0/3	0/3	1/23	0/23	0/3	0/3
7	<100	0/3	0/3	4/23	3/22	6/23	0/3
8	<100	0/3	0/3	18/23	3/3	1/3	0/3
9	<100	0/23	2/22	5/23	12/23	1/3	0/3
10	<100	0/3	0/3	5/23	0/23	0/3	0/3

* Only data on samples with discrepancies between 1:16 MP HIV-HCV TMA and 1:24 MP HCV PCR or between neat HIV-HCV TMA and neat HCV PCR are presented. Samples with discrepancies between other assays (neat or diluted) are not shown.

TABLE 2. Replicate results on samples from HCV panels that yielded discrepancies between HIV-HCV TMA and HCV PCR*

Sample	Number of RNA copies/mL	Number reactive/number of replicates: HIV-HCV TMA 1:16	HCV PCR 1:24	HIV-HCV TMA neat	HCV PCR neat	d-HCV TMA neat	d-HCV TMA 1:16
		A. Discrepancy between 1:16 MP HIV-HCV TMA and 1:24 HCV PCR					
1	<1620	1/23	0/23	5/23	0/3	0/3	0/3
2	<1620	1/23	0/23	1/23	0/3	0/3	0/3
3	<1620	4/23	2/23	22/22	19/22	3/3	0/3
4	<1620	19/23	18/23	3/3	3/3	3/3	3/3
5	<1620	21/23	16/23	3/3	3/3	3/3	3/3
6	3600	23/23	21/23	3/3	3/3	3/3	3/3
		B. Discrepancy between neat HIV-HCV TMA and neat HCV PCR					
1	<1620	0/3	0/3	0/23	1/23	0/3	0/3
2	<1620	0/3	0/3	19/23	7/23	6/23	0/3
3	<1620	0/3	0/3	5/23	4/23	1/3	0/3
4	<1620	0/3	0/3	3/23	2/23	0/3	0/3
5	<1620	1/23	0/23	5/23	0/3	0/3	0/3
6	<1620	1/23	0/23	1/23	0/3	0/3	0/3
7	<1620	0/3	0/3	5/23	8/23	0/3	0/3
8	<1620	0/3	0/3	8/23	11/23	1/3	0/3
9	<1620	4/23	2/23	22/22	19/22	3/3	0/3
10	<1620	0/3	0/3	7/23	1/23	0/3	0/3
11	<1620	2/23	2/23	18/23	3/3	3/3	0/3
12	<1620	0/3	0/3	11/23	6/23	7/23	0/3
13	<1620	0/3	0/3	3/23	0/23	0/3	0/3
14	<1620	0/3	0/3	7/23	2/23	1/3	0/3
15	<1620	0/3	0/3	13/23	5/23	0/3	0/3
16	<1620	0/3	0/3	5/23	8/23	0/3	0/3

* Only data on samples with discrepancies between 1:16 MP HIV-HCV TMA and 1:24 MP HCV PCR or between neat HIV-HCV TMA and neat HCV PCR are presented. Samples with discrepancies between other assays (neat or diluted) are not shown.

TABLE 3. ORs and 95 percent CIs comparing assays performed neat or in MPs

Assay comparison	HIV	HCV
1:16 MP HIV-HCV TMA vs. 1:24 MP PCR	1.8 (1.3-2.5)	4.0 (2.8-5.8)
Neat HIV-HCV TMA vs. neat PCR	1.0 (0.72-1.4)	2.3 (1.6-3.2)
HIV-HCV TMA vs. d-TMA	1.2 (0.72-2.1)	1.2 (0.76-1.8)
Neat vs. 1:16 MP (HIV-HCV TMA)	45.3 (20.9-97.9)	52.3 (23.5-116.6)
Neat vs. 1:24 MP (PCR)	79.1 (32.7-191.5)	93.4 (37.3-233.8)

these two assays were conducted neat and 6 samples with minor discrepancies between results when testing was conducted in MPs (all these were nonquantifiable).

Assays conducted neat were 52 and 93 times more likely to detect HCV RNA than assays conducted on MPs of 16 and 24 samples, respectively (Table 3).

Implications of differential sensitivity of NAT assays on window-period closure and yield of viremic donations

To translate the slight differential sensitivity between NAT assays into meaningful variables with respect to blood safety, we estimated the gain in window-period closure and the number of additional interdicted viremic donations that could potentially result if TMA (rather than PCR) or ID-NAT (rather than MP-NAT) were conducted (see Materials and methods). Sensitivity differences documented in this study (Table 4) for the Roche and Gen-Probe assays performed on MP-diluted specimens translated into a projected HIV window-period difference of approximately 12 hours (95% CI, 5-19) and differential yield of 1 unit per 33 million donations (95% CI, 1 unit per 20 million to 1 unit per 100 million) and into a projected HCV window-period difference of approximately 14 hours (95% CI, 10-19 hr) and differential yield of 1 unit per 20 million donations (95% CI, 1 unit per 14 million to 1 unit per 33 million). The differences in sensitivity of the assays applied to neat specimens are even smaller, with essentially no difference in time to detection of HIV RNA, and approximately a 10-hour window-period difference for HCV (95% CI, 5-14 hr), which translates into a theoretical differential of about 1 HCV-infected donation per 33 million donations (95% CI, 1 unit per 25 million to 1 unit per 100 million). In contrast, comparisons of results for each manufacturer's assay performed on neat versus MP-diluted samples were highly significant for both viruses. For example, with a MP size of 16, the HIV differential window period is 3.4 days (95% CI, 3.0-3.8) and differential yield is 1 unit per 5 million donations (95% CI, 1 unit per 4 million to 1 unit per 7 million; Table 4).

TABLE 4. Differential window periods* (ΔWP) in days and differential yields of viremic donations† per 10,000,000 donations with 95 percent CIs for comparisons of NAT assays performed neat or on MP dilutions of study panel members

	HIV		HCV	
Assay comparison	ΔWP (days)	ΔYield per 10^7 donations	ΔWP (days)	ΔYield per 10^7 donations
1:16 MP HIV-HCV TMA vs. 1:24 MP PCR	0.5 (0.2 to 0.8)	0.3 (0.1 to 0.5)	0.6 (0.4 to 0.8)	0.5 (0.3 to 0.7)
Neat HIV-HCV TMA vs. neat PCR	0.0 (–0.3 to 0.3)	0.0 (–0.2 to 0.2)	0.4 (0.2 to 0.6)	0.3 (0.1 to 0.4)
HIV-HCV TMA vs. d-TMA	0.2 (–0.3 to 0.7)	0.1 (–0.2 to 0.4)	0.1 (–0.1 to 0.3)	0.1 (–0.1 to 0.2)
Neat vs. 1:16 MP (HIV-HCV TMA)	3.4 (3.0 to 3.8)	2.0 (1.5 to 2.6)	1.8 (1.6 to 2.0)	1.4 (1.1 to 1.7)
Neat vs. 1:24 MP (PCR)	3.9 (3.4 to 4.4)	2.3 (1.7 to 2.9)	2.1 (1.9 to 2.3)	1.6 (1.2 to 2.0)

* Based on doubling times of 20.5 hours for HIV and 10.8 hours for HCV.
† Based on HIV incidence rate of 2.16 per 100,000 person-years and HCV incidence rate of 2.80 per 100,000 person-years.[18]

DISCUSSION

This head-to-head evaluation of FDA-licensed NAT assays focused on frequently collected serial samples from HIV-1- and HCV-infected plasma donor panels. This allowed assessment of relative sensitivity of assays and of the impact of MP dilutions on sensitivity during the critical early viremia phase that is the predominant basis for residual risk of viral transmission by NAT-screened blood components. The study documented little difference in rates of detection of window-phase viremia when the Roche assay (with the multiprep procedure) and the Gen-Probe NAT assay were applied to neat samples, with identical detection rates for HIV and borderline enhanced detection by the Gen-Probe assay for HCV-positive samples with very low levels of virus (OR, 2.3; 95% CI, 1.6-3.2). These findings are consistent with results of analytical sensitivity studies conducted by the manufacturers during licensure trials.[3,4] These analytical sensitivity studies, which used replicate testing of dilutions of viral reference standards and probit analyses, indicated that both assay systems are highly sensitive, with 50 percent detection limits of 14 HIV-1 and 12 HCV copies per mL and 95 percent detection limits that range from 30 to 60 copies per mL for HIV-1 and HCV. A recently published multicenter proficiency study evaluated the analytical sensitivities of the Roche COBAS AmpliScreen test (performed in three laboratories) and Gen-Probe/Chiron Procleix assays (performed in five laboratories) based on analysis of coded panels containing half-log dilution series of HIV-1 and HCV reference standards (two genotypes per virus).[5] The study documented 50 percent (95%) detection limits for HIV-1 clade B RNA of 3.6 copies per mL (31 copies/mL) for Gen-Probe and 7.1 copies/mL (92 copies/mL) for Roche with the Multiprep procedure. Corresponding 50 percent (95%) detection limits for HCV genotype 1 were 7.9 copies per mL (85 copies/mL) for the Procleix assay and 9.9 copies per mL (77 copies/mL) for the COBAS AmpliSceen HCV Version 2.0 test. By analysis of alternative extraction methods, that study demonstrated that the small differences in detection rates were attributable to the different volumes of sample subjected to amplification (500 µL for Gen-Probe/Chiron Procleix TMA assays relative to 250 µL for Roche COBAS AmpliScreen PCR assays) rather than to differences in efficiency of amplification or detection of target nucleic acids.[5]

In comparison to results with neat samples, differences in detection rates were larger and significant when performance of the Roche and Gen-Probe assays was evaluated in the context of diluted samples consistent with MP screening (OR 1.8, 95% CI 1.3–2.5 for HIV; and OR 4.0, 95% CI 2.8-5.8 for HCV, favoring the Gen-Probe assay for both viruses). These results reflect the impact of the larger dilution factor inherent in MPs used by Roche (24 samples per pool) versus Gen-Probe (16 samples per pool). Even these differences in MP sensitivity, however, translated into extremely small window-period differences of approximately 12 and 14 hours for HIV and HCV, respectively. These latter differences, in turn, translated into projected differential detection rates of one infected donation per 20 million units (HCV) or 33 million units (HIV) tested, a theoretical difference in yield of infected units that is well below a level that could be measured clinically. The overall findings from this study are thus reassuring with respect to the comparability of licensed NAT systems when performed in either MP or ID contexts and support results from another study that found similar detection rates of HIV or HCV RNA–positive, antibody-negative donations among users of the Gen-Probe TMA or Roche PCR assays in the first 3 years of screening in the United States.[1]

In contrast, this study documented highly significant differences in detection rates and window periods when comparing results for each manufacturer's assay performed on neat versus MP-diluted samples. These differences translate into the additional detection of approximately 2.0 HIV and 1.5 HCV potentially infectious donations per 10 million donations if screening were conducted on individual donations (IDs) rather than in MPs, similar to our previous projections based both on the incidence window-period model[21] and on extrapolations from HIV-1 and HCV NAT yield and HIV-1 less-sensitive

enzyme immunoassay data.[18] This low projected incremental yield of ID-NAT over MP-NAT is due to the very low incidence of HIV and HCV infection in volunteer donors (two to three per 100,000 person-years) and is consistent with the very small number of MP-NAT "breakthrough" cases that have been documented in the United States and several other countries, most of which have been attributed to units with low-level viremia that would likely have been detected had ID-NAT been performed.[22-26] Despite this low projected yield and the correspondingly low projected incremental cost-effectiveness of ID-NAT,[27,28] some blood organizations are considering converting to ID-NAT once adequate automation is available, both for purposes of HIV-1 and HCV safety and to enhance NAT detection of hepatitis B virus (HBV) and West Nile virus, agents for which ID-NAT sensitivity is particularly important (owing to a slower ramp-up phase for HBV and very high regional and temporal incidence with prolonged low-level viremia following seroconversion for West Nile virus).[17,29-31] An alternative strategy would be to consider intermediate pool sizes (e.g., 4-6 samples per MP rather than 16-24). The incremental yield associated with intermediate-sized pools, however, would be small, because pre-ramp-up viremia is typically so low level it is not detectable by even very small MPs[13,14,17] and the ramp-up phase is exponential, and consequently a fourfold reduction in MP size (e.g., from pools of 16-4 for Gen-Probe or 24-6 for Roche) would result in only 50 percent of the window-period closure and yield that would be achieved by converting to ID-NAT.[29]

The findings from our study have several implications for pool resolution and supplemental testing of NAT-reactive donations. With respect to pool resolution, our data demonstrating 45- to 90-fold higher odds of detection for window-phase samples tested neat compared to results at MP dilutions support manufacturer-recommended and FDA-approved algorithms for MP-NAT resolution testing.[3,4,32] According to these algorithms, samples represented in reactive MPs that subsequently did not react when tested neat are considered negative, and corresponding blood components may be released for transfusion. Our data demonstrating the equivalent sensitivity of the discriminatory HIV-1 and HCV TMA assays, compared to the multiplex HIV-1 and HCV Procleix assay on corresponding neat samples, also support the use of these assays for resolution of multiplex TMA-reactive donations identified subsequent to MP-NAT screening. These data are consistent with analytical sensitivity data comparing the multiplex and discriminatory assays,[3,6] as well as a clinical study that further evaluated a large number of donation samples and followed donors who had tested multiplex-reactive with various patterns of discriminatory TMA results.[10]

Finally, the very similar sensitivities of the Roche and Gen-Probe assays on neat testing of early window-phase samples support use of these assays to confirm HIV or HCV RNA in reactive donation samples detected through MP-NAT screening with the alternate manufacturer's system. This approach of use of an alternate manufacturer's licensed assay in supplemental testing has been adopted for serologic screening[33] and recently applied successfully to reactive individual donor samples detected by MP-NAT screening.[34] This strategy works because MP-NAT screening is able to detect truly infected persons only if their viral loads are high enough to overcome the dilution factor of the pool size. As a result, true-positive samples that are resolved at the ID level will have sufficient viremia to be reliably and reproducibly detected by both manufacturers' screening NAT assays and the relevant discriminatory assay.

This "alternate NAT" supplemental testing strategy, however, may not be as definitive for reactive samples detected by ID-NAT. The results of this study demonstrate that rare window-phase donations with very-low-level viremia may be detected at variable rates (stochastically) by the licensed screening and discriminatory assays. In the setting of routine ID-NAT screening, it is possible that a donation with very-low-level viremia may be detected by one screening assay, but would not be reactive on a single analysis by the alternate screening NAT assay or the discriminatory TMA tests owing to stochastic sampling issues. Because low-level viremic units are known to be infectious,[22-26] we believe that to avoid potential for infusion of a very-low-level viremic unit that was originally detected as reactive by the primary screening NAT assay, all reactive units screened by ID-NAT should be discarded, and donors who are NAT-reactive and seronegative should be recalled and further tested to definitively establish their true infection status.

ACKNOWLEDGMENTS

The investigators thank Yiling Xu (Westat) for her valuable contribution vis-à-vis data compilation and programming.

The National Heart, Lung, and Blood Institute Nucleic Acid Test Study is the responsibility of the following investigators:

National Heart, Lung, and Blood Institute, NIH: George J. Nemo

Blood Centers of the Pacific and Blood Systems Inc.: Michael P. Busch, Principal Investigator of the NAT Study Group

Westat: George B. Schreiber, Melissa King, Steven H. Kleinman, Simone A. Glynn

American Red Cross: Susan L. Stramer

America's Blood Centers: Sally Caglioti, D. Michael Strong

Association of Independent Blood Centers: Richard Gammon

Center for Biologics Evaluation and Research, FDA: Indira K. Hewlett

Roche: James Gallarda, Yanfeng Yang

Gen-Probe Inc.: Larry Mimms, Cristina Giachetti, Sherrol McDonough

Chiron Corp.: Bruce H. Phelps

REFERENCES

1. Stramer SL, Glynn SA, Kleinman SH, et al. Detection of HIV and HCV infections among antibody-negative US blood donors by nucleic acid amplification testing. N Engl J Med 2004;351:760-8.
2. Grant PR, Busch MP. Nucleic acid amplification technology methods used in blood donor screening [review]. Transfus Med 2002;12:229-42.
3. Procleix® HIV-1/HCV assay [package insert]. IN0076-01, Rev A. San Diego: Gen-Probe, Inc.; c2001-2004. Available from: http://www.fda.gov/cber/label/hivhcvgen060404LB.pdf
4. COBAS® AmpliScreen HIV-1 test [package insert]. Version 1.5. Pleasanton (CA): Roche Molecular Systems Inc.; 2003. Available from: http://www.fda.gov/cber/label/hiv1roc121903LB.pdf
5. Lelie PN, van Drimmelen AA, Cuypers HT, et al. Sensitivity of HCV RNA and HIV RNA blood screening assays. Transfusion 2002;42:527-36.
6. Giachetti C, Linnen JM, Kolk DP, et al. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. J Clin Microbiol 2002;40:2408-19.
7. Gallarda JL, Dragon E. Blood screening by nucleic acid amplification technology: current issues, future challenges. Mol Diagn 2000;5:11-22.
8. Stramer SL, Caglioti S, Strong DM. NAT of the United States and Canadian blood supply [special report]. Transfusion 2000;40:1165-8.
9. Galel SA, Strong DM, Tegtmeier GE, et al. Comparative yield of HCV RNA testing in blood donors screened by 2.0 versus 3.0 antibody assays. Transfusion 2002;42:1507-13.
10. McAuley JD, Caglioti S, Williams RC, et al. Clinical significance of nondiscriminated reactive results with the Chiron Procleix® HIV-1 and HCV assay. Transfusion 2004;44:91-6.
11. Fiebig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS 2003;17:1871-9.
12. Smith R, Heldebrant C. Large scale PCR screening of pooled plasma samples for HIV-1 and HCV. Dev Biol (Basel) 2000;102:109-11.
13. Fiebig EW, Heldebrant C, Smith R, et al. Intermittent low-level viremia in very early primary HIV-1 infection. J AIDS 2005;39:133-7.
14. Glynn SA, Wright DJ, Kleinman SH, et al. Dynamics of viremia in early hepatitis C virus infection. Transfusion 2005;45:994-1002.
15. COBAS® AMPLICOR HCV MONITOR test [package insert]. Version 2.0, Revision 4.0. HCM for research use only: not for use in diagnostic procedures. Pleasanton (CA): Roche Molecular Systems Inc.; 2003.
16. SAS Institute Inc. SAS/STAT user's guide. Version 8. Cary (NC): The Institute; 1999.
17. Biswas R, Tabor E, Hsia CC, et al. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion 2003;43:788-98.
18. Busch MP, Glynn SA, Stramer SL, et al. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005;45:254-64.
19. Dodd RY, Notari IV, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 2002;42:975-9.
20. Karlin S, Taylor HM. A first course in stochastic processes. New York: Academic Press; 1975.
21. Glynn SA, Kleinman SH, Wright DJ, Busch MP. International application of the incidence rate/window period model. Transfusion 2002;42:966-72.
22. Schuttler CG, Caspari G, Jursch CA, et al. Hepatitis C virus transmission by a blood donation negative in nucleic acid amplification tests for viral RNA [letter]. Lancet 2000;355:41-2.
23. Delwart EL, Kalmin ND, Jones TS, et al. First case of HIV transmission by an RNA-screened blood donation. Vox Sang 2004;86:771-7.
24. Phelps R, Robbins K, Liberti T, et al. Window-period human immunodeficiency virus transmission to two recipients by an adolescent blood donor. Transfusion 2004;44:929-33.
25. Taylor C, Price TH, Strong DM. Possible HCV transmission from blood screened by pooled nucleic acid testing [Abstract S30-030e]. Transfusion 2002;42(9 Suppl):9s.
26. Stramer SL, Chambers L, Page PL, Wagner AG, Gibble J. Third reported US case of HIV transmission from NAT screened blood [Abstract S134-040k]. Transfusion 2003;43:40A.
27. Jackson BR, Busch MP, Stramer SL, AuBuchon JP. The cost-effectiveness of NAT for HIV, HCV and HBV in whole-blood donations. Transfusion 2003;43:721-9.
28. Marshall DA, Kleinman SH, Wong JB, et al. Cost-effectiveness of nucleic acid test screening of volunteer blood donations for hepatitis B, hepatitis C and human immunodeficiency virus in the United States. Vox Sang 2004;86:28-40.
29. Busch MP, Dodd RY. Nucleic acid amplification testing and blood safety: what is the paradigm? [editorial]. Transfusion 2000;40:1157-60.
30. Dodd RY. Emerging infections, transfusion safety, and epidemiology [perspective]. N Engl J Med 2003;349:1205-6.
31. Custer B, Tomasulo PA, Murphy EL, et al. Triggers for switching from minipool testing by nucleic acid technology to individual-donation nucleic acid testing for West Nile virus: analysis of 2003 data to inform 2004 decision making. Transfusion 2004;44:1547-54.
32. Guidance for industry: use of nucleic acid tests on pooled

and individual samples from donors of whole blood and blood components for transfusion to adequately and appropriately reduce the risk of transmission of HIV-1 and HCV. 67 FR 17077. Rockville (MD): Center for Biologics Evaluation and Research (CBER), Food and Drug Administration; 2002 Mar.
33. Stramer SL. Viral diagnostics in the arena of blood donor screening [plenary presentation # W09 01]. Vox Sang 2004;87(Suppl 2):s180-3.
34. Caglioti S, Robertson G, Winkelman V, et al. NAT cross-supplemental role assessment of the COBAS Ampliscreen and Procleix TMA HIV-1 and HCV assays using initial reactive donor specimens [Abstract SP-188]. Transfusion 2004;44(S9):87a-8a.