Clinical specificity and sensitivity of a blood screening assay for detection of HIV-1 and HCV RNA

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BACKGROUND: An HIV-1 and HCV NAT blood screening assay (Procleix HIV-1/HCV, Gen-Probe, Inc.) simultaneously detecting HIV-1 and HCV RNA) has been implemented. Donor plasma samples reactive in the Procleix HIV-1/HCV assay are tested with the HIV-1 and HCV discriminatory assays to resolve whether HIV-1 RNA, HCV RNA, or both are present.

STUDY DESIGN AND METHODS: To determine the specificity of the Procleix HIV-1/HCV assay, data were analyzed for samples from 192,288 donations, tested in 16-member pools. To determine sensitivity, data were analyzed for 2014 commercial samples known to contain HIV-1, HCV, or both, as well as 10 HIV-1 and 10 HCV commercial seroconversion panels.

RESULTS: The specificity of the Procleix HIV-1/HCV assay was 99.7 percent. The HIV-1 and HCV discriminatory assays showed similar specificity. The sensitivity of the Procleix HIV-1/ HCV assay was 99.9, 99.6, and 100 percent, respectively, for samples containing HIV-1, HCV, or both. The Procleix discriminatory assays were comparably sensitive. The Procleix discriminatory assays detected all tested samples of known HIV-1 subtype or HCV genotype. Procleix HIV-1/HCV testing of seroconversion panels showed that the median times to a positive reaction for HIV-1 and HCV were reduced by 3 and 25 days, respectively, compared to serologic tests.

CONCLUSION: These studies support the use of the Procleix HIV-1/HCV assay for routine blood donor screening.

An HIV-1 and HCV assay (Procleix; Gen-Probe, Inc. San Diego, CA) was developed in response to requirements for a highly sensitive, NAT blood screening technology that could reduce the window period between infection and the detectability of infection by standard serologic tests and therefore increase the chance of interdicting infectious donations. The Committee for Proprietary Medicinal Products of the European Union requires the use of NAT to screen blood and plasma donations.[1] Since 1999, considerable clinical experience in blood banks with the Procleix HIV-1/HCV assay has been obtained in the United States.[2-4]

The Procleix assay comprises three processes in the same test tube: sample preparation (target capture), transcription-mediated amplification (TMA) of HIV-1 and HCV target RNA sequences, and detection of amplification products (amplicons) with a hybridization protection assay (HPA). The assay method has been described in detail.[5,6]

During sample preparation, viral RNA is isolated from plasma by target capture. Oligonucleotides ("capture oligonucleotides") homologous to highly conserved regions of the HIV-1 and HCV genomes are hybridized to

ABBREVIATIONS: HPA = hybridization protection assay; NGI = National Genetics Institute; p24Ag = p24 antigen; TMA = transcription-mediated amplification; TP = true positive.

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any HIV-1 and/or HCV target RNA sequences present in the sample. Subsequent target amplification by TMA uses two enzymes, Moloney murine leukemia virus reverse transcriptase and T7 RNA polymerase. The reverse transcriptase generates DNA copies of the target RNA sequences, and the T7 RNA polymerase produces multiple copies of RNA amplicons from the DNA templates. Finally, amplicons are detected with the HPA, by use of complementary, single-stranded, nucleic acid probes containing a chemiluminescent label. During the HPA, the chemiluminescent signals produced by the hybridized probes are measured in a luminometer and reported in relative light units.

The Procleix HIV-1 and HCV discriminatory assays use the same processes and procedures as does the Procleix HIV-1/HCV assay, except that HIV-1-specific or HCV-specific probes are used in place of the Procleix HIV-1/HCV assay probe reagent. The Procleix HIV-1/HCV assay and the two discriminatory assays can detect as few as 100 copies per mL of viral RNA.[5]

The results reported in this paper were collected during studies that took place between August 2000 and November 2000. These studies were conducted to define the specificity and sensitivity of the Procleix HIV-1/HCV assay in a blood bank setting.

To determine the specificity of the Procleix HIV-1/HCV assay, test data were analyzed from 192,288 prospectively collected blood donations. Before testing, plasma samples from these donations were combined into 11,950 pools of 16 samples each. Specificity of the HIV-1 and HCV discriminatory assays was determined by testing unlinked plasma samples from a subset of the prospectively collected donations. These samples were previously found to not react in the Procleix HIV-1/HCV assay. A total of 2508 and 2443 samples, respectively, were used to assess the specificity of the HIV-1 and HCV discriminatory assays.

To determine the sensitivity of the Procleix HIV-1/HCV assay, the HIV-1 discriminatory assay, and the HCV discriminatory assay, test data were analyzed from 2014 plasma or serum samples known to be positive for HIV-1 and/or HCV. A subset of these known positive samples was characterized with respect to HIV-1 subtype or HCV genotype. Procleix assay test results were compared to the samples' known viral status as determined by alternative NATs for HIV-1 and HCV.

MATERIALS AND METHODS

Clinical specificity

Procleix HIV-1/HCV assay results for the specificity analysis were collected at five study sites: American Red Cross (Detroit, MI); Blood Center of Southeastern Wisconsin (Milwaukee, WI); Blood Systems Laboratories (Tempe, AZ); Citrus Regional Blood Center (Association of Independent Blood Centers, Lakeland, FL); and Florida Blood Services (St. Petersburg, FL). Three of these sites (American Red Cross, Blood Systems Laboratories, and Florida Blood Services) also collected HIV-1 and HCV discriminatory assay results for the specificity analysis of the Procleix discriminatory assays. Each site used three assay kit lots.

Plasma samples for the specificity study were all obtained from consenting voluntary allogeneic blood donors at 92 participating blood donation sites during the study period. Demographic data for these donors were not collected. However, because of the number and geographic diversity of the donation sites, the donors' demographics were considered to be representative of those in the general US blood donor population. All donors who provided plasma samples for the study met the donation sites' standard donor eligibility requirements. Accordingly, a low prevalence of HIV-1 and/or HCV infection was expected in the donor population.

At the blood bank sites participating in the study, routine blood screening for HIV-1 and HCV is performed by testing plasma pools composed of equal volumes of plasma from 16 donor samples. This pooling process is performed with automated pipetting instrumentation. After a pool is made, a weight check is performed to validate that all 16 samples were pipetted into the pool sample. At the study sites, the pool samples were tested with the Procleix HIV-1/HCV assay. When a pool was did not react in the Procleix HIV-1/HCV assay, each individual sample in the pool was also considered to be not reactive for HIV-1 RNA and HCV RNA.

When a plasma sample pool was reactive in the Procleix HIV-1/HCV assay, each of the 16 samples in the pool was retested individually with the Procleix HIV-1/HCV assay. The reactive sample(s) identified by this individual testing were then tested with the Procleix HIV-1 and HCV discriminatory assays to identify the type(s) of viral RNA (HIV-1 and/or HCV) present.

All donations in the specificity study were screened serologically. EIAs for initial serologic screening included an HIV-1/HIV-2 antibody assay (HIV Ab, Abbott (Abbott Park, IL) or Genetic Systems (Redmond, WA)), an assay for HIV-1 p24 antigen (p24Ag, Abbott monoclonal or Coulter (Miami, FL)), and an assay for HCV antibody (Abbott 2.0 or Ortho 3.0, Raritan, NJ). Supplemental serologic tests included a Western blot (Bio-Rad (Hercules, CA), Epitope (Beaverton, OR), or Calypte (Rockville, MD)) or immunofluorescence assay (HIV-1 IFA, Waldheim (Vienna, Austria)) for HIV antibody, a neutralization test (Abbott or Coulter) for HIV-1 p24Ag, and a RIBA (RIBA 3.0 strip immunoblot assay, Chiron, Emeryville, CA) for HCV antibody.

In each of the Procleix specificity study runs, in addition to the standard negative, HIV-1-positive, and HCV-positive run controls, one low-level reactive p24 antigen

external control was included after a 1 in 16 dilution in HIV-1-negative serum (sample after dilution provided by Impath/BioClinical Partners, Franklin, MA). The p24 antigen level in this diluted sample was lower than the p24 antigen assay limit of detection (Coulter). The HIV-1 copy content after dilution was 2312 copies per mL.

Clinical sensitivity

Procleix assay results for the sensitivity analysis were collected at three clinical study sites: American Red Cross, Blood Systems Laboratories, and Johns Hopkins University (Baltimore, MD). Each site used three assay kit lots.

Plasma or serum samples known to be positive for HIV-1 or HCV RNA were obtained by Gen-Probe Inc. from the following vendors: ProMedDx (Norton, MA); Intergen Company (Purchase, NY); Boston Biomedica Inc. (West Bridgewater, MA); and Impath/BioClinical Partners. Ten commercially available HIV-1 and HCV seroconversion panels were obtained from Boston Biomedica, Inc. and Impath/BioClinical Partners. Known positive samples were provided by Gen-Probe to the study sites. Vendor-reported test results for HIV-1-positive samples were obtained on the same samples sent to Gen-Probe or on plasma collected from the same individuals within 90 days of obtaining the study samples. All vendor-reported test results for HCV-positive samples were obtained on the study samples. Additional quantitative PCR test results for some known positive samples were provided by two reference laboratories: Quest (San Juan Capistrano, CA; HIV-1) and National Genetics Institute (NGI, Los Angeles, CA; HCV). Some HIV-1 NAT-positive samples lacking vendor-supplied results of HIV-1 antigen and/or antibody testing were sent to Chiron, where serologic test results were obtained. The tests used by Chiron for p24Ag and HIV antibody were the HIV-1 p24 Ag ELISA system and the Bio-Rad HIV-1/2 peptide test kit HIV-1/HIV-2 peptide EIA (Genetic Systems), respectively.

Known HIV-1-positive samples. Information on the disease status of individuals providing HIV-1-positive samples was available for 1040 samples. A total of 295 samples were obtained from patients diagnosed with AIDS on the basis of prior indicator conditions and/or a CD4+ T lymphocyte count less than 200 per mm^3. An additional 168 samples were obtained from symptomatic patients not considered to have AIDS. A total of 338 samples were obtained from asymptomatic individuals or from patients with persistent generalized lymphadenopathy or an acute HIV-1 infection.[7] The disease status of individuals providing 239 HIV-1-positive samples was not known. The majority of the HIV-1-positive samples were obtained from individuals receiving antiviral medication. HIV-1 subtype (subtypes A-G and group O) was identified in 42 of the known HIV-1-positive samples. The numbers and distribution of HIV-1 subtypes tested are as follows: 9 of A, 10 of B, 10 of C, 2 of D, 5 of E, 3 of F, 2 of G, and 1 of group O.

Known HCV-positive samples. Information on the disease status of individuals providing HCV-positive samples was available for 1015 samples. A total of 887 known HCV-positive samples were obtained from first-time blood donors whose plasma was tested at a donor testing center and found to be antibody reactive for HCV. An additional 53 samples were from patients who were diagnosed with chronic hepatitis C after blood donor screening had shown their donations to be HCV antibody reactive. The disease status of individuals providing 75 HCV-positive samples was not known. HCV genotype (genotypes 1-5) was identified in 41 of the known HCV-positive samples, including 14 serum and 27 plasma samples. The numbers and distribution of HCV genotypes and subtypes tested were as follows: four of type 1, one of type 1a, four of type 1b, one of type 2, one of type 2a/2c, nine of type 2b, seven of type 3, three of type 3a, six of type 4, two of type 4a, one of type 4b/4c, and two of type 5a.

Coinfected samples. A total of 180 of the known HIV-1 NAT-positive samples were reported also to be reactive for HCV RNA or antibody, on the basis, respectively, of HCV NAT and FDA-licensed HCV antibody tests. Seven of the 42 samples of known HIV-1 subtype were positive for antibody to HCV. Twenty-seven known HCV NAT-positive samples were reported also to be reactive for HIV antibody and/or HIV-1 p24Ag, on the basis of FDA-licensed tests. Two of the 41 samples of known HCV genotype were positive for HIV antibody.

Negative samples. To eliminate potential operator bias, 429 samples that were HIV-1 and HCV NAT-negative were provided to Gen-Probe and sent to the study sites along with the known positive samples. Viral status was not shown on any sample label and was accordingly unknown to the operators performing the Procleix assays. Positive and negative samples were placed randomly in each assay run.

Primary testing. Samples were received frozen at Gen-Probe and stored at –70 °C under monitored conditions until shipment. They were shipped frozen to the study sites for testing. The samples were tested neat in the Procleix HIV-1/HCV assay, the Procleix HIV-1 discriminatory assay, and the Procleix HCV discriminatory assay and were also tested after 1 in 16 dilution in the Procleix HIV-1/HCV assay. All dilutions were made with processed human serum known to be negative for HIV-1 and HCV RNA and antibody and for HIV-1 p24Ag. Samples with initial and repeatedly invalid results in the Procleix HIV-1/HCV assay or either discriminatory assay were considered repeatedly invalid in that particular as-

say and were eliminated from the overall sensitivity analysis for that assay.

Viral quantitation. Any known HIV-1-NAT-positive sample that did not react in either the Procleix HIV-1/HCV assay or the HIV-1 discriminatory assay was sent to Quest for viral quantitation with the licensed HIV-1 Amplicor 2.0 ultraquantitative assay (Roche, Basel, Switzerland), which reportedly can detect viral RNA concentrations as low as 50 copies per mL.[8] Samples containing less than 100 copies per mL of HIV-1 RNA according to this quantitative assay were excluded from the overall sensitivity analysis. Procleix assay results for these samples are discussed separately.

Any known HIV-1-NAT-positive sample that was also reactive in the Procleix HCV discriminatory assay was considered potentially coinfected and further evaluated. An HCV discriminatory assay reactive result was considered true positive (TP) if the vendor-reported HCV NAT and/or HCV antibody test result was reactive. The sample was then considered coinfected. If no vendor information on the sample's HCV status was available or if the Procleix assay result disagreed with the vendor-supplied result, the sample was sent to NGI for HCV quantitation with the HCV RNA SuperQuant assay (NGI). A sample that was reactive in this assay was considered positive for HCV, while one that did not react was considered HCV negative. In the latter case, the reactive result in the Procleix HCV discriminatory assay was considered false-positive.

Any known HCV NAT-positive sample that did not react in either the Procleix HIV-1/HCV assay or the HCV discriminatory assay was sent to NGI for HCV viral quantitation with the HCV RNA SuperQuant assay, which reportedly can detect viral RNA concentrations as low as 100 copies per mL. Samples that did not react in this assay were considered to contain less than 100 HCV RNA copies per mL and were excluded from the overall sensitivity analysis. The Procleix assay results for these samples are discussed separately.

Any known HCV-NAT-positive sample also reactive in the Procleix HIV-1 discriminatory assay was considered potentially coinfected and further evaluated. An HIV-1-discriminatory-assay-reactive result was considered TP if the vendor-reported HIV-1 NAT, HIV antibody, and/or p24Ag test result was reactive. The sample was then considered coinfected. If no vendor information on the sample's HIV-1 status was available, or if the Procleix assay result disagreed with the vendor-supplied result, the sample was sent to Quest for HIV-1 quantitation with the Amplicor 2.0 HIV-1 ultraquantitative assay (Roche). A sample reactive in this assay was considered positive for HIV-1, while one that did not react was considered HIV-1 negative. In the latter case, the reactive result in the Procleix HIV-1 discriminatory assay was considered false-positive.

RESULTS

Clinical specificity

Procleix HIV-1 and HCV assay. All laboratory test results required by the study protocol were obtained for 11,950 pools of 16 plasma samples each. Specificity was calculated to be True negative/(True negative + False positive) $\times$ 100 percent or 11,625/(11,625 + 38) $\times$ 100 percent = 99.67 percent. The 95 percent CI for specificity was 99.55 to 99.77 percent.

Of the 11,950 pools, 11,776 (98.5%) did not react and 174 (1.5%) were reactive in the Procleix HIV-1/HCV assay. A total of 136 of the 174 reactive pools contained at least one seropositive or serologically indeterminate sample, and these samples were eliminated from the specificity analysis. The remaining 38 reactive pools contained only seronegative samples. These 38 were considered false positive as the Procleix reactivity could not be confirmed with alternative NAT or follow-up testing. Of these 38 false-positive pools, 37 did not react in Procleix testing at the individual sample level, either in the HIV-1/HCV assay or in both Procleix discriminatory assays, indicating that the source of the false positivity was likely intraassay contamination, not lack of assay specificity. The last of the 38 false-positive pools contained one individual sample that was reactive in the Procleix HIV-1/ HCV and HCV discriminatory assays, as well as in the alternative HCV NAT. The Procleix assay and alternative NAT performed on an alternate index sample and on a follow-up sample were nonreactive, indicating that the false-positive NAT index results were likely due to a contaminated NAT tube.

In the specificity study, there was 100 percent detection of the p24Ag external run controls in the Procleix HIV-1/HCV assay (n = 413) and the Procleix HIV-1 discriminatory assay (n = 27). Of all donor samples tested, five samples were found to be p24Ag repeat reactive and confirmed with neutralization. Of these five, three were also Procleix HIV-1 reactive. Two of the five did not react in the Procleix assay and both of these samples were also HIV seronegative. One of these two donors was entered into the follow-up study and was found to be negative in p24Ag, HIV antibody, and Procleix tests. The index neutralized p24Ag result for this donor was considered false positive based on follow-up results. The other p24Ag-reactive and Procleix-nonreactive donor also tested nonreactive at index in an alternative HIV NAT, and this donor declined follow-up. Thus, the p24Ag-reactive result at index could not be confirmed by other tests or another p24Ag-reactive result on a subsequent sample.

Procleix HIV-1 and HCV discriminatory assays. Specificity of the Procleix HIV-1 and HCV discriminatory assays was determined, respectively, with 2508 and 2443 unlinked donor plasma samples. These samples had been tested in the specificity determination for the Procleix

HIV-1/HCV assay, and all did not react in that assay. Of the samples used to determine discriminatory assay specificity, 0.2 percent (6 of 2508) were false positive in the HIV-1 discriminatory assay and 0.3 percent (7 of 2443) were false positive in the HCV discriminatory assay. The specificity of the Procleix HIV-1 and HCV discriminatory assays was accordingly determined to be 99.8 and 99.7 percent, respectively, across all study sites and assay kit lots. Retesting of the reactive samples was not performed.

Clinical sensitivity

Data accountability. A total of 2174 known positive samples were included in the sensitivity analysis. Complete Procleix assay results, including results for the Procleix HIV-1/HCV assay (neat and diluted 1 in 16) and both discriminatory assays were available for 2130 of these 2174 samples. For the remaining 44 samples, at least 1 of the Procleix assay results was not available, because the result was repeatedly invalid, sufficient material was not available for all required testing, or a protocol deviation occurred.

Procleix HIV-1/HCV assay. The sensitivities of the Procleix HIV-1/HCV assay for known positive samples are summarized in Tables 1 and 2. All samples were positive for HIV-1 and/or HCV RNA, according to their vendors. Samples that were known to contain less than 100 viral RNA copies per mL were excluded from the overall sensitivity analysis (116).

The overall sensitivities of the Procleix HIV-1/HCV assay, regardless of the testing site, clinical lot, or disease status of the individuals who provided the samples, were 99.8 and 99.3 percent, respectively, for samples tested neat and after 1 in 16 dilution. Assay sensitivity was similar across testing sites, clinical lots, and infection status (HIV-1 only, HCV only, or coinfected).

The overall agreement between Procleix HIV-1/HCV assay results for neat and diluted samples, based on a total of 2008 paired results, was 99.2 percent (1990 of 2008). In 13 samples (0.6%) viral RNA was detected when the sample was neat but not upon dilution, and in three samples, viral RNA was detected only upon 1 in 16 dilution. The 13 samples that did not react upon dilution had very low viral RNA concentrations when neat, ranging from less than 50 to 517 copies per mL; therefore, the dilute samples that did not react in Procleix testing contained less than 33 (517 ÷ 16) copies per mL of virus. Three samples whose viral RNA was detected only upon 1 in 16 dilution also contained low levels of virus. When these HCV-seropositive samples were tested neat at Gen-Probe, one was reactive and two had signal-to-cutoff values higher than known negatives but less than the cutoff. When the latter two samples were tested at a 1 in 16 dilution, the signal-to-cutoff values were low, consistent with low-input copy level. Testing sites performing quantitation of these samples reported either RNA levels below the level of detection or inconsistent quantitative results.

Procleix HIV-1 and HCV discriminatory assays. Sensitivity estimates for the Procleix HIV-1 discriminatory assay are shown in Table 2. Sensitivity was determined by testing 1042 samples. These samples included those that contained only HIV-1 RNA as well as those that were coinfected with HCV. Across testing sites, clinical lots, and disease status types, the sensitivity was 100 percent. Table 2 also shows sensitivity estimates for the Procleix HCV discriminatory assay, based on results from 1014 samples. These samples included both those that were only HCV-positive and those that were coinfected with HIV-1. Across testing sites, clinical lots, and disease status types, the clinical sensitivity was 99.6 percent.

Relationship between disease status and assay reactivity. Tables 3 and 4 show the percentage of neat and diluted positive samples reactive in the Procleix HIV-1/HCV and discriminatory assays, when classified by disease status. Serologic test results are also shown. Samples known to contain less than 100 viral RNA copies per mL were excluded.

The percentage of known HIV-1-positive samples reactive in the Procleix HIV-1/HCV assay was comparable across disease status categories for neat and diluted samples, ranging from 96.4 to 100 percent. All samples were reactive in the Procleix HIV-1 discriminatory assay. The percentage of samples positive for HIV antibody (87.9 to 100%) was slightly lower than the percentage reactive in the Procleix assays. Reactivity in an assay for

TABLE 1. Clinical sensitivity of the Procleix HIV-1/HCV assay with known NAT-positive samples

	Neat samples				Samples diluted 1 in 16			
Sample type*	Total	TP	FN†	Sensitivity (%) (95% CI)	Total	TP	FN	Sensitivity (%) (95% CI)
All	2014	2009	5	99.8 (99.4-99.9)	2012	1997	15	99.3 (98.8-99.6)
HIV-1 only	867	866	1	99.9 (99.4-100.0)	866	857	9	99.0 (98.0-99.5)
HCV only	967	963	4	99.6 (98.9-99.9)	966	962	4	99.6 (98.9-99.9)
HIV-1 and HCV	180	180	0	100.0 (98.0-100.0)	180	178	2	98.9 (96.0-99.9)

* Samples with confirmed virus concentrations less than 100 copies per mL were excluded.
† False-negative.

TABLE 2. Clinical sensitivity of the Procleix HIV-1 and HCV discriminatory assays with known NAT-positive samples

Assay	Sample type*	Total	TP	FN†	Sensitivity (%) (95% CI)
HIV-1 discriminatory assay	All	1042	1042	0	100.0 (99.6-100.0)
	HIV-1 only	868	868	0	100.0 (99.6-100.0)
	HIV-1 and HCV	174	174	0	100.0 (97.9-100.0)
HCV discriminatory assay	All	1014	1010	4	99.6 (99.0-99.9)
	HCV only	966	962	4	99.6 (98.9-99.9)
	HIV-1 and HCV	48	48	0	100.0 (92.6-100.0)

* Samples with confirmed virus concentrations less than 100 copies per mL were excluded.
† False-negative.

TABLE 3. Relationship between disease status and number of known HIV-1 positive samples reactive in the Procleix HIV-1/HCV and HIV-1 discriminatory assays

	Total reactive samples/total samples (%)*				
	Procleix HIV-1/HCV assay		Procleix HIV-1 discriminatory assay		
Disease status	Neat samples	Samples diluted 1 in 16	Neat samples	Samples positive for HIV antibody	Samples positive for HIV-1 p24Ag
AIDS	295/295 (100)	295/296 (99.7)	296/296 (100)	296/296 (100)	44/226 (19.5)
Symptomatic†	168/168 (100)	161/167 (96.4)	168/168 (100)	168/168 (100)	14/138 (10.1)
Asymptomatic‡	338/338 (100)	338/338 (100)	338/338 (100)	297/338 (87.9)	73/234 (31.2)
Unknown	239/240 (99.6)	238/240 (99.2)	240/240 (100)	234/236 (99.2)	29/202 (14.4)
Total	1040/1041 (99.9)	1032/1041 (99.1)	1042/1042 (100)	995/1038 (95.9)	160/800 (20.0)

* Samples with confirmed virus concentrations less than 100 copies per mL were excluded.
† Symptomatic patients not considered to have AIDS.
‡ Asymptomatic or generalized lymphadenopathy or acute HIV-1 infection.

TABLE 4. Relationship between subject population and number of known HCV-positive samples reactive in the Procleix HIV-1/HCV and HCV discriminatory assays

	Total reactive samples/total samples (%)*			
	Procleix HIV-1/HCV assay		Procleix HCV discriminatory assay	
Subject population	Neat samples	Samples diluted 1 in 16	Neat samples	Samples positive for HCV antibody
Blood donors†	883/887 (99.5)	882/886 (99.5)	882/886 (99.5)	886/886 (100)
Chronic hepatitis C	53/53 (100)	53/53 (100)	53/53 (100)	53/53 (100)
Unknown	75/75 (100)	73/75 (97.3)	75/75 (100)	20/52 (38.5)
Total	1011/1015 (99.6)	1008/1014 (99.4)	1010/1014 (99.6)	959/991 (96.8)

* Samples with confirmed virus concentrations less than 100 copies per mL were excluded.
† HCV infection (presence of HCV antibody) identified during screening of blood donations.

HIV p24Ag was much less common, ranging from 10.1 to 31.2 percent across disease status categories.

The percentage of known HCV-positive samples reactive in the Procleix HIV-1/HCV assay was also comparable for neat and diluted samples, ranging across disease status categories from 97.3 to 100 percent. In the Procleix HCV discriminatory assay, the percentage of reactive samples ranged across disease status categories from 99.5 to 100 percent. The percentage of samples positive for HCV antibody ranged across disease status categories from 38.5 to 100 percent (Table 4).

Samples containing 49 to 99 virus copies per mL. Data for samples determined to contain 49 to 99 viral RNA copies per mL were analyzed separately. These samples included 16 that were positive only for HIV-1. An additional two samples were coinfected but contained less than 100 HIV-1 copies per mL. No sample was determined to contain 49 to 99 HCV copies per mL.

All the samples with 49 to 99 HIV RNA copies per mL were positive for HIV antibody, while none was positive for HIV-1 p24Ag. Of the 16 samples containing only HIV-1, 15 (93.8%) were reactive in the Procleix HIV-1/HCV assay when tested neat, and 5 (31.2%) were reactive upon dilution. The 11 samples that did not react after dilution included 10 that were reactive when tested neat. These 10 samples contained 51 to 97 HIV-1 copies per mL. The sensitivity of the HIV-1 discriminatory assay for samples

containing 49 to 99 HIV-1 copies per mL was 94.4 percent (17 of 18 samples reactive).

Samples with undetectable virus concentrations. A number of samples designated NAT reactive by the vendors were found to contain an undetectable amount of viral RNA upon confirmatory quantitative NAT by Quest or NGI. Many of the individuals who provided HIV-1-positive samples of this type were taking antiviral drugs, and accordingly, their plasma viral loads could well have been below the limit of detection by the HIV-1 NAT used at Quest. Since the limit of detection was 50 copies per mL for the HIV-1 quantitative assay at Quest and 100 copies per mL for the HCV quantitative assay at NGI, data for samples with undeterminable viral concentrations were analyzed separately.

A total of 111 samples (74 HIV-1-positive and 37 HCV-positive) with undeterminable viral concentrations were tested neat in the Procleix HIV-1/HCV assay, while 115 (76 HIV-1-positive and 39 HCV positive) were tested after 1 in 16 dilution. Of the neat samples, 46.8 percent (52 of 111) were reactive. Of the diluted samples, 17.4 percent (20 of 115) were reactive. Among neat samples, the percentage of assay reactivity was 55.4 percent (41 of 74) for HIV-1 and 29.7 percent (11 of 37) for HCV. After dilution, 22.4 percent of HIV-1-positive samples (17 of 76) and 7.7 percent of HCV-positive samples (3 of 39) were reactive. Such low reactivity rates were expected for samples with viral concentrations less than 100 copies per mL. Of samples tested with the Procleix HIV-1 discriminatory assay, 59.2 percent (58 of 98) were reactive. These 98 included 28 samples with undetectable HIV-1 load, but detectable HCV viral load. Of the 38 samples tested with the HCV discriminatory assay, 28.9 percent (11 of 38) were reactive. These 38 included two samples with undetectable HCV viral load, but detectable HIV-1 viral load.

All samples with confirmed undetectable HIV-1 concentrations were positive for HIV antibody, while 1 of 61 samples tested (1.6%) was positive for HIV-1 p24Ag. This sample was reactive in the Procleix HIV-1/HCV assay (neat and diluted) and in the HIV-1 discriminatory assay. Samples with undetectable HIV-1 concentrations were much more readily detected with the Procleix assay than with a test for HIV-1 p24Ag.

Of 41 samples with undetectable HCV concentrations, 3 (7.3%) were positive for HCV antibody, while 29 percent were reactive in the Procleix assays. Samples with undetectable HCV concentrations were much more often detected with these assays than with a test for HCV antibody.

Samples with known HIV-1 subtype or HCV genotype. All of 42 samples of known HIV-1 types (subtypes A-G and group O) and all of 41 samples of known HCV genotypes (genotypes 1-5) were reactive in the Procleix HIV-1/HCV assay (neat and diluted) and the appropriate Procleix discriminatory assay. Previous work[10] has also shown that the sensitivity of the Procleix HIV-1/HCV assay and the HIV-1 and HCV discriminatory assays is unaffected by HIV-1 subtype or HCV genotype.

Seroconversion panels. To determine the Procleix assay's ability to shorten the window period between the onset and the detectability of infection, 10 commercial seroconversion panels for HIV-1 and 10 for HCV were tested with the Procleix HIV-1/ HCV assay (neat and diluted) and the Procleix HIV-1 and HCV discriminatory assays. Serologic assays also used to test the seroconversion panels included an HIV-1/2 antibody assay (Abbott), an assay for HIV-1 p24Ag (Abbott or Coulter), and an assay for HCV antibody (Ortho anti-HCV 3.0).

The Procleix HIV-1/HCV assay detected HIV-1 RNA a median of 12 days earlier than HIV antibody tests in samples tested neat and a median of 10 days earlier than antibody tests in samples tested after 1 in 16 dilution. Compared to HIV-1 p24Ag, HIV-1 RNA was detected by the Procleix HIV-1/HCV assay a median of 7 days earlier in neat samples and a median of 3 days earlier in samples after dilution. The Procleix HIV-1 discriminatory assay detected HIV-1 RNA a median of 12 days earlier than did the HIV antibody test and a median of 6 days earlier than did tests for p24Ag (Table 5). Both the Procleix HIV-1 and HCV assay (neat or diluted samples) and the HCV discriminatory assay detected HCV RNA a median of 25 days earlier than HCV antibody (Table 6).

Samples tested as part of a pool. To estimate the sensitivity of the Procleix assay for pools of 16 samples, a total of 204 sixteen-member pools containing known positive and negative samples were prepared. Of these pools, 102 contained 1 positive and 15 negative samples. The positive sample contained HIV-1 in 59 pools and HCV in 43. An additional 102 pools each contained 2 positive and 14 negative samples. These pools included 21 with 2 HIV-1-positive samples, 23 with 2 HCV-positive samples, and 58 with 1 HIV-1- and 1 HCV-positive sample. All 204 pools containing 1 or 2 positive samples were reactive in the Procleix HIV-1/HCV assay, showing that sensitivity was unaffected when positive samples were pooled with negative samples.

DISCUSSION

NAT for HIV-1 and HCV during blood and plasma screening is now standard practice in the US, Europe, and Japan.[1-4,11-15] Most samples in the US are tested in 24- or 16-member pools, although some sites with small testing volume are testing individual (unpooled) samples with NAT.

The studies reported here were conducted to determine the specificity and sensitivity of the Procleix HIV-1/HCV assay and the Procleix HIV-1 and HCV discriminatory assays. The specificities of the Procleix HIV-1/HCV

TABLE 5. Earlier detection of HIV-1 RNA in seroconversion panels by use of the Procleix HIV-1/HCV assay

HIV-1 seroconversion panel	Days earlier than HIV antibody test*			Days earlier than HIV p24Ag test†		
	Procleix HIV-/HCV		Procleix HIV discriminatory	Procleix HIV-1/HCV		Procleix HIV discriminatory
	Neat	Diluted 1 in 16	Neat	Neat	Diluted 1 in 16	Neat
6240	12	7	12	7	2	7
6248	14	11	11	7	4	4
PRB923	12	17	17	2	7	7
PRB926	27	25	27	7	5	7
PRB929	11	11	11	0	0	0
PRB932	0	0	0	0	0	0
PRB943	9	9	9	2	2	2
PRB945	13	10	13	13	10	13
PRB946	11	7	7	7	3	3
PRB950	28	10	28	18	0	18
Median	12	10	12	7	3	6

* Abbott HIV 1/2 III or Abbott HIV 1/2.
† Coulter HIV p24Ag, Abbott HIV p24Ag, or Abbott HIV antigen monoclonal.

TABLE 6. Earlier detection of HCV RNA in seroconversion panels by use of the Procleix HIV-1/HCV assay

HCV seroconversion panel	Days earlier than HCV antibody test*		
	Procleix HIV-1/HCV		Procleix HCV discriminatory
	Neat	Diluted 1 in 16	Neat
6213	26	26	26
6225	39	33	39
6226	39	39	39
6228	31	31	31
9045	41	41	41
PHV904	14	14	14
PHV907	21	21	21
PHV908	19	19	19
PHV914	24	24	24
PHV916	23	23	23
Median	25	25	25

* Ortho anti-HCV 3.0 or Abbott anti-HCV 2.0.

assay, HIV-1 discriminatory assay, and HCV discriminatory assay were found to be 99.7, 99.8, and 99.7 percent, respectively. False-positive Procleix results on pooled samples were likely the result of intraassay or NAT tube contamination, rather than lack of assay reagent specificity.

Besides demonstrating high specificity of the Procleix HIV-1/HCV assay in the blood donor testing arena, the specificity study also demonstrated that the Procleix HIV-1/HCV test could replace p24Ag testing without reducing HIV-1 detection. The low-level reactive p24Ag external run control was detected 100 percent of the time in both the Procleix HIV-1/HCV and the HIV-1 discriminatory assays. Also, all p24Ag-reactive results that could be confirmed by another test or another reactive result on a subsequent sample were also detected by the Procleix assays.

The sensitivity study required testing of known HIV-1-positive and/or HCV-positive samples, since positive samples are infrequent among blood donations. The design of this study, the first large-scale sensitivity assessment of the Procleix assay, complemented that of the specificity study. Plasma or serum samples known to be NAT positive for HIV-1 and/or HCV, as well as HIV-1 and HCV seroconversion panels, were tested both neat and diluted 1 in 16 in the Procleix HIV-1/HCV assay. The sensitivities of the Procleix HIV-1/HCV assay for neat samples were 99.9, 99.6, and 100.0 percent, respectively, for HIV-1-positive, HCV-positive, and coinfected samples. With samples tested after 1 in 16 dilution, this assay's sensitivities were 99.0, 99.6, and 98.9 percent, respectively, for HIV-1-positive, HCV-positive, and coinfected samples.

As expected, the difference in reactivity of undiluted versus diluted samples was only apparent for samples with very low virus concentrations (<517 copies per mL undiluted). Reactivity with the Procleix HIV-1/HCV assay on neat samples containing less than 100 copies per mL HCV or less than 50 copies per mL HIV was 46.8 percent (52 of 111). In diluted samples that reactivity was 17.4 percent (20 of 115).

Since highly conserved regions of the HCV and HIV-1 genomes have been targeted for capture, amplification, and detection, the Procleix assays can detect all evaluated HIV-1 subtypes and HCV genotypes.[10] In the present study, all samples of known HIV-1 subtype or HCV genotype were reactive in the Procleix HIV-1/HCV assay (neat and diluted) and in the appropriate discriminatory assay.

When 10 HIV-1 and 10 HCV seroconversion panels were tested neat in the Procleix HIV-1/HCV assay, the median time to appearance of a reactive result was 12, 7, and 25 days shorter, compared to HIV antibody, HIV-1 p24Ag, and HCV antibody tests, respectively. These are similar to predictions based on infection incidence and NAT assay sensitivity.[16-19] Comparable results have been previously predicted and reported.[2-4,11,15,20,21] When the panels were tested in the Procleix HIV-1/HCV assay after 1 in 16 dilution, the median time to appearance of a

reactive result was 10, 3, and 25 days shorter compared to HIV antibody, HIV-1 p24Ag, and HCV antibody tests, respectively. In the HIV-1 seroconversion panel, testing with the Procleix HIV-1/HCV assay, 6 of 10 panels were detected earlier when neat than when diluted. This was true for only 1 of 10 HCV seroconversion panels. The Procleix window period reduction of 25 days for detection of HCV is less than that reported by others. Both of these findings are likely due to the fact that in 8 of the 10 HCV panels, the earliest sample in the panel was Procleix reactive for HCV RNA when tested by Procleix. Differences in results for neat and diluted seroconversion panel members, and for Procleix results versus antibody results, may be more apparent in seroconversion panels with samples collected earlier and more frequently after infection. The earlier detection of HIV-1 and HCV infection with the Procleix assay compared to serologic tests can improve the efficiency of excluding infectious donations from the blood supply.

In the samples tested, the Procleix HIV-1/HCV assay was significantly more sensitive than were assays for HIV antibody, HIV-1 p24Ag, or HCV antibody. All samples that were positive for p24Ag, and confirmed by neutralization, were also reactive in the Procleix HIV-1/HCV assay whether tested neat or after 1 in 16 dilution. The Procleix HIV-1/HCV and HIV-1 discriminatory assays detected 99.9 percent of known HIV-1-positive samples, compared to the 20 percent detected by assays for p24Ag. The Procleix assays could also detect a significant number of known NAT-reactive samples that were not detected by antibody assays for HIV (95.9% reactive) or HCV (96.8% reactive) antibody (Tables 3 and 4).

In conclusion, the high specificity and sensitivity of the Procleix HIV-1/HCV assay confirm that this assay is appropriate for blood and plasma screening.

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