SUMMARY BASIS OF APPROVAL

1.0 GENERAL INFORMATION

Product Trade Name:	Procleix® Ultrio® Assay
Other Name:	Nucleic Acid Test (NAT) for human immunodeficiency virus type 1 (HIV-1) RNA and hepatitis C virus (HCV) RNA and hepatitis B virus (HBV) DNA
Name and Address of Sponsor:	Gen-Probe Incorporated 10210 Genetic Center Drive San Diego, CA 92121
Biologics License Application Tracking Number:	STN BL 125113/0
Date of Submission:	September 28, 2004

2.0 INTENDED USE

The PROCLEIX® ULTRIO® Assay* is a qualitative in vitro nucleic acid assay system to screen for human immunodeficiency virus type I (HIV-1) RNA and hepatitis C virus (HCV) RNA in plasma and serum specimens from individual human donors, including donors of whole blood and blood components, source plasma and other living donors. It is also intended for use in testing plasma and serum specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens from cadaveric (non-heart-beating) donors. The assay is not intended for use on cord blood specimens.

The assay is intended for use in testing individual samples from living donors of whole blood, blood components, or source plasma, other living donors and heart-beating organ donors, and for testing individual blood specimens from cadaveric (non-heart-beating) donors. It is also intended for use in testing pools of human plasma comprised of equal aliquots of not more than 16 individual donations from donors of whole blood, blood components, or source plasma. This assay is intended to be used in conjunction with licensed tests for detecting antibodies to HIV-1 and HCV.

The PROCLEIX ULTRIO Assay is not intended for use to screen donor specimens for HBV DNA. The assay detects HBV DNA in HBV seroconversion panel specimens that are negative for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core antigen (anti-HBc). The assay also detects HBV DNA in donor specimens that are positive for HBsAg and/or anti-HBc. However, detection of HBV DNA in donations negative for both HBsAg and anti-HBc has not been demonstrated in the donor setting.

This assay is not intended for use as an aid in diagnosis of infection with HIV-1, HCV or HBV.

3.0 BRIEF DESCRIPTION OF TEST

Components of the Procleix Ultrio Assay Kit are listed below:

- Internal Control Reagent: A HEPES buffered solution containing detergent and an RNA transcript
- Target Capture Reagent: A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles
- Amplification Reagent: Primers, dNTPs, NTPs and co-factors in TRIS buffered solution containing PROCLIN® 300 as preservative
- Enzyme Reagent: MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffered solution containing 0.05% sodium azide as preservative
- Probe Reagent: Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent
- Selection Reagent: Borate buffered solution containing surfactant
- Procleix Negative Calibrator: Defibrinated normal human plasma (nonreactive for HIV-1/2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.
- Procleix HIV-1 Positive Calibrator: Inactivated HIV-1 positive plasma in defibrinated normal human plasma (nonreactive for HIV-2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives
- Procleix HCV Positive Calibrator: Inactivated HCV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives
- Procleix HBV Positive Calibrator: Inactivated HBV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HCV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives
- Procleix Assay Fluids
  Wash Solution: [redacted] buffered solution
  Oil: [redacted] oil
  Buffer for Deactivation Fluid: [redacted] buffered solution
- Procleix Auto Detect Reagents
  Auto Detect 1: [redacted]
  Auto Detect 2: [redacted] sodium hydroxide

The Procleix HIV-1, HCV, and HBV Discriminatory Assays utilize the same procedure and reagents as the Procleix Ultrio Assay with one difference: HIV-1-specific, HCV-specific, or HBV-specific probe reagents are used in place of the Ultrio Assay Probe Reagent.

- Procleix HIV-1 Discriminatory Probe Reagent: Chemiluminescent oligonucleotide probe in succinate buffered solution containing detergent

- Procleix HCV Discriminatory Probe Reagent: Chemiluminescent oligonucleotide probe in succinate buffered solution containing detergent

- Procleix HBV Discriminatory Probe Reagent: Chemiluminescent oligonucleotide probe in succinate buffered solution containing detergent

4.0 MANUFACTURING AND CONTROLS

A. Manufacturing Overview

The Procleix Ultrio Assay components are manufactured by Gen-Probe Incorporated. The manufactured components of the assay include Oligonucleotides, Calibrators, Internal Control (IC) and Reagents.

Raw materials intended for the manufacture and use in these assay components are subject to appropriate quality control evaluations before they are accepted for use in manufacturing. The quality of the product components is assessed at multiple stages during manufacture using tests to ensure conformance to acceptable specifications. Acceptance criteria and specifications have been established for all kit components. Components are assembled into kits, each lot of which is subjected to a final performance test with an in-house panel of samples containing varying copy levels of HIV-1, HCV and/ or HBV. Meeting the established performance parameters is required for kit release.

B. Stability Studies

The stability of the Procleix Ultrio Assay kit reagents has been established based upon the results of Real Time studies. All reagents are stored at their recommended long-term storage conditions as defined by the procedures in the package insert for the Procleix Ultrio Assay. The results of these stability studies indicate no compromise in product performance and support the [redacted] dating period for the Procleix Ultrio Assay kit.

C. Methods Validation

Production of test kit components is monitored by in-process testing. Product purity and potency are assured through evaluation of multiple parameters including [redacted]. Assay performance of test kits is assessed through laboratory evaluations using an in-house panel of samples containing varying copy levels of HIV-1, HCV and /or HBV.

Three conformance lots of the Procleix Ultrio Assay kit have been submitted to CBER for evaluation. Each master lot of product, along with protocols summarizing pertinent product testing, will be submitted for evaluation and approval by CBER prior to release for distribution.

D. Labeling

The product labeling, including immediate container and package labels and the package insert (directions for use), has been reviewed for compliance with 21 CFR 610.60, 610.61, 610.62, and 809.10 and found to be satisfactory. The package insert for the Procleix Ultrio Assay states that the intended use of the test

is for the detection of human immunodeficiency virus type 1 (HIV-1) RNA and hepatitis C virus (HCV) RNA and hepatitis B virus (HBV) DNA in plasma and serum. The product trade name, Procleix Ultrio Assay, is not known to conflict with other biologic or device trade name.

E. Establishment Inspection

A pre-licensing inspection of the areas where product is manufactured, tested, stored and shipped was performed in January and February 2005. Facilities and procedures are in compliance with current good manufacturing practices (cGMP).

F. Environmental Assessment (EA)

A detailed Environmental Assessment was provided in Section 15.0 of the Biologic License Application (STN BL 125113/0). This product has no significant environmental impact. Gen-Probe Incorporated, in accordance with current Good Manufacturing Practices, performs all production activities in compliance with applicable environmental regulations.

A summary of the procedures taken by the manufacturer to assure that no adverse environment impacts occur is listed below:

1. All biohazardous waste material is managed in accordance with applicable local, State and Federal regulations.

2. Appropriate precautionary statements are included in the labeling and package insert of the product.

3. Product shipping containers are appropriately labeled and are shipped according to applicable regulations.

5.0 BIOLOGICAL PRINCIPLES OF THE TEST

The Procleix Ultrio Assay with associated Discriminatory HIV-1, HCV and HBV Assays is a qualitative *in vitro* assay that utilizes target amplification nucleic acid probe technology for the detection of detection of human immunodeficiency virus type 1 (HIV-1) RNA and hepatitis C virus (HCV) RNA and hepatitis B virus (HBV) DNA in plasma and serum. The three main steps of the Procleix Ultrio Assay are (i) Target Capture-based sample preparation, (ii) Internal Control and target amplification by Transcription-Mediated Amplification (TMA) and (iii) detection of the amplification product (amplicon) by the Hybridization Protection Assay (HPA).

(i) Target Capture-based sample preparation:
During Sample Preparation, viral RNA and DNA are isolated from specimens via the use of target capture. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins and release viral genomic RNA and/or DNA. Oligonucleotides (capture oligonucleotides) that are homologous to highly conserved regions of HIV-1, HCV, and HBV are hybridized to the HIV-1 RNA, HCV RNA, or HBV DNA target, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube. Magnetic separation and wash steps are performed with a target capture system.

(ii) Transcription-Mediated Amplification (TMA):
Target amplification occurs via TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The Procleix Ultrio Assay utilizes the TMA method to amplify regions of HIV-1 RNA, HCV RNA, and/or HBV DNA.

(iii) Hybridization Protection Assay (HPA):
Detection is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured in a luminometer and is reported as Relative Light Units (RLU).

Internal Control is added to each test specimen, control (if used), or assay calibrator tube via the working Target Capture Reagent that contains the Internal Control. The Internal Control in this reagent controls for specimen processing, amplification, and detection steps. Internal Control signal in each tube or assay reaction is discriminated from the HIV-1/HCV/HBV signal by the differential kinetics of light emission from probes with

different labels. Internal Control-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to HIV-1/HCV/HBV is detected using probes with relatively slower kinetics of light emission (glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels. When used for the simultaneous detection of HIV-1, HCV, and HBV, the Procleix Ultrio Assay differentiates between Internal Control and combined HIV-1/HCV/HBV signals but does not discriminate between individual HIV-1, HCV, and HBV signals.

Specimens found to be reactive in the Procleix Ultrio Assay must be run in individual HIV-1, HCV, and/or HBV Discriminatory Assays to determine if they are reactive for HIV-1, HCV, HBV or any combination of the three. The Procleix HIV-1, HCV, and HBV Discriminatory Assays utilize the same three main steps as the Procleix Ultrio Assay (target capture, TMA and HPA); the same assay procedure is followed with one difference: HIV-1-specific, HCV-specific, or HBV-specific probe reagents are used in place of the Procleix Ultrio Assay Probe Reagent.

6.0 CLINICAL DATA

A. REPRODUCIBILITY

Reproducibility of the Procleix Ultrio Assay, HIV-1 Discriminatory Assay, HCV Discriminatory Assay, and HBV Discriminatory Assay was evaluated at three blood center laboratories. For determination of the reproducibility of each assay, 10 members from a reproducibility panel were tested as individual specimens (Tables 1-4). Eight of the panel members were either positive for HIV-1 RNA (150, 2,500 and 10,000 c/mL), HCV RNA (150 and 2,500 c/mL), and/or HBV DNA (50 and 500 IU/mL) and two panel members were HIV-1, HCV, and HBV negative.

The reproducibility panels were tested by a total of seven operators (two to three from each testing site) with three different Clinical Lots over multiple nonconsecutive days, using an automated front end pipettor (Tecan) or manual pipetting of specimen and working Target Capture Reagent (wTCR). Twenty-four valid runs were generated for each assay across three Clinical Lots, with each panel member tested in triplicate per run and each operator performing testing for at least eight days.

The Reproducibility Study assessed intra- and inter-assay, inter-lot and inter-site variability of the Procleix Ultrio Assay and each discriminatory assay.

Reproducibility analyses included evaluation of percent agreement and mean Signal/Cutoff (S/CO) ratios for panel members and mean Relative Light Unit (RLU) values for the Negative, HIV-1 Positive, HCV Positive, and HBV Positive Calibrators and evaluation of standard deviation (SD) and percent coefficient of variation (%CV) of those S/CO ratios and RLU values for each of the four variance factors (Tables 1-4). The mean analyte S/CO ratios were analyzed for the positive panel members and the IC S/CO ratios were analyzed for the negative panel members. The mean analyte RLU values were analyzed for the Positive Calibrators and the IC RLU values were analyzed for the Negative Calibrators. The percent agreement between the assay results and the true status of each panel member was calculated using analyte S/CO for all panel members. Since no significant difference in assay reproducibility was observed between automated Tecan pipettor and manual pipetting, results from testing of individual specimens for the two pipetting methods were combined and are shown in the tables below (Tables 1-4).

For the Procleix Ultrio Assay, results for all individual panel members are shown. For the discriminatory assays, results for negative panel members and panel members containing target(s) that should be nonreactive were combined. Results for panel members containing target that should be reactive are shown individually.

For the Procleix Ultrio Assay and three discriminatory assays, the overall percent agreement of test results was 100% for positive samples and 98.6 - 100% for negative samples. With regard to signal variability, intra-assay (or random error) and inter-assay factors, in most cases, were the largest and second largest contributors to total variance (according to SD values) in the Procleix Ultrio Assay, and the Procleix HIV-1 and HCV Discriminatory Assays. For the Procleix HBV Discriminatory Assay, the inter-assay factor was the largest contributor to total variance (according to SD values), followed by the intra-assay and inter-site factors, which similarly contributed to total variance. It should be noted that while these factors were responsible for the majority of the variance in the assays, the %CV of each of these components by itself did not exceed 11.2% for any positive or negative samples, in any assay. Therefore, the reproducibility of the assays is robust and the variation that is observed can be attributed primarily to random error. Other variance factors, including testing site and Clinical Lot, have zero or very little impact on assay performance (Tables 1-4).

Table 1. Procleix System - Reproducibility of the Procleix Ultrio Assay (analysis of analyte signals, unless noted)

Specimen	N	Concentration*	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive**	1	0	72	98.6	2.00	0.10	4.9	0.08	3.9	0.00	0.0	0.01	0.6
Nonreactive**	1	0	72	100	2.02	0.07	3.4	0.08	4.1	0.00	0.0	0.00	0.0
HIV-1	1	10,000	72	100	17.06	0.48	2.8	0.56	3.3	0.37	2.2	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	72	100	37.6	0.84	2.2	1.55	4.1	0.83	2.2	0.42	1.1
HCV	1	150	72	100	6.06	0.27	4.4	0.27	4.5	0.24	3.9	0.30	4.9
HCV/HBV	1	2,500/500	72	100	21.79	0.50	2.3	0.85	3.9	0.55	2.5	0.43	2.0
HIV-1	1	150	72	100	13.72	1.46	10.6	0.54	4.0	0.68	4.9	0.89	6.5
HBV	1	50	72	100	14.92	0.37	2.5	0.55	3.7	0.55	3.7	0.24	1.6
HIV-1/HBV	1	2,500/500	72	100	31.01	0.76	2.4	1.13	3.6	0.55	1.8	0.21	0.7
HIV-1/HCV	1	2,500/2,500	72	100	23.32	0.49	2.1	0.81	3.5	0.00	0.0	0.00	0.0

Specimen	Number of replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator**	71	N/A	216,522	6,392	3.0	8,055	3.7	9,533	4.4	13,466	6.2
HIV-1 Positive Calibrator	47	N/A	1,242,002	16,313	1.3	25,122	2.0	25,142	2.0	49,452	4.0
HCV Positive Calibrator	48	N/A	611,102	17,099	2.8	23,958	3.9	0.00	0.0	37,381	6.1
HBV Positive Calibrator	48	N/A	1,138,995	26,379	2.3	38,770	3.4	30,233	2.7	72,948	6.4

N = Number of panel members combined for this analysis
* Concentration = copies/mL for HIV-1 and HCV, IU/mL for HBV
** Analysis of internal control signal
*** Per NCCLS guidelines, (EP5-A, page 7), numbers <0 are recorded as 0.

Table 2. Procleix System - Reproducibility of the Procleix HIV-1 Discriminatory Assay (analysis of analyte signals, unless noted)

Specimen	N	Concentration*	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD***	%CV	SD***	%CV	SD***	%CV
Nonreactive**	5	0	359	99.7	1.98	0.09	4.5	0.03	1.5	0.03	1.4	0.00	0.0
HIV-1	1	10,000	72	100	25.80	0.66	2.5	0.80	3.1	0.22	0.8	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	72	100	24.52	0.54	2.2	0.61	2.5	0.00	0.0	0.00	0.0
HIV-1	1	150	72	100	20.51	2.19	10.7	0.42	2.1	0.66	3.2	0.61	3.0
HIV-1/HBV	1	2,500/500	72	100	24.57	0.61	2.5	0.85	3.4	0.31	1.3	0.00	0.0
HIV-1/HCV	1	2,500/2,500	72	100	24.1	1.86	7.7	0.00	0.0	0.00	0.0	0.51	2.1
Specimen			**Number of replicates**	**% Agreement**	**Mean RLU**	**Intra-Assay**		**Inter-Assay**		**Inter-Lot**		**Inter-Site**	
						SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator, ******			166	N/A	220,588	6,334	2.9	9,884	4.5	14,777	6.7	8,168	3.7
HIV-1 Positive Calibrator			71	N/A	1,252,970	31,621	2.5	34,260	2.7	18,887	1.5	86,575	6.9

N = Number of panel members combined for this analysis
* Concentration = copies/mL for HIV-1 and HCV, IU/mL for HBV. For nonreactive specimens, only the HIV-1 concentration is listed.
** Analysis of internal control signal
*** Per NCCLS guidelines, (EP5-A, page 7), numbers <0 are recorded as 0.
**** Analysis of Negative Calibrator and HBV and HCV Positive Calibrators

Table 3. Procleix System - Reproducibility of the Procleix HCV Discriminatory Assay (analysis of analyte signals, unless noted)

Specimen	N	Concentration*	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive**	6	0	413	99.0	2.07	0.08	4.0	0.09	4.2	0.01	0.7	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	69	100	22.18	0.50	2.3	1.27	5.7	0.21	0.9	0.42	1.9
HCV	1	150	69	100	19.08	0.98	5.1	1.01	5.3	0.48	2.5	0.00	0.0
HCV/HBV	1	2,500/500	69	100	22.33	0.58	2.6	1.17	5.3	0.28	1.2	0.59	2.7
HIV-1/HCV	1	2,500/2,500	68	100	21.88	2.45	11.2	1.04	4.8	0.00	0.0	0.57	2.6

Specimen	Number of replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator, ******	113	N/A	220,270	7,527	3.4	9,308	4.2	19,184	8.7	13,180	6.0
HCV Positive Calibrator	69	N/A	1,318,289	26,043	2.0	54,331	4.1	42,644	3.2	79,828	6.1

N = Number of panel members combined for this analysis
* Concentration = copies/mL for HIV-1 and HCV, IU/mL for HBV. For nonreactive specimens, only the HCV concentration is listed.
** Analysis of internal control signal
*** Per NCCLS guidelines, (EP5-A, page 7), numbers <0 are recorded as 0.
**** Analysis of Negative Calibrator and HIV-1 and HBV Positive Calibrators

Table 4. Procleix System - Reproducibility of the Procleix HBV Discriminatory Assay (analysis of analyte signals, unless noted)

Specimen	N	Concentration*	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive**	6	0	430	99.8	2.00	0.12	5.8	0.10	4.9	0.02	0.9	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	72	100	25.17	0.57	2.3	1.55	6.2	0.00	0.0	0.66	2.6
HCV/HBV	1	2,500/500	72	100	25.14	0.66	2.6	1.50	6.0	0.03	0.1	0.69	2.7
HBV	1	50	72	100	25.55	0.69	2.7	1.62	6.3	0.00	0.0	0.92	3.6
HIV-1/HBV	1	2,500/500	72	100	26.06	0.69	2.7	1.79	6.9	0.00	0.0	0.78	3.0

Specimen	Number of replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator, ******	119	N/A	207,762	7,641	3.7	10,093	4.9	18,207	8.8	10,636	5.1
HBV Positive Calibrator	72	N/A	1,083,154	32,781	3.0	40,616	3.8	39,069	3.6	68,573	6.3

N = Number of panel members combined for this analysis
* Concentration = copies/mL for HIV and HCV, IU/mL for HBV. For nonreactive specimens, only the HBV concentration is listed.
** Analysis of internal control signal
*** Per NCCLS guidelines, (EP5-A, page 7), numbers <0 are recorded as 0.
**** Analysis of Negative Calibrator and HIV-1 and HCV Positive Calibrators

B. SPECIFICITY IN NORMAL BLOOD DONORS

The clinical specificity of the Procleix Ultrio Assay was determined on the Procleix System in 16-sample pools made from plasma from either whole blood donations or paid source plasma (PSP) donors and in individual donor samples (IDS) from whole blood donations. The clinical specificity of the HIV-1, HCV, and HBV Discriminatory Assays was determined on the Procleix System in IDS from whole blood donations.

The study was conducted at three blood center testing laboratories and one source plasma center using plasma samples derived from approximately 32 geographically diverse blood donor sites in the United States. During the study, all testing was performed linked using three Clinical Lots of Procleix Ultrio Assay reagent kits. All of the samples collected for the study were tested with the Procleix Ultrio Assay, with the licensed Procleix HIV-1/HCV Assay and with licensed HBsAg serologic tests and, as appropriate, confirmatory tests.

Specificity of the Procleix Ultrio Assay was calculated from 12,028 16-sample plasma pools from whole blood donations, 12,780 IDS from whole blood donations, and 1,198 16-sample plasma pools from PSP donors (Table 7). Specificity of the HIV-1 (n=1,797), HCV (n=1,810), and HBV (n=1,795) Discriminatory Assays was calculated using results of IDS from whole blood donations. Voluntary source plasma (VSP) donations were also collected and included in either the 16-sample pools or IDS from whole blood donations (n=303 and 9, respectively) tested in the Procleix Ultrio Assay. In the specificity evaluation, the results from the Procleix Ultrio Assay and the associated Discriminatory Assays were compared to results from the licensed Procleix HIV-1/HCV Assay and associated Discriminatory Assays and to results from licensed HBsAg serologic tests. The specificity was also based on the results from alternate licensed or validated nucleic acid tests (Alternate NAT), which were performed on IDS or individual samples from pools with discordant results.

Rates of Procleix Ultrio Assay reactivity are presented in Tables 5 and 6 for pools and IDS from whole blood donations that were included in the clinical specificity analyses. The overall clinical specificity results are summarized in Table 7. Table 8 shows clinical specificity by site for pools and IDS from whole blood donations. Pools from PSP donations were tested at only one site.

Table 5. Clinical Specificity Study: Procleix Ultrio Assay Reactivity in 16-Sample Pools

Results	n	Percent (95% CI)
Total pools tested	12,028	100.00%
Nonreactive pools	11,796	98.07% (97.81-98.31%)
Initially reactive pools	232	1.93% (1.69-2.19%)
Pool, individual constituent(s), and reference test reactive (true positive)	167	1.39% (1.19-1.61%)
Pool, individual constituent(s), and discriminatory assay reactive, reference test nonreactive, Alternate NAT reactive (true positive)	6	0.05% (0.02-0.11%)
Pool reactive, individual constituents and reference test nonreactive (false positive)	50	0.42% (0.31-0.55%)
Pool, individual constituent(s), and discriminatory assay reactive, reference test nonreactive, Alternate NAT not available (false positive)	2	0.02% (0.00-0.06%)
Pool and individual constituent(s) reactive, discriminatory assay, individual constituent(s) retest, and reference test nonreactive (false positive)	7	0.06% (0.02-0.12%)

Table 6. Clinical Specificity Study: Procleix Ultrio Assay Reactivity in IDS

Results	n	Percent (95% CI)
Total IDS tested	12,780	100.00%
Nonreactive IDS	12,480	97.65% (97.38-97.91%)
Initially reactive IDS	300	2.35% (2.09-2.62%)
IDS and reference test reactive (true positive)	186	1.46% (1.25-1.68%)
IDS and discriminatory assay reactive, reference test nonreactive, and Alternate NAT reactive (true positive)	7	0.05% (0.02-0.11%)
IDS and discriminatory assay reactive, reference test nonreactive, Alternate NAT nonreactive or unavailable (false positive)	10*	0.08% (0.04-0.14%)
IDS reactive, discriminatory assay, retest, and reference test nonreactive (false positive)	96**	0.75% (0.61-0.92%)
IDS reactive, discriminatory assay nonreactive, retest reactive, and reference test nonreactive (false positive)	1	0.01% (0.00-0.04%)

* Eight IDS had unavailable Alternate NAT results because they were invalidated (n=7) or for other reasons. Two IDS were Alternate NAT nonreactive.
** Includes four initially reactive IDS without discriminatory assay results and with nonreactive reference test results. Also includes 12 IDS without retest results.

Overall Clinical Specificity of the Procleix Ultrio Assay

There were 12,028 pools tested with the Procleix Ultrio Assay and included in the specificity calculations (Table 5). There were 11,796 pools from whole blood donations that tested nonreactive in the Procleix Ultrio Assay (Table 7). Of these, 11,786 pools were considered true negative and 10 pools were considered false negative. Nine of the 10 false negative pools were HBsAg seropositive and 1 pool was reactive in the Procleix HIV-1/HCV Assay. There were 232 pools that tested reactive in the Procleix Ultrio Assay. Of these, 173 pools were considered true positive. Six of these pools were reactive in the Procleix Ultrio Assay and had a constituent sample that was reactive in Alternate NAT, but were nonreactive in the reference test. Fifty-nine pools were considered false positive. The overall specificity of 16-sample pools from whole blood donations was 11,786/11,845 or 99.5% (95% CI: 99.4-99.6%).

There were 12,780 IDS tested with the Procleix Ultrio Assay – from reactive pools or tested as IDS only – and included in the specificity calculations (Table 6). There were 12,480 IDS that tested nonreactive in the Procleix Ultrio Assay. Of these, 12,479 IDS were considered true negative and 1 sample was considered false negative. The false negative sample was Procleix HIV-1/HCV Assay reactive, HCV discriminated. There were 300 IDS that tested reactive in the Procleix Ultrio Assay. Of these, 193 IDS were considered true positive and 107 IDS were considered false positive. The overall specificity of IDS from whole blood donations was 12,479/12,586 or 99.1% (95% CI: 99.0-99.3%).

The specificity of the Procleix Ultrio Assay was also calculated based on the total number of specimens tested either as IDS or in a pool. This included 216,180 donor samples tested either as IDS, IDS after a reactive pool result, or as part of a non-reactive pool (all 16 samples from a non-reactive pool are considered non-reactive). All donor samples tested had valid Procleix Ultrio Assay results, Procleix HIV-1/HCV Assay (including the appropriate discriminatory assay) results and HBsAg serology results. After complete resolution, eleven samples were considered false negative and 193 were considered true positive. Of the remaining 215,976 samples, 107 were considered false positive and 215,869 were considered true negative. The overall specificity from all of the donor samples from whole blood donations was 215,869/215,976 or 99.95% (95% CI: 99.94-99.96%).

There were 1,198 PSP pools tested with the Procleix Ultrio Assay and included in the specificity calculations (Table 7). There were 1,195 PSP pools that tested nonreactive in the Procleix Ultrio Assay and all of these pools were considered true negative. There were three PSP pools that tested reactive in the Procleix Ultrio Assay and all three pools were false positive. The overall specificity of pools from PSP donations was 1,195/1198, or 99.7% (95% CI: 99.3-99.9%).

Overall Clinical Specificity of the PROCLEIX HIV-1, HCV, and HBV Discriminatory Assays

There were 1,785 IDS that tested nonreactive in the Procleix HIV-1 Discriminatory Assay and all were considered true negative (Table 7). There were 12 IDS that tested reactive in the Procleix HIV-1 Discriminatory Assay. Of these, 8 IDS were considered true positive and 4 IDS were considered false positive. The specificity of the Procleix HIV-1 Discriminatory Assay was 99.8% (1,785/1,789; 95% CI: 99.4-99.9%).

There were 1,653 IDS that tested nonreactive in the Procleix HCV Discriminatory Assay and all were considered true negative. There were 157 IDS that tested reactive in the Procleix HCV Discriminatory Assay. Of these, 125 IDS were considered true positive and 32 IDS were considered false positive. The specificity of the Procleix HCV Discriminatory Assay was 98.1% (1,653/1,685; 95% CI: 97.3-98.7%).

There were 1,748 IDS that tested nonreactive in the Procleix HBV Discriminatory Assay and all were considered true negative. There were 47 IDS that tested reactive in the Procleix HBV Discriminatory Assay. Of these, 43 IDS were considered true positive and 4 IDS were considered false positive. The specificity of the Procleix HBV Discriminatory Assay was 99.8% (1,748/1,752; 95% CI: 99.4-99.9%).

Table 7. Procleix System - Clinical Specificity Study: Overall Specificities of the Procleix Ultrio Assay and Discriminatory Assays

Assay	Sample	N	True Negative	False Negative	True Positive	False Positive	Specificity (%)	95% CI
Procleix Ultrio Assay	Pools* from Whole Blood Donations	12,028	11,786	10	173	59	99.5	99.4-99.6
	IDS** from Whole Blood Donations	12,780	12,479	1	193	107	99.1	99.0-99.3
	IDS and Nonreactive Pools	216,180	215,869	11	193	107	99.95	99.94-99.96
	Pools from Paid Source Plasma Donations	1,198	1,195	0	0	3	99.7	99.3-99.9
Procleix HIV-1 Discriminatory Assay	IDS from Whole Blood Donations	1,797	1,785	0	8	4	99.8	99.4-99.9
Procleix HCV Discriminatory Assay	IDS from Whole Blood Donations	1,810	1,653	0	125	32	98.1	97.3-98.7
Procleix HBV Discriminatory Assay	IDS from Whole Blood Donations	1,795	1,748	0	43	4	99.8	99.4-99.9

N = Number of samples (individual donations or pools)
CI = Confidence Interval
*Pools included 303 donor samples from volunteer source plasma donations
**IDS included 9 donor samples from volunteer source plasma donations.

Clinical Specificity of the Procleix Ultrio Assay and Discriminatory Assays by Site

Table 8 shows clinical specificity results for the three blood center testing sites. Clinical specificity of the Procleix Ultrio Assay in 16-sample pools ranged from 99.3% (95% CI: 99.1-99.5%) for Site 2 to 99.8% (95% CI: 99.6-99.9%) for Site 1. Specificity in IDS was significantly lower at Site 2 at 98.5% (95% CI: 98.1-98.8%) than at Sites 1 and 3, which had specificity rates of 99.6% (95% CI: 99.3-99.8%) and 99.5% (95% CI: 99.2-99.7%), respectively. Including all samples tested, whether tested as IDS only, IDS after a reactive pool result, or as part of a nonreactive pool, specificity ranged from 99.930% (95% CI: 99.912-99.945%) for Site 2 to 99.973% (95% CI: 99.955-99.984%) for Site 1.

Clinical specificity of the Procleix HIV-1 Discriminatory Assay ranged from 99.7% (95% CI: 98.7-100%) for Site 3 to 100% (95% CI: 99.3-100%) for Site 1. Specificity of the Procleix HCV Discriminatory Assay was significantly lower at Site 2 at 96.1% (95% CI: 94.3-97.5%) than at Sites 1 and 3, which had specificity rates of 99.2% (95% CI: 98.1-99.8%) and 99.3% (95% CI: 98.1-99.8%), respectively. For the Procleix HBV Discriminatory Assay, specificity ranged from 99.6% (95% CI: 98.7-100%) for Site 3 to 100% (95% CI: 99.3-100%) for Site 1. (Table 8)

Table 8. Procleix System – Clinical Specificity Study: Specificities of the Procleix Ultrio Assay and Discriminatory Assays by Site

Assay	Sample	Site	N	True Negative	False Negative	True Positive	False Positive	Specificity (%)	95% CI
Procleix Ultrio Assay	Pools from Whole Blood Donations	1*	3421	3399	2	13	7	99.8	99.6-99.9
		2	5278	5122	8	114	34	99.3	99.1-99.5
		3	3329	3265	0	46	18	99.5	99.1-99.7
	IDS from Whole Blood Donations	1**	3869	3838	0	15	16	99.6	99.3-99.8
		2	4762	4560	1	130	71	98.5	98.1-98.8
		3	4149	4081	0	48	20	99.5	99.2-99.7
	IDS and Nonreactive Pools	1	58,285	58,252	2	15	16	99.97	99.96-99.98
		2	101,492	101,282	9	130	71	99.93	99.91-99.95
		3	56,403	56,335	0	48	20	99.97	99.95-99.98
Procleix HIV-1 Discriminatory Assay	IDS from Whole Blood Donations	1	532	532	0	0	0	100	99.3-100
		2	690	682	0	6	2	99.7	98.9-100
		3	575	571	0	2	2	99.7	98.7-100
Procleix HCV Discriminatory Assay	IDS from Whole Blood Donations	1	535	522	0	9	4	99.2	98.1-99.8
		2	698	592	0	82	24	96.1	94.3-97.5
		3	577	539	0	34	4	99.3	98.1-99.8
Procleix HBV Discriminatory Assay	IDS from Whole Blood Donations	1	535	530	0	5	0	100	99.3-100
		2	706	674	0	30	2	99.7	98.9-100
		3	554	544	0	8	2	99.6	98.7-100

N = number of samples (individual donations or pools)
CI = Confidence Interval
* Pools included 303 donor samples from volunteer source plasma donations.
** IDS included nine donor samples from volunteer source plasma donations.

False Positive Rates of the Procleix Ultrio Assay in Pools and IDS from Whole Blood Donations

False positive rates for the Procleix Ultrio Assay are shown in Table 9a and Table 9b for pools and IDS, respectively. For the Procleix Ultrio Assay clinical trial, pools and IDS were considered false positive if samples were Procleix Ultrio Assay reactive, reference test (Procleix HIV-1/HCV Assay and HBsAg test) nonreactive, and Alternate NAT nonreactive or not tested.

Table 9a. Clinical Specificity Study: Procleix Ultrio Assay False Positive Rates in 16-Sample Pools

Results	False Positive Rates Procleix Ultrio Assay
Multiplex testing of pools	0.50% (59/11,845)
Pools with 16 multiplex nonreactive IDS	0.42% (50/11,845)
Pools with at least 1 multiplex reactive, discriminatory reactive IDS	0.02% (2/11,845)
Pools with at least 1 multiplex reactive, discriminatory nonreactive IDS	0.06% (7/11,845)

Table 9b. Clinical Specificity Study: Procleix Ultrio Assay False Positive Rates in IDS

Results	False Positive Rates Procleix Ultrio Assay
Multiplex testing of IDS	0.85% (107/12,586)
Multiplex reactive, discriminatory reactive IDS	0.08% (10/12,586)
Multiplex reactive, discriminatory nonreactive IDS	0.77% (97/12,586)*

*6 of the 97 nondiscriminated IDS were not tested in all 3 discriminatory assays

Comparison of the Procleix Ultrio Assay to the Procleix HIV-1/HCV Assay

Table 10 shows the reactivity rates of the Procleix Ultrio Assay and Procleix HIV-1/HCV Assay in HIV-1 and HCV positive donations collected during the clinical specificity study for the Procleix Ultrio Assay. Samples (whether tested in pools or as IDS only) were included in this analysis if they had valid and complete Procleix Ultrio Assay and Procleix HIV-1/HCV Assay results for HIV-1 and HCV detection.

All of the eight HIV-1 positive samples were detected with both the Procleix Ultrio Assay and the Procleix HIV-1/HCV Assay. Of 127 HCV positive samples, 125 samples (98.4%) were detected with the Procleix Ultrio Assay. Two of the 125 samples were reactive for HCV with the Procleix Ultrio Assay and HCV Alternate NAT, but were nonreactive with the Procleix HIV-1/HCV Assay. Likewise, 125 of 127 HCV positive samples (98.4%) were detected with the Procleix HIV-1/HCV Assay. Two of the 125 samples were reactive for HCV with the Procleix HIV-1/HCV Assay but nonreactive with the Procleix Ultrio Assay.

The results demonstrate that the Procleix Ultrio Assay and Procleix HIV-1/HCV Assay detected HIV-1 and HCV equally. Both assays detected the same number of positive samples. Therefore, sensitivity of the Procleix Ultrio Assay is similar to that of the Procleix HIV-1/HCV Assay

Table 10.Comparison of Reactivity Rates between the Procleix Ultrio Assay and the Procleix HIV-1/HCV Assay in HIV-1 and HCV Infected Donations

Target	RNA Positive Donations*	Reactivity Rate	
		Procleix Ultrio Assay	Procleix HIV-1/HCV Assay
HIV-1	8	100% (8/8)	100% (8/8)
HCV	127	98.4% (125/127)**	98.4% (125/127)***

* Number of positive donor samples reactive by the Procleix HIV-1/HCV Assay and/or the Procleix Ultrio Assay and Alternate NAT.
** Two of the 125 positive samples were reactive by Alternate NAT but nonreactive by the Procleix HIV-1/HCV Assay. One of the two samples was also HCV seropositive.
*** Two of the 125 positive donor samples were reactive with the Procleix HIV-1/HCV Assay only.

Comparison of the Procleix Ultrio Assay to HIV-1 and HCV Serology Results

Results generated from the pooled, individual donation and discriminatory assay specificity studies allow comparison of the Procleix Ultrio Assay with serology reactivity (Table 11). All of the specimens included in this analysis were EIA repeat reactive.

HIV-1 Western Blot results were available for 67 samples. Of these, two were HIV-1 Western Blot positive: both samples were Procleix Ultrio Assay reactive (100%). The remaining 65 of 67 had negative or indeterminate Western Blot results: all 65 (100%) were Procleix Ultrio Assay nonreactive.
HCV RIBA Assay results were available for 180 samples. Of these, 44 were RIBA Assay positive: 37 (84.1%) were Procleix Ultrio Assay reactive and seven were Procleix Ultrio Assay nonreactive. The remaining 136 of 180 had RIBA Assay negative or indeterminate results: all 136 (100%) were Procleix Ultrio Assay nonreactive.

Table 11. Clinical Specificity Study: Comparison of HIV-1 and HCV Confirmatory Serology and Procleix Ultrio Assay Results

Serology			Procleix Ultrio Assay *		
	Western Blot Result		Reactive	Non-reactive	
HIV-1 EIA RR (n=75)	Positive	2	2	0	
	Indeterminate	44	0	44	
	Negative	21	0	21	
	Not available	8	0	8	
	RIBA Assay Results				
HCV EIA RR (n=197)	Positive	44	37	7**	
	Indeterminate	51	0	51	
	Negative	85	0	85	
	Not available	17	6	11	

RR: Repeatedly reactive
* Includes samples with dHIV-1 or dHCV results or with nonreactive pool or IDS-only results.
** Seven samples that were HCV positive by the RIBA Assay came from Procleix Ultrio Assay nonreactive pools, so discriminatory testing was not required.

Comparison of the Procleix Ultrio Assay to HBV Serology Results

Table 12 summarizes the HBV results for 218,260 samples that were tested initially in pools or as IDS only and had valid Procleix Ultrio Assay and Procleix HBV Discriminatory Assay results.

Of 218,260 donor samples tested in the clinical specificity study in the Procleix Ultrio Assay and HBV Discriminatory Assay, 216,949 (99.40%) were Procleix Ultrio Assay nonreactive and HBsAg and anti-HBc seronegative, indicating no evidence of previous HBV exposure. One of the 216,949 was Procleix Ultrio Assay nonreactive, HBsAg and anti-HBc serology negative, and HBV Discriminatory Assay reactive. Of the remaining 1,311 of 218,260 specimens, 46 samples were reactive for HBV DNA in the Procleix Ultrio Assay and HBV Discriminatory Assay and 1,265 were nonreactive.

Thirty-eight of 46 IDS samples were HBsAg seropositive (i.e., true positive) and 8 (of the 46 were HBsAg seronegative. Three of these 8 specimens were categorized as false positive as HBV was not detected by Alternate NAT, or Alternate NAT results were unavailable due to insufficient sample volume. Of these three, one was Procleix Ultrio Assay nonreactive and seronegative at follow-up, 2.5 weeks after the index donation. Alternate NAT detected HBV in the remaining 5 (of 8) specimens. Two of these 5 cases were followed-up 3 to 5.5 weeks after the index donation and were both nonreactive in the Procleix Ultrio Assay and HBV Alternate NAT. HBsAg and anti-HBc results were also seronegative at follow-up.

Of the 1,265 samples that showed evidence of HBV exposure by serology but were nonreactive for HBV DNA, 1,254 were anti-HBc seropositive only samples. The sample pattern of HBV DNA and HBsAg non-reactivity and anti-HBc reactivity would be observed in fully resolved HBV infections as well as in samples with false positive anti-HBc serologic test results. While resolved infections may be present in this population, a significant proportion of these results may be due to false positive anti-HBc results.

Among the remaining 11 HBV seropositive, HBV DNA negative samples, seven were HBsAg seropositive and anti-HBc seroreactive [or seropositive] and four were only HBsAg seropositive. Of these four samples, 2 samples were not detected in Alternate NAT, showing consistency with Procleix Ultrio Assay results (i.e., true negative results). Alternate NAT results for the remaining samples are unavailable.

Table 12. Procleix System - Clinical Specificity Study: Comparison of HBV Serology and Procleix Ultrio Assay Results at Index

Line	Initial Results	N	%
1	Anti-HBc + / HBsAg + / DNA +	38	0.017
2	Anti-HBc + / HBsAg + / DNA -	7	0.003
3	Anti-HBc - / HBsAg + / DNA +	0	0.000
4	Anti-HBc - / HBsAg + / DNA -	4	0.002
5	Anti-HBc - / HBsAg - / DNA +	7*	0.003
6	Anti-HBc - / HBsAg - / DNA -	216,949	99.399
7	Anti-HBc + / HBsAg - / DNA +	1	0.001
8	Anti-HBc + / HBsAg - / DNA -	1,254	0.575
Total		218,260	

N = number of samples
Anti-HBc + = seropositive for HBV core antibody
HBsAg + = seropositive for HBsAg
DNA + = Procleix Ultrio Assay reactive, HBV discriminated
Anti-HBc - = seronegative for HBV core antibody
HBsAg - = seronegative for HBsAg
DNA - = nonreactive in the dHBV Assay or Procleix Ultrio Assay nonreactive, dHBV Assay reactive
* Three of 7 donors were followed up. All of the follow-up specimens were Procleix Ultrio Assay nonreactive and HBsAg seronegative.

C. NON-SPECIFICITY STUDIES

Specificity and Sensitivity of the Procleix Ultrio Assay and Discriminatory Assays in the Presence of Donor and Donation Factors

Tables 13 and 14 show all valid initial test results obtained when specimens containing various donor and donation factors were tested with the Procleix Ultrio Assay and Discriminatory Assays. HIV-1, HCV, and HBV positive specimens were created by individually spiking the various donor and donation specimens to a final concentration of 200 copies/mL of HIV-1, 60 IU/mL of HCV, or 30 IU/mL of HBV. Cross-reactivity and interference are defined as greater than 5% unexpected results.

No cross-reactivity (Table 14) or interference (Table 13) was observed for naturally occurring hemolyzed or lipemic specimens or plasma containing the following substances: serum albumin (6 g/dL), hemoglobin (500 mg/dL) and lipids (3,000 mg/dL). No cross-reactivity or interference for detection of HIV-1, HCV, and HBV was observed for naturally occurring icteric specimens or plasma containing bilirubin up to 20 mg/dL. However, this high level of spiked bilirubin produces a slight decrease in HBV analytical sensitivity. This effect was not observed when bilirubin is present at 2.5 mg/dL.

Multiple specimens from each group of patients with the following autoimmune conditions were evaluated: rheumatoid factor, antinuclear antibody, lupus and multiple myeloma. Also tested were samples from flu vaccinees, from hepatitis B vaccinees, from patients with elevated IgM, with elevated IgG, with elevated amino alanine transferase (ALT) and from patients with alcoholic liver cirrhosis. For the majority of these conditions, no cross-reactivity or interference was observed. However, a small portion of these specimens had random, unexpected results in greater than 5% of the samples tested. These occurrences are indicated in bold text in Tables 13 and 14.

No cross-reactivity or interference was observed in the majority of bacterially contaminated plasma specimens or in specimens from patients infected with other bloodborne pathogens. Multiple specimens from each group of patients with the following viral infections were evaluated: herpes simplex virus-1 (HSV 1), herpes simplex virus-2 (HSV 2), CMV, EBV, hepatitis A virus (HAV), HTLV-I, HTLV-II, hepatitis G virus (HGV), rubella, and parvovirus B-19. A small portion of the specimens containing viral infections had random, unexpected results in greater than 5% of the samples tested. These occurrences are indicated in bold text in Tables 13 and 14.

Table 13. Detection of HIV-1, HCV, and HBV in the Presence of Donor and Donation Factors with the Procleix Ultrio Assay and Discriminatory Assays

Donor or Donation Factor	Reactive/Tested*					
	HIV-1 Positive (200 c/mL)		HCV Positive (60 IU/mL)		HBV Positive (30 IU/mL)	
	Procleix Ultrio Assay	dHIV-1	Procleix Ultrio Assay	dHCV	Procleix Ultrio Assay	dHBV
Hemolyzed	21/21	21/21	18/18	18/18	29/30	30/30
Icteric	21/21	21/21	30/30	30/30	30/30	30/30
Lipemic	24/24	24/24	12/12	12/12	30/30	29/30
Normal	39/39	37/37	39/39	39/39	39/39	39/39
Albumin (6 g/dL)	39/39	39/39	39/39	39/39	38/39	39/39
Bilirubin (20 mg/dL)	39/39	39/39	39/39	39/39	38/39	**37/39****
Bilirubin (2.5 mg/dL)	NA	NA	NA	NA	86/87	89/89
Hemoglobin (500 mg/dL)	38/38	39/39	39/39	39/39	39/39	39/39
Lipids (3000 mg/dL)	39/39	39/39	39/39	39/39	38/39	39/39
Alcoholic Cirrhosis	30/30	30/30	30/30	**28/30**	30/30	29/30
Antinuclear Antibody	27/27	27/27	27/27	26/27	27/27	27/27
ALT	30/30	30/30	30/30	30/30	30/30	30/30
Elevated IgG	30/30	**26/30**	**25/30**	30/30	30/30	30/30
Elevated IgM	29/30	**27/30**	29/30	29/30	**26/30**	**27/30**
Lupus	**28/30**	30/30	30/30	30/30	30/30	30/30
Multiple Myeloma	23/23	23/23	23/23	23/23	**21/23**	23/23
Rheumatoid Factor	**27/30**	29/30	29/30	30/30	29/30	30/30
Flu Vaccinee	30/30	30/30	30/30	30/30	30/30	30/30
HBV Vaccinee	30/30	30/30	30/30	30/30	29/30	29/30
C albicans	30/30	30/30	30/30	30/30	30/30	30/30
C diphtheriae	30/30	30/30	30/30	30/30	31/31	31/31
M luteus	30/30	30/30	30/30	30/30	29/30	30/30
P acnes	30/30	30/30	30/30	30/30	29/30	29/30
P carinii	30/30	30/30	30/30	30/30	30/30	30/30
S aureus	30/30	30/30	30/30	30/30	30/30	30/30

S epidermidis	30/30	30/30	30/30	30/30	28/29	29/29
CMV	30/30	30/30	30/30	30/30	**28/30**	30/30
EBV	30/30	30/30	30/30	30/30	30/30	30/30
HAV	30/30	30/30	30/30	29/30	**26/30**	**28/30**
HSV 2	30/30	30/30	30/30	30/30	29/30	30/30
HSV 1	30/30	30/30	30/30	30/30	30/30	30/30
HTLV II	30/30	30/30	**27/30**	**27/30**	**26/30**	28/29
Rubella	30/30	30/30	30/30	30/30	30/30	29/30
HGV	21/21	21/21	21/21	**18/21**	20/21	21/21
Parvovirus B19	30/30	30/30	30/30	30/30	**27/30**	**27/30**
HTLV I	30/30	30/30	30/30	30/30	29/30	**28/30**
Controls	270/270	269/270	270/270	270/270	259/270	263/270

NA = Not tested
* Combined results from three clinical lots of reagents.
** Bolded text indicates greater than 5% nonreactive results.

Table 14. Specificity of the Procleix Ultrio Assay and Discriminatory Assays in the Presence of Donor and Donation Factors

Donor or Donation Factor	Nonreactive/Negative Samples Tested*			
	Procleix Ultrio Assay	dHIV-1	dHCV	dHBV
Hemolyzed	30/30	30/30	30/30	30/30
Icteric	29/30	30/30	29/30	29/30
Lipemic	30/30	30/30	29/30	30/30
Normal	36/36	36/36	36/36	36/36
Albumin (6 g/dL)	36/36	36/36	36/36	36/36
Bilirubin (20 mg/dL)	36/36	36/36	36/36	36/36
Hemoglobin (500 mg/dL)	36/36	35/35	36/36	36/36
Lipids (3000 mg/dL)	36/36	35/35	36/36	36/36
Alcoholic Cirrhosis	30/30	30/30	30/30	30/30
Antinuclear Antibody	**24/27****	27/27	26/27	**19/27**
ALT	29/30	30/30	30/30	30/30
Elevated IgG	**27/30**	30/30	30/30	30/30
Elevated IgM	30/30	30/30	30/30	30/30
Lupus	29/30	30/30	30/30	29/30
Multiple Myeloma	**21/24**	24/24	24/24	**22/24**
Rheumatoid Factor	30/30	30/30	30/30	29/30
Flu Vaccinee	**27/30**	30/30	30/30	30/30
HBV Vaccinee	30/30	30/30	29/30	30/30
C albicans	30/30	30/30	30/30	30/30
C diphtheriae	30/30	30/30	30/30	29/30
M luteus	29/30	30/30	30/30	30/30
P acnes	30/30	30/30	29/30	30/30
P carinii	30/30	30/30	29/30	30/30
S aureus	30/30	30/30	**28/30**	30/30
S epidermidis	30/30	30/30	30/30	30/30
CMV	**28/30**	30/30	30/30	**27/30**
EBV	**30/33**	33/33	33/33	**29/33**

HAV	30/30	30/30	30/30	29/30
HSV 2	26/27	30/30	**28/30**	**27/30**
HSV 1	**26/30**	27/27	27/27	**25/27**
HTLV II	30/30	30/30	29/30	30/30
Rubella	29/30	30/30	30/30	30/30
HGV	15/15	15/15	15/15	15/15
Parvovirus B19	**24/27**	27/27	27/27	27/27
HTLV I	27/27	27/27	27/27	27/27
Controls	269/270	270/270	269/270	270/270

* Combined results from three clinical lots of reagents.
****** Bolded text indicates greater than 5% reactive results.

Specificity and Sensitivity of the Procleix Ultrio Assay and Discriminatory Assays in the Presence of Anticoagulants and Serum

The sensitivity and specificity of the Procleix Ultrio Assay and Discriminatory Assays for serum samples and samples collected in various anticoagulants is shown in Tables 15 and 16. Detection rates were calculated from valid initial results. Cross-reactivity and interference are defined as greater than 5% unexpected results. The anticoagulants tested were ACD (Acid Citrate Dextrose), K_2 EDTA (ethylene diamine tetraacetic acid), K_3 EDTA, PPT (K_2 EDTA Plasma Preparation Tube), sodium citrate, CPD (citrate phosphate dextrose), and sodium heparin. For the majority of anticoagulants, no cross-reactivity or interference for detection of HIV-1, HCV, or HBV was observed. A small portion of the anticoagulants tested had random, unexpected results in greater than 5% of the samples tested. These occurrences are indicated in bold text in Tables 15 and 16.

Table 15. Detection of HIV-1, HCV and HBV in the Presence of Anticoagulants and Serum with the Procleix Ultrio Assay and Discriminatory Assays

Anticoagulant	Reactive/Tested (Percent Reactive)					
	HIV-1 Positive* (200 c/mL)		HCV Positive* (60 IU/mL)		HBV Positive** (up to 30 IU/mL)	
	Procleix Ultrio Assay	dHIV-1	Procleix Ultrio Assay	dHCV	Procleix Ultrio Assay	dHBV
ACD	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	**122/130 (93.8%)*****	125/128 (97.7%)
CPD	29/30 (96.7%)	30/30 (100%)	29/30 (96.7%)	29/30 (96.7%)	124/129 (96.1%)	**122/129 (94.6%)**
K_2 EDTA	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	127/130 (97.7%)	129/130 (99.2%)
K_3 EDTA	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	125/130 (96.2%)	125/130 (96.2%)
Sodium Citrate	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	**122/130 (93.8%)**	126/130 (96.9%)
Sodium Heparin	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	**121/129 (93.8%)**	126/129 (97.7%)
PPT	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	121/126 (96.0%)	**119/126 (94.4%)**
Serum	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	**123/130 (94.6%)**	127/130 (97.7%)

* Combined results from three clinical lots of reagents.
** Combined results from five clinical lots of reagents for ACD, CPD, sodium citrate, sodium heparin, PPT, and serum. Results for K_2 EDTA and K_3 EDTA were from four clinical lots.
*** Bolded text indicates greater than 5% nonreactive results.

Table 16. Specificity of the Procleix Ultrio Assay and Discriminatory Assays in the Presence of Anticoagulants and Serum

Anticoagulant	Nonreactive/Negative Samples Tested* (Percent Nonreactive)			
	Procleix Ultrio Assay	**dHIV-1**	**dHCV**	**dHBV**
ACD	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	29/30 (96.7%)
CPD	**28/30 (93.3%)****	30/30 (100%)	30/30 (100%)	30/30 (100%)
K2 EDTA	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
K3 EDTA	30/30 (100%)	30/30 (100%)	30/30 (100%)	29/30 (96.7%)
Sodium Citrate	30/30 (100%)	30/30 (100%)	30/30 (100%)	29/30 (96.7%)
Sodium Heparin	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
PPT	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
Serum	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)

* Combined results from three clinical lots of reagents.
** Bolded text indicates greater than 5% reactive results.

D. CLINICAL SENSITIVITY

Testing of Known Positive Samples from HIV-1, HCV, and/or HBV Infected Individuals

A combined total of 3,138 specimens known to be positive for HIV-1 RNA, or HCV RNA, or HBV DNA were procured from a vendor and were included in the clinical sensitivity analyses.

These specimens were classified as HIV-1 RNA positives, HCV RNA positives, and HBV DNA positives based on qualitative nucleic acid testing (NAT) results. In addition to NAT, the vendor provided serologic test results to confirm that samples were positive for the appropriate target and that samples were not co-infected. HIV-1 positive samples were seroreactive for HIV-1 antibody and negative for HCV antibody and HBsAg. Likewise, HCV positive samples were serologically reactive for HCV antibody and negative for HIV-1 antibody and HBsAg. All but two of the HBV positive samples were positive for HBsAg and negative for HIV-1 and HCV antibody. Two HBV positive samples included in the clinical sensitivity calculations for the Procleix Ultrio Assay and Procleix HBV Discriminatory Assay were negative for HBsAg but were positive for HBV core antibody (and negative for HIV-1 and HCV antibody).

The clinical sensitivity study was performed at three testing sites using three Clinical Lots of the Procleix Ultrio Assay. The positive samples were tested undiluted (neat) with the Procleix Ultrio Assay, Procleix HIV-1 Discriminatory Assay (dHIV-1), Procleix HCV Discriminatory Assay (dHCV), and Procleix HBV Discriminatory Assay (dHBV) and tested diluted (1:16) with the Procleix Ultrio Assay. All dilutions were made with serum known to be negative for HIV-1 antibody and RNA, HCV antibody and RNA, and HBsAg and HBV DNA. In addition, negative serum samples were tested with the Procleix Ultrio Assay and three discriminatory assays at each clinical site as a control for potential study bias.

Known-positive samples with nonreactive (discordant) results were tested neat with quantitative Alternative NAT, along with some known-positive samples with reactive (concordant) results to control for bias. Known-positive samples with viral loads less than the Alternate NAT's quantitative limit of detection (LOD) when tested neat were excluded from the clinical sensitivity analyses, regardless of whether the Procleix Ultrio Assay results were discordant or concordant. Because the LOD of the Alternate NAT is the same or similar to the Procleix Ultrio Assay sensitivity claim, the viral loads of these samples were considered below or potentially below the Procleix Ultrio Assay sensitivity claim. Therefore, the sensitivities presented below include samples with known HIV-1 RNA, HCV RNA, and HBV DNA concentrations at or above the Procleix Ultrio Assay sensitivity claim when tested neat (results were not corrected for dilution). Also

included are samples with unknown viral concentration; not all samples had viral load quantitation performed.

The sensitivity for the Procleix Ultrio Assay and Procleix HIV-1 Discriminatory Assay for undiluted (neat) HIV-1 positive samples was 100% (95% CI: 99.7-100%) and 99.9% (95% CI: 99.5-100%), respectively (Table 17). The sensitivity for the Procleix Ultrio Assay for diluted (1:16) HIV-1 positive samples was 99.0% (95% CI: 98.2-99.5%).

The sensitivity for the Procleix Ultrio Assay and the Procleix HCV Discriminatory Assay for undiluted (neat) HCV positive samples was 99.7% (95% CI: 99.1-99.9%) and 99.9% (95% CI: 99.5-100%), respectively. The sensitivity for the Procleix Ultrio Assay for diluted (1:16) HCV positive samples was 99.3% (95% CI: 98.6-99.7%).

The sensitivity for the Procleix Ultrio Assay and Procleix HBV Discriminatory Assay for undiluted (neat) HBV positive samples was 98.9% (95% CI: 98.1-99.5%) and 99.3% (95% CI: 98.6-99.7%), respectively. The sensitivity for the Procleix Ultrio Assay for diluted (1:16) HBV positive samples was 90.5% (95% CI: 88.5-92.2%).

The overall clinical sensitivity for the Procleix Ultrio Assay, which takes into account all positive samples tested, was 99.6% (95% CI: 99.3-99.8%) for undiluted (neat) positive samples and 96.3% (95% CI: 95.6-96.9%) for diluted (1:16) positive samples.

Table 17. Procleix System - Sensitivity of the Procleix Ultrio Assay and Discriminatory Assays in Known Positive Samples

Assay	Sample	N	Reactive	Sensitivity (%)	95% CI
Procleix Ultrio Assay (Neat)	All	3,136	3,122	99.6	99.3-99.8
	HIV-1 Only	1,076	1,076	100	99.7-100
	HCV Only	1,028	1,025	99.7	99.1-99.9
	HBV Only	1,032	1,021	98.9	98.1-99.5
Procleix Ultrio Assay (Diluted 1:16)	All	3,138	3,022	96.3	95.6-96.9
	HIV-1 Only	1,077	1,066	99.0	98.2-99.5
	HCV Only	1,029	1,022	99.3	98.6-99.7
	HBV Only	1,032	934	90.5	88.5-92.2
dHIV-1	HIV-1 Only	1,076	1,075	99.9	99.5-100
dHCV	HCV Only	1,029	1,028	99.9	99.5-100
dHBV	HBV Only	1,028	1,021	99.3	98.6-99.7

N = number of samples

CI = Confidence Interval

Testing of Known Positive 16-Sample Pools

The clinical sensitivity of the Procleix Ultrio Assay was evaluated in 190 sixteen-member pools composed of 1 to 3 HIV-1, HCV, and/or HBV known positive samples and 13 to 15 negative samples. All the positive samples were collected throughout the United States. Their viral positivity was identified by commercial HIV-1 RNA and HCV RNA assays and a validated HBV DNA assay. Two clinical sites participated in the study using one Clinical Lot of the Procleix Ultrio Assay. Known-negative pools were tested with the Procleix Ultrio Assay at each clinical site as a control for potential study bias.

Known-positive samples from pools with nonreactive results were tested neat with quantitative Alternate NAT, along with some known-positive samples from pools with reactive results to control for bias. Known-positive pools with viral loads below the Procleix Ultrio Assay sensitivity claim were excluded from the clinical sensitivity analyses. Therefore, the sensitivities presented in Table 18 include pools with confirmed viral loads at or above the Procleix Ultrio Assay sensitivity claim or of unknown viral concentration; not all pools had viral load quantitation performed.

Overall, the sensitivity for the Procleix Ultrio Assay for 190 known-positive pools containing HIV-1 RNA, HCV RNA, and/or HBV DNA was 97.9% (95% CI: 94.7-99.4%) (Table 18). The sensitivity for the Procleix Ultrio Assay for 125 HIV-1 known-positive pools was 100% (95% CI: 97.1-100%). The sensitivity for the Procleix Ultrio Assay for 115 HCV known-positive pools was 100% (95% CI: 96.8-100%). The sensitivity for the Procleix Ultrio Assay for 123 HBV known-positive pools was 96.7% (95% CI: 91.9-99.1%).

Table 18. Procleix System - Sensitivity of the Procleix Ultrio Assay in 16-Sample Pools Containing Known Positive Specimens

Pools*	N	Reactive	Sensitivity (%)	95% CI
All**	190	186	97.9	94.7-99.4
HIV-1	125	125	100	97.1-100
HCV	115	115	100	96.8-100
HBV	123***	119	96.7	91.9-99.1

N = number of samples
CI = Confidence Interval
* Pools with confirmed viral loads greater than or equal to the Procleix Ultrio Assay sensitivity claim or with unknown copy levels.
** All pools containing single analytes or a combination of analytes
*** The neat positive samples from the 4 of 123 pools with Procleix Ultrio Assay nonreactive results had viral loads of 9,700; 3,800; 6,200; and 9,500 copies/mL at initial quantitation. Viral loads were not determined for all positive samples in the remaining 119 pools.

Clinical Sensitivity High-Risk Population Study

Plasma specimens from individuals at high risk for infection with HIV-1, HCV, and/or HBV were evaluated for the clinical sensitivity high-risk population study. Of the total of 503 high-risk subjects included in the study, the majority reported injection drug use as a risk factor. Other risk factors included multiple sex partners, needle stick accident, blood or blood product transfusion, history of a STD, previous diagnosis of HIV-1, HCV, or HBV infection and dialysis. All the specimens from qualified high-risk subjects were aliquoted and tested undiluted (neat) and diluted (1:16) at one clinical site. The neat specimens were tested with the PROCLEIX® ULTRIO® Assay and HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays. The diluted specimens were tested only with the PROCLEIX ULTRIO Assay. Three clinical lots were used for PROCLEIX ULTRIO Assay and discriminatory assay testing.
True status of the samples was based on their completed laboratory results with the licensed PROCLEIX® HIV-1/HCV Assay and corresponding discriminatory assays and results of HBsAg testing. For the Procleix Ultrio Assay, clinical sensitivity was determined by comparing results (neat and diluted) with the true status of the samples (Table 19a). For the discriminatory assays, results were compared to the true status in neat specimens with Procleix Ultrio Assay reactive results. For specimens with discordant results, comparisons were further made to HIV-1, HCV, and HBV Alternate NAT. The Alternate NAT results were used in clinical sensitivity calculations to interpret PROCLEIX ULTRIO Assay results.

Of the 503 high risk specimens tested neat and diluted (1:16 to simulate multiplex testing of pools) in the PROCLEIX ULTRIO Assay, 495 and 502 specimens, respectively, had valid PROCLEIX ULTRIO Assay results and completed laboratory results and were included in the clinical sensitivity calculations (Table 19a). Of the 495 specimens tested neat, 369 were HIV-1, HCV, and/or HBV positive in the reference tests. Of these, all were Procleix Ultrio Assay reactive. Sensitivity was 100% (95% CI: 99.0 - 100%) in neat specimens for HIV-1, HCV, and HBV detection. Of the 502 specimens tested diluted, 373 were HIV-1, HCV, and/or HBV positive in the reference tests. Of these, 370 were Procleix Ultrio Assay reactive and 3 were Procleix Ultrio Assay nonreactive. Sensitivity was 99.2% (95% CI: 97.7 – 99.8%) in diluted specimens for detection of HIV-1, HCV, and HBV.

Of the Procleix ULTRIO Assay neat-reactive specimens, 317 specimens had valid HIV-1 Discriminatory Assay and reference test results (Table 19b). Of these, 158 were HIV-1 positive in the reference tests and all were HIV-1 discriminated. Sensitivity of the HIV-1 Discriminatory Assay was 100% (95% CI: 97.7%-100%) in specimens that tested Procleix Ultrio Assay neat-reactive.

Of the Procleix ULTRIO Assay neat-reactive specimens, 376 specimens had valid HCV Discriminatory Assay and reference test results (Table 19b). Of these, 299 were HCV positive in the reference tests 298 were HCV discriminated and 1 was

HCV Discriminatory Assay nonreactive. Sensitivity of the HCV Discriminatory Assay was 99.7% (95% CI: 98.2%-100%) in specimens that tested Procleix Ultrio Assay neat-reactive.

Of the Procleix ULTRIO Assay neat-reactive specimens, 311 specimens tested neat had valid HBV Discriminatory Assay and reference test results (Table 19b). Of these, 25 were HBV positive in the reference tests and all were HBV discriminated. Sensitivity of the HBV Discriminatory Assay was 100% (95% CI: 86.3%-100%) in specimens that tested Procleix Ultrio Assay neat-reactive.

Several specimens were infected with two or more viruses, based on results of the PROCLEIX HIV-1/HCV and Discriminatory Assays and/or HBsAg serologic tests. Of the 503 subject specimens, 92 were co-infected with HIV-1 and HCV, 9 were co-infected with HIV-1 and HBV, 1 was co-infected with HCV and HBV, and 5 were co-infected with HIV-1, HCV, and HBV. All co-infected specimens tested reactive in the PROCLEIX ULTRIO Assay**.**

Table 19a. Procleix System – Clinical Sensitivity of the Procleix Ultrio Assay in a High Risk Population

Target	Sample	N	ULTRIO Reactive	Reference Test Positive		Reference Test Negative		Sensitivity (%)	95% CI
				TP*	FN*	TN*	FP*		
All	Neat	495	384	369**	0	111	15	100	99.0-100
	Diluted	502	382	370**	3	117	12	99.2	97.7-99.8

N = number of valid specimens with completed lab results, TP = True positive, FN = False negative, TN = True negative, FP = False positive, CI = Confidence interval
*Interpretations of the Procleix Ultrio Assay results (for calculating sensitivity) when compared to the reference test results.
**92 were co-infected with HIV-1 and HCV, 9 were co-infected with HIV-1 and HBV, 1 was co-infected with HCV and HBV, and 5 were co-infected with HIV-1, HCV, and HBV.

Table 19b. Procleix System – Clinical Sensitivity of the Discriminatory Assays in Procleix Ultrio Assay Neat-Reactive Specimens From a High Risk Population

Assay	N	Discriminatory Assay Reactive	Reference Test Positive		Reference Test Negative		Sensitivity (%)	95% CI
			TP*	FN*	TN*	FP*		
dHIV-1	317	159	158**	0	158	1	100	97.7-100
dHCV	376	307	298***	1	68	9	99.7	98.2-100
dHBV	311	31	25****	0	280	6	100	86.3-100

N = number of valid specimens with completed lab results, TP = True positive, FN = False negative, TN = True negative, FP = False positive, CI = Confidence interval, dHIV-1 = Procleix HIV-1 Discriminatory Assay, dHCV = Procleix HCV Discriminatory Assay, dHBV = Procleix HBV Discriminatory Assay

*Interpretations of the HIV-1, HCV, or HBV Discriminatory Assay results (for calculating sensitivity) when compared to the reference test results.
**87 were co-infected with HIV-1 and HCV, 9 were co-infected with HIV-1 and HBV, and 5 were co-infected with HIV-1, HCV, and HBV.
***91 were co-infected with HIV-1 and HCV, 1 was co-infected with HCV and HBV, and 5 were co-infected with HIV-1, HCV, and HBV.
****9 were co-infected with HIV-1 and HBV and 4 were co-infected with HIV-1, HCV, and HBV.

E. ANALYTICAL SENSITIVITY

Analytical sensitivity panels comprised of serially diluted HIV-1 type B virus, HIV-1 WHO standard (97/656), HCV WHO standard (96/790), and HBV WHO standard (97/746) were used to evaluate assay sensitivity. The HIV-1 type B virus panel was prepared by serial dilution of an HIV-1 type B tissue culture supernatant, which was value assigned using an in-house HIV-1 quantitative assay, which is calibrated with the VQA Standard, from the Virology Quality Assurance Laboratory, Rush-Presbyterian St. Luke's Medical Center, Rush University, Chicago, IL. Four operators, testing 30 replicates of each copy level, ran a total of 120 replicates of each target level with three clinical lots using the Procleix System. The S/CO and %CV values are the averages of the values calculated for each clinical lot. The 95% confidence intervals of the positivity rates were based on the exact binomial distribution. Estimations of 50% and 95% detection rates by Probit Analysis are provided.

Detection of HIV-1 type B virus

HIV-1 type B virus detection with the Procleix Ultrio Assay and HIV-1 Discriminatory Assay (dHIV-1) was 100% at 300 copies/mL, 99% at 100 copies/mL and ≥ 92% at 30 copies/mL for both assays. Positivity rates at 10 copies/mL were 53% and 57% for the Procleix Ultrio Assay and dHIV-1 Assay. At 3 copies/mL, the detection rates were 25% and 24% for the Procleix Ultrio Assay and dHIV-1 Assay. Although there was variability between the two assays, the differences were not statistically significant as indicated by overlapping 95% confidence intervals (Table 20). Detection rates were calculated from valid initial results.

Table 20. Procleix System - Detection of HIV-1 Type B in Analytical Sensitivity Panels

Procleix Ultrio Assay							dHIV-1					
HIV-1 B* copies/mL	Number of reactive/tested**	% Positive	95% Confidence Limits		Average S/CO	%CV	Number of reactive/tested**	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper					Lower	Upper		
300	120/120	100	97	100	15.08	6	120/120	100	97	100	21.44	15
100	119/120	99	95	100	13.34	13	119/120	99	95	100	18.75	26
30	110/120	92	85	96	9.53	31	112/118	95	89	98	12.97	44
10	64/120	53	44	62	6.97	56	68/120	57	47	66	8.80	71
3	30/120	25	18	34	6.91	52	29/120	24	17	33	5.87	114
0	0/120	0	0	3	0.11	87	0/119	0	0	3	0.12	70

*HIV-1 B tissue culture supernatant value assigned with VQA standard.
** Invalid reactions were not included.

Detection of HIV-1 WHO Standard (97/656)

Detection of the HIV-1 WHO standard with the dHIV-1 Assay was 100% at 600, 200 and 60 IU/mL. The detection rates at 20 IU/mL and 6 IU/mL were 93% and 61%, respectively (Table 21). Detection rates were calculated from valid initial results. Due to the cross-reactivity of this standard with HBV, [38] only the dHIV-1 Assay was tested.

Table 21. Procleix System - Detection of HIV-1 WHO Standard in Analytical Sensitivity Panels with the Procleix HIV-1 Discriminatory Assay

HIV-1 WHO (97/656) IU/mL	Number of reactive/ tested*	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper		
600	119/119	100	97	100	23.48	13
200	120/120	100	97	100	22.58	12
60	119/119	100	97	100	20.56	17
20	110/118	93	87	97	14.28	43
6	73/120	61	52	70	11.17	57
0	0/120	0	0	3	0.10	59

***** Invalid reactions were not included.

Detection of HCV WHO Standard (96/790)

The detection rate for the HCV WHO standard at 100 and 30 IU/mL was 100% and ≥99% at 10 IU/mL for both the Procleix Ultrio Assay and HCV Discriminatory Assay (dHCV). The detection rate at 3 IU/mL in the Procleix Ultrio Assay was 91%. In the dHCV Assay, the detection rate at 3 IU/mL was 96%. The detection rates for 1 IU/mL were 64% and 67% for the Procleix Ultrio Assay and dHCV Assay. There were no statistically significant differences observed in the positivity rates for the detection of HCV WHO standard with the Procleix Ultrio Assay and dHCV Assay (Table 22). Detection rates were calculated from valid initial results.

Table 22. Procleix System - Detection of HCV WHO Standard in Analytical Sensitivity Panels

	Procleix Ultrio Assay						dHCV					
HCV WHO (96/790) IU/mL	Number of reactive/tested*	% Positive	95% Confidence Limits		Average S/CO	%CV	Number of reactive/tested*	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper					Lower	Upper		
100	118/118	100	97	100	7.45	5	120/120	100	97	100	21.97	9
30	119/119	100	97	100	7.32	5	118/118	100	97	100	21.51	8
10	119/120	99	95	100	7.10	8	120/120	100	97	100	20.84	12
3	109/120	91	84	95	6.52	19	115/120	96	91	99	18.88	21
1	77/120	64	55	73	5.80	28	79/118	67	58	75	17.40	32
0	0/120	0	0	3	0.09	52	0/120	0	0	3	0.11	104

***** Invalid reactions were not included.

Detection of HBV WHO Standard (97/746)

The detection rate of the Procleix Ultrio Assay and the HBV Discriminatory Assay (dHBV) was 100% for HBV WHO standard at 45 IU/mL and ≥ 99% at 15 IU/mL. HBV detection at 5 IU/mL with the Procleix Ultrio Assay and the dHBV Assay was 74% and 77% respectively, at 1.67 IU/mL detection rates were 40% and 41% respectively and 19% and 18% respectively for 0.56 IU/mL. There were no statistically significant differences observed in the positivity rates for the detection of HBV WHO standard with the Procleix Ultrio Assay and dHBV Assay (Table 23). Detection rates were calculated from valid initial results.

Table 23. Procleix System - Detection of HBV WHO Standard in Analytical Sensitivity Panels

	Procleix Ultrio Assay						dHBV					
HBV WHO (97/746) IU/mL	Number of reactive/ tested*	% Positive	95% Confidence Limits		Average S/CO	%CV	Number of reactive/ tested*	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper					Lower	Upper		
45	120/120	100	97	100	14.27	7	119/119	100	97	100	22.70	9
15	119/120	99	95	100	13.91	12	120/120	100	97	100	22.05	13
5	89/120	74	65	82	11.18	36	91/119	77	68	84	17.93	38
1.67	48/120	40	31	49	11.89	32	49/119	41	32	51	17.75	38
0.56	22/119	19	12	27	9.95	48	21/120	18	11	26	16.15	51
0	0/119	0	0	3	0.12	73	0/120	0	0	3	0.07	129

***** Invalid reactions were not included.

Probit Analysis

The predicted 50% and 95% detection rates in copies/mL or IU/mL for each target were determined with Probit Analysis of the analytical sensitivity results. The predicted 95% detection rate for HIV-1 type B was 37.7 copies/mL for the Procleix Ultrio Assay and 35.4 copies/mL for the dHIV-1 Assay. The predicted 95% detection rate for HIV-1 WHO was 18.1 IU/mL for the dHIV-1 Assay. The predicted 95% detection rate for HCV WHO was 3.7 IU/mL and 2.4 IU/mL for the Procleix Ultrio Assay and the dHCV Assay, respectively. The 95% detection rate for HBV was 8.0 IU/mL and 6.8 IU/mL for the Procleix Ultrio Assay and dHBV Assay, respectively (Table 24).

Table 24. Procleix System - Detection Probabilities of HIV-1, HCV, and HBV

Panel Tested	Assay	Detection Probabilities	
		50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
HIV-1 B copies/mL	Procleix Ultrio Assay	13.9 (12.0-15.9)	37.7 (33.6-43.0)
HIV-1 B copies/mL	dHIV-1	12.9 (11.2-14.6)	35.4 (33.8-36.9)
HIV-1 WHO (97/656) IU/mL	dHIV-1	7.5 (6.4-8.7)	18.1 (16.1-20.8)
HCV WHO (96/790) IU/mL	Procleix Ultrio Assay	1.3 (1.0-1.5)	3.7 (3.3-4.2)
HCV WHO (96/790) IU/mL	dHCV	1.0 (0.9-1.2)	2.4 (2.1-2.7)
HBV WHO (97/746) IU/mL	Procleix Ultrio Assay	3.3 (3.0-3.8)	8.0 (7.1-9.3)
HBV WHO (97/746) IU/mL	dHBV	3.0 (2.7-3.4)	6.8 (6.0-7.7)

F. SENSITIVITY OF DETECTION FOR HIV-1, HCV, AND HBV GENETIC VARIANTS

Multiple specimens and tissue culture isolates were tested to determine the sensitivity of detection of the viral genetic variants.

Detection of HIV-1 Genetic Variants with the Procleix Ultrio Assay and HIV-1 Discriminatory Assay

HIV-1 specimens and tissue culture isolates of group M (subtypes A, B, C, D, E, F, and G), N, and O were quantified for HIV-1 RNA concentrations using commercially available quantitative HIV-1 RNA assays or with an in-house quantitative HIV-1 RNA test. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. Diluted specimens were tested in the Procleix Ultrio Assay and dHIV-1 Assay. Fifty-four unique specimens or tissue culture isolates were tested in duplicate using three clinical lots on the Procleix System. Six of the specimens were co-infected with HCV and/or HBV and were therefore only tested in the dHIV-1 Assay. At 300 copies/mL, 287/288 replicates (99.7%) were reactive with the Procleix Ultrio Assay and 324/324 replicates (100%) were reactive with the dHIV-1 Assay. At 100 copies/mL, 286/288 replicates (99.3%) were reactive with the Procleix Ultrio Assay and 320/324 replicates (98.8%) were reactive with the dHIV-1 Assay. At 30 copies/mL, 252/288 replicates (87.5%) were reactive with the Procleix Ultrio Assay and 289/324 replicates (89.2%) were reactive with the dHIV-1 Assay (Table 25). Detection rates were calculated from valid initial results.

Table 25. Procleix System - Detection of HIV-1 Genetic Variants with the Procleix Ultrio Assay and HIV-1 Discriminatory Assay

Genetic Variant	Conc. copies/mL	Procleix Ultrio Assay			HIV-1 Discriminatory Assay		
		Unique Donors*	Reactive/Tested	% Reactive	Unique Donors*	Reactive/Tested	% Reactive
HIV-1 Group M Subtype A	300	8	48/48	100	9	54/54	100
	100		48/48	100		54/54	100
	30		46/48	95.8		48/54	88.9
HIV-1 Group M Subtype B	300	6	36/36	100	7	42/42	100
	100		36/36	100		42/42	100
	30		30/36	83.3		40/42	95.2
HIV-1 Group M Subtype C	300	8	48/48	100	8	48/48	100
	100		48/48	100		47/48	97.9
	30		42/48	87.5		42/48	87.5
HIV-1 Group M Subtype D	300	6	36/36	100	6	36/36	100
	100		36/36	100		36/36	100
	30		34/36	94.4		32/36	88.9
HIV-1 Group M Subtype E	300	8	48/48	100	9	54/54	100

	100		48/48	100		54/54	100
	30		45/48	93.8		51/54	94.4
HIV-1 Group M Subtype F	300	3	18/18	100	5	30/30	100
	100		18/18	100		30/30	100
	30		13/18	72.2		25/30	83.3
HIV-1 Group M Subtype G	300	1	6/6	100	2	12/12	100
	100		6/6	100		12/12	100
	30		6/6	100		12/12	100
HIV-1 Group N	300	1	5/6	83.3	1	6/6	100
	100		4/6	66.7		3/6	50
	30		3/6	50		2/6	33.3
HIV-1 Group O	300	7	42/42	100	7	42/42	100
	100		42/42	100		42/42	100
	30		33/42	78.6		37/42	88.1
All Genotypes	300	48	287/288	99.7	54	324/324	100
	100		286/288	99.3		320/324	98.8

	30		252/288	87.5		289/324	89.2

* Each unique donor was tested in duplicate with three clinical lots of reagents.

Detection of HCV Genotypes with the Procleix Ultrio Assay and HCV Discriminatory Assay

HCV specimens of genotypes 1, 2, 3, 4, 5, and 6 were quantified for HCV RNA using commercially available quantitative HCV RNA assays. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. The diluted specimens were tested with the Procleix Ultrio Assay and dHCV Assay. Sixty-one unique specimens were tested in duplicate using three clinical lots on the Procleix System**.** One specimen was co-infected with HIV-1 and was therefore only tested in the dHCV Assay. At 300 copies/mL, all replicates were reactive with both the Procleix Ultrio Assay and the dHCV Assay. At 100 copies/mL, 354/360 replicates (98.3%) were reactive with the Procleix Ultrio Assay and 357/366 replicates (97.5%) were reactive with the dHCV Assay. At 30 copies/mL, 330/360 replicates (91.7%) were reactive with the Procleix Ultrio Assay and 337/366 replicates (92.1%) were reactive with the dHCV Assay (Table 26). Detection rates were calculated from valid initial results.

Table 26. Procleix System - Detection of HCV Genotypes with the Procleix Ultrio Assay and HCV Discriminatory Assay

Genotype	Conc. copies/mL	Procleix Ultrio Assay			HCV Discriminatory Assay		
		Unique Donors*	Reactive/Tested	% Reactive	Unique Donors*	Reactive/Tested	% Reactive
HCV Genotype 1	300	11	66/66	100	11	66/66	100
	100		66/66	100		66/66	100
	30		59/66	89.4		62/66	93.9
HCV Genotype 2	300	12	72/72	100	13	78/78	100
	100		67/72	93.1		73/78	93.6
	30		62/72	86.1		67/78	85.9
HCV Genotype 3	300	12	72/72	100	12	72/72	100
	100		71/72	98.6		69/72	95.8
	30		65/72	90.3		64/72	88.9
HCV Genotype 4	300	14	84/84	100	14	84/84	100
	100		84/84	100		83/84	98.8
	30		81/84	96.4		80/84	95.2
HCV Genotype 5	300	6	36/36	100	6	36/36	100
	100		36/36	100		36/36	100
	30		35/36	97.2		35/36	97.2

HCV Genotype 6	300	5	30/30	100	5	30/30	100
	100		30/30	100		30/30	100
	30		28/30	93.3		29/30	96.7
All Genotypes	300	60	360/360	100	61	366/366	100
	100		354/360	98.3		357/366	97.5
	30		330/360	91.7		337/366	92.1

* Each unique donor was tested in duplicate with three clinical lots of reagents.

Detection of HBV Genotypes with the Procleix Ultrio Assay and HBV Discriminatory Assay

HBV specimens of genotypes A, B, C, D, E, F, and G were quantified for HBV DNA using commercially available quantitative HBV DNA assays. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. Diluted specimens were tested with the Procleix Ultrio Assay and dHBV Assay. Fifty-seven unique specimens were tested in duplicate using three clinical lots on the Procleix System**.** At 300 copies/mL, 337/342 replicates (98.5%) were reactive with the Procleix Ultrio Assay and 337/342 replicates (98.5%) were reactive with the dHBV Assay. At 100 copies/mL, 324/342 replicates (94.7%) were reactive with the Procleix Ultrio Assay and 312/342 replicates (91.2%) were reactive with the dHBV Assay. At 30 copies/mL, 265/342 replicates (77.5%) were reactive with the Procleix Ultrio Assay and 244/342 replicates (71.3%) were reactive with the dHBV Assay (Table 27). Detection rates were calculated from valid initial results.

Table 27. Procleix System - Detection of HBV Genotype with the Procleix Ultrio Assay and HBV Discriminatory Assay

Genotype	Conc. copies/mL	Procleix Ultrio Assay			HBV Discriminatory Assay		
		Unique Donors*	Reactive/ Tested	% Reactive	Unique Donors*	Reactive/ Tested	% Reactive
HBV Genotype A	300	12	71/72	98.6	12	70/72	97.2
	100		70/72	97.2		67/72	93.1
	30		63/72	87.5		57/72	79.2
HBV Genotype B	300	10	60/60	100	10	60/60	100
	100		57/60	95		56/60	93.3
	30		43/60	71.7		36/60	60
HBV Genotype C	300	10	60/60	100	10	59/60	98.3
	100		52/60	86.7		54/60	90
	30		41/60	68.3		41/60	68.3
HBV Genotype D	300	8	45/48	93.8	8	47/48	97.9
	100		46/48	95.8		44/48	91.7
	30		41/48	85.4		35/48	72.9
HBV Genotype E	300	8	47/48	97.9	8	48/48	100
	100		46/48	95.8		40/48	83.3
	30		32/48	66.7		34/48	70.8
HBV Genotype F	300	8	48/48	100	8	47/48	97.9
	100		47/48	97.9		45/48	93.8

	30		39/48	81.3		35/48	72.9
HBV Genotype G	300	1	6/6	100	1	6/6	100
	100		6/6	100		6/6	100
	30		6/6	100		6/6	100
All Genotypes	300	57	337/342	98.5	57	337/342	98.5
	100		324/342	94.7		312/342	91.2
	30		265/342	77.5		244/342	71.3

* Each unique donor was tested in duplicate with three clinical lots of reagents.

G. PERFORMANCE OF THE PROCLEIX ULTRIO ASSAY IN CADAVERIC BLOOD SPECIMENS FROM TISSUE DONORS

REPRODUCIBILITY

The reproducibility of the Procleix Ultrio Assay and Discriminatory Assays with cadaveric blood specimens was assessed on the Procleix System. Plasma containing HIV-1, HCV or HBV was spiked into 20 cadaveric and 20 control specimens (HIV-1 at 200 copies/mL, HCV at 60 IU/mL and HBV at 45 IU/mL); 10 of each were tested in the Procleix Ultrio Assay and the other 10 of each were tested in the Discriminatory Assays. The specimens were tested with two clinical lots. Specimens were tested in three separate runs for each clinical lot, for a total of six runs. The percent positive, analyte S/CO values, and coefficients of variation (%CVs) are shown in Table 28. Detection rates were calculated from valid initial results. The positivity rates ranged from 98% to 100% on the Procleix System. The %CVs ranged from 28% to 32% for HIV-1 spiked cadaveric specimens, 29% to 36% for the HCV spiked cadaveric specimens, and 7% to 8% for the HBV spiked cadaveric specimens.

Table 28. Procleix System - Reproducibility of Procleix Ultrio Assay and Discriminatory Assays in Cadaveric Blood Specimens

Virus	Assay	Sample Type*	# of Donors	# of Replicates	% Positive	Mean Analyte S/CO	%CV
HIV-1	Procleix Ultrio Assay	Cadaveric	10	60	100% (95-100)	14.80	28
		Control	10	60	98% (91-100)	12.05	21
	dHIV-1	Cadaveric	10	57**	98% (91-100)	15.04	32
		Control	10	60	100% (95-100)	18.89	17
HCV	Procleix Ultrio Assay	Cadaveric	10	60	98% (91-100)	5.93	36
		Control	10	60	100% (95-100)	6.83	8
	dHCV	Cadaveric	10	60	98% (91-100)	16.17	29
		Control	10	60	100% (95-100)	21.96	13
HBV	Procleix Ultrio Assay	Cadaveric	10	60	98% (91-100)	13.55	8
		Control	10	60	98% (91-100)	13.51	7
	dHBV	Cadaveric	10	60	100% (95-100)	23.05	7
		Control	10	60	100% (95-100)	23.74	5

CI = Confidence Interval
* Cadaveric specimens included serum and plasma specimens.
** Three specimens with invalid IC, QNS for retest.

SPECIFICITY

Specificity of Procleix Ultrio Assay and Discriminatory Assays in Cadaveric Blood Specimens on the Procleix System

HIV-1, HCV and HBV seronegative cadaveric blood specimens were tested to determine the specificity of the Procleix Ultrio Assay and dHIV-1, dHCV and dHBV Assays. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots on the Procleix System. The specificity of the Procleix Ultrio Assay and dHIV-1 and dHBV Assays for the cadaveric specimens was 100% (95% confidence interval: 94%-100%) for the Procleix System. The specificity of the dHCV Assay for the cadaveric specimens was 98% (95% confidence interval: 89%-100%) (Table 29). Specificity rates were calculated from all valid initial results.

Table 29. Procleix System - Specificity of Procleix Ultrio Assay and Discriminatory Assays in Cadaveric Blood Specimens

		Control	Cadaveric
Procleix Ultrio Assay	Mean IC S/CO	1.94	1.89
	Mean Analyte S/CO	0.05	0.09
	Specificity rate	100%	100%
	95% CI spec. rate	94-100	94-100
	N	50	47
dHIV-1	Mean IC S/CO	2.00	1.99
	Mean Analyte S/CO	0.14	0.11
	Specificity rate	100%	100%
	95% CI spec. rate	94-100	94-100
	N	50	50
dHCV	Mean IC S/CO	2.07	1.98
	Mean Analyte S/CO	0.14	0.21
	Specificity rate	100%	98*%
	95% CI spec. rate	94-100	89-100
	N	50	49
dHBV	Mean IC S/CO	2.01	2.02
	Mean Analyte S/CO	0.11	0.10
	Specificity rate	100%	100%
	95% CI spec. rate	94-100	94-100
	N	50	49

* One initial reactive, QNS to resolve
CI = Confidence Interval
N = number of samples

SENSITIVITY

Sensitivity for Detection of HIV-1 in Cadaveric Blood Specimens on the Procleix System

HIV-1, HCV, and HBV seronegative cadaveric blood specimens spiked with a low level of HIV-1 virus were tested to determine the sensitivity of the Procleix Ultrio Assay and dHIV-1 Assay. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots on the Procleix System after spiking each with approximately 200 copies/mL of HIV-1. The positivity rate of the Procleix Ultrio Assay and dHIV-1 Assay for the cadaveric specimens was 100% (95% confidence interval: 94%-100%) on the Procleix System (Table 30). Detection rates were calculated from valid initial results.

Table 30. Procleix System - Reactivity of Procleix Ultrio Assay and HIV-1 Discriminatory Assay in Cadaveric Blood Specimens Spiked with HIV-1 Virus

		Control	Cadaveric
Procleix Ultrio Assay	Mean IC S/CO	2.36	2.31
	Mean Analyte S/CO	12.85	12.05
	% positive	100	100
	95% CI (% pos)	94-100	94-100
	N	50	50
dHIV-1	Mean IC S/CO	2.12	2.07
	Mean Analyte S/CO	17.01	15.89
	% positive	100	100
	95% CI (% pos)	94-100	94-100
	N	50	50

CI = Confidence Interval
N = number of samples

Sensitivity for Detection of HCV in Cadaveric Specimens on the Procleix System

HIV-1, HCV and HBV seronegative cadaveric blood specimens spiked with a low level of HCV virus were tested to determine the sensitivity of the Procleix Ultrio Assay and dHCV Assay. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots on the Procleix System after spiking each with approximately 200 copies/mL of HCV. The positivity rate of both the Procleix Ultrio Assay and dHCV Assay for the cadaveric specimens was 100% (95% confidence interval: 94%-100%) on the Procleix System (Table 31). Detection rates were calculated from valid initial results.

Table 31. Procleix System - Reactivity of Procleix Ultrio Assay and HCV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HCV Virus

		Control	Cadaveric
Procleix Ultrio Assay	Mean IC S/CO	2.03	1.95
	Mean Analyte S/CO	7.93	7.05
	% positive	100	100
	95% CI (% pos)	94-100	94-100
	N	50	50
dHCV	Mean IC S/CO	2.03	1.91
	Mean Analyte S/CO	22.06	19.35
	% positive	100	100
	95% CI (% pos)	94-100	94-100
	N	50	50

CI = Confidence Interval
N = number of samples

Sensitivity for Detection of HBV in Cadaveric Blood Specimens on the Procleix System

HIV-1, HCV and HBV seronegative cadaveric blood specimens spiked with a low level of HBV virus were tested to determine the sensitivity of the Procleix Ultrio Assay and dHBV Assay. Seventy cadaveric and 70 normal donor specimens were tested using three clinical lots on the Procleix System after spiking each with approximately 30 IU/mL of HBV. The positivity rate of the Procleix Ultrio Assay for the cadaveric specimens was 100% (95% confidence interval: 96%-100%) on the Procleix System. The positivity rate of the dHBV Assay was 98% (95% confidence interval: 92%-100%) for the Procleix System (Table 32). Detection rates were calculated from valid initial results.

Table 32. Procleix System - Reactivity of Procleix Ultrio Assay and HBV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HBV Virus

		Control	Cadaveric*
Procleix Ultrio Assay	Mean IC S/CO	1.66	1.56
	Mean Analyte S/CO	13.39	12.92
	% positive	100	100
	95% CI (% pos)	96-100	96-100
	N	70	70
dHBV	Mean IC S/CO	1.89	1.72
	Mean Analyte S/CO	21.75	21.54
	% positive	100	98
	95% CI (% pos)	96-100	92-100
	N	70	70

CI = Confidence Interval
N = number of samples
* Included serum and plasma specimens

H. REACTIVITY IN SEROCONVERTING DONORS

Commercially available seroconversion panels collected from plasmapheresis donors were tested to determine the ability of the Procleix Ultrio Assay and HIV-1, HCV, and HBV Discriminatory Assays to shorten the window period of HIV-1, HCV, and HBV detection when compared to antigen and/or antibody tests. Two separate studies were performed, each with a different clinical lot. (Tables 33a and 33b). Both studies tested a similar set of HIV-1 (n=10), HCV (n=10), and HBV (n=10) seroconversion panels at one site. Each seroconversion panel was tested with the Procleix Ultrio Assay (either neat and 1:16 diluted in one study or neat and 1:8 diluted in the other study which used development clinical lots) and with the HIV-1 Discriminatory (neat only), HCV Discriminatory (neat only) and HBV Discriminatory (neat only) Assays. The test results were compared with those of the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay for HIV-1 seroconversion panels, with those of the Ortho HCV 3.0 ELISA test for HCV seroconversion panels, or with those of Ortho Antibody to HBsAg ELISA Test System 3 and Abbott PRISM HBsAg Assay for HBV seroconversion panels.

HIV-1 Detection in Seroconversion Panels

The Procleix Ultrio Assay was able to detect HIV-1 RNA a median of 14 and 7 (or 14 and 8 in the second study) days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested neat (Tables 33a and 33b). The Procleix Ultrio Assay was able to detect HIV-1 RNA a median of 11.5 and 4 days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested at a 1:8 dilution. The Procleix Ultrio Assay was able to detect HIV-1 RNA a median of 11 and 5 days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested at a 1:16 dilution. Similar results were observed with the HIV-1 Discriminatory Assay, as compared to the Procleix Ultrio Assay, when specimens were tested neat in both studies.

Table 33a. Procleix System - Comparison to HIV-1 Antibody and Antigen Tests on Seroconversion Panels Study 1 (Number of Days Earlier Detection)

Panel ID	Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay			Coulter HIV-1 p24 Ag Assay		
	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:16)	dHIV-1 (Neat)	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:16)	dHIV-1 (Neat)
60772	12	7	12	7	2	7
61694	11	8	8	6	3	3
62238	14	20	14	7	13	7
62357	11	7	11	4	0	4
65389	14	12	14	7	5	7
65790*	>11	>7	>11	7	3	7
66048	15	12	15	22	19	22
67485	18	14	18	8	4	8
68106*	14	10	14	>46	>42	>46
68582	14	14	14	7	7	7
Median	14	11	14	7	5	7

* Panel did not show Ab or Ag reactivity.

Table 33b. Procleix System - Comparison to HIV-1 Antibody and Antigen Tests on Seroconversion Panels Study 2 (Number of Days Earlier Detection)

Panel ID	Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay			Coulter HIV-1 p24 Ag Assay		
	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:8)	dHIV-1 (Neat)	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:8)	dHIV-1 (Neat)
60772	12	7	7	7	2	2
62357*	11	7	11	4	0	4
63602	16	9	14	9	2	7
64954*	15	13	15	15	13	15
65790*	12	7	12	8	3	8
66575	14	11	11	10	7	7
67485*	14	14	14	4	4	4
68582	14	14	14	7	7	7
66048*	15	12	15	22	19	22
68106*,**	15	15	19	>54	>54	>54
Median	14	11.5	14	8	4	7

* Intermittent Procleix Ultrio Assay reactivity prior to ramp up is not used for this calculation
** Panel did not show Ag reactivity.

HCV Detection in Seroconversion Panels

In both studies the Procleix Ultrio Assay was able to detect HCV RNA a median of 32 days earlier than the Ortho HCV 3.0 ELISA test when specimens were tested neat, at 1:8 dilution, and at 1:16 dilution (Tables 34a and 34b). The HCV Discriminatory Assay was able to detect HCV RNA a median of 32 days and 34.5 days earlier than the Ortho HCV 3.0 ELISA Assay when specimens were tested neat in the two separate studies.

Table 34a. Procleix System - Comparison to Ortho HCV 3.0 ELISA Assay on Seroconversion Panels Study 1 (Number of Days Earlier Detection)

Panel ID	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:16)	dHCV (Neat)
60779	0	Not Reactive*	0
61067	32	32	32
62286	23	23	23
62680**	>22	>22	>22
62804	20	20	20
62886	31	31	31
62999	39	33	64
63318	32	32	32
63625	62	38	38
790989	46	46	46
Median	32	32	32

* Panel did not have a reactive NAT result
** Panel did not show Ab reactivity

Table 34b. Procleix System - Comparison to Ortho HCV 3.0 ELISA Assay ~~s~~on Seroconversion Panels Study 2 (Number of Days Earlier Detection)

Panel ID	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:8)	dHCV (Neat)
790989	44	44	44
61067	27	27	30
60779	0	0	0
62286	23	23	23
62999	33	33	39
63318	52	34	52
62886*	31	31	31
63625	38	38	38
64150*	46	46	46
64273*	29	29	29
Median	32	32	34.5

* Intermittent Procleix Ultrio Assay reactivity prior to ramp up is not used for this calculation.

HBV Detection in Seroconversion Panels

The Procleix Ultrio Assay was able to detect HBV DNA a median of 19 days and 17 (or 18.5 in the second study) days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested neat (Tables 35a and 35b). The Procleix Ultrio Assay was able to detect HBV DNA a median of 11.5 days earlier than the Abbott PRISM HBsAg Assay when specimens were tested at 1:8 dilution. The Procleix Ultrio Assay was able to detect HBV DNA a median of 9 days and 7 days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested at 1:16 dilution. The HBV Discriminatory Assay was able to detect HBV DNA a median of 16 days and 15 (or 17 in the second study) days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested neat.

Table 35a. Procleix System - Comparison to HBV Surface Antigen Tests on Seroconversion Panels (Number of Days Earlier Detection)

Panel ID	Ortho Antibody to HBsAg ELISA Test System 3			Abbott PRISM HBsAg Assay		
	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:16)	dHBV (Neat)	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:16)	dHBV (Neat)
62675	19	17	19	19	17	19
62825	29	29	29	29	29	29
62967	14	5	14	14	5	14
63133	11	9	11	11	9	11
63568	14	11	14	10	7	10
63659	15	0	0	15	0	0
63997	21	7	14	19	5	12
64006	20	8	18	20	8	18
64121	19	0	27	19	0	27
64132	23	14	23	15	6	15
Median	19	9	16	17	7	15

Table 35b. Procleix System - Comparison to Abbott PRISM HBsAg Assay on Seroconversion Panels (Number of Days Earlier Detection)

Panel ID	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:8)	dHBV (Neat)
62825	17	3	17
62347	11	7	11
62967*	10	3	12
64121*	19	19	17
64006*	23	11	23
66201*	21	21	23
67303*	22	12	19
68029	18	16	16
68105	29	29	29
68739*	15	6	15
Median	18.5	11.5	17

* Intermittent Procleix Ultrio Assay reactivity prior to ramp up is not used for this calculation.

7.0 LIMITATIONS OF THE PROCEDURE

This assay has been approved for use with the Procleix System only.

The Procleix Ultrio Assay may not be used to replace antibody-detection tests such as an EIA test for HIV-1, or HCV.
The clinical sensitivity for the Procleix Ultrio Assay has been demonstrated for specimens with HIV-1 or HCV viral RNA concentrations equal to or greater than 100 copies/mL or HBV viral DNA concentrations equal to or greater than 15 IU/mL. Samples with less than these concentrations may not yield reproducible results.

The results of the HBV genotype studies shown in Table 27 indicated equivalent performance of the Discriminatory HBV Assay to the Procleix Ultrio Assay at a concentration of 300 copies/mL, but the Procleix HBV Discriminatory Assay was less sensitive than the Procleix Ultrio Assay at the lower concentrations of 30 and 100 copies/mL. some HBV true positive specimens reactive in Procleix Ultrio Assay individual donation screening may test non reactive by the Procleix HBV Discriminatory Assay.

Assays must be performed and results interpreted according to procedures provided.

Deviation from these procedures, adverse shipping and/or storage conditions, or use of outdated calibrators and/or reagents may produce unreliable results.

Various donor and donation factors were evaluated for interference and cross-reactivity in the assays. A small portion had unexpected results in greater than 5% of the samples tested (Tables 13 to 16).

8.0 Package Inserts

A copy of the package insert is attached:

- IN0166EN-FDA, revision 7