Procleix® HIV-1/HCV Assay

For *In Vitro* Diagnostic Use
1000 Test Kit, 5000 Test Kit

CONTENTS

INTENDED USE

The PROCLEIX® HIV-1/HCV Assay* is a qualitative *in vitro* nucleic acid assay system for the detection of human immunodeficiency virus type 1 and/or hepatitis C virus RNA in plasma 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 specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens.

The assay is intended for use in screening individual donor samples. 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 and blood components, and source plasma. It is also intended for use in testing pools of human plasma comprised of equal aliquots of not more than 16 individual specimens from donors of hematopoietic stem/progenitor cells (HPCs) sourced from bone marrow, peripheral blood or cord blood, and donor lymphocytes for infusion (DLI).[1] This assay is intended to be used in conjunction with licensed tests for detecting antibodies to HIV-1 and HCV.

The PROCLEIX® HIV-1 Discriminatory Assay may be used as an aid in the diagnosis of HIV-1 infection. The PROCLEIX HIV-1/HCV Assay can be considered a supplemental test that confirms HIV-1 infection for specimens that are repeatedly reactive on a licensed donor screening test for antibodies to HIV-1, and reactive on both the PROCLEIX HIV-1/HCV Assay and on the PROCLEIX HIV-1 Discriminatory Assay.

The PROCLEIX HIV-1/HCV Assay can be considered a supplemental test that confirms HCV infection for specimens that are repeatedly reactive on a licensed donor screening test for antibodies to HCV, and reactive on both the PROCLEIX HIV-1/HCV Assay and on the PROCLEIX HCV Discriminatory Assay.

SUMMARY AND EXPLANATION OF THE TEST

Epidemiological studies identified human immunodeficiency virus type 1 (HIV-1) as the etiological agent of acquired immunodeficiency syndrome (AIDS)[2-8] and hepatitis C virus (HCV)[9-14] as the etiological agent for most blood-borne non-A, non-B hepatitis (NANBH). Both viruses are transmitted primarily by exposure to infected blood or blood products, certain body fluids or tissues, and from mother to fetus or child.

Current detection of HIV-1 infection in the blood bank setting is based on serologic screening for anti-viral antibodies by enzyme immunoassay (EIA) with confirmation by supplemental antibody tests such as Western blot or immunofluorescence assays. Although sensitivity of HIV-1 antibody detection has increased in the last few years and sensitive tests for p24 antigen (p24Ag) have been developed and implemented, a window period between infection and detectable serological markers still exists.[15,17,18] The screening of blood with current EIA tests results, on average, in a 22-day seronegative window.[18] Although addition of the p24Ag test allows earlier detection of HIV-1 infection, implementation of the p24Ag test in the U.S. yielded a very modest number of Ab(-)/Ag(+) donors with no significant reduction in the risk of infection. Several studies suggest that addition of nucleic acid-based amplification tests would reduce the window period of detection by 6–11 days, preventing more than half of the HIV-1 infections by blood transfusion.[15]

Nucleic Acid Testing (NAT) of whole blood donations has been in place in the United States since early 1999. Stramer et al., have reported the results of testing small pools of 16, 24, and 128 plasma samples[16,33]

* Developed and manufactured by Gen-Probe Incorporated; distributed by Novartis Vaccines and Diagnostics, Inc.

under IND (Investigational New Drug Application) clearances from the FDA. As of November 2001, the major programs (pooled and individual donation testing) in the U.S. have tested a total of 24.9 million donations for HCV RNA and HIV-1 RNA. A total of 89 donations (1:280,000 screened units) were confirmed to be positive for HCV RNA and negative in serological testing. Similarly, nine HIV-1 RNA positive, serologically negative donations (1:2,767,000) were identified. During this testing period, two specimens were reactive for HIV-1 RNA and p24Ag but nonreactive by HIV-1 antibody testing.

Detection of HCV is based on serologic screening for anti-viral antibodies with enzyme-linked immunosorbent assays (ELISA) or enzyme immunoassays (EIA) and confirmation with a Strip Immunoblot Assay (e.g., CHIRON® RIBA® SIA). Even though the development of these tests has significantly reduced the incidence of post transfusion HCV infection in the U.S., risk of contracting HCV through transfusion still exists.[15,17,18] Recent studies indicate that nucleic acid-based amplification tests for HCV RNA will allow detection of HCV infection approximately 59 days earlier than the current antibody-based tests.[18]

The PROCLEIX® HIV-1/HCV Assay utilizes target amplification nucleic acid probe technology for the detection of HIV-1 and HCV RNA in voluntary blood donors.[19] The assay contains reagents which may be used for simultaneous detection of both viruses or individual viruses, HIV-1 and HCV. All three assays incorporate an Internal Control for monitoring assay performance in each individual specimen.

PRINCIPLES OF THE PROCEDURE

The PROCLEIX® HIV-1/HCV Assay involves three main steps which take place in a single tube: sample preparation; HIV-1 and HCV RNA target amplification by Transcription-Mediated Amplification (TMA)[20]; and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[21]

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

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 RNA sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The PROCLEIX HIV-1/HCV Assay utilizes the TMA method to amplify regions of HIV-1 RNA and of HCV RNA.

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 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 signal by the differential kinetics of light emission from probes with different labels.[22] Internal Control specific amplicon is detected using a probe with rapid emission of light (termed flasher signal). Amplicon specific to HIV-1/HCV is detected using probes with relatively slower kinetics of light emission (termed glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels.[22] When used for the simultaneous detection of HIV-1 and HCV, the PROCLEIX HIV-1/HCV Assay differentiates between Internal Control and combined HIV-1/HCV signals but does not discriminate between individual HIV-1 and HCV signals.

Specimens found to be reactive in the PROCLEIX HIV-1/HCV Assay must be run in individual HIV-1 and HCV Discriminatory Assays to determine if they are reactive for HIV-1, HCV, or both.

The PROCLEIX HIV-1 and HCV Discriminatory Assays utilize the same three main steps as the PROCLEIX HIV-1/HCV Assay (target capture, TMA and HPA); the same assay procedure is followed with one difference: HIV-1-specific or HCV-specific probe reagents are used in place of the PROCLEIX HIV-1/HCV Assay Probe Reagent.

MATERIALS PROVIDED

PROCLEIX® HIV-1/HCV Assay — 1000 Test Kit P/N 301031; 5000 Test Kit P/N 301030

- Internal Control Reagent
- Target Capture Reagent
- Amplification Reagent
- Enzyme Reagent
- Probe Reagent
- Selection Reagent
- PROCLEIX® Negative Calibrator
- PROCLEIX® HIV-1 Positive Calibrator
- PROCLEIX® HCV Positive Calibrator

MATERIALS REQUIRED, SOLD SEPARATELY

PROCLEIX® HIV-1 and HCV Discriminatory Probe Reagents — P/N 301026

- HIV-1 Discriminatory Probe Reagent
- HCV Discriminatory Probe Reagent

PROCLEIX® Assay Fluids — P/N 301116

- Wash Solution
- Oil
- Buffer for Deactivation Fluid

PROCLEIX® Auto Detect Reagents — P/N 301120

- Auto Detect 1
- Auto Detect 2

Disposables

(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)

- Ten-Tube Units (TTUs) — P/N TU0040
- Ten Tip Cassettes — P/N 104578
- Sealing Cards — P/N 102085

Equipment/Software

PROCLEIX® System:

- TECAN GENESIS RSP instrument (front end pipettor)
- PROCLEIX® Assay Software and operator's manual; or PROCLEIX® Worklist Editor software and operator's manual
- PROCLEIX® TCS (target capture system) and operator's manual
- PROCLEIX® HC+ Luminometer, PROCLEIX® System Software, and operator's manual
- Multi-tube Vortex Mixer (Vortexer)
- Water bath

Dedicated fixed or adjustable repeat pipettor capable of delivering 400 µL of Target Capture Reagent with a ± 5% accuracy and a precision of ≤ 5% CV. (Only required for manual sample pipetting method.)

Dedicated single channel pipettor capable of delivering 500 µl of specimen with a ± 5% accuracy and a precision of ≤ 5% CV. (Only required for manual sample pipetting method.)

Other

PROCLEIX®System Quick Reference Guide (PROCLEIX® System QRG)

Any applicable technical bulletins

OTHER MATERIALS AVAILABLE FROM CHIRON FOR USE WITH PROCLEIX® HIV-1/HCV ASSAY

PROCLEIX® HIV-1/HCV Assay Calibrators P/N 301036

HIV-1 Positive Calibrator

HCV Positive Calibrator

Negative Calibrator

PROCLEIX® CPT (Correlated Pipetting Transfer) Pooling Software (Only required for pooling)

The PROCLEIX CPT Pooling Software, used in combination with the TECAN GENESIS RSP pipettor instrument, performs sample scanning and pooling operations that combine aliquots from 16 individual samples into a single Master Pool Tube, which may be used for further testing.

PROCLEIX® Reagent Preparation Incubator (RPI), independent temperature monitor (ITM), and operator's manual

MATERIALS REQUIRED BUT NOT PROVIDED

Eppendorf COMBITIPS repeat pipettor tips (12.5 mL, 5.0 mL, 1.25 mL) or equivalent

Disposable 1000 µL filter tips in rack

Bleach

For use in final concentration of 5% sodium hypochlorite and 0.5% sodium hypochlorite

Bleach alternative (optional). Contact Chiron Technical Support for a list of bleach alternatives and instructions for use.

Sterile, polypropylene conical tubes with sealing caps

Freestanding tubes are recommended in two different sizes (5 mL to 10 mL tube and ≥ 30 mL tube). The tubes must be able to accommodate the diameter of an Eppendorf Repeat pipettor tip

TECAN GENESIS disposable 1000 µL conductive filter tips

TECAN 100 mL reagent troughs

REAGENTS

PROCLEIX® HIV-1/HCV Assay Kit:

P/N 301031 – 1000 Test Kit
P/N 301030 – 5000 Test Kit

Each Kit Contains:	**Number of vials/ Volume per vial**	
Reagent Name	**1000 Test Kit**	**5000 Test Kit**
Internal Control Reagent	**2 x 5 mL**	**10 x 5 mL**
A HEPES buffered solution containing detergent and an RNA transcript		
*Store **unopened reagent** at –15° to –35°C.*		
Target Capture Reagent	**2 x 280 mL**	**10 x 280 mL**
A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles.		
Store at 2° to 8°C. (Do not freeze) Internal Control Reagent must be added to Target Capture Reagent before use in the assay.		
Amplification Reagent	**3 x 32 mL**	**15 x 32 mL**
Primers, dNTPs, NTPs and co-factors in TRIS buffered solution containing PROCLIN 300 as preservative.		
*Store **unopened reagent** at –15° to –35°C.*		
Enzyme Reagent	**2 x 18 mL**	**10 x 18 mL**
MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffered solution containing 0.05% sodium azide as preservative.		
*Store **unopened reagent** at –15° to –35°C.*		
Probe Reagent	**2 x 75 mL**	**10 x 75 mL**
Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.		
*Store **unopened reagent** at –15° to –35°C.*		
Selection Reagent	**2 x 180 mL**	**10 x 180 mL**
Borate buffered solution containing surfactant.		
Store at 15° to 30°C.		
PROCLEIX® Negative Calibrator	**30 x 2 mL**	**90 x 2 mL**
Defibrinated normal human plasma, nonreactive for hepatitis B surface antigen (HBsAg), HIV-1 p24Ag, antibodies to human immunodeficiency virus type 1 (anti-HIV-1) and type 2 (anti-HIV-2), and antibodies to human hepatitis C virus (anti-HCV) when tested by FDA-licensed assays, containing gentamicin and 0.2% sodium azide as preservatives.		
Store at –15° to –35°C.		
PROCLEIX® HIV-1 Positive Calibrator	**30 x 2 mL**	**90 x 2 mL**
Inactivated HIV-1 positive plasma in defibrinated normal human plasma, nonreactive for hepatitis B surface antigen (HBsAg), and antibodies to human hepatitis C virus (anti-HCV) when tested by FDA-licensed assays, containing gentamicin and 0.2% sodium azide as preservatives.		
Store at –15° to –35°C.		

PROCLEIX® HCV Positive Calibrator **30 x 2 mL** **90 x 2mL**

Inactivated HCV positive plasma in defibrinated normal human plasma, nonreactive for hepatitis B surface antigen (HBsAg), and antibodies to human immunodeficiency virus type 1 (anti-HIV-1) and type 2 (anti-HIV-2), when tested by FDA-licensed assays, containing gentamicin and 0.2% sodium azide as preservatives.

C2

Store at –15° to –35°C.

STORAGE INSTRUCTIONS

A. Room temperature is defined as 15° to 30°C.

B. ⚠ The PROCLEIX® HIV-1/HCV Assay Probe Reagent and the Discriminatory Probe Reagents are light sensitive. Protect these reagents from light during storage and preparation for use.

Target Capture Reagent (TCR) is stable when stored unopened at 2° to 8°C until the expiration date. Do not use after expiration date. If a precipitate forms in the Target Capture Reagent during storage, see instructions under REAGENT PREPARATION. DO NOT VORTEX. DO NOT FREEZE Target Capture Reagent.

NOTE: If after removing the TCR from storage at 2° to 8°C, the precipitate is allowed to settle to the bottom of the container, the likelihood of the formation of a gelatinous precipitate is increased substantially.

C. Selection Reagent is stable when stored unopened at room temperature until the expiration date. Do not use after expiration date. Mix thoroughly prior to use.

D. The following reagents are stable when stored unopened at room temperature until the expiration date.

Wash Solution
Oil
Auto Detect 1
Auto Detect 2
Buffer for Deactivation Fluid

Do not use after expiration date.

E. Once opened, Wash Solution, Oil, Selection Reagent, Buffer for Deactivation Fluid, Auto Detect 1 and Auto Detect 2 are stable for 30 days when stored at room temperature.

F. The following reagents are stable when stored unopened at -15° to -35°C until the expiration date:

Internal Control Reagent
Amplification Reagent
Enzyme Reagent
Probe Reagent
PROCLEIX® Negative Calibrator
PROCLEIX® HIV-1 Positive Calibrator
PROCLEIX® HCV Positive Calibrator
PROCLEIX® HIV-1 Discriminatory Probe Reagent
PROCLEIX® HCV Discriminatory Probe Reagent

Do not use after expiration date.

G. After thawing, the Amplification Reagent, Enzyme Reagent, Probe Reagent, HIV-1 Discriminatory Probe Reagent, and HCV Discriminatory Probe Reagent are stable when stored at 2° to 8°C for 30 days. Once completely thawed, these reagents may be kept at room temperature up to 8 hours per 24 hour period while in use, not to exceed 80 hours at room temperature. Do not refreeze Amplification, Enzyme, Probe, HIV-1 and HCV Discriminatory Probe Reagents after the initial thaw.

H. After thawing, Negative, HIV-1 and HCV Positive Calibrators may be kept at room temperature up to 8 hours. These are single use vials and must be discarded after use.

I. After addition of Internal Control Reagent, the working Target Capture Reagent is stable when stored at 2° to 8°C for 30 days and may be kept at room temperature up to 8 hours per 24 hour period while in use, not to exceed 80 hours at room temperature.

J. If precipitate forms in the Wash Solution, Amplification Reagent, Probe Reagent, or HIV-1 and HCV Discriminatory Probe Reagents, warm to 15° to 30°C and mix thoroughly prior to use. See instructions under REAGENT PREPARATION.

K. If precipitate forms in the Selection Reagent during storage, see instructions under REAGENT PREPARATION.

L. Changes in the physical appearance of the reagent supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed (e.g., obvious changes in reagent color or cloudiness apparent with microbial contamination), they should not be used.

PRECAUTIONS

For *In Vitro* Diagnostic Use.

A. Specimens may be infectious. Use Universal Precautions[23,27] when performing the assay. Proper handling and disposal methods should be established according to local, state and federal regulations.[24-26] Only personnel qualified as proficient in the use of the PROCLEIX® HIV-1/HCV Assay, the use of the TECAN GENESIS RSP instrument and/or manual sample/TCR pipetting, and trained in handling infectious materials should perform this type of diagnostic procedure.

B. CAUTION: Some components of this kit contain human blood products. The HIV-1 Positive Calibrator in this kit contains human plasma that is HIV-1 positive and has been heat-treated to inactivate the virus. The HCV Positive Calibrator contains human plasma that is HCV positive and has been heat-treated to inactivate the virus. The Negative Calibrator has been assayed by FDA licensed tests and found non-reactive for the presence of hepatitis B surface antigen (HBsAg), HIV-1 p24Ag and antibodies to HIV-1/-2 and HCV. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All human blood sourced materials should be considered potentially infectious and should be handled with Universal Precautions.[23,27] If spillage occurs, immediately disinfect, then wipe up with a 0.5% (final concentration) sodium hypochlorite solution (diluted bleach) or follow appropriate site procedures. A BLEACH ALTERNATIVE MAY BE USED IN PRE-AMPLIFICATION AREAS ONLY. DO NOT USE BLEACH ALTERNATIVES IN AMPLIFICATION AREAS OR IN AREAS SUSPECTED TO BE CONTAMINATED WITH AMPLIFICATION PRODUCTS.

C. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

D. This product contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

E. Avoid contact of Auto Detect Reagents 1 and 2 with skin, eyes, and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping dry and follow appropriate site procedures.

F. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations.[24,25] Thoroughly clean and disinfect all work surfaces.

G. Use only supplied or specified required disposables.

H. Do not use this kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

I. Avoid microbial and ribonuclease contamination of reagents.

J. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See STORAGE INSTRUCTIONS and REAGENT PREPARATION.

K. Do not combine any assay reagents or fluids without specific instruction.

L. Some reagents of this kit are labeled with risk and safety symbols according to the European Directive 1999/45/EC and should be handled accordingly.

Material Safety Data Sheets are available upon request.

The following reagents contain 0.2% sodium azide as a preservative:

PROCLEIX® Negative Calibrator
PROCLEIX® HIV-1 Positive Calibrator
PROCLEIX® HCV Positive Calibrator

Xn. Harmful
R22/R32/S2
S13/S36/S46

Biological Risk

R22 Harmful if swallowed
R32 Contact with acid liberates very toxic gas
S2 Keep out of reach of children
S13 Keep away from food, drink, and animal feeding stuffs
S36 Wear suitable protective clothing
S46 If swallowed, seek medical advice immediately and show this container or label

M. Refer to PRECAUTIONS in other PROCLEIX Assay package inserts, operator's manuals, and the PROCLEIX® System QRG.

REAGENT PREPARATION

This step should be performed prior to beginning Target Capture in an area that is free of template and amplicon.

1. Warm all reagents to room temperature and mix thoroughly prior to use. A dedicated water bath at room temperature or the PROCLEIX® Reagent Preparation Incubator (RPI) may be used to aid this process. If using the RPI to warm the TCR, Probe Reagents, Enzyme Reagent, and Amplification Reagent, refer to the PROCLEIX® System QRG. Ensure that precipitates are dissolved. Do not use a reagent if precipitate or cloudiness is present. See step 7 for Target Capture Reagent preparation.
2. DO NOT heat Probe Reagent, HIV-1 Discriminatory Probe Reagent or HCV Discriminatory Probe Reagent above 30°C **if** using a water bath. Do not heat Probe Reagents above 35°C if using the RPI. Refer to the PROCLEIX System QRG.
3. Thaw reagents upright.
4. If necessary, thaw Amplification, HIV-1 Discriminatory Probe, HCV Discriminatory Probe, Probe, and Enzyme Reagents at room temperature or at 2° to 8°C. Internal Control, Amplification, HIV-1 Discriminatory Probe, HCV Discriminatory Probe, and Probe Reagents may be mixed by vortexing. Enzyme Reagent should be mixed thoroughly by gentle inversion taking care to avoid excessive foaming. Once completely thawed, these reagents may be kept at room temperature up to 8 hours per 24 hour period while in use. These reagents are stable for 30 days when stored at 2° to 8°C. Record date of thaw (THAW DATE) for Amplification, HIV-1 Discriminatory Probe, HCV Discriminatory Probe, Probe, and Enzyme Reagents in the space provided on the label.
5. Precipitate will form in the HIV-1 Discriminatory Probe, HCV Discriminatory Probe, and Probe Reagent when stored at 2° to 8°C. Probe Reagents may be warmed in a water bath to facilitate dissolution of precipitate, but temperature in the bath should not exceed 30°C. The Probe Reagents may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate if thawing is conducted on the lab bench. Alternatively, use the RPI to thaw the Probe Reagents at an average temperature of 32° ± 2°C, not to exceed 35°C. Refer to the PROCLEIX System QRG. Ensure that precipitates in the Probe Reagents are dissolved. Do not use if precipitate or cloudiness is present.
6. Selection Reagent is stored at room temperature. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls below 15°C, precipitate may form. If precipitate forms in the Selection Reagent during storage, heat at 60° ± 1° C for no more than 45 minutes, shaking the bottle frequently (every 5 to 10 minutes). Once all precipitate has gone back into solution, place the bottle in a room temperature water bath and allow the bottle to equilibrate for at least 1 hour. Alternatively, use the RPI as described in the PROCLEIX System QRG. Do not use the Selection Reagent until it has equilibrated. The Selection Reagent must be at room temperature before use. Do not use if precipitate or cloudiness is present.
7. Prepare working Target Capture Reagent: thaw one vial of Internal Control Reagent at room temperature or 2° to 8°C. Do not use the RPI to thaw Internal Control Reagent. Mix the Internal Control Reagent thoroughly by inversion. Remove Target Capture Reagent (TCR) from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX. After mixing, place the TCR bottle at 22° to 30°C. Approximately every 10 minutes shake the bottle until all precipitate has disappeared. TCR precipitate should normally dissolve in about 30 minutes. Alternatively, use the RPI to thaw the TCR at an average temperature of 32° ± 2°C, not to exceed 35°C. Refer to the PROCLEIX System QRG. If a gel is observed after performing this procedure, a new bottle must be used according to the handling recommendations above. Return the bottle with gel back to 2° to 8°C storage for subsequent use. When the Internal Control Reagent and TCR have reached room temperature, mix TCR thoroughly by inversion. Pour the entire vial of Internal Control Reagent into the TCR bottle. The total time for each of these reagents at room temperature must not exceed 8 hours, in the first 24-hour period. This is now the working Target Capture Reagent. Mix thoroughly. Use the space indicated on the TCR bottle to record the date Internal Control Reagent was added and lot number used (IC LOT). Record the expiration date of the working TCR in the space provided on the label.
8. Thaw calibrators at room temperature. **Do not use the RPI to thaw calibrators.** These are single use vials and must be thawed prior to each run. Once thawed, use calibrators within 8 hours. Mix thoroughly by inversion.
9. Wash Solution is shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution during shipment or during storage when temperatures fall below 15°C. Wash Solution may be incubated in a warm water bath to facilitate dissolution of precipitate. **Do not use the RPI to warm the Wash Solution**. Temperature in the bath should not exceed 30°C. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.
10. Once opened, Wash Solution, Oil, Selection Reagent, Buffer for Deactivation Fluid, Auto Detect 1 and Auto Detect 2 are stable for 30 days when stored at room temperature. Record the date the

reagent was first opened (OPEN DATE) in the space provided on the label.

11. To prepare Deactivation Fluid, mix one part Buffer for Deactivation Fluid with one part 5% sodium hypochlorite. Deactivation Fluid is stable for 30 days when stored at room temperature.

SPECIMEN COLLECTION, STORAGE AND HANDLING

NOTE: Handle all specimens as if they are potentially infectious agents.

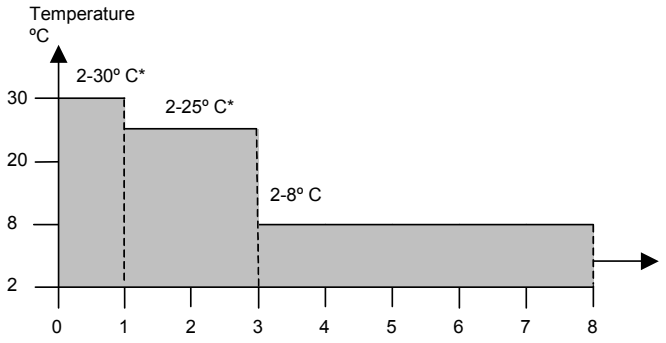
NOTE: Take care to avoid cross-contamination during the sample handling steps. For example, discard used material without passing over open tubes.

Living Donor Blood Specimens

A. Plasma collected in glass or plastic tubes may be used.

B. Plasma collected in K_2EDTA, K_3EDTA or in Becton-Dickinson EDTA Plasma Preparation Tubes (PPT) may be used. Specimen stability is affected by elevated temperature. Whole blood or plasma from pooled or individual donor specimens may be stored for up to 72 hours from time of draw at ≤ 25°C; temperatures not to exceed 30°C are acceptable for no more than 24 hours. Specimens may be stored an additional five days at 2° to 8°C following centrifugation. Plasma separated from the cells may be stored for longer periods of time at ≤ -20°C before testing.

Do not freeze whole blood.



*The 2-30° and 2-25°C periods indicated above may occur at any time.

C. Additional specimens taken from blood or plasma units collected in ACD or sodium citrate according to the collection container manufacturer's instructions may be used. ACD or sodium citrate whole blood or plasma may be stored as in B above.

D. Additional specimens may be taken from whole blood or plasma units containing CPD, CP2D, or CPDA-1 anticoagulants collected according to the collection container manufacturer's instructions. Whole blood (not plasma units) collected in these anticoagulants may be stored for up to 13 days at 2° to 8°C prior to centrifugation. At any time within this 13 day period, the whole blood unit may have been stored for up to one day at 30°C and up to two days at 25°C. Following centrifugation, the plasma may be stored for an additional five days at 2° to 8°C before testing. Plasma separated from the cells may be stored for longer periods of time at ≤ -20°C before testing.

E. No adverse effect on assay performance was observed when plasma was subjected to three freeze-thaw cycles.

F. Mix thawed plasma thoroughly and centrifuge for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed PPT tubes must be validated by the user.

G. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[26]

H. False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.

I. Specimen Pooling

The PROCLEIX® CPT Pooling Software, used in combination with the TECAN GENESIS RSP instrument, performs sample scanning and pooling operations that combine aliquots from 16 individual samples into a single Master Pool Tube, which may be used for further testing.

Cadaveric Blood Specimens

Note: A serum or plasma specimen collected pre-mortem from a non-heart beating (cadaveric) organ/cell/tissue donor may be tested instead of a cadaveric blood specimen using instructions for cadaveric donors.

A. Cadaveric blood specimens can be collected in clot or EDTA anti-coagulant tubes. Follow sample tube manufacturer's instructions.

B. For collection of specimens from cadaveric donors, follow general standards and/or regulations. Specimen stability is affected by elevated temperature.

C. Whole blood (EDTA collection tube) or plasma may be stored for up to 72 hours at 2° to 8°C; temperatures not to exceed 25°C are acceptable for no more than 24 hours. Specimens may be stored an additional 5 days at 2° to 8°C following centrifugation. Long-term storage for plasma at ≤ -20°C has not been established. Do not freeze whole blood.

D. Whole blood (clot tube) or serum may be stored for up to 72 hours at 2° to 8°C; temperatures not to exceed 25°C are acceptable for no more than 24 hours. Specimens may be stored for an additional two days at 2° to 8°C following centrifugation. Long-term storage for serum at ≤ -20°C has not been established. Do not freeze whole blood.

E. No adverse effect on assay performance was observed when plasma and serum were subjected to three freeze-thaw cycles.

F. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000 x g prior to testing. Do not test specimens that do not have sufficient sample volume above the gel separator or red cell interface.

G. Mix thawed plasma or serum thoroughly and centrifuge for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed BD PPT tubes must be validated by the user.

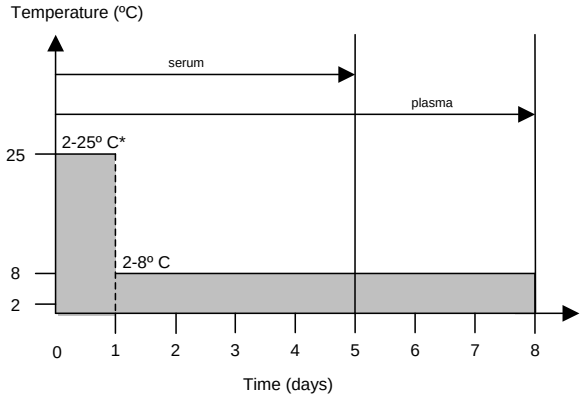
H. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[26]

I. False positive results may occur if cross-contamination of specimens is not adequately controlled during specimen handling and processing.

J. Cadaveric blood specimens may be diluted to overcome potential sample inhibitory substances or specimen shortage. Plasma and/or serum may be diluted 1:5 in saline (0.9% sodium chloride), i.e. 100 µL sample plus 400 µL saline. Diluted specimens should be inverted several times to mix and then may be used in the standard assay procedure by pipetting the 500 µL of the diluted specimen into the TTU containing TCR.

Note: If the TECAN GENESIS RSP instrument will be used to pipette the samples, the minimum volume for the diluted sample should be 1100 µL (220 µL neat sample plus 880 µL saline).

Note: Studies performed to validate these conditions were performed on negative cadaveric specimens spiked with virus. The stability of HIV-1 and HCV *in vivo* post-mortem was not assessed.



*The 2°-25°C period indicated above may occur at any time.

PROCEDURAL NOTES

A. RUN SIZE

When the average run size is 55 tests or more, P/N 301030 should yield 5000 tests per kit. P/N 301031 should yield 1000 tests per kit. Smaller run sizes will result in a lower yield. Each run of up to 100 tests must contain 3 replicates each of the Negative Calibrator, the HIV-1 Positive Calibrator and the HCV Positive Calibrator.

B. EQUIPMENT PREPARATION

1. Three dedicated circulating water baths must be used: one for target capture and pre-amplification (60° ± 1°C), one for amplification (41.5° ± 1°C) and one for hybridization and selection (60° ± 1°C). An additional water bath is required to be maintained at 23° ± 4°C for the step preceding detection.
2. Equilibrate circulating water baths to 60° ± 1°C for target capture and 41.5° ± 1°C for amplification incubations.
3. Prepare the TECAN GENESIS RSP instrument for use according to instructions in the PROCLEIX® System QRG.
4. Prepare the PROCLEIX TCS for use according to instructions in the PROCLEIX System QRG.
5. Wipe work surfaces and pipettors daily with diluted bleach (0.5% sodium hypochlorite in water). Allow bleach to contact surfaces and pipettors for at least 15 minutes and then follow with a water rinse. A BLEACH ALTERNATIVE MAY BE USED IN PRE-AMPLIFICATION AREAS ONLY. DO NOT USE BLEACH ALTERNATIVES IN AMPLIFICATION AREAS OR IN AREAS SUSPECTED TO BE CONTAMINATED WITH AMPLIFICATION PRODUCTS. DO NOT USE DEACTIVATION FLUID ON SURFACES.
6. Equilibrate a circulating water bath to 60° ± 1°C for hybridization and selection incubations. Prepare an additional container of water at 23° ± 4°C for cool down prior to detection.
7. Setup procedures for the PROCLEIX® HC+ Luminometer are given in the PROCLEIX System QRG.

C. REAGENTS

1. Add all reagents using an Eppendorf repeat pipettor (or equivalent) capable of delivering specified volume with ± 5% accuracy and a precision of ≤ 5% CV. Check pipettor functionality monthly and calibrate regularly.
2. To minimize waste of Amplification, Oil, Enzyme, HIV-1 Discriminatory Probe, HCV Discriminatory Probe, Probe, and Selection Reagents, aliquot each reagent for a given run size. Aliquoting must be performed after reagent preparation using sterile, polypropylene conical tubes with sealing caps in an area that is template and amplicon free. The aliquoting area must be wiped down with diluted bleach (0.5% sodium hypochlorite in water) before and after the aliquoting process. A BLEACH ALTERNATIVE MAY BE USED IN PRE-AMPLIFICATION AREAS ONLY. DO NOT USE BLEACH ALTERNATIVES IN AMPLIFICATION AREAS OR IN AREAS SUSPECTED TO BE CONTAMINATED WITH AMPLIFICATION PRODUCTS. The aliquoted reagents must be used the same day the aliquoting was performed. DO NOT store reagents in the aliquot conical tubes.

D. WORK FLOW

1. To minimize the possibility of laboratory areas from becoming contaminated with amplicon, the laboratory area should be arranged with a uni-directional workflow. Proceed from reagent preparation to sample preparation to amplification and then to detection areas. Samples, equipment and reagents should not be returned to the area where a previous step was performed. Also, personnel may not move from the dedicated HPA area back into previous work areas without proper anti-contamination safeguards.
2. Perform reagent preparation in a template free area.
3. Perform Target Capture and Pre-Amplification steps in an amplicon-free area.
4. Perform Hybridization Protection Assay in an area separate from the reagent preparation and amplification areas.

E. TEMPERATURE

1. The Target Capture, Amplification, Hybridization and Selection reactions are temperature dependent. Therefore, it is imperative that the water baths are maintained within the specified temperature range. Use a calibrated thermometer.
2. Room temperature is defined as 15**°** to 30**°**C.
3. Detection is sensitive to temperature. The laboratory temperature in the detection area must be 21**°** to 27**°**C.
4. The operational conditions of the room in which the RPI runs must be within a temperature of 15° to 25°C.

F. TIME

The Target Capture, Amplification, and Hybridization Protection Assay steps are all time dependent. Adhere to specific times outlined in INSTRUCTIONS FOR USE. Use a calibrated timer.

G. VORTEXING

Proper vortexing is important to the successful performance of the PROCLEIX® HIV-1/HCV Assay. Vortex equipment speed settings may vary. Start the vortexer at low speed and then adjust upward to allow reaction mixture to reach and maintain a height within the upper half of all tubes. The reaction mixture should never touch the sealing cards. **It is critical to have a homogeneous mixture after the additions of the HIV-1 Discriminatory Probe, HCV Discriminatory Probe, or Probe Reagent and Selection Reagent.**

H. PIPETTING

1. All pipettors used in the Amplification and HPA steps must be dedicated. All pipettors used for manual pipetting in the Target Capture steps must be dedicated.
2. Take care to deliver reagents, excluding working TCR, to each tube without inserting pipette tip into the tube or touching the rim of the tube to minimize the chance of carryover from one tube to another.

I. MANUAL SPECIMEN PIPETTING

1. When using the manual sample/TCR pipetting method, improper pipetting technique will affect the results of the assay. See PROCEDURAL NOTES, Section H. In order to avoid the loss of Positive ID Tracking, verification of correct sample ID by a second individual is recommended.

2. Ensure that the TTU is oriented in the rack with the pointed end on the left side and the rounded end on the right side of the rack. Pipette the first calibrator into the first tube next to the pointed end of the TTU. Samples are pipetted from left to right.
3. Use a new pipette tip for each sample and dispose of the tip in a biological waste container after use. Take care to avoid cross-contamination by pipetting the specimens and discarding the used pipette tips without passing over open tubes or touching laboratory surfaces or other pieces of equipment.
4. To avoid the risk of contamination, clean and decontaminate manual sample pipettors between assay runs.
5. Ensure proper sample placement into the correct TTU position as indicated on the manual work list record.

J. DECONTAMINATION

1. The extremely sensitive nature of the test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces, and pipettes must be decontaminated daily with diluted bleach (0.5% sodium hypochlorite in water). Allow bleach to contact surfaces for at least 15 minutes and then follow with a water rinse. Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment to avoid pitting.
2. A BLEACH ALTERNATIVE MAY BE USED IN PRE-AMPLIFICATION AREAS ONLY. DO NOT USE BLEACH ALTERNATIVES IN AMPLIFICATION AREAS OR IN AREAS SUSPECTED TO BE CONTAMINATED WITH AMPLIFICATION PRODUCTS.
3. Reactions must be decontaminated with Deactivation Fluid as described in the detection procedure.

K. SEALING CARDS

1. When applying sealing cards, cover the TTUs with the sealing card and press gently to ensure complete contact with all of the tubes. Always use a new sealing card. DO NOT reuse sealing cards.
2. When removing sealing cards, carefully lift and peel in one continuous motion to avoid aerosols and cross contamination. Immediately dispose of card in appropriate waste container.

INSTRUCTIONS FOR USE

PROCLEIX® HIV-1/HCV Assay on individual donor specimens or on pooled specimens

All specimens (individual donations or pooled specimens) should be run in singlet in the initial PROCLEIX HIV-1/HCV Assay.

Specimens from other living donors (except whole blood, blood components, source plasma, or HPC) and from cadaveric donors must be tested neat using the individual donor testing method only. If the initial test result from a cadaveric blood specimen is invalid, the specimen may be diluted to overcome potential inhibitory substances as described in Specimen Collection, Storage and Handling, Cadaveric Blood Specimens, and retested in singlet.

PROCLEIX® HIV-1/HCV Assay Calibrators and Discriminatory Probe Reagents are to be used with the corresponding master lot of the PROCLEIX HIV-1/HCV Assays. The operator must check to ensure that the PROCLEIX HIV-1/HCV Assay Calibrators and Discriminatory Probe Reagents are used with the corresponding master lot of kit reagents as indicated on the master lot sheet in use.

To run the PROCLEIX HIV-1/HCV Assay for the detection of HIV-1 and HCV RNA, follow the steps below for Target Capture, Amplification and Hybridization Protection Assay. To run the PROCLEIX HIV-1/HCV Assay for discrimination between HIV-1 and HCV RNA, see Section D, below, prior to proceeding.

NOTE: Continuous Process Flow:
All process steps described below are intended to be completed in a continuous flow with a minimal, if any, delay between steps.

A. TARGET CAPTURE

The PROCLEIX HIV-1/HCV Assay has been validated using the TECAN GENESIS RSP 150/8 Pipettor. The use of manual pipetting requires additional operator training and demonstration of proficiency. Repeat pipettors used in this step must be dedicated for use only in the TARGET CAPTURE steps.

IF USING THE TECAN GENESIS RSP 150/8 PIPETTOR:

1. Start the PROCLEIX Assay Software. Refer to the PROCLEIX® System QRG for software operating instructions.
2. Place TTU Carriers on the TECAN GENESIS RSP deck according to the deck layout indicated on the screen. Load sufficient Ten Tube Units (TTUs) for the run into TTU Carriers.
3. Mix working Target Capture Reagent thoroughly to resuspend microparticles. This is important before putting into the TECAN GENESIS RSP reagent trough. Put sufficient working Target Capture Reagent into the reagent trough and place on the TECAN GENESIS RSP deck as indicated on the deck layout screen. If pipetting can not be completed within 2 hours, remix prior to use.
4. Place Calibrators and samples into TECAN 16-Tube Strip Racks. Place TECAN 16-Tube Strip Racks on the TECAN GENESIS RSP deck according to the deck layout indicated on the screen.
5. Reference the PROCLEIX® System QRG for instructions on pipetting. The TECAN GENESIS RSP instrument will read bar codes of all carriers, TTUs, Calibrators, and samples. The TECAN GENESIS RSP instrument will pipette 400 µL of working TCR into each reaction tube and then pipette 500 µL each of calibrators and test samples into assigned reaction tubes. An electronic work list will be created.
6. When all samples have been pipetted, transfer the TTUs to a TTU rack. Cover the TTUs with sealing cards. See PROCEDURAL NOTES.
7. Vortex the rack of TTUs a minimum of 20 seconds and until magnetic microparticles are resuspended. See PROCEDURAL NOTES.
8. Rack may remain at room temperature up to 75 minutes prior to proceeding to the 60°C ± 1°C incubation.
9. Incubate the tubes in a water bath at 60° ± 1°C for 20 minutes ± 1 minute.
10. Remove the rack of TTUs and transfer to target capture area.
11. Incubate the rack of TTUs on the lab bench at room temperature for 14 minutes to 20 minutes.
12. Transfer the rack of TTUs to the PROCLEIX TCS separation bay for 9 to 20 minutes.
13. Carefully remove and dispose of the sealing cards. See PROCEDURAL NOTES.
14. Aspirate the solution from each tube according to the PROCLEIX System QRG.
15. Add 1 mL of Wash Solution to each tube. Cover the TTUs with sealing cards. See PROCEDURAL NOTES. Remove the rack of TTUs from the PROCLEIX TCS separation bay and vortex to resuspend the microparticle pellets. See PROCEDURAL NOTES.
16. Place the rack of TTUs on the PROCLEIX TCS separation bay for 4 to 10 minutes.
17. Carefully remove and dispose of the sealing cards. See PROCEDURAL NOTES.
18. Aspirate the solution from each tube according to the PROCLEIX System QRG.
19. Add 1 mL of Wash Solution to each tube. Cover the TTUs with sealing cards. Remove the rack of TTUs from the Target Capture System separation bay and vortex to resuspend the microparticle pellets. See PROCEDURAL NOTES.
20. Place the rack of TTUs on the PROCLEIX TCS separation bay for 4 to 10 minutes.

21. Carefully remove and dispose of the sealing cards. See PROCEDURAL NOTES.
22. Completely aspirate the solution from each tube according to the PROCLEIX System QRG. Cover the TTUs with a sealing card.
23. Remove the rack of TTUs from the PROCLEIX TCS separation bay and proceed directly to Amplification.

IF USING THE MANUAL SAMPLE PIPETTING METHOD:

The assay results within the run report will be marked "M" indicating that the specimens were manually pipetted.

1. For sample tracking, an electronic worklist must be created using the PROCLEIX Worklist Editor software. Refer to the PROCLEIX System QRG for instructions. Verification of correct sample ID on the worklist with the specimen tubes and with the detailed assay run report by a second individual is recommended.
2. Load sufficient Ten Tube Units (TTUs) for the run into a TTU rack.
3. Thoroughly mix working TCR immediately before use to resuspend microparticles.
4. Refer to the worklist and carefully pipette 400 µL of working TCR to each reaction tube that will contain a specimen. **To dispense, insert the tip approximately one quarter of the way into the tube at an angle and pipette working TCR down the side of the tube. Always pipette the working TCR first, followed by the specimen.**
5. Pipette specimens.
    a. Refer to the worklist to identify the TTU number with the corresponding calibrator and test specimen identification numbers.
    b. Aspirate 500 µL of each calibrator, external quality control or test sample from its collection tube using a single channel pipettor with corresponding filtered disposable tip. Insert only the end of the pipette tip into the specimen. Do not disturb the sediment, if any.
    c. To dispense, insert the pipette tip halfway into the tube taking care not to touch the sides of the upper half of the tube with the pipette tip. At an angle, pipette the specimen down the side of the bottom half of the tube. Hold down the plunger of the pipettor while removing it from the tube. Take care to avoid touching the rim or the side of the tube with the pipette tip when removing it from the tube.
6. Replace pipette tip with a new tip and repeat Step 5 until all specimens have been pipetted.
7. Visually inspect tubes to ensure proper specimen volume and working TCR volume have been dispensed.
8. Cover the TTUs with sealing cards. See PROCEDURAL NOTES. Proceed to Step 7 of section titled "If Using the TECAN GENESIS RSP 150/8 Pipettor", above.

B. AMPLIFICATION

The repeat pipettors used in this step must be dedicated for use only in AMPLIFICATION steps. ***DO NOT USE BLEACH ALTERNATIVES IN THIS AREA.***

1. Carefully remove and dispose of the sealing cards. See PROCEDURAL NOTES.
2. Deliver 75 µL of Amplification Reagent to the bottom of each tube using the dedicated repeat pipettor. Take care to deliver the reagent to the bottom of each tube without inserting the pipette tip into the tube or touching the rim of the tube.
3. Add 200 µL of Oil to each reaction tube using the dedicated repeat pipettor. Angle the pipette tip toward the sides of the tubes, not straight to the bottom, to avoid splashback.
4. Cover the TTUs with sealing cards. See PROCEDURAL NOTES.
5. Vortex the rack of TTUs a minimum of 20 seconds and until all microparticles are resuspended. Ensure that magnetic particles are no longer adhering to the walls of the tube, and are evenly dispersed in the aqueous phase.
6. Incubate the TTUs in a water bath at 60° ± 1°C for 10 minutes ± 1 minute, then at 41.5° ± 1°C for 9 to 20 minutes.
7. Leaving the rack of TTUs at 41.5° ± 1°C, carefully remove and dispose of the sealing cards. See PROCEDURAL NOTES. Proceed immediately to enzyme addition. Add 25 µL of the Enzyme Reagent into each tube using the dedicated repeat pipettor. Take care to deliver the reagent to the bottom of each tube without inserting the pipette tip into the tube or touching the rim of the tube. Place new sealing cards over the TTUs. See PROCEDURAL NOTES. Remove the rack of TTUs from the water bath and shake to mix. DO NOT VORTEX. Minimize the time the tubes are out of the water bath.
8. Incubate the rack of TTUs in the water bath at 41.5° ± 1°C for 60 minutes ± 5 minutes.
9. Remove the rack of TTUs from the water bath and transfer it to the Hybridization Protection Assay area. Rack may remain at room temperature for up to 30 minutes prior to the addition of Probe Reagent.

C. HYBRIDIZATION PROTECTION ASSAY (HPA)

The repeat pipettor used in this step must be dedicated for use only in HYBRIDIZATION PROTECTION ASSAY.

A separate, dedicated location for the Hybridization Protection Assay (HPA) step is recommended to minimize amplicon contamination in the assay. This dedicated area should be on a separate bench in a separate area from the reagent and sample preparation and amplification areas. **DO NOT USE BLEACH ALTERNATIVES IN THIS AREA.**

1. Hybridization
    a. Carefully remove and dispose of the sealing cards. See PROCEDURAL NOTES.
    b. Add 100 µL of Probe Reagent into each tube using the dedicated repeat pipettor. Take care to deliver the reagent to the bottom of each tube without inserting the pipette tip into the tube or touching the rim of the tube. Angle the pipette tip toward the sides of the tubes, not straight to the bottom, to avoid splashback.
    c. Cover the TTUs with sealing cards. See PROCEDURAL NOTES.
    d. Vortex the rack of TTUs a minimum of 20 seconds and until a homogeneous solution is achieved. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. See PROCEDURAL NOTES.
    e. Incubate the rack of TTUs in a dedicated water bath at 60° ± 1°C for 15 minutes ± 1 minute.
2. Selection
    a. Remove the rack of TTUs from the 60° ± 1°C water bath. Carefully remove and dispose of the sealing cards. See PROCEDURAL NOTES.
        (1) Add 250 µL of Selection Reagent to each tube using a repeat pipettor. Take care to deliver the reagent to the bottom of each tube without inserting the pipette tip into the tube or touching the rim of the tube. Angle the pipette tip toward the sides of the tubes, not straight to the bottom, to avoid splashback.
        (2) Cover the TTUs with sealing cards. See PROCEDURAL NOTES. Vortex the rack of TTUs a minimum of 20 seconds and until a homogeneous solution is achieved. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. See PROCEDURAL NOTES.

(3) Return the rack of TTUs to the 60° ± 1°C water bath for 10 minutes ± 1 minute.

(4) Remove the rack of TTUs from the 60° ± 1°C water bath.

(5) Cool the rack of TTUs in a 23° ± 4°C container of water for a minimum of 10 minutes while preparing for Detection (step 3a).

(6) Remove the rack of TTUs from the 23° ± 4°C container of water onto absorbent material.

3. Detection

a. Prepare the PROCLEIX® HC+ Luminometer for operation as indicated in the PROCLEIX System QRG. Ensure that there are sufficient volumes of Auto Detect 1 and Auto Detect 2 to complete the tests.

b. Select the "HIV-1-HCV" assay protocol from the PROCLEIX System Software menu.

c. Carefully remove and dispose of the sealing cards. See PROCEDURAL NOTES.

d. Before transferring TTUs to the luminometer, wipe the outside of the tubes using an absorbent tissue dampened with deionized water. This will ensure that no residue is present on the outside of the tubes and will help reduce static electricity that may affect luminometer readings.

e. Transfer TTUs to the luminometer according to the software instructions. Note: Tube reading should be completed within 75 minutes after completing the selection reaction. See step 2a(4) in Selection procedure.

f. When the analysis is complete, remove the TTUs from the luminometer.

g. After removing the TTUs from the luminometer, add at least 1 mL Deactivation Fluid to each tube. Allow to sit at room temperature for at least 30 minutes before disposing the contents of the tubes. This will help to prevent contamination of the laboratory environment with amplicon.

h. TTU racks should be decontaminated by complete immersion in diluted bleach (0.5% sodium hypochlorite in water) for a minimum of 15 minutes. The bleach should then be rinsed off with water and the rack may be allowed to air dry or may be wiped dry.

D. PROCLEIX HIV-1 AND HCV DISCRIMINATORY ASSAYS

1. All reactive individual specimens should be run in singlet in the HIV-1 and/or HCV Discriminatory Assays.
2. To perform the discriminatory assays, make the following modifications to the procedure above:

a. Perform all TARGET CAPTURE and AMPLIFICATION steps exactly as they are outlined above. Set up separate runs for HIV-1 and HCV Discriminatory Assays. Both Discriminatory Assays use the same Calibrators that are used in the PROCLEIX HIV-1/HCV Assay.

b. Substitute HIV-1 or HCV Discriminatory Probe Reagent for Probe Reagent, in step C.1.b, when performing HYBRIDIZATION PROTECTION ASSAY.

c. Choose the appropriate PROCLEIX System Software protocol: "dHIV-1" for HIV-1 DISCRIMINATORY ASSAY or "dHCV" for HCV DISCRIMINATORY ASSAY, in step C.3.b, when performing the HYBRIDIZATION PROTECTION ASSAY.

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX® HIV-1/HCV ASSAY AND PROCLEIX® HIV-1 AND HCV DISCRIMINATORY ASSAYS

Run Validity Criteria

A. A run is valid if the minimum number of calibrators is valid and calibrators meet acceptance criteria (see II.A. below). In a run, no more than 2 of the 9 calibrators may be invalid. The PROCLEIX System Software will invalidate a run if more than 2 calibrators are invalid in a run. Cutoff values will be calculated for Internal Control (flasher) and Analyte (glower) in valid runs (see II.A. below). For Positive Calibrators or samples which are reactive for Analyte (glower signal), an Internal Control signal below the cutoff is not used to invalidate the result. All specimens in an invalid run are to be retested, except as noted in step I.B. below.

B. For each run, an alert prints on the run report when more than 10% of the calibrators and specimens in a run are invalid (see the PROCLEIX® System QRG for details). Specimens that are invalid solely due to insufficient sample or wTCR are not included in the calculation of the 10% invalid rate.

C. An assay run or an individual sample may be invalidated by an operator if specific technical/operator/instrument difficulties were observed and documented. If individual samples in a run are invalidated by an operator, then the percent invalid rate must be manually calculated.

D. For runs that exceed the 10% invalid rate, further evaluation of the run is recommended. Review package insert procedures to identify operator errors. In addition, the run report should be reviewed using the criteria described below:

1. If the invalid specimens are all from the same TTU, those specimens contributing to the 10% invalid rate may have been inadequately washed, or erroneous reagent addition may have occurred. All nonreactive and invalid specimens in the affected TTU must be repeated.
2. If the invalid specimens are randomly located throughout the run, a specific cause that explains the invalid results can be identified, and the remaining valid results have consistent Internal Control RLU values, only the individual invalid specimens must be retested.
3. If the invalid specimens are randomly located throughout the run, and no specific cause can be identified, all of the nonreactive and invalid specimens in the run must be retested.

Note: Specimens with an overall interpretation of Reactive, as determined by the software, must become the test of record. The specimens should be resolved according to the resolution algorithm for the reactive specimens, as explained in the Interpretation of Results section.

II. ACCEPTANCE CRITERIA FOR THE CALIBRATION AND CALCULATION OF CUTOFF

A. PROCLEIX® HIV-1/HCV Assay

Negative Calibrator Acceptance Criteria

Each individual Negative Calibrator (NC) must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 300,000 RLU. Each individual Negative Calibrator must also have an Analyte value less than or equal to 40,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid due to an IC value or an Analyte value outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator values (NC_x) for Internal Control [NC_x (Internal Control)].

Example:

Negative Calibrator	Internal Control Relative Light Units
1	124,000
2	126,000
3	125,000
Total Internal Control RLU =	375,000

$$NC_x \text{ (Internal Control)} = \frac{\text{Total Internal Control RLU}}{3} = 125{,}000$$

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)].

Example:

Negative Calibrator	Analyte Relative Light Units
1	14,000
2	16,000
3	15,000
Total Analyte RLU =	45,000

$$NC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 15{,}000$$

HIV-1 Positive Calibrator Acceptance Criteria

Individual HIV-1 Positive Calibrator (PC) Analyte values must be less than or equal to 1,800,000 RLU and greater than or equal to 300,000 RLU. If one of the HIV-1 Positive Calibrator values is outside these limits, the HIV-1 Positive Calibrator mean (HIV-1 PC_x) will be recalculated based upon the two acceptable HIV-1 Positive Calibrator values. The run is invalid and must be repeated if two or more of the three HIV-1 Positive Calibrator Analyte values are outside of these limits. IC values may not exceed 475,000 RLU.

Determination of the mean of the HIV-1 Positive Calibrator (HIV-1 PC_x) values for Analyte [HIV-1 PC_x (Analyte)].

Example:

HIV-1 Positive Calibrator	Analyte Relative Light Units
1	690,000
2	700,000
3	710,000
Total Analyte RLU =	2,100,000

$$\text{HIV-1 } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 700{,}000$$

HCV Positive Calibrator Acceptance Criteria

Individual HCV Positive Calibrator Analyte values must be less than or equal to 900,000 RLU and greater than or equal to 200,000 RLU. If one of the HCV Positive Calibrator values is outside these limits, the HCV Positive Calibrator mean (HCV PC_x) will be recalculated based upon the two acceptable HCV Positive Calibrator values. The run is invalid and must be repeated if two or more of the three HCV Positive Calibrator Analyte values are outside these limits. IC values may not exceed 475,000 RLU.

Determination of the mean of the HCV Positive Calibrator (HCV PC_x) values for Analyte [HCV PC_x (Analyte)].

Example:

HCV Positive Calibrator	Analyte Relative Light Units
1	350,000
2	360,000
3	340,000
Total Analyte RLU =	1,050,000

$$\text{HCV } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 350{,}000$$

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 x [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 x (125,000)

Internal Control Cutoff Value = 62,500 RLU

Calculation of the HIV-1/HCV Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.02 x HIV-1 PC_x (Analyte)]+ [0.04 x HCV PC_x (Analyte)]

Using values given in the Negative Calibrator and Positive Calibrator examples above:

Analyte Cutoff Value = 15,000 + (0.02 x 700,000) + (0.04 x 350,000)

Analyte Cutoff Value = 43,000 RLU

Summary of Acceptance Criteria for PROCLEIX® HIV-1/ HCV Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 300,000 RLU
HIV-1 Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU
HCV Positive Calibrator	
Analyte	≥ 200,000 and ≤ 900,000 RLU
Internal Control	≤ 475,000 RLU

Summary of Cutoff Calculations for PROCLEIX® HIV-1/HCV Assay

Analyte Cutoff =	NC Analyte Mean RLU + 0.02 x (HIV-1 PC Analyte Mean RLU) + 0.04 x (HCV PC Analyte Mean RLU)
Internal Control Cutoff =	0.5 x (Negative Calibrator IC Mean RLU)

B. PROCLEIX® HIV-1 Discriminatory Assay

Negative Calibrator Acceptance Criteria

Each individual Negative Calibrator (NC) must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 300,000 RLU. Each individual Negative Calibrator must also have an Analyte value less than or equal to 40,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid due to an IC value or Analyte value that is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more

of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator (NC_x) values for Internal Control [NC_x (Internal Control)].

Example:

Negative Calibrator	Internal Control Relative Light Units
1	124,000
2	126,000
3	125,000
Total Internal Control RLU =	375,000

$$NC_x \text{ (Internal Control)} = \frac{\text{Total Internal Control RLU}}{3} = 125{,}000$$

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)].

Example:

Negative Calibrator	Analyte Relative Light Units
1	12,000
2	11,000
3	13,000
Total Analyte RLU =	36,000

$$NC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 12{,}000$$

HIV-1 Positive Calibrator Acceptance Criteria

Individual HIV-1 Positive Calibrator (PC) Analyte values must be less than or equal to 1,800,000 RLU and greater than or equal to 300,000 RLU. If one of the HIV-1 Positive Calibrator values is outside these limits, the HIV-1 Positive Calibrator mean (HIV-1 PC_x) will be recalculated based upon the two acceptable HIV-1 Positive Calibrator values. The run is invalid and must be repeated if two or more of the three HIV-1 Positive Calibrator Analyte values are outside these limits. IC values may not exceed 475,000 RLU.

Determination of the mean of the HIV-1 Positive Calibrator (HIV-1 PC_x) values for Analyte [HIV-1 PC_x (Analyte)].

Example:

HIV-1 Positive Calibrator	Analyte Relative Light Units
1	690,000
2	700,000
3	710,000
Total Analyte RLU =	2,100,000

$$\text{HIV-1 } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 700{,}000$$

HCV Positive Calibrator Acceptance Criteria

In the HIV-1 Discriminatory Assay, each individual HCV Positive Calibrator must have Analyte values less than or equal to 40,000 RLU and greater than or equal to 0 RLU. Each HCV Positive Calibrator must also have IC values greater than or equal to 75,000 RLU and less than or equal to 300,000 RLU. The run is invalid and must be repeated if two or more of the three calibrator values have IC values or Analyte values that are outside these limits.

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 x [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 x (125,000)

Internal Control Cutoff Value = 62,500 RLU

Calculation of the Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.04 x HIV-1 PCx (Analyte)]

Using values given in the Negative Calibrator and HIV-1 Positive Calibrator examples above:

Analyte Cutoff Value = 12,000 + (0.04 x 700,000)

Analyte Cutoff Value = 40,000 RLU

Summary of Acceptance Criteria for the PROCLEIX® HIV-1 Discriminatory Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 300,000 RLU
HIV-1 Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU
HCV Positive Calibrator*	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 300,000 RLU

*** Note that the HCV Positive Calibrator performs similarly to the Negative Calibrator in the HIV-1 Discriminatory Assay.**

Summary of Cutoff Calculations for the PROCLEIX® HIV-1 Discriminatory Assay

Analyte Cutoff =	NC Analyte Mean RLU + 0.04 x (HIV-1 PC Analyte Mean RLU)
Internal Control Cutoff =	0.5 x (Negative Calibrator IC Mean RLU)

C. PROCLEIX® HCV Discriminatory Assay

Negative Calibrator Acceptance Criteria

Each individual Negative Calibrator must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 300,000 RLU. Each individual Negative Calibrator must also have an Analyte value less than or equal to 40,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid or an IC or Analyte value is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator values (NC_x) for Internal Control [NC_x (Internal Control)].

Example:

Negative Calibrator	Internal Control Relative Light Units
1	124,000
2	126,000
3	125,000
Total Internal Control RLU =	375,000

$$NC_x \text{ (Internal Control)} = \frac{\text{Total Internal Control RLU}}{3} = 125{,}000$$

Determination of the Analyte mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)].

Example:

Negative Calibrator	Analyte Relative Light Units
1	20,000
2	22,000
3	18,000
Total Analyte RLU =	60,000

$$NC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 20{,}000$$

HIV-1 Positive Calibrator Acceptance Criteria

In the HCV Discriminatory Assay, each individual HIV-1 Positive Calibrator must have Analyte values less than or equal to 40,000 RLU and greater than or equal to 0 RLU. Each HIV-1 Positive Calibrator must also have IC values greater than or equal to 75,000 RLU and less than or equal to 300,000 RLU. The run is invalid and must be repeated if two or more of the three calibrator values have IC values or Analyte values that are outside of these limits.

HCV Positive Calibrator Acceptance Criteria

Individual HCV Positive Calibrator value must be less than or equal to 2,700,000 RLU and greater than or equal to 400,000 RLU. If one of the HCV Positive Calibrator values is outside these limits, the HCV Positive Calibrator mean (HCV PC_x) will be recalculated based upon the two acceptable HCV Positive Calibrator values. The run is invalid and must be repeated if two or more of the three HCV Positive Calibrator values are outside these limits. IC values may not exceed 475,000 RLU.

Determination of the mean of the HCV Positive Calibrator (HCV PC_x) values for Analyte [HCV PC_x (Analyte)].

Example:

HCV Positive Calibrator	Analyte Relative Light Units
1	900,000
2	1,000,000
3	1,100,000
Total Analyte RLU =	3,000,000

$$\text{HCV } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 1{,}000{,}000$$

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 x [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 x (125,000)

Internal Control Cutoff Value = 62,500 RLU

Calculation of the Analyte Cutoff Value

Analyte Cutoff Value = NC_x(Analyte) + [0.04 x HCV PC_x(Analyte)]

Using values given in the Negative Calibrator and HCV Positive Calibrator examples above:

Analyte Cutoff Value = 20,000 + (0.04 x 1,000,000)

Analyte Cutoff Value = 60,000 RLU

Summary of Acceptance Criteria for the PROCLEIX® HCV Discriminatory Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 300,000 RLU
HIV-1 Positive Calibrator*	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 300,000 RLU
HCV Positive Calibrator	
Analyte	≥ 400,000 and ≤ 2,700,000 RLU
Internal Control	≤ 475,000 RLU

*** Note that the HIV-1 Positive Calibrator performs similarly to the Negative Calibrator in the HCV Discriminatory Assay.**

Summary of Cutoff Calculations for the PROCLEIX® HCV Discriminatory Assay

Analyte Cutoff =	NC Analyte Mean RLU + 0.04 x (HCV PC Analyte Mean RLU)
Internal Control Cutoff =	0.5 x (Negative Calibrator IC Mean RLU)

INTERPRETATION OF RESULTS

All calculations described above are performed by the PROCLEIX® System Software. Two cutoffs are determined for each assay: one for the Analyte signal (glower signal) termed the Analyte Cutoff and one for the Internal Control signal (flasher signal) termed the Internal Control Cutoff. The calculation of these cutoffs is shown above. For each sample, an Analyte signal RLU value and Internal Control signal RLU value is determined. Analyte signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/Cutoff (S/CO) on the report.

For a sample with Analyte signal less than the Analyte Cutoff (i.e., Analyte S/CO < 1), the Internal Control (IC) signal must be greater than or equal to the Internal Control Cutoff (IC Cutoff) for the result to be valid. In this case the Internal Control result will be reported as **Valid** and the sample is reported as **Nonreactive**. For a sample with the Analyte signal less than the Analyte Cutoff (i.e., Analyte S/CO < 1) and the Internal Control signal less than the Internal Control Cutoff, the Internal Control Result will be reported as **Invalid** and the sample result is reported as **Invalid**. For all samples, the Internal Control signal may not exceed 475,000 RLU. The sample will automatically be reported as **Invalid** with the PROCLEIX System Software.

Cadaveric blood specimens, when tested neat, may be invalid due to inhibitory substances within the specimen. These invalid samples may be diluted as in, Cadaveric Blood Specimens, Section J, and retested in singlet.

Summary of Sample Validity:

Sample Interpretation	Internal Control Result	
Nonreactive	Valid	Analyte S/CO < 1 and IC ≥ IC Cutoff and IC ≤ 475,000 RLU
Reactive	(Not used)	Analyte S/CO ≥ 1and IC ≤ 475,000 RLU

1. Any specimen with an interpretation of invalid in the PROCLEIX® HIV-1/HCV Assay, PROCLEIX® HIV-1 Discriminatory Assay or PROCLEIX® HCV Discriminatory Assay must be retested in the same assay in singlet, except as noted in step 7. Cadaveric specimens with an interpretation of invalid in any of the PROCLEIX Assays that were previously diluted 1:5 may be retested in singlet, diluted at the 1:5 dilutions, except as noted in step 7.

2. If at any point in the testing algorithm there is insufficient volume to complete the testing then an alternate specimen from the index donation (e.g., plasma unit or serology tube) may be used as long as the storage criteria in the package insert are met.
3. Specimens with a valid internal control and with an S/CO less than 1.00 in the HIV-1/HCV Assay are considered Nonreactive for HIV-1 and HCV RNA. If the Nonreactive specimen is a pool of 16, each of the 16 individual specimens comprising the pool is considered Nonreactive and no further testing is required. Specimens with S/CO greater than or equal to 1.00 are considered Reactive.
4. IF THE REACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool is tested with the HIV-1/HCV Assay.
    a. If an individual specimen tests Nonreactive with the HIV-1/HCV Assay, then the specimen is considered Nonreactive for HIV-1 and HCV RNA and no further testing is required.
    b. If an individual specimen tests Reactive with the HIV-1/HCV Assay, then the specimen must be tested with the HIV-1 Discriminatory and HCV Discriminatory Assays.
        (1) If an individual specimen then tests Reactive with one or both Discriminatory tests, then the specimen is considered Reactive-Discriminated.
        (2) If an individual specimen then tests Nonreactive with both Discriminatory tests, then the specimen is considered Non-Discriminated. For HPC donors, continue to step 6a.
5. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION OF WHOLE BLOOD, BLOOD COMPONENTS OR SOURCE PLASMA, then the specimen must be tested with the HIV-1 Discriminatory and HCV Discriminatory Assays.
    a. If an individual specimen then tests Reactive with one or both Discriminatory tests, then the specimen is considered Reactive-Discriminated.
    b. If an individual specimen then tests Nonreactive with both Discriminatory tests, then the specimen is considered Non-Discriminated.
6. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION FROM ANY OTHER LIVING DONOR (I.E., NOT A BLOOD DONOR) OR FROM A CADAVERIC DONOR, then the specimen must be tested with the PROCLEIX HIV-1 Discriminatory and PROCLEIX HCV Discriminatory Assays.
    a. If an individual specimen tests Reactive with one or both Discriminatory tests, then the specimen is considered Reactive-Discriminated.
    b. If an individual specimen tests Nonreactive with both Discriminatory tests, then the specimen is considered Non-Discriminated. The Non-Discriminated specimen may be retested in the PROCLEIX HIV-1/HCV Assay if sufficient sample is available.
        (1) If the individual specimen tests Nonreactive in the repeated PROCLEIX HIV-1/HCV Assay, then the specimen is considered Nonreactive for HIV-1 and HCV RNA and no further testing is required.
        (2) If the individual specimen tests Reactive in the repeated PROCLEIX HIV-1/HCV Assay, then the specimen is considered Repeatedly Reactive, Non-Discriminated for HIV-1 and HCV RNA. Further clarification of these specimens, for informational purposes, may be obtained by testing an alternate specimen from the index donation with the PROCLEIX Assays and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of cell/tissue donor eligibility.
7. Reactive specimens in an operator invalidated run due to the 10% invalid rate are identified by the PROCLEIX System Software as reactive and must become the test of record. Any reactive result (analyte signal/cutoff $\geq$ 1) serves as the test of record and the sample should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section, Step 4, 5, or 6.
8. HIV seroreactive specimens found to be Reactive- HIV-1 Discriminated in the PROCLEIX Assays may be considered positive for HIV-1 nucleic acid. HCV seroreactive specimens found to be Reactive-HCV Discriminated in the PROCLEIX Assays may be considered positive for HCV nucleic acid. The interpretation of Reactive-Discriminated specimen results on specimens that are Nonreactive by serology is unclear.
9. For specimens that are Reactive-HIV-1 Discriminated in the PROCLEIX Assays, and also repeatedly reactive on a licensed donor screening test for antibodies to HIV-1, the PROCLEIX HIV-1/HCV Assay can be considered a supplemental test that confirms HIV-1 infection.
10. For specimens that are Reactive-HCV Discriminated in the PROCLEIX Assays, and also repeatedly reactive on a licensed donor screening test for antibodies to HCV, the PROCLEIX HIV-1/HCV Assay can be considered a supplemental test that confirms HCV infection.
11. Specimens that are Nonreactive on the PROCLEIX HIV-1/HCV Assay or are Reactive on the PROCLEIX HIV-1/HCV Assay but are not HIV-1 Discriminated, and are also repeatedly reactive on a licensed donor screening test for antibodies to HIV-1, should be further tested using an FDA approved HIV-1 supplemental test (such as Western blot or immunofluorescence assay).
12. Specimens that are Nonreactive on the PROCLEIX HIV-1/HCV Assay or are Reactive on the PROCLEIX HIV-1/HCV Assay but are not HCV Discriminated, and are also repeatedly reactive on a licensed donor screening test for antibodies to HCV, should be further tested using an FDA approved HCV supplemental test (such as RIBA).
13. Persons demonstrating a reactive test with the PROCLEIX HIV-1 or HCV Discriminatory assays, or a repeatedly reactive enzyme immunoassay (EIA) test for HIV-1 or HCV, should be referred for medical evaluation. A clinical diagnosis can be made only if a person meets the case definition(s) established by the Centers for Disease Control.[28,29]

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

PROCLEIX® HIV-1/HCV Assay reproducibility was determined at three blood testing laboratories. The reproducibility study evaluated both automated pipetting using TECAN GENESIS RSP instrument, and manual pipetting of the specimen and Target Capture Reagent (TCR) into the reaction tube. The reproducibility of the HIV-1/HCV Assay was assessed with a seven member reproducibility panel; 16 individual specimens were pipetted with the PROCLEIX® CPT Pooling Software to create each of the seven panel members (each panel member is a 16 member pool). Each pool contained from zero to three HIV-1 and/or HCV RNA positive specimens with the remaining specimens in the pool being HIV-1 and HCV RNA negative (Table 1).

For determination of the reproducibility of the PROCLEIX® HIV-1 Discriminatory Assay and the PROCLEIX® HCV Discriminatory Assay, nine panel members were tested as individual specimens and not in a pool. Eight of these panel members were HIV-1 and/or HCV RNA positive, and one was HIV-1 and HCV RNA negative (Tables 2 and 3).

The reproducibility panels were tested by a total of six operators (two at each site) with three different Clinical Lots over at least 18 nonconsecutive days. Inter- and intra-assay variability and inter-lot variability were determined. Mean S/CO, standard deviation (SD) and coefficient of variation (%CV) results are shown for panel members and for the Negative, HIV-1 Positive and HCV Positive Calibrators. Since no significant difference in assay reproducibility was observed between automated pipetting and manual pipetting, results for the two methods are combined in the tables below (Tables 1, 2, and 3). Also, since HCV RNA positive and HIV-1 RNA positive samples containing 90 copies/mL or greater gave high (saturated) signals in all three assays, results on multiple panel members are combined.

Table 1. Reproducibility of the PROCLEIX® HIV-1/HCV Assay

Specimen	N	Concentration Copies/mL	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot	
						SD	%CV	SD	%CV	SD	%CV
Nonreactive	1	0	320	100.00	0.23	0.054	23.5	0.034	14.7	0.041	17.7
HIV-1	3	190, 620, 720	965	99.90	18.75	1.864	9.9	1.191	6.4	2.351	12.5
HIV-1/HCV	2	620,720/90	641	100.00	27.42	2.374	8.7	1.840	6.7	2.743	10.0
HCV	1	190	321	100.00	8.70	1.074	12.3	0.615	7.1	0.743	8.5
Specimen			**Number of replicates**	**% Agreement**	**Mean RLU**	**Intra-Assay**		**Inter-Assay**		**Inter-Lot**	
						SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator			323	N/A	10363	2023	19.5	1740	16.8	1606	15.5
HIV-1 Positive Calibrator			324	N/A	858644	34660	4.0	55020	6.4	141285	16.5
HCV Positive Calibrator			316	N/A	398939	17511	4.4	15926	4.0	41127	10.3

N = Number of panel members combined for this analysis.

Table 2. Reproducibility of the PROCLEIX® HIV-1 Discriminatory Assay (excludes 10 false positive results)

Specimen	N	Concentration Copies/mL	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot	
						SD	%CV	SD	%CV	SD	%CV
Nonreactive	1	0	322	100.00	0.19	0.050	26.3	0.029	15.3	0.024	12.3
HIV-1	4	150, 500, 1500, 10000	1289	100.00	19.69	2.391	12.1	1.114	5.7	0.883	4.5
HIV-1/HCV	1	500/500	318	100.00	19.44	1.225	6.3	1.373	7.1	1.045	5.4
HCV	3	150, 500, 1500	955	100.00	0.18	0.054	29.7	0.038	20.9	0.029	16.1
Specimen			**Number of replicates**	**% Agreement**	**Mean RLU**	**Intra-Assay**		**Inter-Assay**		**Inter-Lot**	
						SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator			323	N/A	8900	2121	23.8	1824	20.5	1470	16.5
HIV-1 Positive Calibrator			320	N/A	894464	57091	6.4	63756	7.1	30695	3.4
HCV Positive Calibrator			322	N/A	8686	2381	27.4	1572	18.1	783	9.0

N = Number of panel members combined for this analysis.

Table 3. Reproducibility of the PROCLEIX® HCV Discriminatory Assay (excludes 3 false positive results)

Specimen	N	Concentration Copies/mL	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot	
						SD	%CV	SD	%CV	SD	%CV
Nonreactive	1	0	323	100.00	0.13	0.051	39.0	0.031	24.1	0.010	8.0
HIV-1	4	150, 500, 1500, 10000	1288	100.00	0.13	0.073	55.0	0.042	31.5	0.018	13.3
HIV-1/HCV	1	500/500	323	100.00	21.32	1.965	9.2	1.000	4.7	0.510	2.4
HCV	3	150, 500, 1500	966	99.90	21.48	1.884	8.8	1.211	5.6	0.479	2.2

Specimen	Number of replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot	
				SD	%CV	SD	%CV	SD	%CV
Negative Calibrator	320	N/A	8132	2861	35.2	1893	23.3	81	1.0
HIV-1 Positive Calibrator	321	N/A	7937	3089	38.9	2240	28.2	929	11.7
HCV Positive Calibrator	323	N/A	1243692	57818	4.6	92720	7.5	36728	3.0

N = Number of panel members combined for this analysis.

PERFORMANCE OF POOLED SAMPLE TESTING

Specificity of the PROCLEIX® HIV-1/HCV Assay

The HIV-1/HCV Assay was used to screen plasma pools comprised of 16 donor specimens and individual donor specimens (IDS). These specimens were tested at eight volunteer blood donor sites using three Clinical Lots of reagents. This population derived from approximately 103 geographically diverse blood donor sites in the continental US and five others which were US military blood donor sites located in Hawaii (one), Japan (two), Germany (one), and Guam (one). Pools or individual specimens with S/CO<1.0 are considered Nonreactive (NR). Individual specimens that are Reactive (R) by the HIV-1/HCV Assay and Reactive by either the HIV-1 or HCV Discriminatory Assay or both are termed Reactive-Discriminated. Individual specimens that are reactive by the HIV-1/HCV Assay but Nonreactive by both the HIV-1 or HCV Discriminatory Assays are termed Reactive-Non-Discriminated. A non-discriminated specimen which tested again as HIV-1/HCV Reactive was termed Repeatedly Reactive-Non-Discriminated. A non-discriminated specimen which tested again as HIV-1/HCV Assay Nonreactive was considered Nonreactive for HIV-1 and HCV RNA.

At the time the study was performed there was no recognized standard for establishing the presence or absence of HIV-1 RNA or HCV RNA in blood. Specificity was based on testing of blood donations from random volunteer blood donors. For the purpose of specificity calculations, specimens testing seroreactive for anti-HIV-1 and anti-HCV antibody (Ab) or HIV-1 p24 antigen (Ag) were eliminated from the analysis.

In the pooling specificity study, 11,978 pools (191,648 donor specimens) were tested (Tables 4 and 5). Specificity of pool testing relative to serology testing was 99.67% (11,625/11,663). Specificity was defined as number of pools containing all serology and PROCLEIX HIV-1/HCV Assay nonreactive specimens (True negative, TN) divided by the sum of TN and False positive pools. False positive pools were defined as pools Reactive by the HIV-1/HCV Assay and that contained all specimens nonreactive by serology.

There were 175 (1.46%) pools initially reactive by the HIV-1/HCV Assay. All 16 individual donor specimens from each reactive pool were tested. There were 33 pools containing all Nonreactive individual donor specimens. One hundred and sixty-six individual donor specimens derived from the remaining 142 pools were Reactive in the HIV-1/HCV Assay. No significant differences among sites or Clinical Lots were observed.

Of the 166 HIV-1/HCV Assay reactive individual donor specimens, 138 (83.1%) were Reactive in the HCV Discriminatory Assay, 13 (7.8%) were Reactive in the HIV-1 Discriminatory Assay and 15 (9.0%) were Nonreactive in both discriminatory tests. The 15 Reactive-Non-Discriminated specimens were seronegative for HIV-1 and HCV and were Nonreactive when retested in the HIV-1/HCV Assay. The adjusted reactive rate after removal of reactive pools containing true positive samples was 0.31% (37/11,841).

A total of 49,054 specimens (total of pooled and individual donor specimens) were run in the HIV-1/HCV Assay during these specificity studies and 185 (0.38%) specimens tested as initially invalid due to an Internal Control failure. All 185 specimens giving initially invalid results gave valid repeat testing results in the HIV-1/HCV Assay.

Table 4. PROCLEIX® HIV-1/HCV Assay Reactivity in Volunteer Blood Donors

	PROCLEIX® HIV-1/HCV Assay
	Plasma Pool of 16
Samples Tested*	11,978
Initial Reactive	175
Initial Reactive Rate	1.46%
Adjusted Reactive Rate	0.31%
Combined Mean S/CO on Negative Analytes	0.21 ± 0.10

*Combined data across all sites and Clinical Lots.

No significant differences among Clinical Lots of reagents were observed for either the Negative population Analyte mean S/CO or mean Internal Control S/CO in the negative population distribution.

Results of deconvolution of all 16 member pools are shown in Table 5. In these pivotal specificity studies (pooled and individual donation testing), no specimen was identified as a yield specimen based on the HIV-1/HCV Assay reactivity, HIV-1 and/or HCV Discriminatory Assay reactivity, reactivity by Alternative NAT and lack of HCV or HIV-1 antibody reactivity. The finding of no HIV-1 or HCV RNA yield donors in 226,205 specimens is consistent with published yields.[15] Rates of specimens testing reactive for both HCV RNA and HCV antibody in volunteer donors was 1 in 1,508 donors and rates of specimens testing reactive for both HIV-1 RNA and HIV-1 antibody was 1 in 14,138 donors.

Table 5. Deconvoluted Pooling Results

	N (%)	Sero-RR*
Total pools tested	**11,978 (100.0)**	**N/A**
HIV-1/HCV Assay Nonreactive pools	11,803 (98.54)	N/A
HIV-1/HCV Assay Reactive pools	175 (1.46)	N/A
Pools with all HIV-1/HCV Assay Nonreactive IDS	33 (18.9)	N/A
Pools with ≥ 1 HIV-1/HCV Assay Reactive IDS	142 (81.1)	N/A
HIV-1/HCV Assay Reactive IDS	166 (100.0)	120
HIV-1 and HCV Discriminatory Reactive IDS	0 (0.0)	0
HIV-1 Discriminatory Reactive IDS	13 (7.8)	13
HCV Discriminatory Reactive IDS	138 (83.1)	131
HIV-1 and HCV Discriminatory Nonreactive IDS	15 (9.0)	0
Repeat HIV-1/HCV Assay Nonreactive IDS	15 (100.0)	0
Repeat HIV-1/HCV Assay Reactive IDS	0 (0.0)	0

*EIA repeatedly reactive, supplemental serology positive or indeterminate.
IDS = Individual Donor Specimen.

Specificity of the PROCLEIX® HIV-1 and HCV Discriminatory Assays

In the HIV-1 and HCV Discriminatory Assays specificity study, the HIV-1 Discriminatory and HCV Discriminatory assays were run only on individual donor specimens which had previously tested as Nonreactive by the HIV-1/HCV assay. The initial reactive rates for the HIV-1 Discriminatory and HCV Discriminatory Assays were 0.24% (6/2508) and 0.29% (7/2443), respectively. In this study initially reactive specimens were not retested.

Comparison with Serology

Results generated from the pooled and individual donation testing specificity studies allow comparison of the HIV-1/HCV Assay with serology reactivity (Table 6). Sixteen of 17 HIV-1 seroreactive, Western blot positive specimens were also PROCLEIX HIV-1/HCV Assay Reactive (Table 6). The one discordant specimen was Nonreactive when tested as a pool by the PROCLEIX HIV-1/HCV Assay but was Reactive by the PROCLEIX HIV-1/HCV Assay when tested as an individual donor specimen. All of the HIV-1 seroreactive specimens which were Indeterminate or negative by Western Blot (91.7% of total HIV-1 seroreactive specimens) were Nonreactive by the PROCLEIX HIV-1/HCV Assay. Overall agreement between the PROCLEIX HIV-1/HCV Assay and Western blot was 100% (226/226) if testing at an individual donor level is compared.

77.3% (150/194) of HCV seroreactive, CHIRON RIBA HCV 3.0 (RIBA) Positive specimens were Reactive by the HIV-1/HCV Assay. 3.3% (2/60) of HCV seroreactive, RIBA Indeterminate specimens were reactive by the PROCLEIX HIV-1/HCV Assay. All 163 RIBA Negative specimens were also Nonreactive by the PROCLEIX HIV-1/HCV Assay. Overall agreement between the PROCLEIX HIV-1/HCV Assay and RIBA was 89.0% (371/417). These data are consistent with previous reports that about 20% of HCV seropositives will have undetectable HCV RNA[30] and estimates that approximately 20% of HCV seropositive individuals may have resolved infection.[31]

Table 6. Comparison of Serologic and PROCLEIX® HIV-1/HCV Assay Reactives from the Specificity Studies

Serology		PROCLEIX® HIV-1/HCV	
		Reactive	**Nonreactive**
HIV-1 Ab RR Western Blot (N = 228)	POS	16 (7.0%)	1* (0.4%)
	IND	0 (0%)	74 (32.5%)
	NEG	0 (0%)	135 (59.2%)
	N/A	0 (0%)	2 (0.9%)
HCV Ab RR RIBA (N = 419)	POS	150 (35.8%)	44 (10.5%)
	IND	2 (0.5%)	58 (13.8%)
	NEG	0 (0.0%)	163 (38.9%)
	N/A	1 (0.2%)	1 (0.2%)

*Sample contained < 50 copies/mL of HIV-1 RNA and was PROCLEIX HIV-1/HCV negative in pool testing but reactive in individual sample testing.

Non-Specificity Studies

When tested with the PROCLEIX HIV-1/HCV Assay, no cross-reactivity or interference was observed for naturally occurring icteric, hemolyzed or lipemic specimens or plasma containing the following substances: serum albumin (up to 225 g/L), hemoglobin (up to 5000 mg/L), bilirubin (up to 200 mg/L) and lipids (up to 2,752 mg/dL).

No cross-reactivity or interference was observed in specimens from patients with autoimmune diseases or with liver diseases not caused by HCV infection. Autoimmune conditions included rheumatoid arthritis (n = 10), rheumatoid factor (n = 10), antinuclear antibody (n = 10), multiple sclerosis (n = 10), lupus (n = 10) and multiple myeloma (n = 9). Also tested were flu vaccinees (n = 10), hepatitis B vaccinees (n = 10), elevated IgM (n = 6), elevated IgG (n = 11), alcoholic liver cirrhosis (n = 10) and elevated ALT (n = 10).

No cross-reactivity or interference was observed in bacterially contaminated plasmas or in plasmas infected with other blood borne pathogens, including herpes simplex virus-1 (n = 10), herpes simplex virus-2 (n = 1), CMV (n = 10), EBV (n = 10), hepatitis A virus (n = 10), HTLV-I (n = 10), HTLV-II (n = 10), hepatitis B virus (n = 10), HIV-2 (n = 10), rubella (n = 10) and parvovirus B-19 (n = 10).

CLINICAL SENSITIVITY

Testing of Whole Blood Donor Specimens

A total of 24,764,889 donations were screened as part of pooled sample testing since early 1999.[33] As of November 2001, 88 PROCLEIX HCV yield cases (1:281,419) and seven HIV-1 yield cases (1:3,537,841) were identified across the 10 PROCLEIX pooled testing sites. Yield cases were confirmed to be positive for either HIV-1 or HCV RNA, but negative in serology testing. Two of the nine HIV-1 yield cases were reactive for HIV-1 RNA and p24Ag but Nonreactive by HIV-1 antibody testing (Table 7).

Table 7. Summary of PROCLEIX® Assay Yield Cases

Pooled Sample Testing	
Number of Donations Tested	24,764,889
PROCLEIX® HCV Yield Cases	88 (1:281,419)
PROCLEIX® HIV-1 Yield Cases	7 (1:3,537,841)

Testing of Specimens from HIV-1 and/or HCV Infected Individuals

A total of 2014 specimens positive by commercial HIV-1 RNA and HCV RNA assays (sensitivity ≥100 copies/mL) were obtained from four commercial vendors. Three Clinical Lots were used for all testing. These specimens were classified as HIV-1 RNA positives (n = 867), HCV RNA positives (n = 967) and both HIV-1 and HCV RNA (coinfected) positives (n = 180) based on alternate nucleic acid testing (Table 8). These specimens were also classified by disease category as described below and as shown in Table 9. These positive samples were tested undiluted (neat) with the PROCLEIX HIV-1/HCV Assay, HIV-1 Discriminatory Assay and the HCV Discriminatory Assay, and tested diluted 1:16 with the PROCLEIX HIV-1/HCV Assay. All dilutions were made with processed human serum that was negative for HIV-1 RNA and antibody/antigen, and HCV RNA and antibody.

During the study, specimens known to contain <100 copies/mL of viral RNA were excluded from this analysis and therefore the sensitivity presented herein is for samples with viral RNA concentrations equal to or greater than 100 copies/mL, or of unknown viral concentration.

The sensitivity for the PROCLEIX HIV-1/HCV and HIV-1 Discriminatory Assays for undiluted (neat) HIV-1 positive samples was 99.9% (95% CI: 99.4-100%) and 100% (95% CI: 99.6-100%), respectively. The sensitivity for the PROCLEIX HIV-1/HCV Assay for diluted (1:16) HIV-1 positive samples was 99.0% (95% CI: 98.0-99.5%).

The sensitivity for both the PROCLEIX HIV-1/HCV Assay and the HCV Discriminatory Assay for undiluted (neat) HCV positive samples was 99.6% (95% CI: 98.9-99.9%). The sensitivity for the PROCLEIX HIV-1/HCV Assay for diluted (1:16) HCV positives was 99.6% (95% CI: 98.9-99.9).

The sensitivity for the PROCLEIX HIV-1/HCV Assay, HIV-1 Discriminatory Assay and HCV Discriminatory Assay for undiluted HIV-1/HCV coinfected specimens was 100% (95% CI: 98.0-100%), 100% (95% CI: 97.9-100%) and 100% (95% CI: 92.6-100%), respectively. The sensitivity for the PROCLEIX HIV-1/HCV Assay for HIV-1/HCV coinfected specimens was 98.9% (95% CI: 96.0-99.9) when tested at 1:16 dilution.

The overall clinical sensitivity for the PROCLEIX HIV-1/HCV Assay, which takes into account all samples (RNA concentrations ≥ 100 copies/mL or unknown viral concentration) tested, is 99.8% (95% CI: 99.4-99.9), that for the HIV-1 Discriminatory Assay is 100% (95% CI: 99.6-100%), and that for the HCV Discriminatory Assay is 99.6% (95% CI: 99.0-99.9%).

Table 8. The Sensitivity of the PROCLEIX® HIV-1/HCV, HIV-1 and HCV Discriminatory Assays for HIV-1 and HCV Positive Specimens with RNA Concentrations ≥ 100 Copies/mL or Unknown

HIV-1/HCV Assay								
	Sensitivity for Neat Specimens				Sensitivity for 1:16 Diluted Specimens			
Sample	N	TP	%	(95% C. I.)	N	TP	%	(95% C. I.)
All	2014	2009	99.8	(99.4 - 99.9)	2012	1997	99.3	(98.8 - 99.6)
HIV-1 Only	867	866	99.9	(99.4 - 100.0)	866	857	99.0	(98.0 - 99.5)
HCV Only	967	963	99.6	(98.9 - 99.9)	966	962	99.6	(98.9 - 99.9)
HIV-1 & HCV	180	180	100	(98.0 - 100.0)	180	178	98.9	(96.0 - 99.9)

HIV-1 Discriminatory Assay				
	Sensitivity			
Sample	N	TP	%	(95% C. I.)
All	1041	1041	100	(99.6 - 100.0)
HIV-1 Only	867	867	100	(99.6 - 100.0)
HIV-1 & HCV	174	174	100	(97.9 - 100.0)

HCV Discriminatory Assay				
	Sensitivity			
Sample	N	TP	%	(95% C. I.)
All	1014	1010	99.6	(99.0 - 99.9)
HCV Only	966	962	99.6	(98.9 - 99.9)
HIV-1 & HCV	48	48	100	(92.6 - 100.0)

C. I. = Confidence interval.

The data from the above study were further analyzed according to the disease stages of the patients from whom the specimens were obtained as shown in Table 9. A total of 296 samples were from AIDS patients (as defined by AIDS-indicative conditions and/or a CD4 count of <200/mm^3), 338 from asymptomatic patients (asymptomatic, persistent generalized lymphadenopathy, or acute HIV-1 infection), 168 from symptomatic but non-AIDS patients (not AIDS and not asymptomatic) and 239 from individuals with unknown HIV disease state.[32] Approximately half of these patients were on HIV anti-viral medication. The sensitivity for HIV-1 detection with the PROCLEIX HIV-1/HCV Assay ranged from 99.6 to 100% for neat specimens and from 96.4 to 100% for 1:16 diluted specimens. The sensitivity for the HIV-1 Discriminatory Assay was 100%. All HIV-1 p24Ag reactive specimens were also reactive with the PROCLEIX HIV-1/HCV Assay when tested as undiluted (neat) or as 1:16 diluted samples, and with HIV-1 Discriminatory Assay when tested as neat samples. This was also true of all specimens excluded from the study due to low viral RNA concentrations (<100 copies/mL).

Similarly, the specimens from HCV infected patients were segregated as shown in Table 10. A total of 887 specimens were from volunteer blood donors whose donations were HCV reactive with PCR-based NAT, 53 specimens were from patients with chronic HCV infection that was first identified by blood donation screening and 75 specimens were not categorized by the vendor other than being HCV NAT or antibody positive. The sensitivity for HCV testing with the PROCLEIX HIV-1/HCV Assay ranged from 99.5 to 100% (95% CI: 98.9-99.9%) with neat samples and 97.3 to 100% (95% CI: 93.3-100%) with 1:16 diluted samples. The sensitivity for HCV Discriminatory Assay ranged from 99.5 to 100% (95% CI: 95.2-100%) for neat samples.

During this clinical study, in which a total of 1014 HCV RNA-positive samples were tested, two of these samples were consistently reactive when tested at 1:16 dilution, but non-reactive when tested neat.

In the same study, 13 sero-positive samples with low copy numbers of HIV-1 or HCV (or both) were non-reactive when tested at 1:16 dilution, and reactive when tested neat.

In summary, it appears that the disease stages for HIV-1 or HCV infected individuals did not significantly affect the sensitivity for the PROCLEIX Assays, although some low copy number HCV antibody positive specimens (2/967) were non-reactive when tested neat but reactive when tested at 1:16 dilution.

The clinical sensitivity claims of the assay are still met with the inclusion of these specimens.

Table 9. Sensitivity of the PROCLEIX® HIV-1/HCV and HIV-1 Discriminatory Assays for HIV-1 Positive Specimens from Individuals at Various Disease States*

	PROCLEIX® HIV-1/HCV Assay						HIV-1 Discriminatory			HIV-1 Antibody			HIV-1 p24Ag		
	Neat			1:16 Dilution			Neat								
Disease	N Tested	N R	% R	N Tested	N R	% R	N Tested	N R	% R	N Tested	N R	% R	N Tested	N R	% R
AIDS	295	295	100.0	296	295	99.7	296	296	100.0	296	296	100.0	226	44	19.5
Symptomatic**	168	168	100.0	167	161	96.4	168	168	100.0	168	168	100.0	138	14	10.1
Asymptomatic***	338	338	100.0	338	338	100.0	338	338	100.0	338	297	87.9	234	73	31.2
Unknown	239	238	99.6	240	238	99.2	239	239	100.0	239	232	97.1	201	29	14.4
Total	1040	1039	99.9	1041	1032	99.1	1041	1041	100.0	1041	993	95.4	799	160	20.0

N = Number R = Reactive

*Samples with confirmed viral loads < 100 copies/mL excluded.

**Symptomatic (not asymptomatic and not AIDS).

***Asymptomatic (asymptomatic, persistent generalized lymphadenopathy, or acute HIV-1 infection).

Table 10. Sensitivity of the PROCLEIX® HIV-1/HCV and HCV Discriminatory Assays for HCV Positive Specimens From Individuals at Various Disease States*

	PROCLEIX® HIV-1/HCV Assay						HCV Discriminatory Assay			HCV Antibody		
	Neat			1:16 Dilution			Neat					
Disease	N Tested	N R	% R	N Tested	N R	% R	N Tested	N R	% R	N Tested	N R	% R
First Time Blood	887	883	99.5	886	882	99.5	886	882	99.5	886	886	100
Chronic HCV	53	53	100	53	53	100	53	53	100	53	53	100
Unknown	75	75	100	75	73	97.3	75	75	100	52	20	38.5
Total	1015	1011	99.6	1014	1008	99.4	1014	1010	99.6	991	959	96.8

N = Number R = Reactive

*Samples with confirmed viral loads < 100 copies/mL excluded.

Sensitivity for Pooled Samples

The clinical sensitivity of the PROCLEIX HIV-1/HCV Assay with pooled samples was determined by testing 102 sixteen-member pools composed of one HIV-1 or HCV positive sample and 15 negative samples, and 102 sixteen-member pools composed of two HIV-1 and/or HCV positive samples and 14 negative samples. The viral load of the HIV-1 positive samples used to make the pools ranged from 1060 copies/mL to 10,018,200 copies/mL with a median of 27,490 copies/mL. The viral load of the HCV positive samples used to make the pools ranged from 1660 copies/mL to 20,200,000 copies/mL with a median of 327,000 copies/mL. All 204 (100%; 95% CI: 98.2-100%) pools containing at least one HIV-1 and/or HCV RNA positive specimen were reactive with the HIV-1/HCV Assay.

Prospective Study of Individuals at High Risk for HIV-1 and HCV Infection

Specimens from 539 individuals at high risk for infection with HIV-1 and/or HCV were tested as undiluted (neat) samples with the PROCLEIX HIV-1/HCV, HIV-1 Discriminatory and HCV Discriminatory Assays. These samples were also tested at 1:16 dilution with the PROCLEIX HIV-1/HCV Assay. Results are shown in Table 11. 72.5% (391/539) had IV drug use (IVDU) as one of their risk factors. Risk factors other than IVDU included having unprotected sex, men having sex with men, occupational exposure, having sex with positive partner, and having transfusion of blood or blood products. Sensitivity was determined by comparing the PROCLEIX Assays with an HIV-1 or HCV PCR-based assay that has a claimed analytical sensitivity of $\geq$100 copies viral RNA /mL.

Both the PROCLEIX HIV-1/HCV Assay and HIV-1 Discriminatory Assay detected all 23 samples, either undiluted or 1:16 diluted, that were tested reactive for HIV-1 with the PCR NAT test. Of these 23 HIV-1 reactive specimens, 11 were also reactive with the HCV Discriminatory Assay and are considered samples from individuals coinfected with HIV-1 and HCV.

There was one confirmed HIV-1 yield case that appeared to be in the window period. This specimen was reactive with the PROCLEIX HIV-1/HCV and HIV-1 Discriminatory Assays as an undiluted sample, and with the PROCLEIX HIV-1/HCV Assay as a 1:16 diluted sample. The specimen was HIV-1/-2 antibody seronegative, HIV-1 p24Ag positive and was positive with an alternate HIV-1 nucleic acid test (NAT).

There were 268 HCV antibody and/or alternate HCV NAT positive specimens among the 520 high-risk specimens that were tested with PROCLEIX HIV-1/HCV and HCV Discriminatory Assays. When tested as undiluted (neat) samples, 266 of the 268 positive specimens (99.3%, 95% CI: 97.3-99.9%) were reactive with both the PROCLEIX HIV-1/HCV and HCV Discriminatory Assays. When tested as diluted samples, 254 of 259 (98.1%, 95% CI: 95.6-99.4%) were reactive with the PROCLEIX HIV-1/HCV Assay. There were 44 HCV seropositive specimens that were nonreactive in all PROCLEIX Assays. Of the 44, 37 were tested by an alternate HCV NAT and 34 were found to be NAT negative (considered PROCLEIX TN for sensitivity calculations), two were equivocal, and one was positive (PROCLEIX FN).

This study resulted in three confirmed HCV yield cases that were considered true positives for the PROCLEIX assay. These specimens were HCV antibody negative; two of which were alternate HCV NAT positive and one was QNS for alternate NAT. All three subjects later seroconverted.

Table 11. Clinical Sensitivity of the PROCLEIX® HIV-1/HCV Assay in a High Risk Population

							Sensitivity	
Disease	**Specimen**	**Number**	**TP**	**FP**	**TN**	**FN**	**%**	**(95% C. I.)**
HIV-1*	Neat	530	23	0	507	0	100	(85.2 - 100.0)
	Diluted	520	23	0	497	0	100	(85.2 - 100.0)
HCV**	Neat	520	266	3	249	2	99.3	(97.3 – 99.9)
	Diluted	508	254	2	247	5	98.1	(95.6 – 99.4)

*Reactive in the HIV-1/HCV Assay and the HIV-1 Discriminatory Assay
**Reactive in the HIV-1/HCV Discriminatory Assay

Analytical Sensitivity

To determine the analytical sensitivity of the PROCLEIX HIV-1/HCV Assay and HIV-1 and HCV Discriminatory Assays for detection of HIV-1 and HCV viral RNA, HIV-1 panel members were prepared by serial dilution of negative human plasma spiked with HIV-1 (type B isolate) tissue culture supernatant. HCV panel members were made by serial dilution of a patient plasma specimen containing HCV (subtype 1a). The RNA levels in viral stocks used to make the HIV-1 panel and high titer HCV plasma used to make the HCV panel were value assigned using an in-house quantitative HIV-1 assay calibrated to the VQA standard obtained from Dr. James Bremer (Rush-Presbyterian Hospital, Chicago, IL) or quantitative HCV assay compared to HCV RNA WHO standard. (1 WHO IU/mL is equivalent to 2.7 copies/mL.)

The panel members were tested with ten clinical lots of reagents and the test results are presented in Table 12. The PROCLEIX HIV-1/HCV Assay and HIV-1 and HCV Discriminatory Assays achieved 100% detection for panel members containing 300 copies/mL, and > 99% detection for those members containing 100 copies/mL of HIV-1 or HCV RNA. The lower bound of 95% CI for both HIV-1 and HCV at 100 and 300 copies/mL for all assays exceeded 95%, which is consistent with a claimed analytical sensitivity of 100 copies RNA/mL. The PROCLEIX HIV-1/HCV and Discriminatory Assays were able to detect 30 copies/mL of HIV-1 or HCV RNA at a frequency greater than 90%, with the lower bound of 95% CI ranging from 90% to 97.3%.

Table 12. Detection of HIV-1 B RNA and HCV 1a RNA in Analytical Sensitivity Panels

HIV-1 Copies/mL	**PROCLEIX® HIV-1/HCV Assay**				**HIV-1 Discriminatory Assay**			
	Number of reactive/ tested*	**% Positive**	**95% Confidence Limits**		**Number of reactive/ tested***	**% Positive**	**95% Confidence Limits**	
			Lower	**Upper**			**Lower**	**Upper**
300	716/716	100	99.5	100	715/715	100	99.5	100
100	719/719	100	99.5	100	718/718	100	99.5	100
30	707/720	98.2	96.9	99.0	702/713	98.5	97.3	99.2
10	573/718	79.8	76.7	82.7	592/717	82.6	79.6	85.3
3	297/718	41.4	37.7	45.1	305/717	42.5	38.9	46.3
1	112/717	15.6	13.0	18.5	139/718	19.4	16.5	22.4

HCV 1a Copies/mL	**PROCLEIX® HIV-1/HCV Assay**				**HCV Discriminatory Assay**			
	Number of reactive/ tested*	**% Positive**	**95% Confidence Limits**		**Number of reactive/ tested***	**% Positive**	**95% Confidence Limits**	
			Lower	**Upper**			**Lower**	**Upper**
300	718/718	100	99.5	100	720/720	100	99.5	100
100	720/720	100	99.5	100	745/746	99.9	99.3	100
30	669/718	93.2	91.1	94.9	660/716	92.2	90.0	94.0
10	470/719	65.4	61.8	68.9	458/717	63.9	60.2	67.4
3	231/718	32.2	28.8	35.7	258/717	36.0	32.5	39.6
1	70/716	9.8	7.7	12.2	104/719	14.5	11.0	17.3

*Invalid reactions were not retested.

CBER HIV-1 RNA Panel

Panel A (five members) and Panel B (eight members) were tested in duplicate with five Clinical Lots using both the HIV-1/HCV Assay and the HIV-1 Discriminatory Assay. Results for both Panel A and B are shown in Table 13. For Panel A, testing with the HIV-1/HCV Assay showed reproducible detection of HIV-1 RNA at copy levels ranging from 250,000 to 100 copies/mL; the panel member at 0 copies/mL was non-reactive. Results for Panel B demonstrated reproducible detection of HIV-1 RNA at copy levels ranging from 250,000 to 50 copies/mL and non-reactive results with both negative panel members (B4 and B8). Similar results were obtained with the Discriminatory Assays.

CBER HCV RNA Panel

This panel consisted of 10 panel members with copy levels ranging from 100,000 to 0 copies/mL. This panel was tested in duplicate with five Clinical Lots using both the HIV-1/HCV and HCV Discriminatory Assays. Results are shown in Table 13. Reproducible detection of HCV was obtained down to 50 copies/mL with both assays.

Table 13. Detection of HIV-1 RNA and HCV RNA in CBER panel members

Panel members tested and positivity rates													
CBER HIV-1 RNA Panel (copies/mL)	**A1** 250,000	**A2** 25,000	**A3** 1,000	**A4** 100	**A5** 0	**B1** 2,500	**B2** 10	**B3** 250,000	**B4** 0	**B5** 100	**B6** 50	**B7** 25,000	**B8** 0
HIV-1/HCV Assay*	100%	100%	100%	100%	0%	100%	60%	100%	0%	100%	100%	100%	0%
HIV-1 Discriminatory Assay**	100%	100%	100%	100%	0%	100%	100%	100%	0%	100%	100%	100%	0%

Panel members tested and positivity rates										
CBER HCV RNA Panel (copies/mL)	**1** 1,000	**2** 0	**3** 100,000	**4** 10,000	**5** 0	**6** 500	**7** 200	**8** 50	**9** 10	**10** 5
HIV-1/HCV Assay*	100%	0%	100%	100%	0%	100%	100%	100%	90%	30%
HCV Discriminatory Assay**	100%	0%	100%	100%	0%	100%	100%	100%	100%	50%

* n = 10; ** n = 6

WHO International Standard for HIV-1

The WHO International Standard for HIV-1 RNA (NIBSC code 97/656) with a concentration of 100,000 IU/mL was serially diluted and tested with the HIV-1/HCV Assay and the HIV-1 Discriminatory Assay. The results obtained are shown in Table 13.

WHO International Standard for HCV

The WHO International Standard for HCV RNA (96/790) with a concentration of 100,000 international units (IU)/mL was serially diluted and tested with the HIV-1/HCV Assay and the HCV Discriminatory Assay. The results obtained are shown in Table 14.

Table 14. Testing of International Standards for HIV-1 RNA (NIBSC code 97/656) and HCV RNA (NIBSC 96/790)

Concentrations tested and positivity rates							
WHO HIV-1 (97/656)	300 IU/mL	100 IU/mL	33.3 IU/mL	11.1 IU/mL	3.7 IU/mL	1.23 IU/mL	0 IU/mL
HIV-1/HCV Assay*	100%	100%	100%	77.5%	50%	32.5%	0%
HIV-1 Discriminatory Assay **	100%	100%	100%	80%	27.6%	26.6%	0%

Concentrations tested and positivity rates									
WHO HCV (96/790)	110 IU/mL	37 IU/mL	11 IU/mL	3.7 IU/mL	1.1 IU/mL	0.37 IU/mL	0.11 IU/mL	0.04 IU/mL	0.00 IU/mL
HIV-1/HCV Assay*	100%	100%	100%	100%	50%	25%	0%	0%	0%
HCV Discriminatory Assay**	100%	100%	100%	95%	60%	25%	0%	0%	0%

* n = 40; ** n = 30

Subtype Detectability

Since there are no recognized international standards for HCV or HIV-1 other than HCV subtype 1a and HIV-1 subtype B, multiple specimens and isolates (59 different HIV-1 and 53 different HCV specimens) were tested to determine detectability of these viral subtypes.[34] HIV-1 specimens of subtypes A, B, C, D, E, F, and G were quantified for HIV-1 RNA concentrations using commercial quantitative HIV-1 RNA assays or an in-house developed quantitative test, the latter using the same technology as the PROCLEIX Assays. HIV-1 subtypes N and O were quantified with an in-house quantitative HIV-1 RNA test. Specimens were diluted into negative human plasma to target viral concentrations of 300 or 100 copies/mL and diluted specimens were tested in the HIV-1/HCV and HIV-1 Discriminatory Assays. All HIV-1 subtypes were reactive with both the PROCLEIX HIV-1/HCV and HIV-1 Discriminatory Assays at 300 and 100 copies/mL (Table 17).

HCV specimens of subtypes 1, 2, 3, 4, 5, and 6 were quantified for HCV RNA using commercially available quantitative HCV RNA assays. Specimens were diluted into negative human plasma to target viral concentrations of 300 or 100 copies/mL and diluted specimens were tested with the HIV-1/HCV and HCV Discriminatory Assays. All HCV subtypes were reactive by the HIV-1/HCV and HCV Discriminatory Assays at 300 and 100 copies/mL, except one HCV subtype 2 specimen which was reactive at 300 copies/mL, but nonreactive at 100 copies/mL (Table 17).

Table 17. HIV-1 and HCV Subtype Detectability

Specimen	Subtype	Copies/mL	HIV-1/HCV Reactive/Total	HIV-1 Discriminatory Reactive/Total
HIV-1	A*	300	11/11	11/11
		100	9/9	9/9
	B	300	10/10	11/11
		100	10/10	11/11
	C	300	9/9	9/9
		100	9/9	9/9
	D	300	6/6	6/6
		100	6/6	6/6
	E	300	8/8	8/8
		100	8/8	8/8
	F	300	5/5	5/5
		100	5/5	5/5
	G	300	3/3	3/3
		100	3/3	3/3
	N	300	1/1	1/1
		100	1/1	1/1
	O	300	6/6	6/6
		100	6/6	6/6
Specimen	**Subtype**	**Copies/mL**	**HIV-1/HCV Reactive/Total**	**HCV Discriminatory Reactive/Total**
HCV	1	300	10/10	10/10
		100	10/10	10/10
	2	300	13/13	13/13
		100	12/13	13/13
	3	300	11/11	11/11
		100	11/11	11/11
	4	300	10/10	11/11
		100	10/10	11/11
	5	300	4/4	4/4
		100	4/4	4/4
	6	300	4/4	4/4
		100	5/5	5/5

*Two samples were quantified at < 1000 copies/mL and were reactive when tested undiluted and at 1:3 dilution.

PERFORMANCE OF POOLED SAMPLE TESTING - PAID SOURCE PLASMA

Specificity of the PROCLEIX® HIV-1/HCV Assay

The HIV-1/HCV Assay was used to test 1,200 16-member pools of seronegative source plasma from 19,200 donor samples taken from 4,916 paid donors. The clinical study included nine collection centers geographically distributed throughout the U.S. Testing was conducted at one plasma and one blood donor testing laboratory using three reagent lots. One site serves multiple paid source plasma collection centers and the other site routinely tests volunteer whole blood donations.

All 1,200 pools tested were Nonreactive, thus the overall specificity of the HIV-1/HCV Assay in source plasma samples from paid donors across both testing sites and all reagent lots was 100.0% (95% CI: 99.4-100%). Specificity was defined as number of HIV-1/HCV Assay Nonreactive pools containing all seronegative specimens (True negative, TN) divided by the sum of TN and False positive pools. False positive pools were defined as pools Reactive by the HIV-1/HCV Assay and that contained all specimens Nonreactive by serology. Zero samples were found to be reactive in the HIV-1/HCV Assay for an initial false reactive rate of 0% (0/19,200).

A total of 1,200 initial pooled donor test results were generated from all valid HIV-1/HCV Assay runs. Of these, 1,198 (99.8%) results were initially valid. The two invalid results were due to low values for the Internal Control (IC). Both of these samples produced valid HIV-1/HCV results upon retest.

Specificity of the PROCLEIX® HIV-1 and HCV Discriminatory Assays

In the HIV-1 and HCV Discriminatory Assays specificity study a subset (n=1,012) of the 19,200 donor samples tested in the HIV-1/HCV Assay were tested in each of the Discriminatory Assays at the same two testing centers with the same three reagent lots.

The clinical specificity of the HIV-1 Discriminatory Assay was evaluated from 1,012 paid source plasma donors. Of these, 1,007 samples had valid results for inclusion in the specificity analysis. One sample was invalidated at initial testing by the operator due to improper reagent addition, and was designated as QNS for further testing; four were invalid in the initial test due to insufficient volume. Of 1,007 valid HIV-1 Discriminatory Assay results, none were reactive, for a 0% initial false reactive rate. The overall specificity relative to serology of the HIV-1 Discriminatory Assay across both testing sites and all reagent lots was calculated at 100.0% (95% CI: 99.3-100.0%) from 1,007 true negative and zero false positive results.

The clinical specificity of the HCV Discriminatory Assay was evaluated from 1,012 paid source plasma donors. A total of six samples had invalid results. Two samples were invalid at initial testing due to the detection of a clot, and were designated as having insufficient volume (QNS) for further testing; four were invalid in the initial test due to insufficient volume. Two of 1,006 (0.20%) valid HCV Discriminatory Assay results were reactive. Repeat and follow-up testing for these reactive samples was negative in the HCV Discriminatory Assay and/or in the alternative HCV Nucleic Acid Test. Thus, the initial false reactive rate for the HCV Discriminatory Assay was 0.20%. The overall specificity relative to serology of the HCV Discriminatory Assay across both testing sites and all reagent lots was calculated at 99.8% (95% CI: 98.9-100.0%) from 1,004 true negative and two false positive results.

In this specificity study of 19,200 donor samples, there were no yield specimens.

PERFORMANCE OF INDIVIDUAL DONATION TESTING

Clinical Sensitivity

The clinical sensitivity of the HIV-1/HCV Assay, HIV-1 Discriminatory and HCV Discriminatory Assays were evaluated by testing clinical samples without dilution (neat). The HIV-1/HCV Assay was used to test a total of 867 confirmed HIV-1 positive, 967 HCV positive and 180 HIV-1 and HCV positive samples. As summarized in Table 8, the overall sensitivity based on this study was 99.8% (95% CI: 99.4-99.9). Specifically, the sensitivity for HIV-1 positive samples was 99.9% (95% CI: 99.4-100.0) while that for HCV positive samples and HIV-1 and HCV positive samples was 99.6% (95% CI: 98.9-99.9) and 100% (95% CI: 98.0-100.0), respectively. *Three HCV positive samples tested non-reactive as undiluted samples, but reactive at 1:16 dilution. However, one of the three showed a viral load lower than the Limit of Detection for the PROCLEIX® HIV-1/HCV Assay as determined by an alternate nucleic acid test. The other two consistently tested reactive at 1:16 dilution but non-reactive with the undiluted sample and the nature of this discordance is under investigation.*

The sensitivity for the discriminatory assays was evaluated as well. As shown in Table 8, the sensitivity for the HIV-1 Discriminatory Assay was 100% (95% CI: 99.6-100.0) for HIV-1 positive samples and 100% (95% CI: 97.9-100.0) for HIV-1 and HCV positive samples. The HCV Discriminatory Assay showed a sensitivity of 99.6% (95% CI: 98.9-99.9) for HCV positive samples and 100% (95% CI: 92.6-100.0) for HIV-1 and HCV positive samples.

The data in the aforementioned studies were reanalyzed according to the disease stages and the results are presented in Tables 9 (for HIV-1/AIDS) and 10 (for HCV). Overall, the assays showed similar sensitivity for samples from various disease stages.

Seroconversion Panel Testing

When a limited number of HIV-1 seroconversion panel members were tested as undiluted (neat), an average of two days earlier detection was observed as compared to 1:16 dilution (Table 15). No difference was observed between the HIV-1/HCV Assay and HIV-1 Discriminatory Assay. Both assays were more sensitive for detecting window period samples as compared to the HIV-1 p24 Antigen assay.

For HCV seroconversion panels, no differences were observed between the PROCLEIX HIV-1/HCV Assay and the HCV Discriminatory Assay when testing was performed on undiluted samples or 1:16 diluted samples (Table 16). Both assays were able to detect HCV infection on average 25 days earlier than the antibody test.

Clinical Specificity

The clinical specificity for individual donation testing was determined for the HIV-1/HCV Assay by testing individual donor specimens that were never pooled (Table 18). Seventy-one of 34,557 (0.21%) individual donor specimens were initially reactive in the PROCLEIX HIV-1/HCV Assay. Twenty-one of these 71 specimens were also reactive in the HCV Discriminatory Assay and were seropositive for HCV. Three of the 71 were reactive in the HIV-1 Discriminatory Assay and seropositive for HIV-1/HIV-2. Forty-five specimens were nonreactive by both the HCV and HIV-1 Discriminatory Assays and HCV and HIV-1/HIV-2 antibody assays yielding an adjusted (false) reactive rate of 0.13% (45/34,533). One specimen had incomplete assay results and could not be discriminated. One specimen was PROCLEIX HCV Discriminated, HCV EIA repeatedly Reactive with no RIBA available. Seventeen samples with Nonreactive PROCLEIX assay results had incomplete serologic results and were excluded from the specificity calculations, yielding a specificity in individually tested donor samples of 99.87% (34,229/34,274).

Table 18. PROCLEIX® HIV-1/HCV Assay Reactivity in Volunteer Blood Donors

	PROCLEIX® HIV-1/HCV Assay
	Individual Donation
Samples Tested*	34,557
Initial Reactive	71
Initial Reactive Rate	0.21%
Adjusted Reactive Rate	0.13%
Combined Mean S/CO on Negative Analytes	0.17 ± 0.07

*Combined data across all sites and Clinical Lots.

This specificity study was conducted primarily in three military sites. The military donor population may differ from the civilian donor population. However, when sub-analyses were conducted across donor age groups, gender and race, comparable clinical specificity was observed across all categories ranging from 99.7% to 100% (all 95% confidence intervals overlapped). These sub-analyses included evaluation of 5,743 females; 1,102 donors over the age of 50; and at least 2,900 donors in each of the race categories of Black/Non-Hispanic, White/Hispanic, and White/Non-Hispanic. These results suggest that the specificity of the PROCLEIX HIV-1/HCV Assay with individual donations is not affected by race, age or gender.

Table 19. Summary of PROCLEIX® Assay Yield Under IND Testing

Individual Donation Testing	
Number of Donations Tested	103,357
PROCLEIX® HCV Yield Case	1 (1:103,357)
PROCLEIX® HIV-1 Yield Case	0

A total of 103,357 individual donations were screened under IND from April 2000 to November 2001 (Table 19). One PROCLEIX HCV yield case (1:103,357) was identified across the three PROCLEIX individual donation test sites. The yield case was confirmed to be positive for HCV RNA, but negative in serology testing. No HIV-1 yield cases were identified with individual donation testing.

PERFORMANCE OF CADAVERIC BLOOD SPECIMENS FROM TISSUE DONORS

NOTE: All performance evaluations, including reproducibility, specificity, and sensitivity were performed on cadaveric serum specimens.

REPRODUCIBILITY

The inter-assay reproducibility of the PROCLEIX® HIV-1/HCV Assay with cadaveric blood specimens was assessed by determining the %CVs obtained when each of 19-21 cadaveric and 20 control specimens were tested with 3 reagent lots in 3 separate runs for each reagent lot. The S/COs and %CVs are shown in Table 20. For the HIV-1 spiked specimens tested in the PROCLEIX HIV-1/HCV Assay, the %CVs for the cadaveric and control specimens were 40% and 31%, respectively. For the HIV-1 spiked specimens tested in the HIV-1 Discriminatory Assay, the cadaveric and control specimen %CVs were 37% and 33% respectively. For the HCV spiked specimens tested in the PROCLEIX HIV-1/HCV Assay, the %CVs for the cadaveric and control specimens were 14% and 17% respectively. For the HCV spiked specimens tested in the HCV Discriminatory Assay, the % CVs for the cadaveric and control specimens both were 22% and 16% respectively.

The analyte S/CO values were analyzed using a mixed effects model to compare the variation observed with the S/CO values from the cadaveric blood specimens to those from the control specimens. The comparison was adjusted for random effects such as reagent lot, virus stock and assay run. Statistically significant differences were observed between the cadaveric and control specimens for the HIV-1 spiked specimens in the PROCLEIX HIV-1/HCV Assay (p value <0.001), but not the HIV-1 Discriminatory Assay (p value 0.167). No significant differences were observed between the cadaveric and control HCV spiked specimens with both the PROCLEIX HIV-1/HCV Assay and the HCV Discriminatory Assay (p values 0.706 and 0.281 respectively). There were no clinically significant differences between the cadaveric and control specimens as noted by the 95% CI of the positivity rates (Table 20).

Table 20. Reproducibility

PROCLEIX® HIV-1/HCV Assay and HIV-1 Discriminatory Assay with Cadaveric and Control Specimens Spiked with 200 copies/mL of HIV-1

Virus	Assay	Sample	# of donors	Number of replicates	% Positive (95% CI)	Grand Mean Analyte S/CO	% CV
HIV-1	HIV-1/HCV Assay	Cadaveric	19	180	98.3% (95.2-99.7)	15.34	40
		Control	20	180	100% (98.0-100)	16.76	31
		P value				<0.001	
	HIV-1 Discriminatory Assay	Cadaveric	19	180	98.9% (96.0-99.9)	16.00	37
		Control	20	180	98.3% (95.2-99.7)	16.77	33
		P value				0.167	

PROCLEIX® HIV-1/HCV Assay and HCV Discriminatory Assay with Cadaveric and Control Specimens Spiked with 200 copies/mL of HCV

Virus	Assay	Sample	# of donors	Number of replicates	% Positive	Grand Mean Analyte S/CO	% CV
HCV	HIV-1/HCV Assay	Cadaveric	20	180	99.4% (96.9-100)	9.85	14
		Control	20	180	99.4% (96.9-100)	9.75	17
		P value				0.706	
	HCV Discriminatory Assay	Cadaveric	21	185	98.9% (96.2-99.9)	21.64	22
		Control	20	180	99.4% (96.9-100)	22.13	16
		P value				0.281	

SPECIFICITY

The specificity of the PROCLEIX HIV-1/HCV Assay was determined with 52 seronegative cadaveric blood specimens and 52 normal donor specimens using three reagent lots. The mean S/CO ratio for the 52 seronegative cadaveric blood specimens was 0.21 and the mean analyte S/CO for the 52 normal donor specimens was 0.19. These results were not statistically different as determined by Student's t-test (p value 0.29). The specificity of the HIV-1/HCV Assay with cadaveric blood specimens and normal donor specimens was 100% (95% confidence interval: 93.2%-100%) (Table 21).

The specificity of the HIV-1 Discriminatory Assay was determined with 52 seronegative cadaveric blood specimens and 52 normal donor specimens using three reagent lots. The mean analyte S/CO ratio for the 52 seronegative cadaveric blood specimens was 0.17 and the mean analyte S/CO for the 52 normal donor specimens was 0.14. While these values were statistically different (p value 0.03), there were no clinically significant differences as noted by the specificity of the HIV-1 Discriminatory Assay, of 100% (95% confidence interval: 93.2%-100%) for both cadaveric blood specimens and normal donor specimens.

The specificity of the HCV Discriminatory Assay was determined with 50 seronegative cadaveric blood specimens and 50 normal donor specimens using three reagent lots. The mean analyte S/CO ratio for the 50 seronegative cadaveric blood specimens was 0.09 and the mean analyte S/CO for the 50 normal donor specimens was 0.10. These results were not statistically different (p value 0.62). The specificity of the HCV Discriminatory Assay with cadaveric blood specimens and normal donor specimens was 100% (95% confidence interval: 92.9%-100%).

Table 21. Specificity of PROCLEIX® HIV-1/HCV Assay, HIV-1 Discriminatory Assay and HCV Discriminatory Assay in Cadaveric Blood Specimens

		Cadaveric		Controls	
		IC	Analyte	IC	Analyte
HIV-1/HCV Assay	Average S/CO	1.95	0.21	2.04	0.19
	Initial specificity (95% CI)		100% (93.2%-100%)		100% (93.2%-100%)
	N=		52		52
HIV-1 Discriminatory Assay	Average S/CO	1.92	0.17	2.00	0.14
	Initial specificity (95% CI)		100% (93.2%-100%)		100% (93.2%-100%)
	N=		52		52
HCV Discriminatory Assay	Average S/CO	1.95	0.09	1.98	0.10
	Initial specificity (95% CI)		100% (92.9%-100%)		100% (92.9%-100%)
	N=		50		50

SENSITIVITY

Sensitivity for Detection of HIV-1

The sensitivity of the PROCLEIX HIV-1/HCV Assay for the detection of HIV-1 was determined with 50 cadaveric blood and 50 normal donor specimens spiked with a low level of HIV-1 virus (approximately 200 copies/mL) and tested using three reagent lots. The mean analyte S/CO ratio for the 50 cadaveric blood specimens was 16.68 and the mean analyte S/CO for the 50 normal donor specimens was 17.36. There were no significant differences in the S/CO values as determined by Student's t-test (p value 0.44). The initial positivity rate for the cadaveric blood specimens was 96% (95% CI: 86.3%-99.5%) and 100% (95% CI: 92.3%-100%) for the normal donors. The two initial non-reactive cadaveric blood specimens were reactive upon retest.

The sensitivity of the HIV-1 Discriminatory Assay for the detection of HIV-1 was determined with 50 cadaveric blood and 50 normal donor specimens spiked with a low level of HIV-1 virus (approximately 200 copies/mL) and tested using three reagent lots. The mean analyte S/CO ratio for the 50 cadaveric blood specimens was 15.39 and the mean analyte S/CO for the 50 normal donor specimens was 16.76. There were no significant differences in the S/CO values as determined by Student's t-test (p value 0.15). The initial positivity rate for both the cadaveric blood specimens and the normal donor specimens was 98% (95% CI: 89.4%-100%). The one cadaveric and one normal donor specimen that were initially non-reactive were reactive upon retest (Table 22).

Table 22. Reactivity of PROCLEIX® HIV-1/HCV Assay and HIV-1 Discriminatory Assay in Cadaveric Blood Specimens Spiked with HIV-1 virus

		Cadaveric		Controls	
		IC	Analyte	IC	Analyte
HIV-1/HCV Assay	Average S/CO	2.14	16.68	2.03	17.36
	Initial Positivity (95% CI)		96% (86.3%-99.5%)		100% (92.3%-100%)
	N=		50		50
HIV-1 Discriminatory Assay	Average S/CO	2.04	15.39	2.02	16.76
	Initial Positivity (95% CI)		98% (89.4%-100%)		98% (89.4%-100%)
	N=		50		50

Sensitivity for Detection of HCV

The sensitivity of the PROCLEIX HIV-1/HCV Assay for the detection of HCV was determined with 51 cadaveric blood and 50 normal donor specimens spiked with a low level of HCV virus (approximately 200 copies/mL) and tested using three reagent lots. The mean analyte S/CO ratio for the 51 cadaveric blood specimens was 10.50 and the mean analyte S/CO for the 50 normal donor specimens was 10.72. There were no significant differences in the S/CO values as determined by Student's t-test (p value 0.44). The initial positivity rate for the cadaveric blood specimens was 100% (95% CI: 93.0%-100%) and 100% (95% CI: 92.3%- 100%) for normal donors.

The sensitivity of the PROCLEIX HCV Discriminatory Assay for the detection of HCV in the HCV Discriminatory Assay was determined with 51 cadaveric blood and 50 normal donor specimens spiked with a low level of HCV virus (approximately 200 copies/mL) and tested using three reagent lots. The mean analyte S/CO ratio for the 51 cadaveric blood specimens was 23.05 and the mean analyte S/CO for the 50 normal donor specimens was 21.94. There were no significant differences in the S/CO values as determined by Student's t-test (p value 0.16). The initial positivity rate for the cadaveric blood specimens was 100% (95% CI: 93.0%-100%) and 98% (95% CI: 89.4%-100%) for the normal donors. The one normal donor specimen that was initially non-reactive was reactive upon retest (Table 23).

Table 23. Reactivity of PROCLEIX® HIV-1/HCV Assay and HCV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HCV virus

		Cadaveric		Controls	
		IC	Analyte	IC	Analyte
HIV-1/HCV Assay	Average S/CO	2.09	10.50	2.05	10.72
	Initial Positivity (95% CI)		100% (93.0%-100%)		100% (92.3%-100%)
	N=		51		50
HCV Discriminatory Assay	Average S/CO	2.02	23.05	2.03	21.94
	Initial Positivity (95% CI)		100% (93.0%-100%)		98% (89.4%-100%)
	N=		51		50

LIMITATIONS OF THE PROCEDURE

This assay has been evaluated with the PROCLEIX® instrument only.

The concentrations for HIV-1 subtype N and group O virus used for assessing analytical sensitivity were determined by an in-house quantitative test, which used the same technology as the PROCLEIX assays. This may result in inaccurate assessment of analytical sensitivity for these viral subtypes.

The PROCLEIX HIV-1/HCV 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 HIV-1/HCV Assay has been evaluated only for specimens with viral concentrations equal to or greater than 100 copies RNA/mL or those with unknown viral concentration.

CONCLUSIONS

Overall specificity for the PROCLEIX® HIV-1/HCV Assay (16-member pools, single donors), HIV-1 Discriminatory Assay (single donors) and HCV Discriminatory Assay (single donors) is shown in Table 24. Sensitivity for the PROCLEIX HIV-1/HCV Assay (based on known HIV-1 and HCV RNA positives run neat and diluted 1:16), HIV-1 Discriminatory Assay (based on known HIV-1 RNA positives run neat), and HCV Discriminatory Assay (based on known HCV RNA positives run neat) are also shown in Table 24.

Table 24. Overview

		Specificity (95% C. I.)	Sensitivity (95% C. I.)
PROCLEIX® HIV-1/HCV	16-member Pool	99.67% (99.55-99.77%)	99.3% (98.8-99.6%)*
	Individual donation	99.87% (99.83-99.91%)	99.8% (99.4-99.9%)
HIV-1 Discriminatory	Individual donation	99.76% (99.48-99.91%)	100% (99.6-100%)
HCV Discriminatory	Individual donation	99.71% (99.41-99.88%)	99.6% (99.0-99.9%)

C. I. = Confidence intervals.
*Sensitivity in 2,012 known-positive specimens diluted 1:16; Sensitivity was 100% in 204 pools.