Procleix® Ultrio® Assay

For *In Vitro* Diagnostic Use

IVD

Rx Only

1000 Test Kit, 5000 Test Kit

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GENERAL INFORMATION

INTENDED USE

The Procleix Ultrio Assay is a qualitative in vitro nucleic acid assay system to screen for human immunodeficiency virus type I (HIV-1) RNA, hepatitis C virus (HCV) RNA and hepatitis B virus (HBV) DNA in plasma and serum specimens from individual human donors, including donors of whole blood, blood components, and source plasma, and from other living donors. It is also intended for use in testing plasma and serum specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens from cadaveric (non-heart-beating) donors.

The assay is not intended for use on cord blood specimens.

The assay is intended for use in testing individual samples from living donors of whole blood, blood components, and source plasma, other living donors and heart-beating organ donors, and for testing individual blood specimens from cadaveric (non-heart-beating) donors. It is also intended for use in testing pools of human plasma comprised of equal aliquots of not more than 16 individual donations from donors of whole blood, blood components, 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[1], and from donors of donor lymphocytes for infusion (DLI). This assay is intended to be used in conjunction with licensed tests for detecting antibodies to HIV-1, HCV, and hepatitis B core antigen (anti-HBc), and with licensed tests for hepatitis B surface antigen (HBsAg).

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

The Procleix Ultrio Assay can be considered a supplemental test that confirms HIV-1 infection for specimens that are 1) repeatedly reactive on a licensed donor screening test for antibodies to HIV-1, and reactive on both the Procleix Ultrio Assay and on the Procleix HIV-1 Discriminatory Assay and 2) specimens that are repeatedly reactive on a licensed donor screening assay for antibodies to HIV-1, negative in a minipool with the Ultrio Assay and reactive with the Procleix Ultrio HIV-1 Discriminatory Assay.

The Procleix Ultrio Assay can be considered a supplemental test that confirms HCV infection for specimens that are 1) repeatedly reactive on a licensed donor screening test for antibodies to HCV, and reactive on both the Procleix Ultrio Assay and on the Procleix HCV Discriminatory Assay and 2) specimens that are repeatedly reactive on a licensed donor screening assay for antibodies to HCV, negative in a minipool with the Ultrio Assay and reactive with the Procleix Ultrio HCV Discriminatory Assay.

The Procleix Ultrio Assay can be considered a supplemental test that confirms HBV infection for specimens that are 1) repeatedly reactive on a licensed donor screening test for HBsAg, and reactive on both the Procleix Ultrio Assay and on the Procleix HBV Discriminatory Assay and 2) specimens that are repeatedly reactive on a licensed donor screening assay for HBsAg, negative in a minipool with the Procleix Ultrio Assay and reactive with the Procleix Ultrio HBV 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] hepatitis C virus (HCV)[9–14] as the etiological agent for most bloodborne non-A, non-B hepatitis (NANBH), and hepatitis B virus (HBV) as the etiological agent for infectious serum hepatitis. HIV-1, HCV, and HBV 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 Nucleic Acid Testing (NAT) for HIV-1 RNA detection[26, 27, 29, 30] and/or serologic screening for anti-viral antibodies, with confirmation by supplemental antibody tests such as Western blot or immunofluorescence assays. In addition, depending on the NAT assay of use, p24Ag assays followed by confirmation by neutralization are used. The addition of nucleic acid-based amplification tests has reduced the window period of detection by 6 to 11 days, preventing more than half of the HIV-1 infections by blood transfusion.[16-18, 28]

Current detection of HCV infection in the blood bank setting is based on NAT for HCV RNA detection[26, 27, 29, 30] and/or serologic screening for anti-viral antibodies and confirmation with a Strip Immunoblot Assay. The introduction of nucleic acid-based amplification tests for HCV RNA has allowed detection of HCV infection approximately 59 days earlier than the current antibody-based tests.[18, 28]

Current detection of HBV infection in the blood bank setting is based on NAT for HBV DNA detection[35] and/or serological screening for HBsAg, with confirmation by confirmatory neutralization tests, and for anti-HBc. Data from post-transfusion cases indicate that HBsAg is first detected 50 to 60 days following transfusion.[15]

The Procleix Ultrio Assay utilizes target amplification nucleic acid probe technology for the detection of HIV-1 RNA, HCV RNA, and HBV DNA.[19, 26] The assay contains reagents which may be used for simultaneous detection of all three viruses or the individual viruses: HIV-1, HCV, and HBV. The Procleix Assays incorporate an Internal Control for monitoring assay performance in each individual specimen.

PRINCIPLES OF THE PROCEDURE

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

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

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

Internal Control is added to each test specimen, control (if used), or assay calibrator tube via the working Target Capture Reagent that contains the Internal Control. The Internal Control in this reagent controls for specimen processing, amplification, and detection steps. Internal Control signal in each tube or assay reaction is discriminated from the HIV-1/HCV/HBV signal by the differential kinetics of light emission from probes with different labels.[22] Internal Control-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to HIV-1/HCV/HBV is detected using probes with relatively slower kinetics of light emission (glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels.[22] When used for the simultaneous detection of HIV-1, HCV, and HBV, the Procleix Ultrio Assay differentiates between Internal Control and combined HIV-1/HCV/HBV signals but does not discriminate between individual HIV-1, HCV, and HBV signals.

Specimens found to be reactive in the Procleix Ultrio Assay must be run in individual HIV-1, HCV, and/or HBV Discriminatory Assays to determine if they are reactive for HIV-1, HCV, HBV or any combination of the three.

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

Procleix Ultrio Assay Calibrators are used with the Procleix Ultrio Assay and the Procleix HIV-1, HCV, and HBV Discriminatory Assays to determine the assay cutoff and assess assay run validity in each run. All four calibrators contain preserved processed human plasma. Each Positive Calibrator has been spiked to a predetermined concentration of virus. The HIV-1 RNA, HCV RNA, and HBV DNA were obtained from individual units of heat-inactivated plasma found positive for HIV-1, HCV, or HBV.

Procleix Ultrio Tigris Controls are placed periodically throughout the worklist. This practice is known as Control Bracketing. The Procleix Tigris System will automatically analyze control data and determine if results within a bracket are acceptable. A minimum of one set of controls is required per worklist. The control frequency can be configured by a system administrator. See *Procleix Tigris System Quick Reference Guide* for details. The Procleix Ultrio Controls will be valid or invalid as determined by the expected S/CO values and by the assay software.

The Procleix System Fluid Preservative is a system fluid preservative concentrate used to inhibit microbial growth in the System Fluid Bottle and hydraulic pipettor lines of the Procleix Tigris System.

REAGENTS

Procleix Ultrio Assay Reagents

Internal Control Reagent

A HEPES buffered solution containing detergent and an RNA transcript.

*Store **unopened reagent** at –35° to –15°C.*

Target Capture Reagent

A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles.

Internal Control Reagent must be added to Target Capture Reagent before use in the assay.

Store at 2° to 8°C. (Do not freeze)

Amplification Reagent

Primers, dNTPs, NTPs and cofactors in TRIS buffered solution containing ProClin 300 preservative.

*Store **unopened reagent** at –35° to –15°C.*

Enzyme Reagent

MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffered solution containing 0.05% sodium azide as preservative.

*Store **unopened reagent** at –35° to –15°C.*

Probe Reagent

Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.

*Store **unopened reagent** at –35° to –15°C.*

Selection Reagent

Borate buffered solution containing surfactant.

Store at 15° to 30°C.

Procleix Ultrio Assay Calibrators

Negative Calibrator

C0

Defibrinated normal human plasma (nonreactive for HIV-1/2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.

Store at –15° to –35°C.

HIV-1 Positive Calibrator

C1

Inactivated HIV-1 positive plasma in defibrinated normal human plasma (nonreactive for HIV-2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.

Store at –15° to –35°C.

HCV Positive Calibrator

C2

Inactivated HCV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.

Store at –15° to –35°C.

HBV Positive Calibrator

C3

Inactivated HBV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HCV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.

Store at –15° to –35°C.

Procleix Ultrio Discriminatory Probe Reagents

D1

HIV-1 Discriminatory Probe Reagent

Chemiluminescent oligonucleotide probe in a succinate buffered solution containing detergent.

*Store **unopened reagent** at –15° to –35°C*

D2

HCV Discriminatory Probe Reagent

Chemiluminescent oligonucleotide probe in a succinate buffered solution containing detergent.

*Store **unopened reagent** at –15° to –35°C*

D3

HBV Discriminatory Probe Reagent

Chemiluminescent oligonucleotide probe in a succinate buffered solution containing detergent.

*Store **unopened reagent** at –15° to –35°C*

Procleix Ultrio Tigris Controls

T0

Ultrio Tigris Negative Control

Defibrinated normal human plasma (nonreactive for HIV-1/2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.

Store at –15° to –35°C

T1

Ultrio Tigris HIV-1 Control

Inactivated HIV-1 positive plasma in defibrinated normal human plasma (nonreactive for HIV-2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.

Store at –15° to –35°C

T2

Ultrio Tigris HCV Control

Inactivated HCV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.

Store at –15° to –35°C

T3

Ultrio Tigris HBV Control

Inactivated HBV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HCV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.

Store at –15° to –35°C

Procleix Tigris System Reagents

Auto Detect 1

Aqueous solution containing hydrogen peroxide and nitric acid.

*Store **unopened reagent** at 15° to 30°C.*

Auto Detect 2

1.6 N sodium hydroxide.

*Store **unopened reagent** at 15° to 30°C.*

Wash Solution

HEPES buffered solution.

*Store **unopened reagent** at 15° to 30°C.*

Oil

Silicone oil.

*Store **unopened reagent** at 15° to 30°C.*

Buffer for Deactivation Fluid

Sodium bicarbonate buffered solution. Must be mixed 1:1 with bleach (5% sodium hypochlorite) before use.

*Store **unopened reagent** at 15° to 30°C.*

Procleix System Fluid Preservative

Procleix System Fluid Preservative

Procleix System Fluid Preservative contains 2.5% sodium hypochlorite that inhibits microbial growth in aqueous media. The solution is basic.

*Store **unopened reagent** at 15° to 30°C.*

STORAGE AND HANDLING INSTRUCTIONS

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

B. The Procleix Ultrio Assay Probe Reagent and the Discriminatory Probe Reagents are light-sensitive. Protect these reagents from light during storage.

C. Do not use reagents or fluids after the expiration date.

D. Do not use assay-specific reagents from any other Procleix Assay.

E. If a precipitate forms in the Target Capture Reagent (TCR) during storage, see instructions under PROCLEIX SYSTEM USERS or PROCLEIX TIGRIS SYSTEM USERS, REAGENT PREPARATION. DO NOT VORTEX. DO NOT FREEZE TCR.

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.

F. Do not refreeze Internal Control, Amplification, Enzyme, Probe, HIV-1, HCV, and HBV Discriminatory Probe Reagents after the initial thaw.

G. Calibrators are single use vials and must be discarded after use.

H. If precipitate forms in the Wash Solution, Selection Reagent, Probe Reagent, or HIV-1, HCV, or HBV Discriminatory Probe Reagents, see instructions under PROCLEIX SYSTEM USERS or PROCLEIX TIGRIS SYSTEM USERS, REAGENT PREPARATION.

I. 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 once resuspended (e.g., obvious changes in reagent color or cloudiness indicative of microbial contamination), they should not be used.

J. Consult the following table for storage information.

Reagent/Fluid	Unopened Storage	Opened/Thawed Stability (up to expiration date)
Internal Control Reagent (IC)	-15° to -35°C until the expiration date	Prior to combining with TCR, 8 hours at RT*
Target Capture Reagent (TCR)	2° to 8°C until the expiration date	
working Target Capture Reagent (wTCR)		30 days at 2° to 8°C; 80 hours at RT**
Probe Reagents	-15° to -35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT**
Amplification Reagent	-15° to -35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT**
Enzyme Reagent	-15° to -35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT**
Selection Reagent	RT until the expiration date	30 days at RT
Calibrators	-15° to -35°C until the expiration date	8 hours at RT
Controls	-15° to -35°C until the expiration date	8 hours at RT
Auto Detect Reagents	RT until the expiration date	30 days at RT
Buffer for Deactivation Fluid	RT until the expiration date	30 days at RT
Deactivation Fluid	RT until the expiration date	30 days at RT
Oil	RT until the expiration date	30 days at RT
Wash Solution	RT until the expiration date	30 days at RT

* RT = Room Temperature

** The 80 hours must occur within the 30 days

K. The Procleix System Fluid Preservative is stable when stored unopened at 15° to 30°C until the expiration date. Once opened, the Procleix System Fluid Preservative is stable for 30 days. The final System Fluid (Procleix System Fluid Preservative and water for the Procleix Tigris System) is then stable for up to 14 days when on the Procleix Tigris System. For water specifications for the Procleix Tigris System see the *Procleix Tigris System Operator's Manual*.

SPECIMEN COLLECTION, STORAGE, AND HANDLING

Note: Handle all specimens as if they are capable of transmitting 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. Blood specimens collected in glass or plastic tubes may be used.

B. Plasma collected in K_2EDTA, K_3EDTA, ACD, sodium citrate, or in Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT) may be used. Follow sample tube manufacturer's instructions. Specimen stability is affected by elevated temperature.

Whole blood or plasma from pooled or individual donor specimens may be stored for a total of 8 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

For storage above 8°C, specimens may be stored for 72 hours up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example storage temperature chart under step D.

In addition, plasma separated from the cells may be stored for up to 6 months at ≤ -20°C before testing.

Do not freeze whole blood.

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

D. Additional specimens collected in serum tubes or heparin tubes according to the collection container manufacturer's instructions, may be used.

Whole blood, plasma, or serum may be stored for a total of 8 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

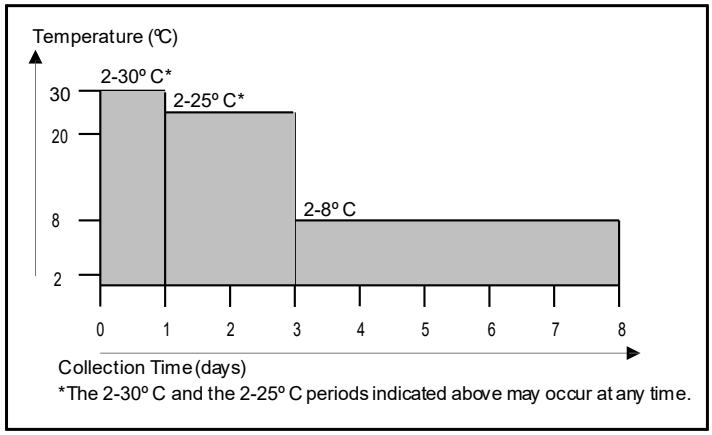
For storage above 8°C, specimens may be stored for 72 hours up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example storage temperature chart below.

Long-term storage of serum and heparinized plasma has not been evaluated.

Do not freeze whole blood.



E. 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) may be stored for a total of 18 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 13 days of draw.

For storage above 8°C, specimens may be stored for 72 hours up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

In addition, plasma separated from the cells may be stored for up to 6 months at ≤ -20°C before testing.

Do not freeze whole blood.

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

G. 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.

H. 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.

I. 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.

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

K. Specimen Pooling

The pooling software, used in combination with a front-end pipettor, performs sample scanning and pooling operations that combine aliquots from individual samples into a single Master Pool Tube, which may be used for further testing.

Note: Only specimens from donors of whole blood, blood components, source plasma, HPCs, or DLI may be pooled. Pooling of serum specimens has not been validated.

CADAVERIC BLOOD SPECIMENS

Note: A serum or plasma specimen collected from a donor prior to death may be tested instead of a cadaveric blood specimen using either the instructions for cadaveric donor specimens or the instructions for living donor blood specimens.

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 a total of 8 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

For storage above 8°C, specimens may be stored for 24 hours at up to 25°C during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example temperature chart below.

In addition, plasma separated from the cells may be stored for up to 14 days at ≤ -70°C before testing.

Do not freeze whole blood.

D. Whole blood (clot tube) or serum may be stored for a total of 5 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

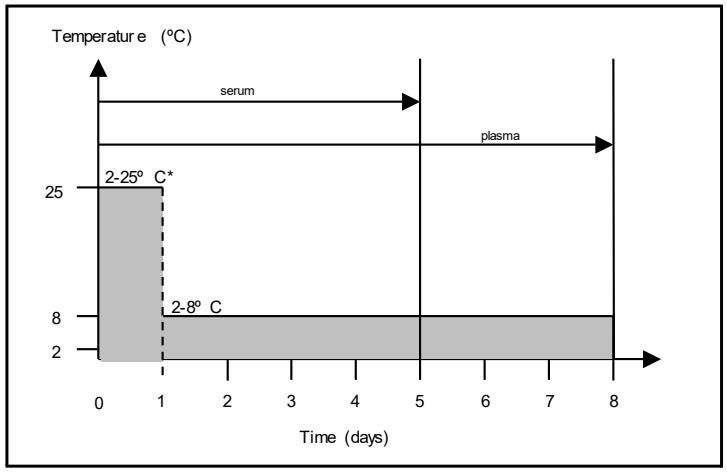
For storage above 8°C, specimens may be stored for 24 hours at up to 25°C during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example temperature chart below.

In addition, serum removed from the clot tube may be stored for up to 14 days at ≤ -70°C before testing.

Do not freeze whole blood.



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

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.

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 standard assay procedure by pipetting the 500 µL of the diluted specimen into the TTU containing TCR.

Note: If a front-end pipettor 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, HCV, and HBV *in vivo* post-mortem was not assessed.

PROCLEIX SYSTEM USERS

MATERIALS REQUIRED

Component	Part Number	Part Number
Procleix Ultrio Assay Reagents	**301103** (1000 Test Kit)	**301105** (5000 Test Kit)
Internal Control Reagent	2 x 5 mL	10 x 5 mL
Target Capture Reagent	2 x 280 mL	10 x 280 mL
Amplification Reagent	2 x 50 mL	10 x 50 mL
Enzyme Reagent	2 x 18 mL	10 x 18 mL
Probe Reagent	2 x 75 mL	10 x 75 mL
Selection Reagent	2 x 180 mL	10 x 180 mL
Procleix Negative Calibrator	30 x 2 mL	90 x 2 mL
Procleix HIV-1 Positive Calibrator	30 x 2 mL	90 x 2 mL
Procleix HCV Positive Calibrator	30 x 2 mL	90 x 2 mL
Procleix HBV Positive Calibrator	30 x 2 mL	90 x 2 mL
Procleix HIV-1, HCV, and HBV Discriminatory Probe Reagents Kit	**PRD-03708** (200 tests)	
Procleix HIV-1 Discriminatory Probe Reagent	2 x 14 mL	
Procleix HCV Discriminatory Probe Reagent	2 x 14 mL	
Procleix HBV Discriminatory Probe Reagent	2 x 14 mL	
Procleix Wash Solution	2 x 2.9 L	303665
Procleix Oil	4 x 260 mL	302441
Procleix Buffer for Deactivation Fluid	2 x 1.4 L	303666
Procleix Auto Detect Reagents	**301120** (1000 tests)	
Auto Detect 1	2 x 240 mL	
Auto Detect 2	2 x 240 mL	

Disposables

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

Ten-Tube Units (TTUs)	TU0040
Ten Tip Cassettes	104578
Sealing Cards	102085

Equipment/Software

Procleix Xpress Pipettor and Software (for pooling only), Tecan Genesis RSP instrument or Procleix AP instrument (front-end pipettors), Procleix Assay Software, and operator's manual; or Procleix Worklist Editor software and operator's manual

Procleix TCS and operator's manual

Procleix HC+ Luminometer, Procleix Ultrio System Software, and operator's manual

Multi-tube Vortex Mixer (Vortexer)

Water bath

Dedicated fixed or adjustable repeat pipettors capable of delivering 25–500 µL of liquid with a ±5% accuracy and a coefficient of variation of ≤ 5%.

Other

Procleix System Quick Reference Guide (Procleix System QRG)

Any applicable Technical Bulletins

OTHER MATERIALS AVAILABLE FROM GRIFOLS FOR USE WITH THE PROCLEIX ULTRIO ASSAY

Procleix Ultrio Assay Calibrators Kit	**301106**	
Procleix Negative Calibrator	30 x 2 mL	
Procleix HIV-1 Positive Calibrator	30 x 2 mL	
Procleix HCV Positive Calibrator	30 x 2 mL	
Procleix HBV Positive Calibrator	30 x 2 mL	
Procleix Ultrio Negative Calibrator Kit	90 x 2 mL	303259
Procleix HIV-1 Discriminatory Probe Reagent Kit	2 x 14 mL	301107
Procleix HCV Discriminatory Probe Reagent Kit	2 x 14 mL	301108
Procleix HBV Discriminatory Probe Reagent Kit	2 x 14 mL	301109
General Equipment/Software		
Procleix Reagent Preparation Incubator (RPI), independent temperature monitor (ITM), and operator's manual		
Procleix CPT Pooling Software and operator's manual		

For instrument specifics and ordering information, contact Grifols Customer Service.

MATERIALS REQUIRED BUT NOT PROVIDED

Repeat pipettor tips (1.25 mL, 5.0 mL, 10.0 mL, 12.5 mL)

If using the Manual Sample Pipetting Method: Filtered fixed pipettor tips capable of delivering 500 µL (for samples) and repeat pipettor tips capable of delivering 400 µL (for wTCR)

If using the Tecan Genesis RSP instrument or the Procleix AP instrument: Disposable conductive filter tips in rack approved for use with equipment and front-end pipettor reagent troughs

Bleach
For use in final concentrations of 5% sodium hypochlorite and 0.5% sodium hypochlorite

Bleach alternative (optional)
Contact Grifols Technical Service 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 a repeat pipettor tip.

PRECAUTIONS

A. **For *in vitro* diagnostic use.**

B. When performing testing with different Procleix Assays using shared instrumentation, ensure appropriate segregation is maintained to prevent mix-up of samples during processing (e.g., use of colored TTU racks). In addition, verify that the correct set of reagents is being used for the assay that is being run.

C. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix Ultrio Assay and the Procleix System QRG prior to performing an assay run.

D. Specimens may be infectious. Use Universal Precautions[23, 25] when performing the assay. Proper handling and disposal methods should be established according to local, state and federal regulations.[24] Only personnel adequately qualified as proficient in the use of the Procleix Ultrio Assay and trained in handling infectious materials should perform this procedure.

E. **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 HBV Positive Calibrator contains human plasma that is HBV positive and has been heat-treated to inactivate the virus. The Negative Calibrator has been assayed by FDA-licensed tests and found nonreactive for HIV-1/2, HCV, and HBV. 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, 25] 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/RPI areas only. Do not use bleach alternatives in Amplification areas or in areas suspected to be contaminated with Amplification products.**

F. 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.

G. 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.

H. 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 and follow appropriate site procedures.

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

J. Use only supplied or specified required disposables.

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

L. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present. See REAGENT PREPARATION for specific instructions.

M. Avoid microbial and ribonuclease contamination of reagents. Use of filtered, disposable pipette tips is strongly recommended.

N. 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 PROCLEIX SYSTEM USERS, REAGENT PREPARATION for specific instructions.

O. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See SPECIMEN COLLECTION, STORAGE, AND HANDLING for specific instructions.

P. Only combine assay reagents or fluids as instructed to by the Procleix Ultrio Assay package insert.

Q. Some reagents of this kit are labeled with risk and safety symbols and should be handled accordingly. Safety Data Sheets are accessible on the manufacturer's website

Procleix Selection Reagent	
 	*Boric Acid 3.63 Weight-%* **DANGER** Harmful if inhaled May damage fertility or the unborn child Obtain special instructions before use Do not handle until all safety precautions have been read and understood Use personal protective equipment as required Avoid breathing dust/fume/gas/mist/vapors/spray Use only outdoors or in a well-ventilated area IF exposed or concerned: Get medical advice/attention IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing Store locked up Dispose of contents/container to an approved waste disposal plant
	*Ethyl Alcohol 2.33 Weight-%* **DANGER** May cause cancer Obtain special instructions before use Do not handle until all safety precautions have been read and understood Use personal protective equipment as required IF exposed or concerned: Get medical advice/attention Store locked up Dispose of contents/container to an approved waste disposal plant
	*Ethyl Alcohol 2.33 Weight-%* **DANGER** May cause cancer Obtain special instructions before use Do not handle until all safety precautions have been read and understood Use personal protective equipment as required IF exposed or concerned: Get medical advice/attention Store locked up Dispose of contents/container to an approved waste disposal plant

Procleix Buffer for Deactivation Fluid	
	*Sodium Hydroxide 1.12 Weight-%* *Sodium Hypochlorite 0.49 Weight-%* **WARNING** Causes skin irritation Causes serious eye irritation Wash face, hands and any exposed skin thoroughly after handling Wear protective gloves/protective clothing/eye protection/face protection IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing If eye irritation persists: Get medical advice/attention IF ON SKIN: Wash with plenty of soap and water If skin irritation occurs: Get medical advice/attention Take off contaminated clothing and wash before reuse
Procleix Auto Detect 2	
	*Sodium Hydroxide 6.04 Weight-%* **DANGER** Causes severe skin burns and eye damage Do not breathe dust/fume/gas/mist/vapors/spray Wash face, hands and any exposed skin thoroughly after handling Wear protective gloves/protective clothing/eye protection/face protection Immediately call a POISON CENTER or doctor/physician IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing Immediately call a POISON CENTER or doctor/physician IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower Wash contaminated clothing before reuse IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing Immediately call a POISON CENTER or doctor/physician IF SWALLOWED: Rinse mouth. Do NOT induce vomiting Store locked up Dispose of contents/container to an approved waste disposal plant

R. Refer to precautions in the appropriate Procleix Assay package inserts, operator's manuals, and the Procleix System QRG..

S. Each Calibrator is designed to be run in duplicate or triplicate and excess material in each vial is to be appropriately discarded according to local, state, and federal regulations.

REAGENT PREPARATION

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

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

B. Verify that reagents have not exceeded the expiration date and/or storage stability times.

C. Remove a bottle of Selection Reagent from room temperature storage.

Note: The Selection Reagent must be at room temperature before use.

1. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls between 2° and 15°C, precipitate may form.
2. If precipitate forms in the Selection Reagent, 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, perform Selection Reagent recovery as described in the *Procleix Reagent Preparation Incubator Operator's Manual*. Do not use the Selection Reagent until it has equilibrated.
3. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
4. Do not use if precipitate or cloudiness persists.
5. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

D. Warm all reagents to room temperature and mix thoroughly prior to use. A dedicated water bath at room temperature or the RPI may be used to aid this process. If using the RPI to warm the TCR, Probe Reagent, Discriminatory Probe Reagents, Enzyme Reagent, and Amplification Reagent, refer to the Procleix System QRG.

1. If using a water bath, thaw reagents upright.
2. Amplification, HIV-1 Discriminatory Probe, HCV Discriminatory Probe, HBV Discriminatory Probe, and Probe Reagents may be mixed by vortexing.

3. Enzyme Reagent should be mixed thoroughly by gentle inversion, taking care to avoid excessive foaming.
4. Record the date of thaw (THAW DATE) for each reagent on the space provided on the label.
5. After thawing, the HIV-1 Discriminatory Probe, HCV Discriminatory Probe, and HBV Discriminatory Probe Reagents are stable when stored at 2° to 8°C for 30 days. Within the 30 days, these reagents may be kept at room temperature up to a total of 80 hours. Do not refreeze these reagents after initial thaw.

E. If using a water bath, DO NOT heat the Probe Reagent, the HIV-1 Discriminatory Probe Reagent, the HCV Discriminatory Probe Reagent or the HBV Discriminatory Probe Reagent above 30°C. If using the RPI, DO NOT heat the Probe Reagent, the HIV-1 Discriminatory Probe Reagent, the HCV Discriminatory Probe Reagent or the HBV Discriminatory Probe Reagent above 35°C. Refer to the Procleix System QRG.

F. The Procleix Ultrio Assay Probe Reagent and the Discriminatory Probe Reagents are light-sensitive. Protect these reagents from light during storage.

G. Probe Reagent and Discriminatory Probe Reagents may be warmed in a water bath to facilitate dissolution of precipitate, but the temperature of the water bath should not exceed 30°C. Precipitate will form in the Probe Reagent and Discriminatory Probe Reagents when stored at 2° to 8°C. If incubation is conducted on the lab bench, Probe Reagent and Discriminatory Probe Reagents may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate. Alternatively, use the RPI to thaw the Probe Reagent and Discriminatory Probe Reagents at an average temperature of 32° ± 2°C, not to exceed 35°C. Refer to the Procleix System QRG. If precipitate is still present after thawing, the Probe Reagent can be incubated at room temperature in a water bath or the RPI to facilitate complete dissolution of precipitate, as long as the total time at room temperature does not exceed 80 hours. Ensure that precipitates in the Probe Reagent and Discriminatory Probe Reagents are dissolved. Do not use if precipitate or cloudiness is present.

H. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. 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.

Note: 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.

3. Thaw one vial of Internal Control Reagent up to 24 hours at 2° to 8°C or up to 8 hours at room temperature. **Do not use the RPI to thaw Internal Control Reagent.**
4. Mix the Internal Control Reagent thoroughly by gentle manual inversion. mechanical inversion using a laboratory rocker, or vortexing.

Note: If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature. To expedite the dissolution of gel, warm the Internal Control Reagent at 25° to 30°C in a water bath. Periodically remove Internal Control Reagent from water bath to gently invert until gel is dissolved. Dry the exterior of the tube prior to opening.

5. 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. This is now the working Target Capture Reagent (wTCR). Mix thoroughly.
6. 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 wTCR in the space provided on the label.

I. Thaw calibrators at room temperature. **Do not use the RPI to thaw Procleix Ultrio Assay Calibrators.**

Note: These are single use vials and must be thawed prior to each run.

1. Mix thoroughly by gentle inversion. Avoid reagent foaming.
2. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
3. Once thawed, use calibrators within 8 hours.

J. 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 between 2° and 15°C. Wash Solution may be warmed in a water bath to facilitate dissolution of precipitate. **Do not use the RPI to warm the Wash Solution**. The temperature in the water 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.

K. Discriminatory Probe reagents can be run with any matched set of reagents (Amplification Reagent, Enzyme Reagent, Probe Reagent, Selection Reagent, and TCR) within each master lot.

L. For Wash Solution, Oil, Selection Reagent, Buffer for Deactivation Fluid, Auto Detect 1, and Auto Detect 2, record the date the reagent was first opened (OPEN DATE) in the space provided on the label. Procleix Assay Fluids and Procleix Auto Detect Reagents are stable for 30 days when stored at room temperature.

M. To prepare Deactivation Fluid, mix one part Buffer for Deactivation Fluid with one part 5% sodium hypochlorite. Record the date the Deactivation Fluid was prepared.

PROCEDURAL NOTES

Note: Refer to the Procleix System QRG for maintenance procedures and information about software operation.

Note: Procleix Assay Fluids and Procleix Auto Detect Reagents may be used with any master lot of Procleix Assay Reagents.

A. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix Ultrio Assay prior to performing an assay run. This package insert must be used with the Procleix System QRG and any applicable Technical Bulletins.

B. RUN SIZE

1. Kit test size is based on an average run size of 55 tests. Smaller run sizes will result in a lower number of tests performed per kit.
2. Each Procleix Ultrio Assay run will yield up to 100 test results, including results for external quality controls (if used), three replicates of the Negative Calibrator, and two replicates each of the HIV-1 Positive Calibrator, the HCV Positive Calibrator, and the HBV Positive Calibrator.
3. Each Procleix HIV-1, HCV, or HBV Discriminatory Assay run will yield up to 100 test results, including results for external quality controls (if used), three replicates of the Negative Calibrator, three replicates of the corresponding Positive Calibrator, and two replicates of each of the other two Positive Calibrators.

C. EQUIPMENT PREPARATION

1. Three dedicated 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 (62° ± 1°C). An additional container of water is required to be maintained at 23° ± 4°C for the step preceding detection.
2. Equilibrate water baths to 60° ± 1°C for target capture and 41.5° ± 1°C for amplification incubations.
3. If using a front-end pipettor, set up according to instructions in the Procleix System QRG.
4. Prepare the target capture system 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/RPI 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 water bath to 62° ± 1°C for hybridization and selection incubations. Equilibrate a container of water at 23° ± 4°C for cool down prior to detection.
7. Prepare the luminometer according to instructions in the Procleix System QRG.

D. REAGENTS

1. Add all reagents using a repeat pipettor 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, HBV 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/RPI 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.
3. A color change will occur in the reaction tube after the addition of each of the following reagents: Amplification, Enzyme, Probe, and Selection.

E. RUN CONFIGURATION

1. Each worklist must have a set of calibrators at the beginning.
2. For the Procleix Ultrio Assay and Discriminatory Assays, a set of calibrators consists of one vial each of Negative Calibrator, HIV-1 Positive Calibrator, HCV Positive Calibrator, and HBV Positive Calibrator.

F. 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 Hybridization Protection Assay (HPA) area back into previous work areas without proper anti-contamination safeguards.
2. Perform reagent preparation in a clean (amplicon- and template-free) area.
3. Perform Sample Preparation, 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.
5. After pipetting specimens (individual or pooled) into TTUs, remove the TTUs from the deck and load them into a TTU rack. If the same specimens will be tested with a different Procleix Assay, the specimens may be left on the deck, but the empty calibrator tubes and TCR trough must be discarded. Change gloves after discarding the used calibrator tubes and TCR trough, then load new TTUs into the TTU carriers. See PROCLEIX SYSTEM USERS, PRECAUTIONS, step B for additional information.

G. ENVIRONMENTAL CONDITIONS

1. The Target Capture, Amplification, Hybridization, and Selection steps 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.
5. Refer to instrument and software operator's manuals for additional environmental conditions requirements.

H. TIME

The Target Capture, Amplification, and Hybridization Protection Assay steps are all time dependent. Adhere to specific times outlined in PROCLEIX SYSTEM USERS, ASSAY PROCEDURE.

I. VORTEXING

Proper vortexing is important to the successful performance of the Procleix Ultrio Assay. Vortex equipment speed settings may vary. The vortexer speed should start at a low level and increase until the speed is adequate to achieve the desired results without allowing the reaction mixture to touch the sealing cards. For each step that requires vortexing, it is critical that the contents of the tubes be well-mixed.

J. PIPETTING

1. Operator pipetting precision is critical to assay performance.
2. All pipettors used in the Target Capture, Amplification, and HPA steps must be dedicated to avoid cross-contamination.
3. Take care to deliver reagents, excluding wTCR, to each tube without inserting the pipette tip into the tube or touching the rim of the tube to minimize the chance of carryover from one tube to another.
4. When adding Oil, Probe Reagent, and Selection Reagent, angle the pipette tip toward the sides of the tube, not straight to the bottom, to avoid splashback.

K. MANUAL SPECIMEN PIPETTING

1. When using the manual sample/wTCR pipetting method, improper pipetting technique will affect the results of the assay.
2. In order to avoid the loss of Positive ID Tracking, verification of correct sample ID by a second individual is recommended.
3. 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.
4. 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.
5. To avoid the risk of contamination, clean and decontaminate manual sample pipettors between assay runs.
6. Ensure proper sample placement into the correct TTU position as indicated on the manual worklist record.

L. 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 0.5% sodium hypochlorite in water (diluted bleach). 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/RPI areas only. Do not use bleach alternatives in Amplification areas or in areas suspected to be contaminated with Amplification products.
3. Reaction tubes must be decontaminated with Deactivation Fluid as described in the Procleix System QRG.
4. Follow instructions provided in the Procleix System QRG for instrument decontamination and maintenance procedures.

M. 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 re-use 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.

ASSAY PROCEDURE

Procleix Ultrio Assay Calibrators must be included in each assay run.

Procleix Ultrio Assay Calibrators and Discriminatory Probe Reagents are to be used with the corresponding master lot of Procleix Ultrio and Discriminatory Assays. The operator must check to ensure that the Procleix Ultrio Assay Calibrators and Discriminatory Probe Reagents are used with the corresponding master lot of kit reagents as indicated on the Procleix Ultrio Assay master lot sheet in use. Procleix Auto Detect Reagents and Procleix Assay Fluids may be used with any master lot of Procleix Assay Reagents.

Specimens from other living donors (except whole blood, blood components, source plasma, HPCs, or DLI) 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, J, and retested in singlet.

To run the Procleix Ultrio Assay for the detection of HIV-1 RNA, HCV RNA, and HBV DNA, follow the steps below for Sample Preparation, Target Capture, Amplification, and the Hybridization Protection Assay. To run the Procleix Discriminatory Assays for discrimination between HIV-1 RNA, HCV RNA, and HBV DNA, see the Procleix HIV-1, HCV, AND HBV DISCRIMINATORY ASSAYS section D, prior to proceeding.

Note: For instrument and software steps, refer to the Procleix System QRG.

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

A. SAMPLE PREPARATION/TARGET CAPTURE

Sample Preparation

The Procleix Ultrio Assay has been validated using manual pipetting and a front-end pipettor. The use of manual pipetting requires additional operator training and demonstration of proficiency.

IF USING THE MANUAL SAMPLE PIPETTING METHOD:

The repeat pipettors used in these steps must be dedicated for use only in SAMPLE PREPARATION steps.

For sample tracking, an electronic worklist must be created using the Procleix Worklist Editor software. Refer to the Procleix System QRG for instructions, or contact Grifols Technical Service. 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. The assay results within the run report will be marked M indicating that the specimens were manually pipetted.

1. Load sufficient Ten Tube Units (TTUs) for the run into a TTU rack.
2. Thoroughly mix the wTCR immediately before use to resuspend microparticles.
3. Refer to the worklist and carefully pipette 400 µL of wTCR to each tube that will contain a sample. To dispense, insert the tip approximately one quarter of the way into the tube at an angle and pipette wTCR down the side of the tube. Take care to avoid touching the rim or the side of the tube with the pipette tip. Always pipette the wTCR first, followed by the sample.
4. Pipette samples.
   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 specimen from its collection tube using a single channel pipettor with corresponding filtered disposable tip. Insert only the end of the pipette tip into the sample. 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 sample 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.
5. Replace the pipette tip with a new tip and repeat step 4 until all samples have been pipetted.
6. Visually inspect tubes to ensure proper sample volume and wTCR volume have been dispensed.
7. Cover the TTUs with sealing cards. See PROCLEIX SYSTEM USERS, PROCEDURAL NOTES.
8. Proceed to the Target Capture section.

IF USING A FRONT-END PIPETTOR:

1. Prepare front-end pipettor for automatic pipetting of calibrators, specimens, and wTCR; refer to the Procleix System QRG.
2. Instrument will add 400 µL of wTCR to reaction tubes.
3. Instrument will add 500 µL each of calibrators and test specimens into assigned reaction tubes.
4. When all samples have been pipetted, transfer the TTUs to a TTU rack. Cover the TTUs with sealing cards. See PROCLEIX SYSTEM USERS, PROCEDURAL NOTES on sealing cards.
5. Proceed to the Target Capture section.

Target Capture

1. Vortex the rack of TTUs a minimum of 20 seconds and until magnetic microparticles are resuspended. See PROCLEIX SYSTEM USERS, PROCEDURAL NOTES on vortexing.
2. The rack may remain at room temperature up to 75 minutes prior to proceeding to the 60° ± 1°C incubation.
3. Incubate the tubes in a water bath at 60° ± 1°C for 20 minutes ± 1 minute.
4. Remove the rack of TTUs and transfer to the Target Capture area.
5. Incubate the rack of TTUs on the lab bench at room temperature for 14 minutes to 20 minutes.
6. Transfer the rack of TTUs to the target capture system (TCS) for 9 to 20 minutes.
7. Carefully remove and dispose of the sealing cards.
8. Perform one Aspiration and Wash step using 1 mL of Wash Solution—refer to the Target Capture section of the Procleix System QRG for instructions.
9. Cover the TTUs with sealing cards.
10. Vortex to resuspend the microparticle pellets, then inspect the reaction tubes to make sure that all of the magnetic particles have been uniformly suspended.
11. Place the rack of TTUs on the TCS for 4 to 10 minutes.

12. Carefully remove and dispose of the sealing cards.
13. Repeat steps 8 through 12.
14. Completely aspirate the solution from each tube. Refer to the Target Capture section of the Procleix System QRG.
15. Cover the TTUs with sealing cards.
16. Proceed directly to Amplification.

B. AMPLIFICATION

Do not use bleach alternatives in this area.

The repeat pipettors used in these steps must be dedicated for use only in AMPLIFICATION steps.

1. Carefully remove and dispose of the sealing cards.
2. Add 75 µL of Amplification Reagent to each tube (a color change can be observed in the reaction tube). See PROCLEIX SYSTEM USERS, PROCEDURAL NOTES on pipetting.
3. Add 200 µL of Oil to each tube.
4. Cover the TTUs with sealing cards.
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 uniformly resuspended.
6. Incubate the TTUs in a water bath at 60° ± 1°C for 10 minutes ± 1 minute.
7. Incubate the TTUs in a water bath at 41.5° ± 1°C for 9 to 20 minutes.
8. Leaving the rack of TTUs at 41.5° ± 1°C, carefully remove and dispose of the sealing cards. Immediately add 25 µL of the Enzyme Reagent into each tube (a color change can be observed in the reaction tube). Place new sealing cards over the TTUs.
9. 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.
10. Incubate the rack of TTUs in the water bath at 41.5° ± 1°C for 60 minutes ± 5 minutes.
11. 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)

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.**

The repeat pipettor used in these steps must be dedicated for use only in HYBRIDIZATION PROTECTION ASSAY steps.

1. Hybridization
   a. Carefully remove and dispose of the sealing cards. See PROCLEIX SYSTEM USERS, PROCEDURAL NOTES.
   b. Add 100 µL of Probe Reagent into each tube (a color change can be observed in the reaction tube). See PROCLEIX SYSTEM USERS, PROCEDURAL NOTES on pipetting.
   c. Cover the TTUs with sealing cards. Vortex the rack of TTUs a minimum of 20 seconds and until contents are well-mixed. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. See PROCLEIX SYSTEM USERS, PROCEDURAL NOTES on vortexing.
   d. Incubate the rack of TTUs in a dedicated water bath at 62° ± 1°C for 15 minutes ± 1 minute.
2. Selection
   a. Remove the rack of TTUs from the 62° ± 1°C water bath. Carefully remove and dispose of the sealing cards.
   b. Add 250 µL of Selection Reagent to each tube (a color change can be observed in the reaction tube).
   c. Cover the TTUs with sealing cards. Vortex the rack of TTUs a minimum of 20 seconds and until contents are well-mixed. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. Return the rack of TTUs to the 62° ± 1°C water bath for 10 minutes ± 1 minute.
   d. Cool the rack of TTUs in a 23° ± 4°C container of water for a minimum of 10 minutes while preparing for Detection.
   e. Remove the rack of TTUs from the 23° ± 4°C container of water onto absorbent material.
3. Detection

   Note: Tube readings should be completed within 75 minutes after completing the selection reaction.

For Detection and decontamination, refer to the Procleix System QRG.

D. PROCLEIX HIV-1, HCV, AND HBV DISCRIMINATORY ASSAYS

1. To perform the Discriminatory Assays, make the following modifications to the procedure above:
   a. Perform all SAMPLE PREPARATION/TARGET CAPTURE and AMPLIFICATION steps exactly as they are outlined above. Set up separate runs for HIV-1, HCV, and HBV Discriminatory Assays. All three Discriminatory Assays use the same calibrators that are used in the Procleix Ultrio Assay.
   b. Substitute HIV-1, HCV, or HBV Discriminatory Probe Reagent for Probe Reagent when performing HYBRIDIZATION PROTECTION ASSAY.
   c. Choose the appropriate protocol in the luminometer software (refer to the Procleix System QRG).

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX ULTRIO ASSAY AND PROCLEIX HIV-1, HCV, AND HBV DISCRIMINATORY ASSAYS

A. A run is valid if the minimum number of calibrator replicates is valid and calibrators meet acceptance criteria.

1. In a Procleix Ultrio Assay run, at least 7 of the 9 calibrator replicates must be valid. At least 2 of the 3 Negative Calibrator replicates and 5 of the 6 Positive Calibrator replicates must be valid.
2. In a Procleix HIV-1, HCV, or HBV Discriminatory Assay run, at least seven of the 10 calibrator replicates must be valid. In addition, at least two of the three Negative Calibrator replicates must be valid, and the Positive Calibrator criteria below must be met:
   a. For the HIV-1 Discriminatory Assay, two of the three HIV-1 Positive Calibrator replicates must be valid.
   b. For the HCV Discriminatory Assay, two of the three HCV Positive Calibrator replicates must be valid.
   c. For the HBV Discriminatory Assay, two of the three HBV Positive Calibrator replicates must be valid.
3. The luminometer software will automatically invalidate the run if less than the minimum number of calibrator replicates is valid. All specimens in an invalid run due to calibrators must be retested.
4. In a valid run, Cutoff values will be automatically calculated for Internal Control (flasher) and Analyte (glower).
5. In a valid run, specimens with an Analyte Signal (glower signal) greater than the Analyte Cutoff are not invalidated even if the Internal Control signal is below the cutoff. Specimens with an Internal Control signal above 550,000 RLU are invalidated by the software and the reactive status cannot be assessed. Positive Calibrators with an Internal Control signal above 475,000 RLU are invalidated by the software.

B. An assay run or an individual sample may also 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 recalculated.

C. 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.

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 retested.
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 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 PROCLEIX SYSTEM USERS, INTERPRETATION OF RESULTS section.

II. ACCEPTANCE CRITERIA FOR CALIBRATION AND CALCULATION OF CUTOFF

A. Procleix Ultrio Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate. Each individual Negative Calibrator replicate must have an Internal Control value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an Internal Control 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 replicate values have Internal Control 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 RLU
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 RLU
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

The HIV-1 Positive Calibrator is run in duplicate in the Procleix Ultrio Assay. 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. Internal Control values may not exceed 475,000 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 the remaining acceptable HIV-1 Positive Calibrator value. The run is invalid and must be repeated if both of the HIV-1 Positive Calibrator Analyte values are outside of these limits.

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 RLU
1		690,000
2		700,000
Total Analyte RLU	=	1,390,000

$$\text{HIV-1 } PC_x\text{(Analyte)} = \frac{\text{Total Analyte RLU}}{2} = 695{,}000$$

HCV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator is run in duplicate in the Procleix Ultrio Assay. Individual HCV Positive Calibrator (PC) Analyte values must be less than or equal to 1,000,000 RLU and greater than or equal to 200,000 RLU. Internal Control values may not exceed 475,000 RLU. If one of the HCV Positive Calibrator values is outside these limits, the HCV Positive Calibrator mean (HCV PC_x) will be the remaining acceptable HCV Positive Calibrator value. The run is invalid and must be repeated if both of the HCV Positive Calibrator Analyte values are outside these limits.

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

Example:

HCV Positive Calibrator		Analyte RLU
1		350,000
2		360,000
Total Analyte RLU	=	710,000

$$\text{HCV } PC_x\text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{2} = 355{,}000$$

HBV Positive Calibrator Acceptance Criteria

The HBV Positive Calibrator is run in duplicate in the Procleix Ultrio Assay. Individual HBV 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. Internal Control values may not exceed 475,000 RLU. If one of the HBV Positive Calibrator values is outside these limits, the HBV Positive Calibrator mean (HBV PC_x) will be the remaining acceptable HBV Positive Calibrator value. The run is invalid and must be repeated if both of the HBV Positive Calibrator Analyte values are outside these limits.

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

Example:

HBV Positive Calibrator		Analyte RLU
1		690,000
2		700,000
Total Analyte RLU	=	1,390,000

$$\text{HBV } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{2} = 695{,}000$$

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/HBV Analyte Cutoff Value

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

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

Analyte Cutoff Value = 15,000 + (0.02 x 695,000) + (0.04 x 355,000) + (0.02 x 695,000)

Analyte Cutoff Value = 57,000 RLU

Summary of Acceptance Criteria for Procleix Ultrio Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 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 ≤ 1,000,000 RLU
Internal Control	≤ 475,000 RLU
HBV Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU

Summary of Cutoff Calculations for Procleix Ultrio Assay

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

B. Procleix HIV-1 Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) must be run in triplicate. Each individual Negative Calibrator replicate must have an Internal Control value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate 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 replicate values have Internal Control 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 RLU
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 RLU
1		12,000
2		11,000
3		13,000
Total Analyte Control RLU	=	36,000

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

HIV-1 Positive Calibrator Acceptance Criteria

The HIV-1 Positive Calibrator is run in triplicate in the Procleix HIV-1 Discriminatory Assay. 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. Internal Control values may not exceed 475,000 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 more than one of the three HIV-1 Positive Calibrator Analyte values is outside of these limits.

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 RLU
1		1,000,000
2		1,100,000
3		1,050,000
Total Analyte RLU	=	3,150,000

$$\text{HIV-1 } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 1{,}050{,}000$$

HCV Positive Calibrator and HBV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator and HBV Positive Calibrator are run in duplicate in the Procleix HIV-1 Discriminatory Assay on the Procleix System only. Each individual HCV Positive Calibrator and HBV Positive Calibrator replicate must have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. Each HCV Positive Calibrator and HBV Positive Calibrator must also have Internal Control values greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. The run is invalid and must be repeated if more than one of the four calibrator values have Internal Control 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 PC_x (Analyte)]

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

Analyte Cutoff Value = 12,000 + (0.04 x 1,050,000)

Analyte Cutoff Value = 54,000 RLU

Summary of Acceptance Criteria for the Procleix HIV-1 Discriminatory Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 RLU
HIV-1 Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU
HCV Positive Calibrator and HBV Positive Calibrator*	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 RLU

***Note that the HCV Positive Calibrator and HBV Positive Calibrator perform similarly to the Negative Calibrator in the Procleix 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 Internal Control Mean RLU)

C. Procleix HCV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) must be run in triplicate. Each individual Negative Calibrator replicate must have an Internal Control value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid or an Internal Control 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 replicate values have Internal Control 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 RLU
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 RLU
1		20,000
2		22,000
3		18,000
Total Analyte RLU	=	60,000

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

HCV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator is run in triplicate in the Procleix HCV Discriminatory Assay. Individual HCV Positive Calibrator values must be less than or equal to 2,700,000 RLU and greater than or equal to 400,000 RLU. Internal Control values may not exceed 475,000 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 more than one of the three HCV Positive Calibrator Analyte values is outside of these limits.

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

Example:

HCV Positive Calibrator		Analyte RLU
1		1,300,000
2		1,200,000
3		1,250,000
Total Analyte RLU	=	3,750,000

$$\text{HCV } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 1{,}250{,}000$$

HIV-1 Positive Calibrator and HBV Positive Calibrator Acceptance Criteria

The HIV-1 Positive Calibrator and the HBV Positive Calibrator are run in duplicate in the Procleix HCV Discriminatory Assay only. Each individual HIV-1 Positive Calibrator and HBV Positive Calibrator must have analyte values less than or equal to 45,000 RLU and greater than or equal to 0 RLU. Each HIV-1 Positive Calibrator and HBV Positive Calibrator must also have an Internal Control value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. The run is invalid and must be repeated if more than one of the four calibrator values have Internal Control values or analyte values that are outside of 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 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 1,250,000)

Analyte Cutoff Value = 70,000 RLU

Summary of Acceptance Criteria for the Procleix HCV Discriminatory Assay

Acceptance Criteria:			
Negative Calibrator			
Analyte	≥ 0	and	≤ 45,000 RLU
Internal Control	≥ 75,000	and	≤ 375,000 RLU
HIV-1 Positive Calibrator and HBV Positive Calibrator*			
Analyte	≥ 0	and	≤ 45,000 RLU
Internal Control	≥ 75,000	and	≤ 375,000 RLU
HCV Positive Calibrator			
Analyte	≥ 400,000	and	≤ 2,700,000 RLU
Internal Control	≤ 475,000 RLU		

***Note that the HIV-1 Positive Calibrator and HBV Positive Calibrator perform similarly to the Negative Calibrator in the Procleix 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 Internal Control Mean RLU)

D. Procleix HBV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) must be run in triplicate. Each individual Negative Calibrator) replicate must have an Internal Control value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an Internal Control value or analyte value that is outside of these limits, the Negative Calibrator mean (NCx) 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 replicate values have Internal Control 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 RLU
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 RLU
1		12,000
2		11,000
3		13,000
Total Analyte RLU	=	36,000

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

HBV Positive Calibrator Acceptance Criteria

The HBV Positive Calibrator is run in triplicate in the Procleix HBV Discriminatory Assay. Individual HBV 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. Internal Control values may not exceed 475,000 RLU. If one of the HBV Positive Calibrator values is outside these limits, the HBV Positive Calibrator mean will be recalculated based upon the two acceptable HBV Positive Calibrator values. The run is invalid and must be repeated if more than one of the three HBV Positive Calibrator Analyte values is outside of these limits.

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

Example:

HBV Positive Calibrator		Analyte RLU
1		1,150,000
2		1,160,000
3		1,170,000
Total Analyte RLU	=	3,480,000

$$\text{HBV } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 1{,}160{,}000$$

HIV-1 Positive Calibrator and HCV Positive Calibrator Acceptance Criteria

The HIV-1 Positive Calibrator and the HCV Positive Calibrator are run in duplicate in the Procleix HBV Discriminatory Assay only. Each individual HIV-1 Positive Calibrator and HCV Positive Calibrator replicate must have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. Each HIV-1 Positive Calibrator and HCV Positive Calibrator must also have Internal Control values greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. The run is invalid and must be repeated if more than one of the four calibrator values have Internal Control 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 HBV PC_x (Analyte)]

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

Analyte Cutoff Value = 12,000 + (0.04 x 1,160,000)

Analyte Cutoff Value = 58,400 RLU

Summary of Acceptance Criteria for the Procleix HBV Discriminatory Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 RLU
HBV Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU
HIV-1 Positive Calibrator and HCV Positive Calibrator*	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 RLU

***Note that the HIV-1 Positive Calibrator and HCV Positive Calibrator perform similarly to the Negative Calibrator in the Procleix HBV Discriminatory Assay.**

Summary of Cutoff Calculations for the Procleix HBV Discriminatory Assay

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

INTERPRETATION OF RESULTS

All calculations described above are performed by the luminometer 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 are determined. Analyte Signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/cutoff (S/CO) on the report.

A specimen is considered Nonreactive if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control Signal is greater than or equal to the Internal Control Cutoff and less than or equal to 550,000 RLU in the Procleix Ultrio Assay, or less than or equal to 375,000 RLU in the Procleix HIV-1, HCV, or HBV Discriminatory Assays. A specimen is considered Reactive if the Analyte Signal is greater than or equal to the Analyte Cutoff (i.e., Analyte S/CO ≥1.00) and the Internal Control Signal is less than or equal to 550,000 RLU in the Procleix Ultrio Assay, or less than or equal to 375,000 RLU in the Procleix HIV-1, HCV, or HBV Discriminatory Assays. Reactive results will be designated by the software. A specimen is considered Invalid if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control Signal is less than the Internal Control Cutoff. A specimen is also considered Invalid if the Internal Control Signal is greater than 550,000 RLU in the Procleix Ultrio Assay, or greater than 375,000 RLU in the Procleix HIV-1, HCV, or HBV Discriminatory Assays.

High titers of non-target analytes may produce invalid results in each of the individual Procleix Ultrio Discriminatory Assays. (For example, a high titer HBV sample may produce an invalid result in the discriminatory assay targeting HIV-1 or HCV.) In such cases, further testing with an alternate test method could be used for discrimination.

Cadaveric blood specimens, when tested neat, may be invalid due to inhibitory substances within the specimen. These invalid specimens may be diluted as in SPECIMEN COLLECTION, STORAGE, AND HANDLING, Cadaveric Blood Specimens, step J, and retested in singlet.

Failure to achieve expected results, as described in this package insert, is an indication of an invalid run. Possible sources of error include test kit deterioration, operator error, faulty performance of equipment, specimen deterioration, or contamination of reagents.

Summary of Specimen Interpretation

Specimen Interpretation	Criteria for the Procleix Ultrio Assay	Criteria for the Procleix HIV-1, HCV, and HBV Discriminatory Assays
Nonreactive	Analyte S/CO < 1.00 and Internal Control ≥ Internal Control Cutoff and Internal Control ≤ 550,000 RLU	Analyte S/CO < 1.00 and Internal Control ≥ Internal Control Cutoff and Internal Control ≤ 375,000 RLU
Reactive	Analyte S/CO ≥ 1.00 and Internal Control ≤ 550,000 RLU*	Analyte S/CO ≥ 1.00 and Internal Control ≤ 375,000 RLU**
Invalid	Internal Control > 550,000 RLU or Analyte S/CO < 1.00 and Internal Control < Internal Control Cutoff	Internal Control > 375,000 RLU or Analyte S/CO < 1.00 and Internal Control < Internal Control Cutoff

* In the Procleix Ultrio Assay, specimens with Internal Control signal greater than 550,000 RLU will be invalidated by the software and the reactive status cannot be assessed.
** In the Procleix HIV-1, HCV, and HBV Discriminatory Assays specimens with Internal Control signal greater than 375,000 RLU will be invalidated by the software and the reactive status cannot be assessed.

1. Any specimen with an interpretation of Invalid in the Procleix Ultrio Assay, Procleix HIV-1 Discriminatory Assay, Procleix HCV Discriminatory Assay, or Procleix HBV Discriminatory Assay must be retested in the same assay in singlet, except as noted in step 8. Cadaveric specimens with an interpretation of Invalid in the Procleix Ultrio Assay, Procleix HIV-1 Discriminatory Assay, Procleix HCV Discriminatory Assay, or Procleix HBV Discriminatory Assay previously diluted 1:5 may be retested in singlet, diluted at the 1:5 dilution, except as noted in step 8.
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 value and with an Analyte S/CO less than 1.00 in the Procleix Ultrio Assay are considered nonreactive for HIV-1 RNA, HCV RNA, and HBV DNA. If the nonreactive specimen is a pool, each of the individual specimens comprising the pool is considered nonreactive and no further testing is required.
4. In the Procleix Ultrio Assay, specimens with an Analyte S/CO greater than or equal to 1.00 and an Internal Control Signal less than or equal to 550,000 RLU are considered **Reactive**. In the Procleix HIV-1, HCV, and HBV Discriminatory Assays, specimens with an Analyte S/CO greater than or equal to 1.00 and an Internal Control signal less than or equal to 375,000 RLU are considered **Reactive.**
5. IF THE REACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool must be tested with the Procleix Ultrio Assay.
    a. If an individual specimen tests nonreactive with the Procleix Ultrio Assay, then the specimen is considered nonreactive for HIV-1 RNA, HCV RNA, and HBV DNA and no further testing is required.
    b. If an individual specimen tests Reactive with the Procleix Ultrio Assay, then the specimen must be tested with the HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays.
        (1) If an individual specimen then tests Reactive with one or more Discriminatory tests, then the specimen is considered Reactive-Discriminated.

(2) If an individual specimen then tests nonreactive with all Discriminatory tests, then the specimen is considered Non-Discriminated. For HPC or DLI donors, continue to step 7b.

6. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION FROM A DONOR OF WHOLE BLOOD, BLOOD COMPONENTS OR SOURCE PLASMA, then the specimen must be tested with the HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays.

   a. If an individual specimen then tests Reactive with one or more Discriminatory tests, then the specimen is considered Reactive-Discriminated.

   b. If an individual specimen then tests nonreactive with all Discriminatory tests, then the specimen is considered Non-Discriminated. Caution: Some HBV true positive specimens reactive by Procleix Ultrio Assay individual donation screening may test nonreactive by the Prodeix HBV Discriminatory Assay. (See statement in LIMITATIONS OF THE PROCEDURE.)

7. 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 HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays.

   a. If an individual specimen then tests Reactive with one or more Discriminatory tests, then the specimen is considered Reactive-Discriminated.

   b. If an individual specimen then tests nonreactive with all Discriminatory tests, then the specimen is considered Non-Discriminated. The Non-Discriminated specimen may be retested in the Procleix Ultrio Assay if sufficient sample is available.

      (1) If the individual specimen tests nonreactive in the repeated Procleix Ultrio Assay, then the specimen is considered nonreactive for HIV-1 RNA, HCV RNA, and HBV DNA and no further testing is required.

      (2) If the individual specimen tests Reactive in the repeated Procleix Ultrio Assay, then the specimen is considered Repeatedly Reactive, Non-Discriminated for HIV-1 RNA, HCV RNA, and HBV DNA. 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 donor eligibility. Caution: Some HBV true positive specimens reactive by Procleix Ultrio Assay individual donation screening may test nonreactive by the Procleix HBV Discriminatory Assay. (See statement in LIMITATIONS OF THE PROCEDURE.)

8. Reactive specimens in an operator invalidated run due to the 10% invalid rate are identified by the luminometer software as reactive, and must become the test of record. Any reactive result serves as the test of record and the sample should be resolved according to the resolution algorithm for reactive specimens, as explained in the PROCLEIX SYSTEM USERS, INTERPRETATION OF RESULTS section.

9. 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. HBV seroreactive specimens found to be Reactive-HBV Discriminated in the Procleix Assays may be considered positive for HBV nucleic acid. The interpretation of Reactive-Discriminated specimen results on specimens that are nonreactive by serology is unclear.

10. For specimens that are repeat reactive on a licensed anti-HIV-1 screening test and reactive on the Procleix Ultrio Assay and reactive on the Procleix HIV-1 Discriminatory Assay, the Procleix Ultrio Assay acts as a supplemental test that confirms HIV-1 infection; it is not necessary to run an HIV-1 Western Blot.

11. For specimens that are repeat reactive on a licensed anti-HCV screening test and reactive on the Procleix Ultrio Assay and reactive on the Procleix HCV Discriminatory Assay, the Procleix Ultrio Assay acts as a supplemental test that confirms HCV infection; it is not necessary to run an additional FDA approved HCV supplemental test.

12. For specimens that are repeat reactive on a licensed HBsAg screening test and reactive on the Procleix Ultrio Assay and reactive on the Procleix HBV Discriminatory Assay, the Procleix Ultrio Assay acts as a supplemental test that confirms HBV infection; it is not necessary to run an HBsAg neutralization test.

13. Specimens that are Nonreactive in the Procleix Ultrio Assay or are Reactive in the Procleix Ultrio Assay but are not HIV-1 Discriminated, and are also repeatedly reactive in 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).

    Specimens that are Nonreactive in the Procleix Ultrio Assay or are Reactive in the Procleix Ultrio Assay but are not HCV Discriminated, and are also repeatedly reactive in a licensed donor screening test for antibodies to HCV, should be further tested using an FDA approved HCV supplemental test.

    Specimens that are Nonreactive in the Procleix Ultrio Assay or are Reactive in the Procleix Ultrio Assay but are not HBV Discriminated, and are also repeatedly reactive in a licensed donor screening test for HBsAg, should be further tested using an FDA approved HBsAg neutralization test.

14. Donors with specimens that are reactive in the Procleix HIV-1, HCV, or HBV Discriminatory Assays and/or repeatedly EIA reactive by licensed serological tests for HIV, HCV or HBV (or any combinations of these), should be referred for medical evaluation. A clinical diagnosis can be made only if the person meets the case definition(s) established by the Centers for Disease Control and Prevention.[31, 32]

PROCLEIX TIGRIS SYSTEM USERS

MATERIALS REQUIRED

Component	Part Number	Part Number
Procleix Ultrio Assay Kits	**301103** (1000 Test Kit)	**301105** (5000 Test Kit)
Internal Control Reagent	2 x 5 mL	10 x 5 mL
Target Capture Reagent	2 x 280 mL	10 x 280 mL
Amplification Reagent	2 x 50 mL	10 x 50 mL
Enzyme Reagent	2 x 18 mL	10 x 18 mL
Probe Reagent	2 x 75 mL	10 x 75 mL
Selection Reagent	2 x 180 mL	10 x 180 mL
Procleix Negative Calibrator	30 x 2 mL	90 x 2 mL
Procleix HIV-1 Positive Calibrator	30 x 2 mL	90 x 2 mL
Procleix HCV Positive Calibrator	30 x 2 mL	90 x 2 mL
Procleix HBV Positive Calibrator	30 x 2 mL	90 x 2 mL
Procleix HIV-1, HCV, and HBV Discriminatory Probe Reagents Kit	**PRD-03708** (200 tests)	
Procleix HIV-1 Discriminatory Probe Reagent	2 x 14 mL	
Procleix HCV Discriminatory Probe Reagent	2 x 14 mL	
Procleix HBV Discriminatory Probe Reagent	2 x 14 mL	
Procleix Ultrio Tigris Controls	**302193 (30 tests)**	
Procleix Ultrio Tigris Negative Control	30 x 1 mL	
Procleix Ultrio Tigris HIV-1 Control	30 x 1 mL	
Procleix Ultrio Tigris HCV Control	30 x 1 mL	
Procleix Ultrio Tigris HBV Control	30 x 1 mL	
Procleix Wash Solution	2 x 2.9 L	303665
Procleix Oil	4 x 260 mL	302441
Procleix Buffer for Deactivation Fluid	2 x 1.4 L	303666
Procleix Auto Detect Reagents	**301120** (1000 tests)	
Auto Detect 1	2 x 240 mL	
Auto Detect 2	2 x 240 mL	
Procleix System Fluid Preservative	1 x 200 mL	301175

Disposables

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

Multi-Tube Units (MTUs)	1 case of 100	104772
Waste Bag Kit (MTU and Tiplet)	30 of each	900907
MTU Waste Cover	1 box of 30	105523
MTU Waste Deflector	1 box of 30	900931

Reagent Spare Caps (TCR, Selection, Probe Reagents)	1 bag of 100	CL0039
Reagent Spare Caps (Amplification Reagent)	1 bag of 100	CL0042

Reagent Spare Caps (Enzyme, Discriminatory Probe Reagents)	1 bag of 100	501619

Equipment

Procleix Tigris System, Procleix Tigris System Software, Procleix Ultrio Assay Software, and operator's manual

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

Other

Procleix Tigris System Maintenance Bottle Kit	105655
Procleix Tigris System Quick Reference Guide (Procleix Tigris System QRG)	
Any applicable Technical Bulletins	

OTHER MATERIALS AVAILABLE FROM GRIFOLS FOR USE WITH THE PROCLEIX ULTRIO ASSAY

Procleix Ultrio Assay Calibrators	**301106**	
Procleix Negative Calibrator	30 x 2 mL	
Procleix HIV-1 Positive Calibrator	30 x 2 mL	
Procleix HCV Positive Calibrator	30 x 2 mL	
Procleix HBV Positive Calibrator	30 x 2 mL	
Procleix Ultrio Negative Calibrator	90 x 2 mL	303259
Procleix Ultrio Tigris Negative Control	165 x 1 mL	303258
Procleix HIV-1 Discriminatory Probe Reagent Kit	2 x 14 mL	301107
Procleix HCV Discriminatory Probe Reagent Kit	2 x 14 mL	301108
Procleix HBV Discriminatory Probe Reagent Kit	2 x 14 mL	301109

General Equipment/Software

For pooling only: Procleix Xpress Pipettor and Software, Tecan Genesis RSP instrument (front-end pipettor), Procleix CPT Pooling Software, and operator's manual

For instrument specifics and ordering information, contact Grifols Customer Service.

MATERIALS REQUIRED BUT NOT PROVIDED

Bleach

For use in final concentrations of 5 to 8.25% sodium hypochlorite and 0.5% sodium hypochlorite

Bleach alternative (optional)

Contact Grifols Technical Service for a list of bleach alternatives and instructions for use.

Alcohol (70% ethanol, 70% isopropyl alcohol solution, or 70% isopropyl alcohol wipes)

Water for the Procleix Tigris System

For water specifications for the Procleix Tigris System, see the *Procleix Tigris System Operator's Manual*.

Disposable 1000 µL conductive filter tips (DiTis) in rack approved for use with the Procleix Tigris System and pooling instrument. Contact Grifols Technical Service for approved tips.

PRECAUTIONS

A. **For *in vitro* diagnostic use.**

B. When performing testing with different Procleix Assays using shared instrumentation, ensure appropriate segregation is maintained to prevent mix-up of samples during processing. In addition, verify that the correct set of reagents is being used for the assay that is being run.

C. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix Ultrio Assay and the Procleix Tigris System QRG prior to performing an assay run.

D. Specimens may be infectious. Use Universal Precautions[23,25] when performing the assay. Proper handling and disposal methods should be established according to local, state and federal regulations.[24] Only personnel adequately qualified as proficient in the use of the Procleix Ultrio Assay and trained in handling infectious materials should perform this procedure.

E. **CAUTION: Some components of this kit contain human blood products. The HIV-1 Positive Calibrator in this kit and the Procleix Ultrio Tigris HIV-1 Control contain human plasma that is HIV-1 positive and has been heat-treated to inactivate the virus. The HCV Positive Calibrator and the Procleix Ultrio Tigris HCV Control contain human plasma that is HCV positive and has been heat-treated to inactivate the virus. The HBV Positive Calibrator and the Procleix Ultrio Tigris HBV Control contain human plasma that is HBV positive and has been heat-treated to inactivate the virus. The Negative Calibrator and the Procleix Ultrio Tigris Negative Control have been assayed by FDA-licensed tests and found nonreactive for the presence of HIV-1/2, HCV, and HBV. 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, 25] 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 the sample preparation/RPI areas only. Do not use bleach altematives on the Procleix Tigris System.**

F. 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.

G. 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.

H. 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 and follow appropriate site procedures.

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

J. Use only supplied or specified required disposables.

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

L. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present. See REAGENT PREPARATION for specific instructions.

M. Avoid microbial and ribonuclease contamination of reagents. Use of filtered, disposable pipette tips is strongly recommended.

N. 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 PROCLEIX TIGRIS SYSTEM USERS, REAGENT PREPARATION for specific instructions.

O. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See SPECIMEN COLLECTION, STORAGE, AND HANDLING for specific instructions.

P. Only combine assay reagents or fluids as instructed to by the Procleix Ultrio Assay package insert. Do not top off reagents or fluids. The Procleix Tigris System verifies reagent levels.

Q. Some reagents of this kit are labeled with risk and safety symbols and should be handled accordingly. Safety Data Sheets are accessible on the manufacturer's website.

Procleix Selection Reagent
   
Boric Acid 3.63 Weight-% **DANGER** Harmful if inhaled May damage fertility or the unborn child Obtain special instructions before use Do not handle until all safety precautions have been read and understood Use personal protective equipment as required Avoid breathing dust/fume/gas/mist/vapors/spray Use only outdoors in a well-ventilated area IF exposed or concerned: Get medical advice/attention IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing Store locked up Dispose of contents/container to an approved waste disposal plant

Procleix Probe Reagent

Ethyl Alcohol 2.33 Weight-% **DANGER** May cause cancer Obtain special instructions before use Do not handle until all safety precautions have been read and understood Use personal protective equipment as required IF exposed or concerned: Get medical advice/attention Store locked up Dispose of contents/container to an approved waste disposal plant

Procleix Ultrio Assay Discriminatory Probe Reagents

Ethyl Alcohol 2.33 Weight-% **DANGER** May cause cancer Obtain special instructions before use Do not handle until all safety precautions have been read and understood Use personal protective equipment as required IF exposed or concerned: Get medical advice/attention Store locked up Dispose of contents/container to an approved waste disposal plant

Procleix Buffer for Deactivation Fluid

SodiumHydroxide 1.12 Weight-% *SodiumHypochlorite 0.49 Weight-%* **WARNING** Causes skin irritation Causes serious eye irritation Wash face, hands and any exposed skin thoroughly after handling Wear protective gloves/protective clothing/eye protection/face protection IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing If eye irritation persists: Get medical advice/attention IF ON SKIN: Wash with plenty of soap and water If skin irritation occurs: Get medical advice/attention Take off contaminated clothing and wash before reuse

Procleix Auto Detect 2	
	*Sodium Hydroxide 6.04 Weight-%* **DANGER** Causes severe skin burns and eye damage Do not breathe dust/fume/gas/mist/vapors/spray Wash face, hands and any exposed skin thoroughly after handling Wear protective gloves/protective clothing/eye protection/face protection Immediately call a POISON CENTER or doctor/physician IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing Immediately call a POISON CENTER or doctor/physician IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower Wash contaminated clothing before reuse IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing Immediately call a POISON CENTER or doctor/physician IF SWALLOWED: Rinse mouth. DO NOT induce vomiting Store locked up Dispose of contents/container to an approved waste disposal plant
Procleix System Fluid Preservative	
	*Sodium Hypochlorite 1–5 Weight-%* **DANGER** Causes severe skin burns and eye damage Do not breathe dust/fume/gas/mist/vapors/spray Wash face, hands and any exposed skin thoroughly after handling Wear protective gloves/protective clothing/ eye protection/face protection Immediately call a POISON CENTER or doctor/physician IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing Immediately call a POISON CENTER or doctor/physician IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower Wash contaminated clothing before reuse IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing Immediately call a POISON CENTER or doctor/physician IF SWALLOWED: rinse mouth. Do NOT induce vomiting Store locked up Dispose of contents/container to an approved waste disposal plant

R. The Procleix Tigris System groups a quadrant of reagents into a matched set the first time that it scans their barcodes during the inventory process and are required to be run as a set in all subsequent worklists. Bottles belonging to a matched set cannot be swapped with bottles in other matched sets of reagents. Refer to the Procleix Tigris System QRG for more information.

S. Resolution of pools is not performed by the Procleix Tigris System. Follow laboratory procedures for resolving pools.

T. Each Calibrator is designed to be run in duplicate or triplicate and excess material in each vial is to be appropriately discarded according to local, state, and federal regulations.

U. Each control is designed for a single use and excess material in each vial is to be appropriately discarded.

V. The undiluted Procleix System Fluid Preservative is corrosive. Avoid contact with skin, eyes, and mucous membranes. Wash with water if contact with this reagent occurs. If a spill of this reagent occurs, dilute with water before wiping dry.

REAGENT PREPARATION

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

B. Choose a new or opened matched set of reagents that will be sufficient to complete testing of the number of samples in a worklist. Do not use reagents that have been used outside the Procleix Tigris System, or on another Procleix Tigris System, as the instrument verifies reagent volumes.

C. Verify that the reagents have not exceeded the expiration date and/or storage stability times, including onboard stability.

1. The Procleix Tigris System does not track the room temperature stability of reagents or fluids. However, it does track the number of hours each reagent and fluid is loaded onboard the analyzer. The Procleix Tigris System will not allow an assay to be run using reagents that have expired or exceeded their onboard stability. Consult the following table for onboard stability information.

Reagent/Fluid	Onboard Stability*
wTCR, Probe Reagents, Enzyme Reagent, Amplification Reagent, Selection Reagent	60 hours**
Wash Solution, Oil, System Fluid, Deactivation Fluid, Auto Detect Reagents	14 days

* The onboard time must occur within the room temperature times listed in GENERAL INFORMATION, STORAGE AND HANDLING INSTRUCTIONS.

** Worklists cannot be queued using reagents that have been onboard for more than 48 hours.

2. Print an Assay Reagent Status Report to check the stability remaining for unexpired reagent sets in the system's database.

D. Remove a bottle of Selection Reagent from room temperature storage.

Note: The Selection Reagent must be at room temperature before use.

1. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls between 2° and 15°C, use the RPI as described in the *Procleix Reagent Preparation Incubator Operator's Manual*, as precipitate may form.
2. If cloudiness or precipitate is present, perform Selection Reagent recovery as described in the *Procleix Reagent Preparation Incubator Operator's Manual*. Do not use if precipitate or cloudiness persists.
3. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
4. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

E. To prepare the following reagents using the RPI, refer to the Procleix Tigris System QRG: TCR, Probe Reagent, Enzyme Reagent, Amplification Reagent, and HIV-1, HCV, and HBV Discriminatory Probe Reagents. Record the date of thaw (THAW DATE) for each reagent on the space provided on the label. If precipitate is still present after thawing, the Probe Reagent can be incubated with File 3 (room temperature) to facilitate complete dissolution of precipitate, as long as the total time at room temperature does not exceed 80 hours.

1. After thawing, the HIV-1 Discriminatory Probe, HCV Discriminatory Probe, and HBV Discriminatory Probe Reagents are stable when stored at 2° to 8°C for 30 days. Within the 30 days, these reagents may be kept at room temperature up to a total of 80 hours.
2. Do not refreeze these reagents after initial thaw.

F. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2°to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. Place TCR into the RPI, and refer to the Prodeix Tigris System QRG for instructions.

Note: 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.

3. Thaw one vial of Internal Control Reagent at up to 24 hours at 2° to 8°C or up to 8 hours at room temperature. **Do not use the RPI to thaw Internal Control Reagent.**
4. Mix the Internal Control Reagent thoroughly by gentle manual inversion. mechanical inversion using a laboratory rocker, or vortexing.

Note: If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature. To expedite the dissolution of gel, warm the Internal Control Reagent at 25° to 30°C in a water bath. Periodically remove Internal Control Reagent from water bath to gently invert until gel is dissolved. Dry the exterior of the tube prior to opening.

5. After unloading TCR from the RPI and warming the Internal Control Reagent to room temperature, pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR). Mix thoroughly.
6. Use the space indicated on the TCR bottle to record the date Internal Control Reagent was added and lot number used (Internal Control Lot). Record the expiration date of the wTCR in the space provided on the label.
7. Retain the Internal Control vial to scan the barcode label into the system.

G. Thaw calibrators at room temperature. **Do not use the RPI to thaw the Procleix Ultrio Assay Calibrators.**

Note: These are single use vials, which must be thawed prior to each run.

1. Mix thoroughly by gentle inversion. Avoid reagent foaming.
2. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
3. Once thawed, use calibrators within 8 hours.

H. Thaw Procleix Ultrio Tigris Controls at room temperature. **Do not use the RPI to thaw Procleix Ultrio Tigris Controls.**

Note: These are single use vials, which must be thawed prior to each run.

1. Mix thoroughly by gentle inversion. Avoid reagent foaming.
2. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
3. Once thawed, treat the controls as samples and use within 8 hours.

I. Discriminatory Probe reagents can be run with any matched set of reagents (Amplification Reagent, Enzyme Reagent, Probe Reagent, Selection Reagent, and wTCR) within each master lot.

J. 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 between 2° and 15ºC. Wash Solution may be warmed in a water bath to facilitate dissolution of precipitate. **Do not use the RPI to warm the Wash Solution.** The temperature in the water bath should not exceed 30ºC. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.

K. For Wash Solution, Oil, Auto Detect 1, and Auto Detect 2, record the date the fluid was first opened and loaded onto the Procleix Tigris System (OPEN DATE) in the space provided on the label. Procleix Assay Fluids and Procleix Auto Detect Reagents are stable for 30 days when stored at room temperature.

L. To prepare Deactivation Fluid, combine Buffer for Deactivation Fluid with 5 to 8.25% sodium hypochlorite in the Deactivation Fluid bottle.

1. Fill the Deactivation Fluid bottle with 5 to 8.25% sodium hypochlorite to between the liquid fill lines.
2. Pour entire contents of one bottle of Buffer for Deactivation Fluid into the Deactivation Fluid bottle.
3. Place the barcode label from the Buffer for Deactivation Fluid bottle on the top of the Deactivation Fluid bottle. This barcode is required to be scanned into the system during Fluid Inventory.
4. Record the date the Deactivation Fluid was prepared on the Buffer for Deactivation Fluid label.

M. To prepare System Fluid, combine Procleix System Fluid Preservative with water for the Procleix Tigris System. in the System Fluid Bottle. For water specifications for the Procleix Tigris System, see the *Procleix Tigris System Operator's Manual.*

1. Remove System Fluid bottle from the Procleix Tigris System.
2. Dispose of any existing System Fluid, following the appropriate institutional policy, local, state, and federal regulations.
3. Fill the System Fluid Bottle to the liquid fill line with water for the Procleix Tigris System.
4. Pour the entire contents of one bottle of Procleix System Fluid Preservative into the System Fluid Bottle.
5. Mix System Fluid Bottle contents completely.
6. Place the barcode label from the Procleix System Fluid Preservative on the top of the System Fluid bottle. This barcode is required to be scanned into the system during Fluid Inventory.
7. Record the date the System Fluid was prepared on the System Fluid Preservative label.
8. Install the System Fluid Bottle by placing it on the Procleix Tigris System and reconnecting the fluid lines. Follow instructions in the *Procleix Tigris System Quick Reference Guide*.

N. Load Fluids on the Procleix Tigris System according to instructions provided in the Procleix Tigris System QRG.

PROCEDURAL NOTES

Note: Refer to the Procleix Tigris System QRG for maintenance procedures and information about software operation.

A. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix Ultrio Assay prior to performing an assay run. This package insert must be used with the Procleix Tigris System QRG and any applicable Technical Bulletins.

B. RUN SIZE

1. Kit size is based on an average run size of 55 tests. Smaller run sizes will result in a lower number of tests performed per kit.
2. For the Procleix Ultrio Assay, each run (also identified as a worklist) may contain up to 500 tests.
3. For the discriminatory assays, the run size is limited by the Probe Reagents. The maximum run size is 100 tests.

C. EQUIPMENT PREPARATION

See the Procleix Tigris System QRG.

D. RUN CONFIGURATION

1. Each run (also identified as a worklist) must have a set of Procleix Ultrio Assay Calibrators at the beginning and a set of Procleix Ultrio Tigris Controls at the end.

   a. **For the Procleix Ultrio Assay**, a set of calibrators consists of one vial each of Procleix Negative Calibrator, Procleix HIV-1 Positive Calibrator, Procleix HCV Positive Calibrator, and Procleix HBV Positive Calibrator. The Procleix Negative Calibrator is run in triplicate and each Procleix Ultrio Assay Positive Calibrator is run in duplicate.

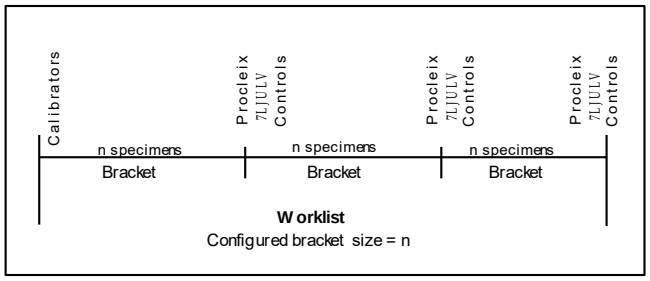
   b. **For the Procleix HIV-1, HCV, and HBV Discriminatory Assays**, a set of calibrators consists of one vial each of Procleix Negative Calibrator and the corresponding Positive Calibrator. Each Prodeix Ultrio Assay calibrator is run in triplicate.

   c. **In the Procleix Ultrio Assay**, a set of Procleix Ultrio Tigris Controls consists of one vial each of Procleix Ultrio Tigris Negative Control, Procleix Ultrio Tigris HIV-1 Control, Procleix Ultrio Tigris HCV Control, and Procleix Ultrio Tigris HBV Control. Each Procleix Ultrio Tigris Control is run in singlet.

   d. **In the Procleix HIV-1, HCV, and HBV Discriminatory Assays**, a set of Procleix Ultrio Tigris Controls consists of one vial each of the Procleix Ultrio Tigris Negative Control and the corresponding Positive Control. Each Procleix Ultrio Tigris Control is run in singlet.

2. Using additional sets of Procleix Ultrio Tigris Controls, each run (worklist) can be divided into smaller subsets called control brackets. A control bracket is defined as a group of specimens within a worklist that have a set of Procleix Ultrio Tigris Controls at each end. The results of each bracket are reported based on the validity criteria of each control (see PROCLEIX TIGRIS SYSTEM USERS, QUALITY CONTROL

PROCEDURES for more details). The default bracket size is 172, but this feature is configurable in the Procleix Tigris System Software. In the first bracket of a worklist, Procleix Ultrio Tigris Controls are not required at the beginning of the bracket.



3. The largest assay run allowable for each of the three discriminatory assays is 100 tests, which is smaller than the default bracket size. Therefore, unless the bracket size is set to a number less than 100, a set of controls is only required at the end of any discriminatory assay worklist regardless of size.
4. A printed worklist report may assist operators in locating the rack and tube position where calibrators and controls are to be placed in a worklist. Refer to the Procleix Tigris System QRG for instructions on how to view/print a worklist report.
5. Calibrator and Procleix Ultrio Tigris Control tube placement is automatically read and verified by the Procleix Tigris System. The Procleix Tigris System will not allow assay processing if a calibrator or Procleix Ultrio Tigris Control is placed in an incorrect tube position in a worklist or has an unreadable or missing barcode.
6. Test results from completed brackets of in-process run (worklist) can be viewed or printed by the operator before processing of the entire run is finished. Refer to the Procleix Tigris System QRG for instruction on how to view/print test results.

E. WORK FLOW

1. Previously opened and unopened reagents must be prepared in a clean (amplicon- and template-free) preparation area.
2. The sample loading area must be amplicon-free.

F. ENVIRONMENTAL CONDITIONS

1. The operational conditions of the room in which the Procleix Tigris System runs must be within a temperature of 15° to 25°C and humidity of 20 to 85%.
2. Refer to instrument operator's manuals for additional environmental conditions requirements.
3. The Procleix System Fluid Preservative must be used within operational conditions defined at 15° to 30°C and 20% to 85% relative humidity.

G. DECONTAMINATION

1. The extremely sensitive nature of the test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces must be decontaminated daily with 0.5% sodium hypochlorite in water (diluted bleach). Allow bleach to contact surfaces for at least 15 minutes, 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 the sample preparation/RPI areas only. **Do not use bleach alternatives on the Procleix Tigris System.**
3. The Procleix Tigris System automates the decontamination step by adding Deactivation Fluid to MTUs prior to disposal.
4. Follow instructions provided in the Procleix Tigris System QRG for instrument decontamination and maintenance procedures.

H. WATER FOR THE PROCLEIX TIGRIS SYSTEM

Water for the Procleix Tigris System is required. For water specifications for the Procleix Tigris System, see the *Procleix Tigris System Operator's Manual*. Excursions up to 100 cfu/mL do not adversely affect assay results. Refer to manufacturer instructions for maintaining the water system.

ASSAY PROCEDURE

Procleix Ultrio Assay Calibrators must be included in each assay run.

Procleix Ultrio Assay Calibrators and Discriminatory Probe Reagents are to be used with the corresponding master lot of Procleix Ultrio Assay and Discriminatory Assays. The operator must check to ensure that the Procleix Ultrio Assay Calibrators and Discriminatory Probe Reagents are used with the corresponding master lot of kit reagents as indicated on the Procleix Ultrio Assay master lot sheet in use. The software will generate an error if calibrators from a different master lot are used. Procleix Auto Detect Reagents and Procleix Assay Fluids may be used with any master lot of Procleix Assay Reagents.

Specimens from other living donors (except whole blood, blood components, source plasma, HPCs, or DLI) 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, J, and retested in singlet.

For equipment preparation, rack setup, and assay procedure information, see instructions in the Procleix Tigris System QRG.

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX ULTRIO ASSAY AND PROCLEIX HIV-1, HCV, AND HBV DISCRIMINATORY ASSAYS

A. Run validity:
A run (also identified as a worklist) is valid if the minimum numbers of calibrators meet their acceptance criteria and are valid (see section II below).

1. **In a Procleix Ultrio Assay run**, at least 7 of the 9 calibrator replicates must be valid. At least 2 of the 3 Negative Calibrator replicates and 5 of the 6 Positive Calibrator replicates must be valid.
2. **In a Procleix HIV-1, HCV, or HBV Discriminatory Assay run**, at least two of the three Negative Calibrator replicates must be valid, and at least two of the three Positive Calibrator replicates must be valid.
3. Calibrator acceptance criteria are automatically verified by the Procleix Tigris System Software. If less than the minimum number of calibrator replicates is valid, the Procleix Tigris System Software will automatically invalidate the run.
4. In a valid run, cutoff values will be automatically calculated for Internal Control (flasher) and analyte (glower).
5. If a run is invalid, sample results are reported as Invalid and all specimens must be retested.

B. Sample validity:

1. In a valid run, a sample result is valid if the IC signal is equal to or above the IC cutoff, with the following exceptions:
   a. Specimens with an analyte signal (glower signal) greater than the analyte cutoff are not invalidated even if the Internal Control (IC) signal is below the cutoff.
   b. **In the Procleix Ultrio Assay**, specimens with an IC signal above 650,000 RLU are invalidated by the software and their reactive status cannot be assessed. The software also automatically invalidates positive Calibrators and Positive Procleix Ultrio Tigris Controls with an IC signal above 475,000 RLU.
   c. **In the Procleix HIV-1, HCV, and HBV Discriminatory Assays**, specimens with an IC signal above 475,000 RLU are invalidated by the software and their reactive status cannot be assessed. The software also automatically invalidates positive Calibrators and Positive Procleix Ultrio Tigris Controls with an IC signal above 475,000 RLU.
2. A sample may also be invalidated due to instrument and results processing errors. Refer to the QRG for details.
3. All individual specimen results that are Invalid in a valid run or control bracket must be retested.

C. Control bracket validity:

1. A valid control bracket requires valid Procleix Ultrio Tigris Control sets at the beginning and end of the bracket (excluding the first bracket which has calibrators at the beginning and Procleix Ultrio Tigris Controls at the end). A valid control set requires that all Procleix Ultrio Tigris Controls in the set be valid. Controls acceptance criteria are automatically verified by the Procleix Tigris System Software. Instructions for handling specimens in brackets with invalid Procleix Ultrio Tigris Control sets are described in item E below.
   a. **In the Procleix Ultrio Assay**, a set of Procleix Ultrio Tigris Controls consists of one vial each of Procleix Ultrio Tigris Negative Control, Procleix Ultrio Tigris HIV-1 Control, Procleix Ultrio Tigris HCV Control, and Procleix Ultrio Tigris HBV Control. Each Procleix Ultrio Tigris Control is run in singlet.
   b. **In the Procleix HIV-1, HCV, and HBV Discriminatory Assays**, a set of Procleix Ultrio Tigris Controls consists of one vial each of the Procleix Ultrio Tigris Negative Control and the corresponding Positive Control. Each Procleix Ultrio Tigris Control is run in singlet.

D. Specimen results interpretation when bracket acceptance criteria are not met:

1. Specimens with an analyte S/CO <1.00 and IC RLU less than the IC cutoff will be marked as Invalid by the Procleix Tigris System Software.
2. **In the Procleix Ultrio Assay**, specimens with an analyte S/CO greater than or equal to 1.00 and with IC signal between 0 and 650,000 RLU will be marked as Reactive by the Procleix Tigris System Software and are the test of record.
3. **In the Procleix HIV-1, HCV, and HBV Discriminatory Assays**, specimens with an analyte S/CO greater than or equal to 1.00 and with IC signal between 0 and 475,000 RLU will be marked as Reactive by the Procleix Tigris System Software and are the test of record.
4. Specimens with an analyte S/CO <1.00 and IC RLU greater than or equal to the IC cutoff will be flagged as Suspect by the Procleix Tigris System Software. For the Procleix Tigris System, the term "Suspect" refers to nonreactive specimens that are not automatically invalid, but must be further evaluated and resolved (see section E).

E. Resolution of Suspect specimens due to invalid Procleix Ultrio Tigris Control sets:

1. Suspect specimens that result from invalid Procleix Ultrio Tigris Control sets are flagged with error code "x" on the Assay Results Run Report. Procleix Ultrio Tigris Controls may be invalid for one of two reasons (see the Procleix Tigris System QRG for definitions):
   a. Instrument processing errors (error codes in UPPERCASE letters)
   b. Results processing errors (error codes in lowercase letters)
2. If Procleix Ultrio Tigris Control sets are invalidated due to instrument processing errors, results from all Suspect specimens should be considered valid non-reactive if the next set of Procleix Ultrio Tigris Controls is valid. If no valid Procleix Ultrio Tigris Control results are available in the subsequent bracket(s), all Suspect specimens should be considered invalid and be retested.
3. If Procleix Ultrio Tigris Control results are invalidated due to results processing errors, all Suspect specimens should be considered invalid and be retested regardless of the status of subsequent Procleix Ultrio Tigris Controls.

Note: See the Procleix Tigris System QRG for a complete list and description of all error codes.

F. Summary of Specimen Result Interpretation for Procleix Ultrio Assay
The following table and flow chart in section H below, summarize results interpretation on the Procleix Tigris System:

Interpretation Assigned by Procleix Tigris Software on Run Report	Status of Procleix Ultrio Tigris Controls for the Bracket	Analyte S/CO	IC Result	User Action Required
Reactive (test of record)	Valid or Invalid	≥ 1.00	0 to 650,000 RLU	Follow instructions in INTERPRETATION OF RESULTS for Procleix Tigris System Users.
Valid, Non-reactive	Valid	< 1.00	≥ IC C/O, ≤ 650,000 RLU	None
Suspect (marked with error code "x")	Invalid	< 1.00	≥ IC C/O, ≤ 650,000 RLU	Follow instructions in section E and flow chart below for Suspect results.
Invalid	NA	NA	NA	Retest

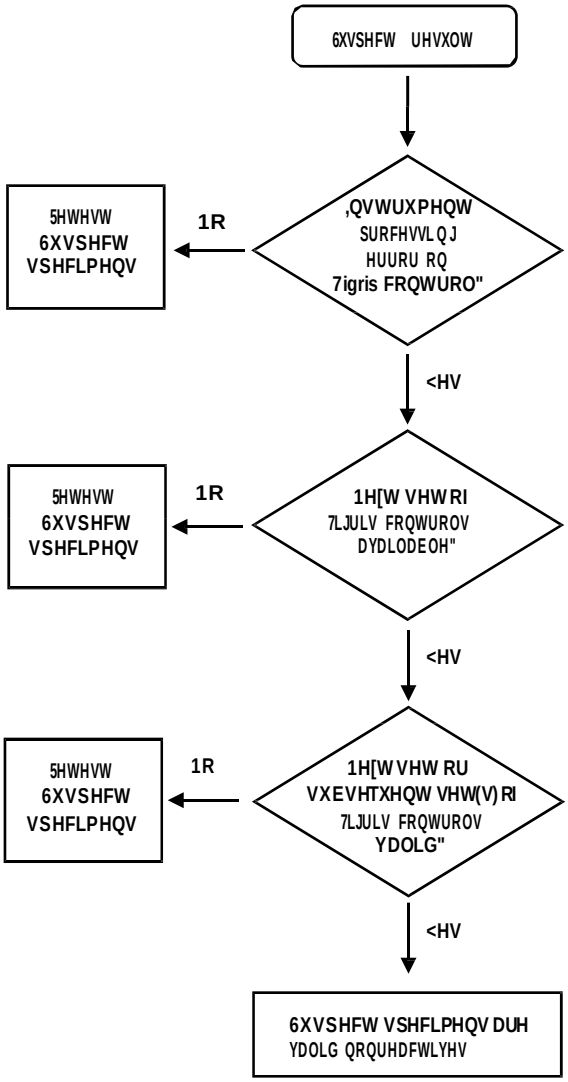
NA = Not applicable.

G. Summary of Specimen Result Interpretation for Procleix HIV-1, HCV, and HBV Discriminatory Assays
The following table and flow chart in section H below, summarize results interpretation on the Procleix Tigris System:

Interpretation Assigned by Procleix Tigris Software on Run Report	Status of Procleix Ultrio Tigris Controls for the Bracket	Analyte S/CO	IC Result	User Action Required
Reactive (test of record)	Valid or Invalid	≥ 1.00	0 to 475,000 RLU	None
Valid, Non-reactive	Valid	< 1.00	≥ IC C/O, ≤ 475,000 RLU	None
Suspect (marked with error code "x")	Invalid	< 1.00	≥ IC C/O, ≤ 475,000 RLU	Follow instructions in section E and flow chart below for Suspect results.
Invalid	NA	NA	NA	Retest

NA = Not applicable.

H. If Suspect results are observed in the Run Report, consult the following chart for direction:



Instrument processing errors are marked with error codes in UPPER CASE letters.

Results processing errors are indicated by error codes in lower case letters.

Note: Specimens with an overall interpretation of Reactive, as determined by the software, must become the test of record. In the Procleix Ultrio Assay, reactive pools or individual specimens should be resolved according to the resolution algorithm, as explained in the PROCLEIX TIGRIS SYSTEM USERS, INTERPRETATION OF RESULTS section.

Note: A run or an individual sample may also be invalidated by an operator if package insert instructions for specimen or reagent handling were not followed.

II. ACCEPTANCE CRITERIA FOR CALIBRATION AND CALCULATION OF CUTOFF

A. Procleix Ultrio Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate. Each individual Negative Calibrator replicate must have an Internal Control value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid due to an Internal Control 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 Internal Control 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 RLUs
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 (NCx) for Analyte [NCx (Analyte)]

Example:

Negative Calibrator		Analyte RLU
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

The HIV-1 Positive Calibrator is run in duplicate in the Procleix Ultrio Assay. 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. Internal Control values may not exceed 475,000 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 the remaining acceptable HIV-1 Positive Calibrator value. The run is invalid and must be repeated if both of the HIV-1 Positive Calibrator Analyte values are outside of these limits.

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 RLU
1		690,000
2		700,000
Total Analyte RLU	=	1,390,000

$$\text{HIV-1 } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{2} = 695{,}000$$

HCV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator is run in duplicate in the Procleix Ultrio Assay. Individual HCV Positive Calibrator (PC) Analyte values must be less than or equal to 1,000,000 RLU and greater than or equal to 200,000 RLU. Internal Control values may not exceed 475,000 RLU. If one of the HCV Positive Calibrator values is outside these limits, the HCV Positive Calibrator mean (HCV PC_x) will be the remaining acceptable HCV Positive Calibrator value. The run is invalid and must be repeated if both of the HCV Positive Calibrator Analyte values are outside these limits.

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

Example:

HCV Positive Calibrator		Analyte RLU
1		350,000
2		360,000
Total Analyte RLU	=	710,000

$$\text{HCV } PC_x\text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{2} = 355{,}000$$

HBV Positive Calibrator Acceptance Criteria

The HBV Positive Calibrator is run in duplicate in the Procleix Ultrio Assay. Individual HBV 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. Internal Control values may not exceed 475,000 RLU. If one of the HBV Positive Calibrator values is outside these limits, the HBV Positive Calibrator mean (HBV PC_x) will be the remaining acceptable HBV Positive Calibrator value. The run is invalid and must be repeated if both of the HBV Positive Calibrator Analyte values are outside these limits.

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

Example:

HBV Positive Calibrator		Analyte RLU
1		690,000
2		700,000
Total Analyte RLU	=	1,390,000

$$\text{HBV } PC_x\text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{2} = 695{,}000$$

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/HBV Analyte Cutoff Value

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

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

Analyte Cutoff Value = 15,000 + (0.02 x 695,000) + (0.04 x 355,000) + (0.02 x 695,000)

Analyte Cutoff Value = 57,000 RLU

Summary of Acceptance Criteria for Procleix Ultrio Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 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 ≤ 1,000,000 RLU
Internal Control	≤ 475,000 RLU
HBV Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU

Summary of Cutoff Calculations for Procleix Ultrio Assay

Analyte Cutoff = NC Analyte Mean RLU
+ 0.02 x (HIV-1 PC Analyte Mean RLU)
+ 0.04 x (HCV PC Analyte Mean RLU)
+ 0.02 x (HBV PC Analyte Mean RLU)

Internal Control Cutoff = 0.5 x (Negative Calibrator Internal Control Mean RLU)

B. Procleix HIV-1 Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate. Each individual Negative Calibrator replicate must have an IC value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid due to an Internal Control 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 Internal Control 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 RLU
1		124,000
2		125,000
3		126,000
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 RLU
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

The HIV-1 Positive Calibrator is run in triplicate in the Procleix HIV-1 Discriminatory Assay. 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. Internal Control values may not exceed 475,000 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 more than one of the three HIV-1 Positive Calibrator Analyte values is outside of these limits.

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 RLU
1		1,000,000
2		1,100,000
3		1,050,000
Total Analyte RLU	=	3,150,000

$$\text{HIV-1 } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 1{,}050{,}000$$

HCV Positive Calibrator and HBV Positive Calibrator Acceptance Criteria

The HCV and HBV Positive Calibrators are not used in the HIV-1 Discriminatory Assay for the Procleix Tigris System. Only the Negative Calibrator and the HIV-1 Positive Calibrator are used.

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 PC_x (Analyte)]

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

Analyte Cutoff Value = 12,000 + (0.04 x 1,050,000)

Analyte Cutoff Value = 54,000 RLU

Summary of Acceptance Criteria for the Procleix HIV-1 Discriminatory Assay

Negative Calibrator	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 RLU
HIV-1 Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU

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 Internal Control Mean RLU)

C. Procleix HCV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate. Each individual Negative Calibrator replicate must have an Internal Control value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid or an Internal Control 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 Internal Control 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 RLU
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 RLU
1		20,000
2		22,000
3		18,000
Total Analyte RLU	=	60,000

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

HCV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator is run in triplicate in the Procleix HCV Discriminatory Assay. Individual HCV Positive Calibrator values must be less than or equal to 2,700,000 RLU and greater than or equal to 400,000 RLU. Internal Control values may not exceed 475,000 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 more than one of the three HCV Positive Calibrator Analyte values is outside of these limits.

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

Example:

HCV Positive Calibrator		Analyte RLU
1		1,300,000
2		1,200,000
3		1,250,000
Total Analyte RLU	=	3,750,000

$$\text{HCV } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 1{,}250{,}000$$

HIV-1 Positive Calibrator and HBV Positive Calibrator Acceptance Criteria

The HIV-1 and HBV Positive Calibrators are not used in the HCV Discriminatory Assay for the Procleix Tigris System. Only the Negative Calibrator and the HCV Positive Calibrator are used.

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 1,250,000)

Analyte Cutoff Value = 70,000 RLU

Summary of Acceptance Criteria for the Procleix HCV Discriminatory Assay

Negative Calibrator	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 RLU
HCV Positive Calibrator	
Analyte	≥ 400,000 and ≤ 2,700,000 RLU
Internal Control	≤ 475,000 RLU

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 Internal Control Mean RLU)

D. Procleix HBV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate. Each individual Negative Calibrator replicate must have an Internal Control value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid due to an Internal Control 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 Internal Control 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 RLU
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 RLU
1		12,000
2		11,000
3		13,000
Total Analyte RLU	=	36,000

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

HBV Positive Calibrator Acceptance Criteria

The HBV Positive Calibrator is run in triplicate in the Procleix HBV Discriminatory Assay. Individual HBV 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. Internal Control values may not exceed 475,000 RLU. If one of the HBV Positive Calibrator values is outside these limits, the HBV Positive Calibrator mean will be recalculated based upon the two acceptable HBV Positive Calibrator values. The run is invalid and must be repeated if more than one of the three HBV Positive Calibrator Analyte values is outside of these limits.

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

Example:

HBV Positive Calibrator		Analyte RLU
1		1,150,000
2		1,160,000
3		1,170,000
Total Analyte RLU	=	3,480,000

$$\text{HBV } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 1{,}160{,}000$$

HIV-1 Positive Calibrator and HCV Positive Calibrator Acceptance Criteria

The HCV and HIV-1 Positive Calibrators are not used in the HBV Discriminatory Assay for the Procleix Tigris System. Only the Negative Calibrator and the HBV Positive Calibrator are used.

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 HBV PC_x(Analyte)]

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

Analyte Cutoff Value = 12,000 + (0.04 x 1,160,000)

Analyte Cutoff Value = 58,400 RLU

Summary of Acceptance Criteria for the Procleix HBV Discriminatory Assay

Negative Calibrator	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 RLU
HBV Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU

Summary of Cutoff Calculations for the Procleix HBV Discriminatory Assay

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

III. ACCEPTANCE CRITERIA FOR PROCLEIX ULTRIO TIGRIS CONTROLS

The Procleix Tigris System requires Procleix Ultrio Tigris Controls for acceptance of brackets within a worklist. For more information, refer to the Procleix Tigris System QRG. All the controls at the beginning and end of a bracket (except the first bracket, which only has controls at the end) must have the correct reactivity status (e.g., Nonreactive for Negative Controls and Reactive for Positive Controls) and be valid for the bracket to be valid.

Acceptance Criteria for Procleix Ultrio Tigris Controls in the Procleix Ultrio Assay

In the Procleix Ultrio Assay, a valid Procleix Ultrio Tigris Negative Control, Procleix Ultrio Tigris HIV-1 Control, Procleix Ultrio Tigris HCV Control, and Procleix Ultrio Tigris HBV Control are required at the beginning and end of a bracket (except the first bracket) for the results for that bracket to be valid. The Negative Control must have an S/CO less than 1.00 (nonreactive) to be accepted. The HIV-1 and HBV Controls must have an S/CO greater than

or equal to 1.00 (reactive) and less than 40.00 to be accepted. The HCV Control must have an S/CO greater than or equal to 1.00 (reactive) and less than 20.00 to be accepted.

Negative Control	
Analyte	≥ 0 and ≤ 150,000 RLU
Analyte S/CO Internal	< 1.00
Control	≥ 75,000 and ≤ 375,000 RLU
Internal Control S/CO	≥ 1.00
HIV-1 Control	
Analyte	≥ 45,000 and ≤ 1,800,000 RLU
Analyte S/CO	≥ 1.00 and < 40.00
Internal Control	≤ 475,000 RLU
HCV Control	
Analyte	≥ 45,000 and ≤ 1,000,000 RLU
Analyte S/CO	≥ 1.00 and < 20.00
Internal Control	≤ 475,000 RLU
HBV Control	
Analyte	≥ 45,000 and ≤ 1,800,000 RLU
Analyte S/CO	≥ 1.00 and < 40.00
Internal Control	≤ 475,000 RLU

Acceptance Criteria for Procleix Ultrio Tigris Controls in the HIV-1 Discriminatory Assay

In the HIV-1 Discriminatory Assay, a valid Procleix Ultrio Tigris Negative Control and Procleix Ultrio Tigris HIV-1 Control are required at the beginning and end of each bracket (except the first bracket) for the results for the specimens in that bracket to be valid. No other controls are required. The Negative Control must have an S/CO less than 1.00 (nonreactive) to be accepted. The HIV-1 Control must have S/CO greater than or equal to 1.00 (reactive) and less than 40.00 to be accepted.

Negative Control	
Analyte	≥ 0 and ≤ 150,000 RLU
Analyte S/CO	< 1.00
Internal Control	≥ 75,000 and ≤ 375,000 RLU
Internal Control S/CO	≥ 1.00
HIV-1 Control	
Analyte	≥ 45,000 and ≤ 1,800,000 RLU
Analyte S/CO	≥ 1.00 and < 40.00
Internal Control	≤ 475,000 RLU

Acceptance Criteria for Procleix Ultrio Tigris Controls in the HCV Discriminatory Assay

In the HCV Discriminatory Assay, a valid Procleix Ultrio Tigris Negative Control and Procleix Ultrio Tigris HCV Control are required at the beginning and end of each bracket (except the first bracket) for the results for the specimens in that bracket to be valid. No other controls are required. The Negative Control must have an S/CO less than 1.00 (nonreactive) to be accepted. The HCV Control must have S/CO greater than or equal to 1.00 (reactive) and less than 40.00 to be accepted.

Negative Control	
Analyte	≥ 0 and ≤ 150,000 RLU
Analyte S/CO	< 1.00
Internal Control	≥ 75,000 and ≤ 375,000 RLU
Internal Control S/CO	≥ 1.00
HCV Control	
Analyte	≥ 45,000 and ≤ 2,700,000 RLU
Analyte S/CO	≥ 1.00 and < 40.00
Internal Control	≤ 475,000 RLU

Acceptance Criteria for Procleix Ultrio Tigris Controls in the HBV Discriminatory Assay

In the HBV Discriminatory Assay, a valid Procleix Ultrio Tigris Negative Control and Procleix Ultrio Tigris HBV Control are required at the beginning and end of each bracket (except the first bracket) for the specimens in that bracket to be valid. No other controls are required. The Negative Control must have an S/CO less than 1.00 (nonreactive) to be accepted. The HBV Control must have S/CO greater than or equal to 1.00 (reactive) and less than 40.00 to be accepted.

Negative Control	
Analyte	≥ 0 and ≤ 150,000 RLU
Analyte S/CO	< 1.00
Internal Control	≥ 75,000 and ≤ 375,000 RLU
Internal Control S/CO	≥ 1.00
HBV Control	
Analyte	≥ 45,000 and ≤ 1,800,000 RLU
Analyte S/CO	≥ 1.00 and < 40.00
Internal Control	≤ 475,000 RLU

INTERPRETATION OF RESULTS

All calculations described above are performed by the Procleix Tigris 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 are determined. Analyte Signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/Cutoff (S/CO) on the report.

A specimen is Nonreactive if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control Signal is greater than or equal to the Internal Control Cutoff and less than or equal to 650,000 RLU in the Procleix Ultrio Assay, or less than or equal to 475,000 RLU in the Procleix HIV-1, HCV, or HBV Discriminatory Assays. A specimen is Reactive if the Analyte Signal is greater than or equal to the Analyte Cutoff (i.e., Analyte S/CO ≥1.00) and the Internal Control signal is less than or equal to 650,000 RLU in the Procleix Ultrio Assay, or less than or equal to 475,000 RLU in the Procleix HIV-1, HCV, or HBV Discriminatory Assays. Reactive results will be designated by the software. A specimen is Invalid if the Analyte Signal is less than the Analyte Cutoff (i.e., analyte S/CO <1.00) and the Internal Control signal is less than the Internal Control Cutoff. A specimen is also considered Invalid if the Internal Control Signal is greater than 650,000 RLU in the Procleix Ultrio Assay, or greater than 475,000 RLU in the Procleix HIV-1, HCV, or HBV Discriminatory Assays.

High titers of non-target analytes may produce invalid results in each of the individual Procleix Ultrio Discriminatory Assays. (For example, a high titer HBV sample may produce an invalid result in the discriminatory assay targeting HIV-1 or HCV.) In such cases, further testing with an alternate test method could be used for discrimination.

Cadaveric blood specimens, when tested neat, may be invalid due to inhibitory substances within the specimen. These invalid specimens may be diluted as in SPECIMEN COLLECTION, STORAGE, AND HANDLING, Cadaveric Blood Specimens, step J, and retested in singlet.

Summary of Specimen Interpretation

Specimen Interpretation	Criteria for the Procleix Ultrio Assay	Criteria for the Procleix HIV-1, HCV, and HBV Discriminatory Assays
Nonreactive	Analyte S/CO < 1.00 and Internal Control ≥ Internal Control Cutoff and Internal Control ≤ 650,000 RLU	Analyte S/CO < 1.00 and Internal Control ≥ Internal Control Cutoff and Internal Control ≤ 475,000 RLU
Reactive	Analyte S/CO ≥ 1.00 and Internal Control ≤ 650,000 RLU*	Analyte S/CO ≥ 1.00 and Internal Control ≤ 475,000 RLU**
Invalid	Internal Control > 650,000 RLU or Analyte S/CO < 1.00 and Internal Control < Internal Control Cutoff	Internal Control > 475,000 RLU or Analyte S/CO < 1.00 and Internal Control < Internal Control Cutoff

* In the Procleix Ultrio Assay, specimens with Internal Control signal greater than 650,000 RLU will be invalidated by the software and the reactive status cannot be assessed.
** In the Procleix HIV-1, HCV, and HBV Discriminatory Assays specimens with Internal Control signal greater than 475,000 RLU will be invalidated by the software and the reactive status cannot be assessed.

1. Any specimen with an interpretation of **Invalid** in the Procleix Ultrio Assay, Procleix HIV-1 Discriminatory Assay, Procleix HCV Discriminatory Assay, or Procleix HBV Discriminatory Assay must be retested in the same assay in singlet, except as noted in step 8. Cadaveric specimens with an interpretation of Invalid in the Procleix Ultrio Assay, Procleix HIV-1 Discriminatory Assay, Procleix HCV Discriminatory Assay, or Procleix HBV Discriminatory Assay previously diluted 1:5 may be retested in singlet, diluted at the 1:5 dilution, except as noted in step 8.

2. Failure to achieve expected results is an indication of an invalid run. Possible sources of error include test kit deterioration, operator error, faulty performance of equipment, specimen deterioration, or contamination of reagents.
3. 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.
4. Specimens with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the Procleix Ultrio Assay are considered nonreactive for HIV-1 RNA, HCV RNA, and HBV DNA. If the nonreactive specimen is a pool, each of the individual specimens comprising the pool is considered nonreactive and no further testing is required.
5. In the Procleix Ultrio Assay, specimens with an Analyte S/CO greater than or equal to 1.00 and an Internal Control signal less than or equal to 650,000 RLU are considered **Reactive**. In the Procleix HIV-1, HCV, and HBV Discriminatory Assays, specimens with an Analyte S/CO greater than or equal to 1.00 and an Internal Control signal less than or equal to 475,000 RLU are considered **Reactive.**
6. IF THE REACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool must be tested with the Procleix Ultrio Assay.
    a. If an individual specimen tests nonreactive with the Procleix Ultrio Assay, then the specimen is considered nonreactive for HIV-1 RNA, HCV RNA, and HBV DNA and no further testing is required.
    b. If an individual specimen tests Reactive with the Procleix Ultrio Assay, then the specimen must be tested with the HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays.
        (1) If an individual specimen then tests Reactive with one or more Discriminatory tests, then the specimen is considered Reactive-Discriminated.
        (2) If an individual specimen then tests nonreactive with all Discriminatory tests, then the specimen is considered Non-Discriminated. For HPC or DLI donors, continue to step 7b.
7. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION FROM A DONOR OF WHOLE BLOOD, BLOOD COMPONENTS OR SOURCE PLASMA, then the specimen must be tested with the HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays.
    a. If an individual specimen then tests Reactive with one or more Discriminatory tests, then the specimen is considered Reactive-Discriminated.
    b. If an individual specimen then tests nonreactive with all Discriminatory tests, then the specimen is considered Non-Discriminated.
8. 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 HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays.
    a. If an individual specimen then tests Reactive with one or more Discriminatory tests, then the specimen is considered Reactive-Discriminated.
    b. If an individual specimen then tests nonreactive with all Discriminatory tests, then the specimen is considered Non-Discriminated. The Non-Discriminated specimen may be retested in the Procleix Ultrio Assay if sufficient sample is available.
        (1) If the individual specimen tests nonreactive in the repeated Procleix Ultrio Assay, then the specimen is considered nonreactive for HIV-1 RNA, HCV RNA, and HBV DNA and no further testing is required.
        (2) If the individual specimen tests Reactive in the repeated Procleix Ultrio Assay, then the specimen is considered Repeatedly Reactive, Non-Discriminated for HIV-1 RNA, HCV RNA, and HBV DNA. 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 donor eligibility.
9. In runs or brackets that the Procleix Tigris System Software has flagged as Suspect, reactive specimens are identified by the software and must become the test of record. Specimens with Reactive results should be resolved according to the resolution algorithm for reactive specimens, as explained in steps 5, 6, and 7 in this section. Nonreactive specimens that have been invalidated or marked by the software as Suspect must be retested in the same assay in singlet.
10. 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. HBV seroreactive specimens found to be Reactive-HBV Discriminated in the Procleix Assays may be considered positive for HBV nucleic acid. The interpretation of Reactive-Discriminated specimen results on specimens that are nonreactive by serology is unclear.
11. For specimens that are repeat reactive on a licensed anti-HIV-1 screening test and reactive on the Procleix Ultrio Assay and reactive on the Procleix HIV-1 Discriminatory Assay, the Procleix Ultrio Assay acts as a supplemental test that confirms HIV-1 infection; it is not necessary to run an HIV-1 Western Blot.
12. For specimens that are repeat reactive on a licensed anti-HCV screening test and reactive on the Procleix Ultrio Assay and reactive on the Procleix HCV Discriminatory Assay, the Procleix Ultrio Assay acts as a supplemental test that confirms HCV infection; it is not necessary to run an additional FDA approved HCV supplemental test.
13. For specimens that are repeat reactive on a licensed HBsAg screening test and reactive on the Procleix Ultrio Assay and reactive on the Procleix HBV Discriminatory Assay, the Procleix Ultrio Assay acts as a supplemental test that confirms HBV infection; it is not necessary to run an HBsAg neutralization test.
14. Specimens that are Nonreactive in the Procleix Ultrio Assay or are Reactive in the Procleix Ultrio Assay but are not HIV-1 Discriminated, and are also repeatedly reactive in 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).

    Specimens that are Nonreactive in the Procleix Ultrio Assay or are Reactive in the Procleix Ultrio Assay but are not HCV Discriminated, and are also repeatedly reactive in a licensed donor screening test for antibodies to HCV, should be further tested using an FDA approved HCV supplemental test.

    Specimens that are Nonreactive in the Procleix Ultrio Assay or are Reactive in the Procleix Ultrio Assay but are not HBV Discriminated, and are also repeatedly reactive in a licensed donor screening test for HBsAg, should be further tested using an FDA approved HBsAg neutralization test.

15. Donors with specimens that are reactive in the Procleix HIV-1, HCV, or HBV Discriminatory Assays and/or repeatedly EIA reactive by licensed serological tests for HIV, HCV or HBV (or any combinations of these), should be referred for medical evaluation. A clinical diagnosis can be made only if the person meets the case definition(s) established by the Centers for Disease Control and Prevention.[31, 32]

GENERAL INFORMATION

LIMITATIONS OF THE PROCEDURE

This assay has been approved for use with the Procleix System and the Procleix Tigris System only. The Procleix Ultrio Tigris System Controls must not be used on systems other than the Procleix Tigris System or with assays other than the Procleix Ultrio Assay.

The Procleix Ultrio Assay may not be used to replace antibody-detection tests such as a test for anti-HIV-1, anti-HCV, or anti-HBc, or a test for HBsAg.

The clinical sensitivity for the Procleix Ultrio Assay has been demonstrated for specimens with HIV-1 or HCV viral RNA concentrations equal to or greater than 100 copies/mL or HBV viral DNA concentrations equal to or greater than 15 IU/mL. Samples with less than these concentrations may not yield reproducible results.

The Procleix Ultrio Assay on the Procleix Tigris System may have reduced sensitivity for detection of HBV DNA of genotypes A, B or D present at equal to or less than 100 copies/mL.

The results of the HBV genotype studies on the Procleix System shown in Table 39a indicated equivalent performance of the Procleix HBV Discriminatory Assay to the Procleix Ultrio Assay at a concentration of 300 copies/mL, but the Procleix HBV Discriminatory Assay was less sensitive than the Procleix Ultrio Assay at the lower concentrations of 30 and 100 copies/mL. Therefore, some HBV true positive specimens reactive in Procleix Ultrio Assay individual donation screening may test nonreactive by the Procleix HBV Discriminatory Assay.

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

Do not use proficiency panels, external quality controls, or Procleix Ultrio Tigris Controls as substitutes for the Procleix Ultrio Assay Calibrators.

Do not use proficiency panels, external quality controls, or the mandatory positive and negative calibrator reagents provided with the Procleix Ultrio Assay kits as substitutes for the Procleix Ultrio Tigris Controls.

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

Various donor and donation factors were evaluated for interference and cross-reactivity in the assays. A small portion had unexpected results in greater than 5% of the samples tested (Tables 19a to 22b).

Procleix System Fluid Preservative inhibits microbial growth for up to 30 days when added to 10 liters of water for the Procleix Tigris System. The two solid lines on the System Fluid Container indicate 10 liters. DO NOT TOP OFF THE SYSTEM FLUID CONTAINER AT ANY TIME. Procleix System Fluid Preservative effectiveness can not be assured unless conditions explicitly stated in this insert are followed.

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

PROCLEIX SYSTEM

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

The reproducibility panels were tested by a total of seven operators (two to three from each testing site) with three different clinical lots over multiple nonconsecutive days, using an automated front-end pipettor (Tecan) or manual pipetting of specimen and working Target Capture Reagent (wTCR). Twenty-four valid runs were generated for each assay across three clinical lots, with each panel member tested in triplicate per run and each operator performing testing for at least eight days. Panel members in an invalid run were retested until a valid run was obtained. Panel members with invalid test results generated in valid assay runs were not retested.

For the Procleix Ultrio Assay, 24 runs were generated on the Procleix System: none were invalid. From the valid assay runs, 720 test results were generated: none were invalid.

For the Procleix HIV-1 Discriminatory Assay, 24 runs were generated on the Procleix System: none were invalid. From the valid assay runs, 720 test results were generated: 1 (0.1%) was invalid due to Internal Control (IC) failure.

For the Procleix HCV Discriminatory Assay, 24 runs were generated on the Procleix System: 1 (4.2%) was invalidated by the operator. From the valid assay runs, 690 test results were generated. Of these, 2 (0.3%) samples had invalid results: 1 was due to IC failure and 1 occurred because the sample was not pipetted correctly.

For the Procleix HBV Discriminatory Assay, 25 runs were generated on the Procleix System: 1 (4.0%) was invalidated by the operator because the wrong probe was used, which resulted in an insufficient number of valid calibrators. From the valid assay runs, 720 test results were generated. Of these, 2 (0.3%) were invalid: 1 was due to IC failure and 1 occurred because the sample was not pipetted correctly.

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

Reproducibility analyses included evaluation of percent agreement and mean Signal/Cutoff (S/CO) ratios for panel members and mean Relative Light Unit (RLU) values for the Negative, HIV-1 Positive, HCV Positive, and HBV Positive Calibrators and evaluation of standard deviation (SD) and percent

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

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

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

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

Specimen	n	Concentration*	Number of Replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive*	1	0	72	98.6	2.00	0.10	4.9	0.08	3.9	0.00	0.0	0.01	0.6
Nonreactive*	1	0	72	100	2.02	0.07	3.4	0.08	4.1	0.00	0.0	0.00	0.0
HIV-1	1	10,000	72	100	17.06	0.48	2.8	0.56	3.3	0.37	2.2	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	72	100	37.6	0.84	2.2	1.55	4.1	0.83	2.2	0.42	1.1
HCV	1	150	72	100	6.06	0.27	4.4	0.27	4.5	0.24	3.9	0.30	4.9
HCV/HBV	1	2,500/500	72	100	21.79	0.50	2.3	0.85	3.9	0.55	2.5	0.43	2.0
HIV-1	1	150	72	100	13.72	1.46	10.6	0.54	4.0	0.68	4.9	0.89	6.5
HBV	1	50	72	100	14.92	0.37	2.5	0.55	3.7	0.55	3.7	0.24	1.6
HIV-1/HBV	1	2,500/500	72	100	31.01	0.76	2.4	1.13	3.6	0.55	1.8	0.21	0.7
HIV-1/HCV	1	2,500/2,500	72	100	23.32	0.49	2.1	0.81	3.5	0.00	0.0	0.00	0.0
Specimen			**Number of Replicates**	**% Agreement**	**Mean RLU**	**Intra-Assay**		**Inter-Assay**		**Inter-Lot**		**Inter-Site**	
						SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator**			71	N/A	216,522	6,392	3.0	8,055	3.7	9,533	4.4	13,466	6.2
HIV-1 Positive Calibrator			47	N/A	1,242,002	16,313	1.3	25,122	2.0	25,142	2.0	49,452	4.0
HCV Positive Calibrator			48	N/A	611,102	17,099	2.8	23,958	3.9	0.00	0.0	37,381	6.1
HBV Positive Calibrator			48	N/A	1,138,995	26,379	2.3	38,770	3.4	30,233	2.7	72,948	6.4

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

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

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

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

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

Specimen	n	Concentration*	Number of Replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive**	6	0	413	99.0	2.07	0.08	4.0	0.09	4.2	0.01	0.7	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	69	100	22.18	0.50	2.3	1.27	5.7	0.21	0.9	0.42	1.9
HCV	1	150	69	100	19.08	0.98	5.1	1.01	5.3	0.48	2.5	0.00	0.0
HCV/HBV	1	2,500/500	69	100	22.33	0.58	2.6	1.17	5.3	0.28	1.2	0.59	2.7
HIV-1/HCV	1	2,500/2,500	68	100	21.88	2.45	11.2	1.04	4.8	0.00	0.0	0.57	2.6
Specimen			Number of Replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator**, ****			113	N/A	220,270	7,527	3.4	9,308	4.2	19,184	8.7	13,180	6.0
HCV Positive Calibrator			69	N/A	1,318,289	26,043	2.0	54,331	4.1	42,644	3.2	79,828	6.1

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

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

Specimen	n	Concentration*	Number of Replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive**	6	0	430	99.8	2.00	0.12	5.8	0.10	4.9	0.02	0.9	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	72	100	25.17	0.57	2.3	1.55	6.2	0.00	0.0	0.66	2.6
HCV/HBV	1	2,500/500	72	100	25.14	0.66	2.6	1.50	6.0	0.03	0.1	0.69	2.7
HBV	1	50	72	100	25.55	0.69	2.7	1.62	6.3	0.00	0.0	0.92	3.6
HIV-1/HBV	1	2,500/500	72	100	26.06	0.69	2.7	1.79	6.9	0.00	0.0	0.78	3.0
Specimen			Number of Replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator**, ****			119	N/A	207,762	7,641	3.7	10,093	4.9	18,207	8.8	10,636	5.1
HBV Positive Calibrator			72	N/A	1,083,154	32,781	3.0	40,616	3.8	39,069	3.6	68,573	6.3

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

REPRODUCIBILITY

PROCLEIX TIGRIS SYSTEM

Reproducibility of the Procleix Ultrio Assay, HIV-1 Discriminatory Assay, HCV Discriminatory Assay, and HBV Discriminatory Assay was evaluated at three blood center laboratories. To determine the reproducibility of each assay, 10 members of a reproducibility panel were tested as individual specimens (Tables 5-8). Eight of the panel members were either positive for HIV-1 RNA (150, 2,500 and 10,000 c/mL), HCV RNA (150 and 2,500 c/mL), and/or HBV DNA (50 and 500 IU/mL) and two panel members were HIV-1, HCV and HBV negative.

The reproducibility panels were tested by a total of six operators (two from each testing site) with three different clinical lots over multiple nonconsecutive days using four Procleix Tigris Systems (Tigris). For each assay, each operator performed three worklists (i.e., runs) per Procleix Ultrio Assay clinical lot on one of the four Procleix Tigris Systems (3 worklists/operator X 6 operators X 3 lots = 54 worklists). The 54 worklists were repeated 3 times, totaling 162 results per panel member. Panel members in an invalid worklist were retested until a valid worklist was obtained. Panel members with invalid test results generated in valid assay worklists were not retested.

For the Procleix Ultrio Assay, 57 worklists were generated on the Procleix Tigris System: 3 (5.3%) were invalidated by the operator because the ambient room temperature was out of range. From the valid assay worklists, 1,620 test results were generated. Of these, 6 (0.4%) test results were invalid: 2 were due to Internal Control (IC) failure and 4 were due to a pump failure or a volume verification error of the sample.

For the Procleix HIV-1 Discriminatory Assay, 58 worklists were generated on the Procleix Tigris System: 3 (5.2%) were invalidated by the operator because the ambient room temperature was out of range. From the valid assay worklists, 1,620 test results were generated. Of these, 4 (0.2%) test results were invalid: 3 were due to IC failure and 1 was due volume verification failure.

For the Procleix HCV Discriminatory Assay, 58 worklists were generated on the Procleix Tigris System: 3 (5.2%) were invalidated by the operator because the ambient room temperature was out of range. From the valid assay worklists, 1,618 test results were generated. Of these, 4 (0.2%) samples had invalid results: 3 were due to IC failure and 1 was due to a pump failure.

For the Procleix HBV Discriminatory Assay, 57 worklists were generated on the Procleix Tigris System: 3 (5.3%) were invalidated by the operator because the ambient room temperature was out of range. From the valid assay worklists, 1,638 test results were generated. Of these, 9 (0.5%) were invalid: 3 were due to IC failure and 6 were due to volume verification failure or a pump failure.

The reproducibility study assessed intra- and inter-worklist, inter-lot and inter-Tigris variability of the Procleix Ultrio Assay and each discriminatory assay. Reproducibility analyses included evaluation of percent agreement and mean signal/cutoff (S/CO) ratios for panel members and mean Relative Light Unit (RLU) values for the Negative, HIV-1 Positive, HCV Positive, and HBV Positive Calibrators and evaluation of standard deviation (SD) and percent coefficient of variation (%CV) of those S/CO ratios and RLU values for each of the four variance factors (Tables 5-8). The mean analyte S/CO ratios were analyzed for the positive panel members and the Internal Control (IC) S/CO ratios were analyzed for the negative panel members. The mean analyte RLU values were analyzed for the Positive Calibrators and the Internal Control RLU values were analyzed for the Negative Calibrators. The percent agreement between the assay results and the true status of each panel member was calculated. For the Procleix Ultrio Assay, results for all individual panel members are shown. For the discriminatory assays, results for panel members with expected results of Nonreactive were combined. Results for panel members with expected results of Reactive are shown individually.

For the Procleix Ultrio Assay and three discriminatory assays, the overall percent agreement of test results was 100% for positive samples and 99.7% - 100% for negative samples. It should be noted that while intra-worklist (or random error), inter-worklist and inter-Tigris factors were responsible for the majority of the variance in the assays, the %CV of any of these components by itself did not exceed 14.9% for any positive or negative samples, in any assay. Therefore, the reproducibility study demonstrated that the assays are robust (Tables 5-8).

Table 5. Procleix Tigris System - Reproducibility of the Procleix Ultrio Assay (analysis of analyte signals, unless noted)

Specimen	n	Concentration*	Number of Replicates	% Agreement	Mean S/CO	Intra-Worklist		Inter-Worklist		Inter-Lot		Inter-Tigris	
						SD	%CV	SD	%CV	SD***	%CV	SD	%CV
Nonreactive**	1	0	162	100	2.00	0.08	4.0	0.07	3.2	0.01	0.7	0.01	0.7
Nonreactive**	1	0	160	100	2.06	0.08	4.0	0.06	2.7	0.00	0.0	0.01	0.6
HIV-1	1	10,000	162	100	16.68	0.32	1.9	0.74	4.4	0.24	1.4	0.92	5.5
HIV-1/ HCV/ HBV	1	2,500/ 2,500 /500	162	100	39.65	1.24	3.1	1.72	4.3	0.43	1.1	2.33	5.9
HCV	1	150	161	100	7.20	1.07	14.9	0.33	4.6	0.00	0.0	0.11	1.6
HCV/HBV	1	2,500/500	162	100	23.10	1.28	5.5	0.95	4.1	0.45	1.9	1.23	5.3
HIV-1	1	150	161	100	12.12	1.37	11.3	0.55	4.5	0.52	4.3	0.27	2.2
HBV	1	50	162	100	16.22	0.58	3.6	0.85	5.3	0.52	3.2	0.89	5.5
HIV-1/HBV	1	2,500/500	162	100	32.20	1.49	4.6	1.43	4.5	0.97	3.0	1.35	4.2
HIV-1/HCV	1	2,500/2,500	160	100	24.18	1.16	4.8	1.02	4.2	0.40	1.6	1.11	4.6

Specimen	Number of Replicates	% Agreement	Mean RLU	Intra-Worklist		Inter-Worklist		Inter-Lot		Inter-Tigris	
				SD	%CV	SD***	%CV	SD***	%CV	SD	%CV
Negative Calibrator**	160	N/A	204,057	8,403	4.1	7,408	3.6	6,426	3.1	13,170	6.5
HIV-1 Positive Calibrator	106	N/A	1,273,698	58,241	4.6	0.00	0.0	12,607	1.0	31,358	2.5
HCV Positive Calibrator	108	N/A	656,712	24,643	3.8	9,642	1.5	21,969	3.3	16,731	2.5
HBV Positive Calibrator	108	N/A	1,210,801	30,664	2.5	18,763	1.6	0.00	0.0	23,218	1.9

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

Table 6. Procleix Tigris System - Reproducibility of the Procleix HIV-1 Discriminatory Assay (analysis of analyte signal, unless noted)

Specimen	n	Concentration*	Number of Replicates	% Agreement	Mean S/CO	Intra-Worklist		Inter-Worklist		Inter-Lot		Inter-Tigris	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive*	5	0	806	99.9	2.05	0.11	5.1	0.05	2.5	0.00	0.0	0.00	0.0
HIV-1	1	10,000	162	100	25.72	0.51	2.0	1.09	4.2	0.25	1.0	2.04	7.9
HIV-1/HCV/ HBV	1	2,500/2,500/ 500	162	100	24.60	0.76	3.1	1.05	4.3	0.00	0.0	1.41	5.7
HIV-1	1	150	162	100	18.85	2.02	10.7	0.81	4.3	0.00	0.0	0.67	3.5
HIV-1/HBV	1	2,500/500	162	100	24.06	1.32	5.5	0.93	3.9	0.00	0.0	1.39	5.8
HIV-1/HCV	1	2,500/2,500	162	100	24.65	1.04	4.2	0.88	3.6	0.00	0.0	1.38	5.6
Specimen			**Number of Replicates**	**% Agreement**	**Mean RLU**	**Intra-Worklist**		**Inter-Worklist**		**Inter-Lot**		**Inter-Tigris**	
						SD	**%CV**	**SD*** **	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator,****			161	N/A	206,552	8,133	3.9	5,135	2.5	9,831	4.8	20,390	9.9
HIV-1 Positive Calibrator			161	N/A	1,314,039	62,321	4.7	0.00	0.0	11,591	0.9	32,355	2.5

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

Table 7. Procleix Tigris System - Reproducibility of the Procleix HCV Discriminatory Assay (analysis of analyte signal, unless noted)

Specimen	n	Concentration*	Number of Replicates	% Agreement	Mean S/CO	Intra-Worklist		Inter-Worklist		Inter-Lot		Inter-Tigris	
						SD	%CV	SD	%CV	SD***	%CV	SD	%CV
Nonreactive**	6	0	967	99.7	2.06	0.13	6.3	0.04	1.9	0.01	0.5	0.05	2.2
HIV-1/ HCV/ HBV	1	2,500/ 2,500/ 500	161	100	22.57	0.69	3.0	1.22	5.4	0.00	0.0	1.37	6.1
HCV	1	150	162	100	20.53	0.48	2.3	1.16	5.6	0.12	0.6	1.27	6.2
HCV/HBV	1	2,500/500	162	100	23.20	0.63	2.7	1.15	5.0	0.00	0.0	1.36	5.9
HIV-1/HCV	1	2,500/2,500	162	100	23.51	0.83	3.5	1.16	4.9	0.00	0.0	1.16	4.9
Specimen			**Number of Replicates**	**% Agreement**	**Mean RLU**	**Intra-Worklist**		**Inter-Worklist**		**Inter-Lot**		**Inter-Tigris**	
						SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator,****			161	N/A	216,937	9,458	4.4	5,318	2.5	14,428	6.7	17,192	7.9
HCV Positive Calibrator			162	N/A	1,415,674	56,763	4.0	31,087	2.2	49,387	3.5	27,551	1.9

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

Table 8. Procleix Tigris System - Reproducibility of the Procleix HBV Discriminatory Assay (analysis of analyte signals, unless noted)

Specimen	n	Concentration*	Number of Replicates	% Agreement	Mean S/CO	Intra-Worklist		Inter-Worklist		Inter-Lot		Inter-Tigris	
						SD	%CV	SD	%CV	SD***	%CV	SD	%CV
Nonreactive**	6	0	968	99.8	2.05	0.10	4.9	0.10	4.7	0.00	0.0	0.03	1.4
HIV-1/HCV/ HBV	1	2,500/ 2,500/ 500	161	100	24.93	0.88	3.5	1.08	4.3	0.58	2.3	1.63	6.5
HCV/HBV	1	2,500/500	160	100	25.42	0.79	3.1	1.08	4.2	0.36	1.4	1.80	7.1
HBV	1	50	160	100	25.62	1.03	4.0	1.15	4.5	0.53	2.0	1.97	7.7
HIV-1/HBV	1	2,500/500	162	100	26.34	0.76	2.9	1.20	4.5	0.41	1.6	1.88	7.1

Specimen	Number of Replicates	% Agreement	Mean RLU	Intra-Worklist		Inter-Worklist		Inter-Lot		Inter-Tigris	
				SD	%CV	SD***	%CV	SD	%CV	SD	%CV
Negative Calibrator**,****	159	N/A	203,545	11,277	5.5	8,361	4.1	18,223	9.0	17,575	8.6
HBV Positive Calibrator	160	N/A	1,200,709	41,895	3.5	0.00	0.0	24,558	2.0	24,922	2.1

n = Number of panel members combined for this analysis.

* Concentration = copies/mL for HIV-1 and HCV, IU/mL for HBV. For nonreactive specimens, only the HBV concentration is listed.

** Analysis of Internal Control signal

*** Per CLSI guidelines (EP5-A, page 7), numbers <0 are recorded as 0.

**** Analysis of Negative Calibrator and HIV-1 and HCV Positive Calibrators

SPECIFICITY IN NORMAL BLOOD DONORS

PROCLEIX SYSTEM

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

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

In some cases, samples collected for the pivotal clinical specificity study were tested with samples from other Procleix System pivotal clinical studies (Testing of Known Positive Samples from HIV-1, HCV, and/or HBV Infected Individuals, Testing of Known Positive 16-Sample Pools, Clinical Sensitivity and Specificity High-Risk Population Study, and Reactivity in Seroconverting Donors). Therefore, the run data presented below were generated from testing samples from all of the Procleix System pivotal clinical studies.

For the Procleix Ultrio Assay, 594 runs were generated from testing of samples from the Procleix System pivotal clinical studies: 7 (1.2%) runs were invalid. Of the 7 invalid runs, 3 were due to an insufficient number of valid calibrators. The remaining 4 of 7 runs were invalidated by the operator: 1 run contained greater than 10% invalid test results and 3 runs were invalidated due to contamination. From the valid assay runs, 13,975 and 13,408 test results were generated for pools and IDS, respectively, from whole blood donations tested in this clinical specificity study. Of the 13,975 pools, 30 (0.2%) had initial invalid results. When 28 of the 30 pools were retested, none were repeat invalid. The remaining 2 pools were not retested and were invalid due to Internal Control (IC) failure. For IDS, 36 (0.3%) samples had initial invalid results. When 24 of the 36 IDS were retested, none were repeat invalid. The remaining 12 IDS were not retested: 8 were invalid due to IC failure and 4 were invalid due to dispense verification failure for the target capture reagent.

Pools and IDS from PSP donors were also tested with the Procleix Ultrio Assay in this clinical specificity study. From the valid assay runs, 1,199 and 48 test results were generated for pools and IDS, respectively, from PSP donors. Of the 1,199 pools, 6 (0.5%) had initial invalid results. When all 6 pools were retested, none were repeat invalid. For IDS, none of the samples had initial invalid results.

For the Procleix HIV-1 Discriminatory Assay, 94 runs were generated from testing of samples from the Procleix System pivotal clinical studies. Of these, 1 (1.1%) run was invalidated by the operator because the run contained greater than 10% invalid test results. From the valid assay runs, 1,853 test results were generated for IDS from whole blood donations tested in this clinical specificity study. Of the 1,853 IDS, 20 (1.1%) had initial invalid results. When 8 of the 20 IDS were retested, 5 were repeat invalid due to IC failure. The remaining 12 of 20 IDS were not retested: 2 were invalid due to IC failure and 10 were invalid due to the specimen not being pipetted correctly.

For the Procleix HCV Discriminatory Assay, 92 runs were generated from testing of samples from the Procleix System pivotal clinical studies. Of these, 1 (1.1%) run was invalid due to an insufficient number of valid calibrators. From the valid assay runs, 1,854 test results were generated for IDS from whole blood donations tested in this clinical specificity study. Of the 1,854 IDS, 10 (0.5%) had initial invalid results. When 4 of the 10 IDS were retested, 2 were repeat invalid due to IC failure. The remaining 6 of 10 IDS were not retested: 2 were invalid due to IC failure and 4 were invalid due to the specimen not being pipetted correctly.

For the Procleix HBV Discriminatory Assay, 96 runs were generated from testing of samples from the Procleix System pivotal clinical studies. Of these, 3 (3.1%) runs were invalid: 2 runs had an insufficient number of valid calibrators and 1 run was invalidated by the operator because the run contained greater than 10% invalid test results. From the valid assay runs, 1,832 test results were generated for IDS from whole blood donations tested in this clinical specificity study. Of the 1,832 IDS, 22 (1.2%) had initial invalid results. When 6 of the 22 IDS were retested, 2 were repeat invalid due to the specimen not being pipetted correctly. The remaining 16 of 22 IDS were not retested: 4 were invalid due to IC failure and 12 were invalid due to the specimen not being pipetted correctly.

Specificity of the Procleix Ultrio Assay was calculated from 12,028 16-sample plasma pools from whole blood donations, 12,780 IDS from whole blood donations, and 1,198 16-sample plasma pools from PSP donors (Table 11). Specificity of the Procleix HIV-1 (n=1,797), HCV (n=1,810), and HBV (n=1,795) Discriminatory Assays was calculated using results of IDS from whole blood donations. Voluntary source plasma (VSP) donations were also collected and included in either the 16-sample pools or IDS from whole blood donations (n=303 and 9, respectively) tested in the Procleix Ultrio Assay. In the specificity evaluation, the results from the Procleix Ultrio Assay and the associated Discriminatory Assays were compared to results from the licensed Procleix HIV-1/HCV Assay and associated Discriminatory Assays and to results from licensed HBsAg serologic tests. The specificity was also based on the results from alternate licensed or validated nucleic acid tests (Alternate NAT), which were performed on IDS or individual samples from pools with discordant results.

Rates of Procleix Ultrio Assay reactivity are presented in Tables 9 and 10 for pools and IDS from whole blood donations that were included in the clinical specificity analyses. The overall clinical specificity results are summarized in Table 11. Table 12 shows clinical specificity by site for pools and IDS from whole blood donations. Pools from PSP donations were tested at only one site.

Table 9. Procleix System - Clinical Specificity Study: Procleix Ultrio Assay Reactivity in 16-Sample Pools

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

CI = Confidence interval, n = number of pools

Table 10. Procleix System - Clinical Specificity Study: Procleix Ultrio Assay Reactivity in IDS

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

CI = Confidence interval, n = number of individual donations

* Eight IDS had unavailable Alternate NAT results because they were invalidated (n=7) or for other reasons. Two IDS were Alternate NAT nonreactive.

** Includes four initially reactive IDS without discriminatory assay results and with nonreactive reference test results. Also includes 12 IDS without retest results.

Overall Clinical Specificity of the Procleix Ultrio Assay

There were 12,028 pools tested with the Procleix Ultrio Assay and included in the specificity calculations (Table 9).There were 11,796 pools from whole blood donations that tested nonreactive in the Procleix Ultrio (Table 11). Of these, 11,786 pools were considered true negative and 10 pools were considered false negative. Nine of the 10 false negative pools were HBsAg seropositive and 1 pool was reactive in the Procleix HIV-1/HCV Assay. There were 232 pools that tested reactive in the Procleix Ultrio Assay. Of these, 173 pools were considered true positive. Six of these pools were reactive in the Procleix Ultrio Assay and had a constituent sample that was reactive in Alternate NAT, but were nonreactive in the reference test. Fifty-nine pools were considered false positive. The overall specificity of 16-sample pools from whole blood donations was 11,786/11,845 or 99.5% (95% CI: 99.4-99.6%).

There were 12,780 IDS tested with the Procleix Ultrio Assay – from reactive pools or tested as IDS only – and included in the specificity calculations (Table 10).There were 12,480 IDS that tested nonreactive in the Procleix Ultrio Assay (Table 11). Of these, 12,479 IDS were considered true negative and 1 sample was considered false negative. The false negative sample was Procleix HIV-1/HCV Assay reactive, HCV discriminated. There were 300 IDS that tested reactive in the Procleix Ultrio Assay. Of these, 193 IDS were considered true positive and 107 IDS were considered false positive. The overall specificity of IDS from whole blood donations was 12,479/12,586 or 99.1% (95% CI: 99.0-99.3%).

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

There were 1,198 PSP pools tested with the Procleix Ultrio Assay and included in the specificity calculations (Table 11). There were 1,195 PSP pools that tested nonreactive in the Procleix Ultrio Assay and all of these pools were considered true negative. There were three PSP pools that tested reactive in the Procleix Ultrio Assay and all three pools were false positive. The overall specificity of pools from PSP donations was 1,195/1,198 or 99.7% (95% CI: 99.3-99.9%).

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

There were 1,785 IDS that tested nonreactive in the Procleix HIV-1 Discriminatory Assay and all were considered true negative (Table 11). There were 12 IDS that tested reactive in the Procleix HIV-1 Discriminatory Assay. Of these, 8 IDS were considered true positive and 4 IDS were considered false positive. The specificity of the Procleix HIV-1 Discriminatory Assay was 99.8% (1,785/1,789; 95% CI: 99.4-99.9%).

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

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

Table 11. Procleix System - Clinical Specificity Study: Overall Specificities of the Procleix Ultrio Assay and Discriminatory Assays

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

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

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

Table 12 shows clinical specificity results for the three blood center testing sites. Clinical specificity of the Procleix Ultrio Assay in 16-sample pools ranged from 99.3% (95% CI: 99.1-99.5%) for Site 2 to 99.8% (95% CI: 99.6-99.9%) for Site 1. Specificity in IDS was significantly lower at Site 2 at 98.5% (95% CI: 98.1-98.8%) than at Sites 1 and 3, which had specificity rates of 99.6% (95% CI: 99.3-99.8%) and 99.5% (95% CI: 99.2-99.7%), respectively. Including all samples tested, whether tested as IDS only, IDS after a reactive pool result, or as part of a nonreactive pool, specificity ranged from 99.930% (95% CI: 99.912-99.945%) for Site 2 to 99.973% (95% CI: 99.955-99.984%) for Site 1.

Clinical specificity of the Procleix HIV-1 Discriminatory Assay ranged from 99.7% (95% CI: 98.7-100%) for Site 3 to 100% (95% CI: 99.3-100%) for Site 1. Specificity of the Procleix HCV Discriminatory Assay was significantly lower at Site 2 at 96.1% (95% CI: 94.3-97.5%) than at Sites 1 and 3, which had specificity rates of 99.2% (95% CI: 98.1-99.8%) and 99.3% (95% CI: 98.1-99.8%), respectively. For the Procleix HBV Discriminatory Assay, specificity ranged from 99.6% (95% CI: 98.7-100%) for Site 3 to 100% (95% CI: 99.3-100%) for Site 1 (Table 12).

Table 12. Procleix System - Clinical Specificity Study: Specificities of the Procleix Ultrio Assay and Discriminatory Assays by Site

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

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

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

False positive rates for the Procleix Ultrio Assay are shown in Table 13a and Table 13b for pools and IDS, respectively. For the Procleix Ultrio Assay clinical trial, pools and IDS were considered false positive if samples were Procleix Ultrio Assay reactive, reference test (Procleix HIV-1/HCV Assay and HBsAg test) nonreactive, and Alternate NAT nonreactive or not tested.

Table 13a. Procleix System - Clinical Specificity Study: Procleix Ultrio Assay False Positive Rates in 16-Sample Pools

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

Table 13b. Procleix System - Clinical Specificity Study: Procleix Ultrio Assay False Positive Rates in IDS

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

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

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

Table 14 shows the reactivity rates of the Procleix Ultrio Assay and Procleix HIV-1/HCV Assay in HIV-1 and HCV positive donations collected during the clinical specificity study for the Procleix Ultrio Assay. Samples (whether tested in pools or as IDS only) were included in this analysis if they had valid and complete Procleix Ultrio Assay and Procleix HIV-1/HCV Assay results for HIV-1 and HCV detection.

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

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

Table 14. Procleix System - Comparison of Reactivity Rates between the Procleix Ultrio Assay and the Procleix HIV-1/HCV Assay in HIV-1 and HCV Infected Donations

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

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

Comparison of the Procleix Ultrio Assay to HBV Serology Results

Table 15 summarizes the HBV results for 218,260 samples that were tested initially in pools or as IDS only and had valid Procleix Ultrio Assay and Procleix HBV Discriminatory Assay results.

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

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

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

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

Table 15. Procleix System - Clinical Specificity Study: Comparison of HBV Serology and Procleix Ultrio Assay Results at Index

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

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

PROCLEIX TIGRIS SYSTEM

The clinical specificity of the Procleix Ultrio Assay on the Procleix Tigris System was determined in 16-sample pools made from plasma from whole blood donations and individual donor samples (IDS) from whole blood donations.

The study was conducted at two blood center testing laboratories using plasma samples derived from approximately 15 geographically diverse blood donor sites in the United States. During this study, all testing was performed linked using one clinical lot of Procleix Ultrio Assay reagent kits. All of the

samples collected for the study were tested with the Procleix Ultrio Assay, the licensed Procleix HIV-1/HCV Assay, licensed HBsAg serologic tests and, as appropriate, confirmatory tests. Alternate licensed or validated nucleic acid test (Alternate NAT) results, if available, were used to resolve discordant Procleix Ultrio Assay and reference test results. Follow-up testing was not performed.

In some cases, samples collected for the pivotal clinical specificity study were tested with samples from other Procleix Tigris System pivotal clinical studies (Testing of Known Positive Samples from HIV-1, HCV, and/or HBV Infected Individuals, Clinical Sensitivity and Specificity High-Risk Population Study, and Reactivity in Seroconverting Donors). Therefore, the worklist data presented below were generated from testing samples from all of the Procleix Tigris System pivotal clinical studies.

For the Procleix Ultrio Assay, 73 worklists were generated from testing of samples from the Procleix Tigris System pivotal clinical studies: 3 (4.1%) worklists were invalid. Of the 3 invalid worklists, all were due to an insufficient number of valid calibrators. From the valid assay worklists, 1,027 and 948 test results were generated for pools and IDS, respectively, from whole blood donations tested in this clinical specificity study. Of the 1,027 pools, 8 (0.8%) had initial invalid results. The 8 pools were not retested and were invalid due to Internal Control (IC) failure. For IDS, none had initial invalid results.

For the Procleix HIV-1 Discriminatory Assay, 28 worklists were generated from testing of samples from the Procleix Tigris System pivotal clinical studies: none were invalid. From the valid assay worklists, 464 test results were generated for IDS from whole blood donations tested in this clinical specificity study: none were invalid.

For the Procleix HCV Discriminatory Assay, 25 worklists were generated from testing of samples from the Procleix Tigris System pivotal clinical studies. Of these, 3 (12.0%) worklists were invalid: 2 were invalid due to an insufficient number of valid calibrators and 1 was invalidated by the operator because of an instrument error. From the valid assay worklists, 95 test results were generated for IDS from whole blood donations tested in this clinical specificity study: none were invalid.

For the Procleix HBV Discriminatory Assay, 23 worklists were generated from testing of samples from the Procleix Tigris System pivotal clinical studies. Of these, 2 (8.7%) worklists were invalid due to an insufficient number of valid calibrators. From the valid assay worklists, 16 test results were generated for IDS from whole blood donations tested in this clinical specificity study: none were invalid.

Specificity of the Procleix Ultrio Assay was calculated from 1,019 16-sample pools made from plasma from whole blood donations (Table 16) and 948 IDS from whole blood donations (Table 17). In the specificity evaluation, the results from the Procleix Ultrio Assay were compared to the results from the licensed Procleix HIV-1/HCV Assay and associated discriminatory assays and to results from licensed HBsAg serologic tests. Rates of Procleix Ultrio Assay reactivity are presented in Tables 16 and 17 for pools and IDS, respectively, from whole blood donations that were included in the clinical specificity analyses. The overall clinical specificity results are summarized in Table 18.

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

Results	n	Percent (95% CI)
Total pools tested	1,019	100.00
Nonreactive pools	1,003	98.43 (97.46-99.10)
Initially reactive pools	16	1.57 (0.90-2.54)
Pool, individual constituent(s), and reference test reactive (true positive)	14	1.37 (0.75-2.29)
Pool and reference test reactive; incomplete results for individual constituents (true positive)	1	0.10 (0.00-0.55)
Pool reactive, individual constituents and reference test nonreactive (false positive)	1	0.10 (0.00-0.55)

n = number of pools, CI = Confidence interval

Table 17. Procleix Tigris System - Clinical Specificity Study: Procleix Ultrio Assay Reactivity in IDS

Results	n	Percent (95% CI)
Total IDS tested	948	100.00
Nonreactive IDS	932	98.31 (97.27-99.03)
Initially reactive IDS	16	1.69 (0.97-2.73)
IDS, discriminatory assay, and reference test reactive (true positive)	13	1.37 (0.73-2.33)
IDS and reference test reactive; discriminatory assay not tested (true positive)	1	0.11 (0.00-0.59)
IDS reactive, discriminatory assay, retest, and reference test nonreactive (false positive)	1	0.11 (0.00-0.59)
IDS reactive, discriminatory assay and reference test nonreactive, retest not available (false positive)	1	0.11 (0.00-0.59)

n = number of individual donations, CI = Confidence interval

Overall Clinical Specificity of the Procleix Ultrio Assay

There were 1,003 pools that tested nonreactive in the Procleix Ultrio Assay. Of these, 1,002 pools were considered true negative and one pool was considered false negative. The false negative pool contained one IDS that was HBsAg seropositive. There were 16 pools that tested reactive in the Procleix Ultrio Assay. Of these, 15 pools were considered true positive and one pool was considered false positive, as the pool contained no samples with Procleix HIV-1/HCV Assay reactive or HBsAg seropositive results. The overall specificity of 16-sample pools from whole blood donations was 99.9% (1,002/1,003; 95% CI: 99.4-100%).

There were 932 IDS that tested nonreactive in the Procleix Ultrio Assay and all were considered true negative. There were 16 IDS that tested reactive in the Procleix Ultrio Assay. Of these, 14 IDS were considered true positive and two IDS were considered false positive, as the samples did not have Procleix HIV-1/HCV Assay reactive or HBsAg seropositive results. The overall specificity of IDS from whole blood donations was 99.8% (932/934; 95%CI: 99.2-100%).

Table 18. Procleix Tigris System - Clinical Specificity Study: Specificity of the Procleix Ultrio Assay in Pools and IDS

Sample	n	True Negative	False Negative	True Positive	False Positive	Specificity (%)	95% CI
Pools from Whole Blood Donations	1,019	1,002	1	15	1	99.9	99.4-100.0
IDS from Whole Blood Donations	948	932	0	14	2	99.8	99.2-100.0

n = number of samples (pools or individual donations)
CI = Confidence Interval

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

False positive rates for the Procleix Ultrio Assay on the Procleix Tigris System were calculated for pools and IDS. In the clinical trial, pools and IDS were considered false positive if samples were Procleix Ultrio Assay reactive but reference test (licensed Procleix HIV-1/HCV Assay and HBsAg serologic test) and/or Alternate NAT negative. One of 1,003 pools (0.10%) with negative reference test and/or Alternate NAT results had a reactive Prodeix Ultrio Assay result (i.e., false positive result). When the 16 samples from this pool were tested individually, all had nonreactive Procleix Ultrio Assay results (i.e., unresolved pool). Two of 934 (0.21%) IDS with negative reference test results had reactive Procleix Ultrio Assay results (i.e., false positive results). Both of these IDS were nonreactive when tested in the discriminatory assays (i.e., nondiscriminated).

NON-SPECIFICITY STUDIES

PROCLEIX SYSTEM AND PROCLEIX TIGRIS SYSTEM

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

Tables 19a, 19b, 20a, and 20b show all valid initial test results obtained when specimens containing various donor and donation factors were tested with the Procleix Ultrio Assay and Discriminatory Assays. HIV-1, HCV, and HBV positive specimens were created by individually spiking the various donor and donation specimens to a final concentration of 200 copies/mL of HIV-1, 60 IU/mL of HCV, or 30 IU/mL of HBV. Cross-reactivity and interference are defined as greater than 5% unexpected results.

No cross-reactivity (Tables 20a and 20b) or interference (Tables 19a and 19b) was observed for naturally occurring hemolyzed or lipemic specimens or plasma containing the following substances: serum albumin (6 g/dL), hemoglobin (500 mg/dL) and lipids (3,000 mg/dL). No cross-reactivity or interference for detection of HIV-1, HCV, and HBV was observed for naturally occurring icteric specimens or plasma containing bilirubin up to 20 mg/dL. However, this high level of spiked bilirubin produces a slight decrease in HBV analytical sensitivity. This effect was not observed when bilirubin is present at 2.5 mg/dL.

Multiple specimens from each group of patients with the following autoimmune conditions were evaluated: rheumatoid factor, antinuclear antibody, lupus and multiple myeloma. Also tested were samples from flu vaccinees, from hepatitis B vaccinees, from patients with elevated IgM, with elevated IgG, with elevated amino alanine transferase (ALT) and from patients with alcoholic liver cirrhosis. For the majority of these conditions, no cross-reactivity or interference was observed. However, a small portion of these specimens had random, unexpected results in greater than 5% of the samples tested. These occurrences are indicated in bold text in Tables 19a, 19b, 20a, and 20b. Additionally, in the Procleix HBV Discriminatory Assay, antinuclear antibody cross-reactivity may be seen on the Procleix System (Table 20a) and interference may be seen on the Procleix Tigris System (Table 19b).

No cross-reactivity or interference was observed in the majority of bacterially contaminated plasma specimens or in specimens from patients infected with other bloodborne pathogens. Multiple specimens from each group of patients with the following viral infections were evaluated: herpes simplex virus-1 (HSV 1), herpes simplex virus-2 (HSV 2), CMV, EBV, hepatitis A virus (HAV), HTLV-I, HTLV-II, hepatitis G virus (HGV), rubella, and parvovirus B-19. A small portion of the specimens containing viral infections had random, unexpected results in greater than 5% of the samples tested. These occurrences are indicated in bold text in Tables 19a, 19b, 20a, and 20b.

Table 19a. Procleix System - Detection of HIV-1, HCV, and HBV in the Presence of Donor and Donation Factors with the Procleix Ultrio Assay and Discriminatory Assays

Donor or Donation Factor	Reactive/Tested*					
	HIV-1 Positive (200 c/mL)		HCV Positive (60 IU/mL)		HBV Positive (30 IU/mL)	
	Procleix Ultrio Assay	Procleix dHIV-1 Assay	Procleix Ultrio Assay	Procleix dHCV Assay	Procleix Ultrio Assay	Procleix dHBV Assay
Hemolyzed	21/21	21/21	18/18	18/18	29/30	30/30
Icteric	21/21	21/21	30/30	30/30	30/30	30/30
Lipemic	24/24	24/24	12/12	12/12	30/30	29/30
Normal	39/39	37/37	39/39	39/39	39/39	39/39
Albumin (6 g/dL)	39/39	39/39	39/39	39/39	38/39	39/39
Bilirubin (20 mg/dL)	39/39	39/39	39/39	39/39	38/39	**37/39**
Bilirubin (2.5 mg/dL)	NA	NA	NA	NA	86/87	89/89
Hemoglobin (500 mg/dL)	38/38	39/39	39/39	39/39	39/39	39/39
Lipids (3000 mg/dL)	39/39	39/39	39/39	39/39	38/39	39/39
Alcoholic Cirrhosis	30/30	30/30	30/30	**28/30**	30/30	29/30
Antinuclear Antibody	27/27	27/27	27/27	26/27	27/27	27/27
ALT	30/30	30/30	30/30	30/30	30/30	30/30
Elevated IgG	30/30	**26/30**	**25/30**	30/30	30/30	30/30
Elevated IgM	29/30	**27/30**	29/30	29/30	**26/30**	**27/30**
Lupus	**28/30**	30/30	30/30	30/30	30/30	30/30
Multiple Myeloma	23/23	23/23	23/23	23/23	**21/23**	23/23
Rheumatoid Factor	**27/30**	29/30	29/30	30/30	29/30	30/30
Flu Vaccinee	30/30	30/30	30/30	30/30	30/30	30/30
HBV Vaccinee	30/30	30/30	30/30	30/30	29/30	29/30
C albicans	30/30	30/30	30/30	30/30	30/30	30/30
C diphtheriae	30/30	30/30	30/30	30/30	31/31	31/31
M luteus	30/30	30/30	30/30	30/30	29/30	30/30
P acnes	30/30	30/30	30/30	30/30	29/30	29/30
P carinii	30/30	30/30	30/30	30/30	30/30	30/30
S aureus	30/30	30/30	30/30	30/30	30/30	30/30
S epidermidis	30/30	30/30	30/30	30/30	28/29	29/29
CMV	30/30	30/30	30/30	30/30	**28/30**	30/30
EBV	30/30	30/30	30/30	30/30	30/30	30/30
HAV	30/30	30/30	30/30	29/30	**26/30**	**28/30**
HSV 2	30/30	30/30	30/30	30/30	29/30	30/30
HSV 1	30/30	30/30	30/30	30/30	30/30	30/30
HTLV II	30/30	30/30	**27/30**	**27/30**	**26/30**	28/29
Rubella	30/30	30/30	30/30	30/30	30/30	29/30
HGV	21/21	21/21	21/21	**18/21**	20/21	21/21
Parvovirus B19	30/30	30/30	30/30	30/30	**27/30**	**27/30**
HTLV I	30/30	30/30	30/30	30/30	29/30	**28/30**
Controls	270/270	269/270	270/270	270/270	259/270	263/270

NA = Not tested

* Combined results from three clinical lots of reagents.

Note: Bolded text indicates greater than 5% nonreactive results.

Table 19b. Procleix Tigris System - Detection of HIV-1, HCV, and HBV in the Presence of Donor and Donation Factors with the Procleix Ultrio Assay and Discriminatory Assays

Donor or Donation Factor	Reactive/Tested*					
	HIV-1 Positive (200 c/mL)		HCV Positive (60 IU/mL)		HBV Positive (30 IU/mL)	
	Procleix Ultrio Assay	Procleix dHIV-1 Assay	Procleix Ultrio Assay	Procleix dHCV Assay	Procleix Ultrio Assay	Procleix dHBV Assay
Hemolyzed	21/21	21/21	18/18	18/18	29/30	30/30
Icteric	21/21	21/21	27/27	27/27	30/30	**28/30**
Lipemic	24/24	24/24	15/15	**14/15**	30/30	30/30
Normal	40/40	41/41	41/41	41/41	41/41	41/41
Albumin (6 g/dL)	41/41	41/41	41/41	41/41	41/41	41/41
Bilirubin (20mg/dL)	41/41	41/41	41/41	41/41	**37/39**	**34/39**
Bilirubin (2.5 mg/dL)	NA	NA	NA	NA	90/90	90/90
Hemoglobin (500 mg/dL)	41/41	41/41	41/41	40/40	41/41	41/41
Lipids (3000 mg/dL)	40/40	40/41	41/41	41/41	40/40	38/40
Alcoholic Cirrhosis	30/30	30/30	30/30	30/30	**28/30**	29/30
Antinuclear Antibody	30/30	30/30	30/30	30/30	**28/30**	**26/30**
ALT	24/24	23/23	24/24	24/24	24/24	24/24
Hyperglobulinemia**	**28/30**	29/30	30/30	30/30	**27/30**	30/30
Lupus	30/30	30/30	30/30	30/30	29/30	**28/30**
Multiple Myeloma	30/30	30/30	30/30	29/30	**26/30**	**27/30**
Rheumatoid Factor	30/30	30/30	29/29	29/29	30/30	29/30
Flu vaccinee	30/30	30/30	30/30	30/30	30/30	30/30
HBV vaccinee	30/30	30/30	30/30	30/30	30/30	30/30
C albicans	29/30	30/30	30/30	30/30	30/30	30/30
C diphtheriae	30/30	30/30	30/30	30/30	30/30	30/30
M luteus	29/30	30/30	31/31	31/31	29/30	30/30
P acnes	30/30	30/30	30/30	30/30	30/30	30/30
P carinii	30/30	30/30	30/30	30/30	30/30	30/30
S aureus	30/30	30/30	29/29	29/29	29/29	30/30
S epidermidis	30/30	30/30	30/30	30/30	30/30	30/30
CMV	29/30	30/30	**27/30**	**27/30**	**27/30**	**26/30**
EBV	30/30	30/30	29/30	30/30	30/30	30/30
HAV	30/30	30/30	30/30	30/30	30/30	30/30
HSV 2	30/30	30/30	**28/30**	29/30	**26/30**	**27/30**
HSV 1	30/30	30/30	29/30	29/30	**26/30**	**27/30**
HTLV II	21/21	21/21	21/21	21/21	21/21	21/21
Rubella	24/24	24/24	30/30	30/30	29/30	30/30
HGV	30/30	30/30	30/30	30/30	30/30	29/30
Parvovirus B19	30/30	30/30	30/30	30/30	29/30	30/30
HTLV I	27/27	27/27	27/27	27/27	27/27	27/27
Controls	270/270	266/267	269/270	268/270	266/269	267/270

NA = not tested
* Combined results from three clinical lots of reagents.
** Specimens containing levels of IgM and IgG > 60gL were not tested due to gel formation within the specimens.
Note: Bolded text indicates greater than 5% nonreactive results.

Table 20a. Procleix System - Specificity of the Procleix Ultrio Assay and Discriminatory Assays in the Presence of Donor and Donation Factors

Donor or Donation Factor	Nonreactive/Negative Samples Tested*			
	Procleix Ultrio Assay	Procleix dHIV-1 Assay	Procleix dHCV Assay	Procleix dHBV Assay
Hemolyzed	30/30	30/30	30/30	30/30
Icteric	29/30	30/30	29/30	29/30
Lipemic	30/30	30/30	29/30	30/30
Normal	36/36	36/36	36/36	36/36
Albumin (6 g/dL)	36/36	36/36	36/36	36/36
Bilirubin (20 mg/dL)	36/36	36/36	36/36	36/36
Hemoglobin (500 mg/dL)	36/36	35/35	36/36	36/36
Lipids (3000 mg/dL)	36/36	35/35	36/36	36/36
Alcoholic Cirrhosis	30/30	30/30	30/30	30/30
Antinuclear Antibody	**24/27**	27/27	26/27	**19/27**
ALT	29/30	30/30	30/30	30/30
Elevated IgG	**27/30**	30/30	30/30	30/30
Elevated IgM	30/30	30/30	30/30	30/30
Lupus	29/30	30/30	30/30	29/30
Multiple Myeloma	**21/24**	24/24	24/24	**22/24**
Rheumatoid Factor	30/30	30/30	30/30	29/30
Flu Vaccinee	**27/30**	30/30	30/30	30/30
HBV Vaccinee	30/30	30/30	29/30	30/30
C albicans	30/30	30/30	30/30	30/30
C diphtheriae	30/30	30/30	30/30	29/30
M luteus	29/30	30/30	30/30	30/30
P acnes	30/30	30/30	29/30	30/30
P carinii	30/30	30/30	29/30	30/30
S aureus	30/30	30/30	**28/30**	30/30
S epidermidis	30/30	30/30	30/30	30/30
CMV	**28/30**	30/30	30/30	**27/30**
EBV	**30/33**	33/33	33/33	**29/33**
HAV	30/30	30/30	30/30	29/30
HSV 2	26/27	30/30	**28/30**	**27/30**
HSV 1	**26/30**	27/27	27/27	**25/27**
HTLV II	30/30	30/30	29/30	30/30
Rubella	29/30	30/30	30/30	30/30
HGV	15/15	15/15	15/15	15/15
Parvovirus B19	**24/27**	27/27	27/27	27/27
HTLV I	27/27	27/27	27/27	27/27
Controls	269/270	270/270	269/270	270/270

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

Table 20b. Procleix Tigris System - Specificity of the Procleix Ultrio Assay and Discriminatory Assays in the Presence of Donor and Donation Factors

Donor or Donation Factor	Nonreactive/Tested*			
	Negative Samples			
	Procleix Ultrio Assay	Procleix dHIV-1 Assay	Procleix dHCV Assay	Procleix dHBV Assay
Hemolyzed	30/30	30/30	30/30	29/30
Icteric	30/30	30/30	30/30	29/30
Lipemic	30/30	30/30	30/30	30/30
Normal	42/42	40/40	38/38	38/38
Albumin (6 g/dL)	40/40	40/40	37/37	38/38
Bilirubin (20 mg/dL)	42/42	40/40	37/37	38/38
Hemoglobin (500 mg/dL)	42/42	40/40	38/38	38/38
Lipids (3000 mg/dL)	40/40	40/40	37/37	38/38
Alcoholic Cirrhosis	30/30	30/30	30/30	30/30
Antinuclear Antibody	30/30	30/30	30/30	30/30
ALT	**22/24**	24/24	24/24	24/24
Hyperglobulinemia	30/30	30/30	30/30	30/30
Lupus	**28/30**	30/30	30/30	30/30
Multiple Myeloma	30/30	30/30	30/30	29/30
Rheumatoid Factor	29/30	30/30	30/30	30/30
Flu vaccinee	**27/30**	30/30	30/30	30/30
HBV vaccinee	29/29	30/30	30/30	29/30
C albicans	30/30	30/30	30/30	30/30
C diphtheriae	29/29	29/29	29/29	29/29
M luteus	27/27	27/27	27/27	27/27
P acnes	31/31	31/31	30/31	31/31
P carinii	30/30	30/30	30/30	30/30
S aureus	31/32	32/32	**30/32**	32/32
S epidermidis	31/31	31/31	31/31	31/31
CMV	29/30	30/30	30/30	30/30
EBV	**25/30**	28/28	28/28	27/28
HAV	26/27	30/30	29/29	29/30
HSV 2	**31/33**	31/31	30/31	31/31
HSV 1	27/27	27/27	26/27	26/27
HTLV II	27/28	28/28	28/28	27/28
Rubella	30/30	30/30	29/30	30/30
HGV	15/15	15/15	15/15	15/15
Parvovirus B19	26/27	26/26	27/27	26/27
HTLV I	26/26	26/26	26/26	25/26
Controls	270/270	270/270	268/270	270/270

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

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

The sensitivity and specificity of the Procleix Ultrio Assay and Discriminatory Assays for serum samples and samples collected in various anticoagulants is shown in Tables 21a and 22a for the Procleix System and in Tables 21b and 22b for the Procleix Tigris System. Detection rates were calculated from valid initial results. Cross-reactivity and interference are defined as greater than 5% unexpected results. The anticoagulants tested were ACD (Acid Citrate Dextrose), K_2EDTA (ethylene diamine tetraacetic acid), K_3EDTA, PPT (K_2EDTA Plasma Preparation Tube), sodium citrate, CPD (citrate phosphate dextrose), and sodium heparin. For the majority of anticoagulants, no cross-reactivity or interference for detection of HIV-1, HCV, or HBV was observed. A small portion of the anticoagulants tested had random, unexpected results in greater than 5% of the samples tested. These occurrences are indicated in bold text in Tables 21a and 22a for the Procleix System and in Tables 21b and 22b for the Procleix Tigris System.

Table 21a. Procleix System - Detection of HIV-1, HCV, and HBV in the Presence of Anticoagulants and Serum with the Procleix Ultrio Assay and Discriminatory Assays

Anticoagulant	Reactive/Tested (Percent Reactive)					
	HIV-1 Positive* (200 c/mL)		HCV Positive* (60 IU/mL)		HBV Positive** (up to 30 IU/mL)	
	Procleix Ultrio Assay	Procleix dHIV-1 Assay	Procleix Ultrio Assay	Procleix dHCV Assay	Procleix Ultrio Assay	Procleix dHBV Assay
ACD	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	**122/130 (93.8%)**	125/128 (97.7%)
CPD	29/30 (96.7%)	30/30 (100%)	29/30 (96.7%)	29/30 (96.7%)	124/129 (96.1%)	**122/129 (94.6%)**
K_2EDTA	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	127/130 (97.7%)	129/130 (99.2%)
K_3EDTA	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	125/130 (96.2%)	125/130 (96.2%)
Sodium Citrate	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	**122/130 (93.8%)**	126/130 (96.9%)
Sodium Heparin	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	**121/129 (93.8%)**	126/129 (97.7%)
PPT	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	121/126 (96.0%)	**119/126 (94.4%)**
Serum	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	**123/130 (94.6%)**	127/130 (97.7%)

* Combined results from three clinical lots of reagents.
** Combined results from five clinical lots of reagents for ACD, CPD, sodium citrate, sodium heparin, PPT, and serum. Results for K_2EDTA and K_3EDTA were from four clinical lots.
Note: Bolded text indicates greater than 5% nonreactive results.

Table 21b. Procleix Tigris System - Detection of HIV-1, HCV, and HBV in the Presence of Anticoagulants and Serum with the Procleix Ultrio Assay and Discriminatory Assays

Anticoagulant	Reactive/Tested (Percent Reactive)*					
	HIV-1 Positive (200 c/mL)		HCV Positive (60 IU/mL)		HBV Positive (30 IU/mL)	
	Procleix Ultrio Assay	Procleix dHIV-1 Assay	Procleix Ultrio Assay	Procleix dHCV Assay	Procleix Ultrio Assay	Procleix dHBV Assay
ACD	**18/20 (90.0%)**	19/20 (95.0%)	19/20 (95.0%)	20/20 (100%)	20/20 (100%)	20/20 (100%)
CPD	19/20 (95.0%)	19/20 (95.0%)	19/20 (95.0%)	20/20 (100%)	19/20 (95.0%)	19/20 (95.0%)
K_2EDTA	19/20 (95.0%)	19/20 (95.0%)	20/20 (100%)	19/20 (95.0%)	**17/20 (85.0%)**	**16/20 (80.0%)**
K_3EDTA	19/20 (95.0%)	20/20 (100%)	20/20 (100%)	19/20 (95.0%)	**15/20 (75.0%)**	**17/20 (85.0%)**
Na Citrate	20/20 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)
Na Heparin	20/20 (100%)	19/20 (95.0%)	19/20 (95.0%)	20/20 (100%)	**18/20 (90.0%)**	19/20 (95.0%)
PPT	19/20 (95.0%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	**18/20 (90.0%)**	19/20 (95.0%)
Serum	**17/20 (85.0%)**	**18/20 (90.0%)**	**18/20 (90.0%)**	**18/20 (90.0%)**	**18/20 (90.0%)**	20/20 (100%)

* Combined results from three clinical lots of reagents, except HBV positive samples were tested with four clinical lots for K_2EDTA and K_3EDTA and five clinical lots for the other anticoagulants.
Note: Bolded text indicates greater than 5% nonreactive results.

Table 22a. Procleix System - Specificity of the Procleix Ultrio Assay and Discriminatory Assays in the Presence of Anticoagulants and Serum

Anticoagulant	Nonreactive/Negative Samples Tested* (Percent Nonreactive)			
	Procleix Ultrio Assay	Procleix dHIV-1 Assay	Procleix dHCV Assay	Procleix dHBV Assay
ACD	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	29/30 (96.7%)
CPD	**28/30 (93.3%)**	30/30 (100%)	30/30 (100%)	30/30 (100%)
K_2EDTA	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
K_3EDTA	30/30 (100%)	30/30 (100%)	30/30 (100%)	29/30 (96.7%)
Sodium Citrate	30/30 (100%)	30/30 (100%)	30/30 (100%)	29/30 (96.7%)
Sodium Heparin	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
PPT	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
Serum	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)

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

Table 22b. Procleix Tigris System - Specificity of the Procleix Ultrio Assay and Discriminatory Assays in the Presence of Anticoagulants and Serum

Anticoagulant	Nonreactive/Negative Samples Tested* (Percent Nonreactive)			
	Procleix Ultrio Assay	Procleix dHIV-1 Assay	Procleix dHCV Assay	Procleix dHBV Assay
ACD	20/20 (100%)	20/20 (100%)	20/20 (100%)	19/20 (95.0%)
CPD	**17/20 (85.0%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)
K_2EDTA	20/20 (100%)	20/20 (100%)	20/20 (100%)	19/20 (95.0%)
K_3EDTA	20/20 (100%)	20/20 (100%)	19/20 (95.0%)	20/20(100%)
Na Citrate	19/20 (95.0%)	20/20 (100%)	20/20 (100%)	**18/20 (90.0%)**
Na Heparin	20/20 (100%)	20/20 (100%)	20/20 (100%)	19/20 (95.0%)
PPT	20/20 (100%)	20/20 (100%)	19/20 (95.0%)	19/20 (95.0%)
Serum	19/20 (95.0%)	20/20 (100%)	20/20 (100%)	19/20 (95.0%)

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

CLINICAL SENSITIVITY

PROCLEIX SYSTEM

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

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

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

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

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

In some cases, known-positive samples were tested in the same runs with the samples from the Procleix System pivotal clinical studies, including the clinical specificity study described earlier (see Specificity in Normal Blood Donors in the Procleix System section for run statistics). For the Procleix Ultrio Assay, from the valid assay runs, 3,220 and 3,221 test results were generated for samples prepared neat and diluted, respectively, tested in this known-positive sample study. Of the 3,220 neat samples, 17 (0.5%) had initial invalid results. When 16 of the 17 neat samples were retested, none were repeat invalid. The remaining neat sample was not retested and was invalid due to Internal Control (IC) failure. For diluted samples, 8 (0.2%) samples had initial invalid results. When all 8 diluted samples were retested, none were repeat invalid.

For the Procleix HIV-1 Discriminatory Assay, from the valid assay runs, 1,080 test results were generated for samples tested in this known-positive sample study. Of the 1,080 samples, 86 (8.0%) had initial invalid results: 83 had initial invalid results due to the specimen not being pipetted correctly. When 85 of the 86 samples were retested, none were repeat invalid. The remaining sample was not retested and was invalid due to IC failure.

For the Procleix HCV Discriminatory Assay, from the valid assay runs, 1,080 test results were generated for samples tested in this known-positive sample study. Of the 1,080 samples, 51 (4.7%) had initial invalid results: 49 were due to the specimen not being pipetted correctly. When all 51 samples were retested, 2 were repeat invalid due to IC failure, although both samples had valid results when retested further.

For the Procleix HBV Discriminatory Assay, from the valid assay runs, 1,061 test results were generated for samples tested in this known-positive sample study. Of the 1,061 samples, 9 (0.8%) had initial invalid results. When 5 of the 9 samples were retested, none were repeat invalid. The remaining 4 of 9 samples were not retested and were invalid due to IC failure.

The sensitivity for the Procleix Ultrio Assay and Procleix HIV-1 Discriminatory Assay for undiluted (neat) HIV-1 positive samples was 100% (95% CI: 99.7-100%) and 99.9% (95% CI: 99.5-100%), respectively (Table 23). The sensitivity for the Procleix Ultrio Assay for diluted (1:16) HIV-1 positive samples was 99.0% (95% CI: 98.2-99.5%).

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

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

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

Table 23. Procleix System - Sensitivity of the Procleix Ultrio Assay and Discriminatory Assays in Known Positive Samples

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

n = number of samples
CI = Confidence Interval

Testing of Known Positive 16-Sample Pools

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

Known-positive samples from pools with nonreactive results were tested neat with quantitative Alternate NAT, along with some known-positive samples from pools with reactive results to control for bias. Known-positive pools with viral loads below the Procleix Ultrio Assay sensitivity claim were excluded from the clinical sensitivity analyses. Therefore, the sensitivities presented in Table 24 include pools with confirmed viral loads at or above the Procleix Ultrio Assay sensitivity claim or of unknown viral concentration; not all pools had viral load quantitation performed.

In some cases, known-positive pools were tested in the same runs with the samples from the Procleix System pivotal clinical studies, including the clinical specificity study described earlier (see Specificity in Normal Blood Donors in the Procleix System section for run statistics). For the Procleix Ultrio Assay, from the valid assay runs, 198 test results were generated for pooled samples tested in this known-positive pool study. Of the 198 neat samples, none had initial invalid results.

Overall, the sensitivity for the Procleix Ultrio Assay for 190 known-positive pools containing HIV-1 RNA, HCV RNA, and/or HBV DNA was 97.9% (95% CI: 94.7-99.4%) (Table 24). The sensitivity for the Procleix Ultrio Assay for 125 HIV-1 known-positive pools was 100% (95% CI: 97.1-100%). The sensitivity for the Procleix Ultrio Assay for 115 HCV known-positive pools was 100% (95% CI: 96.8-100%). The sensitivity for the Procleix Ultrio Assay for 123 HBV known-positive pools was 96.7% (95% CI: 91.9-99.1%).

Table 24. Procleix System - Sensitivity of the Procleix Ultrio Assay in 16-Sample Pools Containing Known Positive Specimens

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

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

PROCLEIX TIGRIS SYSTEM

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

A combined total of 2,426 specimens known to be positive for HIV-1 RNA, HCV RNA, or HBV DNA were procured from a vendor and included in the clinical sensitivity analyses.

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

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

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

In some cases, known-positive samples were tested in the same worklists with the samples from the Procleix Tigris System pivotal clinical studies, including the clinical specificity study described earlier (see Specificity in Normal Blood Donors in the Procleix Tigris System section for worklist statistics). For the Procleix Ultrio Assay, from the valid assay worklists, 2,477 and 2,479 test results were generated for samples prepared neat and diluted, respectively, tested in this known-positive sample study. Of the 2,477 neat samples, 5 (0.2%) had initial invalid results. When 3 of the 5 neat samples were retested, none were repeat invalid. The remaining 2 of 5 samples were not retested and were invalid due to Internal Control (IC) failure. For diluted samples, 1 (1/2,479) sample had an initial invalid result. When the diluted sample was retested, it was not repeat invalid.

For the Procleix HIV-1 Discriminatory Assay, from the valid assay worklists, 840 test results were generated for samples tested in this known-positive sample study. Of the 840 samples, 5 (0.6%) had initial invalid results. When all 5 samples were retested, none were repeat invalid.

For the Procleix HCV and HBV Discriminatory Assays, from the valid assay worklists, 803 and 833 test results, respectively, were generated for samples tested in this known-positive sample study: none were invalid.

The sensitivity for the Procleix Ultrio Assay and Procleix HIV-1 Discriminatory Assay for undiluted (neat) HIV-1 positive samples was 100% (95% CI: 99.6-100%) and 99.8% (95% CI: 99.1-100%), respectively (Table 25). The sensitivity for the Procleix Ultrio Assay for diluted (1:16) HIV-1 positive samples was 98.1% (95% CI: 96.9-98.9%).

The sensitivity for the Procleix Ultrio Assay and the Procleix HCV Discriminatory Assay for neat HCV positive samples was 99.7% (95% CI: 99.1-100%) and 99.6% (95% CI: 98.9-99.9%), respectively (Table 25). The sensitivity for the Procleix Ultrio Assay for diluted (1:16) HCV positive samples was 99.4% (95% CI: 98.5-99.8%).

The sensitivity for the Procleix Ultrio Assay and Procleix HBV Discriminatory Assay for neat HBV positive samples was 98.4% (95% CI: 97.3-99.1%) and 98.3% (95% CI: 97.1-99.0%), respectively (Table 25). The sensitivity for the Procleix Ultrio Assay for diluted (1:16) HBV positive samples was 86.7% (95% CI: 84.1-89.0%).

The overall clinical sensitivity for the Procleix Ultrio Assay, which takes into account all positive samples tested, was 99.4% (95% CI: 99.0-99.7%) for neat positive samples and 94.7% (95% CI: 93.8-95.6%) for diluted (1:16) positive samples (Table 25).

Table 25. Procleix Tigris System - Sensitivity of the Procleix Ultrio Assay and Discriminatory Assays in Known Positive Samples

Assay	Sample	n	Reactive	Sensitivity (%)	95% CI
Procleix Ultrio Assay (Neat)	All	2,422	2,407	99.4	99.0-99.7
	HIV-1 Only	837	837	100	99.6-100
	HCV Only	780	778	99.7	99.1-100
	HBV Only	805	792	98.4	97.3-99.1
Procleix Ultrio Assay (Diluted 1:16)	All	2,426	2,298	94.7	93.8-95.6
	HIV-1 Only	838	822	98.1	96.9-98.9
	HCV Only	784	779	99.4	98.5-99.8
	HBV Only	804	697	86.7	84.1-89.0
Procleix dHIV-1 Assay	HIV-1 Only	838	836	99.8	99.1-100
Procleix dHCV Assay	HCV Only	780	777	99.6	98.9-99.9
Procleix dHBV Assay	HBV Only	805	791	98.3	97.1-99.0

n = number of samples, CI = Confidence Interval

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

HIV-1, HCV, and HBV seropositive samples were obtained from a commercial vendor. The samples were tested neat in triplicate with an appropriate qualitative licensed nucleic acid test (NAT). One thousand fifty-four (1,054) HIV-1, 591 HCV, and 850 HBV seropositive samples had consistently reactive licensed NAT results and were tested neat and diluted with the Procleix Ultrio Assay and tested neat with the appropriate discriminatory assay in singlicate on the Procleix Tigris System. Three HIV-1, 9 HCV, and 4 HBV seropositive samples had inconsistently reactive or nonreactive licensed NAT results and were tested neat in triplicate with the Procleix Ultrio Assay and appropriate discriminatory assay on the Procleix Tigris System.

For the Procleix Ultrio Assay, 47 worklists were generated from testing of neat and diluted samples. Of these, 1 (2.1%) worklist was invalid due to an insufficient number of valid calibrators. From the valid assay worklists, 2,543 and 2,494 test results were generated from neat and diluted samples, respectively. For the neat samples, 60 (2.4%) had initial invalid results; after retesting, 1 (1/2,543) had a repeat invalid result due to internal control failure. For the diluted samples, 13 (0.5%) had initial invalid results; after retesting, none had repeat invalid results.

For the Procleix HIV-1 Discriminatory Assay, 20 worklists were generated from testing of neat samples: none were invalid. From the valid assay worklists, 1,063 test results were generated from neat samples. Eight (0.8%) had initial invalid results; after retesting, none had repeat invalid results.

For the Procleix HCV Discriminatory Assay, 17 worklists were generated from testing of neat samples: none were invalid. From the valid assay worklists, 618 test results were generated from neat samples. Three (0.5%) had initial invalid results; after retesting, 1 (0.2%) had a repeat invalid result due to the presence of a clot in the sample.

For the Procleix HBV Discriminatory Assay, 16 worklists were generated from testing of neat samples: none were invalid. From the valid assay worklists, 862 test results were generated from neat samples. Seven (0.8%) had initial invalid results; after retesting, none had repeat invalid results.

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

One thousand fifty-four (1,054) HIV-1, 591 HCV, and 850 HBV samples were considered to have a positive true status because the samples were reactive in 3 of 3 replicates and were included in the clinical sensitivity analyses in Table 26. Three HIV-1, 9 HCV, and 4 HBV seropositive samples' true status could not be determined because the samples were inconsistently reactive or nonreactive (less than 3 of 3 replicates were reactive). These samples were included in the evaluation in Table 27.

All of the 1,054 HIV-1, 591 HCV, and 850 HBV consistently NAT-reactive samples were tested neat with the Procleix Ultrio Assay and the appropriate discriminatory assay on the Procleix Tigris System. Testing was performed at three clinical sites using two clinical lots. Plasma samples negative for HIV-1 and HCV RNA, HBsAg, and HBV DNA were tested with the positive samples to minimize bias.

For each of the four assays, results were compared to the true status to estimate clinical sensitivity (Table 26). Overall, sensitivities of the Procleix Ultrio Assay and discriminatory assays were 99.9% or higher when tested on the Procleix Tigris System.

Table 26. Procleix Tigris System - Clinical Sensitivity of the Procleix Ultrio Assay and Discriminatory Assays in Known NAT-Reactive Samples Prepared Neat

Assay	Sample	n	True Positive	False Negative	Sensitivity (%)	95% CI
Procleix Ultrio Assay	All	2,495	2,492	3	99.9	99.6 – 100
	HIV-1	1,054	1,054	0	100	99.7 – 100
	HCV	591	589	2	99.7	98.8 – 100
	HBV	850	849	1	99.9	99.3 – 100
Procleix dHIV-1 Assay	HIV-1	1,054	1,054	0	100	99.7 – 100
Procleix dHCV Assay	HCV	590*	590	0	100	99.4 – 100
Procleix dHBV Assay	HBV	850	849	1	99.9	99.3 – 100

CI = confidence interval, n = number of samples
* One of 591 HCV samples had a final invalid result and was excluded from this analysis.

For each of the 3 HIV-1, 9 HCV, and 4 HBV seropositive samples with inconsistently reactive or nonreactive licensed NAT results, 3 replicates (prepared neat) were tested with the Procleix Ultrio Assay and with the appropriate discriminatory assay on the Procleix Tigris System. Testing was performed at one clinical site using one clinical lot. Control negative plasma samples were tested with the seropositive samples to minimize bias.

Reactive rates are summarized in Table 27 for the licensed NATs, Procleix Ultrio Assay, and discriminatory assays. The reactive rates of the Procleix Ultrio Assay were higher than those of the licensed NATs in HIV-1, HCV, and HBV samples (Table 27). For HCV and HBV samples, the difference was significant (*P* values < 0.05).

In 1 of 3 HIV-1 samples, 3 of 9 HCV samples, and 3 of 4 HBV samples with inconsistently reactive or nonreactive licensed NAT results, the Procleix Ultrio Assay was reactive in 3 of 3 replicates. Discriminatory assay results were similar to those of the Procleix Ultrio Assay

Table 27. Procleix Tigris System - Summary of Reactive Rates in Seropositive Samples with Inconsistently Reactive or Nonreactive Licensed NAT Results.

Sample Type	n	Reactive Rates, n(%)		
		Licensed NAT*	Procleix Ultrio Assay	Discriminatory Assay
All**	48***	9 (18.8%)	26 (55.3%)	N/A
HIV-1	9***	2 (22.2%)	4 (50.0%)	3 (33.3%)
HCV****	27	5 (18.5%)	11 (40.7%)	13 (48.1%)
HBV****	12	2 (16.7%)	11 (91.7%)	11 (91.7%)

n = number of replicates (3 HIV-1, 9 HCV, and 4 HBV samples tested in triplicate)
* Three licensed NATs used: 1 for HIV-1 detection, 1 for HCV detection, and 1 for HBV detection.
** The difference in reactive rates between the licensed NATs and the Procleix Ultrio Assay was statistically significant (P value <0.05 by Score test from conditional logistic regression).
*** One of 9 HIV-1 replicates had an invalid Procleix Ultrio Assay result.
**** The differences in reactive rates between the licensed NAT and the Procleix Ultrio Assay and HCV and HBV discriminatory assays were statistically significant (P value <0.05 by Score test from conditional logistic regression).

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

Aliquots of the HIV-1, HCV, and HBV NAT-reactive samples included in the clinical sensitivity analyses in Table 26 were diluted with plasma known to be negative for HIV-1 and HCV RNA, HBsAg, and HBV DNA. Diluted samples were tested with the Procleix Ultrio Assay on the Procleix Tigris System at three clinical sites and with the appropriate licensed NAT. Samples tested with the Procleix Ultrio Assay and with the HIV-1 and HCV licensed NAT were diluted 1:16 (per the package insert's instructions). Samples tested with the HBV licensed NAT were diluted 1:24 (per the package insert's instructions). Testing was performed using two clinical lots of the Procleix Ultrio Assay. Control negative plasma samples were tested with the diluted samples to minimize bias.

For the Procleix Ultrio Assay and licensed NAT, reactive rates were calculated (Table 28). In addition, agreement of results between the two assays was calculated. The reactive rates of the Procleix Ultrio Assay were similar to those of the licensed NAT in HIV-1 and HCV diluted samples. In diluted HBV samples, the reactive rate of the Procleix Ultrio Assay (95.3%) was lower than the reactive rate of the licensed NAT (99.3%). However, agreement between Procleix Ultrio Assay and licensed NAT results for HBV detection was 95.0%.

Because the sensitivity of the Procleix Ultrio Assay was lower for HBV detection, further investigation simulating smaller pool sizes was performed with the HBV samples (n=40) that had nonreactive Procleix Ultrio Assay results when tested in a 1:16 dilution. Of the 40 HBV samples, 34 (85.0%) were reactive when tested in a 1:8 dilution and 39 (97.5%) were reactive when tested in a 1:4 dilution.

Table 28. Procleix Tigris System - Agreement Between Procleix Ultrio Assay and Licensed NAT* Results in Positive Samples Prepared Diluted**

Sample	n	Licensed NAT Reactive		Licensed NAT Nonreactive		Reactive Rates (95% CI)		Agreement (95% CI)
			Procleix Ultrio Assay Nonreactive	Procleix Ultrio Assay Reactive	Procleix Ultrio Assay Nonreactive	Licensed NAT**	Procleix Ultrio Assay	
All	2,489	2,427	46	9	7	99.4 (99.0–99.6)	97.9 (97.2–98.4)	97.8 (97.1–98.3)
HIV-1	1,054	1,043	6	3	2	99.5 (98.9–99.8)	99.2 (98.5–99.7)	99.1 (98.4–99.6)
HCV	591	584	2	2	3	99.2 (98.0–99.7)	99.2 (98.0–99.7)	99.3 (98.3–99.8)
HBV	844***	800	38	4	2	99.3 (98.5–99.7)	95.3 (93.6–96.6)	95.0 (93.3–96.4)

CI = confidence interval, n = number of samples
* Two licensed NATs used: 1 for HIV-1 and HCV detection and 1 for HBV detection.
** Samples tested with the Procleix Ultrio Assay and with the HIV-1 and HCV licensed NAT were diluted 1:16. Samples tested with the HBV licensed NAT were diluted 1:24.
*** Five samples with reactive Procleix Ultrio Assay results were excluded from this analysis because they were not tested with the licensed NAT. Another sample was excluded from this analysis because it was not tested with licensed NAT or with the Procleix Ultrio Assay.

PROCLEIX SYSTEM

Clinical Sensitivity and Specificity High-Risk Population Study

Plasma specimens from individuals at high risk for infection with HIV-1, HCV, and/or HBV were evaluated for the clinical sensitivity and specificity high-risk population study. Of the total of 503 high-risk subjects included in the study, the majority reported injection drug use as a risk factor. Other risk factors included multiple sex partners, needle stick accident, blood or blood product transfusion, history of a STD, previous diagnosis of HIV-1, HCV, or HBV infection and dialysis. All the specimens from qualified high-risk subjects were aliquoted and tested undiluted (neat) and diluted (1:16) at one clinical site. The neat specimens were tested with the Procleix Ultrio Assay and with the HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays. The diluted specimens were tested only with the Procleix Ultrio Assay. Three clinical lots were used for Procleix Ultrio Assay and discriminatory assay testing.

True status of the samples was based on their completed laboratory results with the licensed Procleix HIV-1/HCV Assay and corresponding discriminatory assays and results of HBsAg testing. For the Procleix Ultrio Assay, clinical sensitivity and specificity were determined by comparing results (neat and diluted) with the true status of the samples (Table 29a). For the discriminatory assays, results were compared to the true status in neat specimens with Procleix Ultrio Assay reactive results (Table 29b). For specimens with discordant results, comparisons were further made to HIV-1, HCV, and HBV Alternate NAT. The Alternate NAT results were used in clinical sensitivity and specificity calculations to interpret Procleix Ultrio Assay results.

In some cases, high-risk population study samples were tested in the same runs with the samples from the Procleix System pivotal clinical studies, including the clinical specificity study described earlier (see Specificity in Normal Blood Donors in the Procleix System section for run statistics). For the Procleix Ultrio Assay, from the valid assay runs, 503 test results were generated for samples prepared neat and diluted and tested in this high-risk population study. Of the 503 neat samples, 20 (4.0%) had initial invalid results. When 18 of the 20 neat samples were retested, 6 were repeat invalid due to Internal Control (IC) failure. The remaining 2 of 20 samples were not retested: 1 was invalid due to IC failure and the other was invalid due to the sample not being pipetted correctly. For diluted samples, 1 (0.2%) sample had an initial invalid result. When the diluted sample was retested, it was not repeat invalid.

For the Procleix HIV-1 Discriminatory Assay, from the valid assay runs, 503 test results were generated for samples prepared neat and tested in this high-risk population study. Of the 503 samples, 108 (21.5%) had initial invalid results. When 99 of the 108 samples were retested, 64 were repeat invalid due to IC failure. The remaining 9 of 108 samples were not retested and were invalid due to IC failure.

For the Procleix HCV Discriminatory Assay, from the valid assay runs, 503 test results were generated for samples prepared neat and tested in this high-risk population study. Of the 503 samples, 18 (3.6%) had initial invalid results. When 16 of the 18 samples were retested, 5 were repeat invalid due to IC failure. The remaining 2 of 18 samples were not retested: 1 was invalid due to IC failure and the other was due to dispense verification failure.

For the Procleix HBV Discriminatory Assay, from the valid assay runs, 503 test results were generated for samples prepared neat and tested in this high-risk population study. Of the 503 samples, 124 (24.7%) had initial invalid results. When 117 of the 124 samples were retested, 67 were repeat invalid due to IC failure. The remaining 7 of 124 samples were not retested and were invalid due to IC failure.

Of the 503 high risk specimens tested neat in the Procleix Ultrio Assay, 495 specimens had valid Procleix Ultrio Assay results and completed laboratory results and were included in the clinical sensitivity and specificity calculations (Table 29a). Of the 495 specimens tested neat, 369 were HIV-1, HCV, and/or HBV positive in the reference tests. Of these, all were Procleix Ultrio Assay reactive. Sensitivity was 100% (95% CI: 99.0-100%) for HIV-1, HCV, and HBV detection. The remaining 126 of 495 specimens were HIV-1, HCV, and HBV negative in the reference tests. Of these, 111 were Procleix Ultrio Assay nonreactive and 15 were Procleix Ultrio Assay reactive. Specificity was 88.1% (95% CI: 81.1-93.2%).

Of the 503 high risk specimens tested diluted (1:16 to simulate multiplex testing of pools) in the Procleix Ultrio Assay, 502 specimens had valid Procleix Ultrio Assay results and completed laboratory results and were included in the clinical sensitivity and specificity calculations (Table 29a). Of the 502 specimens tested diluted, 373 were HIV-1, HCV, and/or HBV positive in the reference tests. Of these, 370 were Procleix Ultrio Assay reactive and 3 were Procleix Ultrio Assay nonreactive. Sensitivity was 99.2% (95% CI: 97.7–99.8%) for detection of HIV-1, HCV, and HBV. The remaining 129 of 502 specimens were HIV-1, HCV, and HBV negative in the reference tests. Of these, 117 were Procleix Ultrio Assay nonreactive and 12 were Procleix Ultrio Assay reactive. Specificity was 90.7% (95% CI: 84.3-95.1%).

Of the Procleix Ultrio Assay neat-reactive specimens, 317 specimens had valid HIV-1 Discriminatory Assay and reference test results (Table 29b). Of these, 158 were HIV-1 positive in the reference tests and all were HIV-1 discriminated. Sensitivity of the HIV-1 Discriminatory Assay was 100% (95% CI: 97.7-100%) in specimens that tested Procleix Ultrio Assay neat-reactive. The remaining 159 of 317 specimens were HIV-1 negative in the reference tests. Of these, 1 was HIV-1 discriminated and 158 were HIV-1 Discriminatory Assay nonreactive. Specificity of the HIV-1 Discriminatory Assay was 99.4% (95% CI: 96.5-100%) in specimens that tested Procleix Ultrio Assay neat-reactive.

Of the Procleix Ultrio Assay neat-reactive specimens, 376 specimens had valid HCV Discriminatory Assay and reference test results (Table 29b). Of these, 299 were HCV positive in the reference tests: 298 were HCV discriminated and 1 was HCV Discriminatory Assay nonreactive. Sensitivity of the HCV Discriminatory Assay was 99.7% (95% CI: 98.2-100%) in specimens that tested Procleix Ultrio Assay neat-reactive. The remaining 77 of 376 specimens were HCV negative in the reference tests. Of these, 9 were HCV discriminated and 68 were HCV Discriminatory Assay nonreactive. Specificity of the HCV Discriminatory Assay was 88.3% (95% CI: 79.0-94.5%) in specimens that tested Procleix Ultrio Assay neat-reactive.

Of the Procleix Ultrio Assay neat-reactive specimens, 311 specimens tested neat had valid HBV Discriminatory Assay and reference test results (Table 29b). Of these, 25 were HBV positive in the reference tests and all were HBV discriminated. Sensitivity of the HBV Discriminatory Assay was 100% (95% CI: 86.3-100%) in specimens that tested Procleix Ultrio Assay neat-reactive. The remaining 286 of 311 specimens were HBV negative in the reference tests. Of these, 6 were HBV discriminated and 280 were HBV Discriminatory Assay nonreactive. Specificity of the HBV Discriminatory Assay was 97.9% (95% CI: 95.5-99.2%) in specimens that tested Procleix Ultrio Assay neat-reactive.

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

Table 29a. Procleix System - Clinical Sensitivity and Specificity of the Procleix Ultrio Assay in a High Risk Population

Target	Sample	n	Ultrio Reactive	Reference Test Positive		Reference Test Negative		Sensitivity (95% CI)	Specificity (95% CI)
					False Negative*	True Negative*	False Positive*		
All	Neat	495	384	369**	0	111	15	100 (99.0-100)	88.1 (81.1-93.2)
	Diluted	502	382	370**	3	117	12	99.2 (97.7-99.8)	90.7 (84.3-95.1)

n = number of valid specimens with completed lab results, CI = Confidence interval
* Interpretations of the Procleix Ultrio Assay results (for calculating sensitivity and specificity) when compared to the reference test results.
** 92 were co-infected with HIV-1 and HCV, 9 were co-infected with HIV-1 and HBV, 1 was co-infected with HCV and HBV, and 5 were co-infected with HIV-1, HCV, and HBV.

Table 29b. Procleix System - Clinical Sensitivity and Specificity of the Discriminatory Assays in Procleix Ultrio Assay Neat-Reactive Specimens From a High Risk Population

Assay	n	Discriminatory Assay Reactive	Reference Test Positive		Reference Test Negative		Sensitivity (95% CI)	Specificity (95% CI)
				False Negative*	True Negative*	False Positive*		
Procleix dHIV-1 Assay	317	159	158**	0	158	1	100 (97.7-100)	99.4 (96.5-100)
Procleix dHCV Assay	376	307	298***	1	68	9	99.7 (98.2-100)	88.3 (79.0-94.5)
Procleix dHBV Assay	311	31	25****	0	280	6	100 (86.3-100)	97.9 (95.5-99.2)

n = number of valid specimens with completed lab results, CI = Confidence interval
* Interpretations of the HIV-1, HCV, or HBV Discriminatory Assay results (for calculating sensitivity and specificity) when compared to the reference test results.
** 87 were co-infected with HIV-1 and HCV, 9 were co-infected with HIV-1 and HBV, and 5 were co-infected with HIV-1, HCV, and HBV.
*** 91were co-infected with HIV-1 and HCV, 1 was co-infected with HCV and HBV, and 5 were co-infected with HIV-1, HCV, and HBV.
**** 9 were co-infected with HIV-1 and HBV and 4 were co-infected with HIV-1, HCV, and HBV.

PROCLEIX TIGRIS SYSTEM

Clinical Sensitivity and Specificity High-Risk Population Study

Plasma specimens from individuals at high risk for infection with HIV-1, HCV, and/or HBV were evaluated for the clinical sensitivity and specificity high-risk population study on the Procleix Tigris System. Of the 150 high-risk subjects included in the study, the majority reported injection drug use as a risk factor. Other risk factors included multiple sex partners, needle stick accident, blood or blood product transfusion, history of a STD, previous diagnosis of HIV-1, HCV, or HBV infection and dialysis. All the specimens from qualified high-risk subjects were aliquoted and tested undiluted (neat) and diluted (1:16) at one clinical site. The neat specimens were tested with the Procleix Ultrio Assay and with the HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays if the specimens were Procleix Ultrio Assay reactive. The diluted specimens were tested only with the Procleix Ultrio Assay. Three clinical lots were used for Procleix Ultrio Assay and discriminatory assay testing.

True status of the samples was based on their completed laboratory results with the licensed Procleix HIV-1/HCV Assay and corresponding discriminatory assays and results of HBsAg testing. For the Procleix Ultrio Assay, clinical sensitivity and specificity were determined by comparing results

(neat and diluted) with the true status of the samples (Table 29c). For the discriminatory assays, results were compared to the true status in neat specimens with Procleix Ultrio Assay reactive results (Table 29d). For specimens with discordant results, comparisons were further made to HIV-1, HCV, and HBV Alternate NAT. The Alternate NAT results were used to interpret Procleix Ultrio Assay results for calculating clinical sensitivity and specificity.

In some cases, high-risk population study samples were tested in the same worklists with the samples from the Procleix Tigris System pivotal clinical studies, including the clinical specificity study described earlier (see Specificity in Normal Blood Donors in the Procleix Tigris System for worklist statistics). For the Procleix Ultrio Assay, from the valid assay worklists, 150 test results were generated for samples prepared neat and diluted and tested in this high-risk population study: none were invalid.

For the Procleix HIV-1 Discriminatory Assay, from the valid assay worklists, 99 test results were generated for samples prepared neat and tested in this high-risk population study. Of the 99 samples, 4 (4.0%) had initial invalid results. When 3 of the 4 samples were retested, all 3 were repeat invalid due to failures in process validation because of insufficient sample volume. The remaining 1 of 4 samples was not retested and was invalid due to Internal Control (IC) failure.

For the Procleix HCV Discriminatory Assay, from the valid assay worklists, 99 test results were generated for samples prepared neat and tested in this high-risk population study. Of the 99 samples, 3 (3.0%) had initial invalid results. When all 3 samples were retested, 2 were repeat invalid due to failures in process validation because of insufficient sample volume.

For the Procleix HBV Discriminatory Assay, from the valid assay worklists, 99 test results were generated for samples prepared neat and tested in this high-risk population study. Of the 99 samples, 5 (5.1%) had initial invalid results. When all 5 samples were retested, all were repeat invalid due to failures in process validation because of insufficient sample volume.

Of the 150 high risk specimens tested neat in the Procleix Ultrio Assay on the Procleix Tigris System, all specimens had valid Procleix Ultrio Assay results and completed laboratory results and were included in the clinical sensitivity and specificity calculations (Table 29c). Of the 150 specimens tested neat, 98 were HIV-1, HCV, and/or HBV positive in the reference tests. Of these, all were Procleix Ultrio Assay reactive. Sensitivity was 100% (95% CI: 96.3% - 100%) for HIV-1, HCV, and HBV detection. The remaining 52 of 150 specimens were HIV-1, HCV, and HBV negative in the reference tests. Of these, 51 were Procleix Ultrio Assay nonreactive and 1 was Procleix Ultrio Assay reactive. Specificity was 98.1% (95% CI: 89.7% - 100%).

Of the 150 high risk specimens tested diluted (1:16 to simulate multiplex testing of pools) in the Procleix Ultrio Assay on the Procleix Tigris System, 149 specimens had valid Procleix Ultrio Assay results and completed laboratory results and were included in the clinical sensitivity and specificity calculations (Table 29c). Of the 149 specimens tested diluted, 97 were HIV-1, HCV, and/or HBV positive in the reference tests. Of these, 92 were Procleix Ultrio Assay reactive and 5 were Procleix Ultrio Assay nonreactive. Sensitivity was 94.8% (95% CI: 88.4% – 98.3%) for detection of HIV-1, HCV, and HBV. The remaining 52 of 149 specimens were HIV-1, HCV, and HBV negative in the reference tests. Of these, 51 were Procleix Ultrio Assay nonreactive and 1 was Procleix Ultrio Assay reactive. Specificity was 98.1% (95% CI: 89.7% - 100%).

Of the Procleix Ultrio Assay neat-reactive specimens, 95 specimens had valid HIV-1 Discriminatory Assay and reference test results (Table 29d). Of these, 16 were HIV-1 positive in the reference tests and all were HIV-1 discriminated. Sensitivity of the HIV-1 Discriminatory Assay was 100% (95% CI: 79.4%-100%) in specimens that tested Procleix Ultrio Assay neat-reactive. The remaining 79 of 95 specimens were HIV-1 negative in the reference tests. Of these, all were HIV-1 Discriminatory Assay nonreactive. Specificity of the HIV-1 Discriminatory Assay was 100% (95% CI: 95.4%-100%) in specimens that tested Procleix Ultrio Assay neat-reactive.

Of the Procleix Ultrio Assay neat-reactive specimens, 97 specimens had valid HCV Discriminatory Assay and reference test results (Table 29d). Of these, 87 were HCV positive in the reference tests: 86 were HCV discriminated and 1 was HCV Discriminatory Assay nonreactive. Sensitivity of the HCV Discriminatory Assay was 98.9% (95% CI: 93.8%-100%) in specimens that tested Procleix Ultrio Assay neat-reactive. The remaining 10 of 97 specimens were HCV negative in the reference tests. Of these, 1 was HCV discriminated and 9 were HCV Discriminatory Assay nonreactive. Specificity of the HCV Discriminatory Assay was 90.0% (95% CI: 55.5%-99.7%) in specimens that tested Procleix Ultrio Assay neat-reactive.

Of the Procleix Ultrio Assay neat-reactive specimens, 94 specimens tested neat had valid HBV Discriminatory Assay and reference test results (Table 29d). Of these, 6 were HBV positive in the reference tests and all were HBV discriminated. Sensitivity of the HBV Discriminatory Assay was 100% (95% CI: 54.1%-100%) in specimens that tested Procleix Ultrio Assay neat-reactive. The remaining 88 of 94 specimens were HBV negative in the reference tests. Of these, all were HBV Discriminatory Assay nonreactive. Specificity of the HBV Discriminatory Assay was 100% (95% CI: 95.9%-100%) in specimens that tested Procleix Ultrio Assay neat-reactive.

Several specimens were infected with two or more viruses, based on results of the Procleix HIV-1/HCV and Discriminatory Assays and/or HBsAg serologic tests. Of the 150 subject specimens, 8 were co-infected with HIV-1 and HCV, 1 was co-infected with HIV-1 and HBV, and 1 was co-infected with HIV-1, HCV, and HBV. All co-infected specimens tested reactive in the Procleix Ultrio Assay when tested neat.

Table 29c. Procleix Tigris System - Clinical Sensitivity and Specificity of the Procleix Ultrio Assay in a High Risk Population

Target	Sample	n	Ultrio Reactive	Reference Test Positive		Reference Test Negative		Sensitivity (95% CI)	Specificity (95% CI)
					False Negative*	True Negative*	False Positive*		
All	Neat	150	99	98**	0	51	1	100 (96.3-100)	98.1 (89.7-100)
	Diluted	149	93	92***	5	51	1	94.8 (88.4-98.3)	98.1 (89.7-100)

n = number of valid specimens with completed lab results, CI = Confidence interval
* Interpretations of the Procleix Ultrio Assay results (for calculating sensitivity and specificity) when compared to the reference test results.
** 8 were co-infected with HIV-1 and HCV, 1 was co-infected with HIV-1 and HBV, and 1 was co-infected with HIV-1, HCV, and HBV.
*** 7 were co-infected with HIV-1 and HCV, 1 was co-infected with HIV-1 and HBV, and 1 was co-infected with HIV-1, HCV, and HBV.

Table 29d. Procleix Tigris System - Clinical Sensitivity and Specificity of the Discriminatory Assays in Procleix Ultrio Assay Neat-Reactive Specimens From a High Risk Population

Assay	n	Discriminatory Assay Reactive	Reference Test Positive		Reference Test Negative		Sensitivity (95% CI)	Specificity (95% CI)
				False Negative*	True Negative*	False Positive*		
Procleix dHIV-1 Assay	95	16	16**	0	79	0	100 (79.4-100)	100 (95.4-100)
Procleix dHCV Assay	97	87	86***	1	9	1	98.9 (93.8-100)	90.0 (55.5-99.7)
Procleix dHBV Assay	94	6	6****	0	88	0	100 (54.1-100)	100 (95.9-100)

n = number of valid specimens with completed lab results, CI = Confidence interval
* Interpretations of the HIV-1, HCV, or HBV Discriminatory Assay results (for calculating sensitivity and specificity) when compared to the reference test results.
** 8 were co-infected with HIV-1 and HCV, 1 was co-infected with HIV-1 and HBV, and 1 was co-infected with HIV-1, HCV, and HBV.
*** 8 were co-infected with HIV-1 and HCV and 1 was co-infected with HIV-1, HCV, and HBV.
**** 1 was co-infected with HIV-1 and HCV; this specimen was HBV seronegative but HBV Discriminatory Assay and HBV Alternate NAT reactive. 1 was co-infected with HIV-1 and HBV.

CONFIRMED HBV YIELD CASES IN NORMAL BLOOD DONORS

Two multi-center, prospective, post-marketing clinical studies were conducted in the U.S. blood bank setting to demonstrate detection of confirmed HBV yield cases. An HBV yield case is defined as an individual whose HBV infection was not detected by licensed serologic methods at index (HBsAg and anti-HBc test seronegative) but was correctly identified using the Procleix Ultrio Assay (as determined by seroconversion or by consistent reactivity in Procleix Ultrio Assay and another licensed HBV nucleic acid test in follow-up samples).

Index donations were tested per the sites' standard operating procedures and the Procleix Ultrio Assay package insert instructions. In the first study, sites primarily tested 8-sample pools, though a small proportion of samples was tested as IDS only. Some sites tested using the PROCLEIX System and others tested using the Procleix Tigris System. In the second study, the majority of samples were tested in 16-sample pools on the Procleix Tigris System, though about one-fourth of the samples were tested as IDS only.

For both studies, if an index donation was Procleix Ultrio Assay and Procleix HBV Discriminatory Assay reactive, but HBsAg and anti-HBc seronegative, the donor was asked to enroll into follow-up in which further testing with the Procleix Ultrio Assay, HBV serologic tests, and Alternate NAT was performed. Index donation samples and follow-up samples from those donors were tested with the Procleix Ultrio Assay on the alternate instrument platform.

In the first study, 2 confirmed yield cases were identified in 8-sample pools (in approximately 350,000 donations tested). One confirmed yield case was identified on the Procleix System and one was identified on the Procleix Tigris System. Both cases were also reactive in IDS. Both cases were also confirmed as yields in 8-sample pools and IDS on the alternate instrument platform. Follow-up samples collected from the prospective donors 12 and 20 days after the respective index donations were HBsAg seropositive, indicating seroconversion.

In the second study, 3 confirmed yield cases were identified in 16-sample pools (in approximately 945,000 donations tested) tested on the Procleix Tigris System. All 3 cases were also reactive in IDS. All 3 cases were reactive in IDS on the Procleix System, as well. A sample from 1 of the 3 yield cases was reactive when tested in a 16-sample pool on the Procleix System. The remaining 2 yield case samples were nonreactive when tested in 16 sample pools on the Procleix System. These inconsistent (nonreactive) results were most likely due to low copy levels after pooling. For 1 of these 3 prospective donors, a follow-up sample collected 70 days after the index donation was anti-HBc seroreactive, indicating seroconversion. The remaining 2 prospective donors had been vaccinated for HBV and had anti-HBs seroreactive results in plasma samples collected at the index donation. Their follow-up samples were persistently HBsAg and anti-HBc seronegative and anti-HBs seroreactive, and reactive with another licensed HBV nucleic acid test. These 2 cases appear to represent breakthrough HBV infections following vaccination.

COMPARISON OF THE PROCLEIX ULTRIO ASSAY TO HIV-1, HCV, AND HBSAG SEROLOGY RESULTS: BASIS FOR THE SUPPLEMENTAL TEST CLAIMS

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

Results generated from the specificity study of the Procleix Ultrio Assay in pooled and individual donations and/or the Procleix HIV-1, HCV, and HBV Discriminatory Assays on the Procleix System and results obtained from Procleix Ultrio Assay donor screening on the Procleix Tigris System at 3 US blood testing sites allow comparison of the Procleix Ultrio Assay with serology reactivity (Table 30). All of the samples included in this analysis were EIA repeat reactive.

HIV-1 Western Blot results were available for 283 samples. Of these, 20 were HIV-1 Western Blot positive: 18 samples were Procleix Ultrio Assay reactive for HIV-1 (90.0%) and 2 samples were Procleix Ultrio Assay nonreactive for HIV-1 (10.0%). Of these 2 samples, 1 was tested in a 16-sample pool that had a nonreactive Procleix Ultrio Assay result and 1 was Procleix Ultrio Assay reactive, HBV discriminated and HBV seropositive. The remaining 263 of 283 had negative or indeterminate HIV-1 Western Blot results: all 263 (100%) were Procleix Ultrio Assay nonreactive for HIV-1. Overall agreement between Procleix Ultrio Assay and HIV-1 Western Blot results was 99.3% (281/283). All of the 18 HIV-1 EIA repeat reactive samples that were Procleix Ultrio Assay reactive and Procleix HIV-1 Discriminatory Assay reactive were HIV-1 Western Blot positive, confirming HIV-1 infection. Therefore, when a sample is repeat reactive on a licensed anti-HIV-1 screening test and reactive on the Procleix Ultrio Assay and reactive on the Procleix HIV-1 Discriminatory Assay, the Procleix Ultrio Assay acts as a supplemental test that confirms HIV-1 infection; it is not necessary to run an HIV-1 Western Blot.

HCV RIBA results were available for 638 samples. Of these, 240 were HCV RIBA positive: 174 (72.5%) were Procleix Ultrio Assay reactive for HCV and 66 (27.5%) were Procleix Ultrio Assay nonreactive for HCV. The remaining 398 of 638 had HCV RIBA negative or indeterminate results: 395 (99.2%) were Procleix Ultrio Assay nonreactive for HCV and 3 (0.8%) were Procleix Ultrio Assay reactive for HCV. Overall agreement between Procleix Ultrio Assay and HCV RIBA results was 89.2% (569/638). It has been reported that about 20% of HCV RIBA positive samples will have undetectable HCV RNA due to a resolved HCV infection.[33] All of the 174 HCV EIA repeat reactive samples that were Procleix Ultrio Assay reactive and Procleix HCV Discriminatory Assay reactive were HCV RIBA positive, confirming HCV infection. The 3 HCV EIA repeat reactive samples that were Procleix Ultrio Assay reactive and Procleix HCV Discriminatory Assay reactive and were HCV RIBA indeterminate most likely represent true HCV infection. It has been reported that HCV EIA repeat reactive results and reactive NAT results indicate true HCV infection, even if the HCV RIBA results are indeterminate or negative.[33] Therefore, when a sample is repeat reactive on a licensed anti-HCV screening test and reactive on the Procleix Ultrio Assay and reactive on the Procleix HCV Discriminatory Assay, the Procleix Ultrio Assay acts as a supplemental test that confirms HCV infection; it is not necessary to run an HCV RIBA.

Table 30. Procleix System and Procleix Tigris System – Comparison of HIV-1 and HCV Confirmatory Serology and Procleix Ultrio Assay Results

Serology			Procleix Ultrio Assay*	
	HIV-1 Western Blot		Reactive	Nonreactive
HIV-1 EIA RR (n=291)	Positive	20	18 (6.2%)	2 (0.7%)**
	Indeterminate	130	0 (0.0%)	130 (44.7%)
	Negative	133	0 (0.0%)	133 (45.7%)
	Not available	8	0 (0.0%)	8 (2.7%)
	HCV RIBA			
HCV EIA RR (n=656)	Positive	240	174 (26.5%)	66*** (10.1%)
	Indeterminate	148	3 (0.5%)	145 (22.1%)
	Negative	250	0 (0.0%)	250 (38.1%)
	Not available	18	6 (0.9%)	12 (1.8%)

RR: Repeatedly reactive
*Includes samples with Procleix HIV-1 or HCV Discriminatory Assay results or with nonreactive pool or IDS-only results.
** 1 sample was tested in a 16-sample pool that had a nonreactive Procleix Ultrio Assay result and 1 was Procleix Ultrio Assay reactive, HBV discriminated and HBV seropositive.
*** 66 samples that were HCV positive by the RIBA came from Procleix Ultrio Assay nonreactive pools, so discriminatory testing was not required.

Comparison of the Procleix Ultrio Assay to HBsAg Serology Results

Results obtained from Procleix Ultrio Assay donor screening on the Procleix Tigris System at 5 US blood testing sites and from HBsAg neutralization testing at 1 laboratory allow comparison of the Procleix Ultrio Assay with HBsAg screening and neutralization test reactivity (Table 31). All of the samples included in this analysis were HBsAg screening test repeat reactive.

Procleix Ultrio Assay and HBsAg neutralization test results were available for 641 samples. Overall agreement between Procleix Ultrio Assay and HBsAg neutralization test results was 86.9% (557/641; 95% CI: 84.1% to 89.3%). Of the 641 samples, 541 samples were Procleix Ultrio Assay nonreactive for HBV and 100 samples were Procleix Ultrio Assay reactive for HBV. Of the 541 samples that were Procleix Ultrio Assay nonreactive for HBV, 84 samples had reactive HBsAg neutralization test results and 457 had nonreactive HBsAg neutralization test results. The results for the 84 samples that were HBsAg neutralization test reactive and Procleix Ultrio Assay nonreactive are not unexpected, as HBsAg may be present in particles that do not contain nucleic acids[36] or after vaccination with a vaccine derived from HBsAg. All of the 100 HBsAg screening test repeat reactive samples that were Procleix Ultrio Assay reactive and Procleix HBV Discriminatory Assay reactive were HBsAg neutralization test reactive, confirming HBV infection. Therefore, when a sample is repeat reactive on a licensed HBsAg screening test and reactive on the Procleix Ultrio Assay and reactive on the Procleix HBV Discriminatory Assay, the Procleix Ultrio Assay acts as a supplemental test that confirms HBV infection; it is not necessary to run an HBsAg neutralization test.

Table 31. Procleix Tigris System – Comparison of HBsAg Neutralization Test and Procleix Ultrio Assay Results

Serology			Procleix Ultrio Assay	
	HBsAg Neutralization Test		Reactive	Nonreactive
HBsAg Screening Test RR (n=815)	Reactive	184	100	84*
	Nonreactive	457	0	457
	Not available	174	162	12

RR= repeatedly reactive
*83 samples that were HBV positive by the neutralization test came from Procleix Ultrio Assay nonreactive pools or IDS, so discriminatory assay testing was not required; the remaining 1 sample was Procleix Ultrio Assay reactive, nondiscriminated.

ANALYTICAL SENSITIVITY

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

Procleix System - Detection of HIV-1 type B virus

HIV-1 type B virus detection with the Procleix Ultrio Assay and HIV-1 Discriminatory Assay (dHIV-1) was 100% at 300 copies/mL, 99% at 100 copies/mL and ≥92% at 30 copies/mL for both assays. Positivity rates at 10 copies/mL were 53% and 57% for the Procleix Ultrio Assay and dHIV-1 Assay. At 3 copies/mL, the detection rates were 25% and 24% for the Procleix Ultrio Assay and dHIV-1 Assay. Although there was variability between the two assays, the differences were not statistically significant as indicated by overlapping 95% confidence intervals (Table 32a). Detection rates were calculated from valid initial results.

Table 32a. Procleix System - Detection of HIV-1 Type B in Analytical Sensitivity Panels

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

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

Procleix Tigris System - Detection of HIV-1 type B virus

HIV-1 type B virus detection with the Procleix Ultrio Assay and dHIV-1 Assay was 100% at 300 copies/mL, 100% at 100 copies/mL and ≥ 88% for both assays. Positivity rates at 30 copies/mL were 92% and 88% for the Procleix Ultrio Assay and dHIV-1 Assay. Positivity rates at 10 copies/mL were 60% and 57% for the Procleix Ultrio Assay and dHIV-1 Assay. At 3 copies/mL, the detection rates were 18% and 26% for the Procleix Ultrio Assay and

dHIV-1 Assay. Although there was variability between the two assays, the differences were not statistically significant as indicated by overlapping 95% confidence intervals (Table 32b). Detection rates were calculated from valid initial results.

Table 32b. Procleix Tigris System - Detection of HIV-1 Type B in Analytical Sensitivity Panels*

Procleix Ultrio Assay							Procleix dHIV-1 Assay					
HIV-1 B* Copies/mL	Number of Reactive/ Tested**	% Positive	95% Confidence Limits		Average S/CO	%CV	Number of Reactive/ Tested**	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper					Lower	Upper		
300	119/119	100	97	100	14.98	9	118/118	100	97	100	22.77	5
100	118/118	100	97	100	12.52	15	120/120	100	97	100	19.00	18
30	108/118	92	85	96	8.42	44	104/118	88	81	93	12.28	49
10	71/119	60	50	69	6.82	54	68/120	57	47	66	9.62	58
3	21/120	18	11	26	4.11	79	31/118	26	19	35	9.81	58
0	0/120	0	0	3	0.05	109	0/120	0	0	3	0.03	142

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

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

Detection of the HIV-1 WHO standard with the dHIV-1 Assay was 100% at 600, 200 and 60 IU/mL. The detection rates at 20 IU/mL and 6 IU/mL were 93% and 61%, respectively (Table 33a). Detection rates were calculated from valid initial results. Due to the cross reactivity of this standard with HBV,[34] only the dHIV-1 Assay was tested.

Table 33a. Procleix System - Detection of HIV-1 WHO Standard in Analytical Sensitivity Panels with the Procleix HIV-1 Discriminatory Assay

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

***** Invalid reactions were not included.

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

Detection of the HIV-1 WHO standard with the dHIV-1 Assay was 100% at 600, 200 and 60 IU/mL. The detection rates at 20 IU/mL and 6 IU/mL were 90% and 58%, respectively (Table 33b). Detection rates were calculated from valid initial results. Due to the cross reactivity of this standard with HBV,[34] only the dHIV-1 Assay was tested.

Table 33b. Procleix Tigris System - Detection of HIV-1 WHO Standard in Analytical Sensitivity Panels with the Procleix HIV-1 Discriminatory Assay

HIV-1 WHO (97/656) IU/mL	Number of Reactive/ Tested*	% Positive	95% Confidence Limits		Average S/CO	%CV
				Upper		
600	120/120	100	97	100	24.48	5
200	119/119	100	97	100	22.52	5
60	118/118	100	97	100	20.07	14
20	108/120	90	83	95	14.64	39
6	69/120	58	48	67	10.32	52
0	0/120	0	0	3	0.03	151

***** Invalid reactions were not included.

Procleix System - Detection of HCV WHO Standard (96/790)

The detection rate for the HCV WHO standard at 100 and 30 IU/mL was 100% and ≥99% at 10 IU/mL for both the Procleix Ultrio Assay and HCV Discriminatory Assay (dHCV). The detection rate at 3 IU/mL in the Procleix Ultrio Assay was 91%. In the dHCV Assay, the detection rate at 3 IU/mL was 96%. The detection rates for 1 IU/mL were 64% and 67% for the Procleix Ultrio Assay and dHCV Assay. There were no statistically significant

differences observed in the positivity rates for the detection of HCV WHO standard with the Procleix Ultrio Assay and dHCV Assay (Table 34a). Detection rates were calculated from valid initial results.

Table 34a. Procleix System - Detection of HCV WHO Standard in Analytical Sensitivity Panels

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

***** Invalid reactions were not included.

Procleix Tigris System - Detection of HCV WHO Standard (96/790)

The detection rate for the HCV WHO standard at 100, 30 and 10 IU/mL was 100% for both the Procleix Ultrio Assay and dHCV Assay. The detection rate at 3 IU/mL in the Procleix Ultrio Assay was 90%. In the dHCV Assay, the detection rate at 3 IU/mL was 88%. The detection rates for 1 IU/mL were 59% and 56% for the Procleix Ultrio Assay and dHCV Assay. There were no statistically significant differences observed in the positivity rates for the detection of HCV WHO standard with either the Procleix Ultrio Assay or dHCV Assay (Table 34b). Detection rates were calculated from valid initial results**.**

Table 34b. Procleix Tigris System - Detection of HCV WHO Standard in Analytical Sensitivity Panels

Procleix Ultrio Assay							Procleix dHCV Assay					
HCV WHO (96/790) IU/mL	Number of Reactive/ Tested*	% Positive	95% Confidence Limits		Average S/CO	%CV	Number of Reactive/ Tested*	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper					Lower	Upper		
100	115/115	100	97	100	7.67	5	119/119	100	97	100	23.45	6
30	119/119	100	97	100	7.55	5	119/119	100	97	100	22.93	6
10	120/120	100	97	100	7.38	8	119/119	100	97	100	22.48	8
3	108/120	90	83	95	6.63	21	106/120	88	81	94	19.88	27
1	70/118	59	50	68	6.11	34	66/118	56	47	65	16.99	37
0	0/120	0	0	3	0.05	109	0/119	0	0	3	0.03	186

***** Invalid reactions were not included.

Procleix System - Detection of HBV WHO Standard (97/746)

The detection rate of the Procleix Ultrio Assay and the HBV Discriminatory Assay (dHBV) was 100% for HBV WHO standard at 45 IU/mL and ≥99% at 15 IU/mL. HBV detection at 5 IU/mL with the Procleix Ultrio Assay and the dHBV Assay was 74% and 77% respectively, at 1.67 IU/mL detection rates were 40% and 41% respectively and 19% and 18% respectively for 0.56 IU/mL. There were no statistically significant differences observed in the positivity rates for the detection of HBV WHO standard with the Procleix Ultrio Assay and dHBV Assay (Table 35a). Detection rates were calculated from valid initial results.

Table 35a. Procleix System - Detection of HBV WHO Standard in Analytical Sensitivity Panels

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

***** Invalid reactions were not included.

Procleix Tigris System - Detection of HBV WHO Standard (97/746)

The detection rate of the Procleix Ultrio Assay and the dHBV Assay was 100% for HBV WHO standard at 45 IU/mL. The detection rate 15 IU/mL was 97% and 98% for the Procleix Ultrio Assay and dHBV Assay. HBV detection at 5 IU/mL with the Procleix Ultrio Assay and the dHBV Assay was 75% and 77% respectively. The detection rates were 31% and 33%, respectively, for 1.67 IU/mL and 15% and 12%, respectively, for 0.56 IU/mL. There were no statistically significant differences observed in the positivity rates for the detection of HBV WHO standard with either the Procleix Ultrio Assay or dHBV Assay (Table 35b). Detection rates were calculated from valid initial results.

Table 35b. Procleix Tigris System - Detection of HBV WHO Standard in Analytical Sensitivity Panels

	Procleix Ultrio Assay						Procleix dHBV Assay					
HBV WHO (97/746) IU/mL	Number of Reactive/ Tested*	% Positive	95% Confidence Limits		Average S/CO	%CV	Number of Reactive/ Tested*	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper					Lower	Upper		
45	120/120	100	97	100	14.97	5	119/119	100	97	100	24.00	8
15	116/120	97	92	99	14.57	11	116/118	98	94	100	23.48	12
5	88/118	75	66	82	13.03	27	90/117	77	68	84	21.49	18
1.67	37/120	31	23	40	10.90	27	39/120	33	24	42	17.10	45
0.56	18/120	15	9	23	11.63	38	14/119	12	7	19	10.90	101
0	0/119	0	0	3	0.04	103	0/119	0	0	3	0.02	227

***** Invalid reactions were not included.

Procleix System and Procleix Tigris System - Testing to Detect HBV DNA at 10 Copies/mL with Greater than 95% Probability

To detect HBV DNA at 10 copies/mL (approximately 2 IU/mL) at greater than 95% probability, Procleix Ultrio Assay testing should be performed using 6 replicates. A reactive result in at least 1 of the 6 replicates indicates the sample is HBV DNA positive.

Procleix System - Probit Analysis

The predicted 50% and 95% detection rates in copies/mL or IU/mL for each target were determined with Probit Analysis of the analytical sensitivity results. The predicted 95% detection rate for HIV-1 type B was 37.7 copies/mL for the Procleix Ultrio Assay and 35.4 copies/mL for the dHIV-1 Assay. The predicted 95% detection rate for HIV-1 WHO was 18.1 IU/mL for the dHIV-1 Assay. The predicted 95% detection rate for HCV WHO was 3.7 IU/mL and 2.4 IU/mL for the Procleix Ultrio Assay and the dHCV Assay, respectively. The 95% detection rate for HBV was 8.0 IU/mL and 6.8 IU/mL for the Procleix Ultrio Assay and dHBV Assay, respectively (Table 36a).

Table 36a. Procleix System - Detection Probabilities of HIV-1, HCV, and HBV

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

Procleix Tigris System - Probit Analysis

The predicted 50% and 95% detection rates in copies/mL or IU/mL for each target were determined with Probit Analysis of the analytical sensitivity results. The predicted 95% detection rate for HIV-1 type B was 28.8 copies/mL for the Procleix Ultrio Assay and 32.2 copies/mL for the dHIV-1 Assay. The predicted 95% detection rate for HIV-1 WHO was 20.3 IU/mL for the dHIV-1 Assay. The predicted 95% detection rate for HCV WHO was 3.0 IU/mL and 3.2 IU/mL for the Procleix Ultrio Assay and the dHCV Assay, respectively. The 95% detection rate for HBV was 10.4 IU/mL and 8.5 IU/mL for the Procleix Ultrio Assay and dHBV Assay, respectively (Table 36b).

Table 36b. Procleix Tigris System - Detection Probabilities of HIV-1, HCV, and HBV

Panel Tested	Assay	Detection Probabilities	
		50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
HIV-1 B copies/mL	Procleix Ultrio Assay	12.4 (11.0-14.0)	28.8 (25.8-32.7)
HIV-1 B copies/mL	Procleix dHIV-1 Assay	12.7 (11.1-14.5)	32.2 (28.7-36.7)
HIV WHO (97/656) IU/mL	Procleix dHIV-1 Assay	8.4 (7.2-9.6)	20.3 (18.1-23.1)
HCV WHO (96/790) IU/mL	Procleix Ultrio Assay	1.3 (1.1-1.5)	3.0 (2.7-3.4)
HCV WHO (96/790) IU/mL	Procleix dHCV Assay	1.4 (1.2-1.6)	3.2 (2.8-3.6)
HBV WHO (97/746) IU/mL	Procleix Ultrio Assay	4.3 (3.7-4.9)	10.4 (9.2-12.2)
HBV WHO (97/746) IU/mL	Procleix dHBV Assay	3.7 (3.3-4.2)	8.5 (7. 6-9.8)

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

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

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

HIV-1 specimens and tissue culture isolates of group M (subtypes A, B, C, D, E, F, and G), N, and O were quantified for HIV-1 RNA concentrations using commercially available quantitative HIV-1 RNA assays or with an in-house quantitative HIV-1 RNA test. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. Diluted specimens were tested in the Procleix Ultrio Assay and dHIV-1 Assay. Fifty-four unique specimens or tissue culture isolates were tested in duplicate using three clinical lots on the Procleix System. Six of the specimens were co-infected with HCV and/or HBV and were therefore only tested in the dHIV-1 Assay. At 300 copies/mL, 287/288 replicates (99.7%) were reactive with the Procleix Ultrio Assay and 324/324 replicates (100%) were reactive with the dHIV-1 Assay. At 100 copies/mL, 286/288 replicates (99.3%) were reactive with the Procleix Ultrio Assay and 320/324 replicates (98.8%) were reactive with the dHIV-1 Assay. At 30 copies/mL, 252/288 replicates (87.5%) were reactive with the Procleix Ultrio Assay and 289/324 replicates (89.2%) were reactive with the dHIV-1 Assay (Table 37a). Detection rates were calculated from valid initial results.

Table 37a. Procleix System - Detection of HIV-1 Genetic Variants with the Procleix Ultrio Assay and HIV-1 Discriminatory Assay*

Genetic Variant							
HIV-1 Group M Subtype A	300	8	48/48	100	9	54/54	100
	100		48/48	100		54/54	100
	30		46/48	95.8		48/54	88.9
HIV-1 Group M Subtype B	300	6	36/36	100	7	42/42	100
	100		36/36	100		42/42	100
	30		30/36	83.3		40/42	95.2
HIV-1 Group M Subtype C	300	8	48/48	100	8	48/48	100
	100		48/48	100		47/48	97.9
	30		42/48	87.5		42/48	87.5
HIV-1 Group M Subtype D	300	6	36/36	100	6	36/36	100
	100		36/36	100		36/36	100
	30		34/36	94.4		32/36	88.9
HIV-1 Group M Subtype E	300	8	48/48	100	9	54/54	100
	100		48/48	100		54/54	100
	30		45/48	93.8		51/54	94.4
HIV-1 Group M Subtype F	300	3	18/18	100	5	30/30	100
	100		18/18	100		30/30	100
	30		13/18	72.2		25/30	83.3
HIV-1 Group M Subtype G	300	1	6/6	100	2	12/12	100
	100		6/6	100		12/12	100
	30		6/6	100		12/12	100
HIV-1 Group N	300	1	5/6	**83.3**	1	6/6	100
	100		4/6	**66.7**		3/6	**50**
	30		3/6	50		2/6	33.3
HIV-1 Group O	300	7	42/42	100	7	42/42	100
	100		42/42	100		42/42	100
	30		33/42	78.6		37/42	88.1
All Genotypes	300	48	287/288	99.7	54	324/324	100
	100		286/288	99.3		320/324	98.8
	30		252/288	87.5		289/324	89.2

* The same panels were used for testing on the Procleix System and the Procleix Tigris System.
** Each unique donor was tested in duplicate with three clinical lots of reagents.
Note: Bolded text indicates reactive rates less than 95% for specimens at or above 100 copies/mL.

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

HIV-1 specimens and tissue culture isolates of group M (subtypes A, B, C, D, E, F, and G), N, and O were quantified for HIV-1 RNA concentrations using commercially available quantitative HIV-1 RNA assays or with an in-house quantitative HIV-1 RNA test. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. Diluted specimens were tested in the Procleix Ultrio Assay and dHIV-1 Assay. Fifty-four unique specimens or isolates were tested in duplicate using three clinical lots on the Procleix Tigris System. Six of the specimens were co-infected with HCV and/or HBV and were therefore only tested in the dHIV-1 Assay. At 300 copies/mL, 288/288 replicates (100%) were reactive with the Procleix Ultrio Assay and 324/324 replicates (100%) were reactive with the dHIV-1 Assay. At 100 copies/mL, 287/288 replicates (99.7%) were reactive with the Procleix Ultrio Assay and 320/324 replicates (98.8%) were reactive with the dHIV-1 Assay. At 30 copies/mL, 259/288 replicates (89.9%) were reactive with the Procleix Ultrio Assay and 298/324 replicates (92.0%) were reactive with the dHIV-1 Assay (Table 37b). Detection rates were calculated from valid initial results.

Table 37b. Procleix Tigris System - Detection of HIV-1 Genetic Variants with the Procleix Ultrio Assay and HIV-1 Discriminatory Assay*

Genetic Variant	Conc. Copies/mL	Procleix Ultrio Assay			Procleix HIV-1 Discriminatory Assay		
			Reactive/ Tested	% Reactive	Unique Donors**	Reactive/ Tested	% Reactive
HIV-1 Group M Subtype A	300	8	48/48	100	9	54/54	100
	100		48/48	100		53/54	98.1
	30		47/48	97.9		51/54	94.4
HIV-1 Group M Subtype B	300	6	36/36	100	7	42/42	100
	100		36/36	100		42/42	100
	30		30/36	83.3		40/42	95.2
HIV-1 Group M Subtype C	300	8	48/48	100	8	48/48	100
	100		48/48	100		48/48	100
	30		43/48	89.6		39/48	81.3
HIV-1 Group M Subtype D	300	6	36/36	100	6	36/36	100
	100		36/36	100		36/36	100
	30		36/36	100		36/36	100
HIV-1 Group M Subtype E	300	8	48/48	100	9	54/54	100
	100		48/48	100		54/54	100
	30		48/48	100		54/54	100
HIV-1 Group M Subtype F	300	3	18/18	100	5	30/30	100
	100		17/18	**94.4**		28/30	**93.3**
	30		17/18	94.4		25/30	83.3
HIV-1 Group M Subtype G	300	1	6/6	100	2	12/12	100
	100		6/6	100		12/12	100
	30		6/6	100		12/12	100
HIV-1 Group N	300	1	6/6	100	1	6/6	100
	100		6/6	100		5/6	**83.3**
	30		1/6	16.7		3/6	50.0
HIV-1 Group O	300	7	42/42	100	7	42/42	100
	100		42/42	100		42/42	100
	30		31/42	73.8		38/42	90.5
All Genotypes	300	48	288/288	100	54	324/324	100
	100		287/288	99.7		320/324	98.8
	30		259/288	89.9		298/324	92.0

* The same panels were used for testing on the Procleix System and the Procleix Tigris System.
** Each unique donor was tested in duplicate with three clinical lots of reagents.
Note: Bolded text indicates reactive rates less than 95% for specimens at or above 100 copies/mL.

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

HCV specimens of genotypes 1, 2, 3, 4, 5, and 6 were quantified for HCV RNA using commercially available quantitative HCV RNA assays. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. The diluted specimens were tested with the Procleix Ultrio Assay and dHCV Assay. Sixty-one unique specimens were tested in duplicate using three clinical lots on the Procleix System. One specimen was co-infected with HIV-1 and was therefore only tested in the dHCV Assay. At 300 copies/mL, all replicates were reactive with both the Procleix Ultrio Assay and the dHCV Assay. At 100 copies/mL, 354/360 replicates (98.3%) were reactive with the Procleix Ultrio Assay and 357/366 replicates (97.5%) were reactive with the dHCV Assay. At 30 copies/mL, 330/360 replicates (91.7%) were reactive with the Procleix Ultrio Assay and 337/366 replicates (92.1%) were reactive with the dHCV Assay (Table 38a). Detection rates were calculated from valid initial results.

Table 38a. Procleix System - Detection of HCV Genotypes with the Procleix Ultrio Assay and HCV Discriminatory Assay*

Genotype	Conc. Copies/mL	Procleix Ultrio Assay			Procleix HCV Discriminatory Assay		
			Reactive/ Tested	% Reactive	Unique Donors**	Reactive/ Tested	% Reactive
HCV Genotype 1	300	11	66/66	100	11	66/66	100
	100		66/66	100		66/66	100
	30		59/66	89.4		62/66	93.9
HCV Genotype 2	300	12	72/72	100	13	78/78	100
	100		67/72	**93.1**		73/78	**93.6**
	30		62/72	86.1		67/78	85.9
HCV Genotype 3	300	12	72/72	100	12	72/72	100
	100		71/72	98.6		69/72	95.8
	30		65/72	90.3		64/72	88.9
HCV Genotype 4	300	14	84/84	100	14	84/84	100
	100		84/84	100		83/84	98.8
	30		81/84	96.4		80/84	95.2
HCV Genotype 5	300	6	36/36	100	6	36/36	100
	100		36/36	100		36/36	100
	30		35/36	97.2		35/36	97.2
HCV Genotype 6	300	5	30/30	100	5	30/30	100
	100		30/30	100		30/30	100
	30		28/30	93.3		29/30	96.7
All Genotypes	300	60	360/360	100	61	366/366	100
	100		354/360	98.3		357/366	97.5
	30		330/360	91.7		337/366	92.1

* The same panels were used for testing on the Procleix System and the Procleix Tigris System.
** Each unique donor was tested in duplicate with three clinical lots of reagents.
Note: Bolded text indicates reactive rates less than 95% for specimens at or above 100 copies/mL.

Procleix Tigris System - Detection of HCV Genotypes with the Procleix Ultrio Assay and HCV Discriminatory Assay

HCV specimens of genotypes 1, 2, 3, 4, 5, and 6 were quantified for HCV RNA using commercially available quantitative HCV RNA assays. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. The diluted specimens were tested with the Procleix Ultrio Assay and dHCV Assay. Sixty-one unique specimens were tested in duplicate using three clinical lots on the Procleix Tigris System. One specimen was co-infected with HIV-1 and was therefore only tested in the dHCV Assay. At 300 copies/mL, 360/360 replicates (100%) were reactive with the Procleix Ultrio Assay and 366/366 replicates (100%) were reactive with the dHCV Assay. At 100 copies/mL, 353/360 replicates (98.1%) were reactive with the Procleix Ultrio Assay and 363/366 replicates (99.2%) were reactive with the dHCV Assay. At 30 copies/mL, 339/360 replicates (94.2%) were reactive with the Procleix Ultrio Assay and 346/366 replicates (94.5%) were reactive with the dHCV Assay (Table 38b). Detection rates were calculated from valid initial results.

Table 38b. Procleix Tigris System - Detection of HCV Genotypes with the Procleix Ultrio Assay and HCV Discriminatory Assay*

Genotype	Conc. Copies/mL	Procleix Ultrio Assay			Procleix HCV Discriminatory Assay		
			Reactive/ Tested	% Reactive	Unique Donors**	Reactive/ Tested	% Reactive
HCV Genotype 1	300	11	66/66	100	11	66/66	100
	100		66/66	100		66/66	100
	30		64/66	97.0		66/66	100
HCV Genotype 2	300	12	72/72	100	13	78/78	100
	100		66/72	**91.7**		75/78	96.2
	30		65/72	90.3		69/78	88.5
HCV Genotype 3	300	12	72/72	100	12	72/72	100
	100		72/72	100		72/72	100
	30		67/72	93.1		65/72	90.3
HCV Genotype 4	300	14	84/84	100	14	84/84	100
	100		84/84	100		84/84	100
	30		81/84	96.4		83/84	98.8
HCV Genotype 5	300	6	36/36	100	6	36/36	100
	100		36/36	100		36/36	100
	30		34/36	94.4		34/36	94.4
HCV Genotype 6	300	5	30/30	100	5	30/30	100
	100		29/30	96.7		30/30	100
	30		28/30	93.3		29/30	96.7
All Genotypes	300	60	360/360	100	61	366/366	100
	100		353/360	98.1		363/366	99.2
	30		339/360	94.2		346/366	94.5

* The same panels were used for testing on the Procleix System and the Procleix Tigris System.
** Each unique donor was tested in duplicate with three clinical lots of reagents.
Note: Bolded text indicates reactive rates less than 95% for specimens at or above 100 copies/mL.

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

HBV specimens of genotypes A, B, C, D, E, F, and G were quantified for HBV DNA using commercially available quantitative HBV DNA assays. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. Diluted specimens were tested with the Procleix Ultrio Assay and dHBV Assay. Fifty-seven unique specimens were tested in duplicate using three clinical lots on the Procleix Ultrio System. At 300 copies/mL, 337/342 replicates (98.5%) were reactive with the Procleix Ultrio Assay and 337/342 replicates (98.5%) were reactive with the dHBV Assay. At 100 copies/mL, 324/342 replicates (94.7%) were reactive with the Procleix Ultrio Assay and 312/342 replicates (91.2%) were reactive with the dHBV Assay. At 30 copies/mL, 265/342 replicates (77.5%) were reactive with the Procleix Ultrio Assay and 244/342 replicates (71.3%) were reactive with the dHBV Assay (Table 39a). Detection rates were calculated from valid initial results.

Table 39a. Procleix System - Detection of HBV Genotypes with the Procleix Ultrio Assay and HBV Discriminatory Assay*

Genotype	Conc. Copies/mL	Procleix Ultrio Assay			Procleix HBV Discriminatory Assay		
			Reactive/ Tested	% Reactive	Unique Donors**	Reactive/ Tested	% Reactive
HBV Genotype A	300	12	71/72	98.6	12	70/72	97.2
	100		70/72	97.2		67/72	**93.1**
	30		63/72	87.5		57/72	79.2
HBV Genotype B	300	10	60/60	100	10	60/60	100
	100		57/60	95		56/60	**93.3**
	30		43/60	71.7		36/60	60
HBV Genotype C	300	10	60/60	100	10	59/60	98.3
	100		52/60	**86.7**		54/60	**90**
	30		41/60	68.3		41/60	68.3
HBV Genotype D	300	8	45/48	**93.8**	8	47/48	97.9
	100		46/48	95.8		44/48	**91.7**
	30		41/48	85.4		35/48	72.9
HBV Genotype E	300	8	47/48	97.9	8	48/48	100
	100		46/48	95.8		40/48	**83.3**
	30		32/48	66.7		34/48	70.8
HBV Genotype F	300	8	48/48	100	8	47/48	97.9
	100		47/48	97.9		45/48	**93.8**
	30		39/48	81.3		35/48	72.9
HBV Genotype G	300	1	6/6	100	1	6/6	100
	100		6/6	100		6/6	100
	30		6/6	100		6/6	100
All Genotypes	300	57	337/342	98.5	57	337/342	98.5
	100		324/342	**94.7**		312/342	**91.2**
	30		265/342	77.5		244/342	71.3

* The same panels were used for testing on the Procleix System and the Procleix Tigris System.
** Each unique donor was tested in duplicate with three clinical lots of reagents.
Note: Bolded text indicates reactive rates less than 95% for specimens at or above 100 copies/mL.

Procleix Tigris System - Detection of HBV Genotypes with the Procleix Ultrio Assay and HBV Discriminatory Assay

HBV specimens of genotypes A, B, C, D, E, F, and G were quantified for HBV DNA using commercially available quantitative HBV DNA assays. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. Diluted specimens were tested with the Procleix Ultrio Assay and dHBV Assay. Fifty-eight unique specimens were tested in duplicate using three clinical lots on the Procleix Tigris System. At 300 copies/mL, 341/348 replicates (98%) were reactive with the Procleix Ultrio Assay and 345/348 replicates (99.1%) were reactive with the dHBV Assay. At 100 copies/mL, 300/348 replicates (86.2%) were reactive with the Procleix Ultrio Assay and 324/348 replicates (93.1%) were reactive with the dHBV Assay. At 30 copies/mL, 220/348 replicates (63.2%) were reactive with the Procleix Ultrio Assay and 256/348 replicates (73.6%) were reactive with the dHBV Assay (Table 39b). Detection rates were calculated from valid initial results.

Table 39b. Procleix Tigris System - Detection of HBV Genotypes with the Procleix Ultrio Assay and HBV Discriminatory Assay*

Genotype	Conc. Copies/mL	Procleix Ultrio Assay			Procleix HBV Discriminatory Assay		
			Reactive/ Tested	% Reactive	Unique Donors**	Reactive/ Tested	% Reactive
HBV Genotype A	300	12	71/72	98.6	12	71/72	98.6
	100		61/72	**84.7**		70/72	97.2
	30		45/72	62.5		54/72	75.0
HBV Genotype B	300	10	60/60	100	10	60/60	100
	100		51/60	**85.0**		58/60	96.7
	30		28/60	46.7		47/60	78.3
HBV Genotype C	300	10	59/60	98.3	10	60/60	100
	100		53/60	**88.3**		54/60	**90**
	30		40/60	66.7		42/60	70.0
HBV Genotype D	300	9	52/54	96.3	9	54/54	100
	100		45/54	**83.3**		49/54	**90.7**
	30		37/54	68.5		36/54	66.7
HBV Genotype E	300	8	46/48	95.8	8	47/48	97.9
	100		39/48	**81.3**		41/48	**85.4**
	30		26/48	54.2		29/48	60.4
HBV Genotype F	300	8	47/48	97.9	8	47/48	97.9
	100		45/48	**93.8**		46/48	95.8
	30		38/48	79.2		42/48	87.5
HBV Genotype G	300	1	6/6	100	1	6/6	100
	100		6/6	100		6/6	100
	30		6/6	100		6/6	100
All Genotypes	300	58	341/348	98.0	58	345/348	99.1
	100		300/348	**86.2**		324/348	**93.1**
	30		220/348	63.2		256/348	73.6

* The same panels were used for testing on the Procleix System and the Procleix Tigris System.
** Each unique donor was tested in duplicate with three clinical lots of reagents.
Note: Bolded text indicates reactive rates less than 95% for specimens at or above 100 copies per mL.

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

REPRODUCIBILITY

The reproducibility of the Procleix Ultrio Assay and Discriminatory Assays with cadaveric blood specimens was assessed on the Procleix System and the Procleix Tigris System. Plasma containing HIV-1, HCV or HBV was spiked into cadaveric plasma and serum specimens and control plasma specimens (HIV-1 at 200 copies/mL, HCV at 60 IU/mL and HBV at 45 IU/mL); 20 specimens were tested in the Procleix Ultrio Assay and 20 specimens were tested in each Discriminatory Assay. The specimens were tested with three clinical lots, one of which was tested only on the Procleix System, the second on both the Procleix System and the Procleix Tigris System, and the third on just the Procleix Tigris System. Specimens were tested in three separate runs for each clinical lot, for a total of six runs. The percent positive, analyte S/CO values, and coefficients of variation (%CVs) are shown in Tables 40a and 40b. The positivity rates ranged from 96% to 100% on the Procleix System and 98% to 100% on the Procleix Tigris System. The %CVs ranged from 16% to 35% for HIV-1 spiked cadaveric specimens, 6% to 34% for the HCV spiked cadaveric specimens, and 4% to 17% for the HBV spiked cadaveric specimens.

Table 40a. Procleix System - Reproducibility of Procleix Ultrio Assay and Discriminatory Assays in Cadaveric Blood Specimens

Virus	Assay	Sample Type*	# of Donors	# of Replicates	% Positive** (95% CI)	Mean Analyte S/CO***	%CV
HIV-1	Procleix Ultrio Assay	Cadaveric	20	119	99 (95-100)	2.91	30
		Control	20	120	99 (95-100)	12.02	19
	Procleix dHIV-1 Assay	Cadaveric	20	117****	96 (91-100)	14.60	35
		Control	20	120	100 (98-100)	18.12	21
HCV	Procleix Ultrio Assay	Cadaveric	20	120	98 (94-100)	5.77	34
		Control	20	120	100 (98-100)	6.92	7
	Procleix dHCV Assay	Cadaveric	20	119	98 (95-100)	16.83	27
		Control	20	120	100 (98-100)	21.74	10
HBV	Procleix Ultrio Assay	Cadaveric	20	120	99 (95-100)	13.60	9
		Control	20	120	99 (95-100)	13.63	12
	Procleix dHBV Assay	Cadaveric	20	120	100 (98-100)	21.75	17
		Control	20	120	100 (98-100)	23.11	8

CI = Confidence Interval
* Cadaveric specimens included serum and plasma specimens.
** The percent positive results were determined from the initial neat result. If the initial neat result was invalid, the result from a retest neat result was used. If both the initial and repeat neat results were invalid, the valid results from testing diluted samples were used. The 95% CI are computed based on the assumption that all the outcomes are independent.
*** S/CO analysis reflects only valid neat results.
**** Three specimens with invalid IC, QNS for retest.

Table 40b. Procleix Tigris System - Reproducibility of Procleix Ultrio Assay and Discriminatory Assays in Cadaveric Blood Specimens

Virus	Assay	Sample Type*	# of Donors	# of Replicates	% Positive** (95% CI)	Mean Analyte S/CO***	%CV
HIV-1	Procleix Ultrio Assay	Cadaveric	20	119	98 (94-100)	13.46	23
		Control	20	120	99 (95-100)	13.10	16
	Procleix dHIV-1 Assay	Cadaveric	20	120	100 (98-100)	18.27	18
		Control	20	120	100 (98-100)	19.03	19
HCV	Procleix Ultrio Assay	Cadaveric	20	120	100 (98-100)	7.16	23
		Control	20	120	100 (98-100)	7.65	7
	Procleix dHCV Assay	Cadaveric	20	120	100 (98-100)	20.57	18
		Control	20	120	100 (98-100)	22.67	6
HBV	Procleix Ultrio Assay	Cadaveric	20	120	100 (98-100)	15.39	4
		Control	20	120	100 (98-100)	15.29	5
	Procleix dHBV Assay	Cadaveric	20	120	100 (98-100)	24.42	5
		Control	20	120	100 (98-100)	24.42	4

CI = Confidence Interval
* Cadaveric specimens included serum and plasma specimens.
** The percent positive results were determined from the initial neat result. If the initial neat result was invalid, the result from a retest neat result was used. If both the initial and repeat neat results were invalid, the valid results from testing diluted samples were used. The 95% CI are computed based on the assumption that all the outcomes are independent.
*** S/CO analysis reflects only valid neat results.

SPECIFICITY

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

HIV-1, HCV and HBV seronegative cadaveric blood specimens were tested to determine the specificity of the Procleix Ultrio Assay and dHIV-1, dHCV and dHBV Assays. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots on the Procleix System and the Procleix Tigris System. The specificity of the Procleix Ultrio Assay and dHIV-1 and dHBV Assays for the cadaveric specimens was 100% (95% confidence interval: 93%-100%) for the Procleix System and the Procleix Tigris System. The specificity of the dHCV Assay for the cadaveric specimens was 98% (95% confidence interval: 89%-100%) (Tables 41a and 41b). Specificity rates were calculated from all valid initial results.

Table 41a. Procleix System - Specificity of Procleix Ultrio Assay and Discriminatory Assays in Cadaveric Blood Specimens

		Control	Cadaveric
Procleix Ultrio Assay	Mean IC S/CO	1.94	1.89
	Mean Analyte S/CO	0.05	0.09
	Specificity Rate	100%	100%
	95% CI Specificity Rate	93-100	92-100
	n	50	47
Procleix dHIV-1 Assay	Mean IC S/CO	2.00	1.99
	Mean Analyte S/CO	0.14	0.11
	Specificity Rate	100%	100%
	95% CI Specificity Rate	93-100	93-100
	n	50	50
Procleix dHCV Assay	Mean IC S/CO	2.07	1.98
	Mean Analyte S/CO	0.14	0.21
	Specificity Rate	100%	98*%
	95% CI Specificity Rate	93-100	89-100
	n	50	49
Procleix dHBV Assay	Mean IC S/CO	2.01	2.02
	Mean Analyte S/CO	0.11	0.10
	Specificity Rate	100%	100%
	95% CI Specificity Rate	93-100	93-100
	n	50	49

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

Table 41b. Procleix Ultrio Tigris System - Specificity of Procleix Ultrio Assay and Discriminatory Assays in Cadaveric Blood Specimens

		Control	Cadaveric
Procleix Ultrio Assay	Mean IC S/CO	2.05	2.07
	Mean Analyte S/CO	0.05	0.07
	Specificity Rate	100%	100%
	95% CI Specificity Rate	93-100	93-100
	n	50	48
Procleix dHIV-1 Assay	Mean IC S/CO	2.04	2.04
	Mean Analyte S/CO	0.03	0.03
	Specificity Rate	100%	100%
	95% CI Specificity Rate	93-100	93-100
	n	50	49
Procleix dHCV Assay	Mean IC S/CO	2.02	2.03
	Mean Analyte S/CO	0.03	0.10
	Specificity Rate	100%	98*%
	95% CI Specificity Rate	93-100	89-100
	n	50	49
Procleix dHBV Assay	Mean IC S/CO	2.02	2.02
	Mean Analyte S/CO	0.03	0.02
	Specificity Rate	100%	100%
	95% CI Specificity Rate	93-100	93-100
	n	50	50

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

SENSITIVITY

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

HIV-1, HCV, and HBV seronegative cadaveric blood specimens spiked with a low level of HIV-1 were tested to determine the sensitivity of the Procleix Ultrio Assay and dHIV-1 Assay. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots on the Procleix System and the Procleix Tigris System after spiking each with approximately 200 copies/mL of HIV-1. The positivity rate of the Procleix Ultrio Assay and dHIV-1 Assay for the cadaveric specimens was 100% (95% confidence interval: 93%-100%) on the Procleix System and the Procleix Tigris System (Tables 42a and 42b). Detection rates were calculated from valid initial results.

Table 42a. Procleix System - Reactivity of Procleix Ultrio Assay and HIV-1 Discriminatory Assay in Cadaveric Blood Specimens Spiked with HIV-1

		Control	Cadaveric
Procleix Ultrio Assay	Mean IC S/CO	2.36	2.31
	Mean Analyte S/CO	12.85	12.05
	% Positive	100	100
	95% CI (% Positive)	93-100	93-100
	n	50	50
Procleix dHIV-1 Assay	Mean IC S/CO	2.12	2.07
	Mean Analyte S/CO	17.01	15.89
	% Positive	100	100
	95% CI (% Positive)	93-100	93-100
	n	50	50

CI = Confidence Interval
n = number of samples

Table 42b. Procleix Tigris System - Reactivity of Procleix Ultrio Assay and HIV-1 Discriminatory Assay in Cadaveric Blood Specimens Spiked with HIV-1

		Control	Cadaveric
Procleix Ultrio Assay	Mean IC S/CO	2.17	2.21
	Mean Analyte S/CO	12.70	12.29
	% Positive	100	100
	95% CI (% Positive)	93-100	93-100
	n	49	52
Procleix dHIV-1 Assay	Mean IC S/CO	1.84	2.02
	Mean Analyte S/CO	20.28	21.28
	% Positive	100	100
	95% CI (% Positive)	93-100	93-100
	n	51	50

CI = Confidence Interval
n = number of samples

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

HIV-1, HCV and HBV seronegative cadaveric blood specimens spiked with a low level of HCV were tested to determine the sensitivity of the Procleix Ultrio Assay and dHCV Assay. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots on the Procleix System and the Procleix Tigris System after spiking each with approximately 200 copies/mL of HCV. The positivity rate of both the Procleix Ultrio Assay and dHCV Assay for the cadaveric specimens was 100% (95% confidence interval: 93%-100%) on the Procleix System and the Procleix Tigris System (Tables 43a and 43b). Detection rates were calculated from valid initial results.

Table 43a. Procleix System - Reactivity of Procleix Ultrio Assay and HCV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HCV

		Control	Cadaveric
Procleix Ultrio Assay	Mean IC S/CO	2.03	1.95
	Mean Analyte S/CO	7.93	7.05
	% Positive	100	100
	95% CI (% Positive)	93-100	93-100
	n	50	50
Procleix dHCV Assay	Mean IC S/CO	2.03	1.91
	Mean Analyte S/CO	22.06	19.35
	% Positive	100	100
	95% CI (% Positive)	93-100	93-100
	n	50	50

CI = Confidence Interval
n = number of samples

Table 43b. Procleix Tigris System - Reactivity of Procleix Ultrio Assay and HCV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HCV

		Control	Cadaveric
Procleix Ultrio Assay	Mean IC S/CO	2.09	2.06
	Mean Analyte S/CO	7.89	7.69
	% Positive	100	100
	95% CI (% Positive)	93-100	93-100
	n	50	50
Procleix dHCV Assay	Mean IC S/CO	1.88	1.91
	Mean Analyte S/CO	23.21	23.32
	% Positive	100	100
	95% CI (% Positive)	93-100	93-100
	n	50	50

CI = Confidence Interval
n = number of samples

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

HIV-1, HCV and HBV seronegative cadaveric blood specimens spiked with a low level of HBV were tested to determine the sensitivity of the Procleix Ultrio Assay and dHBV Assay. Seventy cadaveric and 70 normal donor specimens were tested using three clinical lots on the Procleix System and the Procleix Tigris System after spiking each with approximately 30 IU/mL of HBV. The positivity rate of the Procleix Ultrio Assay for the cadaveric specimens was 100% (95% confidence interval: 95%-100%) on the Procleix System. The positivity rate of the dHBV Assay was 98% (95% confidence interval: 92%-100%) for the Procleix System (Table 44a). On the Procleix Tigris System, the positivity rate of the Procleix Ultrio Assay and the dHBV Assay was 96% (95% confidence interval: 88%-99%) (Table 44b). Detection rates were calculated from valid initial results.

Table 44a. Procleix System - Reactivity of Procleix Ultrio Assay and HBV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HBV

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

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

Table 44b. Procleix Tigris System - Reactivity of Procleix Ultrio Assay and HBV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HBV

		Control	Cadaveric*
Procleix Ultrio Assay	Mean IC S/CO	1.78	1.89
	Mean Analyte S/CO	14.58	14.41
	% Positive	96	96
	95% CI (%pos)	88-99	88-99
	n	70	70
Procleix dHBV Assay	Mean IC S/CO	2.06	2.17
	Mean Analyte S/CO	22.62	23.54
	% Positive	84	96
	95% CI (% Positive)	74-92	88-99
	n	70	70

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

REACTIVITY IN SEROCONVERTING DONORS

PROCLEIX SYSTEM

Commercially available seroconversion panels collected from plasmapheresis donors were tested to determine the ability of the Procleix Ultrio Assay and HIV-1, HCV, and HBV Discriminatory Assays to shorten the window period of HIV-1, HCV, and HBV detection when compared to antigen and/or antibody tests. Two separate studies were performed, each with a different clinical lot (Tables 45a and 45b). Both studies tested a similar set of HIV-1 (n=10), HCV (n=10), and HBV (n=10) seroconversion panels at one site. Each seroconversion panel was tested with the Procleix Ultrio Assay (either neat and 1:16 diluted in one study or neat and 1:8 diluted in the other study which used development clinical lots) and with the HIV-1 Discriminatory (neat only), HCV Discriminatory (neat only) and HBV Discriminatory (neat only) Assays. The test results were compared with those of the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay for HIV-1 seroconversion panels, with those of the Ortho HCV 3.0 ELISA test for HCV seroconversion panels, or with those of Ortho Antibody to HBsAg ELISA Test System 3 and Abbott PRISM HBsAg Assay for HBV seroconversion panels.

In some cases, seroconversion study samples were tested in the same runs with the samples from the Procleix System pivotal clinical studies, including the clinical specificity study described earlier (see Specificity in Normal Blood Donors in the Procleix System section for run statistics). When 378, 143, and 127 samples were tested neat in the Procleix Ultrio Assay and HIV-1 and HCV Discriminatory Assays, respectively, none of the samples had initial invalid test results in the valid assay runs. For the 378 samples tested diluted in the Procleix Ultrio Assay, 2 (0.5%) had initial invalid results. These samples were not retested: 1 was invalid due to an Internal Control (IC) failure and the other was invalid due to the sample not being pipetted correctly. For the 108 samples tested in the HBV Discriminatory Assay, 1 (0.9%) had an initial invalid result. This sample was not retested: it was invalid due to dispense verification failure.

HIV-1 Detection in Seroconversion Panels

The Procleix Ultrio Assay was able to detect HIV-1 RNA a median of 14 and 7 (or 14 and 8 in the second study) days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested neat (Tables 45a and 45b). The Procleix Ultrio Assay was able to detect HIV-1 RNA a median of 11.5 and 4 days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested at a 1:8 dilution. The Procleix Ultrio Assay was able to detect HIV-1 RNA a median of 11 and 5 days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were

tested at a 1:16 dilution. Similar results were observed with the HIV-1 Discriminatory Assay, as compared to the Procleix Ultrio Assay, when specimens were tested neat in both studies.

Table 45a. Procleix System - Comparison to HIV-1 Antibody and Antigen Tests on Seroconversion Panels Study 1 (Number of Days Earlier Detection)

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

* Panel did not show Ab or Ag reactivity.

Table 45b. Procleix System - Comparison to HIV-1 Antibody and Antigen Tests on Seroconversion Panels Study 2 (Number of Days Earlier Detection)

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

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

HCV Detection in Seroconversion Panels

In both studies the Procleix Ultrio Assay was able to detect HCV RNA a median of 32 days earlier than the Ortho HCV 3.0 ELISA test when specimens were tested neat, at 1:8 dilution, and at 1:16 dilution (Tables 46a and 46b). The HCV Discriminatory Assay was able to detect HCV RNA a median of 32 days and 34.5 days earlier than the Ortho HCV 3.0 ELISA Assay when specimens were tested neat in the two separate studies.

Table 46a. Procleix System - Comparison to Ortho HCV 3.0 ELISA Assay on Seroconversion Panels Study 1 (Number of Days Earlier Detection).

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

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

Table 46b. Procleix System - Comparison to Ortho HCV 3.0 ELISA Assay on Seroconversion Panels Study 2 (Number of Days Earlier Detection)

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

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

HBV Detection in Seroconversion Panels

The Procleix Ultrio Assay was able to detect HBV DNA a median of 19 days and 17 (or 18.5 in the second study) days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested neat (Tables 47a and 47b). The Procleix Ultrio Assay was able to detect HBV DNA a median of 11.5 days earlier than the Abbott PRISM HBsAg Assay when specimens were tested at 1:8 dilution. The Procleix Ultrio Assay was able to detect HBV DNA a median of 9 days and 7 days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested at 1:16 dilution. The HBV Discriminatory Assay was able to detect HBV DNA a median of 16 days and 15 (or 17 in the second study) days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested neat.

Table 47a. Procleix System - Comparison to HBV Surface Antigen Tests on Seroconversion Panels (Number of Days Earlier Detection)

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

Table 47b. Procleix System - Comparison to Abbott PRISM HBsAg Assay on Seroconversion Panels (Number of Days Earlier Detection)

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

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

PROCLEIX TIGRIS SYSTEM

Commercially available seroconversion panels collected from plasmapheresis donors were tested to determine the ability of the Procleix Ultrio Assay and the Procleix HIV-1, HCV, and HBV Discriminatory Assays on the Procleix Tigris System to shorten the window period of HIV-1, HCV, and HBV detection when compared to antigen and/or antibody tests. Testing was performed using HIV-1 (n=10), HCV (n=10), and HBV (n=10) seroconversion panels using one clinical lot. Each seroconversion panel was tested with the Procleix Ultrio Assay (neat, and 1:16 diluted) and with the HIV-1 Discriminatory (neat only), HCV Discriminatory (neat only) and HBV Discriminatory (neat only) Assays. The test results were compared with those of the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay for HIV-1 seroconversion panels, with those of the Ortho HCV 3.0 ELISA test for HCV seroconversion panels, or with those of Ortho Antibody to HBsAg ELISA Test System 3 and Abbott PRISM HBsAg Assay for HBV seroconversion panels.

In some cases, seroconversion study samples were tested in the same worklists with the samples from the Procleix Tigris System pivotal clinical studies, including the clinical specificity study described earlier (see Specificity in Normal Blood Donors in the Procleix Tigris System section for worklist statistics). For the Procleix Ultrio Assay, from the valid assay worklists, 378 test results were generated from neat and from diluted samples. For the neat samples, 2 (0.5%) had initial invalid results. These samples were not retested: both were invalid due to clots in the samples. For the diluted samples, 1 (0.3%) had an initial invalid result. This sample was not retested: it was invalid due to a clot in the sample. For the HIV-1, HCV, and HBV Discriminatory Assays, from the valid assay worklists, 143, 127, and 108 test results, respectively, were generated: none were invalid.

HIV-1 Detection in Seroconversion Panels

The Procleix Ultrio Assay was able to detect HIV-1 RNA a median of 14 and 7 days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested neat (Table 48). The Procleix Ultrio Assay was able to detect HIV-1 RNA a median of 11 and 6 days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested at a 1:16 dilution. Similar results were observed with the HIV-1 Discriminatory Assay when specimens were tested neat.

Table 48. Procleix Tigris System - Comparison to HIV-1 Antibody and Antigen Tests on Seroconversion Panels (Number of Days Earlier Detection)

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

* Panel did not show Ab or Ag reactivity.

HCV Detection in Seroconversion Panels

The Procleix Ultrio Assay was able to detect HCV RNA a median of 32 days earlier than the Ortho HCV 3.0 ELISA test when specimens were tested neat, and at 1:16 dilution. Similar results were observed with the HCV Discriminatory Assay when specimens were tested neat (Table 49).

Table 49. Procleix Tigris System - Comparison to Ortho HCV 3.0 Assay on Seroconversion Panels (Number of Days Earlier Detection)

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

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

HBV Detection in Seroconversion Panels

The Procleix Ultrio Assay was able to detect HBV DNA a median of 18 days and 17 days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested neat (Table 50). The Procleix Ultrio Assay was able to detect HBV DNA a median of 8 days and 6 days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested at 1:16 dilution. The HBV Discriminatory Assay was able to detect HBV DNA a median of 16 days and 14 days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested neat.

Table 50. Procleix Tigris System - Comparison to HBV Surface Antigen Tests on Seroconversion Panels (Number of Days Earlier Detection)

Panel ID	Ortho Antibody to HBsAg ELISA Test System 3			Abbott PRISM HBsAg		
	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:16)	Procleix dHBV Assay (Neat)	Procleix Ultrio Assay (Neat)	Procleix Ultrio Assay (Diluted 1:16)	Procleix dHBV Assay (Neat)
62675	19	12	19	19	12	19
62825	29	29	29	29	29	29
62967	12	7	14	12	7	14
63133	11	9	11	11	9	11
63568	14	7	14	10	3	10
63659	15	13	13	15	13	13
63997	23	7	14	21	5	12
64006	20	0	20	20	0	20
64121	19	0	25	19	0	25
64132	17	8	17	9	0	9
Median	18	8	16	17	6	14

COMPARABILITY OF THE PROCLEIX TIGRIS SYSTEM AND THE PROCLEIX SYSTEM

The comparability of the Procleix Tigris System and the Procleix System was evaluated in panels comprised of HIV-1, HCV, and/or HBV positive and negative samples. The panel members tested in the Procleix Ultrio Assay (n=729), the HIV-1 (n=194), the HCV (n=189), and the HBV (n=198) Discriminatory Assays contained positive members with low copy levels, genetic variants and co-infected samples and negative members with various anticoagulants, interfering substances, and bloodborne pathogens. Three replicates of each panel were tested on the Procleix Tigris System at three sites and on the Procleix System at one site. The contents of the panels were masked during testing to control for bias. Testing was performed using one Procleix Ultrio Assay clinical lot. Panel members from a valid assay run with initial invalid test results were not retested.

Of the 27 Procleix Ultrio Assay runs generated on the Procleix System, 1 (3.7%) was invalidated because it contained more than 10% invalid test results. From the valid assay runs, 4 of 2,184 (0.2%) test results were invalid on the Procleix System; all were due to Internal Control [IC] failures. For the Procleix Tigris System, 1 of 23 (4.3%) Procleix Ultrio Assay worklists was invalid due to instrument error. From the valid assay worklists, 16 of 6,513 (0.2%) test results were invalid on the Procleix Tigris System: 1 was due to IC failure and 15 were due to other reasons such as instrument failures, software failures, chemistry errors, and clots in the samples.

Of the 9 Procleix HIV-1 Discriminatory Assay runs generated on the Procleix System, 1 (11.1%) was invalidated because it contained more than 10% invalid test results. From the valid assay runs, 14 of 562 (2.5%) test results were invalid on the Procleix System; 6 were due to IC failures and 8 were due to improperly pipetted samples. For the Procleix Tigris System, 23 HIV-1 Discriminatory Assay worklists were generated: none were invalid. From the valid assay worklists, 18 of 1,695 (1.1%) test results were invalid on the Procleix Tigris System: 13 were due to IC failure and 5 were due to other reasons such as instrument failures, software failures, chemistry errors, and clots in the samples.

Of the 8 Procleix HCV Discriminatory Assay runs generated on the Procleix System, none were invalid. From the valid assay runs, 555 test results were generated on the Procleix System: none were invalid. For the Procleix Tigris System, 1 of 24 (4.2%) HCV Discriminatory Assay worklists was invalid due to instrument error. From the valid assay worklists, 21 of 1,678 (1.3.%) test results were invalid on the Procleix Tigris System: 3 were due to IC failure and 18 were due to other reasons such as instrument failures, software failures, chemistry errors, and clots in the samples.

Of the 8 Procleix HBV Discriminatory Assay runs generated on the Procleix System, none were invalid. From the valid assay runs, 5 of 594 (0.8%) test results were invalid on the Procleix System; all were due to IC failures. For the Procleix Tigris System, 24 HBV Discriminatory Assay worklists were generated: none were invalid. From the valid assay worklists, 14 of 1,781 (0.8%) test results were invalid on the Procleix Tigris System: all were due to other reasons such as instrument failures, software failures, chemistry errors, clots in the samples, and insufficient sample volume.

To assess the relative performance of the Procleix Tigris System and the Procleix System, the accuracy was calculated for each system using the Procleix Ultrio Assay and Procleix HIV-1, HCV, and HBV Discriminatory Assays. The accuracies of the two systems were compared for all positive samples, all negative samples, and all samples combined. In addition, analysis of the S/CO values (IC for negative samples and analyte for positive samples) was performed for each system using each of the four assays. The S/CO values of the two systems were compared for all positive samples, the subcategories of the positive samples and all negative samples.

PROCLEIX ULTRIO ASSAY

Performance of the Procleix Ultrio Assay on the Procleix Tigris System was not significantly different from that on the Procleix System. The accuracy for all sample types was 99.3% (95% CI: 98.8%-99.6%) for the Procleix System and 99.6% (95% CI: 99.5%-99.8%) for the Procleix Tigris System. Accuracy rates were also similar between the two systems in the positive samples and in the negative samples (Table 51a). The mean analyte S/CO values for the positive samples tested with the Procleix Ultrio Assay were 16.32 for the Procleix Ultrio System and 16.50 for the Procleix Tigris System. The mean analyte S/CO values were also similar between the two systems for the various positive sample subcategories. The mean IC S/CO values for the negative samples were 1.99 for the Procleix Ultrio System and 2.08 for the Procleix Ultrio Tigris System (Table 51b).

Table 51a. Comparison of Procleix Ultrio Assay Performance on the Procleix Tigris System and the Procleix System - Analysis of Accuracy*

Sample Type	Procleix System			Procleix Tigris System		
	Correct	Total	Accuracy (%) (95% CI)**	Correct	Total	Accuracy (%) (95% CI)**
All	2,126	2,141	99.3 (98.8 - 99.6)	6,359	6,382	99.6 (99.5 - 99.8)
Positive	909	909	100 (99.6 - 100)	2,707	2,710	99.9 (99.7 - 100)
Negative	1,217	1,232	98.8 (98.0 - 99.3)	3,652	3,672	99.5 (99.2 - 99.7)

CI = Confidence interval
* HIV-1, HCV, and/or HBV positive samples with viral loads ≥100 copies/mL for HIV-1 and HCV or 15 IU/mL for HBV or with unknown copy levels.
** The 95% CI are computed based on the assumption that all the outcomes are independent.

Table 51b. Comparison of the Procleix Ultrio Assay Signal to Cutoff Ratio Values* for the Procleix Tigris System and the Procleix System**

Sample Type	n		Mean S/CO		SD		%CV	
	PS	TS	PS	TS	PS	TS	PS	TS
Positive	943	2,796	16.32	16.50	9.06	9.25	55.51	56.08
HIV-1	228	670	13.65	13.11	4.47	4.84	32.73	36.88
HCV	211	624	7.32	7.43	1.27	1.36	17.37	18.30
HBV	234	699	14.24	14.90	1.30	1.14	9.13	7.65
HIV-1 + HCV + HBV	90	268	35.92	36.43	3.35	4.40	9.34	12.08
HIV-1 + HCV	60	177	22.58	23.42	7.92	7.85	35.05	33.51
HIV-1 + HBV	60	178	26.46	25.72	6.47	6.33	24.44	24.60
HCV + HBV	60	180	20.50	21.16	4.21	4.52	20.54	21.38
Negative	1,217	3,652	1.99	2.08	0.14	0.11	7.13	5.22

n = Number of replicates
S/CO = Signal to cutoff ratio
SD = Standard deviation CV
= Coefficient of variation PS
= Procleix System
TS = Procleix Tigris System
* Analyte S/CO for positive panel members and Internal Control S/CO for negative panel members
** Samples with false negative and false positive results were excluded from this analysis.

PROCLEIX HIV-1 DISCRIMINATORY ASSAY

Performance of the Procleix HIV-1 Discriminatory Assay on the Procleix Tigris System was not significantly different from that on the Procleix System. The accuracy for all sample types was 99.0% (95% CI: 97.8%-99.7%) for the Procleix System and 99.1% (95% CI: 98.5%-99.5%) for the Procleix Tigris System. Accuracy rates were also similar between the two systems in the HIV-1 positive samples and in the HIV-1 negative samples (Table 52a). The mean analyte S/CO values for the HIV-1 positive samples tested with the Procleix HIV-1 Discriminatory Assay were 17.61 for the Procleix System and 20.06 for the Procleix Tigris System. The mean analyte S/CO values were also similar between the two systems for the various HIV-1 positive sample subcategories. The mean IC S/CO values for all HIV-1 negative samples were 2.13 for the Procleix System and 2.07 for the Procleix Tigris System (Table 52b).

Table 52a. Comparison of Procleix HIV-1 Discriminatory Assay Performance on the Procleix Tigris System and the Procleix System - Analysis of Accuracy*

Sample Type	Procleix System			Procleix Tigris System		
	Correct	Total	Accuracy (%) (95% CI)**	Correct	Total	Accuracy (%) (95% CI)**
All	520	525	99.0 (97.8 - 99.7)	1,593	1,607	99.1 (98.5 - 99.5)
HIV-1 Positive	293	298	98.3 (96.1 - 99.5)	887	901	98.4 (97.4 - 99.1)
HIV-1 Negative	227	227	100 (98.4 - 100)	706	706	100 (99.5 - 100)

CI = Confidence interval
* HIV-1 positive samples with viral loads ≥100 copies/mL or with unknown copy levels.
** The 95% CI are computed based on the assumption that all the outcomes are independent.

Table 52b. Comparison of the Procleix HIV-1 Discriminatory Assay Signal to Cutoff Ratio Values* for the Procleix Tigris System and the Procleix System**

Sample Type	n		Mean S/CO		SD		%CV	
	PS	TS	PS	TS	PS	TS	PS	TS
HIV-1 Positive	313	940	17.61	20.06	4.99	5.49	28.31	27.38
HIV-1 only	228	675	16.76	18.89	4.29	5.09	25.57	26.97
HIV-1 + HCV	28	87	20.45	24.28	7.27	6.39	35.56	26.33
HIV-1 + HBV	25	72	18.69	21.36	7.18	6.43	38.41	30.10
HIV-1 + HCV + HBV	32	106	20.31	23.17	2.92	2.70	14.36	11.63
HIV-1 Negative	227	706	2.13	2.07	0.32	0.12	15.20	5.64

n = Number of replicates
S/CO = Signal to cutoff ratio
SD = Standard deviation CV
= Coefficient of variation PS
= Procleix System
TS = Procleix Tigris System
* Analyte S/CO for HIV-1 positive panel members and Internal Control S/CO for negative panel members
** Samples with false negative and false positive results were excluded from this analysis.

PROCLEIX HCV DISCRIMINATORY ASSAY

Performance of the Procleix HCV Discriminatory Assay on the Procleix Tigris System was not significantly different from that on the Procleix System. The accuracy for all sample types was 98.7% (95% CI: 97.3%-99.5%) for the Procleix System and 99.1% (95% CI: 98.5%-99.5%) for the Procleix Tigris System. Accuracy rates were also similar between the two systems in the HCV positive samples and in the HCV negative samples (Table 53a). The mean analyte S/CO values for the HCV positive samples tested with the Procleix HCV Discriminatory Assay were 22.18 for the Procleix System and 22.81 for the Procleix Tigris System. The mean analyte S/CO values were also similar between the two systems for the various HCV positive sample subcategories. The mean IC S/CO values for the HCV negative samples were 1.98 for the Procleix System and 2.06 for the Procleix Tigris System (Table 53b).

Table 53a. Comparison of Procleix HCV Discriminatory Assay Performance on the Procleix Tigris System and the Procleix System - Analysis of Accuracy*

Sample Type	Procleix System			Procleix Tigris System		
	Correct	Total	Accuracy (%) (95% CI)**	Correct	Total	Accuracy (%) (95% CI)**
All	527	534	98.7 (97.3 - 99.5)	1,580	1,594	99.1 (98.5 - 99.5)
Positive	289	289	100 (98.7 - 100)	872	872	100 (99.6 - 100)
Negative	238	245	97.1 (94.2 - 98.8)	708	722	98.1 (96.8 - 98.9)

CI = Confidence interval
* HCV positive samples with viral loads ≥100 copies/mL or with unknown copy levels.
** The 95% CI are computed based on the assumption that all the outcomes are independent.

Table 53b. Comparison of the Procleix HCV Discriminatory Assay Signal to Cutoff Ratio Values* for the Procleix Tigris System and the Procleix System**

Sample Type	n		Mean S/CO		SD		%CV	
	PS	TS	PS	TS	PS	TS	PS	TS
HCV Positive	305	911	22.18	22.81	2.77	3.06	12.50	13.41
HCV only	207	618	21.48	22.11	2.32	3.24	10.80	14.67
HCV + HIV-1	33	99	24.15	24.62	1.53	1.57	6.33	6.38
HCV + HBV	29	90	23.41	24.55	4.02	1.71	17.19	6.96
HCV + HIV-1 + HBV	36	104	23.42	23.68	3.26	2.29	13.93	9.69
HCV Negative	241	717	1.98	2.06	0.15	0.13	7.56	6.19

n = Number of replicates
S/CO = Signal to cutoff ratio
SD = Standard deviation CV
= Coefficient of variation PS
= Procleix System
TS = Procleix Tigris System
* Analyte S/CO for HCV positive panel members and IC S/CO for negative panel members
** Samples with false negative and false positive results were excluded from this analysis.

PROCLEIX HBV DISCRIMINATORY ASSAY

Performance of the Procleix HBV Discriminatory Assay on the Procleix Tigris System was not significantly different from that on the Procleix System. The accuracy for all sample types was 98.6% (95% CI: 97.3%-99.4%) for the Procleix System and 98.8% (95% CI: 98.2%-99.3%) for the Procleix Tigris System. Accuracy rates were also similar between the two systems in the HBV positive samples and in the HBV negative samples (Table 54a). The mean analyte S/CO values for the HBV positive samples tested with the Procleix HBV Discriminatory Assay were 24.64 for the Procleix System and 23.95 for the Procleix Tigris System. The mean analyte S/CO values were also similar between the two systems for the various HBV positive sample subcategories. The mean IC S/CO values for the HBV negative samples were 1.94 for the Procleix System and 2.06 for the Procleix Tigris System (Table 54b).

Table 54a. Comparison of Procleix HBV Discriminatory Assay Performance on the Procleix Tigris System and the Procleix System - Analysis of Accuracy*

Sample Type	Procleix System			Procleix Tigris System		
	Correct	Total	Accuracy (%) (95% CI)**	Correct	Total	Accuracy (%) (95% CI)**
All	**570**	**578**	**98.6 (97.3 - 99.4)**	**1,711**	1,731	98.8 (98.2 - 99.3)
Positive	335	335	100 (98.9 - 100)	997	1,002	99.5 (98.8 - 99.8)
Negative	235	243	96.7 (93.6 - 98.6)	714	729	97.9 (96.6 - 98.8)

CI = Confidence interval
* HBV positive samples with viral loads ≥15 IU/mL or with unknown copy levels.
** The 95% CI are computed based on the assumption that all the outcomes are independent.

Table 54b. Comparison of the Procleix HBV Discriminatory Assay Signal to Cutoff Ratio Values* for the Procleix Tigris System and the Procleix System**

Sample Type	n		Mean S/CO		SD		%CV	
	PS	TS	PS	TS	PS	TS	PS	TS
HBV Positive	344	1,017	24.64	23.95	3.03	2.15	12.28	8.97
HBV only	249	742	24.69	23.92	2.73	2.04	11.05	8.55
HBV + HIV-1	30	90	25.03	24.60	1.12	1.54	4.49	6.24
HBV + HCV	30	87	24.68	23.85	2.64	2.23	10.70	9.34
HBV + HIV-1 + HCV	35	98	23.98	23.68	5.53	3.06	23.04	12.92
HBV Negative	237	723	1.94	2.06	0.21	0.12	10.86	5.90

n = Number of replicates
S/CO = Signal to cutoff ratio
SD = Standard deviation CV
= Coefficient of variation PS
= Procleix System
TS = Procleix Tigris System
* Analyte S/CO for HBV positive panel members and IC S/CO for negative panel members
** Samples with false negative and false positive results were excluded from this analysis.

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