Procleix® Ultrio Plus® Assay

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

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INTENDED USE

The Procleix Ultrio Plus Assay is a qualitative *in vitro* nucleic acid amplification test for use on the Procleix TIGRIS System to screen for human immunodeficiency virus type 1 (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 Plus Assay can be considered a supplemental test that confirms HIV-1 infection for specimens that are repeatedly reactive on a licensed donor screening test for antibodies to HIV-1, and reactive on both the Procleix Ultrio Plus Assay and on the Procleix Ultrio Plus HIV-1 Discriminatory Assay.

The Procleix Ultrio Plus Assay can be considered a supplemental test that confirms HCV infection for specimens that are repeatedly reactive on a licensed donor screening test for antibodies to HCV, and reactive on both the Procleix Ultrio Plus Assay and on the Procleix Ultrio Plus HCV Discriminatory Assay.

The Procleix Ultrio Plus Assay can be considered a supplemental test that confirms HBV infection for specimens that are repeatedly reactive on a licensed donor screening test for HBsAg, and reactive on both the Procleix Ultrio Plus Assay and on the Procleix Ultrio Plus 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 blood-borne 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[30, 31, 33, 34] and/or serologic screening for anti-viral antibodies by enzyme immunoassay (EIA) with confirmation by supplemental antibody tests such as Western blot or immunofluorescence assays. In addition, depending on the NAT assay of use, p24 Ag 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.[20–22, 32]

Current detection of HCV infection in the blood bank setting is based on NAT for HCV RNA detection[30, 31, 33, 34, 41] and/or serologic screening for anti-viral antibodies with enzyme-linked immunoabsorbent assays (ELISA) or enzyme immunoassays (EIA) and confirmation with a Strip Immunoblot Assay (e.g., CHIRON RIBA HCV 3.0 SIA). 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.[20–22, 32]

Current detection of HBV infection in the blood bank setting is based on NAT for HBV DNA detection[38] and/or serological screening for antibodies to HBc and for HBsAg by enzyme immunoassay (EIA) with confirmation by confirmatory neutralization tests. Data from post-transfusion cases indicate that HBsAg is first detected 50 to 60 days following transfusion.[15] Studies indicate that nucleic acid-based amplification assays for HBV DNA will allow detection of HBV infection several weeks before HBsAg detection.[16-19] The introduction of NAT for HIV-1, HCV, and HBV has improved blood safety.[37, 39] However, the advent of HBV NAT has raised new issues. HBV replicates more slowly during the pre-seroconversion window period than HIV-1 and HCV, and low levels of HBV DNA can be found in serologically negative samples during early stages of infection and in HBc antibody-positive/HBsAg-negative samples during later stages of infection. As a result, some low-copy HBV positive donations may go undetected by current serological and NAT methods. To address this risk, the Procleix Ultrio Plus Assay with enhanced sensitivity for HBV was developed.[40]

The Procleix Ultrio Plus Assay utilizes target amplification nucleic acid probe technology for the detection of HIV-1 RNA, HCV RNA, and HBV DNA.[23, 30, 38, 40] 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 Plus 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)[24]; and detection of the Amplification products (amplicon) by the Hybridization Protection Assay (HPA).[25, 36]

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. In the Procleix Ultrio Plus Assay, Target Enhancer Reagent is added to each reaction tube after the addition of the sample to enhance the disruption of the HBV viral particles. Following the addition of Target Enhancer Reagent, the hybridized target is captured onto magnetic microparticles which are then 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 Plus 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, or assay calibrator tube via the working Target Capture Reagent (wTCR) 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.[26] Internal Control-specific amplicon is detected using a probe with rapid emission of light (termed a "flasher signal"). Amplicon specific to HIV-1/HCV/HBV is detected using probes with relatively slower kinetics of light emission (termed a "glower signal"). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels.[26] When used for the simultaneous detection of HIV-1, HCV, and HBV, the Procleix Ultrio Plus 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 Plus Assay may be run in individual HIV-1, HCV, and HBV Discriminatory Assays to determine if they are reactive for HIV-1, HCV, HBV or any combination of the three.

DISCRIMINATORY TESTING

The Procleix Ultrio Plus HIV-1, HCV, and HBV Discriminatory Assays utilize the same three main steps as the Procleix Ultrio Plus 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 Plus Assay Probe Reagent.

REAGENTS

Procleix Ultrio Plus Assay Kit:

Each kit contains:	Number of vials/Volume per vial	
Reagent Name	**1000 Test Kit**	**5000 Test Kit**
Internal Control Reagent *A HEPES buffered solution containing detergent and an RNA transcript.* *Store **unopened reagent** at –15° to –35°C.*	**2 x 5 mL**	**10 x 5 mL**
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)*	**2 x 280 mL**	**10 x 280 mL**
Amplification Reagent *Primers, dNTPs, NTPs, and co-factors in TRIS buffered solution containing PROCLIN 300 as preservative.* *Store **unopened reagent** at –15° to –35°C.*	**2 x 50 mL**	**10 x 50 mL**
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 –15° to –35°C.*	**2 x 18 mL**	**10 x 18 mL**
Probe Reagent *Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.* *Store **unopened reagent** at –15° to –35°C.*	**2 x 75 mL**	**10 x 75 mL**
Selection Reagent *Borate buffered solution containing surfactant.* *Store at 15° to 30°C.*	**2 x 180 mL**	**10 x 180 mL**
Target Enhancer Reagent *A concentrated solution of lithium hydroxide.* *Store **unopened reagent** at 15° to 30°C.*	**2 x 75 mL**	**10 x 75 mL**
Negative Calibrator *Defibrinated normal human plasma (nonreactive for HIV-1/2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.* *Store at –15° to –35°C.*	**30 x 2 mL**	**90 x 2 mL**
HIV-1 Positive Calibrator *Inactivated HIV-1 positive plasma in defibrinated normal human plasma (nonreactive for HIV-2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.* *Store at –15° to –35°C.*	**30 x 2 mL**	**90 x 2 mL**
HCV Positive Calibrator *Inactivated HCV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.* *Store at –15° to –35°C.*	**30 x 2 mL**	**90 x 2 mL**
HBV Positive Calibrator *Inactivated HBV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HCV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.* *Store at –15° to –35°C.*	**30 x 2 mL**	**90 x 2 mL**

STORAGE INSTRUCTIONS

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

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

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 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 Discriminatory Probe, HCV Discriminatory Probe, or HBV Discriminatory Probe Reagents after the initial thaw.

G. Negative, HIV-1, HCV, and HBV Positive Calibrators are single use vials and must be discarded after use.

H. If precipitate forms in the Wash Solution, Amplification Reagent, Selection Reagent, Target Enhancer Reagent, Probe Reagent, or HIV-1, HCV, or HBV Discriminatory Probe Reagents, see instructions under 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
Target Enhancer Reagent	RT until the expiration date	30 days at RT
Calibrators	-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

SPECIMEN COLLECTION, STORAGE, AND HANDLING

Warning: **Handle all specimens as if they are capable of transmitting infectious agents.**

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, 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, plasma, or serum may be stored for a total of 13 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 at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

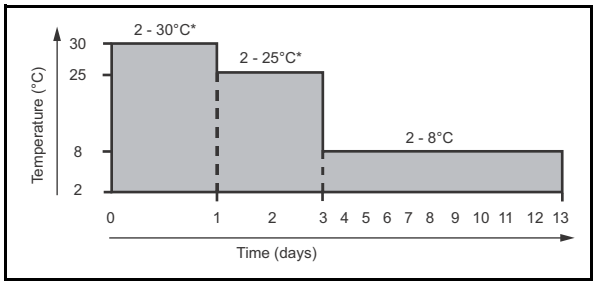
Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example storage temperature chart below.

In addition, plasma separated from the cells may be stored for up to 15 months at ≤ -20°C before testing.

Do not freeze whole blood.

Note: The Greiner K_2EDTA Sep VACUETTE Blood Collection Tube does not affect assay sensitivity and specificity, but specimen stability in this tube has not been validated.



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

C. Additional specimens taken from blood or plasma units collected in ACD or sodium citrate according to the collection container manufacturer's instructions may be used. ACD or sodium citrate whole blood or plasma may be stored as in 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 13 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 above.

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 at 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 15 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.

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.

CADAVERIC BLOOD SPECIMENS

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

Note: Serum or plasma specimens collected pre-mortem from a cadaveric organ/tissue donor must be collected, handled, and tested using instructions for cadaveric donors.

B. Specimens should be collected within 24 hours of death if the cadaver was refrigerated (1° to 10°C) within 12 hours of death. Specimens should be collected within 15 hours of death if the cadaver was not refrigerated (1° to 10°C). 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.

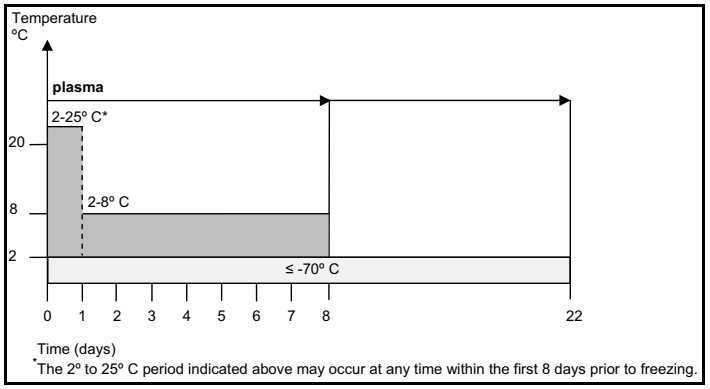
For storage above 8°C, specimens may be stored for 24 hours at up to 25°C during the 72 hours.

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 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.

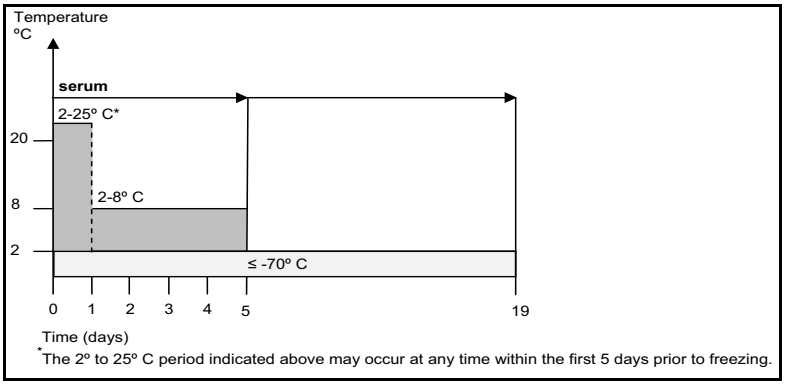
For storage above 8°C, specimens may be stored for 24 hours at up to 25°C during the 72 hours.

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 may be stored for up to 14 days at ≤ -70°C before testing.

Do not freeze whole blood.



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

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

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

H. Other cadaveric blood specimen collection, handling, and storage conditions must be validated by the user. 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., 220 µL sample plus 880 µL saline. Diluted specimens should be inverted several times to mix and then may be used in standard assay procedure.

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.

MATERIALS PROVIDED

Procleix Ultrio Plus Assay — 1000 Test Kit P/N 302573; 5000 Test Kit P/N 302574

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

MATERIALS REQUIRED BUT PROVIDED SEPARATELY

Procleix Ultrio Plus HIV-1, HCV, and HBV Discriminatory Probe Reagents

HIV-1 Discriminatory Probe Reagent	P/N 302571
HCV Discriminatory Probe Reagent	P/N 302577
HBV Discriminatory Probe Reagent	P/N 302576

Procleix Assay Fluids — P/N 301116

- Wash Solution
- Oil
- Buffer for Deactivation Fluid

Procleix Auto Detect Reagents — P/N 301120

- Auto Detect 1
- Auto Detect 2

Procleix System Fluid Preservative — P/N 301175

Procleix Ultrio Plus TIGRIS Controls — P/N 302572

- Procleix Ultrio Plus TIGRIS Negative Control
- Procleix Ultrio Plus TIGRIS HIV-1 Control
- Procleix Ultrio Plus TIGRIS HCV Control
- Procleix Ultrio Plus TIGRIS HBV Control

Disposables

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

Multi-Tube Units (MTUs) – case of 100	P/N 104772
Waste Bag Kit (MTU and Tiplet) – 30 of each	P/N 900907
MTU Waste Cover	P/N 105523
MTU Waste Deflector	P/N 900931
Reagent Spare Caps (TCR, Target Enhancer, Selection, and Probe Reagents)	P/N CL0039
Reagent Spare Caps (Amplification Reagent)	P/N CL0042
Reagent Spare Caps (Enzyme, Discriminatory Probe Reagents)	P/N 501619
Procleix TIGRIS System Maintenance Bottle Kit	P/N 105655

Equipment

Procleix TIGRIS System, Procleix TIGRIS System Software, Procleix Ultrio Plus Assay Software, and operator's manual

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

Other

Procleix TIGRIS System Quick Reference Guide (Procleix TIGRIS System QRG)

OTHER MATERIALS AVAILABLE FROM NOVARTIS DIAGNOSTICS FOR USE WITH PROCLEIX ULTRIO PLUS ASSAY

Procleix Ultrio Plus Assay Calibrators — P/N 302575

- Procleix Ultrio Plus Negative Calibrator
- Procleix Ultrio Plus HIV-1 Positive Calibrator
- Procleix Ultrio Plus HCV Positive Calibrator
- Procleix Ultrio Plus HBV Positive Calibrator

Procleix Ultrio Plus Negative Calibrator — P/N 303260

Procleix Ultrio Plus TIGRIS Negative Control — P/N 303261

Procleix Oil — P/N 302441

General Equipment/Software

Tecan Genesis RSP instrument (for pooling only), Procleix CPT Pooling Software, and operator's manual

Disposable 1000 µL conductive filter tips (DiTis) in rack approved for use with equipment (for pooling only)

For instrument specifics and ordering information, contact Novartis Diagnostics Customer Service.

MATERIALS REQUIRED BUT NOT PROVIDED

Bleach

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

Bleach alternative (optional)

Contact Novartis Diagnostics 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 in rack approved for use with the Procleix TIGRIS System. Contact Novartis Diagnostics Technical Service for approved tips.

PRECAUTIONS

A. For *in vitro* diagnostic use.

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

C. 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.

D. Specimens may be infectious. Use Universal Precautions[27, 29] when performing the assay. Proper handling and disposal methods should be established according to local, state and federal regulations.[28] Only personnel adequately qualified as proficient in the use of the Procleix Ultrio Plus 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 Plus 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 Plus 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 Plus 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 Plus TIGRIS Negative Control have been assayed by FDA-licensed tests and found non-reactive 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.[27, 29] 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 alternatives 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.[27, 28] 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. Avoid microbial and ribonuclease contamination of reagents.

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

N. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Procleix TIGRIS System verifies reagent levels.

O. Material Safety Data Sheets are available upon request.

The following reagents contain 0.2% sodium azide as a preservative. They may also pose a potential biological risk.
Negative Calibrator
HIV-1 Positive Calibrator
HCV Positive Calibrator
HBV Positive Calibrator
Procleix Ultrio Plus TIGRIS Negative Control
Procleix Ultrio Plus TIGRIS HIV-1 Control
Procleix Ultrio Plus TIGRIS HCV Control
Procleix Ultrio Plus TIGRIS HBV Control

Xn. Harmful 

Biological Risk 

R22/R32/S2/
S13/S36/S46

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

The Target Enhancer Reagent is corrosive:

C. Corrosive 

R35 S1/2 S26
S37/39 S45

R35 Causes severe burns
S1/2 Keep in a locked place and out of reach of children
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S37/39 Wear suitable gloves and eye/face protection
S45 In case of accident or sickness, seek medical advice immediately and show this container or label

P. 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.

Q. Refer to precautions in the appropriate Procleix Assay package inserts and the Procleix TIGRIS System QRG.

R. Do not use the RPI to prepare Target Enhancer Reagent.

REAGENT PREPARATION

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

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

C. Verify that the reagents have not exceeded their 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, Target Enhancer 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 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 kits in the system's database.

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

1. The Selection Reagent must be at room temperature before use.
2. 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.
3. If cloudiness or precipitate is present, use the RPI as described in the *Procleix Reagent Preparation Incubator Operator's Manual.* Do not use if precipitate or cloudiness persists.
4. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
5. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

E. Remove a bottle of Target Enhancer Reagent from room temperature storage.

1. The Target Enhancer Reagent must be at room temperature before use.
2. Record the date that it was first opened (OPEN DATE) on the space provided on the label.
3. Do not use the RPI to prepare Target Enhancer Reagent.

F. 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, probe reagents can be incubated with RPI File 3 (room temperature) to facilitate complete dissolution of precipitate, as long as the total time at room temperature does not exceed 80 hours.

G. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present (refer to instructions in step I.4 below).

H. Refer to the Procleix TIGRIS System QRG for RPI temperature parameters.

I. 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 Procleix TIGRIS System QRG for instructions.
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 inversion 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°C to 30°C in a water bath. Periodically remove Internal Control Reagent from water bath to gently invert until gel is dissolved.
5. After unloading TCR from the RPI and warming the IC 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 (IC LOT).
7. Retain the IC vial to scan the barcode label into the system.

J. Thaw calibrators at room temperature.

1. These are single use vials, which must be thawed prior to each run.
2. Mix calibrators gently by inversion to avoid foaming.
3. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

K. Follow instructions provided in the Procleix Ultrio Plus TIGRIS Controls package insert for preparation of Procleix Ultrio Plus TIGRIS Controls.

1. Avoid reagent foaming.
2. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

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

M. Wash Solution and Target Enhancer Reagent are shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution and Target Enhancer Reagent during shipment or during storage when temperatures fall between 2° and 15ºC. Wash Solution and Target Enhancer Reagent may be warmed to facilitate dissolution of precipitate. Do not use the RPI to warm the Wash Solution or Target Enhancer Reagent. Temperature should not exceed 30ºC. Ensure that precipitates in the Wash Solution and Target Enhancer Reagent are dissolved prior to use. Do not use if precipitate or cloudiness is present.

N. 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.

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

1. Fill the Deactivation Fluid bottle with 5% 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.

P. 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. Fill the System Fluid Bottle to the liquid-fill line with water for the Procleix TIGRIS System.
2. Pour the entire contents of one bottle of Procleix System Fluid Preservative into the System Fluid Bottle.
3. Mix System Fluid Bottle contents completely.
4. 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.
5. Record the date the System Fluid was prepared on the System Fluid Preservative label.

Q. 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 Plus 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. EQUIPMENT PREPARATION

See the Procleix TIGRIS System QRG.

C. 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 Plus Assay, each 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.

D. RUN CONFIGURATION

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

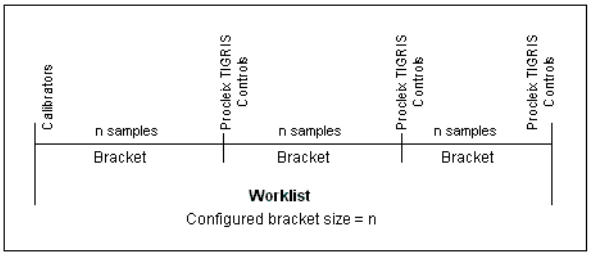
   a. For the Procleix Ultrio Plus Assay, a set of calibrators consists of one vial each of Negative Calibrator, HIV-1 Positive Calibrator, HCV Positive Calibrator, and HBV Positive Calibrator. The Negative Calibrator is run in triplicate, and each Positive Calibrator is run in duplicate.

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

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

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

2. Using additional sets of Procleix Ultrio Plus 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 Plus TIGRIS Controls at each end. The results of each bracket are reported based on the validity criteria of each control (see 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 Plus 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 Plus 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 Plus 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. Perform reagent preparation in a clean (amplicon- and template-free) area.
2. The sample loading area must be amplicon-free.

F. ENVIRONMENTAL CONDITIONS

The operational conditions of the room in which the Procleix TIGRIS System (including the RPI) runs must be within a temperature of 15° to 25°C and humidity of 20 to 85%.

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/ reagent preparation incubator 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, 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 Plus Assay Calibrators and Discriminatory Probe Reagents are to be used with the corresponding master lot of Procleix Ultrio Plus and Discriminatory Assays. The operator must check to ensure that the Procleix Ultrio Plus Assay Calibrators and Discriminatory Probe Reagents are used with the corresponding master lot of kit reagents as indicated on the Procleix Ultrio Plus Assay master lot sheet in use.

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 PLUS ASSAY AND PROCLEIX ULTRIO PLUS 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 Plus Assay run**, at least seven of the nine calibrator replicates must be valid. At least two of the three Negative Calibrator replicates and five of the six positive calibrator replicates must be valid.
2. **In a Procleix Ultrio Plus 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 Plus 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 Ultrio Plus 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 Plus TIGRIS Control sets at the beginning and end of the bracket (excluding the first bracket which has calibrators at the beginning and Procleix Ultrio Plus TIGRIS Controls at the end). A valid control set requires that all Procleix Ultrio Plus 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 Plus TIGRIS Control sets are described in item E below.
   a. **In the Procleix Ultrio Plus Assay**, a set of Procleix Ultrio Plus TIGRIS Controls consists of one vial each of Procleix Ultrio Plus TIGRIS Negative Control, Procleix Ultrio Plus TIGRIS HIV-1 Control, Procleix Ultrio Plus TIGRIS HCV Control, and Procleix Ultrio Plus TIGRIS HBV Control. Each Procleix Ultrio Plus TIGRIS Control is run in singlet.
   b. **In the Procleix Ultrio Plus HIV-1, HCV, and HBV Discriminatory Assays**, a set of Procleix Ultrio Plus TIGRIS Controls consists of one vial each of the Procleix Ultrio Plus TIGRIS Negative Control and the corresponding positive control. Each Procleix Ultrio Plus 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 Plus 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 Ultrio Plus 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 Plus TIGRIS Control sets:

1. Suspect specimens that result from invalid Procleix Ultrio Plus TIGRIS Control sets are flagged with error code ”x” on the Assay Results Run Report. Procleix Ultrio Plus 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 UPPER CASE letters)
   b. Results processing errors (error codes in lower case letters)
2. If Procleix Ultrio Plus 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 or subsequent set(s) of Procleix Ultrio Plus TIGRIS Controls is valid. If no valid Procleix Ultrio Plus TIGRIS Control results are available in the subsequent bracket(s), all Suspect specimens should be considered invalid and be retested.
3. If Procleix Ultrio Plus 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 Plus 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 Plus 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 Plus 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.
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

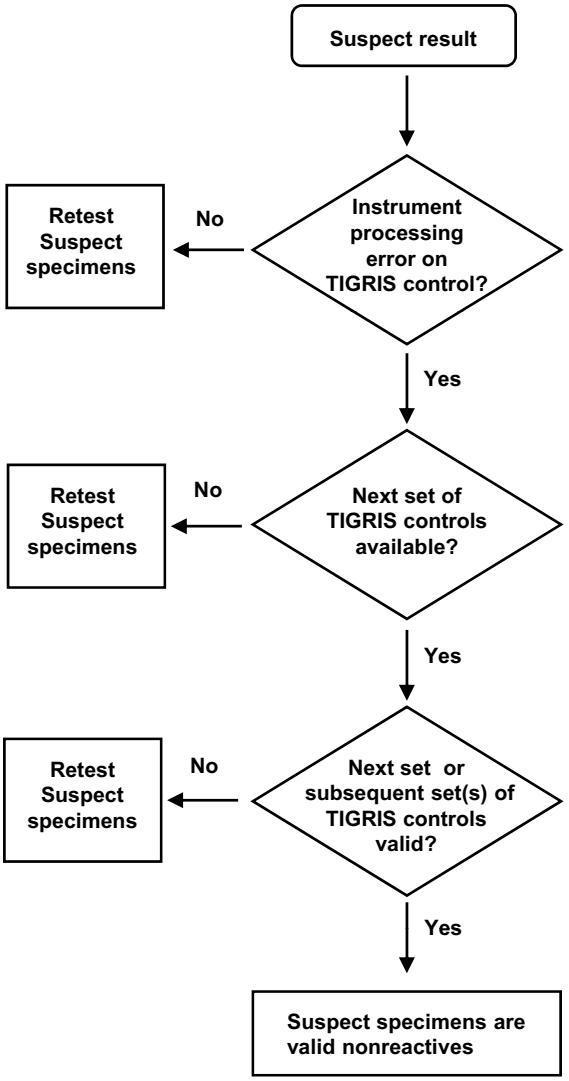
NA = Not applicable.

G. Summary of Specimen Result Interpretation for Procleix Ultrio Plus 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 Plus 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 Plus Assay, reactive pools or individual specimens should be resolved according to the resolution algorithm, as explained in the 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 Plus Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate. Each individual Negative Calibrator replicate must have an Internal Control (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 IC value or an Analyte value outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

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

Example:

Negative Calibrator	Internal Control 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 (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 Plus 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. 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. IC values may not exceed 475,000 RLU.

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

Example:

HIV-1 Positive Calibrator		Analyte 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 Plus 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. 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. IC values may not exceed 475,000 RLU.

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 Plus 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. 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. IC values may not exceed 475,000 RLU.

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 Plus 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 Plus 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 IC Mean RLU)

B. Procleix Ultrio Plus HIV-1 Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator must be run in triplicate. Each individual Negative Calibrator (NC) must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator 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 IC value or Analyte value that is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

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

Example:

Negative Calibrator		Internal Control 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 Ultrio Plus 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. 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. IC values may not exceed 475,000 RLU.

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

Example:

HIV-1 Positive Calibrator		Analyte 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

These calibrators are not run in the HIV-1 Discriminatory Assay on the Procleix TIGRIS System.

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

The HCV and HBV Positive Calibrators are not used in the HIV-1 Discriminatory Assay for the Procleix TIGRIS System. Only the three replicates of the Negative Calibrator and the three replicates of the HIV-1 Positive Calibrator are used. This means that testing is not required for all of the Procleix Ultrio Plus positive calibrators for discriminatory assays, with the exception of the actual discriminatory assay positive calibrator. This increases system output by eliminating tests not required.

Summary of Acceptance Criteria for the Procleix Ultrio Plus 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		

Summary of Cutoff Calculations for the Procleix Ultrio Plus HIV-1 Discriminatory Assay

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

C. Procleix Ultrio Plus HCV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator must be run in triplicate. Each individual Negative Calibrator must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator 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 IC or Analyte value is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator (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 Ultrio Plus 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. 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. IC values may not exceed 475,000 RLU.

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

These calibrators are not run on the HCV Discriminatory Assay on the Procleix TIGRIS System.

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

The HIV-1 and HBV Positive Calibrators are not used in the HCV Discriminatory Assay for the Procleix TIGRIS System. Only the three replicates of the Negative Calibrator and the three replicates of the HCV Positive Calibrator are used. This means that testing is not required for all of the Procleix Ultrio Plus positive calibrators for discriminatory assays, with the exception of the actual discriminatory assay positive calibrator. This increases system output by eliminating tests not required.

Summary of Acceptance Criteria for the Procleix Ultrio Plus HCV Discriminatory Assay

Acceptance Criteria:			
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 Ultrio Plus HCV Discriminatory Assay

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

D. Procleix Ultrio Plus HBV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator must be run in triplicate. Each individual Negative Calibrator (NC) must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator 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 IC value or Analyte value that is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.
Determination of the mean of the Negative Calibrator (NC_x) values for Internal Control [NC_x (Internal Control)].

Example:

Negative Calibrator		Internal Control 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. 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. IC values may not exceed 475,000 RLU.

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

These calibrators are not run on the HBV Discriminatory Assay on the Procleix TIGRIS System.

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

The HCV and HIV-1 Positive Calibrators are not used in the HBV Discriminatory Assay for the Procleix TIGRIS System. Only the three replicates of the Negative Calibrator and the three replicates of the HBV Positive Calibrator are used. This means that testing is not required for all of the Procleix Ultrio Plus positive calibrators for discriminatory assays, with the exception of the actual discriminatory assay positive calibrator. This increases system output by eliminating tests not required.

Summary of Acceptance Criteria for the Procleix Ultrio Plus 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

Summary of Cutoff Calculations for the Procleix Ultrio Plus 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 IC Mean RLU)

Acceptance Criteria for Procleix Ultrio Plus TIGRIS Controls

The Procleix TIGRIS System requires Procleix Ultrio Plus 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., Non-reactive for Negative controls and Reactive for positive controls) and be valid for the bracket to be valid.

Acceptance Criteria for Procleix Ultrio Plus TIGRIS Controls in the Procleix Ultrio Plus Assay

In the Procleix Ultrio Plus Assay, a valid Procleix Ultrio Plus TIGRIS Negative Control, Procleix Ultrio Plus TIGRIS HIV-1 Control, Procleix Ultrio Plus TIGRIS HCV Control, and Procleix Ultrio Plus 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 (non-reactive) to be accepted. All other controls (HIV-1, HCV and HBV) must have S/CO greater than or equal to 1.00 (reactive) to be accepted.

Acceptance Criteria:	
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
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 Plus TIGRIS Controls in the HIV-1 Discriminatory Assay

In the HIV-1 Discriminatory Assay, a valid Procleix Ultrio Plus TIGRIS Negative Control and Procleix Ultrio Plus 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 (non-reactive) to be accepted. The HIV-1 Control must have S/CO greater than or equal to 1.00 (reactive) to be accepted.

Acceptance Criteria:	
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 Plus TIGRIS Controls in the HCV Discriminatory Assay

In the HCV Discriminatory Assay, a valid Procleix Ultrio Plus TIGRIS Negative Control and Procleix Ultrio Plus TIGRIS HCV 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 (non-reactive) to be accepted. The HCV Control must have S/CO greater than or equal to 1.00 (reactive) to be accepted.

Acceptance Criteria:	
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 Plus TIGRIS Controls in the HBV Discriminatory Assay

In the HBV Discriminatory Assay, a valid Procleix Ultrio Plus TIGRIS Negative Control and Procleix Ultrio Plus TIGRIS HBV Control are required at the beginning and end of each 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 (non-reactive) to be accepted. The HBV Control must have S/CO greater than or equal to 1.00 (reactive) to be accepted.

Acceptance Criteria:	
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 Plus Assay, or less than or equal to 475,000 RLU in the Procleix Ultrio Plus 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 Plus Assay, or less than or equal to 475,000 RLU in the Procleix Ultrio Plus 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 Plus Assay, or greater than 475,000 RLU in the Procleix Ultrio Plus HIV-1, HCV, or HBV Discriminatory Assays.

High titers of non-target analytes may produce invalid results in each of the individual Procleix Ultrio Plus 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 Plus Assay	Criteria for the Procleix Ultrio Plus 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 Plus 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 Ultrio Plus 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 Plus Assay, Procleix Ultrio Plus HIV-1 Discriminatory Assay, Procleix Ultrio Plus HCV Discriminatory Assay, or Procleix Ultrio Plus 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 Plus Assay, Procleix Ultrio Plus HIV-1 Discriminatory Assay, Procleix Ultrio Plus HCV Discriminatory Assay, or Procleix Ultrio Plus 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 Plus 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 Plus 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 Ultrio Plus 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.**
5. IF THE REACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool must be tested with the Procleix Ultrio Plus Assay.

a. If an individual specimen tests nonreactive with the Procleix Ultrio Plus 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 Plus 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.

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 Plus Assay if sufficient sample is available.

(1) If the individual specimen tests nonreactive in the repeated Procleix Ultrio Plus 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 Plus 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.

8. 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.

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 Plus Assay and reactive on the Procleix Ultrio Plus HIV-1 Discriminatory Assay, the Procleix Ultrio Plus 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 Plus Assay and reactive on the Procleix Ultrio Plus HCV Discriminatory Assay, the Procleix Ultrio Plus Assay acts as a supplemental test that confirms HCV infection; it is not necessary to run an HCV RIBA.

12. For specimens that are repeat reactive on a licensed HBsAg screening test and reactive on the Procleix Ultrio Plus Assay and reactive on the Procleix Ultrio Plus HBV Discriminatory Assay, the Procleix Ultrio Plus 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 Plus Assay or are Reactive in the Procleix Ultrio Plus 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 Plus Assay or are Reactive in the Procleix Ultrio Plus 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 (such as RIBA).

Specimens that are Nonreactive in the Procleix Ultrio Plus Assay or are Reactive in the Procleix Ultrio Plus 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 Ultrio Plus 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.[42, 43]

LIMITATIONS OF THE PROCEDURE

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

The Procleix Ultrio Plus 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 sensitivity for the Procleix Ultrio Plus 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 5 IU/mL. Samples with less than these concentrations may not yield reproducible results.

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

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

PERFORMANCE CHARACTERISTICS

SPECIFICITY

Specificity of the Procleix Ultrio Plus and Discriminatory Assays in Individual Normal Blood Donations

Fresh and frozen normal blood donor plasma specimens which had previously tested negative for HIV-1, HCV, and HBV nucleic acids using licensed commercial assays were tested in the Procleix Ultrio Plus Assay and the three Procleix Ultrio Plus Discriminatory Assays (dHIV-1, dHCV and dHBV) on the Procleix TIGRIS System. All testing was performed in-house. Initially reactive specimens were retested in the Procleix Ultrio Plus Assay and/or the relevant Procleix Ultrio Plus Discriminatory Assays, and were categorized as defined in Table 1. All 3 specimens that were initially reactive were non-reactive upon retest, indicating that the initially reactive test was a false positive result. The reactivity and specificity rates for each of the 4 assays are shown in Table 1.

Tests that were invalid due to instrument hardware errors were not retested, and are excluded from the data analysis. There were no invalid results due to assay chemistry errors, for an initial invalid rate of 0.00% for each of the 4 assays.

Table 1. Specificity of Procleix Ultrio Plus and Procleix Ultrio Plus Discriminatory Assays in Fresh and Frozen Normal Blood Donor Plasma Specimens*

	Ultrio Plus	dHIV-1 Assay	dHCV Assay	dHBV Assay
Valid Results (N)	3043	578	717	714
Initially Reactive (N)	1	0	0	2
Initially Reactive Rate (%)	0.03	0.00	0.00	0.28
True Positive After Repeat Testing** (N)	0	NA	NA	0
False Positive After Repeat Testing*** (N)	1	NA	NA	2
Unresolved After Repeat Testing**** (N)	0	NA	NA	0
False Positive Rate After Repeat Testing (%)	0.03	0.00	0.00	0.28
Specificity After Repeat Testing (%)	99.97	100.00	100.00	99.72
Combined Mean Analyte S/CO of Negative Specimens	0.07 ± 0.04	0.10 ± 0.05	0.04 ± 0.04	0.05 ± 0.04

N = Number of specimens; NA = Not Applicable; S/CO = Signal to Cutoff ratio
*Two different reagent lots were used during testing.
**Specimens determined to be True Positives were repeat reactive in either the Ultrio Plus Assay or the relevant Ultrio Plus Discriminatory Assay.
***Specimens determined to be False Positives were non-reactive upon retesting in either the Ultrio Plus Assay or the relevant Ultrio Plus Discriminatory Assay.
****Specimens determined to be Unresolved were inconsistently reactive in the Ultrio Plus Assay, but were reactive in one of the Ultrio Plus Discriminatory Assays.

The results from testing yielded an overall specificity for the Procleix Ultrio Plus Assay of 99.97% (N = 3043) in the study. For the Procleix Ultrio Plus Discriminatory Assays, the overall specificity for the Procleix Ultrio Plus HIV-1 Discriminatory Assay was 100.00% (N = 578), the overall specificity for the Procleix Ultrio Plus HCV Discriminatory Assay was 100.00% (N = 717) in the study, and the overall specificity for the Procleix Ultrio Plus HBV Discriminatory Assay was 99.72% (N = 714) in the study.

Specificity in Pooled Voluntary Blood and Paid Source Plasma Donations

A prospective, multisite clinical trial was conducted to establish the specificity of the Procleix Ultrio Plus Assay in 16-sample pools made from plasma from either voluntary blood donations or paid source plasma (SP) donations. The study was conducted using plasma samples from approximately 22 blood and SP collection sites in the Midwestern and Southern United States. Samples were tested at two blood center testing laboratories and one laboratory that tested source plasma.

Individual donations were combined into 16-sample pools. Pools were tested with the Procleix Ultrio Plus Assay and the licensed Procleix Ultrio Assay in accordance with package insert instructions. Alternate licensed or validated nucleic acid test (Alternate NAT) results were used to resolve discordant results when the Procleix Ultrio Plus Assay was reactive and the licensed Procleix Ultrio Assay was nonreactive. Follow-up testing was not performed.

Specificity of the Procleix Ultrio Plus Assay was calculated from 2,104 16-sample plasma pools from voluntary blood donations (Table 2a) and 1,025 16-sample plasma pools from SP donations (Table 3). Specificity of the Procleix Ultrio Plus Assay was determined by comparing the result from the pooled sample to the results from the licensed Procleix Ultrio Assay and associated discriminatory assays and, if appropriate, alternate NAT results. Licensed Procleix Ultrio Assay results were interpreted in accordance with the package insert instructions. A pool was classified as nonreactive if the pool was nonreactive with the licensed assay or if the pool was reactive but all 16 individual samples were nonreactive with the licensed assay. A pool was classified as reactive if the pool was reactive and contained at least one sample that was reactive, discriminated with the licensed assay.

Rates of Procleix Ultrio Plus Assay reactivity are presented in Table 2a for the 2,104 pools from voluntary blood donations that were included in the clinical specificity analyses. Of the 2,104 pools, 2,090 (99.3%) had nonreactive Procleix Ultrio Plus Assay results; all 2,090 pools had true negative results. There were 14 pools with reactive Procleix Ultrio Plus Assay results. Of these, 12 pools (12/2,104; 0.6%) had true positive results. Two pools (2/2,104; 0.1%) had false positive results. Rates of the Ultrio Plus Discriminatory Assays are presented in Table 2b.

Table 2a. Clinical Specificity Study: Procleix Ultrio Plus Assay Reactivity in 16-Sample Pools From Voluntary Blood Donations

Results	N	(95% CI)[1]
Total pools tested	2,104	100%
Nonreactive pools	2,090	99.3% (98.9 - 99.6%)
Initially reactive pools	14	0.7% (0.4 - 1.1%)
Pool, individual constituent(s), and discriminatory assay reactive; Procleix Ultrio Assay reactive; True Positive	12	0.6% (0.3 - 1.0%)
Pool reactive, individual constituent(s) nonreactive; Procleix Ultrio Assay nonreactive; Alternate NAT not performed; False Positive	2	0.1% (0.0 - 0.3%)

[1]SCORE confidence interval
N = number of pools

Table 2b. Clinical Specificity Study: Procleix Ultrio Plus Discriminatory Assays[1]

Assay	N	Specificity (%)	(95% CI)[2]
Procleix Ultrio Plus dHIV-1 Assay	578	100	99.5-100
Procleix Ultrio Plus dHCV Assay	717	100	99.6-100
Procleix Ultrio Plus dHBV Assay	714	99.72	99.0-99.9

[1] Testing done in-house
N = number of specimens
[2] SCORE confidence interval

Rates of Procleix Ultrio Plus Assay reactivity are presented in Table 3 for the 1,025 pools from SP donations that were included in the clinical specificity analyses. Of the 1,025 pools, 1,013 (98.8%) had nonreactive Procleix Ultrio Plus Assay results; all 1,013 pools had true negative results. There were 12 pools with reactive Procleix Ultrio Plus Assay results; all 12 pools (12/1,025; 1.2%) had true positive results. One of the pools with true positive results was nonreactive in the licensed Procleix Ultrio Assay. The pool was considered true positive (and a presumed HBV yield case) because one of the samples in this pool was Procleix Ultrio Plus Assay reactive, HBV discriminated and was reactive in an Alternate NAT for HBV detection.

For the presumptive yield case, further testing of samples from the donor's two previous donations and current donation were nonreactive when tested in 16-sample pools with the site's standard licensed HBV NAT. A sample from the donation 2 days subsequent to the yield case was positive with the site's HBV NAT when tested in a 16-sample pool, although the Procleix Ultrio Plus Assay and licensed Procleix Ultrio Assay results were nonreactive. All of the donor's samples were HBsAg seronegative.

For further investigational testing, samples from the plasma units from 3 of the donations (presumptive yield case donation, donation 10 days previous, and subsequent donation) were tested in 10 replicates with the Procleix Ultrio Plus Assay and licensed Procleix Ultrio Assay at Gen-Probe. All replicates were Procleix Ultrio Plus Assay reactive (10/10, 10/10, 10/10); 1 replicate from the yield case donation was nonreactive with the licensed Procleix Ultrio Assay and the remaining replicates were reactive with the licensed Procleix Ultrio Assay (9/10, 10/10, 10/10). Samples from these 3 donations were anti-HBc seronegative.

No follow-up samples from this donor were tested to demonstrate seroconversion and to prove HBV infection in this presumptive yield case.

Table 3. Clinical Specificity Study: Procleix Ultrio Plus Assay Reactivity in 16-Sample Pools From SP Donations

Results	N	Percentage (95% CI)[1]
Total pools tested	1,025	100%
Nonreactive pools	1,013	98.8% (98.0 - 99.3%)
Initially reactive pools	12	1.2% (0.7 - 2.0%)
Pool, individual constituent(s), and discriminatory assay reactive; Procleix Ultrio Assay reactive; True Positive	11	1.1% (0.6 - 1.9%)
Pool, individual constituent(s), and discriminatory assay reactive; Procleix Ultrio Assay nonreactive; Alternate NAT confirmed reactive; True Positive	1	0.1% (0.0 - 0.6%)

[1]SCORE confidence interval
N = number of pools

The overall clinical specificity of the Procleix Ultrio Plus Assay in pools from voluntary blood donations and in pools from SP donations is summarized in Table 4. Specificity results for the two blood center testing sites are also shown separately. Pools from SP donations were tested at only one site. The overall specificity in 16-sample pools from voluntary blood donations was 99.9% (2,090/2,092; 95% CI: 99.7%-100%) in this study. The overall specificity in 16-sample pools from SP donations was 100% (1,013/1,013; 95% CI: 99.6%-100%) in this study.

Table 4. Clinical Specificity Study: Specificity of the Procleix Ultrio Plus Assay in 16-Sample Pools From Voluntary Blood and SP Donations

Pool Type	N	True Negative	False Negative	True Positive	False Positive	Specificity (%)	95% CI[1]
Voluntary Blood	2,104	2,090	0	12	2	99.9	99.7 - 100
Site 1	1,067	1,056	0	9	2	99.8	99.3 - 99.9
Site 2	1,037	1,034	0	3	0	100	99.6 - 100
SP	1,025	1,013	0	12	0	100	99.6 - 100

[1]SCORE confidence interval
N = number of pools

SPECIFICITY AND SENSITIVITY OF THE PROCLEIX ULTRIO PLUS AND THE PROCLEIX ULTRIO PLUS DISCRIMINATORY ASSAYS IN THE PRESENCE OF DONOR AND DONATION FACTORS

Tables 5 and 6 show all valid test results obtained when specimens containing various donor and donation factors were tested with the Procleix Ultrio Plus Assay and Discriminatory Assays. Initially invalid or suspect reactions were retested when sufficient volume was available, and the valid retests were used in analysis. HIV-1, HCV, and HBV positive specimens were created by individually spiking the various donor and donation specimens and control specimens to a final concentration of 150 copies/mL of HIV-1, 150 copies/mL of HCV, or 15 IU/mL of HBV. Cross-reactivity and interference are defined as greater than 5% unexpected results.

When tested with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus Discriminatory Assays, no interference (Table 5) or cross reactivity (Table 6) was observed for naturally occurring icteric, hemolyzed, or lipemic specimens or plasma containing the following substances: albumin (60 g/L), hemoglobin (5,000 mg/L), bilirubin (200 mg/L), and lipids (30,000 mg/L).

No interference (Table 5) or cross reactivity (Table 6) was observed in specimens from patients with autoimmune and other diseases not caused by HIV- 1, HCV, or HBV infection. Multiple specimens from each group of patients with the following autoimmune and other conditions were evaluated: rheumatoid factor, antinuclear antibody, systemic lupus erythematosus, multiple myeloma, multiple sclerosis, rheumatoid arthritis, hyperglobulinemia (elevated IgG and/or IgM), alcoholic cirrhosis, and elevated alanine aminotransferase; specimens from donors with these conditions were associated with a higher rate of invalid results due to TIGRIS magnetic wash station errors.

No interference (Table 5) or cross reactivity (Table 6) was observed in bacterially contaminated plasma or in specimens from subjects infected with other blood-borne pathogens or those that had received HBV and flu vaccines. The following microorganisms that were spiked into plasma specimens were evaluated: Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Corynebacterium diphtheriae, Propionibacterium acnes, Candida albicans, and Pneumocystis carinii. Multiple specimens from each group of patients with the following viral infections were evaluated: herpes simplex virus 1 or 2, Human T-cell Lymphotrophic Virus Type I and II, hepatitis A virus, cytomegalovirus, Epstein-Barr virus, rubella virus, parvovirus B-19, and West Nile Virus (WNV). Specimens spiked with the following viruses were also evaluated: HIV-2, WNV, and Dengue virus (serotypes 1-4).

Table 5. Detection of HIV-1, HCV, and HBV in the Presence of Donor and Donation Factors with the Procleix Ultrio Plus Assay and Procleix Ultrio Plus Discriminatory Assays

Donor or Donation Factor	Reactive/Tested*					
	HIV-1 Positive (150 copies/mL)		HCV Positive (150 copies/mL)		HBV Positive (15 IU/mL)	
	Procleix Ultrio Plus	dHIV-1	Procleix Ultrio Plus	dHCV	Procleix Ultrio Plus	dHBV
Normal	26/26	26/26	26/26	26/26	26/26	26/26
Albumin (60 g/L)	26/26	26/26	26/26	26/26	26/26	26/26
Bilirubin (200 mg/L)	26/26	26/26	26/26	26/26	26/26	25/26
Hemoglobin (5000 mg/L)	26/26	26/26	26/26	26/26	26/26	26/26
Lipids (30,000 mg/L)	26/26	26/26	26/26	26/26	26/26	26/26
Hemolyzed	16/16	16/16	16/16	16/16	16/16	16/16
Icteric	20/20	20/20	20/20	20/20	20/20	20/20
Lipemic	16/16	16/16	16/16	16/16	16/16	16/16
Antinuclear Antibody	8/8	7/7	6/6	6/6	10/10	10/10
Alcoholic Cirrhosis	10/10	10/10	10/10	10/10	10/10	10/10
ALT	10/10	10/10	10/10	10/10	10/10	10/10
Hyperglobulinemia	10/10	10/10	10/10	10/10	10/10	10/10
Lupus	10/10	10/10	8/8	8/8	10/10	10/10
Multiple Myeloma	8/8	8/8	8/8	8/8	8/8	8/8
Multiple Sclerosis	10/10	10/10	10/10	10/10	10/10	10/10
Rheumatoid Arthritis	10/10	10/10	10/10	10/10	10/10	10/10
Rheumatoid Factor	10/10	10/10	10/10	10/10	10/10	10/10
C. albicans	10/10	10/10	10/10	10/10	10/10	10/10
C. diphtheriae	10/10	10/10	10/10	10/10	10/10	10/10
M. luteus	10/10	10/10	10/10	10/10	10/10	10/10
P. acnes	10/10	10/10	10/10	10/10	10/10	10/10
P. carinii	10/10	10/10	10/10	10/10	10/10	10/10
S. aureus	10/10	10/10	10/10	10/10	10/10	10/10
S. epidermidis	10/10	10/10	10/10	10/10	10/10	10/10
CMV	10/10	10/10	10/10	10/10	10/10	11/11
Dengue	8/8	8/8	8/8	8/8	8/8	8/8
EBV	10/10	10/10	10/10	10/10	10/10	10/10
Flu Vaccinee	10/10	10/10	10/10	10/10	10/10	10/10
HAV	10/10	10/10	10/10	10/10	10/10	10/10
HBV Vaccinee	10/10	10/10	10/10	10/10	10/10	10/10
HIV-2	10/10	10/10	10/10	10/10	10/10	10/10
HSV I/II	10/10	10/10	10/10	10/10	10/10	10/10
HTLV I/II	10/10	10/10	10/10	10/10	10/10	10/10
Parvovirus B19	10/10	10/10	10/10	10/10	10/10	10/10
Rubella	10/10	10/10	10/10	10/10	10/10	10/10
WNV	12/12	12/12	12/12	12/12	12/12	12/12
Controls	90/90	90/90	90/90	90/90	90/90	90/90

*Combined results from two pilot lots of reagents

Table 6. Specificity of the Procleix Ultrio Plus Assay and Procleix Ultrio Plus Discriminatory Assays in the Presence of Donor and Donation Factors

Donor or Donation Factor	Nonreactive/Tested*			
	Procleix Ultrio Plus	dHIV-1	dHCV	dHBV
Normal	24/24	24/24	24/24	24/24
Albumin (60 g/L)	24/24	24/24	24/24	24/24
Bilirubin (200 mg/L)	24/24	24/24	24/24	24/24
Hemoglobin (5000 mg/L)	24/24	24/24	24/24	24/24
Lipids (30,000 mg/L)	24/24	24/24	24/24	24/24
Hemolyzed	16/16	16/16	16/16	16/16
Icteric	20/20	20/20	20/20	20/20
Lipemic	16/16	16/16	16/16	16/16
Antinuclear Antibody	6/6	NT	10/10	10/10
Alcoholic Cirrhosis	10/10	10/10	10/10	10/10
ALT	10/10	10/10	10/10	10/10
Hyperglobulinemia	10/10	10/10	9/9	10/10
Lupus/ SLE	20/20	20/20	10/10	10/10
Multiple Myeloma	7/7	7/7	6/6	5/5
Multiple Sclerosis	10/10	10/10	10/10	10/10
Rheumatoid Arthritis	15/15	10/10	10/10	10/10
Rheumatoid Factor	18/18	10/10	10/10	10/10
C. albicans	10/10	10/10	10/10	10/10
C. diphtheriae	10/10	10/10	10/10	10/10
M. luteus	10/10	10/10	10/10	10/10
P. acnes	10/10	10/10	10/10	10/10
P. carinii	10/10	10/10	10/10	10/10
S. aureus	10/10	10/10	10/10	10/10
S. epidermidis	10/10	10/10	10/10	10/10
CMV	10/10	10/10	10/10	10/10
Dengue	8/8	8/8	8/8	8/8
EBV	10/10	10/10	10/10	10/10
Flu Vaccinee	10/10	10/10	10/10	10/10
HAV	10/10	10/10	10/10	10/10
HBV Vaccinee	10/10	10/10	10/10	10/10
HIV-2	10/10	10/10	10/10	10/10
HSV I/II	10/10	10/10	10/10	10/10
HTLV I/II	10/10	10/10	10/10	10/10
Parvovirus B19	10/10	10/10	10/10	10/10
Rubella	10/10	10/10	10/10	10/10
WNV	12/12	12/12	12/12	12/12
Controls	92/92	90/90	90/90	90/90

*Combined results from two pilot lots of reagents
NT= Not tested

Specificity and Sensitivity in Serum and Plasma Specimens Collected in Various Anticoagulants

The sensitivity and specificity of the Procleix Ultrio Plus Assay and Discriminatory Assays for serum samples and samples collected in various anticoagulants and spiked with HIV-1, HCV, or HBV is shown in Table 7 and without spiking, as shown in Table 8. Detection rates were calculated from valid results. The anticoagulants tested were ACD (Acid Citrate Dextrose), CPD (citrate phosphate dextrose), CP2D (Citrate Phosphate Double Dextrose), CPDA (citrate phosphate dextrose adenine), K2EDTA (ethylene diamine tetraacetic acid), K2EDTA Sep (K2EDTA separation tube), K3EDTA, LiH (lithium heparin), NaC (sodium citrate), PPT (K2EDTA Plasma Preparation Tube), and a serum collection tube.

For all anticoagulants as well as serum, no interference or cross reactivity for detection of HIV-1, HCV, or HBV was observed.

Table 7. Detection of HIV-1, HCV, and HBV in the Presence of Anticoagulants and Serum

Anticoagulant	Reactive/Tested (Percent Reactive)*					
	HIV-1 Positive (150 copies/mL)		HCV Positive (150 copies/mL)		HBV Positive (15 IU/mL)	
	Ultrio Plus	dHIV-1	Ultrio Plus	dHCV	Ultrio Plus	dHBV
ACD	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
CPD	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
CP2D	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
CPDA-1	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
K2EDTA	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
K2EDTA Sep	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
K3EDTA	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
LiH	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
NaC	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
PPT	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Serum	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)

*Combined results from 2 pilot lots of reagents.

Table 8. Specificity of Procleix Ultrio Plus and Procleix Ultrio Plus Discriminatory Assays in the Presence of Anticoagulants and Serum

Anticoagulant	Nonreactive/Negative Samples Tested (Percent Nonreactive)*			
	Ultrio Plus	dHIV-1	dHCV	dHBV
ACD	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
CPD	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
CP2D	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
CPDA-1	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
K2EDTA	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
K2EDTA Sep	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
K3EDTA	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
LiH	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
NaC	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
PPT	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Serum	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)

*Combined results from 2 pilot lots of reagents.

TESTING OF SPECIMENS FROM HIV-1, HCV, OR HBV INFECTED INDIVIDUALS

A combined total of 624 HIV-1, HCV, or HBV NAT-positive plasma specimens were obtained from a commercial vendor. Two different reagent lots were used for all testing; all testing was performed in-house. Each sample was tested neat (undiluted) and diluted 1:16 in negative donor plasma samples with the Procleix Ultrio Plus Assay. Each sample was also tested neat with the corresponding Procleix Ultrio Plus Discriminatory (dHIV-1, dHCV, or dHBV) Assay. Initially invalid reactions were retested; the valid retest results were used for the data analysis. There were 10 initially invalid reactions out of 1,248 (0.80%) for the Procleix Ultrio Plus Assay, 5 initially invalid reactions out of 200 (2.5%) for the Procleix Ultrio Plus dHIV-1 Assay, and 1 initially invalid reaction out of 200 (0.5%) for the Procleix Ultrio Plus dHCV Assay.

HIV-1 Detection in Known Positive Samples. Both the Procleix Ultrio Plus and the Procleix Ultrio Plus dHIV-1 Assays for neat and diluted (1:16) HIV-1 positive samples (n = 200) detected 100% (95% Confidence Interval [CI]: 98.2-100%) of the samples.

HCV Detection in Known Positive Samples. Both the Procleix Ultrio Plus and the Procleix Ultrio Plus dHCV Assays for neat and diluted (1:16) HCV positive samples (n = 200) detected 100% (95% CI: 98.2-100%) and 99.0% (95% CI: 96.4-99.9%) of the samples, respectively.

HBV Detection in Known Positive Samples. Both the Procleix Ultrio Plus and the Procleix Ultrio Plus dHBV Assays for neat and diluted (1:16) HBV positive samples (n = 224) detected 100% (95% CI: 98.4-100%) of the samples.

Overall Detection in Known Positive Samples. The overall detection rate for the Procleix Ultrio Plus Assay and all 3 Procleix Ultrio Plus Discriminatory Assays for all 624 specimens tested neat was 100% (624/624). The overall detection rate for the Procleix Ultrio Plus Assay for all 624 specimens tested in a 1:16 dilution was 99.7% (622/624). Although there was variability in the detection of the diluted samples between the Procleix Ultrio Plus dHCV Assay and the Procleix Ultrio Plus dHIV-1 and Procleix Ultrio Plus dHBV Assays, the difference observed was not statistically significant, as indicated by the overlapping 95% confidence intervals (Table 9).

Table 9. Sensitivity of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus Discriminatory Assays in Known Positive Samples

Assay	Sample	Valid Tests (N)	Reactive (N)	Sensitivity (%)	95% CI
Procleix Ultrio Plus Assay (Neat)	**All**	624	624	100	99.4-100
	HIV-1	200	200	100	98.2-100
	HCV	200	200	100	98.2-100
	HBV	224	224	100	98.4-100
Procleix Ultrio Plus Assay (Diluted 1:16)	**All**	624	622	99.7	98.9-100
	HIV-1	200	200	100	98.2-100
	HCV	200	198	99.0	96.4-99.9
	HBV	224	224	100	98.4-100
Procleix Ultrio Plus dHIV-1 Assay	**HIV-1**	200	200	100	98.2-100
Procleix Ultrio Plus dHCV Assay	**HCV**	200	200	100	98.2-100
Procleix Ultrio Plus dHBV Assay	**HBV**	224	224	100	98.4-100

N = Number of specimens; CI = Confidence Interval

REACTIVITY IN SEROCONVERTING DONORS

Commercially available seroconversion panels were tested to determine the ability of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HIV-1, HCV, and HBV Discriminatory Assays (dHIV-1, dHCV, and dHBV) to reduce the pre-seroconversion window period of HIV-1, HCV, and HBV detection when compared to antigen and/or antibody tests. The Procleix Ultrio Plus Assay was used to test each seroconversion panel neat, diluted 1:8, and diluted 1:16. All testing was performed in-house. Each seroconversion panel was also tested neat with the Procleix Ultrio Plus dHIV-1, dHCV, or dHBV Assay. The test results were compared with those of the Abbott Anti-HIV 1/2 antibody test for the detection of anti-HIV-1/2 antibody (anti-HIV-1/2 Ab), and either the Coulter HIV-1 p24 Ag test, the Roche Elecsys HIV p24 Ag test, or the ZeptoMetrix p24 Ag test for the detection of HIV-1 p24 antigen (HIV-1 p24 Ag) for HIV-1 seroconversion panels; the Ortho Anti-HCV 3.0 (SAVe), the Ortho ELISA Anti-HCV 3.0, or the Abbott Murex Anti-HCV 4.0 antibody test for the detection of anti-HCV antibody (anti-HCV Ab) for HCV seroconversion panels; and the Abbott PRISM HBsAg test and Ortho HBsAg ELISA Test System 3 for the detection of HBV surface antigen (HBsAg) for HBV seroconversion panels.

HIV-1 Detection in Seroconversion Panels

When compared to the Anti-HIV-1/2 Ab test and the HIV-1 p24 Ag test the Procleix Ultrio Plus Assay was able to detect HIV-1 RNA an average of 14.5 and 8.6 days earlier in neat samples, 11.7 and 5.8 days earlier in 1:8 dilutions, and 12.5 and 6.6 days earlier in 1:16 dilutions. The Procleix Ultrio Plus dHIV-1 Assay was able to detect HIV-1 RNA an average of 14.1 and 8.2 days earlier than the Anti-HIV-1/2 Ab test and the HIV-1 p24 Ag test, respectively (Table 10).

Table 10. Detection of HIV-1 RNA in HIV-1 Seroconversion Panels

Panel ID	Days Earlier Detection Than HIV-1/2 Antibody				Days Earlier Detection Than HIV-1 p24 Ag			
	Procleix Ultrio Plus Assay			dHIV-1 Assay	Procleix Ultrio Plus Assay			dHIV-1 Assay
	Neat	1:8	1:16	Neat	Neat	1:8	1:16	Neat
6244	11	8	8	11	6	3	3	6
6247	16	14	14	14	7	5	5	5
9020	17	14	14	14	7	4	4	4
9021	14	14	14	14	4	4	4	4
9030	14	14	14	14	7	7	7	7
9031	15	12	15	15	22	19	22	22
9032*	14	10	10	17	14	10	10	17
9076	14	8	8	14	8	2	2	8
9077**	16	16	16	16	4	4	4	4
9079**	14	7	12	12	7	0	5	5
Mean	14.5	11.7	12.5	14.1	8.6	5.8	6.6	8.2

For Anti-HIV-1/2 Antibody, all panels were compared to the Abbott Anti-HIV 1/2 test.

For HIV-1 p24 Antigen, all panels were compared to the Coulter HIV-1 p24 Ag test, with the following exceptions:

*Panel 9032 was compared to Roche Elecsys HIV p24 Ag test because seroconversion was not demonstrated with the Coulter HIV-1 p24 Ag test.

**Panels 9077 and 9079 were compared to ZeptoMetrix p24 Ag test, as there were no Coulter HIV-1 p24 Ag test results reported

HCV Detection in Seroconversion Panels

When compared to anti-HCV antibody tests the Procleix Ultrio Plus Assay was able to detect HCV RNA an average of 32.6 days earlier in neat samples, 31.8 days earlier in 1:8 dilutions, and 32.1 days earlier in 1:16 dilutions. The Procleix Ultrio Plus dHCV Assay was able to detect HCV RNA an average of 32.6 days earlier than the anti-HCV antibody tests (Table 11). In 5 of the 12 seroconversion panels (6214, 6226, 6228, 9045, and 9047), the first available bleed in the series was already reactive with both the Procleix Ultrio Plus Assay and Procleix Ultrio Plus dHCV Assay, so the number of days of window closure may underestimate the true window closure period for the assays.

Table 11. Detection of HCV RNA in HCV Seroconversion Panels

Panel ID	Days Earlier Detection Than HCV Antibody			
	Procleix Ultrio Plus Assay			dHCV Assay
	Neat	1:8	1:16	Neat
6213	26	26	26	26
6214	30	30	30	30
6222	23	23	23	23
6225*	39	33	33	39
6226	39	39	39	39
6227*	32	32	32	32
6228	31	31	31	31
9041	38	38	38	38
9045	37	37	37	37
9047	28	28	28	28
9054	30	30	30	30
9055**	38	34	38	38
Mean	32.6	31.8	32.1	32.6

All panels were compared to the Ortho Anti-HCV 3.0 (SAVe) test with the following exceptions:
* Panels 6225 and 6227 were compared to the Ortho ELISA Anti-HCV 3.0 test as there were no Ortho Anti-HCV 3.0 (SAVe) results reported.
** Panel 9055 was compared to the Abbott Murex Anti-HCV 4.0 test because seroconversion was not demonstrated with the Ortho Anti-HCV 3.0 (SAVe) test.

HBV Detection in Seroconversion Panels

When compared to the Abbott PRISM HBsAg test and the Ortho HbsAg Test System 3 the Procleix Ultrio Plus Assay was able to detect HBV DNA an average of 23.6 and 27.0 days earlier in neat samples, 13.5 and 16.8 days earlier in 1:8 dilutions, and 10.5 and 13.9 days earlier in 1:16 dilutions. The Procleix Ultrio Plus dHBV Assay was able to detect HBV DNA an average of 23.7 and 27.1 days earlier than the Abbott PRISM HBsAg test and the Ortho HbsAg Test System 3, respectively (Table 12).

Table 12. Detection of HBV DNA in HBV Seroconversion Panels

Panel ID	Days Earlier Detection Than Hepatitis B Surface Antigen, Abbott PRISM HBsAg Test				Days Earlier Detection Than Hepatitis B Surface Antigen, Ortho HBsAg ELISA Test System 3			
	Procleix Ultrio Plus Assay			dHBV Assay	Procleix Ultrio Plus Assay			dHBV Assay
	Neat	1:8	1:16	Neat	Neat	1:8	1:16	Neat
6283	18	10	10	21	18	10	10	21
6289	20	13	0	15	20	13	0	15
6290	21	14	7	21	23	16	9	23
6292	27	8	8	29	27	8	8	29
9073	21	10	10	21	25	14	14	25
9074	14	14	11	18	17	17	14	21
11006	28	14	14	28	30	16	16	30
11007	21	5	7	14	28	12	14	21
11008	21	11	7	21	31	21	17	31
11015	36	34	27	44	36	34	27	44
11024	33	15	15	29	42	24	24	38
Mean	23.6	13.5	10.5	23.7	27.0	16.8	13.9	27.1

ANALYTICAL SENSITIVITY

Analytical Sensitivity of the Procleix Ultrio Plus Assay

Analytical sensitivity panels were prepared from the following World Health Organization (WHO) International Standards: HIV-1 (97/650), HCV (06/100), and HBV (97/750). A total of 6 Procleix Ultrio Plus Assay kit lots were used to test the HIV-1 and HBV panels (2 kit lots were used to test each of 3 unique HIV-1 and HBV WHO panel preparations). Each of the 6 Procleix Ultrio Plus Assay kit lots was tested in 60 replicates at each HIV-1 and HBV concentration to yield a total of 360 replicates at each level. A total of 4 Procleix Ultrio Plus Assay kit lots were used to test the HCV panel (2 Procleix Ultrio Plus Assay kit lots were used to test each of 2 unique HCV panel preparations). Each of the 4 Procleix Ultrio Plus Assay kit lots was tested in 60 replicates at each HCV concentration for a total of 240 replicates at each level. The panels were tested with the Procleix Ultrio Plus Assay and the 3 Procleix Ultrio Plus Discriminatory (dHIV-1, dHCV and dHBV) Assays. The SCORE method was used to calculate the 95% confidence intervals using SAS version 9.2 (Cary, NC). SAS version 9.2 was also used to perform probit and Pearson chi-square analysis.

Detection of HIV-1 WHO Standard (97/650)

HIV-1 WHO panel detection with the Procleix Ultrio Plus Assay and Procleix Ultrio Plus dHIV-1 Assay was 100% at 600, 200, and 60 IU/mL. The conversion factor for HIV-1 is estimated to be 0.6 copies per IU.[45, 46] The average analyte S/CO values for the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus Discriminatory (dHIV-1) Assay were greater than 10 and 18, respectively, at these panel concentrations. The detection rate for both assays was about 94% and 95% at 20 IU/mL, with an average analyte S/CO of 8.56 and 15.78 for the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHIV-1 Assay, respectively. The detection rate decreased to approximately 60% for both assays at 6 IU/mL, with average analyte S/CO values of 6.57 and 12.47 for the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHIV-1 Assay, respectively (Table 13).

Table 13. Detection of HIV-1 WHO Standard with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHIV-1 Assay

	Procleix Ultrio Plus Assay						Procleix Ultrio Plus dHIV-1 Assay					
HIV-1 WHO (97/650) IU/mL	Number of reactive / tested	% Reactive	95% Confidence Limits		Average S/CO	%CV	Number of reactive / tested	% Reactive	95% Confidence Limits		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
600	360/360	100	99	100	10.73	7	360/360	100	99	100	19.37	13
200	360/360	100	99	100	10.66	7	360/360	100	99	100	19.29	12
60	360/360	100	99	100	10.47	10	360/360	100	99	100	18.86	16
20	337/360	94	91	96	8.56	37	343/360	95	93	97	15.78	37
6	214/360	59	54	64	6.57	52	221/359	62	56	66	12.47	52
0	0/360	0	0	1	0.12	50	0/360	0	0	1	0.18	48

95% Confidence Limit = SCORE Confidence Interval
S/CO = Signal to Cutoff ratio
CV = Coefficient of Variation

Detection of HCV WHO Standard (06/100)

HCV WHO panel detection with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHCV Assay was 100% at 100 and 30 IU/mL, and 98% and 100% at 10 IU/mL, respectively. The conversion factor for HCV is estimated to be 3.4 copies per IU.[47] The average analyte S/CO values for the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHCV Assay were less than 10 and 24, respectively, at these panel concentrations. The detection rate for both assays was 86% and 87% at 3 IU/mL, with an average analyte S/CO of 9.03 and 22.49 for the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHCV Assay, respectively. The detection rate decreased to 40% and 48% for both assays at 1IU/mL, with average analyte S/CO values of 8.56 and 21.59 for the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHCV Assay, respectively (Table 14).

Table 14. Detection of HCV WHO with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHCV Assay

	Procleix Ultrio Plus Assay						Procleix Ultrio Plus dHCV Assay					
HCV WHO (06/100) IU/mL	Number of reactive / tested	% Reactive	95% Confidence Limits		Average S/CO	% CV	Number of reactive / tested	% Reactive	95% Confidence Limits		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
100	240/240	100	98	100	9.35	5	240/240	100	98	100	23.77	6
30	240/240	100	98	100	9.40	5	239/239	100	98	100	23.79	6
10	236/240	98	96	99	9.40	6	239/239	100	98	100	23.80	7
3	207/240	86	81	90	9.03	13	208/240	87	82	90	22.49	17
1	97/240	40	34	47	8.56	22	114/240	48	41	54	21.59	23
0	0/240	0	0	2	0.12	38	0/240	0	0	2	0.06	112

95% Confidence Limit = SCORE Confidence Interval
S/CO = Signal to Cutoff ratio
CV = Coefficient of Variation

Detection of HBV WHO Standard (97/750)

HBV WHO panel detection with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHBV Assay was 100% at 45 and 15 IU/mL and 99% and 97% at 5 IU/mL, respectively. The conversion factor for HBV is estimated to be 5 copies per IU.[45] The average analyte S/CO values for the Procleix Ultrio Plus and the Procleix Ultrio Plus dHBV Assay were approximately 15 and 24, respectively, at these panel concentrations. The detection rate for both assays was less than 80% at 1.67 IU/mL, with an average analyte S/CO of 13.93 and 21.12 for the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHBV Assay, respectively. The detection rate decreased to approximately 40% for both assays at 0.56 IU/mL, with average analyte S/CO values of 13.12 and 20.41 for the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHBV Assay, respectively (Table 15).

Table 15. Detection of HBV WHO with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHBV Assay

Procleix Ultrio Plus Assay							Procleix Ultrio Plus dHBV Assay					
HBV WHO (97/750) IU/mL	Number of reactive / tested	% Reactive	95% Confidence Limits		Average S/CO	% CV	Number of reactive / tested	% Reactive	95% Confidence Limits		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
45	360/360	100	99	100	14.97	6	360/360	100	99	100	23.74	4
15	360/360	100	99	100	14.91	6	360/360	100	99	100	23.74	4
5	356/360	99	97	100	14.66	9	350/360	97	95	99	23.27	8
1.67	282/360	78	74	82	13.93	17	271/360	75	71	80	21.12	25
0.56	156/360	43	38	49	13.12	24	138/359	38	34	44	20.41	27
0	0/360	0	0	1	0.10	39	0/360	0	0	1	0.05	118

95% Confidence Limit = SCORE Confidence Interval
S/CO = Signal to Cutoff ratio
CV = Coefficient of Variation

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 Plus Assay testing should be performed using 3 replicates. A reactive result in at least 1 of the 3 replicates indicates the sample is HBV DNA positive.

Probit Analysis of Analytical Sensitivity Data

Table 16 shows the predicted 50% and 95% detection levels (IU/mL or copies/mL) for each panel member for both the Procleix Ultrio Plus Assay and the respective discriminatory assays. The 95% probability for detection of HIV-1 WHO was similar between the Procleix Ultrio Plus Assay and Procleix Ultrio Plus dHIV-1 Assay (21.2 and 18.9 IU/mL, respectively). The 95% probability for detection of HCV WHO was also similar between the Procleix Ultrio Plus Assay and Procleix Ultrio Plus dHCV assay (5.4 and 4.4 IU/mL, respectively). The 95% probability of detection of HBV WHO was 3.4 and 4.1 IU/mL for the Procleix Ultrio Plus Assay and Procleix Ultrio Plus dHBV Assay, respectively. There were no significant differences ($p>0.05$) in the 95% limit of detection between the Procleix Ultrio Plus Assay and the respective discriminatory assays for all 3 panels.

Table 16. Detection of HIV-1 WHO (97/650), HCV WHO (06/100), and HBV WHO (97/750) Standards – Probit Analysis

Panel Tested	Procleix Assay	Detection Probabilities		p Values from Comparison of the Discriminatory Assays and the Ultrio Plus Assay Limits of Detection
		50% Limit of Detection (95% Fiducial Limits) IU/mL	95% Limit of Detection (95% Fiducial Limits) IU/mL	
HIV-1 WHO (97/650)	Ultrio Plus Assay	4.7 (4.0 - 5.3)	21.2 (18.2 - 25.7)	0.96
	Ultrio Plus dHIV-1 Assay	4.7 (4.0 - 5.3)	18.9 (16.3 - 22.9)	
HCV WHO (06/100)	Ultrio Plus Assay	1.2 (1.1 - 1.4)	5.4 (4.5 - 6.7)	0.77
	Ultrio Plus dHCV Assay	1.1 (0.9 - 1.2)	4.4 (3.7 - 5.6)	
HBV WHO (97/750)	Ultrio Plus Assay	0.7 (0.6 - 0.8)	3.4 (3.0 - 4.1)	0.99
	Ultrio Plus dHBV Assay	0.8 (0.7 - 0.9)	4.1 (3.5 - 4.9)	

Probit and Pearson chi-square analysis were performed with SAS version 9.2.

DETECTION OF HIV-1, HCV, AND HBV IN LOW TITER SAMPLES

A total of 144 samples from individuals who were known to be infected with HIV-1, HCV or HBV were tested in the Procleix Ultrio Assay and Procleix Ultrio Plus Assay at Gen-Probe Incorporated.

For the purposes of this study, “low titer” indicates samples with the lowest titers that were commercially available and had sufficient volume for testing multiple replicates. All the samples were positive for HIV-1, HCV, or HBV in FDA licensed test methods, though some of the samples were below the validated level of quantitation of the various assays used to assign HIV-1, HCV, or HBV titer. Each sample was tested in triplicate, neat and diluted 1:16 in pools of negative plasma, in both the Ultrio and Ultrio Plus Assays. Table 17 details the samples tested.

Table 17. Low Titer Specimens

Analyte	Titer Range	Number of Unique Donors
HIV-1	BLQ -1,760 copies/mL	60
HCV	BLQ -9,421 copies/mL	34
HBV	BLQ -231 IU/mL	50
Total specimens tested		144

BLQ = titer below the level of quantitation.

HIV-1 Positive Samples

The Procleix Ultrio and Procleix Ultrio Plus Assays were used to screen 60 HIV-1 positive samples. There was no significant difference in overall reactivity between the two assays, diluted or undiluted, as indicated by p-values greater than 0.05 (0.81 for neat samples, 0.40 for diluted) (Table 18).

HCV Positive Samples

There were 34 HCV positive samples that were tested with the Procleix Ultrio and Procleix Ultrio Plus Assays. There was no significant difference in overall reactivity between the two assays, diluted or undiluted, as indicated by p-values greater than 0.05 (0.42 for neat samples, 1.00 for diluted) (Table 18).

HBV Positive Samples

A total of 50 HBV positive samples were tested with the Procleix Ultrio and Procleix Ultrio Plus Assays. There was a significant difference in HBV sensitivity between the two assays (p-value<0.0001 for diluted and undiluted testing). Procleix Ultrio Plus detected 98.7% (148/150) of the undiluted replicates compared to 75.3% detection (113/150) with Procleix Ultrio for the identical samples. When tested at a 1:16 dilution, Procleix Ultrio Plus detected 78.0% (117/150) compared to 42.0% (63/150) with Procleix Ultrio (Table 18).

Table 18. Low Titer Sample Testing Summary

Analyte	Dilution	Assay	# Reactive	# Valid Replicates	% Reactive	p-value
HIV-1	Neat	Procleix Ultrio	170	180	94.4%	0.81
		Procleix Ultrio Plus	172	180	95.6%	
	1:16	Procleix Ultrio	128	180	71.1%	0.40
		Procleix Ultrio Plus	136	180	75.6%	
HCV	Neat	Procleix Ultrio	74	102	72.5%	0.42
		Procleix Ultrio Plus	80	102	78.4%	
	1:16	Procleix Ultrio	64	102	62.7%	1.00
		Procleix Ultrio Plus	63	102	61.8%	
HBV	Neat	Procleix Ultrio	113	150	75.3%	<0.0001
		Procleix Ultrio Plus	148	150	98.7%	
	1:16	Procleix Ultrio	63	150	42.0%	<0.0001
		Procleix Ultrio Plus	117	150	78.0%	

p-value determined by Fisher’s Exact test, SAS v. 9.2

The Procleix Ultrio Plus and Procleix Ultrio Assays showed comparable detection of low titer HIV-1 and HCV specimens. The Ultrio Plus Assay showed significantly better low titer HBV detection than the Ultrio Assay.

COMPARISON OF THE DETECTION RATE OF THE ULTRIO AND ULTRIO PLUS ASSAYS IN HIV-1, HCV, OR HBV YIELD AND SEROPOSITIVE SPECIMENS

A total of 2,330 donor specimens were evaluated that were either seronegative and previously nucleic acid test (NAT) reactive for HIV-1, HCV or HBV when tested with the Procleix HIV-1/HCV Assay or the Procleix Ultrio Assay (yield specimens) and samples that were seropositive by standard, licensed serological testing. These specimens consisted of 23 HIV-1 yield samples, 156 HCV yield samples and 13 HBV yield samples in addition to 1,388 HIV-1, HCV or HBV serologically positive samples and 750 anti-HBc reactive/HBsAg negative samples. A summary of the yield and serologically confirmed positive samples and number of replicates tested is listed in Table 19a and Table 19b, respectively. Samples were obtained from a repository of positive plasma units from blood donors that is maintained by the American Red Cross (ARC) Scientific Support Office (Gaithersburg, MD). All NAT-positive units were from blood donors identified as positive for HIV-1, HCV, or HBV by the Procleix HIV-1/HCV Assay or the Procleix Ultrio Assay. Seropositive plasma units were determined by the following criteria:

Anti-HIV-1 confirmed positive: Donations reactive by a screening enzyme immunoassay (HIV-1/HIV-2 rDNA EIA; Abbott Laboratories, Abbott Park, IL) and positive by either HIV-1 western blot (Calypte Biomedical, Rockville, MD) or an immunofluorescent assay (IFA, Sanochemia, Vienna, Austria).

Anti-HCV confirmed positive: Donations reactive by a screening EIA (Ortho v3.0 ELISA; Ortho Diagnostics, Raritan, NJ) and positive by a recombinant immunoblot (RIBA 3.0, Novartis, Emeryville, CA).

HBsAg confirmed positive: Donations reactive by a screening ChLIA (chemiluminescent immunoassay; Abbott PRISM) and positive by neutralization.

Anti-HBc positive: Donations reactive by a screening ChLIA for anti-HBc (Abbott PRISM) but non-reactive for HBsAg.

All plasma units were aliquotted and diluted 1:16 in negative CPD plasma at the ARC Scientific Support Office. HBV plasma units were also diluted 1:8. Neat and diluted samples were shipped to Gen-Probe Incorporated where they were stored frozen. Prior to testing with the Procleix Ultrio Plus Assay at Gen-Probe Incorporated, all samples were tested with the Procleix Ultrio Assay by Creative Testing Solutions (CTS, St. Petersburg, FL) except 8 HBV yield specimens that were received at Gen-Probe Incorporated after completion of the CTS testing.

Table 19a. Description of Yield Samples and Test Count

# of Specimens	Description	Dilution	Replicates	Total Tests
23	HIV-1 Yield	Neat	3	69
		1:16		69
156	HCV Yield	Neat	3	468
		1:16		468
13	HBV Yield	Neat	3	39
		1:8		39
		1:16		39
192	Totals			1,191

Table 19b. Description of Serologically Confirmed Positive Samples and Test Count

# of Specimens	Description	Dilution	Replicates	Total Tests
292	HIV-1 Ab Confirmed Positive	Neat	1	292
490	HCV Ab Confirmed Positive	Neat	1	490
606	HBsAg Confirmed Positive	Neat	1	606
750	Anti-HBc Reactive/HBsAg Negative	Neat	1	750
2,138	Totals			2,138

HIV-1 Yield Samples

All 23 HIV-1 yield samples were reactive in all 3 replicates (100% reactive) when tested neat with both the Procleix Ultrio and Procleix Ultrio Plus Assays. Of the samples diluted 1:16, 64/69 replicates (92.8%) were reactive when tested with the Procleix Ultrio Assay and 61/69 (88.4%) were reactive when tested with the Procleix Ultrio Plus Assay. There was no statistically significant difference in performance between the Procleix Ultrio and Procleix Ultrio Plus Assays with the HIV-1 yield samples diluted 1:16 (p=0.56, Table 20).

Four samples had discrepant results between assays when the 1:16 dilutions were initially tested (Table 21). Three of these samples were reactive in only 1 or 2 replicates when tested in the Procleix Ultrio Plus Assay. To determine if the discrepant results were reproducible, fresh 1:16 pools were made from the original neat aliquots by diluting into equal aliquots of 15 negative samples. All 3 samples were reactive in 3 out of 3 replicates upon testing fresh 1:16 pools. Overall reactivity for Procleix Ultrio Plus increased to 100% after new 1:16 dilutions were tested.

Additional HIV-1 Yield Samples

Additional HIV-1 yield testing was performed to verify the detection rate of the two assays was comparable. The ARC provided two sets (one for each assay) of 17 HIV-1 yield specimens diluted 1:8 and 1:16. There were three aliquots/set, which allowed for 15 replicates per diluted sample, per assay.

All 17 samples were 100% reactive (255/255) in both the Procleix Ultrio Assay and the Procleix Ultrio Plus Assay at the 1:8 dilutions. At 1:16, Ultrio was 98.4% reactive (251/255) and Ultrio Plus was 100% reactive (255/255). All initially invalid reactions were retested and the valid retest results were used in analysis. This data is summarized in Table 22.

HCV Yield Samples

Overall reactivity was 99.8% for the Procleix Ultrio Assay and 99.6% for the Procleix Ultrio Plus Assay when samples were tested neat. At the 1:16 dilution, 464/468 (99.1%) and 466/468 (99.6%) replicates were reactive when tested with the Procleix Ultrio and Procleix Ultrio Plus Assays, respectively. There was no statistically significant difference in performance between the Procleix Ultrio and Procleix Ultrio Plus Assays when tested in pools of 16 (p=0.69, Table 20).

One sample (033GE77315) was unreliably detected by both assays (Table 23). Neat testing resulted in 2 reactive replicates in the Procleix Ultrio Assay and 1 reactive in the Procleix Ultrio Plus Assay. At the 1:16 dilution, this same sample was nonreactive in all 3 replicates when tested with the Procleix Ultrio Assay and reactive in all replicates when tested with the Ultrio Plus Assay. Neat and 1:16 aliquots were retested in Procleix Ultrio Plus gave similar results: 0/3 replicates were positive when tested neat, and 3/3 replicates were positive when tested 1:16. A fresh 1:16 dilution was also prepared and tested from the neat sample and no replicates were reactive, suggesting this sample may have been mislabeled prior to shipping to Gen-Probe.

HBV Yield Samples

Of the 13 HBV yield samples, 12 samples were reactive in all 3 replicates when tested neat with both assays. A total of 36/39 and 37/39 replicates from testing the neat samples were reactive in the Procleix Ultrio and Procleix Ultrio Plus Assays, respectively (92.3% vs. 94.9% reactive). A total of 25/39 and 33/39 replicates were reactive when 1:8 dilutions were tested with the Procleix Ultrio and Procleix Ultrio Plus Assays, respectively (64.1% vs. 84.6% reactive). At 1:16, a total of 22/39 and 30/39 replicates were reactive with the Procleix Ultrio and the Procleix Ultrio Plus Assays, respectively (56.4% vs. 76.9% reactive). Although substantially more replicates were reactive when samples were tested with the Procleix Ultrio Plus Assay, these differences did not reach a level of statistical significance (p=0.06 and 0.09 for 1:8 and 1:16 dilutions, respectively). In addition, there was no statistical difference seen within each assay when comparing the rate of detection of the 1:8 dilutions to the 1:16 dilutions (p= 0.64 and 0.57 for the Procleix Ultrio and the Procleix Ultrio Plus Assays respectively). These data are summarized in Table 20.

Eight samples had discrepant results between the two assays (Table 24). One sample had discrepant results neat, six samples had discrepant results at 1:8 dilutions, and five samples had discrepant results at 1:16 dilutions. In all cases except one (sample 042FM54241P at 1:8), more replicates were reactive in the Procleix Ultrio Plus Assay than in the Procleix Ultrio Assay.

Table 20. HIV-1, HCV and HBV Yield Samples: Summary of Reactivity

	HIV-1		HCV		HBV	
	Ultrio	**Ultrio Plus**	**Ultrio**	**Ultrio Plus**	**Ultrio**	**Ultrio Plus**
Neat	69/69 100% (96.2-100.0)	69/69 100% (96.2-100.0)	467/468 99.8% (98.8-100.0)	466/468 99.6% (98.5-99.9)	36/39 92.3% (79.7-97.4)	37/39 94.9% (83.1-98.6)
1:8	NT	NT	NT	NT	25/39 64.1% (48.4-77.3)	33/39 84.6% (70.3-92.8)
1:16	64/69 92.8% (84.1-96.9)	61/69* 88.4% (78.8-94.0)	464/468 99.1% (97.8-99.7)	466/468 99.6% (98.5-99.9)	22/39 56.4% (41-70.7)	30/39 76.9% (61.7-87.4)
Inter-assay p-value (1:16)	0.56		0.69		0.06**/ 0.09	
Intra-assay p-value (1:8 vs. 1:16)	NT		NT		0.64	0.57

95% CI was calculated using the SCORE method.
NT= Not Tested.
Fisher's Exact Test, SAS v9.2 was used to calculate p-value.
*Results were 69/69 after testing a fresh 1:16 dilution.
**1:8 p-value

Table 21. Discrepant HIV-1 Yield Results Summary (Number Reactive/Number Tested)

Sample	Ultrio		Ultrio Plus		
	Neat	1:16	Neat	1:16	1:16*
003K 16030	3/3	**2/3**	3/3	**1/3**	3/3
029KM27572	3/3	**2/3**	3/3	**3/3**	NT
013FY89120	3/3	**3/3**	3/3	**2/3**	3/3
035FH89864	3/3	**3/3**	3/3	**1/3**	3/3

NT = Not Tested.
*Results after testing a new 1:16 dilution.
Discrepant results are in BOLD.

Table 22. Additional HIV-1 Yield Testing (Number Reactive/Number Tested)

Sample	Viral Load	1:8 Dilution		1:16 Dilution	
		Ultrio	Ultrio Plus	Ultrio	Ultrio Plus
022GH05568	200	15/15	15/15	15/15	15/15
003R 43191	260	15/15	15/15	**13/15**	15/15
032FP28078	370	15/15	15/15	15/15	15/15
022LQ82592	390	15/15	15/15	15/15	15/15
004F 25254	570	15/15	15/15	15/15	15/15
003K 16030	790	15/15	15/15	**13/15**	15/15
035FH89864	850	15/15	15/15	15/15	15/15
013FY89120	910	15/15	15/15	15/15	15/15
036FL20959	1,300	15/15	15/15	15/15	15/15
003GP52279	1,500	15/15	15/15	15/15	15/15
029KM27572	2,300	15/15	15/15	15/15	15/15
006LY61030	3,800	15/15	15/15	15/15	15/15
013FX50837	4,200	15/15	15/15	15/15	15/15
053GM60877	5,800	15/15	15/15	15/15	15/15
013GP13645	9,800	15/15	15/15	15/15	15/15
011GS88902	46,000	15/15	15/15	15/15	15/15
054KC25532	81,000	15/15	15/15	15/15	15/15
Total		255/255	255/255	251/255	255/255
% Reactive and CI		100% reactive (98.9-100 CI)	100% reactive (98.9-100 CI)	98.4% reactive (96.0-99.4 CI)	100% reactive (98.9-100 CI)

CI = Confidence Interval
Discrepant results are in **BOLD**.

Table 23. Discrepant HCV Yield and Follow-Up Testing (Number Reactive/Number Tested)

Sample	Ultrio		Ultrio Plus		Ultrio Plus Retest		
	Neat	1:16	Neat	1:16	Neat	1:16	1:16*
033GE77315	2/3	0/3	1/3	3/3	0/3	3/3	0/3

*Results after testing a new 1:16 dilution.

Table 24. Discrepant HBV Yield Results Summary (Number Reactive/Number Tested)

Sample	Ultrio			Ultrio Plus		
	Neat	1:8	1:16	Neat	1:8	1:16
003FQ75130P	3/3	0/3	0/3	3/3	**3/3**	**3/3**
011KC34573P	3/3	0/3	1/3	3/3	**2/3**	1/3
042FM54241P	3/3	2/3	1/3	3/3	**1/3**	**2/3**
055N 30971P	3/3	2/3	1/3	3/3	**3/3**	**3/3**
W036809309033P	3/3	2/3	1/3	3/3	**3/3**	**2/3**
W036809309764P	0/3	0/3	1/3	**1/3**	0/3	**0/3**
W036810026839P	3/3	1/3	1/3	3/3	**3/3**	1/3
W036810154240P	3/3	3/3	1/3	3/3	3/3	**3/3**

Discrepant results are in **BOLD**.

HIV-1 Detection of HIV-1 Ab Confirmed Positive Samples

Of the 292 HIV-1 Antibody confirmed positive samples tested in single replicates, 254 samples were reactive with the Procleix Ultrio Assay (87.0%) and 258 were reactive with the Procleix Ultrio Plus Assay (88.4%). Although slightly more samples were detected with the Procleix Ultrio Plus Assay, the difference was not statistically significant (p=0.61, Table 25).

HCV Detection of HCV Ab Confirmed Positive Samples

A total of 500 and 490 HCV Ab confirmed positive samples were tested in single replicates with the Procleix Ultrio and Procleix Ultrio Plus Assays, respectively. The rates of detection by the Procleix Ultrio Assay and the Procleix Ultrio Plus Assay were very similar, with a total of 392 samples reactive with the Ultrio Assay (78.4%) and 387 samples reactive with the Procleix Ultrio Plus Assay (79.0%). This did not represent a statistically significant difference in detection rate (p=0.82, Table 25).

HBV Detection of HBsAg Confirmed Positive Samples

606 HBsAg confirmed positive samples were tested in single replicates. 489 were reactive with the Procleix Ultrio Assay (80.7%) and 552 samples were reactive with the Procleix Ultrio Plus Assay (91.1%). The increased detection rate of the Procleix Ultrio Plus Assay compared to the Procleix Ultrio Assay in this population was statistically significant (p<0.0001, Table 25).

Anti-HBc Reactive /HBsAg Negative Samples

Of the 750 anti-HBc reactive/HBsAg negative samples tested in single replicates, 27 samples were reactive with the Procleix Ultrio Assay (3.6%) and 46 samples were reactive with the Procleix Ultrio Plus Assay (6.1%). The increased sensitivity of the Procleix Ultrio Plus Assay resulted in a statistically significant difference in assay sensitivity compared to the Procleix Ultrio Assay (p=0.02, Table 25) in this population.

Table 25. Confirmed Positive and Anti-HBc Reactive/ HBsAg Negative Specimen Summary of Reactivity

Description of Specimen	Ultrio	Ultrio Plus	p-value*
HIV-1 Ab	254/292 87.0% (82.6-90.4)	258/292 88.4% (84.2-91.5)	0.61
HCV Ab	392/500 78.4% (74.6-81.8)	387/490 79.0% (75.2-82.4)	0.82
HBsAg	489/606 80.7% (77.4-83.6)	552/606 91.1% (88.6-93.1)	**<0.0001**
Anti-HBc Reactive/ HBsAg Negative	27/750 3.6% (2.5-5.2)	46/750 6.1% (4.6-8.1)	**0.02**

*Chi-Square Analysis, SAS v9.2.
95% CI was calculated using the SCORE method.
Significant differences are in **BOLD**.

The Procleix Ultrio and Procleix Ultrio Plus Assays demonstrated comparable detection of HIV-1 yield samples and HIV-1 Ab-positive samples. Comparable detection was also demonstrated for HCV yield samples and HCV Ab-positive samples. For HBV yield samples initially identified by testing with the Procleix Ultrio Assay, substantially more replicates were detected with the Procleix Ultrio Plus Assay but the increased detection did not reach the level of statistical significance possibly due to the low number of samples and replicates tested. However, significantly better detection by the Procleix Ultrio Plus Assay was observed with samples that were initially identified as HBsAg-positive ($p < 0.0001$) or Anti-HBc-reactive/HBsAg negative ($p=0.02$).

Overall, these results show that the Procleix Ultrio Plus Assay has comparable detection rates for HIV-1 and HCV and significantly better detection of HBV when compared to the Procleix Ultrio Assay.

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

Results obtained from Procleix Ultrio Plus Assay donor screening on the Procleix TIGRIS System at 1 in-house testing site and from serologic testing at 1 laboratory allow comparison of the Procleix Ultrio Plus Assay with HIV-1, HCV, and HBsAg screening and confirmatory test reactivity (Table 26). All of the samples included in this analysis were confirmed seropositive (screening test repeat reactive and confirmatory test reactive); there were no repeat reactive samples with nonreactive or indeterminate confirmatory results tested with the Procleix Ultrio Plus Assay.

Procleix Ultrio Plus Assay and HIV-1 serology results were available for 292 samples. Agreement between Procleix Ultrio Plus Assay and Western blot or immunofluorescent assay positive results was 88.4% (258/292; 95% CI: 84.2% to 91.5%). Of the 292 confirmed seropositive samples, 34 samples were Procleix Ultrio Plus Assay nonreactive for HIV-1 and 258 samples were Procleix Ultrio Plus Assay reactive for HIV-1. Therefore, when a sample is repeat reactive on a licensed anti-HIV-1 screening test and Procleix Ultrio Plus Assay reactive, the Procleix Ultrio Plus Assay acts as a supplemental test that confirms HIV-1 infection; it is not necessary to run an HIV-1 western blot or immunofluorescent assay.

Procleix Ultrio Plus Assay and HCV serology results were available for 490 samples. Agreement between Procleix Ultrio Plus Assay and recombinant immunoblot assay positive results was 79.0% (387/490; 95% CI: 75.2% to 82.4%). Of the 490 confirmed seropositive samples, 103 samples were Procleix Ultrio Plus Assay nonreactive for HCV and 387 samples were Procleix Ultrio Plus Assay reactive for HCV. Approximately 20% of RIBA positive samples will have undetectable HCV RNA due to a resolved HCV infection[41]. Therefore, when a sample is repeat reactive on a licensed anti-HCV screening test and Procleix Ultrio Plus Assay reactive, the Procleix Ultrio Plus Assay acts as a supplemental test that confirms HCV infection; it is not necessary to run an HCV recombinant immunoblot assay.

Procleix Ultrio Plus Assay and HBsAg serology results were available for 606 samples. Agreement between Procleix Ultrio Plus Assay and HBsAg neutralization test positive results was 91.1% (552/606; 95% CI: 88.6% to 93.1%). Of the 606 samples, 54 samples were Procleix Ultrio Plus Assay nonreactive for HBV and 552 samples were Procleix Ultrio Plus Assay reactive for HBV. The results for the 54 samples that were HBsAg neutralization test reactive and Procleix Ultrio Plus Assay nonreactive are not unexpected, as HBsAg may be present in particles that do not contain nucleic acids[44] or after vaccination with a vaccine derived from HBsAg. Therefore, when a sample is repeat reactive on a licensed HBsAg screening test and reactive on the Procleix Ultrio Plus Assay, the Procleix Ultrio Plus Assay acts as a supplemental test that confirms HBV infection; it is not necessary to run an HBsAg neutralization test.

Table 26. Comparison of HIV-1, HCV, and HBsAg Serology and Procleix Ultrio Plus Assay Results

Serology		Procleix Ultrio Plus Assay	
Specimen Type	**N**	**Reactive**	**Nonreactive**
HIV-1 Screening Test RR and WB or IFA Reactive*	292	258	34
HCV Screening Test RR and RIBA Reactive*	490	387	103
HBsAg Screening Test RR and Neutralization Test Reactive*	606	552	54

IFA = immunofluorescent assay, RIBA = recombinant immunoblot assay, RR = repeatedly reactive, WB = Western blot

* Only WB, IFA, RIBA, and Neutralization Test Reactives have been tested in this study. Testing of repeat reactive samples that are indeterminate or negative has not been performed.

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

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

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

HIV-1 specimens and tissue culture isolates of group M (subtypes A, B, C, D, E, F, G and H), 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 HIV-1/HCV/HBV NAT negative human serum to target viral concentrations of 300, 100, and 30 copies/mL. Three specimens had insufficient volume to test at the 300c/mL level, and so were only tested at 100 and 30c/mL. Diluted specimens were tested in the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HIV-1 Discriminatory (dHIV-1) Assay. Fifty-six unique specimens or isolates were tested in duplicate using two pilot lots of reagents on the Procleix TIGRIS System. Three additional specimens were co-infected with HCV and/or HBV and were therefore only tested in the Procleix Ultrio Plus dHIV-1 Assay. At 300 copies/mL, 216/216 replicates (100%) were reactive with the Procleix Ultrio Plus Assay and 224/224 replicates (100%) were reactive with the Procleix Ultrio Plus dHIV-1 Assay. At 100 copies/mL, 223/224 replicates (99.6%) were reactive with the Procleix Ultrio Plus Assay and 236/236 replicates (100%) were reactive with the Procleix Ultrio Plus dHIV-1 Assay. At 30 copies/mL, 212/224 replicates (94.6%) were reactive with the Procleix Ultrio Plus Assay and 227/236 replicates (96.2%) were reactive with the Procleix Ultrio Plus dHIV-1 Assay (Table 27). All specimens yielded valid results upon initial testing.

Table 27. Detection of HIV-1 Genetic Variants

Genetic Variant	Copies / mL	Procleix Ultrio Plus Assay			Procleix Ultrio Plus HIV-1 Discriminatory Assay		
		Unique Donors	Reactive / Tested	% Reactive	Unique Donors	Reactive / Tested	% Reactive
HIV-1 Group M Subtype A	300	10	40/40	100	11	44/44	100
	100		40/40	100		44/44	100
	30		40/40	100		41/44	93.2
HIV-1 Group M Subtype B	300	8	32/32	100	9	36/36	100
	100		32/32	100		36/36	100
	30		31/32	96.9		36/36	100
HIV-1 Group M Subtype C	300	8	32/32	100	8	32/32	100
	100		32/32	100		32/32	100
	30		31/32	96.9		32/32	100
HIV-1 Group M Subtype D	300	7	28/28	100	7	28/28	100
	100		28/28	100		28/28	100
	30		26/28	92.9		25/28	89.3
HIV-1 Group M Subtype E	300	9	36/36	100	9	36/36	100
	100		35/36	97.2		36/36	100
	30		28/36	77.8		34/36	94.4
HIV-1 Group M Subtype F	300	5	20/20	100	5	20/20	100
	100		20/20	100		20/20	100
	30		20/20	100		19/20	95.0
HIV-1 Group M Subtype G*	300	1	4/4	100	2	4/4	100
	100		4/4	100		8/8	100
	30		4/4	100		8/8	100
HIV-1 Group M Subtype H	300	1	4/4	100	1	4/4	100
	100		4/4	100		4/4	100
	30		4/4	100		4/4	100
HIV-1 Group N	300	1	4/4	100	1	4/4	100
	100		4/4	100		4/4	100
	30		4/4	100		4/4	100
HIV-1 Group O*	300	6	16/16	100	6	16/16	100
	100		24/24	100		24/24	100
	30		24/24	100		24/24	100
All Genotypes	300	56	216/216	100	59	224/224	100
	100		223/224	99.6		236/236	100
	30		212/224	94.6		227/236	96.2

* Insufficient volume to test all specimens at 300 copies/mL.

Detection of HCV Genotypes with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus 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 HIV-1/HCV/HBV NAT negative human serum to target viral concentrations of 300, 100, and 30 copies/mL. The diluted specimens were tested with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HCV Discriminatory (dHCV) Assay. Sixty-one unique specimens were tested in duplicate using two pilot lots of reagents on the Procleix TIGRIS System. One additional specimen was co-infected with HIV-1 and HBV and was therefore only tested in the Procleix Ultrio Plus dHCV Assay. At 300 copies/mL, 243/244 replicates (99.6%) were reactive with the Procleix Ultrio Plus Assay and 248/248 replicates (100%) were reactive with the Procleix Ultrio Plus dHCV Assay. At 100 copies/mL, 241/244 replicates (98.8%) were reactive with the Procleix Ultrio Plus Assay and 246/248 replicates (99.2%) were reactive with the Procleix Ultrio Plus dHCV Assay. At 30 copies/mL, 229/244 replicates (93.9%) were reactive with the Procleix Ultrio Plus Assay and 234/248 replicates (94.4%) were reactive with the Procleix Ultrio Plus dHCV Assay (Table 28). Specimens that were initially invalid were retested; all specimens were valid upon retest, and only the retest result is included in the data analysis.

Table 28. Detection of HCV Genotypes

Genotype	Copies / mL	Procleix Ultrio Plus Assay			Procleix Ultrio Plus HCV Discriminatory Assay		
		Unique Donors	Reactive / Tested	% Reactive	Unique Donors	Reactive / Tested	% Reactive
HCV Genotype 1	300	11	44/44	100	11	44/44	100
	100		44/44	100		44/44	100
	30		44/44	100		43/44	97.7
HCV Genotype 2	300	13	51/52	98.1	14	56/56	100
	100		**49/52**	**94.2**		54/56	96.4
	30		42/52	80.8		44/56	78.6
HCV Genotype 3	300	12	48/48	100	12	48/48	100
	100		48/48	100		48/48	100
	30		45/48	93.8		48/48	100
HCV Genotype 4	300	14	56/56	100	14	56/56	100
	100		56/56	100		56/56	100
	30		55/56	98.2		55/56	98.2
HCV Genotype 5	300	6	24/24	100	6	24/24	100
	100		24/24	100		24/24	100
	30		24/24	100		24/24	100
HCV Genotype 6	300	5	20/20	100	5	20/20	100
	100		20/20	100		20/20	100
	30		19/20	95.0		20/20	100
Total	300	61	243/244	99.6	62	248/248	100
	100		241/244	98.8		246/248	99.2
	30		229/244	93.9		234/248	94.4

Note: Bolded text indicates reactive rates less than 95% for specimens at or above 100 copies/mL.

Detection of HBV Genotypes with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus 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 HIV-1/HCV/HBV NAT negative human serum to target viral concentrations of 300, 100, and 30 copies/mL. Diluted specimens were tested with the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HBV Discriminatory (dHBV) Assay. Fifty-eight unique specimens were tested in duplicate using two pilot lots of reagents on the Procleix TIGRIS System. At 300 copies/mL, 228/228 replicates (100%) were reactive with the Procleix Ultrio Plus Assay and 227/228 replicates (99.6%) were reactive with the Procleix Ultrio Plus dHBV Assay. At 100 copies/mL, 230/232 replicates (99.1%) were reactive with the Procleix Ultrio Plus Assay and 231/232 replicates (99.6%) were reactive with the Procleix Ultrio Plus dHBV Assay. At 30 copies/mL, 220/232 replicates (94.8%) were reactive with the Procleix Ultrio Plus Assay and 228/232 replicates (98.3%) were reactive with the Procleix Ultrio Plus dHBV Assay (Table 29). Specimens that were initially invalid were retested; all specimens were valid upon retest, and only the retest result is included in the data analysis.

Table 29. Detection of HBV Genotypes

Genotype	Copies / mL	Procleix Ultrio Plus Assay			Procleix Ultrio Plus HBV Discriminatory Assay		
		Unique Donors	Reactive / Tested	% Reactive	Unique Donors	Reactive / Tested	% Reactive
HBV Genotype A	300	12	48/48	100	12	47/48	97.9
	100		46/48	95.8		47/48	97.9
	30		38/48	79.2		44/48	91.7
HBV Genotype B	300	10	40/40	100	10	40/40	100
	100		40/40	100		40/40	100
	30		40/40	100		40/40	100
HBV Genotype C	300	10	40/40	100	10	40/40	100
	100		40/40	100		40/40	100
	30		40/40	100		40/40	100
HBV Genotype D	300	9	36/36	100	9	36/36	100
	100		36/36	100		36/36	100
	30		36/36	100		36/36	100
HBV Genotype E*	300	8	28/28	100	8	28/28	100
	100		32/32	100		32/32	100
	30		31/32	96.9		32/32	100
HBV Genotype F	300	8	32/32	100	8	32/32	100
	100		32/32	100		32/32	100
	30		31/32	96.9		32/32	100
HBV Genotype G	300	1	4/4	100	1	4/4	100
	100		4/4	100		4/4	100
	30		4/4	100		4/4	100
All Genotypes	300	58	228/228	100	58	227/228	99.6
	100		230/232	99.1		231/232	99.6
	30		220/232	94.8		228/232	98.3

* Insufficient volume to test all specimens at 300 copies/mL.

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

Cadaveric and living (control) donor blood specimens were tested to determine the specificity and sensitivity of the Procleix Ultrio Assay and the Procleix Ultrio HIV-1, HCV and HBV Discriminatory (dHIV-1, dHCV and dHBV) Assays (Tables 30a, 31a, 32a and 33a). To confirm the similar performance of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HIV-1, HCV and HBV Discriminatory (dHIV-1, dHCV and dHBV) Assays, testing was performed on a smaller number of cadaveric blood specimens (Tables 30b, 31b, 32b and 33b).

SPECIFICITY

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

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%). The specificity of the dHCV Assay for the cadaveric specimens was 98% (95% confidence interval: 89%-100%) (Table 30a). Specificity rates were calculated from all valid initial results.

Table 30a. Specificity of Procleix Ultrio Assay and Procleix Ultrio 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 Ultrio 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 Ultrio 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 Ultrio 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
N = Number of samples
CI = Confidence Interval
IC = Internal Control
S/CO = Signal to Cutoff ratio

Specificity of Procleix Ultrio Plus Assay and Procleix Ultrio Plus Discriminatory Assays in Cadaveric Blood Specimens

HIV-1, HCV and HBV seronegative cadaveric blood specimens were tested to determine the specificity of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HIV-1, HCV and HBV Discriminatory (dHIV-1, dHCV, and dHBV) Assays. Thirty-two cadaveric specimens were tested using two reagent lots. The specificity of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HIV-1, HCV, and HBV Discriminatory Assays for the cadaveric specimens was 100% (95% confidence interval: 94%-100%). (Table 30b).

Table 30b. Specificity of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus Discriminatory Assays in Cadaveric Blood Specimens

		Cadaveric
Procleix Ultrio Plus Assay	Mean IC S/CO	2.10
	Mean Analyte S/CO	0.09
	Specificity Rate (%)	100
	95% CI, Specificity Rate	89.3-100
	N*	32
Procleix Ultrio Plus dHIV-1 Assay	Mean IC S/CO	2.01
	Mean Analyte S/CO	0.13
	Specificity Rate (%)	100
	95% CI, Specificity Rate	89.3-100
	N*	32
Procleix Ultrio Plus dHCV Assay	Mean IC S/CO	2.02
	Mean Analyte S/CO	0.12
	Specificity Rate (%)	100
	95% CI, Specificity Rate	89.3-100
	N*	32
Procleix Ultrio Plus dHBV Assay	Mean IC S/CO	2.03
	Mean Analyte S/CO	0.10
	Specificity Rate (%)	100
	95% CI, Specificity Rate	89.3-100
	N*	32

N = Number of specimens
CI = Confidence Interval
IC = Internal Control
S/CO = Signal to Cutoff ratio
* Sixteen unique cadaveric plasma specimens and 16 unique cadaveric serum specimens were each tested in singlet. Testing was done using 2 reagent lots.

SENSITIVITY

Sensitivity of the Procleix Ultrio Assay and Procleix Ultrio Discriminatory Assays and the Procleix Ultrio Plus Assay and Procleix Ultrio Plus Discriminatory Assays in Cadaveric Blood Specimens

Procleix Ultrio Assay - Sensitivity for Detection of HIV-1

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 Procleix Ultrio dHIV-1 Assay. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots after spiking each with approximately 200 copies/mL of HIV-1. The positivity rate of the Procleix Ultrio Assay and Procleix Ultrio dHIV-1 Assay for the cadaveric specimens was 100% (95% confidence interval: 93%-100%) (Table 31a). Detection rates were calculated from valid initial results.

Table 31a. Reactivity of Procleix Ultrio Assay and Procleix Ultrio 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 (% pos)	93-100	93-100
	N	49	52
Procleix Ultrio dHIV-1 Assay	Mean IC S/CO	1.84	2.02
	Mean Analyte S/CO	20.28	21.28
	% positive	100	100
	95% CI (% pos)	93-100	93-100
	N	51	50

N = Number of samples
CI = Confidence Interval
S/CO = Signal to Cutoff ratio

Procleix Ultrio Plus Assay - Sensitivity for Detection of HIV-1

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 Plus Assay and the Procleix Ultrio Plus HIV-1 Discriminatory (dHIV-1) Assay. Sixteen cadaveric specimens were tested in duplicate using two reagent lots after spiking each specimen with approximately 150 copies/mL of HIV-1. The reactivity rate of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHIV-1 Assay for the cadaveric specimens was 100% (95% confidence interval: 89.3%-100%) (Table 31b).

Table 31b. Reactivity of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HIV-1 Discriminatory Assay in Cadaveric Blood Specimens Spiked with HIV-1

		Cadaveric
Procleix Ultrio Plus Assay	Mean Analyte S/CO	9.45
	Reactive Rate (%)	100
	95% CI, Reactive Rate	89.3-100
	N*	32
Procleix Ultrio Plus dHIV-1 Assay	Mean Analyte S/CO	16.38
	Reactive Rate (%)	100
	95% CI, Reactive Rate	89.3-100
	N*	32

N = Number of samples
CI = Confidence Interval
S/CO = Signal to Cutoff ratio
* Eight unique cadaveric plasma specimens and 8 unique cadaveric serum specimens were each tested in duplicate. Testing was done using 2 reagent lots.

Procleix Ultrio Assay - Sensitivity for Detection of HCV

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 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%) (Table 32a). Detection rates were calculated from valid initial results.

Table 32a. Reactivity of Procleix Ultrio Assay and Procleix Ultrio 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 (% pos)	93-100	93-100
	N	50	50
Procleix Ultrio dHCV Assay	Mean IC S/CO	1.88	1.91
	Mean Analyte S/CO	23.21	23.32
	% positive	100	100
	95% CI (% pos)	93-100	93-100
	N	50	50

N = Number of samples
CI = Confidence Interval
S/CO = Signal to Cutoff ratio

Procleix Ultrio Plus Assay - Sensitivity for Detection of HCV

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 Plus Assay and the Procleix Ultrio Plus HCV Discriminatory (dHCV) Assay. Sixteen cadaveric specimens were tested in duplicate using two reagent lots after spiking each specimen with approximately 150 copies/mL of HCV. The reactivity rate of both the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHCV Assay for the cadaveric specimens was 100% (95% confidence interval: 89.3%-100%) (Table 32b).

Table 32b. Reactivity of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HCV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HCV

		Cadaveric
Procleix Ultrio Plus Assay	Mean Analyte S/CO	8.31
	Reactive Rate (%)	100
	95% CI, Reactive Rate	89.3-100
	N*	32
Procleix Ultrio Plus dHCV Assay	Mean Analyte S/CO	22.21
	Reactive Rate (%)	100
	95% CI, Reactive Rate	89.3-100
	N*	32

N = Number of samples
CI = Confidence Interval
S/CO = Signal to Cutoff ratio
* Eight unique cadaveric plasma specimens and 8 unique cadaveric serum specimens were each tested in duplicate. Testing was done using 2 reagent lots.

Procleix Ultrio Assay - Sensitivity for Detection of HBV

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 after spiking each with approximately 30 IU/mL of HBV. The positivity rate of the Procleix Ultrio Assay and the dHBV Assay was 96% (95% confidence interval: 88%-99%) (Table 33a). Detection rates were calculated from valid initial results.

Table 33a. Reactivity of Procleix Ultrio Assay and Procleix Ultrio 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 Ultrio dHBV Assay	Mean IC S/CO	2.06	2.17
	Mean Analyte S/CO	22.62	23.54
	% positive	84	96
	95% CI (% pos)	74-92	88-99
	N	70	70

N = Number of samples
CI = Confidence Interval
S/CO = Signal to Cutoff ratio
* Included serum and plasma specimens

Procleix Ultrio Plus Assay - Sensitivity for Detection of HBV

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 Plus Assay and the Procleix Ultrio Plus HBV Discriminatory (dHBV) Assay. Sixteen cadaveric specimens were tested in duplicate using two reagent lots after spiking each specimen with approximately 15 IU/mL of HBV. The reactivity rate of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus dHBV Assay for the cadaveric specimens was 100% (95% confidence interval: 89.3%-100%) (Table 33b).

Table 33b. Reactivity of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HBV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HBV

		Cadaveric
Procleix Ultrio Plus Assay	Mean Analyte S/CO	14.10
	Reactive Rate (%)	100
	95% CI, Reactive Rate	89.3-100
	N*	32
Procleix Ultrio Plus dHBV Assay	Mean Analyte S/CO	22.82
	Reactive Rate (%)	100
	95% CI, Reactive Rate	89.3-100
	N*	32

N = Number of samples
CI = Confidence Interval
S/CO = Signal to Cutoff ratio
* Eight unique cadaveric plasma specimens and 8 unique cadaveric serum specimens were each tested in duplicate. Testing was done using 2 reagent lots.

REPRODUCIBILITY

Reproducibility of the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HIV-1, HCV, and HBV Discriminatory Assays on the Procleix TIGRIS System was evaluated. For determination of the reproducibility of each assay, 7 positive quality control panels were tested as individual specimens (Tables 34-37). Six of the panel members were positive for HIV-1 RNA (100 and 30 copies/mL), HCV RNA (100 and 30 copies/mL), or HBV DNA (32 and 11 IU/mL), and 1 panel member was HIV-1, HCV and HBV negative.

The reproducibility panels were tested by a total of 3 operators with 3 different pilot lots and 3 Procleix TIGRIS instruments over multiple days. Nine valid runs were generated with the Procleix Ultrio Plus Assay and each Procleix Ultrio Plus Discriminatory Assay. In the Procleix Ultrio Plus Assay, each of the 7 panel members were tested in 360 replicates (120 replicates for each lot, and 40 replicates on each day). In each of the Procleix Ultrio Plus Discriminatory Assays, each of the 6 positive panel members were tested in 360 replicates, and the negative panel member was tested in 108 replicates.

Invalid runs were retested, while invalid results for panel members in valid worklists runs were not retested. On 1 day on 1 Procleix TIGRIS instrument, an operation failure occurred which invalidated 1 queued worklist of the Procleix Ultrio Plus Assay, as indicated below. This run was repeated to yield a valid result. The validity data for each assay is described below:

For the Procleix Ultrio Plus Assay, 9 worklists were generated on the Procleix TIGRIS System: 1 was invalidated by a system failure, and was repeated to give an invalid run rate of 1/10 (10%). From the valid assay worklists, 2,520 test results were generated: none were invalid.

For the Procleix Ultrio Plus HIV-1 Discriminatory Assay, 9 worklists were generated on the Procleix TIGRIS System. From the valid assay worklists, 828 test results were generated: none were invalid.

For the Procleix Ultrio Plus HCV Discriminatory Assay, 9 worklists were generated on the Procleix TIGRIS System. From the valid assay worklists, 828 test results were generated: none were invalid.

For the Procleix Ultrio Plus HBV Discriminatory Assay, 9 worklists were generated on the Procleix TIGRIS System. From the valid assay worklists, 828 test results were generated: 5 (0.6%) were invalid when a reagent addition error was detected.

Reproducibility analyses included evaluation of percent agreement and mean Signal to Cutoff (S/CO) ratios for panel members, evaluation of 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 the S/CO ratios and RLU values for each of the five variance factors (Tables 34-37). The mean analyte S/CO ratios were analyzed for the positive and negative 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 and Negative 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 the analyte S/CO for all panel members. For the Procleix Ultrio Plus and the Procleix Ultrio Plus Discriminatory Assays, results for each panel member are shown individually.

For the Procleix Ultrio Plus Assay and the 3 Procleix Ultrio Plus Discriminatory Assays, the overall percent agreement of test results was 93.1 to 100% for positive panel members and 100% for the negative panel member. With regard to signal variability, intra-assay (or random error) and inter-instrument factors, in most cases, were the largest and second largest contributors to total variance (according to SD values) in the Procleix Ultrio Plus Assay and the Procleix Ultrio Plus HIV-1, HCV, and HBV Discriminatory Assays. It should be noted that while these factors were responsible for the majority of the variance in the assays, the total %CV did not exceed 6.8% for any positive panel members at 100 copies/mL (HIV-1 and HCV) or 32 IU/mL (HBV), and did not exceed 25.5% for any positive panel members at 30 copies/mL (HIV-1 and HCV) or 11 IU/mL (HBV), in any assay (Tables 34-37).

Table 34. Reproducibility of the Procleix Ultrio Plus Assay (analysis of analyte signals, unless noted)***

Sample	Titer*	N	Agreement (%)	Mean S/CO	Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Day		Intra-Run	
					SD	% CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative, IC**	0	360	100%	2.11	0.00	0.0	0.05	2.4	0.00	0.0	0.04	1.9	0.10	4.7
Negative, Analyte				0.09	0.02	22.2	0.00	0.0	0.00	0.0	0.00	0.0	0.04	44.4
HIV-1	100	360	100%	11.90	0.40	3.4	0.00	0.0	0.13	1.1	0.32	2.7	0.81	6.8
	30	360	98.1%	10.32	0.38	3.7	0.21	2.0	0.00	0.0	0.00	0.0	2.27	22.0
HCV-1a	100	360	100%	8.70	0.25	2.9	0.15	1.7	0.06	0.7	0.13	1.5	0.28	3.2
	30	360	95.8%	8.60	0.32	3.7	0.20	2.3	0.00	0.0	0.14	1.6	0.50	5.8
HBV A	32	360	100%	14.63	0.64	4.4	0.30	2.1	0.55	3.8	0.29	2.0	0.37	2.5
	11	360	99.7%	14.56	0.60	4.1	0.29	2.0	0.56	3.9	0.31	2.1	0.38	2.6

Sample	N	Agreement (%)	Mean RLU	Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Day		Intra-Run	
				SD	% CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator, IC**	27	N/A	151,240	2,950	2.0	2,180	1.4	13,300	8.8	2,680	1.8	5,340	3.5
Negative Calibrator, Analyte			10,490	3,240	30.9	170	1.6	0	0.0	0	0.0	5,070	48.3
HIV-1 Positive Calibrator	18	N/A	1,070,200	57,340	5.4	21,480	2.0	4,330	0.4	31,910	3.0	29,910	2.8
HCV Positive Calibrator	18	N/A	764,310	41,400	5.4	8,260	1.1	8,700	1.1	0	0.0	25,200	3.3
HBV Positive Calibrator	18	N/A	1,299,600	60,590	4.7	0	0.0	50,450	3.9	0	0.0	41,560	3.2

N = Number of panel members combined for this analysis; S/CO = Signal to Cutoff ratio; IC = Internal Control

* 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 35. Reproducibility of the Procleix Ultrio Plus HIV-1 Discriminatory Assay (analysis of analyte signal, unless noted)***

Sample	Titer*	N	Agreement (%)	Mean S/CO	Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Day		Intra-Run	
					SD	% CV	SD	% CV	SD	% CV	SD	%CV	SD	% CV
Negative, IC**	0	108	100%	2.07	0.00	0.0	0.05	2.4	0.00	0.0	0.03	1.5	0.09	4.4
Negative, Analyte				0.14	0.03	21.4	0.00	0.0	0.00	0.0	0.00	0.0	0.07	50.0
HIV-1	100	360	100%	21.30	1.13	5.3	0.55	2.6	0.00	0.0	0.26	1.2	0.93	4.4
	30	360	98.9%	18.02	0.94	5.2	0.00	0.0	0.00	0.0	0.84	4.7	4.61	25.5
Sample		**N**	**Agreement (%)**	**Mean RLU**	**Inter-Instrument**		**Inter-Operator**		**Inter-Lot**		**Inter-Day**		**Intra-Run**	
					SD	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**%CV**	**SD**	**% CV**
Negative Calibrator, IC**		27	N/A	149,300	0	0.0	2,300	1.5	8,370	5.6	4,000	2.7	4,890	3.3
Negative Calibrator, Analyte				7,570	3,080	40.6	1,590	21.0	1,120	14.7	0	0.0	2,530	33.4
HIV-1 Positive Calibrator		27	N/A	1,103,000	39,370	3.6	14,310	1.3	22,360	2.0	0	0.0	31,900	2.9

N = Number of panel members combined for this analysis; S/CO = Signal to Cutoff ratio; IC = Internal Control

* 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 36. Reproducibility of the Procleix Ultrio Plus HCV Discriminatory Assay (analysis of analyte signal, unless noted)***

Sample	Titer*	N	Agreement (%)	Mean S/CO	Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Day		Intra-Run	
					SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Negative, IC**	0	108	100	2.04	0.00	0.0	0.02	1.0	0.01	0.5	0.04	2.0	0.11	5.4
Negative, Analyte				0.1	0.03	30.0	0.01	10.0	0.02	20.0	0.01	10.0	0.06	60.0
HCV-1a	100	360	100	23.46	1.03	4.4	0.00	0.0	0.69	2.9	0.44	1.9	0.79	3.4
	30	360	93.1	22.39	1.26	5.6	0.19	0.9	0.84	3.8	0.00	0.0	2.93	13.0
Sample		**N**	**Agreement (%)**	**Mean RLU**	**Inter-Instrument**		**Inter-Operator**		**Inter-Lot**		**Inter-Day**		**Intra-Run**	
					SD	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**
Negative Calibrator, IC**		27	N/A	147,200	3,750	2.6	2,120	1.4	7,030	4.8	2,560	1.7	6,470	4.4
Negative Calibrator, Analyte				4,370	2,460	56.3	0	0.0	1,190	27.2	850	19.4	1,530	35.0
HCV Positive Calibrator		27	N/A	1,223,000	51,940	4.3	0	0.0	47,460	3.9	8,570	0.7	24,620	2.0

N = Number of panel members combined for this analysis; S/CO = Signal to Cutoff ratio; IC = Internal Control

* 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 37. Reproducibility of the Procleix Ultrio Plus HBV Discriminatory Assay (analysis of analyte signals, unless noted)***

Sample	Titer*	N	Agreement (%)	Mean S/CO	Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Day		Intra-Run	
					SD	% CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative, IC**	0	108	100	2.06	0.04	1.9	0.03	1.5	0.00	0.0	0.00	0.0	0.12	5.8
Negative, Analyte				0.07	0.02	28.6	0.00	0.0	0.00	0.0	0.00	0.0	0.05	71.4
HBV A	32	360	100	23.15	1.05	4.5	0.39	1.7	0.00	0.0	0.28	1.2	0.51	2.2
	11	355	100	23.01	1.03	4.5	0.37	1.6	0.00	0.0	0.30	1.3	0.59	2.6

Sample	N	Agreement (%)	Mean RLU	Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Day		Intra-Run	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator, IC**	27	N/A	149,600	5,400	3.6	1,540	1.0	0	0.0	0	0.0	6,090	4.1
Negative Calibrator, Analyte			5,220	3,100	59.4	320	6.1	0	0.0	0	0.0	3,180	60.9
HBV Positive Calibrator	27	N/A	1,436,000	42,020	2.9	0	0.0	8,580	0.6	13,250	0.9	39,900	2.8

N = Number of panel members combined for this analysis; S/CO = Signal to Cutoff ratio; IC = Internal Control

* 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.

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502432 Rev. A
2012-07

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U.S. patent no. 5,656,207, 5,658,737, 5,696,251, 5,714,596, 5,756,709, 5,780,219, 5,840,873, 6,074,816, 6,090,591, 6,245,519, 6,623,920, and 7,097,979.