Procleix® Ultrio Elite Assay

For *In Vitro* Diagnostic Use

IVD

Rx Only

1000 Test Kit, 5000 Test Kit

CONTENTS

INTENDED USE

The Procleix® Ultrio Elite Assay is a qualitative *in vitro* nucleic acid amplification test to screen for human immunodeficiency virus type 1 (HIV-1) RNA, hepatitis C virus (HCV) RNA, and/or hepatitis B virus (HBV) DNA, and detect human immunodeficiency virus type 2 (HIV-2) RNA 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 to screen organ and tissue donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens from cadaveric (non-heart-beating) donors. This assay is not intended for use on cord blood specimens.

It is also intended for use in testing pools of human plasma and pools of human serum composed of equal aliquots of not more than 16 individual specimens from donors of whole blood, blood components, hematopoietic stem/progenitor cells sourced from bone marrow, peripheral blood or cord blood, and from donors of donor lymphocytes for infusion. It is also intended for use in testing pools of human plasma composed of equal aliquots of not more than 96 individual donations from donors of source plasma. This assay is intended to be used in conjunction with licensed tests for detecting antibodies to HIV-1, HIV-2, HCV, and hepatitis B core antigen, 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, HIV-2, HCV or HBV.

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

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

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

SUMMARY AND EXPLANATION OF THE TEST

Epidemiological studies identified HIV-1 and HIV-2 as the etiological agents of acquired immunodeficiency syndrome (AIDS),[1-7] hepatitis C virus (HCV),[8-13] and hepatitis B virus (HBV) as causative agents of transfusion-associated hepatitis.[14] HIV, 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.

HIV-1 and HIV-2 Summary and Explanation

Current detection of HIV-1 infection in the blood bank setting is based on nucleic acid testing (NAT) for HIV RNA detection and serologic screening for anti-viral antibodies with confirmation by additional more specific supplemental antibody tests.[15-18] The addition of NATs has reduced the window period of detection by 6 to 11 days in donations tested individually, significantly reducing the risk of HIV transmission by transfusion.[19-22]

Diagnosed cases of HIV-2 are observed primarily in West Africa or where exposure through immigration or travel has occurred.[23] Assays that detect the antibodies against both HIV-1 and HIV-2 are commonly used for screening blood donations worldwide. HIV-1 and HIV-2 may be discriminated using rapid immunoassays.[24-25] The residual risk for potential HIV-2 transfusion is estimated to be extremely low, but it has not been possible to confirm these estimates directly.[23, 26] Screening for HIV-2 RNA should reduce the risk even further.

HCV Summary and Explanation

Current detection of HCV infection in the blood bank setting is based on NAT for HCV RNA detection[15-17] and serologic screening for anti-viral antibodies.[27] The introduction of NATs for HCV RNA has allowed detection of HCV infection approximately 59 days earlier than the current antibody-based tests.[22, 15, 28]

HBV Summary and Explanation

Current detection of HBV infection in the blood bank setting is based on NAT for HBV DNA detection[15-17] and serological screening for HBsAg by enzyme immunoassay (EIA) with confirmation by neutralization tests and anti-hepatitis B core antigen (anti-HBc) assays. A model based on HBV doubling time was used to develop an estimate of approximately 38 to 44 days between infection and HBsAg detection using current tests.[29] Studies indicate that NATs for HBV DNA will allow detection of HBV infection several weeks before HBsAg detection.[30-33, 15] NAT with enhanced sensitivity for HBV can detect low levels of HBV DNA in serologically negative samples during early stages of infection and in HBc antibody-positive/HBsAg-negative samples during later stages of infection.

PRINCIPLES OF THE PROCEDURE

The Procleix Ultrio Elite Assay is performed on the fully automated Procleix Panther System.

The Procleix Ultrio Elite Assay involves three main steps which take place in a single tube on the Procleix Panther System: 1) Sample preparation/ target capture 2) HIV RNA, HCV RNA, and HBV DNA target amplification by Transcription-Mediated Amplification (TMA)[34] and 3) Detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[35-36] The Procleix assays incorporate an Internal Control for monitoring assay performance in each individual reaction tube.

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, HCV, and HBV are hybridized to the HIV RNA, HCV RNA, or HBV DNA target, if present, in the test specimen. Target Enhancer Reagent (TER) is added to each reaction tube after the addition of the sample to create a transient alkaline shock which enhances the disruption of the viral particles and denaturation of nucleic acids. Following the addition of TER, 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.

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.

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 and 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/HCV/HBV signal by the differential kinetics of light emission from probes with different labels.[37] Internal Control-specific amplicon is detected using a probe with rapid emission of light (termed a “flasher signal”). Amplicon specific to HIV/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.[37] When used for the simultaneous detection of HIV, HCV, and HBV, the Procleix Ultrio Elite Assay differentiates between Internal Control and combined HIV/HCV/HBV signals but does not discriminate between individual HIV, HCV, and HBV signals.

The Procleix Ultrio Elite Assay Calibrators are used to determine the assay cutoff and assess assay run validity in each run. (See QUALITY CONTROL PROCEDURES for details.)

DISCRIMINATORY TESTING

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

The Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays utilize the same three main steps as the Procleix Ultrio Elite Assay (sample preparation/target capture, TMA and HPA); the same assay procedure is followed with one difference: HIV-specific, HCV-specific, or HBV-specific probe reagents are used in place of the Procleix Ultrio Elite Assay Probe Reagent. The Procleix Ultrio Elite HIV Discriminatory Assay will not distinguish between samples reactive for HIV-1 and those reactive for HIV-2.

REAGENTS

Procleix Ultrio Elite Assay Reagents

Internal Control Reagent
A HEPES buffered solution containing detergent and an RNA transcript.
*Store **unopened reagent** at –35° to –15°C.*

Target Capture Reagent
*A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles. **Note**: Internal Control Reagent must be added to Target Capture Reagent before use in the assay.*
Store at 2° to 8°C. (Do not freeze)

Amplification Reagent
Primers, dNTPs, NTPs, and cofactors in TRIS buffered solution containing ProClin 300 preservative.
*Store **unopened reagent** at –35° to –15°C.*

Enzyme Reagent
MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffered solution containing 0.05% sodium azide as preservative.
*Store **unopened reagent** at –35° to –15°C.*

Probe Reagent
Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.
*Store **unopened reagent** at –35° to –15°C.*

Selection Reagent
Borate buffered solution containing surfactant.
Store at 15° to 30°C.

Target Enhancer Reagent
A concentrated solution of lithium hydroxide.
Store at 15° to 30°C.

Procleix Ultrio Elite Assay Calibrators

C0 **Negative Calibrator**
A HEPES buffered solution containing detergent.
Store at –35° to –15°C.

C1 **HIV Positive Calibrator**
A HEPES buffered solution containing detergent and an HIV RNA transcript.
Store at –35° to –15°C.

C2 **HCV Positive Calibrator**
A HEPES buffered solution containing detergent and an HCV RNA transcript.
Store at –35° to –15°C.

C3 **HBV Positive Calibrator**
A HEPES buffered solution containing detergent and HBV-specific DNA sequences.
Store at –35° to –15°C.

Procleix Ultrio Elite Discriminatory Probe Reagents

D1 **HIV Discriminatory Probe Reagent**
Chemiluminescent oligonucleotide probe in succinate buffered solution containing detergent.
*Store **unopened reagent** at –35° to –15°C.*

D2 **HCV Discriminatory Probe Reagent**
Chemiluminescent oligonucleotide probe in succinate buffered solution containing detergent.
*Store **unopened reagent** at –35° to –15°C.*

D3 **HBV Discriminatory Probe Reagent**
Chemiluminescent oligonucleotide probe in succinate buffered solution containing detergent.
*Store **unopened reagent** at –35° to –15°C.*

Procleix Panther System Reagents

R1	**Auto Detect 1** *Aqueous solution containing hydrogen peroxide and nitric acid.* *Store **unopened reagent** at 15° to 30°C.*
R2	**Auto Detect 2** *1.6 N sodium hydroxide.* *Store **unopened reagent** at 15° to 30°C.*
W	**Wash Solution** *HEPES buffered solution.* *Store **unopened reagent** at 15° to 30°C.*
O	**Oil** *Silicone oil.* *Store **unopened reagent** at 15° to 30°C.*
DF	**Buffer for Deactivation Fluid** *Sodium bicarbonate buffered solution.* *Store **unopened reagent** at 15° to 30°C.*

STORAGE AND HANDLING INSTRUCTIONS

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

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

C. 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.

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

E. Do not refreeze Internal Control, Amplification, Enzyme, Probe, or Procleix Ultrio Elite Discriminatory Probe Reagents after the initial thaw.

F. Negative, HIV, HCV, and HBV Positive Calibrators are single use vials and must be discarded after use. Do not refreeze calibrators after the initial thaw.

G. If precipitate forms in the Wash Solution, Selection Reagent, Target Enhancer Reagent, Probe Reagent, or Procleix Ultrio Elite Discriminatory Probe Reagents, see instructions under REAGENT PREPARATION.

H. 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.

I. Consult the following table for storage information.

Reagent/Fluids	Unopened Reagent	Opened Reagent (Opened/Thawed Stability)*		
	Storage Temperature	Room Temperature	Onboard Stability	Storage Temperature
Internal Control Reagent (IC)	-35° to -15°C	Up to 8 hours at RT prior to combining with TCR		
Target Capture Reagent (TCR)	2° to 8°C			
working Target Capture Reagent (wTCR)		72 hours	60 hours	30 days at 2° to 8°C
Amplification Reagent	-35° to -15°C	72 hours	60 hours	30 days at 2° to 8°C
Enzyme Reagent	-35° to -15°C	72 hours	60 hours	30 days at 2° to 8°C
Probe Reagents	-35° to -15°C	72 hours	60 hours	30 days at 2° to 8°C
Selection Reagent	RT	30 days	60 hours	30 days at RT
Target Enhancer Reagent	RT	30 days	60 hours	30 days at RT
Calibrators	-35° to -15°C	8 hours, single-use reagent		
Auto Detect Reagents	RT	60 days at RT		
Buffer for Deactivation Fluid	RT	60 days at RT		
Oil	RT	60 days at RT		
Wash Solution	RT	60 days at RT		

RT = Room Temperature

RT stability includes onboard stability time on the Procleix Panther System.

- The RT stability period starts as soon as the reagents are removed from the RPI 250 after the preparation program is completed.
- If opened reagents are placed in the RPI 250 at the room temperature program, the time duration is included in the total RT stability.
- The RT stability time must occur within 30 days, which includes onboard stability. See REAGENT PREPARATION, Item C for more information.

* If using Panther System Software version 7.2 and higher:

- RT stability (wTCR and Amplification, Enzyme, and Probe Reagents) is 84 hours.
- Onboard stability (wTCR: Amplification, Enzyme, and Probe Reagents; Selection Reagent; and TER) is 72 hours.

If using RPI 250 File 3 for thawing unopened reagents (TCR and Amplification, Enzyme, and Probe Reagents), reagents must remain in the RPI 250 for 4 to 20 hours. Refer to the *Procleix RPI 250 Operator's Manual* for additional information.

Caution: ⚠ Maintain reagents at the appropriate storage condition when not in use. Return reagents to their appropriate storage conditions without delay unless they are on the Procleix RPI 250 or the Procleix Panther System.

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, Greiner K_2EDTA Sep Vacuette Blood Collection Tubes, 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.

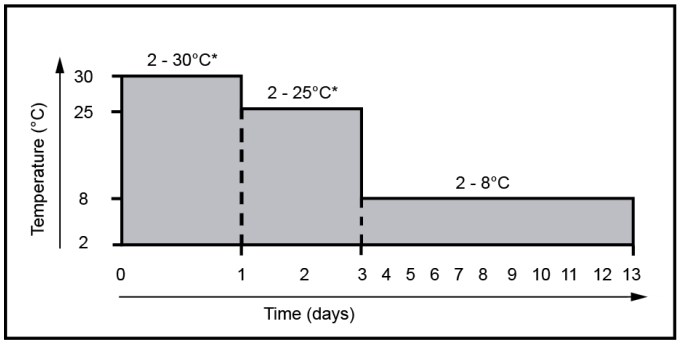
For storage above 8°C, specimens may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

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.



*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, heparin, or sodium citrate according to the collection container manufacturer's instructions may be used. ACD, heparin, or sodium citrate whole blood or plasma may be stored as in step B., above.

D. Additional blood specimens collected in serum tubes according to the collection container manufacturer's instructions may be used. Serum may be stored as in step B., above.

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 must 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, as necessary, for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed BD PPT tubes must be validated by the user.

I. Other collection and storage conditions should 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.

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

K. Specimen Pooling

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, hematopoetic progenitor cells (HPC), or donor lymphocyte infusions (DLI) may be pooled.

CADAVERIC BLOOD SPECIMENS

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

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

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.

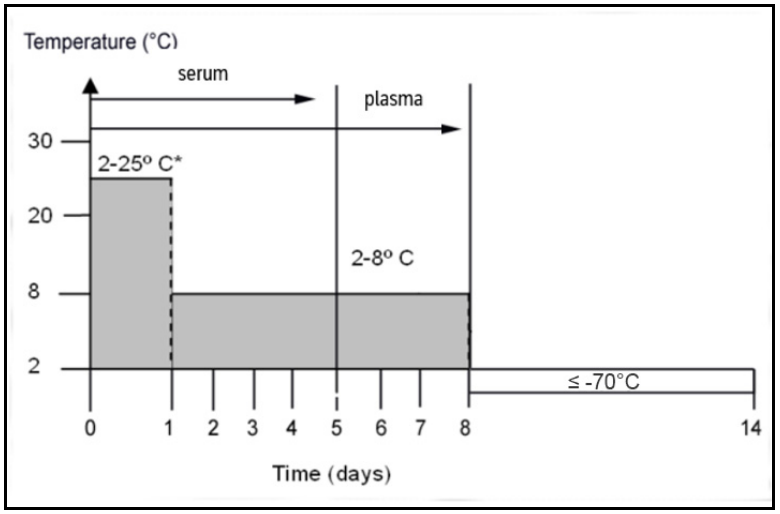
For storage above 8°C, specimens may be stored for 24 hours at up to 25°C during the 72 hours.

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

Do not freeze whole blood.



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

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

F. Specimens with visible precipitates or fibrinous material must 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, as necessary, 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., 100 µL sample plus 400 µL saline. Diluted specimens should be inverted several times to mix and then may be used in standard assay procedure.

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

MATERIALS REQUIRED

Component	Part Number	Part Number
Procleix Ultrio Elite Assay Kits	**303330** (1000 Test Kit)	**303715** (5000 Test Kit)
Internal Control Reagent	4 x 2.8 mL	20 x 2.8 mL
Target Capture Reagent	4 x 161 mL	20 x 161 mL
Amplification Reagent	4 x 26 mL	20 x 26 mL
Enzyme Reagent	4 x 13.4 mL	20 x 13.4 mL
Probe Reagent	4 x 34.7 mL	20 x 34.7 mL
Selection Reagent	4 x 91 mL	20 x 91 mL
Procleix Ultrio Elite Assay Target Enhancer Reagent Kit	**303331** (1000 Test Kit)	**303722** (5000 Test Kit)
Target Enhancer Reagent	4 x 46 mL	20 x 46 mL
Procleix Ultrio Elite Assay Calibrators Kit	**303719** (15 sets)	**303723** (75 sets)
Negative Calibrator	30 x 2.2 mL	90 x 2.2 mL
HIV Positive Calibrator	15 x 2.2 mL	75 x 2.2 mL
HCV Positive Calibrator	15 x 2.2 mL	75 x 2.2 mL
HBV Positive Calibrator	15 x 2.2 mL	75 x 2.2 mL
Procleix Ultrio Elite Assay HIV, HCV, and HBV Discriminatory Probe Reagents Kit	**303334** (200 tests)	
HIV Discriminatory Probe Reagent	2 x 14 mL	
HCV Discriminatory Probe Reagent	2 x 14 mL	
HBV Discriminatory Probe Reagent	2 x 14 mL	
Procleix Assay Fluids Kit	**303344** (1000 tests)	
Wash Solution	1 x 2.9 L	
Oil	1 x 260 mL	
Buffer for Deactivation Fluid	1 x 1.4 L	
Procleix Auto Detect Reagents Kit	**303345** (1000 tests)	
Auto Detect 1	1 x 245 mL	
Auto Detect 2	1 x 245 mL	

Disposables (Disposables are single use only, do not reuse. Use of other disposables is not recommended.)	**Quantity**	**Part Number**
Multi-Tube Units (MTUs)	1 case of 100	104772
Waste Bag Kit	1 box of 10	902731
MTU Waste Cover	1 box of 10	504405
Reagent Spare Caps (TCR and Selection Reagents)	1 bag of 100	CL0039
Reagent Spare Caps (Amplification and Probe Reagents)	1 bag of 100	CL0042
Reagent Spare Caps (Enzyme, Procleix Ultrio Elite Discriminatory Probe Reagents)	1 bag of 100	501619
Reagent Spare Caps (Target Enhancer Reagent)	1 bag of 100	903302

Equipment		
Procleix Panther System and operator's manual, Procleix Reagent Preparation Incubator 250 (RPI 250), independent temperature monitor (ITM), and operator's manual		
Other		
Advanced Cleaning Solution	1 bottle (255 mL)	PRD-04550

Note: Individual catalog numbers can be ordered separately as needed in order to meet individual site testing requirements.

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

Procleix Ultrio Elite Assay Negative Calibrators	30 sets	303333
Procleix Ultrio Elite Assay Positive Calibrators	15 sets	303332

General Equipment/Software

For pooling only: Procleix Xpress Pipettor and 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 Grifols Customer Service.

MATERIALS REQUIRED BUT NOT PROVIDED

Bleach:

For use in final concentrations of 5 to 8.25% sodium hypochlorite and 0.5 to 0.7% sodium hypochlorite

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

Disposable 1000 µL conductive filter tips in rack approved for use with the Procleix Panther System and pooling instrument. Contact Grifols 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 Elite Assay and the *Procleix Panther System Operator's Manual* prior to performing an assay.

C. Specimens may be infectious. Use Universal Precautions[38-39] when performing the assay. Proper handling and disposal methods should be established according to local regulations.[40] Only personnel adequately qualified as proficient in the use of the Procleix Ultrio Elite Assay and trained in handling infectious materials should perform this procedure.

D. 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.

E. The Enzyme Reagent 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.

F. 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.

G. Dispose of all materials that have come in contact with specimens and reagents according to local, state and federal regulations.[38, 40] Thoroughly clean and disinfect all work surfaces.

H. Use only specified disposables.

I. Do not use kit after expiration date.

J. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

K. Avoid microbial and nuclease contamination of reagents.

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

M. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present. See 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 Panther System verifies reagent levels.

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

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

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

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

Procleix Target Enhancer Reagent

Lithium Hydroxide, Monohydrate 6.78 Weight-%
DANGER

Harmful if swallowed
Causes severe skin burns and eye damage

Wash face, hands and any exposed skin thoroughly after handling
Do not eat, drink or smoke when using this product
Do not breathe dust/fume/gas/mist/vapors/spray
Wear protective gloves/protective clothing/eye protection/face protection
Immediately call a POISON CENTER or doctor
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
Immediately call a POISON CENTER or doctor
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
Wash contaminated clothing before reuse
IF INHALED: Remove person to fresh air and keep comfortable for breathing
Immediately call a POISON CENTER or doctor
IF SWALLOWED: Call a POISON CENTER or doctor if you feel unwell
Rinse mouth
Do NOT induce vomiting

Store locked up
Dispose of contents/container to an approved waste disposal plant

Procleix Buffer for Deactivation Fluid

Sodium Hydroxide 1.12 Weight-%
Sodium Hypochlorite 0.49 Weight-%
WARNING

Causes skin irritation
Causes serious eye irritation

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

Procleix Auto Detect 2

Sodium Hydroxide 6.04 Weight-%
DANGER

Causes severe skin burns and eye damage

Do not breathe dust/fume/gas/mist/vapors/spray
Wash face, hands and any exposed skin thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection
Immediately call a POISON CENTER or doctor/physician
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
Immediately call a POISON CENTER or doctor/physician
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
Wash contaminated clothing before reuse
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Immediately call a POISON CENTER or doctor/physician
IF SWALLOWED: rinse mouth. Do NOT induce vomiting

Store locked up
Dispose of contents/container to an approved waste disposal plant

P. The Procleix Panther System groups a kit 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 Panther System Operator's Manual* for more information.

Q. Refer to precautions in the appropriate Procleix assay package inserts and the *Procleix Panther System Operator's Manual*.

R. Do not use the RPI 250 to prepare Target Enhancer Reagent.

S. DO NOT heat the Probe Reagent or the Procleix Ultrio Elite Discriminatory Probe Reagents above 35°C when using the RPI 250. Refer to the *Procleix RPI 250 Operator's Manual*.

T. Each calibrator is designed to be run in duplicate or triplicate, and excess material in each vial is to be appropriately discarded.

REAGENT PREPARATION

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

B. Choose a new or opened matched set of reagents. An open set of reagents must be used on either the same Procleix Panther System as used previously or a Procleix Panther System that is connected to that system via Data Sharing. Do not use reagents that have been used outside the Procleix Panther System, as the instrument verifies reagent volumes.

C. Verify that the reagents have not exceeded their storage stability times.

The Procleix Panther System tracks the number of hours each reagent and fluid is loaded onboard the analyzer. The Procleix Panther System will not start pipetting specimens if reagents 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, Buffer for Deactivation Fluid, Auto Detect Reagents	60 days

*If using Panther System Software version 7.2 and higher, onboard stability is 72 hours.

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

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

1. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls between 2° and 15°C, precipitate may form.
2. If cloudiness or precipitate is present, perform Selection Reagent recovery as described in the *Procleix RPI 250 Operator's Manual*. Do not use if precipitate or cloudiness persists.
3. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
4. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

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

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

Record the date that it was first opened (OPEN DATE) on the space provided on the label.

Note: Do not use the RPI 250 to prepare Target Enhancer Reagent.

F. 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 to between 2° and 15ºC. Wash Solution and Target Enhancer Reagent may be warmed to facilitate dissolution of precipitate. Do not use the RPI 250 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.

G. Precipitate will form in the Procleix Ultrio Elite Assay Probe and the Procleix Ultrio Elite Discriminatory Probe Reagents when stored at 2° to 8°C. To facilitate dissolution of precipitate, use the RPI 250 to thaw all probe reagents at an average temperature of 32° ± 2°C not to exceed 35°C. Refer to the *Procleix RPI 250 Operator's Manual*. Ensure that precipitates in all probe reagents are dissolved. Do not use if precipitate or cloudiness is present.

H. Refer to the *Procleix RPI 250 Operator's Manual* to prepare the following reagents using the RPI 250*:* TCR, Probe Reagent, Enzyme Reagent, Amplification Reagent, and the Procleix Ultrio Elite Discriminatory Probe Reagents.

Record the date of thaw (THAW DATE) for each reagent on the space provided on the label.

Note: If precipitate is still present after thawing, Probe Reagent can be incubated with RPI File 3 (room temperature) to facilitate complete dissolution of precipitate. The Probe Reagent may also be warmed in a water bath to facilitate dissolution of precipitate, but temperature in the water bath should not exceed 30°C. If thawing is conducted on the lab bench, Probe Reagent may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate.

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 250, and refer to the *Procleix RPI 250 Operator's Manual*.
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 250 to thaw Internal Control Reagent.
4. Mix the Internal Control Reagent thoroughly by gentle manual inversion or mechanical inversion using a laboratory rocker.

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

5. Unload TCR from the RPI 250 and warm the Internal Control Reagent to room temperature.
6. Pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR).
7. Mix thoroughly.
8. Record the date Internal Control Reagent was added, wTCR expiration date (date Internal Control Reagent was added plus 30 days), and lot number used (IC LOT), in the space indicated on the TCR bottle.
9. Retain the IC vial to scan the barcode label into the system.

J. Thaw calibrators at room temperature. **Do not use the RPI 250 to thaw Procleix Ultrio Elite Assay Calibrators.**

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

1. Mix calibrators gently by inversion to avoid foaming.
2. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

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 calibrators at 25° to 30°C in a water bath. Periodically remove calibrators from water bath to gently invert until gel is dissolved.

K. Record the date Wash Solution, Oil, Buffer for Deactivation Fluid, Auto Detect 1, and Auto Detect 2 were first opened and loaded onto the Procleix Panther System (OPEN DATE) in the space provided on the label.

PROCEDURAL NOTES

Note: Refer to the *Procleix Panther System Operator's Manual* for operating instructions.

A. The Procleix Ultrio Elite Discriminatory Probe Reagents can be run with any matched set of reagents (Amplification Reagent, Enzyme Reagent, Probe Reagent, Selection Reagent, TCR, and Target Enhancer Reagent) within each master lot.

B. Procleix Ultrio Elite Assay Calibrators are master lotted with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite Discriminatory Probe Reagents. The operator must ensure that the Procleix Ultrio Elite Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the master lot sheet enclosed with each shipment of Procleix Ultrio Elite Assay Calibrators.

C. Proficiency panel members or external quality controls must not be used as substitutes for the Procleix Ultrio Elite Assay Calibrators.

D. Procleix Ultrio Elite Discriminatory Probe Reagents are master lotted with the Procleix Ultrio Elite Assay reagents. The operator must check to ensure that the Procleix Ultrio Elite Discriminatory Probe Reagents are used with the corresponding master lot of kit reagents as indicated on the Procleix Ultrio Elite Assay master lot sheet in use. Procleix Ultrio Elite Discriminatory Probe reagents can be run with any matched set of reagents (Amplification Reagent, Enzyme Reagent, Probe Reagent, Selection Reagent, TCR, and Target Enhancer Reagent) within each master lot.

E. Replace bottles in the Universal Fluids Drawer when notified by the system. Refer to the *Procleix Panther System Operator's Manual.*

Note: Procleix Auto Detect Reagents and Procleix Assay Fluids may be used with any master lot of Procleix Assay Reagents that are run on the Procleix Panther System.

F. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix Ultrio Elite Assay prior to performing an assay run. This package insert must be used with the Procleix Panther System *Operator's Manual* and any applicable Technical Bulletins.

G. EQUIPMENT PREPARATION

See the Procleix Panther System *Operator's Manual*.

H. RUN SIZE

1. For the Procleix Ultrio Elite Assay, each worklist may contain up to 250 tests, including Procleix Ultrio Elite Assay Calibrators.
2. For the discriminatory assays, the run size is limited by the Probe Reagents. The maximum run size is 100 tests, including Procleix Ultrio Elite Assay Calibrators.

I. RUN CONFIGURATION

Each run (also identified as a worklist) must have a set of Procleix Ultrio Elite Assay Calibrators.

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

J. WORK FLOW

1. Prepare reagents in a clean area.
2. The sample loading area must be amplicon-free.

K. DECONTAMINATION

1. The extremely sensitive detection of analytes by this test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces must be decontaminated daily with 0.5 to 0.7% 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. Follow instructions provided in the *Procleix Panther System Operator's Manual* for instrument decontamination and maintenance procedures.

ASSAY PROCEDURE

Procleix Ultrio Elite Assay Calibrators and Procleix Ultrio Elite Discriminatory Probe Reagents are to be used with the corresponding master lot of Procleix Ultrio Elite and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays. The operator must check to ensure that the Procleix Ultrio Elite Assay Calibrators and Procleix Ultrio Elite Discriminatory Probe Reagents are used with the corresponding master lot of kit reagents as indicated on the Procleix Ultrio Elite Assay master lot sheet in use.

Plasma and serum specimens from donors of whole blood, blood components, source plasma, hematopoietic stem/progenitor cells sourced from bone marrow, peripheral blood or cord blood, and from donors of donor lymphocytes for infusion can be tested using the individual donor testing method or in pools. Plasma and serum specimens from other living donors and from cadaveric (non-heart-beating) donors must be tested using the individual donor testing method only. Cadaveric blood specimens can be tested either neat or diluted, as described in SPECIMEN COLLECTION, STORAGE, AND HANDLING, CADAVERIC BLOOD SPECIMENS.

For equipment preparation and further assay processing information, see instructions in the *Procleix Panther System Operator's Manual*.

QUALITY CONTROL PROCEDURES

Note: All Quality Control procedures described below are performed by the Procleix Ultrio Elite Assay software.

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX ULTRIO ELITE ASSAY AND PROCLEIX ULTRIO ELITE HIV, 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 Elite 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 Elite HIV, 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 Panther System Software. If less than the minimum number of calibrator replicates is valid, the Procleix Panther 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 IC signal is below the cutoff.
   b. **In the Procleix Ultrio Elite 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 with an IC signal above 475,000 RLU.
   c. **In the Procleix Ultrio Elite HIV, 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 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 *Procleix Panther System Operator's Manual* for details.
3. All individual specimen results that are Invalid in a valid run must be retested.

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 Elite Assay

Negative Calibrator Acceptance Criteria

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

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

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

Example:

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

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

HCV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator is run in duplicate in the Procleix Ultrio Elite Assay. Individual HCV Positive Calibrator (PC) Analyte values must be less than or equal to 1,400,000 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 Elite 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/HCV/HBV Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.02 x HIV 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 Elite Assay

Acceptance Criteria:			
Negative Calibrator			
Analyte	≥ 0	and	≤ 45,000 RLU
Internal Control	≥ 75,000	and	≤ 375,000 RLU
HIV Positive Calibrator			
Analyte	≥ 300,000	and	≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU		
HCV Positive Calibrator			
Analyte	≥ 200,000	and	≤ 1,400,000 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 Elite Assay

Analyte Cutoff =	NC Analyte Mean RLU + 0.02 x (HIV 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 Elite HIV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator must be run in triplicate. Each individual Negative Calibrator (NC) must have an IC value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator 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 Positive Calibrator Acceptance Criteria

The HIV Positive Calibrator is run in triplicate in the Procleix Ultrio Elite HIV Discriminatory Assay. Individual HIV 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 Positive Calibrator values is outside these limits, the HIV Positive Calibrator mean (HIV PC_x) will be recalculated based upon the two acceptable HIV Positive Calibrator values. The run is invalid and must be repeated if more than one of the three HIV Positive Calibrator Analyte values is outside of these limits. IC values may not exceed 475,000 RLU.

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

Example:

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

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

HCV Positive Calibrator and HBV Positive Calibrator Acceptance Criteria

These calibrators are not run in the Procleix Ultrio Elite HIV Discriminatory Assay on the Procleix Panther 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 PC_x (Analyte)]

Using values given in the Negative Calibrator and HIV 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 Procleix Ultrio Elite HIV Discriminatory Assay for the Procleix Panther System. Only the three replicates of the Negative Calibrator and the three replicates of the HIV Positive Calibrator are used. This means that testing is not required for all of the Procleix Ultrio Elite 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 Elite HIV Discriminatory Assay

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

Summary of Cutoff Calculations for the Procleix Ultrio Elite HIV Discriminatory Assay

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

C. Procleix Ultrio Elite HCV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator must be run in triplicate. Each individual Negative Calibrator must have an IC value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator 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 Elite 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 Positive Calibrator and HBV Positive Calibrator Acceptance Criteria

These calibrators are not run on the Procleix Ultrio Elite HCV Discriminatory Assay on the Procleix Panther 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 and HBV Positive Calibrators are not used in the Procleix Ultrio Elite HCV Discriminatory Assay for the Procleix Panther 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 Elite 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 Elite 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 Elite 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 Elite HBV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator must be run in triplicate. Each individual Negative Calibrator (NC) must have an IC value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator 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 Ultrio Elite 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 Positive Calibrator and HCV Positive Calibrator Acceptance Criteria

These calibrators are not run on the Procleix Ultrio Elite HBV Discriminatory Assay on the Procleix Panther 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 Positive Calibrators are not used in the Procleix Ultrio Elite HBV Discriminatory Assay for the Procleix Panther 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 Elite 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 Elite 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 Elite 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)

INTERPRETATION OF RESULTS

All calculations described above are performed by the Procleix Panther 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 Elite Assay**,** or less than or equal to 475,000 RLU in the Procleix Ultrio Elite HIV, 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 Elite Assay, or less than or equal to 475,000 RLU in the Procleix Ultrio Elite HIV, 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 Elite Assay, or greater than 475,000 RLU in the Procleix Ultrio Elite HIV, HCV, or HBV Discriminatory Assays.

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

Cadaveric (non-heart-beating) blood specimens, when tested neat, may be invalid due to inhibitory substances within the specimen. These invalid samples may be diluted as explained in SPECIMEN COLLECTION, STORAGE, AND HANDLING, Cadaveric Blood Specimens, and repeated in singlet.

Summary of Specimen Interpretation

Specimen Interpretation	Criteria for the Procleix Ultrio Elite Assay	Criteria for the Procleix Ultrio Elite HIV, 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 Elite 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 Elite HIV, 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.

IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION FROM ANY OTHER LIVING DONOR (I.E., NOT A BLOOD DONOR) OR FROM A CADAVERIC DONOR, then the specimen must be tested with the Procleix Ultrio Elite HIV, HCV, 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 Elite Assay if sufficient sample is available.

   1. If the individual specimen tests Nonreactive in the repeated Procleix Ultrio Elite Assay, then the specimen is considered Nonreactive for HIV RNA, HCV RNA, and HBV DNA and no further testing is required.
   2. If the individual specimen tests Reactive in the repeated Procleix Ultrio Elite Assay, then the specimen is considered Repeatedly Reactive, Non-Discriminated for HIV 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.

LIMITATIONS OF THE PROCEDURE

A. This assay has been developed for use with the Procleix Panther System only.

B. The Procleix Ultrio Elite HIV Discriminatory Assay will not distinguish between samples reactive for HIV-1 and those reactive for HIV-2.

C. The Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assay primers and probes target highly conserved regions of the HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA genomes. However, in rare instances, mutations in these regions may affect the sensitivity for the detection of HIV-1, HIV-2, HCV, or HBV.

D. The sensitivity of the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays has been demonstrated for specimens with HIV-1 viral RNA concentrations equal or greater than 100 copies/mL, HIV-2 viral RNA concentrations equal or greater than 100 copies/mL, HCV viral RNA concentrations equal or greater than 30 IU/mL, or HBV viral DNA concentrations equal or greater than 6 IU/mL. Samples with less than these concentrations may not yield reproducible results.

E. Certain substances may interfere with the performance of the assay. See SPECIFICITY AND SENSITIVITY OF THE PROCLEIX ULTRIO ELITE ASSAY IN THE PRESENCE OF DONOR AND DONATION FACTORS section.

F. Test results may be affected by improper specimen collection, storage, or specimen processing.

G. Cross-contamination of samples can cause false positive results.

H. Assays must be performed, and results interpreted, according to the procedures provided. Deviations from these procedures, adverse shipping and/or storage conditions, or use of outdated calibrators and/or reagents may produce unreliable results.

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

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

Reproducibility was evaluated on the Procleix Panther System at 3 US sites. Two operators performed testing at each site. Each operator performed 2 runs with each assay per day over 9 days, using 3 reagent lots over the course of testing. Each run had 2 replicates of each panel member.

The negative panel members were made from HIV-1, HIV-2, HCV, and HBV negative plasma. The positive panel members were created by spiking the negative plasma with clinical plasma specimens (positive for HIV-1, HCV, or HBV) and tissue culture samples (positive for HIV-2). For each analyte, high negative (target concentration below 95% LOD of the assay [approximately 7 IU/mL for HIV-1, 10 IU/mL for HIV-2, 1 IU/mL for HCV and HBV], low positive (≥2x LOD and ≤15x LOD) and moderate positive (≥30x LOD) panel members were prepared for testing.

Agreement values were 100% in the negative panel member for the Procleix Ultrio Elite Assay and ≥94.91% in the moderate positive and low positive panel members for HIV-1, HIV-2, HCV, and HBV for the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays. For both the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays, high-negative panel members for HIV-1, HIV-2, HCV, and HBV had positive agreement values ≥36.57% and ≤57.41%; this was expected, as the panel members were spiked with concentrations of target below the 95% LODs of the assays. For each assay, the total %CV was ≤10.39 and ≤25.68% for the moderate positive and low positive panel members, respectively. The high negative panel members had total %CVs ≤141.75% due to the inconsistent results that are expected when testing samples with concentrations below the LODs of the assays. Note: For each assay, values are shown for panel members containing the applicable analyte(s).

Table 1–Table 4 show the reproducibility and precision of the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assay results for each panel member containing the analyte(s) detected by the assay between sites, between operators, between lots, between days, between runs, within runs, and overall.

Table 1. Reproducibility of the Procleix Ultrio Elite Assay on the Procleix Panther System

Panel Concentration	n	Mean S/CO	Agrmt (%)	Between Sites		Between Operators		Between Lots		Between Days		Between Runs		Within Runs		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	214	<0.1	100	<0.1	24.47	0.0	0.0	0.0	0.0	<0.1	30.18	0.0	0.0	<0.1	40.90	<0.1	56.41
HIV-1 Positive Samples																	
6.8 IU/mL (HN)	216	2.94	56.94	0.0	0.0	0.0	0.0	0.13	4.34	0.53	17.98	1.45	49.55	3.67	124.87	3.98	135.61
68.0 IU/mL (LP)	216	10.77	99.54	0.45	4.18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.09	19.42	2.14	19.87
590.7 IU/mL (MP)	216	12.80	100	0.36	2.83	0.0	0.0	0.25	1.95	0.38	2.97	0.32	2.51	0.46	3.56	0.81	6.29
HIV-2 Positive Samples																	
9.75 IU/mL (HN)	216	2.96	38.43	0.21	6.97	0.0	0.0	0.49	16.39	0.0	0.0	0.71	23.86	2.50	84.50	2.65	89.59
116.95 IU/mL (LP)	216	6.40	100	0.20	3.05	0.0	0.0	0.18	2.79	0.13	2.07	0.27	4.20	0.75	11.79	0.85	13.34
353.4 IU/mL (MP)	216	6.63	100	0.24	3.55	0.0	0.0	0.26	3.90	0.22	3.30	0.18	2.74	0.33	5.04	0.56	8.46
HCV Positive Samples																	
1.1 IU/mL (HN)	216	4.57	46.30	0.0	0.0	0.0	0.0	0.0	0.0	0.94	20.48	0.63	13.75	4.16	90.98	4.31	94.27
15.5 IU/mL (LP)	216	8.91	100	0.23	2.60	0.0	0.0	0.21	2.40	0.27	3.06	0.25	2.79	0.29	3.27	0.57	6.36
265.2 IU/mL (MP)	216	8.91	100	0.27	3.06	0.0	0.0	0.19	2.13	0.25	2.78	0.23	2.54	0.27	3.04	0.54	6.11
HBV Positive Samples																	
1.0 IU/mL (HN)	216	5.39	56.94	2.08	38.66	1.11	20.59	0.0	0.0	0.0	0.0	0.0	0.0	6.16	114.32	6.60	122.43
9.5 IU/mL (LP)	215	13.16	95.35	0.97	7.40	0.0	0.0	0.52	3.95	0.78	5.96	0.0	0.0	2.82	21.44	3.13	23.79
164.6 IU/mL (MP)	216	14.09	100	0.46	3.25	0.0	0.0	0.63	4.48	0.45	3.20	0.33	2.33	0.37	2.64	1.03	7.30

Agrmt = Agreement, CV = Coefficient of Variation, HN = High Negative, LP = Low Positive, MP= Moderate Positive, S/CO = Signal to Cutoff ratio, SD = Standard Deviation

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.0. At each of the 3 sites, approximately 936 samples (13 panel members × 2 replicates × 18 days × 2 operators) were tested with each assay, for a total of 2808 samples tested overall. Each panel member yielded approximately 216 test results (2808 samples tested divided by 13 panel members).

Table 2. Reproducibility of the Procleix Ultrio Elite HIV Discriminatory Assay on the Procleix Panther System

Panel Concentration	n	Mean S/CO	Agrmt (%)	Between Sites		Between Operators		Between Lots		Between Days		Between Runs		Within Runs		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	214	0.11	100	<0.1	32.97	0.0	0.0	0.0	0.0	<0.1	42.16	<0.1	13.63	<0.1	33.71	<0.1	64.70
HIV-1 Known-Positive Samples																	
6.8 IU/mL (HN)	215	4.64	55.81	1.74	37.59	0.0	0.0	0.0	0.0	0.58	12.40	2.11	45.43	5.96	128.31	6.58	141.75
68.0 IU/mL (LP)	216	18.10	99.54	1.59	8.79	0.33	1.82	0.42	2.34	0.22	1.21	0.87	4.83	3.30	18.24	3.81	21.06
590.7 IU/mL (MP)	216	21.45	100	1.03	4.81	0.0	0.0	0.88	4.12	0.83	3.88	0.60	2.79	0.72	3.37	1.85	8.62
HIV-2 Known-Positive Samples																	
9.75 IU/mL (HN)	216	4.51	36.57	0.0	0.0	0.61	13.44	1.04	23.07	0.0	0.0	1.18	26.27	3.96	87.91	4.31	95.56
116.95 IU/mL (LP)	215	10.62	100	0.57	5.33	0.0	0.0	0.62	5.87	0.0	0.0	0.49	4.64	1.28	12.07	1.61	15.17
353.4 IU/mL (MP)	215	11.02	100	0.54	4.92	0.0	0.0	0.60	5.45	0.53	4.83	0.31	2.81	0.53	4.79	1.15	10.39

Agrmt = Agreement, CV = Coefficient of Variation, HN = High Negative, LP = Low Positive, MP= Moderate Positive, S/CO = Signal to Cutoff ratio, SD = Standard Deviation

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.0. At each of the 3 sites, approximately 936 samples (13 panel members × 2 replicates × 18 days × 2 operators) were tested with each assay, for a total of 2808 samples tested overall. Each panel member yielded approximately 216 test results (2808 samples tested divided by 13 panel members).

Table 3. Reproducibility of the Procleix Ultrio Elite HCV Discriminatory Assay on the Procleix Panther System

Panel Concentration	n	Mean S/CO	Agrmt (%)	Between Sites		Between Operators		Between Lots		Between Days		Between Runs		Within Runs		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	214	<0.1	100	<0.1	87.33	0.0	0.0	<0.1	2.10	<0.1	175.63	0.0	0.0	<0.1	162.92	<0.1	254.99
HCV Known-Positive Samples																	
1.1 IU/mL (HN)	216	10.63	53.24	1.48	13.95	0.0	0.0	0.0	0.0	1.65	15.51	0.0	0.0	11.40	107.23	11.62	109.24
15.5 IU/mL (LP)	216	23.71	100	0.69	2.93	0.12	0.49	0.46	1.93	1.04	4.40	0.65	2.76	0.73	3.09	1.66	7.01
265.2 IU/mL (MP)	216	23.76	100	0.60	2.51	0.0	0.0	0.53	2.23	0.92	3.86	0.77	3.24	0.67	2.81	1.59	6.68

Agrmt = Agreement, CV = Coefficient of Variation, HN = High Negative, LP = Low Positive, MP= Moderate Positive, S/CO = Signal to Cutoff ratio, SD = Standard Deviation

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.0. At each of the 3 sites, approximately 936 samples (13 panel members × 2 replicates × 18 days × 2 operators) were tested with each assay, for a total of 2808 samples tested overall. Each panel member yielded approximately 216 test results (2808 samples tested divided by 13 panel members).

Table 4. Reproducibility of the Procleix Ultrio Elite HBV Discriminatory Assay on the Procleix Panther System

Panel Concentration	n	Mean S/CO	Agrmt (%)	Between Sites		Between Operators		Between Lots		Between Days		Between Runs		Within Runs		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	214	<0.1	100	<0.1	106.34	0.0	0.0	<0.1	15.48	<0.1	171.37	<0.1	39.88	<0.1	159.53	<0.1	260.69
HBV Known-Positive Samples																	
1.0 IU/mL (HN)	216	9.32	57.41	1.15	12.30	3.25	34.88	0.0	0.0	2.79	29.88	4.86	52.18	9.40	100.91	11.48	123.15
9.5 IU/mL (LP)	216	22.42	94.91	1.77	7.89	1.81	8.07	0.57	2.56	0.81	3.63	3.53	15.76	3.64	16.24	5.76	25.68
164.6 IU/mL (MP)	216	24.08	100	0.66	2.73	0.0	0.0	0.58	2.43	0.69	2.88	0.76	3.17	0.58	2.40	1.47	6.12

Agrmt = Agreement, CV = Coefficient of Variation, HN = High Negative, LP = Low Positive, MP= Moderate Positive, S/CO = Signal to Cutoff ratio, SD = Standard Deviation

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.0. At each of the 3 sites, approximately 936 samples (13 panel members × 2 replicates × 18 days × 2 operators) were tested with each assay, for a total of 2808 samples tested overall. Each panel member yielded approximately 216 test results (2808 samples tested divided by 13 panel members).

CLINICAL SPECIFICITY IN NORMAL BLOOD DONORS

A prospective, multicenter clinical trial was conducted. Three blood testing laboratories performed testing: 2 laboratories tested plasma samples from voluntary whole blood donors in 16-sample pools or individually and 1 laboratory tested plasma samples from source plasma donors in 96-sample pools. All three laboratories performed testing using two Procleix Panther System instruments each. At least three reagent kit lots were used by each laboratory.

Sixteen-sample pools were created by combining aliquots from 16 individual donations. Ninety-six-sample pools were created by combining six 16-sample pools. Ninety-six-sample pools of source plasma donations, 16-sample pools of whole blood donations, and individual whole blood donations were tested with the Procleix Ultrio Elite Assay on the Procleix Panther System.

Individual donations were tested with licensed serologic tests for HIV-1, HIV-2, HCV, and HBV. At the two laboratories that tested samples from voluntary whole blood donors, 16-sample pools and individual donations were tested with the licensed Procleix Ultrio Plus Assay on the licensed Procleix Tigris System in accordance with package insert instructions. At the laboratory that tested samples from source plasma donors, 16-sample pools and individual donations were tested with the licensed Procleix Ultrio Assay on the licensed Procleix Tigris System in accordance with package insert instructions.

Pools with reactive results were resolved by testing the six 16-sample pools (for 96-sample pools) or the individual donations (for 16-sample pools) within the reactive pool. Individual donations with discordant result(s) were tested with alternate licensed or validated HIV-1, HIV-2, HCV, and/or HBV NATs, if available. All alternate NAT testing was performed on the index donation.

The HIV-1, HCV, and HBV status for the specificity analyses was based on testing with the licensed Procleix Ultrio Plus Assay or Procleix Ultrio Assay on the Procleix Tigris System and serology assays and/or HIV-1, HCV, or HBV licensed NAT or serologic test results from follow-up testing. The HIV-2 status for the specificity analyses was based on HIV-2 serologic testing and/or validated HIV-2 NAT results, and, if available, licensed NAT and/or serologic test results from subsequent donations.

Of the 351 Procleix Ultrio Elite Assay runs on the Procleix Panther System, 3 runs (0.9%, 3/351) were invalid. Of the 37 runs performed with each of the Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays runs on the Procleix Panther System, all were valid. Of the 14,545 sixteen-sample and 96-sample pools and 15,400 individual donations (includes samples that were never pooled and the constituent samples from a reactive pool) processed in valid Procleix Ultrio Elite Assay runs, 7 pools (<0.1%; 7/14,545) and 159 individual donations (1.0%, 159/15,400) had final invalid Procleix Ultrio Elite Assay results. Of the 182, 209, and 181 individual donations processed in valid Procleix Ultrio Elite HIV Discriminatory Assay, Procleix Ultrio Elite HCV Discriminatory Assay, and Procleix Ultrio Elite HBV Discriminatory Assay runs, 11 (6.0%, 11/182), 1 (0.5%, 1/209), and 17 (9.4%, 17/181) had final invalid results, respectively. Final invalid results were excluded from the analyses. Note: Most of the final invalid Procleix Ultrio Elite Assay results occurred in pools (7/7) or individual donations (158/159) that had initial invalid results and were never retested. Most of the final invalid Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assay results occurred in individual donations (9/11 Procleix Ultrio Elite HIV Discriminatory Assay results, 1/1 Procleix Ultrio Elite HCV Discriminatory Assay result, and 17/17 Procleix Ultrio Elite HBV Discriminatory Assay results) that had initial invalid results and were never retested.

Additional 16-sample pools (n=120) and individual donations (n=27) from whole blood donations and 96-sample pools (n=89) from source plasma donations, respectively were excluded from the specificity analyses due to invalid or unknown licensed NAT result interpretations or final unknown status for the specificity analyses.

Rates of Procleix Ultrio Elite Assay reactivity are presented in Table 5 and Table 6 for 16-sample pools and individual donations from whole blood donations and in Table 7 for 96-sample pools from source plasma donations that were included in the clinical specificity analyses.

Table 5. Procleix Ultrio Elite Assay Reactivity in 16-Sample Pools from Whole Blood Donations

	n	95% CI
Total Pools with Specificity Results	10,546	100.00
Nonreactive Pools	10,468	99.26 (99.08–99.41)
Reactive Pools	78	0.74 (0.59–0.92)
Pools with True Positive Procleix Ultrio Elite Assay Results	78	0.74 (0.59–0.92)
Pool, Individual Constituent(s), and Discriminatory Assay Reactive; Licensed Assay Reactive; HIV-1/HCV/HBV Serology Confirmed Reactive	72	0.68 (0.53–0.86)
Pool and Individual Constituent(s) Reactive, Discriminatory Assay Missing; Licensed Assay Reactive; HIV-1/HCV/HBV Serology Confirmed Reactive	4	0.04 (0.01–0.10)
Pool and Individual Constituent(s) Reactive, Discriminatory Assay Missing; Licensed Assay Reactive; HIV-1/HCV/HBV Serology Incomplete; HIV-2 Serology Reactive	1	0.01 (<0.01–0.05)
Pool and Individual Constituent(s) Reactive; Discriminatory Assay Reactive; Licensed Assay Unknown; HIV-1/HCV/HBV Serology Confirmed Reactive	1	0.01 (<0.01–0.05)

n = Number of Specimens, CI = Clopper-Pearson Confidence Interval[43.], Discriminatory Assay = Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays

Table 6. Procleix Ultrio Elite Assay Reactivity in Individual Whole Blood Donations

	n	95% CI
Total IDS with Specificity Results	11,941	100.00
Nonreactive IDS	11,939	99.98 (99.94–100.00)
Reactive IDS	2	0.02 (<0.01–0.06)
IDS with True Positive Procleix Ultrio Elite Assay Results	2	0.02 (<0.01–0.06)
IDS Procleix Ultrio Elite Assay and Discriminatory Assay Reactive; Licensed Assay Reactive; HIV-1/HCV/HBV Serology Confirmed Reactive	2	0.02 (<0.01–0.06)

n = Number of Specimens, CI = Clopper-Pearson Confidence Interval, IDS = Individual Donor Samples, Discriminatory Assay = Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays

Table 7. Procleix Ultrio Elite Assay Reactivity in 96-Sample Pools from Source Plasma Donations

	n	95% CI
Total Pools with Specificity Results	2925	100.00
Nonreactive Pools	2804	95.86 (95.08–96.56)
Reactive Pools	121	4.14 (3.44–4.92)
Pools with True Positive Procleix Ultrio Elite Assay Results	120	4.10 (3.41–4.89)
Pool, Individual Constituent(s), and Discriminatory Assay Reactive; Licensed Assay Reactive; HIV-1/HCV/HBV Serology Confirmed Reactive or Follow-Up Result Reactive	37	1.26 (0.89–1.74)
Pool and Individual Constituent(s) Reactive, Discriminatory Assay Missing; Licensed Assay Reactive; HIV-1/HCV/HBV Serology Confirmed Reactive or Follow-Up Result Reactive	2	0.07 (0.01–0.25)
Pool Reactive with Incomplete Individual Constituent(s) Testing; Licensed Assay Reactive, HIV-1/HCV/HBV Serology Confirmed Reactive or Follow-Up Result Reactive	80	2.74 (2.17–3.39)
Pool Reactive with Incomplete Individual Constituent(s) Testing; Licensed Assay Nonreactive, HIV-1/HCV/HBV Serology Confirmed Reactive or Follow-Up Result Reactive	1	0.03 (<0.01–0.19)
Pools with False Positive Procleix Ultrio Elite Assay Results	1	0.03 (<0.01–0.19)
Pool reactive; All 6 16-Sample Pools Nonreactive; Licensed Assay Nonreactive; HIV-1/HCV/HBV Serology or Follow-Up Result Nonreactive	1	0.03 (<0.01–0.19)

n = Number of Specimens, CI = Clopper-Pearson Confidence Interval, Discriminatory Assay = Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays

Table 8 shows the specificity of the Procleix Ultrio Elite Assay on the Procleix Panther System in 10,546 sixteen-sample pools and 11,941 individual donations from whole blood donations and 2925 ninety-six-sample pools from source plasma donations. Specificity was >99.96% in 16-sample pools and individual donations from whole blood donations and 96-sample pools from source plasma donations.

Table 8. Specificity of the Procleix Ultrio Elite Assay on the Procleix Panther System in 16-Sample Pools, 96-Sample Pools, and Individual Donations

Source	Sample Type	n	True Negative	False Negative	True Positive	False Positive	Specificity (%)	95% CI
Whole Blood Donations	16-Sample Pool	10,546	10,467	1*	78	0	100	99.96–100
	Site 1	5219	5218	0	1	0	100	99.93–100
	Site 2	5327	5249	1*	77	0	100	99.93–100
	Individual Donation	11,941	11,939	0	2	0	100	99.97–100
	Site 1	5420	5420	0	0	0	100	99.93–100
	Site 2	6521	6519	0	2	0	100	99.94–100
Source Plasma Donations	96-Sample Pool	2925	2800	4**	120	1***	99.96	99.80–>99.99

n = Number of Specimens, CI = Clopper-Pearson Confidence Interval

*The 16-sample pool with a false negative result contained an individual donation that had a Procleix Ultrio Plus Assay reactive, HBV discriminated result and a reactive HCV serologic test result. The HCV alternate NAT result detected HCV in the individual donation (<15 IU/mL); there was insufficient volume for testing with the HBV alternate NAT.

**Of the 4 ninety-six sample pools with false negative results, 2 had reactive HIV-2 serologic test results; 1 of the samples also had an indeterminate HIV-1 serologic test result. The remaining 2 ninety-six sample pools with false negative results each contained 1 individual donation that was Procleix Ultrio Assay reactive, HBV discriminated, and had a reactive HBV serologic test result. The HBV alternate NAT detected HBV in these individual donations (63.3 IU/mL and 217 IU/mL). One (1) of the pools also contained an individual donation that was Procleix Ultrio Assay reactive, nondiscriminated; the serologic test results for this individual donation were all nonreactive.

***All 6 sixteen-sample pools comprising the false positive 96-sample pool were Procleix Ultrio Elite Assay and Procleix Ultrio Assay nonreactive; the serologic test results for the 96 individual donations were all nonreactive.

CLINICAL SENSITIVITY IN KNOWN-POSITIVE SAMPLES

Two-thousand seven hundred and twenty-three (2723) known-positive plasma and serum samples, consisted of 986 HIV-1, 207 HIV-2, 1008 HCV, and 522 HBV samples, were procured from clinical specimen suppliers. The positive samples were prepared neat (ie, undiluted, n=2723) and in a 1:16 dilution (n=2804, includes 81 co-infected samples) and tested at three laboratories (two external and one in-house). Single-infected samples were tested neat with the Procleix Ultrio Elite Assay and the appropriate Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assay. Diluted single-infected and co-infected samples were tested with the Procleix Ultrio Elite Assay only. Single-infected samples with known concentrations of ≥100 HIV-1 RNA copies/mL, ≥30 HCV RNA IU/mL, and/or ≥6.25 HBV DNA IU/mL were included in the sensitivity analyses. For HIV-2, separate analyses were performed for samples with known concentrations of ≥100 and ≥10 HIV-2 RNA copies/mL. Results were compared to the known viral status and clinical sensitivity was calculated (Table 9).

Of the 47 Procleix Ultrio Elite Assay runs performed, 1 (1/47, 2.1%) was invalid. Of the 21 Procleix Ultrio Elite HIV Discriminatory Assay runs performed, 1 (1/21, 4.8%) was invalid. Of the 22 Procleix Ultrio Elite HCV Discriminatory Assay runs performed, 2 (2/22, 9.1%) were invalid. Of the 22 Procleix Ultrio Elite HBV Discriminatory Assay runs performed, 1 (1/22, 4.5%) was invalid. Of the 2723 Procleix Ultrio Elite Assay results for neat samples, 12 were invalid. Of the 2804 Procleix Ultrio Elite Assay results for diluted samples, all were valid. Of the 1193 Procleix Ultrio Elite HIV Discriminatory Assay results, 10 were invalid. Of the 1008 Procleix Ultrio Elite HCV Discriminatory Assay results, 9 were invalid. All 522 Procleix Ultrio Elite HBV Discriminatory Assay results were valid.

Samples with undetectable viral loads or results less than the lower limit of quantitation with validated quantitative NATs and samples with final invalid results were excluded from the sensitivity analyses:

For the Procleix Ultrio Elite Assay analyses, a) 55 HIV-1, 23 HCV, and 19 HBV known-positive neat samples, b) 181 (≥100 RNA copies/mL cutoff) and 150 (≥10 HIV-2 RNA copies/mL cutoff) HIV-2 known-positive neat samples, c) 222 HIV-1, 49 HCV, and 94 HBV known-positive diluted samples, and d) 201 (≥100 RNA copies/mL cutoff) and 149 (≥10 RNA copies/mL cutoff) HIV-2 known-positive diluted samples were excluded.

For the Procleix Ultrio Elite Discriminatory Assay analyses, a) 57 HIV-1, 28 HCV, and 19 HBV known-positive neat samples and b) 181 (≥100 RNA copies/mL cutoff) HIV-2 known-positive neat samples were excluded.

Sensitivity of the Procleix Ultrio Elite and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays in neat and diluted samples is shown in Table 9.

The sensitivity of the Procleix Ultrio Elite Assay in neat (undiluted) HIV-1, HCV, or HBV known-positive single-infected samples was: 100% (931/931, 95% confidence interval (CI): 99.60% to 100%) for HIV-1 ≥100 copies/mL, 100% (985/985, 95% CI: 99.63% to 100%) for HCV ≥30 IU/mL, and 100% (503/503, 95% CI: 99.27% to 100%) for HBV ≥6.25 IU/mL. For neat HIV-2 samples, sensitivity was 100% (26/26, 95% CI: 86.77% to 100%) in samples with viral loads ≥100 copies/mL, and 91.23% (52/57; 95% CI: 80.70% to 97.09%) in samples with viral loads ≥10 copies/mL. Of the 5 HIV-2 samples with false negative results, 1 was Procleix Ultrio Elite HIV Discriminatory Assay reactive. All 5 HIV-2 samples had low neat viral loads ≤37 copies/mL; see Table 9).

The sensitivity of the Procleix Ultrio Elite Assay in diluted (1:16) known-positive single-infected samples was 100% for HIV-1 (764/764; 95% CI: 99.52% to 100%) and HCV (959/959, 95% CI: 99.62% to 100%) and 99.07% for HBV (428/432; 95% CI: 97.65% to 99.75%). The 4 HBV samples with false negative results had low viral loads ≤128 IU/mL when tested neat. For diluted HIV-2 samples, sensitivity was 100% (6/6, 95% CI: 54.07% to 100%) in samples with viral loads ≥100 copies/mL, and 67.24% (39/58; 95% CI: 53.66% to 78.99%) in samples with viral loads ≥10 copies/mL. Both the low number of HIV-2 NAT-reactive samples and the low viral load levels are not unexpected based on reports of viral load levels and clinical progression associated with HIV-2 infection.[41, 42, 23] The 19 HIV-2 diluted samples with false negative results had low viral loads ≤41 copies/mL when tested neat. The sensitivity of the Procleix Ultrio Elite Assay in 1:16 diluted co-infected samples was 100% (27/27; 95% CI: 87.23% to 100%).

Table 9. Clinical Sensitivity of the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays in HIV-1, HIV-2, HCV, and HBV Known-Positive Samples

Assay	Sample (Viral Load)	n	TP	FN	Sensitivity (%)	95% CI
Ultrio Elite (Neat)	HIV-1 (≥100 copies/mL)	931	931	0	100	99.60–100
	HIV-2 (≥100 copies/mL)	26	26	0	100	86.77–100
	HIV-2 (≥10 copies/mL)	57	52	5	91.23	80.70–97.09
	HCV (≥30 IU/mL)	985	985	0	100	99.63–100
	HBV (≥6.25 IU/mL)	503	503	0	100	99.27–100
Ultrio Elite (Diluted)	HIV-1 (≥100 copies/mL)	764	764	0	100	99.52–100
	HIV-2 (≥100 copies/mL)	6	6	0	100	54.07–100
	HIV-2 (≥10 copies/mL)	58	39	19	67.24	53.66–78.99
	HCV (≥30 IU/mL)	959	959	0	100	99.62–100
	HBV (≥6.25 IU/mL)	432	428	4*	99.07	97.65–99.75
	Co-Infected	27	27	0	100	87.23–100
dHIV (Neat)	HIV-1 (≥100 copies/mL)	929	929	0	100	99.60–100
	HIV-2 (≥100 copies/mL)	26	26	0	100	86.77–100
dHCV (Neat)	HCV (≥30 IU/mL)	980	980	0	100	99.62–100
dHBV (Neat)	HBV (≥6.25 IU/mL)	503	503	0	100	99.27–100

dHBV = Procleix Ultrio Elite HBV Discriminatory Assay, dHCV = Procleix Ultrio Elite HCV Discriminatory Assay, dHIV = Procleix Ultrio Elite HIV Discriminatory Assay, FN = False Negative, n = Number of Specimens, TP = True Positive, Ultrio Elite = Procleix Ultrio Elite Assay, CI = Clopper-Pearson Confidence Interval

*Diluted samples had approximate neat concentrations ≤128 IU/mL HBV DNA.

CLINICAL SENSITIVITY FOR HIV-1, HCV, AND HBV IN A HIGH-RISK POPULATION

Five hundred and twenty (520) prospectively collected plasma samples from US men and women at high risk of HIV-1, HCV, and/or HBV infection with unknown infection status were procured from a vendor. The samples were tested in-house with the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays on the Procleix Panther System. Three clinical reagent kit lots were used. The samples were also tested with the Procleix Ultrio Plus Assay on the Procleix Tigris System; Procleix Ultrio Plus Assay reactive samples were tested with the Procleix Ultrio Plus HIV, HCV, and HBV Discriminatory Assays. Samples with reactive Procleix Ultrio Elite Assay and/or Procleix Ultrio Plus Assay results were tested with serologic tests for HIV-1/HIV-2, HCV, and/or HBV. Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assay results were compared to the Procleix Ultrio Plus Assay, Procleix Ultrio Plus HIV, HCV, and HBV Discriminatory Assay, and serologic test results and clinical sensitivity was calculated (Table 10). Samples with discordant results were tested with FDA-approved alternate quantitative NATs.

Of the 7 Procleix Ultrio Elite Assay runs performed, 1 (1/7, 14.3%) was invalid. Of the 11 Procleix Ultrio Elite HIV Discriminatory Assay runs performed, 2 (2/11, 18.2%) were invalid. Of the 8 Procleix Ultrio Elite HCV Discriminatory Assay and 10 Procleix Ultrio Elite HBV Discriminatory Assay runs performed, all were valid. All 520 samples tested with the Procleix Ultrio Elite Assay, Procleix Ultrio Elite HIV Discriminatory Assay, Procleix Ultrio Elite HCV Discriminatory Assay, and Procleix Ultrio Elite HBV Discriminatory Assay had final valid results.

One sample was excluded from the sensitivity analyses because it did not meet the study inclusion criteria.

The sensitivity of the Procleix Ultrio Elite Assay as shown in Table 10 was 100% for detection of HIV-1 (6/6), 98.86% (87/88) for detection of HCV, and 100% (2/2) for detection of HBV.

Table 10. Clinical Sensitivity of the Procleix Ultrio Elite Assay in Samples From an HIV-1, HCV, and HBV High-Risk Population

Target	n	TN	FP	TP	FN
HIV-1	519	513	0	6	0
HCV	519	427	4	87	1*
HBV	519	515	2	2	0

FN = False Negative, FP = False Positive, n = Number of Specimens, TN = True Negative, TP = True Positive
*This sample was Procleix Ultrio Plus Assay reactive, HCV discriminated, and HCV serologic test repeat reactive. HCV was not detected by the alternate quantitative NAT in this sample.

CLINICAL SENSITIVITY FOR HIV-2 IN A HIGH-RISK POPULATION

Five hundred and twenty (520) prospectively collected plasma samples from men and women from Côte d'Ivoire at high risk of HIV-2 infection but with unknown infection status were procured from a vendor. The samples were tested in-house with the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay on the Procleix Panther System; samples with reactive Procleix Ultrio Elite Assay results were also tested with the Procleix Ultrio Elite HCV Discriminatory Assay and Procleix Ultrio Elite HBV Discriminatory Assay, volume permitting. Three clinical reagent kit lots were used. All samples were tested with the Abbott Architect HIV Ag/Ab Combo Assay that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen. Repeat reactive samples with repeat reactive HIV-1/2 antigen/antibody combination immunoassay were tested with the Bio-Rad Multispot HIV-1/HIV-2 Rapid Test that detects and differentiates HIV-1 antibodies from HIV 2 antibodies. Samples with nonreactive or HIV undifferentiated results were tested with the Procleix Ultrio Plus HIV 1 Discriminatory Assay and a validated quantitative HIV-2 NAT. Samples with HIV-2 reactive results were tested with a validated quantitative HIV-2 NAT. All samples were also tested with the Procleix Ultrio Plus Assay on the Procleix Tigris System; samples with reactive Procleix Ultrio Plus Assay results were tested with the Procleix Ultrio Plus HIV, HCV, and HBV Discriminatory Assays.

Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay results were compared to an HIV-2 interpretation based on immunoassay and NAT results and clinical sensitivity for detection of HIV 2 was calculated (Table 11). Clinical sensitivity of the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay was also calculated relative to the validated HIV-2 NAT results alone (Table 12).

Of the 6 Procleix Ultrio Elite Assay runs, 9 Procleix Ultrio Elite HIV Discriminatory Assay runs, 5 Procleix Ultrio Elite HCV Discriminatory Assay, and 4 Procleix Ultrio Elite HBV Discriminatory Assay runs performed, all were valid. All 520 samples tested with the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay and all 133 samples tested with the Procleix Ultrio Elite HCV Discriminatory Assay and Procleix Ultrio Elite HBV Discriminatory Assay had final valid results.

Ten (10) samples were withdrawn because they did not meet the study inclusion criteria.

For the analyses of sensitivity relative to the HIV-2 interpretation based on immunoassay and NAT results, the sensitivity of the Procleix Ultrio Elite Assay as shown in Table 11 for detection of HIV-2 was 66.67% (6/9) and the sensitivity of the Procleix Ultrio Elite HIV Discriminatory Assay for detection of HIV-2 was 55.56% (5/9).

Table 11. Clinical Sensitivity of the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays Relative to the HIV-2 Interpretation Based on Immunoassay and NAT Results in Samples From an HIV-2 High-Risk Population

Assay	n	TP	FN
Ultrio Elite	9	6	3*
dHIV	9	5	4**

dHIV = Procleix Ultrio Elite HIV Discriminatory Assay, FN = False Negative, n = Number of Specimens, TP = True Positive, Ultrio Elite = Procleix Ultrio Elite Assay
*All 3 samples were Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay nonreactive and had HIV-2 reactive immunoassay results; 1 sample did not have HIV-2 detected by the HIV-2 NAT and 2 samples had ≤10 copies/mL detected.
**Three (3) of these samples were Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay nonreactive and 1 sample was Procleix Ultrio Elite Assay reactive and Procleix Ultrio Elite HIV Discriminatory Assay nonreactive. All 4 samples had HIV-2 reactive immunoassay results. Two (2) of the samples did not have HIV-2 detected by the HIV-2 NAT and 2 samples had ≤10 copies/mL detected.

The sensitivity of the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays was 100% (1/1) relative to HIV-2 NAT results when samples with HIV-2 NAT results <100 copies/mL were excluded. For the analyses of sensitivity relative to HIV-2 NAT results including all samples, the sensitivity of the Procleix Ultrio Elite Assay as shown in Table 12 for detection of HIV-2 was 50.00% (2/4) and the sensitivity of the Procleix Ultrio Elite HIV Discriminatory Assay for detection of HIV-2 was 50.00% (2/4).

Table 12. Clinical Sensitivity of the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays Relative to the HIV-2 NAT Results in Samples From an HIV-2 High-Risk Population

Assay	n	TP	FN*
Ultrio Elite	4	2	2
dHIV	4	2	2

dHIV = Procleix Ultrio Elite HIV Discriminatory Assay, FN = False Negative, n = Number of Specimens, TP = True Positive, Ultrio Elite = Procleix Ultrio Elite Assay

*The 2 samples with false negative Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay results had HIV-2 reactive immunoassay results; both samples had ≤10 copies/mL detected by the HIV-2 NAT.

SPECIFICITY OF THE PROCLEIX ULTRIO ELITE ASSAY AND THE PROCLEIX ULTRIO ELITE DISCRIMINATORY ASSAYS IN NORMAL BLOOD DONOR SERUM SPECIMENS

Frozen normal blood donor serum specimens, which were previously tested and shown to be negative for HIV-1, HCV, and HBV nucleic acids using licensed commercial assays, were tested in the Procleix Ultrio Elite Assay and the three Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays on the Procleix Panther System. Initial reactive specimens were retested in the Procleix Ultrio Elite Assay and/or the relevant Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays and were categorized as defined in Table 13. The reactivity and specificity rates for each of the 4 assays are shown in Table 13.

Tests that were invalid due to instrument hardware errors were retested. Only the valid retest results are included in the data analysis. All specimens were valid and non-reactive upon retest, indicating that none of the specimens exhibited inhibitory effects on the assay reaction. For the Procleix Ultrio Elite Assay, there were 0 initial invalid results due to assay chemistry errors, for an initial invalid rate of 0.00% (0/3000). Three different reagent lots were used during testing. For the Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays, there were 0 initial invalid results due to assay chemistry errors, for an initial invalid rate of 0.00% for each assay.

Table 13. Specificity of the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays in Frozen Normal Blood Donor Serum Specimens

	Ultrio Elite	dHIV	dHCV	dHBV
Valid Results (n)	3000	211	210	210
Initial Reactive (n)	1	1	0	0
Initial Reactive Rate (%)	0.03	0.47	0.00	0.00
True Positive After Repeat Testing* (n)	0	0	0	0
False Positive After Repeat Testing** (n)	1	1	0	0
False Positive Rate After Repeat Testing (%)	0.03	0.47	0.00	0.00
Specificity After Repeat Testing (%) and 95% CI	99.97 (99.81–99.99)	99.53 (97.36–99.92)	100.00 (98.20–100)	100.00 (98.20–100)
Combined Mean Analyte S/CO of Negative Specimens ± standard deviation	0.04 + 0.02	0.06 + 0.03	0.00 + 0.00	0.00 + 0.01

dHBV = Procleix Ultrio Elite HBV Discriminatory Assay, dHCV = Procleix Ultrio Elite HCV Discriminatory Assay, dHIV = Procleix Ultrio Elite HIV Discriminatory Assay, n = Number of Specimens, CI = Score Confidence Interval, S/CO = Signal to Cutoff ratio, Ultrio Elite = Procleix Ultrio Elite Assay

*Specimens determined to be True Positives were repeat reactive in either the Procleix Ultrio Elite Assay or the relevant Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assay.

**Specimens determined to be False Positives were non-reactive upon retesting in either the Procleix Ultrio Elite Assay or the relevant Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assay.

SPECIFICITY AND SENSITIVITY OF THE PROCLEIX ULTRIO ELITE ASSAY IN THE PRESENCE OF DONOR AND DONATION FACTORS

When tested with the Procleix Ultrio Elite Assay, no cross-reactivity or interference was observed for naturally occurring icteric, hemolyzed, or lipemic specimens or plasma containing the following substances: albumin (60 g/L), hemoglobin (5000 mg/L), bilirubin (200 mg/L), and lipids (30,000 mg/L), with the exception of one lipemic donor with a false positive result for an overall specificity of 95% (95% confidence interval: 76.4%-99.1%).

No cross-reactivity or interference was observed in specimens from patients with autoimmune and other diseases not caused by HIV-1, HIV-2, 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 Panther System magnetic wash station errors.

No cross-reactivity or interference 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 or II, hepatitis A virus, cytomegalovirus, Epstein-Barr virus, rubella virus, parvovirus B-19, and West Nile virus. Specimens spiked with dengue virus (types 1–4) were also evaluated.

SEROCONVERSION PANELS

Reactivity in Seroconverting Donors

Commercially available seroconversion panels were tested to determine the ability of the Procleix Ultrio Elite Assay 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 Elite Assay was used to test each seroconversion panel neat, diluted 1:8, and diluted 1:16. 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 Elite Assay was able to detect HIV-1 RNA an average of 13.9 and 9.4 days earlier in neat samples, 11.0 and 6.5 days earlier in 1:8 dilutions, and 10.4 and 5.9 days earlier in 1:16 dilutions (Table 14).

Table 14. Detection of HIV-1 RNA in HIV-1 Seroconversion Panels

Panel	Days Earlier Detection Than Anti-HIV 1/2 Antibody			Days Earlier Detection Than HIV p24 Antigen		
	Ultrio Elite			Ultrio Elite		
	Neat	1:8	1:16	Neat	1:8	1:16
1	16	14	9	7	5	0
2	14	10	10	4	0	0
3	15	11	11	8	4	4
4	14	14	14	7	7	7
5	12	12	12	19	19	19
6*	14	10	14	14	10	14
7	14	8	8	14	8	8
8**	11	11	11	4	4	4
9	15	13	8	7	5	0
10	14	7	7	10	3	3
Mean	**13.9**	**11.0**	**10.4**	**9.4**	**6.5**	**5.9**
Median	**14.0**	**11.0**	**10.5**	**7.5**	**5.0**	**4.0**

Ultrio Elite = Procleix Ultrio Elite Assay

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 results, with the following exceptions:

*Panel 6 was compared to Roche Elecsys HIV p24 Ag test because seroconversion was not demonstrated with the Coulter HIV-1 p24 Ag test.

**Panel 8 was compared to ZeptoMetrix p24 Ag test, as there were no Coulter HIV-1 p24 Ag results reported.

HCV Detection in Seroconversion Panels

When compared to the Anti-HCV 3.0 antibody tests the Procleix Ultrio Elite Assay was able to detect HCV RNA an average of 33.1 days earlier in neat samples, 32.1 days earlier in 1:8 dilutions, and 32.1 days earlier in 1:16 dilutions (Table 15). In 5 of the 12 seroconversion panels (2, 5, 7, 9, and 10), the first available bleed in the series was already reactive with the Procleix Ultrio Elite Assay, so the number of days of window closure may underestimate the true window closure period for the assays.

Table 15. Detection of HCV RNA in HCV Seroconversion Panels

Panel	Days Earlier Detection Than HCV Antibody		
	Ultrio Elite		
	Neat	1:8	1:16
1	26	26	26
2	30	30	30
3	23	23	23
4*	39	33	33
5*	39	39	39
6	32	32	32
7	31	31	31
8	38	38	38
9*	41	41	41
10*	28	28	28
11	36	30	30
12**	34	34	34
Mean	**33.1**	**32.1**	**32.1**
Median	**33.0**	**31.5**	**31.5**

Ultrio Elite = Procleix Ultrio Elite Assay

All panels were compared to the Ortho Anti-HCV 3.0 (SAVe) test results, with the following exceptions:

*Panels 4, 5, 9, and 10 were compared to the Ortho ELISA Anti-HCV 3.0 test as there were no Ortho Anti-HCV 3.0 (SAVe) results reported.

**Panel 12 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 Elite Assay was able to detect HBV DNA an average of 13.8 and 23.6 days earlier in neat samples, 6.2 and 16.0 days earlier in 1:8 dilutions, and 2.1 and 11.9 days earlier in 1:16 dilutions (Table 16). Substantial closure of the seroconversion window compared to the Abbott PRISM HBsAg tests and Ortho HBsAg was observed with the Procleix Ultrio Elite Assay in 10 of the 11 seroconversion panels tested. In panel 8, HBV detection was 27 days after detection with Abbott PRISM HBsAg in a diluted 1:16 sample. For this panel, there was a 27 day period between the draws (no additional draws were taken during this time). The window period closure calculation for this panel reflects the frequency and spacing of the draw dates of the panel. Panel 10 had low PRISM S/CO values (1.37, 1.18, 1.00, 2.13, 1.77) in the first 5 draws beginning with the 6th draw at 17 days after the first collection. The panel was investigated by submitting each draw to the PRISM assay at an independent reference lab to reveal that of these first five reactive bleeds, only the last two (and all subsequent draws) were again reactive. The window period closure calculation for this panel may reflect extremely low viral titer, or potentially, panel contamination since the Abbott PRISM HBsAg Test was first to detect HBsAg and no other serological marker is positive during those first 5 weeks.

Table 16. Detection of HBV DNA in HBV Seroconversion Panels

Panel	Days Earlier Detection Than HBV Surface Antigen, Abbott PRISM HBsAg Test			Days Earlier Detection Than HBV Surface Antigen, Ortho HBsAg ELISA Test System 3		
	Ultrio Elite			Ultrio Elite		
	Neat	1:8	1:16	Neat	1:8	1:16
1	26	26	26	29	29	29
2	22	0	0	22	0	0
3	7	-7	-7	23	9	9
4	20	8	8	20	8	8
5	14	14	14	18	18	18
6	21	11	14	24	14	17
7	19	12	5	26	19	12
8	9	0	-27	36	27	0
9	25	15	15	34	24	24
10	-14	-14	-21	14	14	7
11	3	3	-4	14	14	7
Mean	**13.8**	**6.2**	**2.1**	**23.6**	**16.0**	**11.9**
Median	**19**	**8**	**5**	**23**	**14**	**9**

Ultrio Elite = Procleix Ultrio Elite Assay

ANALYTICAL SENSITIVITY

Analytical sensitivity panels consisting of serially diluted HIV-1 WHO standard (97/650), HIV-2 WHO standard (08/150), HCV WHO standard (06/100), and HBV WHO standard (97/750) were used to evaluate assay sensitivity. The panels were tested with the Procleix Ultrio Elite Assay and the three Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays on the Procleix Panther System. The average analyte S/CO ratio and percent coefficient of variation (%CV) for samples containing viral RNA or DNA were calculated from concordant results only (S/CO > 1.0). The 95% confidence intervals of the reactivity rates were based on the Score method;[44] estimations of 50% and 95% detection rates were determined through Probit Analysis.

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

The detection rate for the HIV-1 WHO standard at 600, 200, and 60 IU/mL was 100% with both the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV Discriminatory Assay. The detection rate at 20 IU/mL with the Procleix Ultrio Elite Assay was 96%. With the Procleix Ultrio Elite HIV Discriminatory Assay, the detection rate at 20 IU/mL was 97%. The detection rates for 6 IU/mL were 56% and 57% with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV Discriminatory Assay, respectively (Table 17).

Table 17. Detection of HIV-1 WHO Standard in Analytical Sensitivity Panels

Ultrio Elite							dHIV					
HIV-1 WHO (97/650) IU/mL	Number of Reactive / Tested	% Reactive	95% CI		Average S/CO	% CV	Number of Reactive / Tested	% Reactive	95% CI		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
600	182/182	100	98	100	10.26	5	180/180	100	98	100	18.44	6
200	182/182	100	98	100	10.15	5	180/180	100	98	100	18.24	6
60	182/182	100	98	100	9.76	13	180/180	100	98	100	17.90	8
20	175/182	96	92	98	8.37	29	174/180	97	93	98	15.15	31
6	102/182	56	49	63	6.69	47	103/180	57	50	64	11.85	47
0	0/182	0	0	2	0.09	55	0/182	0	0	2	0.10	75

CI = Score Confidence Interval, CV = Coefficient of Variation, dHIV = Procleix Ultrio Elite HIV Discriminatory Assay, S/CO = Signal to Cutoff ratio in concordant replicates only, Ultrio Elite = Procleix Ultrio Elite Assay

Detection of HIV-2 WHO Standard (08/150)

The detection rate for the HIV-2 WHO standard at 30 IU/mL was 100% with both the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV Discriminatory Assay. The detection rate at 10 IU/mL with the Procleix Ultrio Elite Assay was 94%. With the Procleix Ultrio Elite HIV Discriminatory Assay, the detection rate at 10 IU/mL was 96%. The detection rates for 3, 1, and 0.3 IU/mL were 57%, 20%, and 7% with the Procleix Ultrio Elite Assay, respectively. With the Procleix Ultrio Elite HIV Discriminatory Assay, the detection rates at 3, 1, and 0.3 IU/mL were 58%, 31%, and 8%, respectively (Table 18).

Table 18. Detection of HIV-2 WHO Standard in Analytical Sensitivity Panels

Ultrio Elite							dHIV					
HIV-2 WHO (08/150) IU/mL	Number of Reactive / Tested	% Reactive	95% CI		Average S/CO	% CV	Number of Reactive / Tested	% Reactive	95% CI		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
30	180/180	100	98	100	7.24	11	180/180	100	98	100	13.20	9
10	169/180	94	89	97	6.68	22	173/180	96	92	98	11.87	24
3	103/180	57	50	64	6.18	30	105/180	58	51	65	10.45	37
1	36/180	20	15	26	6.16	36	56/180	31	25	38	9.15	47
0.3	13/180	7	4	12	4.41	47	15/180	8	5	13	8.82	50
0	0/180	0	0	2	0.10	54	0/180	0	0	2	0.12	97

CI = Score Confidence Interval, CV = Coefficient of Variation, dHIV = Procleix Ultrio Elite HIV Discriminatory Assay, S/CO = Signal to Cutoff ratio in concordant replicates only, Ultrio Elite = Procleix Ultrio Elite Assay

Detection of HCV WHO Standard (06/100)

The detection rate for the HCV WHO standard at 100, 30, and 10 IU/mL was 100% with both the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite dHCV Assay. The detection rate at 3 IU/mL with the Procleix Ultrio Elite Assay was 95%. With the Procleix Ultrio Elite dHCV Assay, the detection rate at 3 IU/mL was 97%. The detection rates for 1 IU/mL were 55% and 71% with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite dHCV Assay, respectively (Table 19).

Table 19. Detection of HCV WHO Standard in Analytical Sensitivity Panels

Ultrio Elite							dHCV					
HCV WHO (06/100) IU/mL	Number of Reactive / Tested	% Reactive	95% CI		Average S/CO	% CV	Number of Reactive / Tested	% Reactive	95% CI		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
100	183/183	100	98	100	8.60	4	180/180	100	98	100	22.49	7
30	180/180	100	98	100	8.62	5	180/180	100	98	100	22.49	7
10	180/180	100	98	100	8.52	5	180/180	100	98	100	22.32	7
3	171/180	95	91	97	8.28	9	175/180	97	94	99	20.67	18
1	100/182	55	48	62	7.55	21	127/180	71	64	77	19.48	24
0	0/180	0	0	2	0.09	52	0/180	0	0	2	0.07	114

CI = Score Confidence Interval, CV = Coefficient of Variation, dHCV = Procleix Ultrio Elite HCV Discriminatory Assay, S/CO = Signal to Cutoff ratio in concordant replicates only, Ultrio Elite = Procleix Ultrio Elite Assay

Detection of HBV WHO Standard (97/750)

The detection rate for the HBV WHO standard at 45 and 15 IU/mL was 100% with both the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HBV Discriminatory Assay. HBV detection at 5 IU/mL was 97% with both the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HBV Discriminatory Assay. The detection rates for 1.67 IU/mL were 70% and 65% with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HBV Discriminatory Assay, respectively. At 0.56 IU/mL the HBV detection rate was 34% with both the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HBV Discriminatory Assay (Table 20).

Table 20. Detection of HBV WHO Standard in Analytical Sensitivity Panels

Ultrio Elite							dHBV					
HBV WHO (97/750) IU/mL	Number of Reactive / Tested	% Reactive	95% CI		Average S/CO	% CV	Number of Reactive / Tested	% Reactive	95% CI		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
45	360/360	100	99	100	14.10	8	360/360	100	99	100	23.49	8
15	360/360	100	99	100	14.03	8	360/360	100	99	100	23.29	9
5	348/360	97	94	98	13.56	15	349/360	97	95	98	22.46	15
1.67	253/360	70	65	75	13.05	22	235/360	65	60	70	21.35	22
0.56	122/360	34	29	39	12.32	27	124/360	34	30	39	20.61	24
0	0/360	0	0	1	0.07	69	0/360	0	0	1	0.05	133

CI = Score Confidence Interval, CV = Coefficient of Variation, dHBV = Procleix Ultrio Elite HBV Discriminatory Assay, S/CO = Signal to Cutoff ratio in concordant replicates only, Ultrio Elite = Procleix Ultrio Elite Assay

Probit Analysis

The predicted 50% and 95% detection rates in IU/mL for each target were determined through Probit Analysis[45] of the analytical sensitivity results. The predicted 95% detection rate for the HIV-1 WHO was 18.0 IU/mL for the Procleix Ultrio Elite Assay and 17.3 IU/mL for the Procleix Ultrio Elite HIV Discriminatory Assay. The predicted 95% detection rate for the HIV-2 WHO was 10.4 IU/mL for the Procleix Ultrio Elite Assay and 9.6 IU/mL for the Procleix Ultrio Elite HIV Discriminatory Assay The predicted 95% detection rate for the HCV WHO was 3.0 IU/mL for the Procleix Ultrio Elite Assay and 2.4 IU/mL for the Procleix Ultrio Elite HCV Discriminatory Assay. The predicted 95% detection rate for the HBV WHO was 4.3 IU/mL for the Procleix Ultrio Elite Assay and 4.5 IU/mL for the Procleix Ultrio Elite HBV Discriminatory Assay (Table 21).

Table 21. Detection Probabilities of HIV-1, HIV-2, HCV, and HBV

Panel Tested	Procleix Assay	Detection Probabilities (IU/mL)	
		50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
HIV-1 WHO (97/650)	Ultrio Elite	5.4 (4.5 to 6.1)	18.0 (15.0 to 23.5)
HIV-1 WHO (97/650)	dHIV	5.3 (4.4 to 6.0)	17.3 (14.4 to 22.6)
HIV-2 WHO (08/150)	Ultrio Elite	2.6 (2.3 to 3.0)	10.4 (8.9 to 12.6)
HIV-2 WHO (08/150)	dHIV	2.2 (1.9 to 2.5)	9.6 (8.1 to 11.8)
HCV WHO (06/100)	Ultrio Elite	0.9 (0.8 to 1.0)	3.0 (2.5 to 3.9)
HCV WHO (06/100)	dHCV	0.7 (0.5 to 0.8)	2.4 (2.0 to 3.3)
HBV WHO (97/750)	Ultrio Elite	0.9 (0.8 to 1.1)	4.3 (3.8 to 5.0)
HBV WHO (97/750)	dHBV	1.0 (0.9 to 1.1)	4.5 (4.0 to 5.3)

dHBV = Procleix Ultrio Elite HBV Discriminatory Assay, dHCV = Procleix Ultrio Elite HCV Discriminatory Assay, dHIV = Procleix Ultrio Elite HIV Discriminatory Assay, Ultrio Elite = Procleix Ultrio Elite Assay

COMPARISON OF THE PROCLEIX ULTRIO ELITE ASSAY TO HIV-1, HCV, AND HEPATITIS B SURFACE ANTIGEN CONFIRMATORY SEROLOGY RESULTS: BASIS FOR THE SUPPLEMENTAL TEST CLAIMS

Results obtained from Procleix Ultrio Elite Assay testing on the Procleix Panther System at one in-house testing site and from donor screening serologic testing at one laboratory allow comparison of the Procleix Ultrio Elite Assay with HIV-1, HCV, and HBsAg confirmatory test reactivity (Table 22). All of the samples included in this analysis were screening serologic test repeat reactive with positive, negative, or indeterminate (as applicable) confirmatory serology results.

Procleix Ultrio Elite Assay and HIV-1 serology results were available for 159 samples. Of the 159 samples, 59 had confirmed seropositive test results and 100 had negative or indeterminate confirmatory serologic test results. Agreement between Procleix Ultrio Elite Assay and Western blot or immunofluorescent assay positive results was 94.9% (56/59; 95% CI: 86.1% to 98.3%). Confidence interval was determined using the Score method.[44] Of the 59 confirmed seropositive samples, 3 samples were Procleix Ultrio Elite Assay nonreactive for HIV and 56 samples were Procleix Ultrio Elite Assay reactive. One of the Procleix Ultrio Elite nonreactive samples was Procleix Ultrio Elite Assay reactive upon retest, but nonreactive in all discriminatory assays. The remaining 100 of 159 samples with HIV-1 serology results had negative (n=50) or indeterminate (n=50) confirmatory test results. Agreement between Procleix Ultrio Elite Assay and immunofluorescent assay negative results was 100.0% (50/50; 95% CI: 92.9% to 100.0%). Of the 50 confirmed seronegative samples, all samples were Procleix Ultrio Elite Assay nonreactive. For the 50 samples with indeterminate confirmatory test results, 50 were Procleix Ultrio Elite Assay nonreactive for HIV (100.0%, 50/50; 95% CI: 92.9% to 100.0%). Two of these samples were initially Procleix Ultrio Elite Assay reactive but were nonreactive in the Procleix Ultrio Elite HIV Discriminatory Assay; these two samples were then retested with nonreactive results in the Procleix Ultrio Elite Assay and all discriminatory assays. Therefore, when a sample is repeat reactive on a licensed anti-HIV-1 screening test and Procleix Ultrio Elite Assay reactive, the Procleix Ultrio Elite 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 Elite Assay and HCV serology results were available for 113 samples. Of the 113 samples, 61 had confirmed seropositive test results, and 52 had negative confirmatory serologic test results. Agreement between Procleix Ultrio Elite Assay and recombinant immunoblot assay positive results was 75.4% (46/61; 95% CI: 63.3% to 84.5%). Of the 61 confirmed seropositive samples, 46 samples were Procleix Ultrio Elite Assay nonreactive for HCV and 15 samples were Procleix Ultrio Elite Assay reactive. Two of the Procleix Ultrio Elite Assay nonreactive samples initially had Procleix Ultrio Elite Assay reactive results but were nonreactive in the dHCV assay; these two samples were then retested with nonreactive results in the Procleix Ultrio Elite Assay and all discriminatory assays. Approximately 20% of recombinant immunoblot assay positive samples are expected to have undetectable HCV RNA due to a resolved HCV infection.[46] The remaining 52 of 113 samples with HCV serology status had negative confirmatory test results. Agreement between Procleix Ultrio Elite Assay and enzyme-linked immunosorbent assay negative results was 100.0% (52/52; 95% CI: 93.1% to 100.0%). One of these samples was initially Procleix Ultrio Elite Assay reactive but was nonreactive in the dHCV assay; this sample was then retested with nonreactive results in the Procleix Ultrio Elite Assay and all discriminatory assays. Therefore, when a sample is repeat reactive on a licensed anti-HCV screening test and Procleix Ultrio Elite Assay reactive, the Procleix Ultrio Elite Assay acts as a supplemental test that confirms HCV infection; it is not necessary to run an HCV enzyme-linked immunosorbent assay.

Procleix Ultrio Elite Assay and HBsAg serology results were available for 119 samples. Of the 119 samples, 60 had seropositive test results and 59 had negative HBsAg neutralization test results. Agreement between Procleix Ultrio Elite Assay and HBsAg neutralization test positive results was 91.7% (55/60; 95% CI: 81.9% to 96.4%). Of the 60 samples, 5 samples were Procleix Ultrio Elite Assay nonreactive for HBV and 55 samples were Procleix Ultrio Elite Assay reactive. One of the Procleix Ultrio Elite Assay nonreactive samples initially had a Procleix Ultrio Elite Assay reactive result but was nonreactive in the Procleix Ultrio Elite HBV Discriminatory Assay; this sample was then retested with nonreactive results in the Procleix Ultrio Elite Assay and all discriminatory assays. The results for the 5 samples that were HBsAg neutralization test reactive and Procleix Ultrio Elite Assay

nonreactive are not unexpected, as HBsAg may be present in particles that do not contain nucleic acids[47] or after vaccination with a vaccine derived from HBsAg.[48]

The remaining 59 of 119 samples with HBV serology results had negative HBsAg neutralization test results. Agreement between Procleix Ultrio Elite Assay and HBsAg neutralization negative test results was 100.0% (59/59; 95% CI: 93.9% to 100.0%). For the 59 samples with negative neutralization test results, all were Procleix Ultrio Elite Assay nonreactive for HBV. One sample was initially Procleix Ultrio Elite Assay reactive but was nonreactive in the Procleix Ultrio Elite HBV Discriminatory Assay; this sample was then retested with nonreactive results in the Procleix Ultrio Elite Assay, Procleix Ultrio Elite HIV Discriminatory Assay and Procleix Ultrio Elite HBV Discriminatory Assay but with a reactive result in the Procleix Ultrio Elite HCV Discriminatory Assay. Therefore, when a sample is repeat reactive on a licensed HBsAg screening test and reactive on the Procleix Ultrio Elite Assay, the Procleix Ultrio Elite Assay acts as a supplemental test that confirms HBV infection; it is not necessary to run an HBsAg neutralization test.

Table 22. Comparison of Procleix Ultrio Elite Assay and HIV-1, HCV, and HBsAg Confirmatory Serology Results

Serology			Ultrio Elite	
			Reactive	Nonreactive
HIV-1 Screening Test Repeat Reactive	**HIV-1 WB or IFA**			
	Positive	59[a]	56	3[b]
	Indeterminate	50	0	50[c]
	Negative	50	0	50
HCV Screening Test Repeat Reactive	**HCV RIBA or ELISA**			
	Positive	61	46	15[d]
	Negative	52	0	52[e]
HBsAg Screening Test Repeat Reactive	**HBsAg Neutralization**			
	Positive	60	55	5[f]
	Negative	59[g]	0	59[h]

ELISA = Enzyme-Linked Immunosorbent Assay, IFA = Immunofluorescent Assay, RIBA = Recombinant Immunoblot Assay, WB = Western Blot, Ultrio Elite = Procleix Ultrio Elite Assay

[a] One sample was repeatedly invalid in the Procleix Ultrio Elite Assay and removed from analysis.

[b] One sample was nonreactive in initial Procleix Ultrio Elite Assay screening. A second aliquot was retested in the Procleix Ultrio Elite Assay and all Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays; the result was reactive in the Procleix Ultrio Elite Assay but nonreactive in the Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays. This sample was included in analysis as nonreactive.

[c] Two samples were initially reactive in the Procleix Ultrio Elite Assay. A second aliquot was tested in the Procleix Ultrio Elite HIV Discriminatory Assay with nonreactive results. A third aliquot was tested in the Procleix Ultrio Elite Assay and all Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays with nonreactive results. These samples were included in analysis as nonreactive.

[d] Two samples were initially reactive in the Procleix Ultrio Elite Assay but were nonreactive when a second and third aliquot were tested in the Procleix Ultrio Elite HCV Discriminatory Assay, and in the Procleix Ultrio Elite Assay and all Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays, respectively. Two samples were initially nonreactive in the Procleix Ultrio Elite Assay but were reactive when a second aliquot was tested in the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HCV Discriminatory Assay. These samples were included in analysis as nonreactive.

[e] One sample was initially reactive in the Procleix Ultrio Elite Assay. A second and third aliquot were tested with all nonreactive results in the Procleix Ultrio Elite HCV Discriminatory Assay, and in the Procleix Ultrio Elite Assay and all Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays, respectively. This sample was included in analysis as nonreactive.

[f] One sample was initially reactive in the Procleix Ultrio Elite Assay but nonreactive when a second and third aliquot were tested in the Procleix Ultrio Elite HBV Discriminatory Assay, and in the Procleix Ultrio Elite Assay and all Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays, respectively. These samples were included in analysis as nonreactive.

[g] One sample was reactive in 2 of 2 replicates in the Procleix Ultrio Elite Assay and in 2 of 2 replicates of the Procleix Ultrio Elite HBV Discriminatory Assay, and was nonreactive in 1 replicate each of the Procleix Ultrio Elite HIV Discriminatory Assay and the Procleix Ultrio Elite HCV Discriminatory Assay. This sample was removed from analysis.

[h] One sample was initially reactive in the Procleix Ultrio Elite Assay but nonreactive when a second and third aliquot were tested in the Procleix Ultrio Elite HBV Discriminatory Assay, and in the Procleix Ultrio Elite Assay and all Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays, respectively. One of the two samples was also reactive in the Procleix Ultrio Elite HCV Discriminatory Assay. This sample was included in analysis as nonreactive.

ABILITY TO DETECT HIV-1, HIV-2, 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 Elite Assay and the Procleix Ultrio Elite HIV Discriminatory Assay

HIV-1 specimens and tissue culture isolates of group M (subtypes A, B, C, D, E, F, G, H, K, CRF01_AE, CRF02_AG, and CRF03_AB), 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/HCV/HBV NAT negative human serum to target viral concentrations of 100 and 30 copies/mL. Diluted specimens were tested in the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV Discriminatory Assay. Seventy one unique specimens or tissue culture isolates were tested in duplicate using two lots of reagents on the Procleix Panther System. At 100 copies/mL, 286/286 replicates (100%) were reactive with the Procleix Ultrio Elite Assay and 284/284 replicates (100%) were reactive with the Procleix Ultrio Elite HIV Discriminatory Assay. At 30 copies/mL, 272/284 replicates (95.8%) were reactive with the Procleix Ultrio Elite Assay and 273/286 replicates (95.5%) were reactive with the Procleix Ultrio Elite HIV Discriminatory Assay (Table 23). 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 23. Detection of HIV-1 Genetic Variants*

Genetic Variant	Copies/mL	Unique Donors	Ultrio Elite		dHIV	
			Reactive / Tested	% Reactive	Reactive / Tested	% Reactive
HIV-1 Group M Subtype A	100	11	44/44	100	44/44	100
	30		41/44	93.2	41/44	93.2
HIV-1 Group M CRF01_AE	100	2	8/8	100	8/8	100
	30		8/8	100	8/8	100
HIV-1 Group M CRF02_AG	100	2	8/8	100	8/8	100
	30		8/8	100	8/8	100
HIV-1 Group M CRF03_AB	100	2	8/8	100	8/8	100
	30		8/8	100	8/8	100
HIV-1 Group M Subtype B	100	10	40/40	100	40/40	100
	30		40/40	100	39/40	97.5
HIV-1 Group M Subtype C	100	8	32/32	100	32/32	100
	30		31/32	96.9	31/32	96.9
HIV-1 Group M Subtype D	100	10	40/40	100	40/40	100
	30		38/40	95.0	37/40	92.5
HIV-1 Group M Subtype E	100	10	40/40	100	40/40	100
	30		36/40	90.0	38/40	95.0
HIV-1 Group M Subtype F	100	5	20/20	100	20/20	100
	30		19/20	95.0	18/20	90.0
HIV-1 Group M Subtype G	100	2	8/8	100	8/8	100
	30		8/8	100	8/8	100
HIV-1 Group M Subtype H	100	1	4/4	100	4/4	100
	30		4/4	100	4/4	100
HIV-1 Group M Subtype K	100	1	4/4	100	4/4	100
	30		4/4	100	4/4	100
HIV-1 Group N	100	1	4/4	100	4/4	100
	30		3/4	75.0	3/4	75.0
HIV-1 Group O***	100	6	26/26**	100	24/24	100
	30		24/24	100	26/26**	100
Total	100	71	286/286	100	284/284	100
	30		272/284	95.8	273/286	95.5

dHIV = Procleix Ultrio Elite HIV Discriminatory Assay, Ultrio Elite = Procleix Ultrio Elite Assay

*The same panels were used for testing with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV Discriminatory Assay.

**One specimen from this group had 2 additional replicates tested (4 reps total).

***In a multicenter performance evaluation of the Procleix Ultrio Elite Assay the 50% and 95% LOD for HIV-1 Group O was determined to be either 0.5 copies/mL (95% CI: 0.3-0.8) and 3.7 copies/mL (95% CI: 2.1-13.5), respectively, or 1.3 copies/mL (95% CI: 0.9-1.8) and 10.4 copies/mL (95% CI: 6.5-18.2), respectively, based on the calibration of the assay.[49]

Detection of HIV-2 Genetic Variants with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV Discriminatory Assay

HIV-2 tissue culture isolates of subtypes A and B were quantified for HIV-2 RNA by the vendor. The concentrations of HIV-2 RNA transcripts were determined using ultraviolet (UV) absorbance. Specimens were diluted with HIV/HCV/HBV NAT negative human serum or a HEPES buffered solution to target viral concentrations of 100 and 30 copies/mL. Diluted specimens were tested in the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV Discriminatory Assay. Six unique tissue culture isolates or in-vitro transcripts were tested in duplicate using two lots of reagents on the Procleix Panther System. At 100 copies/mL, 21/24 replicates (87.5%) were reactive with the Procleix Ultrio Elite Assay and 23/24 replicates (95.8%) were reactive with the Procleix Ultrio Elite HIV Discriminatory Assay. At 30 copies/mL, 21/24 replicates (87.5%) were reactive with the Procleix Ultrio Elite Assay and 20/24 replicates (83.3%) were reactive with the Procleix Ultrio Elite HIV Discriminatory Assay (Table 24). One HIV-2 subtype A specimen was not 100% reactive at 100 copies/mL, therefore this specimen was tested at 1000 and 300 copies/mL and was 100% reactive at these levels. 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 24. Detection of HIV-2 Genetic Variants*

Genetic Variant	Copies/mL	Unique Donors	Ultrio Elite		dHIV	
			Reactive / Tested	% Reactive	Reactive / Tested	% Reactive
HIV-2 Subtype A	1000	5	4/4	100	4/4	100
	300		4/4	100	4/4	100
	100		17/20	85.0	19/20	95.0
	30		17/20	85.0	16/20	80.0
HIV-2 Subtype B	100	1	4/4	100	4/4	100
	30		4/4	100	4/4	100
Total	1000	6	4/4	100	4/4	100
	300		4/4	100	4/4	100
	100		21/24	87.5	23/24	95.8
	30		21/24	87.5	20/24	83.3

dHIV = Procleix Ultrio Elite HIV Discriminatory Assay, Ultrio Elite = Procleix Ultrio Elite Assay

*The same panels were used for testing with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV Discriminatory Assay.

Detection of HCV Genotypes with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite 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. The HCV genotypes tested included subtypes 1a, 1b, 2a/c, 2b, 3a, 3b, 3e, 4a, 4b/c, 5a, and 6a. Specimens were diluted with HIV/HCV/HBV NAT negative human serum to target viral concentrations of 100 and 30 copies/mL. The diluted specimens were tested with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HCV Discriminatory Assay. Fifty-nine unique specimens were tested in duplicate using two lots of reagents on the Procleix Panther System. At 100 copies/mL, 236/236 replicates (100%) were reactive with both the Procleix Ultrio Elite Assay the Procleix Ultrio Elite HCV Discriminatory Assay. At 30 copies/mL, 217/236 replicates (91.9%) were reactive with the Procleix Ultrio Elite Assay and 213/236 replicates (90.3%) were reactive with the Procleix Ultrio Elite HCV Discriminatory Assay (Table 25). 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 25. Detection of HCV Genotypes*

Genotype	Copies/mL	Unique Donors	Ultrio Elite		dHCV	
			Reactive / Tested	% Reactive	Reactive / Tested	% Reactive
HCV Genotype 1	100	10	40/40	100	40/40	100
	30		37/40	92.5	35/40	87.5
HCV Genotype 2	100	14	56/56	100	56/56	100
	30		45/56	80.4	42/56	75.0
HCV Genotype 3	100	11	44/44	100	44/44	100
	30		39/44	88.6	40/44	90.9
HCV Genotype 4	100	13	52/52	100	52/52	100
	30		52/52	100	52/52	100
HCV Genotype 5	100	5	20/20	100	20/20	100
	30		20/20	100	20/20	100
HCV Genotype 6	100	6	24/24	100	24/24	100
	30		24/24	100	24/24	100
Total	100	59	236/236	100	236/236	100
	30		217/236	91.9	213/236	90.3

dHCV = Procleix Ultrio Elite HCV Discriminatory Assay, Ultrio Elite = Procleix Ultrio Elite Assay

*The same panels were used for testing with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HCV Discriminatory Assay.

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

HBV specimens of genotypes A, B, C, D, E, F, G and H were quantified for HBV DNA using commercially available quantitative HBV DNA assays. Specimens were diluted with HIV/HCV/HBV NAT negative human serum to target viral concentrations of 100 and 30 copies/mL. Diluted specimens were tested with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HBV Discriminatory Assay. Fifty-nine unique specimens were tested in duplicate using two lots of reagents on the Procleix Panther System. At 100 copies/mL, 235/236 replicates (99.6%) were reactive with the Procleix Ultrio Elite Assay and 234/236 replicates (99.2%) were reactive with the Procleix Ultrio Elite HBV Discriminatory Assay. At 30 copies/mL, 220/236 replicates (93.2%) were reactive with the Procleix Ultrio Elite Assay and 219/236 replicates (92.8%) were reactive with the Procleix Ultrio Elite HBV Discriminatory Assay. Three specimens were not 100% reactive at 100 copies/mL, therefore they were tested at 300 copies/mL and were 100% reactive at this level (Table 26). 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 26. Detection of HBV Genotypes*

Genotype	Copies/mL	Unique Donors	Ultrio Elite		dHBV	
			Reactive / Tested	% Reactive	Reactive / Tested	% Reactive
HBV Genotype A	100	11	44/44	100	44/44	100
	30		40/44	90.9	43/44	97.7
HBV Genotype B	100	10	40/40	100	40/40	100
	30		38/40	95.0	39/40	97.5
HBV Genotype C	300	10	8/8	100	8/8	100
	100		39/40	97.5	39/40	97.5
	30		32/40	80.0	31/40	77.5
HBV Genotype D	100	9	36/36	100	36/36	100
	30		34/36	94.4	34/36	94.4
HBV Genotype E	100	9	36/36	100	36/36	100
	30		36/36	100	34/36	94.4
HBV Genotype F	300	8	4/4	100	4/4	100
	100		32/32	100	31/32	96.9
	30		32/32	100	30/32	93.8
HBV Genotype G	100	1	4/4	100	4/4	100
	30		4/4	100	4/4	100
HBV Genotype H	100	1	4/4	100	4/4	100
	30		4/4	100	4/4	100
Total	300	59	12/12	100	12/12	100
	100		235/236	99.6	234/236	99.2
	30		220/236	93.2	219/236	92.8

dHBV = Procleix Ultrio Elite HBV Discriminatory Assay, Ultrio Elite = Procleix Ultrio Elite Assay

*The same panels were used for testing with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HBV Discriminatory Assay.

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

SPECIFICITY

Specificity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens

HIV-1, HIV-2, HCV and HBV seronegative cadaveric blood specimens were tested to determine the specificity of the Procleix Ultrio Elite Assay. Twenty-five cadaveric and 25 normal donor specimens were tested using three reagent lots, with the exception of 50 normal donor specimens tested in the Procleix Ultrio Elite HIV Discriminatory Assay. The specificity of the Procleix Ultrio Elite Assay for the cadaveric specimens was 100% in both plasma and serum specimens (95% confidence interval: 86.3%-100%) (Table 27). The specificity of the Procleix Ultrio Elite HIV Discriminatory Assay for the cadaveric specimens was 100% in both plasma and serum specimens (95% confidence interval: 86.3%-100%) (Table 28). The specificity of the Procleix Ultrio Elite HCV Discriminatory Assay for the cadaveric specimens was 100% in both plasma and serum specimens (95% confidence interval: 86.3%-100%) (Table 29). The specificity of the Procleix Ultrio Elite HBV Discriminatory Assay for the cadaveric specimens was 100% in both plasma and serum specimens (95% confidence interval: 86.3%-100%) (Table 30).

Table 27. Specificity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	2.00	1.90	2.01	1.91
Mean Analyte S/CO	0.06	0.04	0.05	0.04
Specificity rate (%)	100	100	100	100
95% CI, Specificity Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Table 28. Specificity of the Procleix Ultrio Elite HIV Discriminatory Assay in Cadaveric Blood Specimens

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	2.00	1.95	2.02	1.91
Mean Analyte S/CO	0.07	0.06	0.06	0.05
Specificity rate (%)	100	100	100	100
95% CI, Specificity Rate	86.3 - 100	86.3 - 100	92.9 - 100	86.3 - 100
n	25	25	50*	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
*N = 50 due to inclusion of all specimens used in sensitivity build

Table 29. Specificity of the Procleix Ultrio Elite HCV Discriminatory Assay in Cadaveric Blood Specimens

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	1.95	1.83	1.99	1.90
Mean Analyte S/CO	0.00	0.00	0.00	0.00
Specificity rate (%)	100	100	100	100
95% CI, Specificity Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Table 30. Specificity of the Procleix Ultrio Elite HBV Discriminatory Assay in Cadaveric Blood Specimens

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	2.00	1.88	2.00	1.92
Mean Analyte S/CO	0.00	0.00	0.00	0.00
Specificity rate (%)	100	100	100	100
95% CI, Specificity Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

SENSITIVITY

Sensitivity for Detection of HIV-1

HIV-1, HIV-2, 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 Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay. Twenty-five cadaveric and 25 normal donor specimens were tested using three reagent lots after spiking each specimen with approximately 150 copies/mL of HIV-1. The reactivity rate of both the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay for the cadaveric specimens was 100% for both plasma and serum specimens (95% confidence interval: 86.3%-100%) (Table 31 and Table 32).

Table 31. Reactivity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens Spiked with HIV-1

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	1.99	1.80	1.98	1.87
Mean Analyte S/CO	12.07	9.47	11.20	10.07
Reactive rate (%)	100	100	100	100
95% CI, Reactive Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Table 32. Reactivity of the Procleix Ultrio Elite HIV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HIV-1

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	1.76	1.64	1.75	1.68
Mean Analyte S/CO	20.23	17.29	19.41	17.29
Reactive rate (%)	100	100	100	100
95% CI, Reactive Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Sensitivity for Detection of HIV-2

HIV-1, HIV-2, HCV and HBV seronegative cadaveric blood specimens spiked with a low level of HIV-2 were tested to determine the sensitivity of the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay. Twenty-five cadaveric and 25 normal donor specimens were tested using three reagent lots after spiking each specimen with approximately 150 copies/mL of HIV-2. The reactivity rate of both the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HIV Discriminatory Assay for the cadaveric specimens was 100% for both plasma and serum specimens (95% confidence interval: 86.3%-100%) (Table 33 and Table 34).

Table 33. Reactivity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens Spiked with HIV-2

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	1.24	1.24	1.27	1.34
Mean Analyte S/CO	6.80	6.10	6.74	5.57
Reactive rate (%)	100	100	100	100
95% CI, Reactive Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Table 34. Reactivity of the Procleix Ultrio Elite HIV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HIV-2

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	0.99	1.07	0.97	1.13
Mean Analyte S/CO	11.71	10.39	11.85	9.70
Reactive rate (%)	100	100	100	100
95% CI, Reactive Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Sensitivity for Detection of HCV

HIV-1, HIV-2, HCV and HBV seronegative cadaveric blood specimens spiked with a low level of HCV were tested to determine the sensitivity of the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HCV Discriminatory Assay. Twenty-five cadaveric and 25 normal donor specimens were tested using three reagent lots after spiking each specimen with approximately 150 copies/mL of HCV. The reactivity rate of both the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HCV Discriminatory Assay for the cadaveric specimens was 100% for both plasma and serum specimens (95% confidence interval: 86.3%-100%) (Table 35 and Table 36).

Table 35. Reactivity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens Spiked with HCV

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	2.45	2.08	2.48	1.51
Mean Analyte S/CO	9.05	7.92	8.94	7.42
Reactive rate (%)	100	100	100	100
95% CI, Reactive Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Table 36. Reactivity of the Procleix Ultrio Elite HCV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HCV

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	2.05	1.95	1.99	2.02
Mean Analyte S/CO	23.86	21.43	23.54	21.68
Reactive rate (%)	100	100	100	100
95% CI, Reactive Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Sensitivity for Detection of HBV

HIV-1, HIV-2, HCV and HBV seronegative cadaveric blood specimens spiked with a low level of HBV were tested to determine the sensitivity of the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HBV Discriminatory Assay. Twenty-five cadaveric and 25 normal donor specimens were tested using three reagent lots after spiking each specimen with approximately 15 IU/mL of HBV. The reactivity rate of both the Procleix Ultrio Elite Assay and Procleix Ultrio Elite HBV Discriminatory Assay for the cadaveric specimens was 100% for both plasma and serum specimens (95% confidence interval: 86.3%-100%) (Table 37 and Table 38).

Table 37. Reactivity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens Spiked with HBV

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	1.70	1.68	1.81	1.72
Mean Analyte S/CO	13.89	14.39	13.76	13.09
Reactive rate (%)	100	100	100	100
95% CI, Reactive Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Table 38. Reactivity of the Procleix Ultrio Elite HBV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HBV

	Plasma		Serum	
	Control	Cadaveric	Control	Cadaveric
Mean IC S/CO	1.79	1.76	1.97	1.80
Mean Analyte S/CO	24.43	23.78	24.37	21.85
Reactive rate (%)	100	100	100	100
95% CI, Reactive Rate	86.3 - 100	86.3 - 100	86.3 - 100	86.3 - 100
n	25	25	25	25

n = Number of samples
IC = Internal Control
CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

REPRODUCIBILITY IN CADAVERIC BLOOD SPECIMENS

The inter-assay reproducibility of the Procleix Ultrio Elite Assay with cadaveric blood specimens was assessed by determining the %CVs obtained when each of 10 cadaveric plasma, 10 control plasma, 10 cadaveric serum, and 10 control serum specimens spiked with approximately 150 copies/mL of HIV-1, 150 copies/mL of HIV-2, 150 copies/mL of HCV, or 15 IU/mL of HBV were tested with 3 clinical reagent kit lots. The reactive rates, S/COs, and %CVs are shown in Table 39. The %CVs for the HIV-1 cadaveric plasma, control plasma, cadaveric serum, and control serum specimens were 13%, 5%, 20%, and 8%, respectively. The %CVs for the HIV-2 cadaveric plasma, control plasma, cadaveric serum, and control serum specimens were 17%, 4%, 36%, and 16%, respectively. The %CVs for the HCV cadaveric plasma, control plasma, cadaveric serum, and control serum specimens were 9%, 7%, 5%, and 6%, respectively. The %CVs for the HBV cadaveric plasma, control plasma, cadaveric serum, and control serum specimens were 12%, 6%, 16%, and 5%, respectively. The percent reactive rates for the HIV-1 cadaveric plasma, control plasma, cadaveric serum, and control serum specimens in this study were 99.4%, 100%, 100%, and 100%, respectively. The percent reactive rates for the HIV-2 cadaveric plasma, control plasma, cadaveric serum, and control serum specimens in this study were 99.4%, 100%, 98.9%, and 100%, respectively. The percent reactive rates for the HCV cadaveric plasma, control plasma, cadaveric serum, and control serum specimens in this study were 100%, 100%, 100%, and 98.9%, respectively. The percent reactive rates for the HBV cadaveric plasma, control plasma, cadaveric serum, and control serum specimens in this study were 98.3%, 100%, 97.8%, and 99.4%, respectively.

Table 39. Reproducibility of the Procleix Ultrio Elite Assay in Cadaveric and Control Specimens Spiked with Approximately 150 copies/mL of HIV-1, 150 copies/mL of HIV-2, 150 copies/mL of HCV, or 15 IU/mL of HBV

Virus	Sample	# Donors	# Replicates	% Reactivity (95% CI)	Mean Analyte S/CO	%CV
HIV-1	Cadaveric Plasma	10	180	99.4 (96.9–100)	10.55	13
	Control Plasma	10	180	100 (98.0–100)	11.84	5
	Cadaveric Serum	10	180	100 (98.0–100)	10.52	20
	Control Serum	10	180	100 (98.0–100)	11.50	8
HIV-2	Cadaveric Plasma	10	180	99.4 (96.9–100)	6.17	17
	Control Plasma	10	180	100 (98.0-100)	6.88	4
	Cadaveric Serum	10	180	98.9 (96.0-99.9)	5.09	36
	Control Serum	10	180	100 (98.0–100)	6.95	16
HCV	Cadaveric Plasma	10	180	100 (98.0–100)	8.50	9
	Control Plasma	10	180	100 (98.0-100)	8.97	7
	Cadaveric Serum	9*	162	100 (97.7-100)	8.80	5
	Control Serum	10	180	98.9 (96.0–99.9)	8.93	6
HBV	Cadaveric Plasma	10	180	98.3 (95.2–99.7)	14.06	12
	Control Plasma	10	180	100 (98.0-100)	14.22	6
	Cadaveric Serum	10	180	97.8 (94.4-99.4)	13.58	16
	Control Serum	10	180	99.4 (96.9–100)	14.19	5

CI = Clopper-Pearson Exact method 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
% CV = Coefficient of Variation
*One donor was excluded due to HBV reactivity.

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GDSS-IFU-000006 v. 7.0
2022-04

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