Procleix Ultrio Elite Assay

For *In Vitro* Diagnostic Use

IVD

1000 Test Kit, 5000 Test Kit

CONTENTS

INTENDED USE

The Procleix Ultrio Elite Assay is a qualitative *in vitro* nucleic acid amplification test for the detection of human immunodeficiency virus type 1 and human immunodeficiency virus type 2 (HIV) RNA, hepatitis C virus (HCV) RNA, and/or hepatitis B virus (HBV) DNA in plasma and serum specimens from human donors, tested individually or in pools. It is also intended for use in testing plasma and serum to screen organ and tissue donors, including cadaveric (non-heart-beating) donors. It is not intended for use on samples of cord blood.

This assay is not intended for use as an aid in diagnosis.

SUMMARY AND EXPLANATION OF THE TEST

Epidemiological studies identified human immunodeficiency virus type 1 and human immunodeficiency virus type 2 (HIV) as the etiological agents of acquired immunodeficiency syndrome (AIDS),[2–8] hepatitis C virus (HCV),[9–14] and hepatitis B virus (HBV) as causative agents of transfusion-associated hepatitis.[34] 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/or serologic screening for anti-viral antibodies by chemiluminescent immunoassay (ChLIA) with confirmation by supplemental antibody tests such as immunofluorescence assays.[35, 36, 37, 38] In certain instances, depending on the NAT assay of use, p24Ag assays followed by confirmation by neutralization are appropriate.[36] The addition of nucleic acid-based amplification tests 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.[20-22, 32]

Diagnosed cases of HIV-2 are observed primarily in West Africa or where exposure through immigration or travel has occurred.[40] 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.[39, 43] 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.[40, 41] 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[35, 36, 37] and/or serologic screening for anti-viral antibodies with enzyme-linked immunoabsorbent assays (ELISA) and confirmation with a Recombinant Immunoblot Assay (RIBA).[36] The introduction of nucleic acid-based amplification tests for HCV RNA has allowed detection of HCV infection approximately 59 days earlier than the current antibody-based tests.[32, 35, 42]

HBV Summary and Explanation

Current detection of HBV infection in the blood bank setting is based on NAT for HBV DNA detection[35, 36, 37] and/or serological screening for HBsAg by enzyme immunoassay (EIA) with confirmation by neutralization tests. Additional algorithms in non-endemic regions may also include use of 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.[44] Studies indicate that nucleic acid-based amplification assays for HBV DNA will allow detection of HBV infection several weeks before HBsAg detection.[16–19, 35] 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 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)[24] and 3) Detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[25, 50] The Procleix assays incorporate an Internal Control for monitoring assay performance in each individual specimen.

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 Target Enhancer Reagent, the hybridized target is captured onto magnetic microparticles which are then separated from the specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube.

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.[26] 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.[26] 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 of the three.

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 type 1 and those reactive for HIV type 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 **unopened reagent** 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 at 15° to 30°C.*
R2	**Auto Detect 2** *1.6 N sodium hydroxide.* *Store at 15° to 30°C.*
W	**Wash Solution** *HEPES buffered solution.* *Store at 15° to 30°C.*
O	**Oil** *Silicone oil.* *Store at 15° to 30°C.*
DF	**Buffer for Deactivation Fluid** *Sodium bicarbonate buffered solution.* *Store 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 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 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 HIV, HCV, or HBV 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 or RES after the preparation program is completed.
- If opened reagents are placed in the RPI 250 or RES 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 B for more information.

* If using Panther System Software 7.2 and higher:

- RT stability (wTCR, 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 or RES Room Temperature program for thawing unopened reagents (TCR and Amplification, Enzyme, and Probe Reagents), reagents must remain in the RPI 250 or RES for 4 to 20 hours. Refer to the *Procleix RPI 250 Operator's Manual* or the *Procleix RES Operator's Manual*, as applicable, 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, Procleix RES, 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, 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.

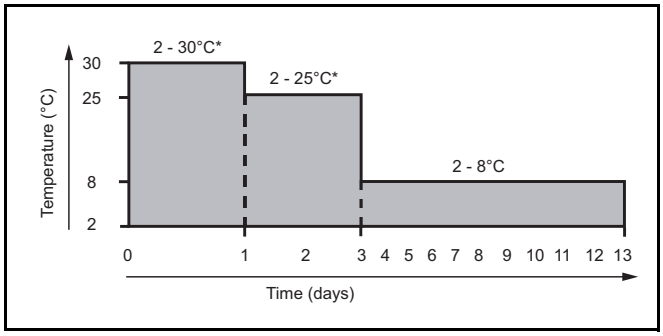
For storage above 8°C, specimens may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

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 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.

CADAVERIC BLOOD SPECIMENS

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

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

B. Specimens should be collected within 24 hours of death if the cadaver was refrigerated (1° to 10°C) within 12 hours of death. Specimens should be collected within 15 hours of death if the cadaver was not refrigerated (1° to 10°C). Specimen stability is affected by elevated temperature.

C. Whole blood (EDTA collection tube) or plasma may be stored for a total of 8 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

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

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

Refer to the example temperature chart below.

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

Do not freeze whole blood.

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

Specimens must be centrifuged within 72 hours of draw.

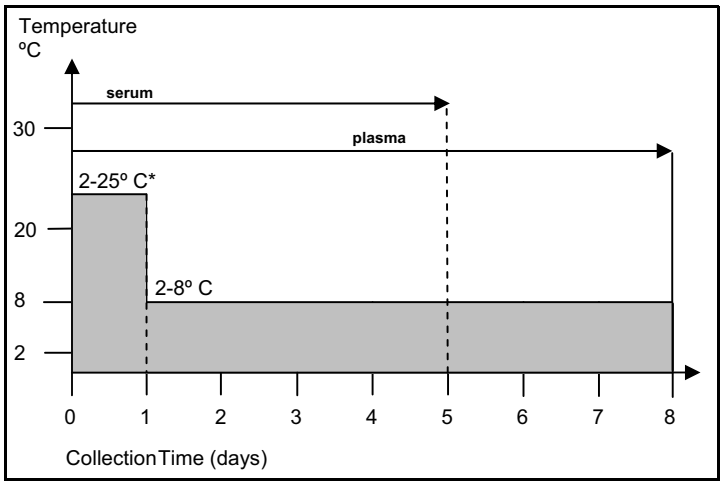
For storage above 8°C, specimens may be stored for 24 hours at up to 25°C during the 72 hours.

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

Refer to the example temperature chart below.

In addition, serum removed from the clot 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 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**	**303723**
Negative Calibrator	30 x 2 mL	90 x 2 mL
HIV Positive Calibrator	15 x 2 mL	75 x 2 mL
HCV Positive Calibrator	15 x 2 mL	75 x 2 mL
HBV Positive Calibrator	15 x 2 mL	75 x 2 mL
Procleix Ultrio Elite Assay HIV, HCV, and HBV Discriminatory Probe Reagents Kit	**303334**	
HIV Discriminatory Probe Reagent	200 tests	
HCV Discriminatory Probe Reagent	200 tests	
HBV Discriminatory Probe Reagent	200 tests	
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, 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 or the Procleix Reagent Equilibration System (RES) 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: front end pipettor, pooling software, operator's manual, and quick reference guide (if applicable)

Disposable 1000 µL or 1100 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. 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[27, 29] when performing the assay. Proper handling and disposal methods should be established according to local regulations.[28] 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.[27, 28] 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 Amplification Reagent

ProClin 300 0.023 Weight-%
Glycerol 4.802 Weight-%
WARNING

H317 - May cause an allergic skin reaction
H412 - Harmful to aquatic life with long lasting effects

P280 - Wear eye protection/face protection

Procleix Selection Reagent

Boric Acid 3.63 Weight-%
Sodium Hydroxide 0.71 Weight-%
WARNING

H315 - Causes skin irritation
H319 - Causes serious eye irritation
H332 - Harmful if inhaled
H333 - May be harmful if inhaled

Procleix Target Enhancer Reagent

Lithium Hydroxide, Monohydrate 6.78 Weight-%
DANGER

H302 - Harmful if swallowed
H314 - Causes severe skin burns and eye damage
H303 - May be harmful if swallowed

P260 - Do not breathe dust/fume/gas/mist/vapours/spray
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310 - Immediately call a POISON CENTER or doctor
P280 - Wear eye protection/face protection

Procleix Auto Detect 2

Sodium Hydroxide 6.04 Weight-%
DANGER

H314 - Causes severe skin burns and eye damage

P260 - Do not breathe dust/fume/gas/mist/vapours/spray
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310 - Immediately call a POISON CENTER or doctor
P280 - Wear eye protection/ face protection

Procleix Buffer for Deactivation Fluid

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

H315 - Causes skin irritation
H319 - Causes serious eye irritation
H411 - Toxic to aquatic life with long lasting effects

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 or the RES to prepare Target Enhancer Reagent.

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

B. Verify that the reagents have not exceeded their storage stability times, including onboard stability.

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 7.2 and higher, onboard stability is 72 hours.

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

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

1. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls between 2° and 15°C, precipitate may form.
2. If cloudiness or precipitate is present, perform Selection Reagent recovery as described in the *Procleix RPI 250 Operator's Manual* or the *Procleix RES 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.

D. 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 or the RES to prepare Target Enhancer Reagent.

E. 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 15°C. Wash Solution and Target Enhancer Reagent may be warmed to facilitate dissolution of precipitate. Do not use the RPI 250 or the RES to warm the Wash Solution or Target Enhancer Reagent. Temperature should not exceed 30°C. 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.

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

G. Refer to the *Procleix RPI 250 Operator's Manual* if using the RPI 250 or the *Procleix RES Operator's Manual* if using the RES to prepare the following reagents: TCR, Probe Reagent, Enzyme Reagent, Amplification Reagent, and HIV, HCV, and HBV 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) or the RES Room Temperature program 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.

H. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. Place TCR into the RPI 250 or the RES, and refer to the applicable *Procleix RPI 250 Operator's Manual* or *Procleix RES 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 or the RES 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 or the RES 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.

I. Thaw calibrators at room temperature.

Note: These are single use vials.

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.

J. 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. 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 Assay 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 Assay HIV, HCV, and HBV Discriminatory Probe Reagents are master lotted with the Procleix Ultrio Elite reagents. The operator must check to ensure that the Procleix Ultrio Elite Assay 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. 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 reagent 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 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.

Specimens from organ/tissue donors, including 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

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 Internal Control (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 Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an Analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid due to an IC value or an Analyte value outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

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

Example:

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

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

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

Example:

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

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

HIV 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 Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator must also have an Analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid due to an IC value or Analyte value that is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

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

Example:

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

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

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

Example:

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

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

HIV 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 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 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 Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator must also have an Analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid or an IC or Analyte value is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

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

Example:

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

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

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

Example:

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

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

HCV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator is run in triplicate in the Procleix Ultrio 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 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 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 Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator must also have an Analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator values is invalid due to an IC value or Analyte value that is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

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

Example:

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

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

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

Example:

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

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

HBV Positive Calibrator Acceptance Criteria

The HBV Positive Calibrator is run in triplicate in the Procleix 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 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 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 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.

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 type 1 and those reactive for HIV type 2.

C. 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.

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

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

F. 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.

G. 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

SPECIFICITY

Specificity of the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite Discriminatory Assays in Normal Blood Donors

Fresh and frozen normal blood donor plasma 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 Discriminatory Assays (dHIV, dHCV, and dHBV) on the Procleix Panther System. Initial reactive specimens were retested in the Procleix Ultrio Elite Assay and/or the relevant Procleix Ultrio Elite Discriminatory Assays and were categorized as defined in Table 1. The reactivity and specificity rates for each of the 4 assays are shown in Table 1.

Tests that were invalid due to instrument hardware errors were retested. Only the valid retest results are included in the data analysis. Tests that were invalid due to assay chemistry errors were retested; in all cases the initial invalid result was due to the Internal Control Signal to Cutoff ratio being less than 1.0. 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 was 1 initial invalid result due to assay chemistry errors, for an initial invalid rate of 0.01% (1/8012). Two different reagent lots were used during testing. For the Procleix Ultrio Elite dHIV, dHCV, and dHBV Assays, there were 0 initial invalid results due to assay chemistry errors, for an initial invalid rate of 0.00% (0/500).

Table 1. Specificity of the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite Discriminatory Assays in Fresh and Frozen Normal Blood Donor Plasma Specimens

	Procleix Ultrio Elite Assay	Procleix Ultrio Elite dHIV Assay	Procleix Ultrio Elite dHCV Assay	Procleix Ultrio Elite dHBV Assay
Valid Results (n)	8011	500	500	500
Initial Reactive (n)	8	0	0	0
Initial Reactive Rate (%)	0.10	0.00	0.00	0.00
True Positive After Repeat Testing* (n)	0	NA	NA	NA
False Positive After Repeat Testing** (n)	8	NA	NA	NA
False Positive Rate After Repeat Testing (%)	0.10	NA	NA	NA
Specificity After Repeat Testing (%) and CI	99.90 (99.80–99.95)	100.00 (99.24–100)	100.00 (99.24–100)	100.00 (99.24–100)
Combined Mean Analyte S/CO of Negative Specimens ± standard deviation	0.06 ± 0.05	0.05 ± 0.04	0.02 ± 0.03	0.05 ± 0.08

n = Number of specimens; NA = Not Applicable; CI = SCORE method, 95% Confidence Interval; S/CO = Signal to Cutoff ratio

* Specimens determined to be True Positives were repeat reactive in either the Ultrio Elite Assay or the relevant Ultrio Elite Discriminatory Assay.

** Specimens determined to be False Positives were non-reactive upon retesting in either the Ultrio Elite Assay or the relevant Ultrio Elite 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 (5,000 mg/L), bilirubin (200 mg/L), and lipids (30,000 mg/L).

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.

CLINICAL SENSITIVITY

Testing of Specimens from HIV-1, HIV-2, HCV, or HBV Infected Individuals

A combined total of 620 HIV-1, HCV, or HBV NAT-positive plasma specimens were obtained from a commercial vendor. Two different reagent lots were used for all testing. Each sample was tested neat (undiluted) and diluted 1:16 in negative donor plasma samples with the Procleix Ultrio Elite Assay. Each sample was also tested neat with the corresponding Procleix Ultrio Elite Discriminatory (dHIV, dHCV, or dHBV) Assay. In addition, 100 HIV-2 specimens with positive serological results were acquired and tested neat with the Procleix Ultrio Elite Assay. Initially invalid reactions due to hardware or software errors were retested and the valid retest results were used for the data analysis. There were no assay chemistry invalid results. The sensitivity results described below are summarized in Table 2.

HIV-1 Sensitivity. The sensitivity for both the Procleix Ultrio Elite and the Procleix Ultrio Elite dHIV Assays for neat HIV-1 positive samples (Ultrio Elite n = 214; dHIV n = 213) was 100% (95% Confidence Interval [CI]: 98.3–100%). The sensitivity for the Procleix Ultrio Elite Assay for diluted (1:16) HIV-1 positive samples was 100% (95% CI: 98.3–100%).

HIV-2 Sensitivity. The rate of reactive results for the Procleix Ultrio Elite Assay for neat HIV-2 seropositive samples (n = 100) was 54.0% (95% Confidence Interval [CI]: 43.7–64.0%). These specimens were not tested diluted 1:16 in the Procleix Ultrio Elite and the Procleix Ultrio Elite dHIV Assays.

HCV Sensitivity. The sensitivity for both the Procleix Ultrio Elite and the Procleix Ultrio Elite dHCV Assays for neat HCV positive samples (n = 203) was 100% (95% CI: 98.2–100%). The sensitivity for the Procleix Ultrio Elite Assay for diluted (1:16) HCV positive samples was 100% (95% CI: 98.2–100%).

HBV Sensitivity. The sensitivity for both the Procleix Ultrio Elite and the Procleix Ultrio Elite dHBV Assays for neat HBV positive samples (n = 203) was 100% (95% CI: 98.2–100%). The sensitivity for the Procleix Ultrio Elite Assay for diluted (1:16) HBV positive samples was 100% (95% CI: 98.2–100%).

Overall Sensitivity. The overall clinical sensitivity for the Procleix Ultrio Elite Assay and all 3 Procleix Ultrio Elite Discriminatory Assays for all 620 HIV-1, HCV and HBV specimens tested neat was 100% (620/620). The overall clinical sensitivity of the HIV-2 specimens tested neat was 54.0%. The overall clinical sensitivity for the Procleix Ultrio Elite Assay for all 620 specimens tested in a 1:16 dilution was 100% (620/620) (Table 2).

Table 2. Sensitivity of the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite Discriminatory Assays in Known Positive Samples

Assay	Sample	Valid Tests (n)	Reactive (n)	Sensitivity (%)	95% CI
Procleix Ultrio Elite Assay (Neat)	**All**	720	674	93.6	91.6–95.2
	HIV-1	214	214	100	98.3–100
	HIV-2	100	54	54	43.7–64.0
	HCV	203	203	100	98.2–100
	HBV	203	203	100	98.2–100
Procleix Ultrio Elite Assay (Diluted 1:16)	**All**	620	620	100	99.4–100
	HIV-1	214	214	100	98.3–100
	HIV-2	NA	NA	NA	NA
	HCV	203	203	100	98.2–100
	HBV	203	203	100	98.2–100
Procleix Ultrio Elite dHIV Assay	**HIV-1**	213	213	100	98.3–100
	HIV-2	NA	NA	NA	NA
Procleix Ultrio Elite dHCV Assay	**HCV**	203	203	100	98.2–100
Procleix Ultrio Elite dHBV Assay	**HBV**	203	203	100	98.2–100

n = Number of specimens; CI = Exact Binomial Confidence Interval;[45] NA = Not Applicable

DIAGNOSTIC SENSITIVITY

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 3).

Table 3. 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		
	Procleix Ultrio Elite Assay			Procleix Ultrio Elite Assay		
	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**

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 4). 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 4. Detection of HCV RNA in HCV Seroconversion Panels

Panel	Days Earlier Detection Than HCV Antibody		
	Procleix Ultrio Elite Assay		
	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**

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 5). 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 5. 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		
	Procleix Ultrio Elite Assay			Procleix Ultrio Elite Assay		
	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**

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 Discriminatory (dHIV, dHCV, and dHBV) 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;[47] 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 dHIV Assay. The detection rate at 20 IU/mL with the Procleix Ultrio Elite Assay was 96%. With the Procleix Ultrio Elite dHIV 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 dHIV Assay, respectively (Table 6).

Table 6. Detection of HIV-1 WHO Standard in Analytical Sensitivity Panels

Procleix Ultrio Elite Assay							Procleix Ultrio Elite dHIV Assay					
HIV-1 WHO (97/650) IU/mL	Number of Reactive / Tested	% Reactive	95% Confidence Limits*		Average S/CO	% CV	Number of Reactive / Tested	% Reactive	95% Confidence Limits*		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
600	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

S/CO = Signal to Cutoff ratio in concordant replicates only
CV = Coefficient of Variation
*SCORE method, 95% Confidence Interval

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 dHIV Assay. The detection rate at 10 IU/mL with the Procleix Ultrio Elite Assay was 94%. With the Procleix Ultrio Elite dHIV 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 dHIV Assay, the detection rates at 3, 1, and 0.3 IU/mL were 58%, 31%, and 8%, respectively (Table 7).

Table 7. Detection of HIV-2 WHO Standard in Analytical Sensitivity Panels

Procleix Ultrio Elite Assay							Procleix Ultrio Elite dHIV Assay					
HIV-2 WHO (08/150) IU/mL	Number of Reactive / Tested	% Reactive	95% Confidence Limits*		Average S/CO	% CV	Number of Reactive / Tested	% Reactive	95% Confidence Limits*		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
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

S/CO = Signal to Cutoff ratio in concordant replicates only
CV = Coefficient of Variation
*SCORE method, 95% Confidence Interval

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 8).

Table 8. Detection of HCV WHO Standard in Analytical Sensitivity Panels

Procleix Ultrio Elite Assay							Procleix Ultrio Elite dHCV Assay					
HCV WHO (06/100) IU/ mL	Number of Reactive / Tested	% Reactive	95% Confidence Limits*		Average S/CO	% CV	Number of Reactive / Tested	% Reactive	95% Confidence Limits*		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
100	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

S/CO = Signal to Cutoff ratio in concordant replicates only
CV = Coefficient of Variation
*SCORE method, 95% Confidence Interval

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 dHBV Assay. HBV detection at 5 IU/mL was 97% with both the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite dHBV Assay. The detection rates for 1.67 IU/mL were 70% and 65% with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite dHBV 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 dHBV Assay (Table 9).

Table 9. Detection of HBV WHO Standard in Analytical Sensitivity Panels

Procleix Ultrio Elite Assay							Procleix Ultrio Elite dHBV Assay					
HBV WHO (97/750) IU/ mL	Number of Reactive / Tested	% Reactive	95% Confidence Limits*		Average S/CO	% CV	Number of Reactive / Tested	% Reactive	95% Confidence Limits*		Average S/CO	% CV
			Lower	Upper					Lower	Upper		
45	360/360	100	99	100	14.10	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

S/CO = Signal to Cutoff ratio in concordant replicates only
CV = Coefficient of Variation
*SCORE method, 95% Confidence Interval

Probit Analysis

The predicted 50% and 95% detection rates in IU/mL for each target were determined through Probit Analysis[46] 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 dHIV 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 dHIV 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 dHCV 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 dHBV Assay (Table 10).

Table 10. 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 Assay	5.4 (4.5 to 6.1)	18.0 (15.0 to 23.5)
HIV-1 WHO (97/650)	Ultrio Elite dHIV Assay	5.3 (4.4 to 6.0)	17.3 (14.4 to 22.6)
HIV-2 WHO (08/150)	Ultrio Elite Assay	2.6 (2.3 to 3.0)	10.4 (8.9 to 12.6)
HIV-2 WHO (08/150)	Ultrio Elite dHIV Assay	2.2 (1.9 to 2.5)	9.6 (8.1 to 11.8)
HCV WHO (06/100)	Ultrio Elite Assay	0.9 (0.8 to 1.0)	3.0 (2.5 to 3.9)
HCV WHO (06/100)	Ultrio Elite dHCV Assay	0.7 (0.5 to 0.8)	2.4 (2.0 to 3.3)
HBV WHO (97/750)	Ultrio Elite Assay	0.9 (0.8 to 1.1)	4.3 (3.8 to 5.0)
HBV WHO (97/750)	Ultrio Elite dHBV Assay	1.0 (0.9 to 1.1)	4.5 (4.0 to 5.3)

SENSITIVITY OF DETECTION FOR 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 and H), N, and O were quantified for HIV-1 RNA concentrations using commercially available quantitative HIV-1 RNA assays or with an in-house quantitative HIV-1 RNA test. Specimens were diluted with HIV/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 (dHIV) Assay. Sixty-four unique specimens or tissue culture isolates were tested in duplicate using two lots of reagents on the Procleix Panther System. At 100 copies/mL, 258/258 replicates (100%) were reactive with the Procleix Ultrio Elite Assay and 256/256 replicates (100%) were reactive with the Procleix Ultrio Elite dHIV Assay. At 30 copies/mL, 244/256 replicates (95.3%) were reactive with the Procleix Ultrio Elite Assay and 245/258 replicates (95.0%) were reactive with the Procleix Ultrio Elite dHIV Assay (Table 11). 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 11. Detection of HIV-1 Genetic Variants*

Genetic Variant	Copies/mL	Unique Donors	Procleix Ultrio Elite Assay		Procleix Ultrio Elite dHIV Assay	
			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 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 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	64	258/258	100	256/256	100
	30		244/256	95.3	245/258	95.0

* The same panels were used for testing with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite dHIV Assay.
** One specimen from this group had 2 additional reps tested (4 reps total).

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 (dHIV) 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 dHIV 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 dHIV Assay (Table 12). 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 12. Detection of HIV-2 Genetic Variants*

Genetic Variant	Copies/mL	Unique Donors	Procleix Ultrio Elite Assay		Procleix Ultrio Elite dHIV Assay	
			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

* The same panels were used for testing with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite dHIV 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 (dHCV) 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 dHCV 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 dHCV Assay (Table 13). 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 13. Detection of HCV Genotypes*

Genotype	Copies/mL	Unique Donors	Procleix Ultrio Elite Assay		Procleix Ultrio Elite dHCV Assay	
			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

* The same panels were used for testing with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite dHCV 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 (dHBV) 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 dHBV 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 dHBV 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 14). 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 14. Detection of HBV Genotypes*

Genotype	Copies/mL	Unique Donors	Procleix Ultrio Elite Assay		Procleix Ultrio Elite dHBV Assay	
			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

* The same panels were used for testing with the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite dHBV 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. Sixteen cadaveric and 16 normal donor specimens were tested using one reagent lot. The specificity of the Procleix Ultrio Elite Assay for the cadaveric specimens was 100% (95% confidence interval: 80.6–100%) (Table 15).

Table 15. Specificity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens

	Control	Cadaveric
Mean IC S/CO	1.96	1.90
Mean Analyte S/CO	0.05	0.09
Specificity rate (%)	100	100
95% CI, Specificity Rate	80.6–100	80.6–100
n*	16	16

n = Number of samples
CI = SCORE method, 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
* Eight unique cadaveric plasma specimens and 8 unique cadaveric serum specimens were tested.

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. Sixteen cadaveric and 16 normal donor specimens were tested using one reagent lot after spiking each specimen with approximately 150 copies/mL of HIV-1. The reactivity rate of the Procleix Ultrio Elite Assay for the cadaveric specimens was 100% (95% confidence interval: 80.6–100%) (Table 16).

Table 16. Reactivity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens Spiked with HIV-1

	Control	Cadaveric
Mean IC S/CO	2.12	1.95
Mean Analyte S/CO	9.94	9.07
Reactive rate (%)	100	100
95% CI, Reactive Rate	80.6–100	80.6–100
n*	16	16

n = Number of samples
CI = SCORE method, 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
* Eight unique cadaveric plasma specimens and 8 unique cadaveric serum specimens were tested.

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. Sixteen cadaveric and 16 normal donor specimens were tested using one reagent lot after spiking each specimen with approximately 150 copies/mL of HIV-2. The reactivity rate of the Procleix Ultrio Elite Assay for the cadaveric specimens was 100% (95% confidence interval: 80.6–100%) (Table 17).

Table 17. Reactivity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens Spiked with HIV-2

	Control	Cadaveric
Mean IC S/CO	1.03	1.31
Mean Analyte S/CO	7.89	5.68
Reactive rate (%)	100	100
95% CI, Reactive Rate	80.6–100	80.6–100
n*	16	16

n = Number of samples
CI = SCORE method, 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
* Eight unique cadaveric plasma specimens and 8 unique cadaveric serum specimens were tested.

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. Sixteen cadaveric and 16 normal donor specimens were tested using one reagent lot after spiking each specimen with approximately 150 copies/mL of HCV. The reactivity rate of the Procleix Ultrio Elite Assay for the cadaveric specimens was 100% (95% confidence interval: 80.6–100%) (Table 18).

Table 18. Reactivity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens Spiked with HCV

	Control	Cadaveric
Mean IC S/CO	2.31	2.46
Mean Analyte S/CO	7.91	8.48
Reactive rate (%)	100	100
95% CI, Reactive Rate	80.6–100	80.6–100
n*	16	16

n = Number of samples
CI = SCORE method, 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
* Eight unique cadaveric plasma specimens and 8 unique cadaveric serum specimens were tested.

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. Sixteen cadaveric and 16 normal donor specimens were tested using one reagent lot after spiking each specimen with approximately 15 IU/mL of HBV. The reactivity rate of the Procleix Ultrio Elite Assay for the cadaveric specimens was 100% (95% confidence interval: 80.6–100%) (Table 19).

Table 19. Reactivity of the Procleix Ultrio Elite Assay in Cadaveric Blood Specimens Spiked with HBV

	Control	Cadaveric
Mean IC S/CO	1.78	1.67
Mean Analyte S/CO	12.18	13.14
Reactive rate (%)	100	100
95% CI, Reactive Rate	80.6–100	80.6–100
n*	16	16

n = Number of samples
CI = SCORE method, 95% Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
* Eight unique cadaveric plasma specimens and 8 unique cadaveric serum specimens were tested.

REPRODUCIBILITY

Reproducibility of the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays on the Procleix Panther System was evaluated. For determination of the reproducibility of each assay, 9 quality control panels were tested as individual specimens (Tables 20–23). Eight of the panel members were positive for HIV-1 RNA (100 and 30 copies/mL), HIV-2 RNA (105 and 30 copies/mL), HCV RNA (100 and 30 copies/mL), or HBV DNA (approximately 11 and 4 IU/mL prepared by diluting the 32 IU/mL quality control panel 1:3 and 1:9 respectively), and 1 panel member was HIV-1, HIV-2, HCV, and HBV negative.

The reproducibility panels were tested by a total of 3 operators with 3 different reagent lots and 3 Procleix Panther Systems over 3 days. Nine valid runs were generated with the Procleix Ultrio Elite Assay and each Procleix Ultrio Elite Discriminatory Assay. In the Procleix Ultrio Elite Assay, each of the positive panel members were tested in 72 replicates (8 replicates for each of 3 reagent lots, on each of 3 days). The negative panel was tested in 144 replicates (16 replicates in each of 3 lots, on each of 3 days). In each of the Procleix Ultrio Elite Discriminatory Assays, each relevant positive panel member was tested in 72 replicates, and the negative panel member was tested in 144 replicates.

Invalid runs were retested, while invalid results for panel members in valid runs were not retested. Valid run data for each assay on the Procleix Panther System is described below:

Procleix Ultrio Elite Assay: 9 runs generated 720 test results of which none were invalid.

Procleix Ultrio Elite HIV Discriminatory Assay: 9 runs generated 432 test results of which none were invalid.

Procleix Ultrio Elite HCV Discriminatory Assay: 9 runs generated 288 test results of which none were invalid.

Procleix Ultrio Elite HBV Discriminatory Assay: 9 runs generated 288 test results of which none were invalid.

Reproducibility analyses included evaluation of percent agreement and mean S/CO ratios for panel members, evaluation of mean Relative Light Unit (RLU) values for the Negative, HIV Positive, HCV Positive, and HBV Positive Calibrators, and evaluation of standard deviation (SD) and percent coefficient of variation (%CV) of the S/CO ratios and RLU values for each of the five variance factors (Tables 20–23). The mean analyte S/CO ratios were analyzed for the positive and negative panel members and the Internal Control S/CO ratios were analyzed for the negative panel members. The mean analyte RLU values were analyzed for the Positive and Negative Calibrators and the Internal Control RLU values were analyzed for the Negative Calibrators. The percent agreement between the assay results and the true status of each panel member was calculated using the analyte S/CO for all panel members. For the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite Discriminatory Assays, results for each panel member are shown individually.

For the Procleix Ultrio Elite Assay and the 3 Procleix Ultrio Elite Discriminatory Assays, the overall percent agreement of test results was 93.1 to 100% for positive panel members and 100% for the negative panel member. There was no correlation of reactive rate to the factors tested in this study. With regard to signal variability, intra-run and inter-instrument factors, in most cases, were the largest contributors to total variance (according to SD values) in the Procleix Ultrio Elite Assay and the Procleix Ultrio Elite HIV, HCV, and HBV Discriminatory Assays. It should be noted that while these factors were responsible for the majority of the variance in the assays, the total %CV was low for all panel members, in all four assays (Tables 20, 21, 22, 23).

Table 20. Reproducibility of the Procleix Ultrio Elite Assay (analysis of analyte signals, unless noted)

Sample	Titer*	n	Agreement (%)	Mean S/CO	Total %CV	Inter-Instrument		Inter-Operator		Inter-Day		Inter-Lot		Intra-Run	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative - IC**	0	144	100	2.00	4	0.01	1	0.01	0	0.01	1	0.07	3	0.05	3
Negative - Analyte				0.08	60	0.02	30	0.01	6	0.00	4	0.00	5	0.04	47
HIV-2 A	105	72	100	7.72	11	0.46	6	0.03	0	0.10	1	0.83	11	0.24	3
	30	72	94.4	7.49	15	0.55	7	0.18	2	0.12	2	0.95	13	0.67	9
HIV-1 B	100	72	100	11.86	7	0.66	6	0.15	1	0.20	2	0.25	2	0.56	5
	30	72	98.6	10.29	18	0.37	4	0.04	0	0.09	1	0.14	1	0.27	3
HCV-1a	100	72	100	9.04	7	0.56	6	0.06	1	0.15	2	0.23	3	0.29	3
	30	72	100	8.85	6	0.82	9	0.10	1	0.21	2	0.32	4	0.28	3
HBV A	11 ‡	72	100	14.15	6	0.94	7	0.06	0	0.15	1	0.35	2	0.39	3
	4 †	72	94.4	13.67	10	0.54	4	0.15	1	0.18	1	0.31	2	1.00	7

Sample	n	Agreement (%)	Mean RLU	Total %CV	Inter-Instrument		Inter-Operator		Inter-Day		Inter-Lot		Intra-Run	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator - IC**	27	N/A	185,911	9	5,358	3	1,835	1	1,206	1	12,046	6	4,871	3
Negative Calibrator - Analyte			9,643	55	3,755	39	656	7	627	7	741	8	2,684	28
HIV Positive Calibrator	18	N/A	1,241,756	9	22,318	2	19,986	2	20,013	2	64,117	5	68,599	6
HCV Positive Calibrator	18	N/A	868,036	5	19,816	2	7,670	1	8,065	1	12,478	1	23,089	3
HBV Positive Calibrator	18	N/A	1,371,496	9	12,251	1	27,644	2	26,733	2	38,903	3	108,806	8

n = Number of panel members combined for this analysis; S/CO = Signal to Cutoff ratio in concordant replicates only; IC = Internal Control

* Concentration = copies/mL for HIV-1, HIV-2 and HCV, IU/mL for HBV.

** Analysis of Internal Control signal

‡ HBV A (appx. 32 IU/mL) diluted 1:3

† HBV A (appx. 32 IU/mL) diluted 1:9

Table 21. Reproducibility of the Procleix Ultrio Elite HIV Discriminatory Assay (analysis of analyte signal, unless noted)

Sample	Titer*	n	Agreement (%)	Mean S/CO	Total %CV	Inter-Instrument		Inter-Operator		Inter-Day		Inter-Lot		Intra-Run	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative - IC**	0	144	100	1.99	4	0.01	0	0.02	1	0.05	2	0.04	2	0.06	3
Negative - Analyte				0.12	66	0.06	47	0.01	7	0.02	13	0.02	16	0.06	50
HIV-2 A	105	72	100	13.13	11	0.42	3	0.25	2	0.87	7	1.41	11	0.40	3
	30	72	98.6	11.53	25	0.42	4	0.77	7	0.45	4	0.58	5	2.89	25
HIV-1 B	100	72	100	19.97	8	0.41	2	0.77	4	0.85	4	0.65	3	1.01	5
	30	72	95.8	16.27	27	0.75	5	0.74	5	1.09	7	0.79	5	5.03	31
Sample		**n**	**Agreement (%)**	**Mean RLU**	**Total %CV**	**Inter-Instrument**		**Inter-Operator**		**Inter-Day**		**Inter-Lot**		**Intra-Run**	
						SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator - IC**		27	N/A	178,249	6	6,787	4	2,127	1	3,358	2	8,440	5	4,491	3
Negative Calibrator - Analyte				7,994	54	1,126	14	1,270	16	3,185	40	1,002	13	2,975	37
HIV Positive Calibrator		27	N/A	1,288,506	6	13,220	1	14,345	1	14,856	1	33,882	3	53,933	4

n = Number of panel members combined for this analysis; S/CO = Signal to Cutoff ratio in concordant replicates only; IC = Internal Control
* Concentration = copies/mL for HIV-1, HIV-2 and HCV, IU/mL for HBV.
** Analysis of Internal Control signal

Table 22. Reproducibility of the Procleix Ultrio Elite HCV Discriminatory Assay (analysis of analyte signal, unless noted)

Sample	Titer*	n	Agreement (%)	Mean S/CO	Total %CV	Inter-Instrument		Inter-Operator		Inter-Day		Inter-Lot		Intra-Run	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative - IC**	0	144	100	1.99	4	0.01	0	0.03	1	0.00	0	0.01	1	0.06	3
Negative - Analyte				0.06	137	0.05	89	0.02	32	0.03	53	0.01	19	0.06	104
HCV-1a	100	72	100	22.96	7	1.32	6	0.91	4	0.59	3	0.66	3	0.67	3
	30	72	100	22.49	7	1.44	6	0.92	4	0.23	1	0.61	3	0.76	3
Sample		**n**	**Agreement (%)**	**Mean RLU**	**Total %CV**	**Inter-Instrument**		**Inter-Operator**		**Inter-Day**		**Inter-Lot**		**Intra-Run**	
						SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator - IC**		27	N/A	174,106	8	5,010	3	3,401	2	1,285	1	7,880	5	5,038	3
Negative Calibrator - Analyte				3,930	133	2,951	75	2,343	60	1,318	34	1,138	29	3,920	100
HCV Positive Calibrator		27	N/A	1,377,630	5	17,123	1	11,520	1	10,692	1	27,140	2	45,574	3

n = Number of panel members combined for this analysis; S/CO = Signal to Cutoff ratio in concordant replicates only; IC = Internal Control
* Concentration = copies/mL for HIV-1, HIV-2 and HCV, IU/mL for HBV.
** Analysis of Internal Control signal

Table 23. Reproducibility of the Procleix Ultrio Elite HBV Discriminatory Assay (analysis of analyte signal, unless noted)

Sample	Titer*	n	Agreement (%)	Mean S/CO	Total %CV	Inter-Instrument		Inter-Operator		Inter-Day		Inter-Lot		Intra-Run	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative - IC**	0	144	100	1.97	4	0.01	1	0.01	1	0.01	0	0.01	0	0.07	3
Negative - Analyte				0.06	137	0.05	96	0.01	13	0.02	42	0.02	32	0.06	103
HBV A	11 ‡	72	100	23.22	10	0.80	3	1.56	7	1.36	6	1.15	5	0.84	4
	4 †	72	93.1	22.52	16	1.05	5	1.93	9	1.20	5	1.16	5	2.88	13

Sample	n	Agreement (%)	Mean RLU	Total %CV	Inter-Instrument		Inter-Operator		Inter-Day		Inter-Lot		Intra-Run	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator - IC**	27	N/A	172,971	7	7,237	4	2,589	1	1,164	1	10,335	6	7,127	4
Negative Calibrator - Analyte			4,044	182	2,794	69	3,669	91	3,586	89	2,715	67	5,023	124
HBV Positive Calibrator	27	N/A	1,505,037	5	15,202	1	15,798	1	14,580	1	23,951	2	62,297	4

n = Number of panel members combined for this analysis; S/CO = Signal to Cutoff ratio in concordant replicates only; IC = Internal Control
* Concentration = copies/mL for HIV-1, HIV-2 and HCV, IU/mL for HBV.
** Analysis of Internal Control signal
‡ HBV A (appx. 32 IU/mL) diluted 1:3
† HBV A (appx. 32 IU/mL) diluted 1:9

BIBLIOGRAPHY

1. **American Association of Blood Banks.** Standards for Cellular Therapy Product Services, current edition.
2. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouziuuz, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for Acquired Immune Deficiency Syndrome (AIDS). Science. **220**:868–871.
3. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo**. 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science. **224**:497–500.
4. **Gallo R. C., S. Z. Salahuddin, M. Popovic, G. M. Strearer, M. Kaplan, D. F. Haynas, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV III) from patients with AIDS and at risk for AIDS. Science. **224**:500–503.
5. **Piot P., F. A. Plummer, F. S. Mhalu, J-L. Lamboray, J. Chin, and J. M. Mann.** 1988. AIDS: An international perspective. Science. **239**:573–579.
6. **Sarngadharan, J. G., M. Popovic, L. Broch, J. Scupbach, and R. C. Gallo.** 1984. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science. **224**:506–508.
7. **Gallo, D., J. S. Kimpton, and P. J. Dailey**. 1987. Comparative studies on use of fresh and frozen peripheral blood lymphocyte specimens for isolation of human immunodeficiency virus and effects of cell lysis on isolation efficiency. J Clin Microbiol. **25**:1291–1294.
8. **Clavel, F., D. Guetard, F. Brun-Vezinet, S. Chamaret, M. Rey, M. O. Santos-Ferraira, A. G. Laurent, C. Dauguet, C. Katlama, C. Rouzioux, D. Klatzmann, J. L. Champalimaud, and L. Montagnier.** 1986. Isolation of a new human retrovirus from West African patients with AIDS. Science. **233**:343–346.
9. **Alter, H. J., R. H. Purcell, J. W. Shih, J. C. Melpolder, M. Houghton, Q-L. Choo, and G. Kuo.** 1989. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med. **321**:1494–1500.
10. **Esteban, J. I., A. Gonzalez, J. M. Hernandez, et al.** 1990. Evaluation of antibodies to hepatitis C virus in a study of transfusion-associated hepatitis. N Engl J Med. **323**:1107–1120.
11. **Van der Poel, C. L., H. W. Reesink, P. N. Lelie, A. Leentvaar-Kuypers, Q-L. Choo, G. Kuo, and M. Houghton.** 1989. Anti-hepatitis C antibodies and non-A, non-B post-transfusion hepatitis in the Netherlands. Lancet. **2**:297–298.
12. **Choo, Q-L., G. Kuo, A. J. Weiner, et al.** 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science. **244**:362–364.
13. **Alter, H. J., P. V. Holland, Ag. Morrow, et al.** 1975. Clinical and serological analysis of transfusion associated hepatitis. Lancet. **2**:838–841.
14. **Kuo, G., Q-L. Choo, H. J. Alter, et al.** 1989**.** An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science. **244**:1494–1500.
15. **Mimms, L. T., J. W. Mosley, F.B. Hollinger, et al.** 1993. Effect of concurrent acute infection with hepatitis C virus on acute hepatitis B virus infection. Brit Med J. **307**:1095–1097.
16. **Kuhns, M. C., A. L. McNamara, B. Peterson, et al.** 1998. Detection of hepatitis B seroconversion by highly sensitive assays for surface antigen and HBV DNA. [Abstract S-342] Transfusion. **38 (10-Suppl)**: 91s.
17. **Ulrich, P. P., R. A. Bhat, B. Seto, et al.** 1989. Enzymatic amplification of hepatitis B virus DNA in serum compared with infectivity testing in chimpanzees. J Infect Dis. **160**:37–43.
18. **Peddada, L., C. Heldebrant, R. Smith, et al.** 2000. HBV viremia preceding HBsAg positivity: implications for minipool (MP) and individual donation (ID) HBV nucleic acid testing (NAT). Abstract 100608 (submitted). American Assn of Blood Banks, 53rd Annual Meeting, Washington DC.
19. **Rawal, B. D., S. H. Kleinman, M. C. Kuhns, M. P. Busch.** 1998. Infectious HBV window period and its projected reduction by genome amplification testing. (Abstract S-343). Transfusion. **38 (10-Suppl)**: 91s.
20. **Busch, M. P., S. L. Stramer, and S. H. Kleinman.** 1997. Evolving applications of nucleic acid amplification assays for prevention of virus transmission by blood components and derivatives. In: Garrity G (ed): Applications of Molecular Biology to Blood Transfusion Medicine. AABB. Bethesda, MD. 123–176.
21. **Busch, M. P., L. L. L. Lee, G. A. Satten, D. R. Henrard, H. Farzadegan, K. E. Nelson, S. Read, R. Y. Dodd, and L. R. Petersen.** 1995. Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. Transfusion. **35**:91–97.
22. **Schreiber, G. B., M. P. Busch, S. H. Kleinman, and J. J. Korelitz.** 1996. For the Retrovirus Epidemiology Study: The risk of transfusion-transmitted viral infections. N Engl J Med. **334**:1685–1690.
23. **McDonough, S., C. Giachetti, Y. Yang, D. Kolk, B. Billyard, and L. Mimms.** 1998. High throughput assay for the simultaneous or separate detection of human immunodeficiency virus (HIV-1) and hepatitis C virus (HCV). Infusion Therapy and Transfusion Medicine. **25**:164–169.
24. **Kacian, D. L. and T. J. Fultz.** 1995. Nucleic acid sequence amplification methods. U. S. Patent 5, 399, 491.
25. **Arnold, L. J., P. W. Hammond, W. A. Wiese, and N. C. Nelson.** 1989. Assay formats involving acridinium-ester-labeled DNA probes. Clin Chem. **35**:1588–1594.
26. **Nelson, N. C., A. BenCheikh, E. Matsuda, and M. Becker.** 1996. Simultaneous detection of multiple nucleic acid targets in a homogeneous format. Biochem. **35**:8429–8438.
27. **Centers for Disease Control.** 1987. Recommendations for prevention of HIV transmission in health care settings. *In* United States Morbid. and Mortal. Weekly Rep. 36, Supplement No. 2S.
28. **Clinical and Laboratory Standards Institute.** 2002. Clinical Laboratory Waste Management. CLSI Document GP5-A2. Villanova, PA.
29. **29 CFR Part 1910.1030.** Occupational Exposure to Bloodborne Pathogens; current version.
30. **Giachetti, C., J. Linnen, D. P. Kolk, J. Dockter, M. K. McCormick, M. Ho-Sing-Loy, M. Park, K. Gillotte-Taylor, L. Mimms and S. H. McDonough.** 2002. Highly Sensitive Multiplex Assay for Detection of HIV-1 and HCV RNA. J. of Clin. Microbio. **40**: 2408–2419.
31. **Linnen, J., J. M. Gilker, A. Menez, A. Vaughn, A. Broulik, J. Dockter, K. Gillotte-Taylor, D. P. Kolk, L. T. Mimms, and C. Giachetti.** 2002. Sensitive detection of genetic variants of HIV-1 and HCV with an HIV-1/HCV assay based on Transcription-Mediated Amplification. J. Virol. Methods. **102**:139–155.
32. **Kolk, D., J. Dockter, J. Linnen, M. Ho-Sing-Loy, K. Gillotte-Taylor, S. H. McDonough, L. Mimms and C. Giachetti.** 2002. Significant Closure of the HIV-1 and HCV Pre-seroconversion Detection Windows with a TMA-driven HIV-1/HCV Assay. J. of Clin. Microbio. **40**:1761–1766.

33. **Jackson, J. B., Smith, K., Knott, C., Dorpela, A., Simmons, A., Piwowar-Manning E., McDonough, S., Mimms, L. and Vargo, J.M.** 2002. Sensitivity of the Procleix HIV-1/HCV Assay for detection of HIV-1 and HCV RNA in a High Risk Population. J. of Clin. Microbio. **40**:2387–2391.
34. **Hollinger, F. B., Liang, T. J.** 2002. Hepatitis B Virus. Viral Hepatitis. (page 103). Lippincott Williams & Wilkins, Philadelphia, PA.
35. **Schmidt, M., Seifried, E.** 2010. Improving Blood Donor Screening by Nucleic Acid Technology. ISBT Science Series. **5**:219-229.
36. **Stramer, S.** 2007. Current Risks of Transfusion-Transmitted Agents. Arch. Pathol. Lab Med. **131**:702-707.
37. **Coste, J., Reesink, H. W., Engelfriet, C. P., Laperche, S.** 2005. Implementation of Donor Screening for Infectious Agents Transmitted by Blood by Nucleic Acid Technology: Update to 2003. Vox Sanguinis. **88**:289-303.
38. **Vermeulen, M., Lelie, N., Sykes, W., Crookes, R., Swanevelder, J., Gaggia, L., Le Roux, M., Kuun, E. Gulube, S., Reddy, R.** 2009. Impact of Individual-Donation Nucleic acid Testing on Risk of Human Immunodeficiency Virus, Hepatitis B Virus, and Hepatitis C Virus Transmission by Blood Transfusion in South Africa. Transfusion. **49**:1115-1125.
39. **Franco-Paredes, C., Tellez, I., del Rio, C.** 2006. Rapid HIV testing: A review of the literature and implications for the clinician. Current HIV/AIDS Reports. **3:**159-165.
40. **Torian, L. V., Selik, R. M., Branson, B., Owen, S. M., Granade, T., Shouse, R. L., Joyce, M. P., Pieniazek, D., Kline, R.** 2011. HIV-2 Infection Surveillance—United States, 1987-2009. MMWR. **60**:985-988.
41. **Assal, A., Barlet, V., Deschaseaux, M., Dupont, I., Gallian, P., Guitton, C., Morel, P., David, B., De Micco, P.** 2009. Comparison of the Analytical and Operational Performance of Two Viral Nucleic Acid Blood Screening Systems: Procleix TIGRIS and Cobas s201. Transfusion. **49**:289-300.
42. **Jackson, B. R., Busch, M. P., Stramer, S. L., AuBuchon, J. P.** 2003. The Cost-Effectiveness of NAT for HIV, HCV, and HBV in Whole Blood Donations. Transfusion. **43**:721-729.
43. **Wesolowski, L. G., Delaney, K. P., Hart, C., Dawsom, C., Owen, S. M., Candal, D., Meyer, W.A., Ethridge, S. F., Branson, B. M.** 2011. Performance of an Alternative Laboratory-based Algorithm for Diagnosis of HIV Infection Utilizing a Third Generation Immunoassay, a Rapid HIV-1/HIV-2 Differentiation Test and a DNA or RNA-based Nucleic Acid Amplification Test in Persons with Established HIV-1 Infection and Blood Donors. J. Clin. Virol. **52**:S45-S49.
44. **Kleinman, S. H., Busch, M. P.** 2006. Assessing the Impact of HBV NAT on Window Period Reduction and Residual Risk. J. Clin. Virol. **36**:S23-S29.
45. **Clopper, C. J., Pearson, E. S.** 1934. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrica. **26**:404-413.
46. **Finney, D. J., Tattersfield, F.** 1947. Probit Analysis, A Statistical Treatment of the Sigmoid Response Curve. At The University Press, Cambridge, UK.
47. **Agresti, A., Coull, B. A.** 1998. Approximate is Better than “Exact” for Interval Estimation of Binomial Proportions. The American Statistician. **52(2)**:119-126.
48. **Vargo, J. M., Smith, K., Knott, C., Wang, S., Fang, C., McDonough, S., Giachetti, C., Caglioti, S., Gammon, R., Gilbert, D., Jackson, J. B., Richards, W., Stramer, S. Mimms, L.** 2002. Clinical Specificity and Sensitivity of a Blood Screening Assay for Detection of HIV-1 and HCV RNA. Transfusion. **42**:876–885.
49. **V. Shyamala, J. Cottrell, P. Arcangel, D. Madriaga, J. Linnen, B. Phelps, and D. Chien.** 2004. Detection and Quantitation of HBV DNA in the WHO International Standard for HIV-1 RNA. 2004. J. Virol. Methods. **118**:69–72.
50. **Margaritis A. R., Brown S. M., Seed C.R., Kiely P, D'Agostino B, Keller A. J.** 2007. Comparison of two automated nucleic acid testing systems for simultaneous detection of human immunodeficiency virus and hepatitis C virus RNA and hepatitis B virus DNA. Transfusion. **47**:1783–93.
51. **Stramer S. L.** 2007. Current risks of transfusion-transmitted agents: a review. Arch Pathol Lab Med. **131(5)**:702–7.
52. **McCormick M. K., Dockter J, Linnen J. M., Kolk D, Wu Y, Giachetti C.** 2006. Evaluation of a new molecular assay for detection of human immunodeficiency virus type 1 RNA, hepatitis C virus RNA, and hepatitis B virus DNA. J Clin Virol. **36**:166–76.
53. **Kleinman S.** 2008. Blood donor screening with nucleic acid amplification tests for human immunodeficiency virus, hepatitis C virus and hepatitis B virus. ISBT Science Series. **3**: 191–195.
54. **Lin C. K., Margaritis A. R., Heaton W. A., Linnen J. M.** 2008. Evaluation of the Procleix Ultrio Plus Assay, a second generation multiplexed NAT assay for HIV-1, HCV, and HBV. Vox Sanguinis. (2008) **95 (Suppl. 1)**: 74–326.
55. **Kleinman, S. H., Stramer, S. L., Brodsky, J. P., Caglioti, S., and Busch, M. P**. 2006. Integration of nucleic acid amplification test results into hepatitis C virus supplemental serologic testing algorithms: implications for donor counseling and revision of existing algorithms. Transfusion. 46:695-702.

503049EN Rev. 006
2021-06

Manufactured by:

Grifols Diagnostic Solutions Inc.
4560 Horton Street
Emeryville, CA 94608 USA
+1 (510) 655-8730

CE 1434

EC REP

Diagnostic Grifols, S.A.
Passeig Fluvial, 24
08150 Parets del Vallès, Spain

Grifols Customer Service:

Americas
Telephone (in U.S.): +1 (888) 244-7667
Or: +1 (323) 441-7762
E-mail: DxSCMCustomer.Service@grifols.com

Europe, Middle East, and Africa
E-mail: customer_support_EMEA@grifols.com

Asia/Pacific
E-mail: CustomerServicesTeam@grifols.com

Grifols Technical Service:

Americas
Telephone (in U.S.): +1 (800) 452-6877
E-mail: service.americas@grifols.com

Europe, Middle East, and Africa
E-mail: service.emea@grifols.com

Asia/Pacific
Telephone: +852 2161 3100
E-mail: service.apac@grifols.com

For U.S. Export Only