Summary Basis for Regulatory Action

Date: May 3, 2018

From: Guang Gao, Ph.D., Chair of the Review Committee

BLA/STN#: 125652.0

Applicant Name: Grifols Diagnostic Solution, Inc.

Date of Submission: January 26, 2017

Complete Response Letter: October 19, 2017

Resubmission: November 17, 2017

MDUFA Goal Date: May 19, 2018

Proprietary Name: Procleix® Ultrio Elite Assay on the Procleix® Panther System

Established Name: Procleix® Ultrio Elite Assay

Intended Use:

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

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

antibodies to HIV-1, HIV-2, HCV, and hepatitis B core antigen, and with licensed tests for hepatitis B surface antigen (HBsAg).

This assay is not intended for use as an aid in diagnosis of infection with HIV-1, HIV-2, HCV or HBV.

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

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

The Procleix® Ultrio Elite Assay can be considered a supplemental test that confirms HBV infection for specimens that are repeatedly reactive on a licensed donor screening test for HBsAg, and reactive on both the Procleix® Ultrio Elite Assay and on the Procleix® Ultrio Elite HBV Discriminatory Assay.

Recommended Action: The Review Committee recommends approval of this product.

Review Offices Signatory Authority: Nicole Verdun, M.D., Acting Director, OBRR/CBER

□ **I concur with the summary review.**
□ **I concur with the summary review and include a separate review to add further analysis.**
□ **I do not concur with the summary review and include a separate review.**

The table below indicates the material reviewed when developing the SBRA.

Discipline Reviewed	Reviewer Names
Product Review (product office) • Clinical • Non-Clinical	Pawan Jain Andy Dayton Krishnamurthy Konduru Yongqing Chen Srinivas Gannavaram Jiangqin Zhao
Statistical Review •	Paul Hshieh Chunrong Cheng
CMC Review (product office) • CMC • Facility Review (OCBQ/DMPQ) • Microbiology Review (OCBQ/DMPQ)	Guang Gao Erica Silberstein Jiangqin Zhao Sean Byrd Claire Wernly
Regulatory Project Manager	Vasantha Kumar
Software and Instrumentation Review	Sajjad Syed
Living Organ Donor and Cadaveric Donor Claim	Brychan Clark Bruce Crise
Bioresearch Monitoring Review	Christine Drabick
Lot Release Protocols/ Testing Plans	Maria Anderson Karan Smith Swati Verma
Advertising and Promotional Labeling Review (APLB)	Dana Jones

I. INTRODUCTION

The Procleix® Ultrio Elite Assay is a derivative product, based on the successful development and commercialization of the licensed Procleix® Ultrio Plus Assay (STN BL 125113/48). Final product formulations and container closure systems for the Ultrio Elite Assay utilize several previously developed and validated formulations and manufacturing processes, differing only in reagent labeling and (b) (4) Similar to the Procleix® Ultrio Plus Assay, the Procleix® Ultrio Elite Assay detects HIV-1 RNA, HCV RNA and HBV DNA with the additional capability to detect HIV-2 RNA. Modifications from the Procleix® Ultrio Plus Assay to the Procleix® Ultrio Elite Assay include, the addition of primers to the Amplification Reagent to amplify target sequences of HIV-2, the addition of oligonucleotide sequences within

the Probe Reagent and HIV Discriminatory Probe Reagent to detect HIV-2 as well as other formulation changes to the Amplification Reagent, Target Capture Reagent, and Positive and Negative Calibrators. While the licensed Procleix® Ultrio Plus Assay is used with the Procleix® Tigris System, the Procleix® Ultrio Elite Assay is performed using the automated Procleix® Panther System.

This BLA for the Procleix® Ultrio Elite Assay on the Procleix® Panther System was originally submitted by Hologic, Inc. on January 26, 2017. On February 1, 2017, the licensed and unapproved (pending review at the FDA) blood screening products manufactured by Hologic Inc., including the Procleix® Ultrio Elite Assay original BLA and investigational products were acquired by Grifols Diagnostic Solutions, Inc. (GDI). On April 10, 2017 GDI submitted an amendment to update the ownership change and update the assay draft labeling to change the manufacturer's name, address and license number to the following:

Grifols Diagnostic Solutions, Inc.
4560 Horton Street Emeryville, CA 94608
License #2032
Grifols Diagnostic Solutions
(b) (4)
FDA Registration No: 2032600

An overview of the Procleix® Panther System instrumentation and software is included within this original BLA submission.

The Procleix® Panther System that includes the Panther instrument and system software is also used for the following currently FDA approved/cleared assays with additional assay specific software:

- Aptima HPV Assay (P100042/S001)
- Aptima HPV 16 18/45 Genotype Assay (P120007/S001)
- Aptima Trichomonas Vaginalis Assay (K102911)
- Aptima Combo 2 Assay (K111409)
- Aptima HIV-1 Quant Assay (BP150318)
- (b) (4) Assay (BL (b) (4), Efficacy Supplement)

Chronological Summary of Submission

Date	Action	By

August 4, 2014	Type B Pre-IND meeting	FDA
October 21, 2014	Response to FDA's meeting minutes of Pre-IND meeting	Hologic
October 31, 2014	Submit IND application	Hologic
November 3, 2014	Submit IND amendment	Hologic
November 21, 2014	Comments and Requests for additional information	FDA
November 24, 2014	Respond FDA comments and requests	Hologic
December 30, 2014	Issue Advice/Information Request Letter	FDA
February 11, 2015	Submit IND Amendment	Hologic
February 13, 2015	Submit IND Amendment	Hologic
March 11, 2015	Submit IND Amendment	Hologic
April 8, 2015	Submit IND Amendment	Hologic
July 17, 2015	Submit IND Amendment	Hologic
January 29, 2016	Submit IND Annual Report	Hologic
March 7, 2016	Submit Meeting Minutes for the teleconference of 3/7/2016	Hologic
January 26, 2017	Submit an original BLA	Hologic
March 10, 2017	File BLA	FDA
March 21, 2017	BLA Amendment 1 for Establishment link	Hologic
April 10, 2017	BLA Amendment 2 for ownership transfer	Grifols
May 11, 2017	BLA Amendment 3 to respond DMPQ Information Request	Grifols
May 15, 2017	BLA Amendment 4 to respond Lot Release Protocol issues	Grifols
June 21, 2017	BLA Amendment 5 to respond Lot Release Protocol issues	Grifols
July 6, 2017	Information Request from Midcycle review	FDA
August 10, 2017	BLA Amendment 6 to respond Information Request from Midcycle review	Grifols
September 5, 2017	BLA Amendment 7 for Labeling	Grifols
September 14, 2017	BLA Amendment 8 for revised Known Positive Study Report	Grifols
September 27, 2017	BLA Amendment 9 to Labeling	Grifols
October 13, 2017	BLA Amendment 10 to respond the Information Request for clinical, statistical and cadaveric specimen issues	Grifols

October 19, 2017	Issue a CR Letter	FDA
October 19, 2017	BLA Amendment 11, responses to Information Request for revised Lot Release Testing Protocol	Grifols
November 16, 2017	BLA Amendment 12, response to CR letter for software and instrumentation issues	Grifols
November 21, 2017	Issue Class II Resubmission Acknowledgement Letter	FDA
December 21, 2017	Information Request for software/instrumentation	FDA
January 22, 2018	BLA Amendment 14, response to software and instrumentation issues	Grifols
January 26, 2018	Information Request for software issues	FDA
January 29, 2018	BLA Amendment 16, response to software and instrumentation issues	Grifols
February 21, 2018	BLA Amendment 17 to update 2 clinical reports	Grifols
February 28, 2018	BLA Amendment 18 for revised PI based on FDA comments	Grifols

II. BACKGROUND

Epidemiological studies identified human immunodeficiency virus type 1 (HIV-1) and human immunodeficiency virus type 2 (HIV-2) as the etiological agents of acquired immunodeficiency syndrome (AIDS), while hepatitis C virus (HCV) and hepatitis B virus (HBV) were identified as the causative agents of blood borne hepatitis. HIV, HCV, and HBV are transmitted primarily by exposure to infected blood or blood products, certain body fluids or tissues, from mother to fetus or child, and through sexual transmission.

HIV: Detection of HIV-1 infection in the blood bank setting is currently based on nucleic acid testing (NAT) for HIV RNA detection and serologic screening for anti-HIV antibodies with confirmation by additional, more specific supplemental antibody tests. The addition of NAT has reduced the window period for detection of HIV infection by 6 to 11 days in those donations tested individually.

Diagnosed cases of HIV-2 are observed primarily in West Africa or travel-related exposure in the US. Assays that detect the antibodies against both HIV-1 and HIV-2 are used for screening blood donations in the US. 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. Screening for HIV-2 RNA should further reduce the risk.

HCV: Detection of HCV infection in the blood bank setting is currently based on NAT for HCV RNA detection and serologic screening for anti-HCV antibodies. The introduction of NAT has allowed for the detection of HCV infection approximately 59 days earlier than the antibody based tests.

HBV: Detection of HBV infection in the blood bank setting is currently based on NAT for HBV DNA detection and serological screening for HBsAg with confirmation by neutralization tests and anti-hepatitis B core antigen (anti-HBc) assays. Studies indicate that NAT detects HBV infection several weeks before detection of HBsAg. 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.

III. CHEMISTRY, MANUFACTURING, AND CONTROLS (CMC)

The manufacture of the Procleix® Ultrio Elite Assay is performed in accordance with Current Good Manufacturing Practices (CGMP) in an environmentally controlled facility.

1. Overview of the Assay

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

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

During sample preparation, viral RNA and DNA are isolated from specimens via the use of target capture. The specimen is treated with a detergent to solubilize the viral

envelope, denature proteins, and release viral genomic RNA and/or DNA. Oligonucleotides (capture oligonucleotides) that are homologous to highly conserved regions of HIV, HCV, and HBV are hybridized to the HIV RNA, HCV RNA, or HBV DNA target, if present, in the test specimen. Target Enhancer Reagent (TER) is added to each reaction tube after the addition of the sample to create a transient alkaline shock, which enhances the disruption of the viral particles and denaturation of nucleic acids. Following the addition of TER, the hybridized target is captured by 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 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).

The internal control is added to each specimen reaction tube 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. The internal control signal in each reaction is discriminated from the HIV/HCV/HBV signal by the differential kinetics of light emission from probes with different labels.

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 the flasher and glower labels. When used for the simultaneous detection of HIV, HCV, and HBV, the Procleix® Ultrio Elite Assay differentiates between the 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.

Discriminatory Testing

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

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

2. Kit Components

Six kits are required to perform the Procleix® Ultrio Elite Assay on the Panther System: four assay kits and two ancillary kits. The Procleix® Ultrio Elite Assay Kit is composed of four kits comprised of 14 reagents packaged in six different boxes. The two ancillary kits, Procleix® Assay Fluids and Procleix® Auto Detect Reagents, are provided separately and are common to all Procleix® assays. The Procleix® Ultrio Elite Assay Kits are available in a 1,000 or 5,000 test-kit size.

The Procleix® Ultrio Elite Assay is a derivative product, based on the currently marketed licensed Procleix® Ultrio Plus Assay. Final product formulations and container closure systems for the Procleix® Ultrio Elite Assay utilize several previously developed and validated formulations and manufacturing processes, differing only in reagent labeling and (b) (4)

The Procleix® Ultrio Elite Assay includes the following modifications from the Procleix® Ultrio Plus Assay:

- (b) (4)

Procleix® Ultrio Elite Assay Kit

- Procleix® Ultrio Elite Master Kit:
  - Internal Control Reagent
  - Probe Reagent
  - Amplification Reagent
  - Enzyme Reagent
  - Target Capture Reagent
  - Selection Reagent
- Procleix® Ultrio Elite Target Enhancer Reagent Kit
- Procleix® Ultrio Elite Assay Calibrators Kit
  - Negative Calibrator
  - HIV Positive Calibrator
  - HCV Positive Calibrator
  - HBV Positive Calibrator

Procleix® Ultrio Elite Discriminatory Probe Reagents Kit

- HIV Discriminatory Probe
- HCV Discriminatory Probe
- HBV Discriminatory Probe

3. Description and Classifications of Oligonucleotides

The *in vitro* substances include (b) (4) oligonucleotides used in the Procleix® Ultrio Elite Assay kit and the Procleix® Ultrio Elite HIV, HCV, and HBV Discriminatory Probe

Reagents kit, which are categorized into (b) (4) distinct classes based on structure, function, and chemical composition as follows:

(b) (4)

The manufacturing processes for the (b) (4)

4. **CBER Lot Release**

The lot release protocol templates were submitted to CBER for review and found to be acceptable after revisions. CBER provided a 29-member blinded CBER Lot Release Panel to be tested at Grifols. Data from three lots were submitted to CBER in support of the BLA. All results met the acceptance criteria. For routine lot release, CBER will review lot specific kit performance data reported on the approved Lot Release Protocol.

5. **Facilities Review/Inspection**

Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. The manufacturer of the Procleix Ultrio Elite Assay is Grifols Diagnostics Solutions, Inc. The manufacturer of the Procleix Panther System platform is (b) (4) The activities performed and inspectional histories are noted in the table below and are further described in the paragraphs that follow.

Manufacturing Facilities Table for Procleix Ultrio Elite Assay and Procleix Panther System platform

Name/Address	FEI number	Inspection/ waiver	Justification /Results
Manufacturer of: • *Procleix Ultrio Elite Assay Master Kit (6 components)* • *Procleix Ultrio Elite Target Enhancer Reagent Kit (1 component)* • *Procleix Ultrio Elite Discriminatory Probe Reagents Kit (3 components)* • *Negative Calibrator components (packaged in the Procleix Ultrio Elite Assay Calibrators Kit (8 components))* • *Wash solution and oil components (packaged in the Procleix Assay Fluids Kit (3 components))* *Final packaging and shipment of finished assay kit* Grifols Diagnostics Solutions, Inc.	(b) (4)	Waived	Team Biologics inspection, (b) (4) NAI

(b) (4)			
Manufacturer of: • *HIV, HCV, and HBV positive calibrator components (packaged in the Procleix Ultrio Elite Assay Calibrators Kit (8 components))* • *Buffer for deactivation fluid (packaged in the Procleix Assay Fluids Kit (3 components))* • *Procleix Auto Detect Reagents Kit (2 components)* *Perform quality control release testing* *Procleix Panther System platform acceptance testing* Grifols Diagnostics Solutions, Inc. (b) (4)	(b) (4)	Waived	Team Biologics inspection, (b) (4) VAI

Manufacture of Procleix Panther System platform and final release of finished platform (b) (4)	(b) (4)	Waived	ORA inspection (b) (4) NAI

Team Biologics performed a surveillance inspection of the Grifols Diagnostics Solutions, Inc. manufacturing facility located at (b) (4) No inspectional observations were noted and the inspection was classified as no action indicated (NAI).

Team Biologics performed a surveillance inspection of the Grifols Diagnostics Solutions, Inc. manufacturing facility located at (b) (4) Inspectional observations were noted on FDA Form 483 and the corrective actions were deemed satisfactory. The inspection was classified as voluntary action indicated (VAI).

ORA performed a surveillance inspection of the (b) (4) manufacturing facility from (b) (4) No inspectional observations were noted and the inspection was classified as no action indicated (NAI).

Container/ Closure

Not applicable

7. Stability Program

Based on a formal stability program, the proposed shelf-life for the Procleix® Ultrio Elite Assay Kit is 24 months when stored under appropriate conditions with open kit stability of up to 30 days including time on board the Panther instrument.

The shelf-life of the Procleix® Assay Fluids kit and Procleix® Auto Detect Reagents kit was previously established under formal stability studies. The approved expiration dating for these kits is 24 months when stored under appropriate conditions. Once opened, the Assay Fluids and Auto Detect Reagents are stable for 60 days including time on board the Panther instrument.

Reagent/kit shelf life

Reagent	Shelf life
Finished Kit	24 months
Finished kit (open)	30 days
Auto Detect Reagents Kit	24 months
Auto Detect Reagents Kit (open)	60 days

8. Environmental Assessment

The BLA included a request for categorical exclusion from an Environmental Assessment under 21 CFR 25.31(c). The FDA concluded that this request is justified as the manufacturing of this product will not alter significantly the concentration and distribution of naturally occurring substances and no extraordinary circumstances exist that would require an environmental assessment.

.

IV. SOFTWARE and INSTRUMENTATION

The Procleix® Ultrio Elite donor screening assay operates on Procleix® Panther System.

The Panther System has been reviewed and approved under an Original PMA (BP150318 - Aptima HIV-1 Quant Assay). The Panther System utilizes the same instrumentation as the following FDA approved / cleared Assays:

- Aptima HPV Assay
- Aptima HPV 16 18/45 Genotype Assay
- Aptima Trichomonas vaginalis Assay
- Aptima Combo 2 Assay

Since the Panther System has been reviewed numerous times in submissions noted

above, the software/instrumentation review primarily focused on the Assay Specific Software (also referred to as the Assay Definition Module; ADM), which is specifically developed to perform the Procleix® Ultrio Elite Assay.

The points listed below are a Summary of information provided by the Applicant in the Original BLA 125652 and in the subsequent Amendments.

- ***Versioning:*** The Applicant states that the Procleix® Ultrio Elite Assay will operate on the Panther System Software version 5.3. The ADM version is listed as ver. 2.4.5.
- ***Device Description:*** The Panther System detects nucleic acid from specimens by automating the following assay processing steps: 1) Sample processing; 2) Amplification using Transcription-Mediated Amplification (TMA); 3) Detection; 4) Results Report Generation. The software required to perform the Procleix® Ultrio Elite Assay on the Panther System includes: 1) Panther System Software (PSS), and 2) Assay Software, which is also referred to as the Assay Definition Module (ADM).

The PSS is 'assay-generic' so that it can be used for all assays on the Panther System. The PSS processes samples and provides results for each assay test. The result processing is performed by the ADM and the result data is maintained in the Panther System database. The ADM contains the assay-specific parameters to perform the Procleix® Ultrio Elite Assay. The ADM is provided on a separate software disk and is installed by qualified Field Service Engineers (FSEs). Only FSEs have access to modify and edit ADM software.

- ***Risk Analysis:*** A Risk Analysis was performed by the Applicant on the ADM. The Applicant has provided the Panther System Risk Management Plan for the Ultrio Elite Assay in the original BLA 125652. The Applicant listed numerous risks and their corresponding mitigations. Some of the identified risks include: "incorrect instructions for accessing and loading consumables," "volume insufficiency," "software flags not thrown," etc. To mitigate these risks, the Applicant has provided resolutions such as a report on volume sufficiency testing, software flagging results outside acceptable limits, etc. The Applicant has also conducted a (b) (4) of known or foreseeable assay specific hazards identified for the Panther System when used with the

Procleix® Ultrio Elite Assay. To ensure that the use-related hazards are minimized and controlled, a human factors risk analysis has been performed as part of the risk management process. In subsequent amendments, the Applicant has completed system documentation, by providing updated Risk Management report and Cyber-Security safeguards on the entire PSS.

- ***Testing:*** The Applicant does provide a description of Validation and Verification activities specific to the Procleix® Ultrio Elite Assay. The Applicant outlines the testing activities that were performed to ensure that the Procleix® Ultrio Elite Assay on the Panther System Software Version 5.3 conformed to the user needs and intended uses, and that the requirements implemented through software and hardware were consistently fulfilled. The testing activities include Intended Use Testing, Assay Verification, Instrument Verification & Validation, and Software Verification & Validation. The Applicant has utilized the Panther System with ADM to perform the Procleix® Ultrio Elite Assay in clinical studies. The Applicant has also conducted analytical studies to evaluate the performance of the Procleix® Ultrio Elite Assay, and Discriminatory HIV, HCV, HBV Assays on the Panther System with ADM installed.

The Applicant has explained in their subsequent Amendments that numerous errors which did occur were due to the operator not following Package Instructions. The Panther Systems detected the errors correctly and followed the procedure by invalidating the samples that had generated those errors. The Applicant recommends that to minimize the occurrence of these issues, the Operator should prepare samples per the assay package insert instructions and follow Panther operating procedures listed in the Panther Operators Manual. Operators should also follow mag wash advance cleaning procedures listed in the Panther Operators Manual for optimal Panther System performance.

- ***Unresolved Anomalies:*** The Applicant provided a list of Unresolved Anomalies (UA) in the original documentation. In subsequent Amendments, the Applicant has provided additional information by discussing the anomalies they are

monitoring, the likelihood of occurrence and their mitigation plan. The Applicant has also stated that since version 5.2, they have fixed 62 anomalies in version 5.3.

- ***Development Management:*** The Applicant does provide a summary of their software development life cycle plan, describing the processes that have been put into place to manage the various software development life cycle activities, including a summary of the configuration management and maintenance activities. The Applicant has stated that the Panther System is developed in (b) (4) Development of the Panther software utilizes (b) (4) software that manages the software development source code control and software anomaly management. The Applicant has specified that the Panther System Software (PSS) Version 5.3 operates on Windows 7. The Applicant has clearly identified in subsequent Amendments that they will only launch Procleix® Ultrio Elite Assay on PSS running on Microsoft Windows 7 Operating System. The Applicant also provides a description of the software system partitioned into its functional subsystems. The Applicant has included a Traceability Matrix (TM), which details the links between the requirements, design, implementation, validation and testing. The Applicant has provided also ADM specific TM that pertains to the Procleix® Ultrio Elite Assay.

Please note that the Panther System has been previously reviewed and assessed for its safety, effectiveness, and performance in prior submissions. Hence, the subject submission (BL125652) instrumentation/software review focused on Procleix® Ultrio Elite Assay specific ADM software and its ability to apply the parameters defined, analyze kinetic data and calculate the results. Based on the software documentation provided and supporting analytical and clinical studies conducted with the Panther Systems using ADM, the instrumentation and software areas of the subject submission BL125652 are adequate. Specific issues pertaining to exact anomaly/errors mitigation, submittal of incomplete cyber-security, risk management documentation, PSS/ADM versioning were also addressed by the Applicant during the review and found to be acceptable.

V. NON-CLINICAL STUDIES

Non-clinical studies were performed by the Research and Development Department at Grifols Diagnostic Solutions, Inc., to evaluate the performance of the Procleix® Ultrio Elite Assay, and the Discriminatory HIV, HCV, HBV assays on the Procleix® Panther System.

The analytical studies were conducted in compliance with 21CFR Part 58 (Good Laboratory Practices or GLPs), as applicable, and were performed under controlled pre-approved protocols. All assay results were evaluated using the validity criteria for runs and specimens outlined in the package insert. All study results demonstrated performance that met all pre-determined acceptance criteria and confirmed that the Procleix® Ultrio Elite Assay on the Procleix® Panther System is safe and effective for its intended use.

1. Determination of the Limits of Detection (LODs)

Analytical sensitivity panels comprised of serially diluted HIV-1 WHO standard (97/650), HIV-2 WHO standard (08/150), HCV WHO standard (06/100), HBV WHO standard (97/750) (equivalent to HBV A) were used to evaluate assay sensitivity. For testing on the Panther System, 60-63 replicates of each target level were tested with each reagent lot in the Procleix® Ultrio Elite Assay and the Discriminatory Assays for a total of 180-183 tests per assay for HIV-1, HIV-2, HCV, and HBV.

Development Lot 3, Pilot Lot 1, and Pilot Lot 2 (DL3, PL1, and PL2, respectively) reagents were used for Procleix® Ultrio Elite Assay on the Procleix® Panther System. The average signal/cut-off (S/CO) and %CV values calculated for samples containing viral RNA or DNA are from reactive results only (S/CO values >1.0). Estimations of 50% and 95% detection rates by PROBIT modeling (SAS version 9.2) for detection of WHO standards for HIV-1, HIV-2, HCV, and HBV with the Procleix® Ultrio Elite Assay and the Discriminatory Assays on the Procleix® Panther platform are shown in Tables 1-4.

The analytical sensitivity studies were conducted with the WHO standards for HIV-1, HIV-2, HCV, and HBV. For the HIV-1 and HIV-2 WHO standards, 95% detection based on PROBIT analysis was 18.0 and 10.4 IU/mL, respectively in the Procleix® Ultrio Elite Assay. For HCV and HBV WHO standards, 95% detection was 3.0 IU/mL and 4.3 IU/mL, respectively in the Procleix® Ultrio Elite Assay. Performance was similar for the screening assay and the discriminatory assays. Similar performance was also

observed for each lot tested. This study showed that the Procleix® Ultrio Elite Assay and Discriminatory Assays satisfy the study criteria, having met the design goals of 95% detection of HIV-1 at 200 IU/mL, HIV-2 at 100 IU/mL, HCV at 30 IU/mL, and HBV at 5 IU/mL.

Table 1: Probit Analysis Detection of HIV-1 WHO (97/650) Standard

Lot	Assay	Detection Probabilities (IU/mL) *	
		50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
DL 3	Procleix® Ultrio Elite	6.0 (4.8 - 7.1)	15.2 (11.7 - 26.0)
	Ultrio Elite HIV Discriminatory	5.3 (3.7 - 6.6)	17.3 (13.0 - 30.8)
PL 1	Procleix® Ultrio Elite	4.5 (2.5 - 6.0)	21.1 (15.2 - 40.2)
	Ultrio Elite HIV Discriminatory	5.8 (4.4 - 7.1)	17.5 (13.3 - 29.8)
PL 2	Procleix® Ultrio Elite	5.5 (4.0 - 6.8)	17.4 (13.1 - 30.5)
	Ultrio Elite HIV Discriminatory	4.6 (2.8 - 5.9)	16.9 (12.5 - 32.2)
Combined Lots	Procleix® Ultrio Elite	5.4 (4.5 - 6.1)	18.0 (15.0 - 23.5)
	Ultrio Elite HIV Discriminatory	5.3 (4.4 - 6.0)	17.3 (14.4 - 22.6)

*Probit analysis was performed with SAS version 9.2

Table 2: Probit Analysis Detection of HIV-2 WHO (08/150) Standard

Lot	Assay	Detection Probabilities (IU/mL) *	
		50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
DL 3	Procleix® Ultrio Elite	3.1 (2.4 - 3.9)	13.8 (10.4 - 20.3)
	Ultrio Elite HIV Discriminatory	2.4 (1.9 - 2.9)	7.4 (5.8 - 10.2)
PL 1	Procleix® Ultrio Elite	2.4 (1.9 - 3.0)	9.0 (7.0 - 12.6)
	Ultrio Elite HIV Discriminatory	2.3 (1.9 - 2.9)	8.3 (6.4 - 11.8)
PL 2	Procleix® Ultrio Elite	2.3 (1.9 - 2.9)	17.4 (13.1 - 30.5)
	Ultrio Elite HIV Discriminatory	1.8 (1.4 - 2.3)	8.8 (6.6 - 13.1)
Combined Lots	Procleix® Ultrio Elite	2.6 (2.3 - 3.0)	10.4 (8.9 - 12.6)
	Ultrio Elite HIV Discriminatory	2.2 (1.9 - 2.5)	9.6 (8.1 - 11.8)

*Probit analysis was performed with SAS version 9.2
HIV-2 WHO Standard detection levels were analyzed with Gompertz Distribution

Table 3: Probit Analysis Detection of HCV WHO (96/798) Standard

Lot	Assay	Detection Probabilities (IU/mL) *	
		50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
DL 3	Procleix® Ultrio Elite	0.9 (0.6 – 1.1)	3.0 (2.2 – 5.4)
	Ultrio Elite HCV Discriminatory	0.6 (0.2 – 0.8)	2.5 (1.8 – 5.4)
PL 1	Procleix® Ultrio Elite	0.9 (0.6 – 1.0)	2.3 (1.8 – 4.0)
	Ultrio Elite HCV Discriminatory	0.6 (0.2 – 0.8)	2.5 (1.8 – 5.4)
PL 2	Procleix® Ultrio Elite	1.1 (0.8 – 1.3)	3.6 (2.7 – 6.0)
	Ultrio Elite HCV Discriminatory	0.8 (0.5 – 1.0)	2.2 (1.7 – 4.2)
Combined Lots	Procleix® Ultrio Elite	0.9 (0.8 – 1.0)	3.0 (2.5 – 3.9)
	Ultrio Elite HCV Discriminatory	0.7 (0.5 – 0.8)	2.4 (2.0 – 3.3)

*Probit analysis was performed with SAS version 9.2

Table 4: Probit Analysis Detection of HBV WHO (97/750) Standard

Lot	Assay	Detection Probabilities (IU/mL) *	
		50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
DL 3	Procleix® Ultrio Elite	1.0 (0.8 – 1.2)	4.2 (3.4 – 5.5)
	Ultrio Elite HBV Discriminatory	1.0 (0.8 – 1.2)	4.4 (3.6 – 5.8)
PL 1	Procleix® Ultrio Elite	1.1 (0.8 – 1.3)	5.1 (4.1 – 6.9)
	Ultrio Elite HBV Discriminatory	1.0 (0.8 – 1.2)	4.8 (3.8 – 6.3)
PL 2	Procleix® Ultrio Elite	0.8 (0.7 – 1.0)	3.6 (2.9 – 4.7)
	Ultrio Elite HBV Discriminatory	1.0 (0.8 – 1.1)	4.4 (3.5 – 5.9)
Combined Lots	Procleix® Ultrio Elite	0.9 (0.8 – 1.1)	4.3 (3.8 – 5.0)
	Ultrio Elite HBV Discriminatory	1.0 (0.9 – 1.1)	4.5 (4.0 – 5.3)

*Probit analysis was performed with SAS version 9.2
HBV WHO detection levels were analyzed with Gompertz Distribution.

2. Detection of Subtypes/Genotypes with the Procleix® Ultrio Elite Assay and Discriminatory Assay

HIV-1 and HIV-2

Characterized specimens from patients infected with different HIV-1 and HIV-2 genetic variants were obtained from vendors. Patient specimens diluted to 100 and 30 copies/mL (c/mL) were tested with the Ultrio Elite and the Ultrio Elite HIV Discriminatory Assays (dHIV) using reagent DL3 and PL1. Two replicates of each target copy level for each specimen were tested with the fully automated Procleix® Panther system.

Table 5 shows an overall summary of detection for HIV-1 subtypes. It also shows the results for each reagent lot as well as the overall detection and the % reactivity for each group and subtype.

All HIV-1 groups and subtypes were 100% reactive at the 100 c/mL copy level. At the 30 c/mL copy level, HIV-1 genotype had an overall detection of 95.3% for the Ultrio Elite Assay and 95.0% for the Ultrio Elite dHIV Assay.

Table 5: Detection of HIV-1 Groups and Subtypes with the Ultrio Elite and Ultrio Elite dHIV Assays on Panther (DL 3, PL1)

HIV-1 Group	Copies/ mL	Development Lot 3 # reactive/# tested		Pilot Lot 1 # reactive/# tested		DL3 & PL1 combined Genotype % Reactivity	
Subtype		Ultrio Elite	dHIV	Ultrio Elite	dHIV	Ultrio Elite	dHIV
Group M Subtype A	100 30	22/22 **21/22**	22/22 **21/22**	22/22 **20/22**	22/22 **20/22**	100 **93.2**	100 **93.2**
Group M Subtype B	100 30	20/20 20/20	20/20 **19/20**	20/20 20/20	20/20 20/20	100 100	100 **97.5**
Group M Subtype C	100 30	16/16 16/16	16/16 16/16	16/16 **15/16**	16/16 **15/16**	100 **96.9**	100 **96.9**
Group M Subtype D	100 30	20/20 20/20	20/20 **18/20**	20/20 **18/20**	20/20 **19/20**	100 **95.0**	100 **92.5**
Group M Subtype E	100 30	20/20 **19/20**	20/20 20/20	20/20 **17/20**	20/20 **18/20**	100 **90.0**	100 **95.0**
Group M	100	10/10	10/10	10/10	10/10	100	100

Subtype F	30	10/10	**9/10**	**9/10**	**9/10**	**95.0**	**90.0**
Group M Subtype G	100 30	4/4 4/4	4/4 4/4	4/4 4/4	4/4 4/4	100 100	100 100
Group M Subtype H	100 30	2/2 2/2	2/2 2/2	2/2 2/2	2/2 2/2	100 100	100 100
Group N	100 30	2/2 2/2	2/2 2/2	2/2 **1/2**	2/2 **1/2**	100 **75.0**	100 **75.0**
Group O*	100 30	12/12 12/12	12/12 12/12	14/14* 12/12	12/12 14/14*	100 100	100 100
Overall Detection (%)	100	128/128 (100)	128/128 (100)	130/130 (100)	128/128 (100)	100	100
	30	**126/128 (98.4)**	**123/128 (96.1)**	**118/128 (92.1)**	**122/130 (93.8)**	**95.3**	**95.0**

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

Table 6 shows an overall summary of detection for HIV-2 subtypes. It shows the results for each reagent lot as well as the overall detection and the % reactivity for each group and subtype.

At the 100 c/mL, HIV-2 subtypes had an overall detection of 87.5% for the Ultrio Elite Assay and 95.8% for the Procleix® Ultrio Elite dHIV Assay. At the 30 c/mL copy level, HIV-2 subtypes had an overall detection of 87.5% for the Ultrio Elite Assay and 83.3% for the Procleix® Ultrio Elite dHIV Assay. At 1000 c/mL and 300 c/mL, HIV-2 subtype had 100% overall detection by both assays.

Table 6: Detection of HIV-2 Subtypes with the Ultrio Elite and Ultrio Elite dHIV Assays on Panther (DL 3, PL1)

HIV-2 Group	Copies/ mL	Development Lot 3 # reactive / # tested		Pilot Lot 1 # reactive / # tested		DL3 & PL1 combined % Reactivity	
Subtype		Ultrio Elite	dHIV	Ultrio Elite	dHIV	Ultrio Elite	dHIV
Subtype A	1000 300 100 30	2/2 2/2 **8/10** **9/10**	2/2 2/2 10/10 **8/10**	2/2 2/2 **9/10** **8/10**	2/2 2/2 **9/10** **8/10**	100 100 **85.0** **85.0**	100 100 **95.0** **80.0**
Subtype B	100 30	2/2 2/2	2/2 2/2	2/2 2/2	2/2 2/2	100 100	100 100

Overall Detection (%)	1000	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	100	100
	300	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	100	100
	100	**10/12 (83.3)**	12/12 (100)	**11/12 (91.7)**	**11/12 (91.7)**	**87.5**	**95.8**
	30	**11/12 (91.7)**	**10/12 (83.3)**	**10/12 (83.3)**	**10/12 (83.3)**	**87.5**	**83.3**

HCV

Characterized specimens from patients infected with different HCV genotypes were obtained from vendors. Patient specimens diluted to 100 and 30 copies/mL (c/mL) were tested with Procleix® Ultrio Elite Assay and Procleix® Ultrio Elite HCV Discriminatory Assays (dHCV) reagent DL3 and PL1. Two replicates of each target copy level for each specimen were tested with the fully automated Procleix® Panther system. Table 7 shows an overall summary of detection for HCV Genotypes. It shows the results for each reagent lot as well as the overall detection and the % reactivity for each genotype.

All HCV genotypes were 100% reactive at the 100 c/mL copy level. At the 30 c/mL copy level, HCV genotypes 1-6 had an overall detection of 91.9% for the Ultrio Elite Assay and 90.3% for the Ultrio Elite dHCV Assay.

Table 7: Detection of HCV Genotypes with the Ultrio Elite and dHCV Assays on Panther (DL3, PL1)

HCV Genotype	Copies/ mL	DL 3		PL 1		DL3 & PL1 combined Genotype % Reactivity	
		# reactive / # tested		# reactive / # tested			
		Ultrio Elite	dHCV	Ultrio Elite	dHCV	Ultrio Elite	dHCV
1	100 30	20/20 **19/20**	20/20 **18/20**	20/20 **18/20**	20/20 **17/20**	100 **92.5**	100 **87.5**
2	100 30	28/28 **24/28**	28/28 **21/28**	28/28 **21/28**	28/28 **21/28**	100 **80.4**	100 **75.0**
3	100 30	22/22 **18/22**	22/22 **20/22**	22/22 **21/22**	22/22 **20/22**	100 **88.6**	100 **90.9**

4	100 30	26/26 26/26	26/26 26/26	26/26 26/26	26/26 26/26	100	100
5	100 30	10/10 10/10	10/10 10/10	10/10 10/10	10/10 10/10	100	100
6	100 30	12/12 12/12	12/12 12/12	12/12 12/12	12/12 12/12	100	100
Overall Detection (%)	100	118/118 (100)	118/118 (100)	118/118 (100)	118/118 (100)	100	100
	30	**109/118 (92.4)**	**107/118 (90.7)**	**108/118 (91.5)**	**106/118 (89.8)**	**91.9**	**90.3**

HBV

Characterized specimens from patients infected with different HBV genotypes were obtained from vendors. Patient specimens diluted to 100 and 30 copies/mL (c/mL) were tested with Ultrio Elite and Ultrio Elite HBV Discriminatory Assays (dHBV) reagent Development Lot 3 (DL3) and Pilot Lot 1 (PL1). Two replicates of each target copy level for each specimen were tested with the fully automated Procleix® Panther system.

Table 8: shows an overall summary of detection for HBV Genotypes. It shows the results for each reagent lot as well as the overall detection and the % reactivity for each genotype.

The overall % reactivity for HBV Genotypes at 100 c/mL for both reagent lots combined was 99.6% for the Ultrio Elite Assay, and 99.2% for the Ultrio Elite dHBV Assay. At 30 c/mL for both reagent lots combined, the overall % reactivity was 93.2% for the Ultrio Elite and 92.8% for Ultrio Elite dHBV Assays.

Two HBV Genotype C specimens, IDs 9990591 and 9992087, and one HBV Genotype F specimen ID 27485 were not 100% reactive at 100 c/mL. Therefore, additional testing was conducted. At 300 c/mL these specimens were 100% reactive in both the Ultrio Elite Assay and Ultrio Elite dHBV assay with both DL3 and PL1 reagents.

Table 8: Detection of HBV Genotypes with the Ultrio Elite and Ultrio Elite dHBV Assays on the Panther System (Reagent Lots DL3 and PL1)

HBV Genotype	Copies/ mL	Development Lot 3 # reactive / # tested		Pilot Lot 1 # reactive / # tested		DL3 & PL1 combined Genotype % Reactivity	
		Ultrio Elite	dHBV	Ultrio Elite	dHBV	Ultrio Elite	dHBV
A	100 30	22/22 **21/22**	22/22 22/22	22/22 **19/22**	22/22 **21/22**	100 **90.9**	100 **97.7**
B	100 30	20/20 **18/20**	20/20 20/20	20/20 20/20	20/20 **19/20**	100 **95.0**	100 **97.5**
C	300 100 30	4/4 **19/20** **17/20**	4/4 20/20 **16/20**	4/4 20/20 **15/20**	4/4 **19/20** **15/20**	100 **97.5** **80.0**	100 **97.5** **77.5**
D	100 30	18/15 17/18	18/18 18/18	18/18 17/18	18/18 16/18	100 94.4	100 94.4
E	100 30	18/18 18/18	18/18 **17/18**	18/18 18/18	18/18 **17/18**	100 100	100 **94.4**
F	300 100 30	2/2 16/16 16/16	2/2 **15/16** **14/16**	2/2 16/16 16/16	2/2 16/16 16/16	100 100 100	100 **96.9** **93.8**
G	100 30	2/2 2/2	2/2 2/2	2/2 2/2	2/2 2/2	100 100	100 100
H	100 30	2/2 2/2	2/2 2/2	2/2 2/2	2/2 2/2	100 100	100 100
Overall Detection (%)	300	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)	100	100
	100	**117/118** **(99.2)**	**117/118** **(99.2)**	**118/118** **(100)**	**117/118** **(99.2)**	**99.6**	**99.2**
	30	**111/118** **(94.9)**	**111/118** **(94.1)**	**109/118** **(92.4)**	**108/118** **(91.5)**	**93.2**	**92.8**

3. Detection of HIV-1, HIV-2, HCV and HBV in Panel Co-Spiked with Other Analytes with the Procleix® Ultrio Elite Assay and HIV, HCV and HBV Discriminatory Assays

Co-spiked panels consisted of the following samples: HIV-1 tissue culture supernatant sample, HIV-2 tissue culture sample, HCV clinical specimens, and HBV clinical specimen were purchased and diluted to different virus concentrations. To prepare co-spiked panels used in this study, (b) (4) amounts of HIV-2, HIV-1, HCV and HBV at the appropriate concentration were combined. (b) (4) replicates of each panel member were tested in each reagent lot (PL1 and PL2) and in the Procleix® Ultrio Elite Assay, and the Ultrio Elite HIV, HCV and HBV Discriminatory Assays on the Panther system.
100% of the co-spiked samples were reactive in the Procleix® Ultrio Elite Assay and the Ultrio Elite HIV, HCV, and HBV Discriminatory Assays on the Panther system, except for panel member E which was 95% reactive in the dHBV assay. Based on these results, it can be concluded that the co-presence of viruses, even at high titers, does not affect the performance of the Ultrio Elite Assay. A small impact was observed in the Ultrio Elite Discriminatory HBV Assay under the most challenging, and unlikely scenario. This does not present a risk to blood safety, as these panels were correctly reactive with the Ultrio Elite Assay.

Review Issues:

The review committee observed that under several conditions with one or two analyte (s) at high concentration, the signal for the other analyte is either lost, significantly reduced, or falsely detected. In the Information Request, we requested the sponsor to design and perform a study to evaluate such effects for all combinations of high and low analyte concentrations and describe under which circumstances performance of the discriminatory assays would be affected.

Grifols provided additional testing results in Amendment 8. All co-infected samples were 100% reactive in the Procleix® Ultrio Elite Assay and Ultrio Elite HIV, HCV and HBV Discriminatory Assays on the Panther system, except for condition S (b) (4) cp/ml HIV-1, and HIV-2, (b) (4) IU/mL HBV and (b) (4) cp/mL HCV) which was 86% reactive in the discriminatory HIV assay. A high IC invalid rate was observed in conditions P (b) (4) cp/mL HIV-2, (b) (4) cp/mL HIV-(b) (4) cp/mL HCV and (b) (4) IU/mL HBV) and S, which both contain high titers of HCV and HBV when tested in the Ultrio Elite HIV Discriminatory Assay. Signal suppression was observed in the Ultrio Elite HIV

Discriminatory Assay in conditions containing high titers of HCV and HBV (conditions Q (b) (4) cp/mL HIV-2, (b) (4) cp/mL HIV-1, (b) (4) c/mL HCV, and (b) (4) IU/mL HCV) and R (b) (4) c/mL HIV-2, (b) (4) cp/mL HIV-1, (b) (4) cp/mL HCV and (b) (4) IU/mL HBV).
The review committee believes that it is not a blood safety issue since units reactive for any analyte will be removed from the inventory. This response is acceptable.

4. Detection of HIV-1, HIV-2, HCV, and HBV in Naturally Infected Samples

A total of 620 samples from individuals who were known to be infected with HIV-1, HCV or HBV were tested in the Procleix® Ultrio Elite Assay and the corresponding discriminatory assay at Grifols. A total of 100 samples from individuals who were known to be infected with HIV-2 were tested in the Procleix® Ultrio Elite Assay at Grifols.
The results from the Ultrio Elite Assay testing were compared to the data generated previously for the same samples using the Ultrio Assay on the TIGRIS System and the Ultrio Plus Assay on the TIGRIS System. All samples tested with the Ultrio Elite Assay have data using the Ultrio Assay for comparison, however, only a subset of samples have data with the Ultrio Plus assay. The 95% exact binomial confidence intervals for the percentage of reactive results were calculated using SAS version 9.02.
Of the 214 HIV-1 positive samples tested with the Ultrio Elite Assay, all were reactive when tested undiluted in the Procleix® Ultrio Elite Assay and Ultrio Elite dHIV Assay. All samples diluted 1:16 were reactive with the Ultrio Elite Assay. The data indicate that the Procleix® Ultrio Elite Assay and Ultrio Elite dHIV Assay had equivalent sensitivity for HIV-1 compared to the Ultrio Assay and dHIV-1 Assay on the TIGRIS System.
Of the 203 HCV positive samples tested with the Procleix® Ultrio Elite Assay and Ultrio Elite dHCV Assay, all were reactive when tested undiluted in the Procleix® Ultrio Elite Assay and Procleix® Ultrio Elite dHCV Assay. All samples diluted 1:16 were reactive with the Ultrio Elite Assay. The data indicate that the Ultrio Elite Assay and Ultrio Elite dHCV Assay had equivalent sensitivity for HCV on the Panther compared to the Ultrio and Ultrio Plus Assays and their respective dHCV Assays on the Tigris System.

Of the 203 HBV positive samples tested with the Ultrio Elite Assay and Ultrio Elite dHBV Assay, all were reactive when tested undiluted in the Procleix® Ultrio Elite Assay and Procleix® Ultrio Elite dHBV Assay on the Panther. All samples diluted 1:16 were reactive with the Ultrio Elite Assay on the Panther. The data indicate that The Ultrio Elite Assay had equivalent sensitivity on the Panther for HBV samples compared to the Ultrio and Ultrio Plus Assays and their respective Discriminatory HBV Assays on the Tigris System.

Of the 100 HIV-2 positive samples tested with the Ultrio Elite Assay, 54 were reactive when tested undiluted. These specimens were sent to (b) (4) for testing with the (b) (4) blood screening assay. Undiluted, the (b) (4) assay detected 17 samples that were non-reactive with Ultrio Elite, and failed to detect 9 that were reactive in Ultrio Elite. In a blood screening scenario where samples were tested as simulated minipools of 6, the (b) (4) Assay detected 4 specimens that were non-reactive with Ultrio Elite, and failed to detect 12 specimens that were reactive in Ultrio Elite.

Table 9 below shows the clinical sensitivity of the Procleix® Ultrio Elite Assay and the Ultrio Elite Discriminatory Assays was 100% for all undiluted HIV-1, HCV, and HBV clinical samples. The clinical sensitivity of the Procleix® Ultrio Elite Assay was 100% for all HIV-1, HCV, and HBV samples tested when diluted 1:16. The Ultrio Elite Assay detected 54% of undiluted specimens seropositive for HIV-2, which was similar to results observed for these samples using the (b) (4) Assay.

Table 9: Summary of Percent Reactive Results (95% CI) for the Ultrio Elite Assay on the Panther System Compared to the Ultrio Plus Assay on the Tigris System

Sample Type	Ultrio Elite Assay Percent Reactive (95% CI)			Ultrio Plus Assay Percent Reactive (95% CI)			Ultrio Assay Percent Reactive (95% CI)		
	Panther System			Tigris System			Tigris System		
	Neat	1:16	Discrim Neat	Neat	1:16	Discrim Neat	Neat	1:16	Discrim Neat
HIV-1	100 (98.3 – 100)	100 (98.3 – 100)	100 (98.3 – 100)	100 (93.2 – 100)	100 93.2 – 100)	100 (93.2 – 100)	100 (98.3 – 100)	99 (96.7 – 99.9)	100 (98.3 – 100)

HCV	100 (98.2 – 100)	100 (98.2 – 100)	100 (98.2 – 100)	100 (98.0 – 100)	100 (98.0 – 100)	100 (98.0 – 100)	100 (98.2 – 100)	100 (98.2 – 100)	100 (98.2 – 100)
HBV	100 (98.2 – 100)	100 (98.2 – 100)	100 (98.2 – 100)	100 (97.6 – 100)	100 (97.6 – 100)	100 (97.6 – 100)	100 (98.2 – 100)	100 (98.2 – 100)	100 (98.2 – 100)
HIV-2	54 (43.7 – 64.0)	NT	NT	NT	NT	NT	NT	NT	NT

Ultrio Elite and Ultrio: N = 214 for HIV-1; 203 for HCV; 203 for HBV;
100 for HIV-2 (Ultrio Elite, only)
Ultrio Plus: N = 52 for HIV-1; N = 186 for HCV; N = 154 for HBV
95% CI = 95% exact binomial confidence interval
NT = Not tested

5. Detection of HIV-1, HCV, and HBV Seroconversion Panels

HIV-1

Ten commercially available HIV-1 seroconversion panels were tested. The number of samples per panel ranged from 10 to 29.

Detection of HIV-1 RNA with the Procleix® Ultrio Elite Assay occurred between 11 to 16 days earlier than detection of HIV antibody test in neat samples and 8 to 14 days earlier in samples diluted 1:8 and 1:16 (Table 10). Detection of HIV-1 RNA with the Procleix® Ultrio Elite Assay occurred between 4-19 days earlier than detection of HIV p24 Ag in neat samples and 0-19 days earlier in samples diluted 1:8 and 1:16 (Table 11). The average for HIV-1 RNA detection with the Procleix® Ultrio Elite Assay was 9.6, 7.1, and 7.0 days earlier than p24 Ag detection for specimens tested neat, diluted 1:8, and diluted 1:16, respectively. These results were compared to the Ultrio Plus Assay data and are shown in Tables 1o and 11. The clinical sensitivity of the Procleix® Ultrio Elite Assay and the Procleix® Ultrio Plus Assay are similar.

Table 10: Performance of the Procleix® Ultrio Elite Assay on HIV Seroconversion Panels

Number of Days HIV-1 RNA Detected Before Anti-HIV 1/2						
Panel ID	Ultrio Elite			Ultrio Plus		
	Neat	1:8	1:16	Neat	1:8	1:16
6247	16	14	9	16	14	14
9020	14	10	10	17	14	14
9021	15	11	11	11	11	11
9030	14	14	14	14	14	14
9031	12	12	12	15	12	15
9032	14	10	14	14	10	10
9076	14	8	8	14	8	8
9077	11	11	11	11	11	11
Average‡	**13.8**	**11.3**	**11.1**	**14.0**	**11.8**	**12.1**
Median‡	**14.0**	**11.0**	**11.0**	**14.0**	**11.5**	**12.5**
Range‡	**11-16**	**8-14**	**8-14**	**11-17**	**8-14**	**8-15**

‡ Results include only seroconversion panels that were tested with both the Ultrio Elite and Ultrio Plus Assays

Table 11: Performance of the Procleix® Ultrio Elite Assay on HIV Seroconversion Panels

Number of Days HIV-1 RNA Detected Before HIV p24 Ag						
Panel ID	Ultrio Elite			Ultrio Plus		
	Neat	1:8	1:16	Neat	1:8	1:16
6247	7	5	0	7	5	5
9020	4	0	0	7	4	4
9021	8	4	4	4	4	4
9030	7	7	7	7	7	7
9031	19	19	19	22	19	22
9032	14	10	14	14	10	10
9076	14	8	8	14	8	8
9077	4	4	4	4	4	4
Average‡	**9.6**	**7.1**	**7.0**	**9.9**	**7.6**	**8.0**
Median‡	**7.5**	**6.0**	**5.5**	**7.0**	**6.0**	**6.0**
Range‡	**4-19**	**0-19**	**0-19**	**4-22**	**4-19**	**4-22**

‡ Results include only seroconversion panels that were tested with both Ultrio Elite and Ultrio Plus Assays

HCV

Twelve commercially available HCV seroconversion panels were tested with Pilot Lot 1 reagent of the Procleix® Ultrio Elite Assay. Samples were tested neat, 1:8 and 1:16

dilutions. Testing was performed in singlet on the fully automated Procleix® Panther System.

Detection of HCV RNA with the Procleix® Ultrio Elite Assay and the Ultrio Plus Assay was from 23 to 41 days earlier than detection of HCV antibodies in samples that were neat or diluted 1:8 and 1:16. These results indicate that for this set of seroconversion panels, the seroconversion window for HCV was reduced by an average of 33.1 days for specimens tested neat and 32.1 days for specimens diluted 1:8 and 1:16 with the Ultrio Elite assay and 32.9, 32.1, and 32.4 days in the Procleix® Ultrio Plus Assay for specimens tested neat, diluted 1:8, and diluted 1:16, respectively (Table 12). In 5 of 12 seroconversion panels (6214, 6226, 6228, 9045, 9047), the first available bleed in the series was already reactive with the Procleix® Ultrio Elite Assay as well as the Ultrio Plus Assay, so the number of days of window closure may underestimate the true window closure period for both of the Procleix® Assays. These results indicate that the clinical sensitivity of both nucleic acid assays is similar.

Table 12: Performance of the Procleix® Ultrio Elite Assay on HCV Seroconversion Panels

Number of Days HCV RNA Detected Before Anti-HCV						
Panel ID	Ultrio Elite			Ultrio Plus		
	Neat	1:8	1:16	Neat	1:8	1:16
6213	26	26	26	26	26	26
6214	30	30	30	30	30	30
6222	23	23	23	23	23	23
6225	39	33	33	39	33	33
6226	39	39	39	39	39	39
6227	32	32	32	32	32	32
6228	31	31	31	31	31	31
9041	38	38	38	38	38	38
9045	41	41	41	41	41	41
9047	28	28	28	28	28	28
9054	36	30	30	30	30	30
9055	34	34	34	38	34	38
Average	**33.1**	**32.1**	**32.1**	**32.9**	**32.1**	**32.4**
Median	**33.0**	**31.5**	**31.5**	**31.5**	**31.5**	**31.5**
Range	**23-41**	**23-41**	**23-41**	**23-41**	**23-41**	**23-41**

HBV

Nine commercially available HBV seroconversion panels were tested with Pilot Lot 11 reagent of the Procleix® Ultrio Elite Assay. The samples were tested neat, 1:8 and 1:16 dilutions in singlet on the fully automated Procleix® Panther system.

Detection of HBV DNA with the Procleix® Ultrio Elite Assay was from 7 to 36 days earlier than detection of HBV surface antigen in neat samples and 0 to 29 days earlier in dilute 1:8 samples and 1:16 samples (Table 13). Detection of HBV DNA with the Procleix® Ultrio Elite Assay was from 7 to 26. Compared to detection of HBV with the HBsAg test, detection of HBV DNA ranged from 26 days earlier to 7 days later in neat samples diluted 1:8 and 1:16. These results show that for the entire combined set of panels, the seroconversion window for HBV was reduced by an average of 18.1 days in the Procleix® Ultrio Elite Assay for specimens tested neat and 8.8 or 5.3 days earlier for samples diluted 1:8 or 1:16, respectively. These results were compared to the Ultrio Plus Assay data (for the subset of panels that were available to test using both) shown in Table 13 demonstrate that the clinical sensitivity of the Procleix® Ultrio Elite Assay and the Procleix® Ultrio Plus Assay are similar.

Table 13: Performance of the Procleix® Ultrio Elite Assay on HBV Seroconversion Panels

Number of Days HBV DNA Detected Before HBsAg						
Panel ID	Ultrio Elite			Ultrio Plus		
	Neat	1:8	1:16	Neat	1:8	1:16
6283	26	26	26	15	7	7
6289	22	0	0	20	13	0
6290	7	-7#	-7#	7	-7#	-7#
6292	20	8	8	27	8	8
9073	14	14	14	21	10	10
9074	21	11	14	14	14	11
11007	19	12	5	21	5	7
11015	9	0	-27#	9	7	0
11024	25	15	15	33	15	15
Average‡	**18.1**	**8.8**	**5.3**	**18.6**	**8.8**	**5.7**
Median‡	**20**	**11**	**8**	**20**	**8**	**7**
Range‡	**7-26**	**-7-26**	**-7-26**	**7-33**	**-7-15**	**-7-15**

‡ Results include only seroconversion panels that were tested with both Ultrio Elite and Ultrio Plus Assays.

Indicate detection of HBV DNA later than HBsAg

6. Serum and Plasma Equivalency in the Procleix® Ultrio Elite Assay

(b) (4) replicate testing per matrix was done for each matched pair of plasma and serum specimen using the appropriate HIV-1, HCV or HBV viral load quantitative assay. The calculated viral load based on the average value of the plasma and serum test results for each matched pair sample was used. A total of (b) (4) runs were completed with the Procleix® Ultrio Elite Assay. At certain dilution levels, there was insufficient discrepancy between the matrixes to apply the McNemar's test. Where the McNemar's test was applicable, it showed no significant differences between the matrices. The Fisher's Exact test was also performed to determine statistical differences for the matched sample pairs per dilution level. Where the test was applicable, the p values were above 0.05 indicating no significant differences in assay performance in the two matrices. The positive agreement between plasma and serum across all analytes at concentration levels ≥3x LOD was 99.61% (95% score CI: 98.87% - 99.87%). The Procleix® Ultrio Elite Assay exhibited comparable assay performance between plasma and serum matrix in matched clinical specimens of HIV-1, HCV, and HBV.

7. Evaluation of the Procleix® Ultrio Elite Assay as a Supplemental Test for Samples with Repeat Reactive HIV-1, HCV, or HBV Screening Serologic Test Results

Individual donations with positive, negative or indeterminate confirmatory serologic test results were tested with one master lot of reagent kits (IVD 4) on the Procleix® Panther system to evaluate the comparability of the Procleix® Ultrio Elite and the licensed confirmatory antibody assays for HIV-1, HCV, and HBsAg.

Of 59 HIV-1 confirmed seropositive samples, 56 were reactive and 3 were nonreactive in the Procleix® Ultrio Elite Assay on Panther. Of 50 serological indeterminate samples, all were nonreactive. All of the 50 confirmed seronegative samples were nonreactive. Of 61 HCV confirmed seropositive samples, 46 were reactive and 15 were nonreactive in the Procleix® Ultrio Elite Assay on the Panther. All of the 52 confirmed seronegative samples were nonreactive. Of 60 HBsAg confirmed seropositive samples, 55 were reactive and 5 were nonreactive in the Procleix® Ultrio Elite Assay on the Panther. All of the 59 confirmed seronegative samples were nonreactive. Table 14 lists

the positive and negative percent agreement with confirmatory serology results for each analyte. HIV-1, HCV, and HBV positive agreements were 94.9%, 75.4%, and 91.7%, respectively. The negative agreements for HIV-1, HCV, and HBV were 100% for all.

Thirty-six samples had Procleix® Ultrio Elite Assay and/or Procleix® Ultrio Elite Discriminatory Assay initial testing results which were unexpected based on serology. Twelve of these were seronegative or indeterminate samples which were reactive in Ultrio Elite, but not in discriminatory testing, were interpreted as nonreactive and therefore concordant with serology. Of the 24 serological discordant samples, three were seropositive HIV samples, 15 were seropositive HCV samples, five were seropositive HBV samples and one was a seronegative HBV sample.

Table 14: Comparison to HIV-1, HCV, and HBsAg Confirmatory Serology Results

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

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

[b] One sample was nonreactive in initial Ultrio Elite screening. A second aliquot was retested in Ultrio Elite and all discriminatory assays; the Ultrio Elite result was reactive but nonreactive in all discriminatory assays. These samples were included in analysis as nonreactive.

[c] Two samples were initially reactive in Ultrio Elite. A second aliquot was tested in Ultrio Elite dHIV with nonreactive results. A third aliquot was tested in Ultrio Elite and all discriminatory assays with nonreactive results. These samples were included in analysis as nonreactive.

[d] Two samples were initially reactive in Ultrio Elite but were nonreactive when a second and third aliquot were tested in dHCV, and Ultrio Elite and all discriminatory assays, respectively. Two additional samples were initially nonreactive in Ultrio Elite but were reactive when a second aliquot was tested in Ultrio Elite and dHCV. These samples were included in analysis as nonreactive.

[e] One sample was initially reactive in Ultrio Elite. A second and third aliquot were tested in Ultrio Elite dHCV and Ultrio Elite and all discriminatory assays, respectively, with all nonreactive results. These samples were included in analysis as nonreactive.

[f] One sample was initially reactive in Ultrio Elite but nonreactive when a second and third aliquot were tested in dHBV and Ultrio Elite and all the discriminatory assays respectively. These samples were included in analysis as nonreactive.

[g] One sample was reactive in two replicates in Ultrio Elite and in two replicates of Ultrio Elite dHBV, and was nonreactive in 1 replicate each of Ultrio Elite dHIV and Ultrio Elite dHCV. This sample was removed from analysis.

[h] One sample was initially reactive in Ultrio Elite but nonreactive when a second and third aliquot were tested in dHBV and Ultrio Elite and all the discriminatory assays respectively. One of the two samples was also reactive in dHCV. These samples were included in analysis as nonreactive.

8. Specificity and Sensitivity in Specimens Containing Blood Borne Pathogens Other than HIV-1/2, HCV, or HBV

(b) (4) specimens, except Dengue which was (b) (4) specimens, from each group of patients with the following viral infections were tested to evaluate the specificity and sensitivity of the Procleix® Ultrio Elite Assay in specimens from donors with infections other than HIV, HCV, or HBV, and in specimens from influenza and HBV vaccines.

- Herpes Simplex Virus 1 or 2 (HSV 1 or HSV 2)
- Human T-cell Lymphotrophic Virus Type I or II (HTLV I, HTLV II)
- Hepatitis A Virus (HAV)
- Cytomegalovirus (CMV)
- Epstein-Barr virus (EBV)
- Rubella Virus
- Parvovirus B19
- West Nile virus
- Dengue viruses (Serotypes 1-4)
- HBV and Flu vaccines receipts

The results of testing on the Panther system, using 2 reagent lots, demonstrated that the presence of other blood-borne pathogens or donor exposure to flu or HBV vaccines does not interfere with the specificity or sensitivity of the Ultrio Elite Assay. No differences were noted in the % reactivity between reagent lots. All tests were 100% specific and 100% sensitive, based on retest results, for samples containing blood-borne pathogens other than HIV-1/2, HCV, or HBV or exposed to flu or HBV vaccines.

9. Sensitivity and Specificity in Specimens from Patients with Autoimmune and Other Diseases with the Procleix® Ultrio Elite Assay

(b) (4) specimens each from HIV-1, HCV, and HBV NAT negative and HIV-1, HIV-2, HCV, and HBV serologically negative patients with autoimmune and other diseases, as well as elevated levels of various factors, were tested with the Procleix® Ultrio Elite Assay to evaluate the clinical specificity and analytical sensitivity of the Procleix® Ultrio Elite Assay in specimens from these donors:

- rheumatoid factor (RF)
- antinuclear antibody (ANA)
- lupus, multiple myeloma (MM)
- multiple sclerosis (MS)
- rheumatoid arthritis (RA)
- hyperglobulinemia (elevated IgG and/or IgM)
- alcoholic cirrhosis (AC)
- elevated alanine aminotransferase (ALT)

The results of testing on the Panther system, using two reagent lots, demonstrated that the performance of the Ultrio Elite Assay in samples from donors with autoimmune and other diseases does not interfere with the specificity or sensitivity. No differences were noted in the % reactivity between reagent lots. All tests were 100% specific and 100% sensitive, for samples from donors with autoimmune and other diseases.

This testing also demonstrated that samples from donors with autoimmune and other related disorders, particularly specimens from patients with hyperglobulinemia, were prone to induce a higher rate of magnetic wash station errors and invalids on the Panther. Due to this observed problem, there will be a warning/limitation in the labeling of the Ultrio Elite assay on the Panther, similar to the one in the FDA licensed Procleix® Ultrio assay on Tigris.

10. Specificity and Sensitivity in Specimens Contaminated with Bacteria, Yeast and Fungal Pathogens

Plasma samples from HIV-1, HCV and HBV NAT negative donors were spiked to approximately (b) (4) /mL with one of the following microorganisms:

- *Staphylococcus epidermidis*
- *Staphylococcus aureus*
- *Micrococcus luteus*
- *Corynebacterium diphtheria*

- *Propionibacterium acnes*
- *Candida albicans*
- *Pneumocystis carinii* (b) (4) nuclei/mL)

Each specimen was divided into (b) (4) groups, each group was spiked with none (control), (b) (4) copies/mL HIV-1, HIV-2, HCV, and (b) (4) /mL HBV and tested with the Procleix® Ultrio Elite Assay on the Procleix® Panther system. The results of testing on the fully automated Panther system using two reagent lots, demonstrate that the presence of bacteria, yeast or fungi in specimens does not interfere with the specificity or sensitivity of the Ultrio Elite Assay.

11. Specificity and Sensitivity in Hemolyzed, Icteric, and Lipemic Specimens

Two different panels of (b) (4) specimens (one analytical and one clinical) were tested with the Ultrio Elite Assay. The analytical panel was prepared by spiking a pool of negative human serum to target the following: (a) hemoglobin at 5,000 mg/L; (b) bilirubin at 200 mg/L; (c) lipids at 30,000 mg/L; and (d) albumin at 60 g/L. The initial concentrations of the analytical panels were slightly higher than the target concentration to account for the dilution caused by virus spiking material.

The clinical panel consisted of clinical specimens from HIV-1, HCV, and HBV serology negative patients with icteric, hemolyzed, or lipemic plasma.

Both panels were divided into several groups of (b) (4) and spiked with HIV-1, HIV-2, HCV, and HBV. Each specimen was tested with DL3 and PL 1 reagent lots of the Procleix® Ultrio Elite Assay on the Procleix® Panther system for specificity (without viruses) and sensitivity (spiked with viruses).

The Procleix® Ultrio Elite Assay was 100% specific and 100% sensitive in analytical samples containing concentrations of hemoglobin, bilirubin, albumin, and lipids when tested with PL1 and DL3 reagents on the Panther platform. The Procleix® Ultrio Elite Assay was also 100% specific and 100% sensitive in clinical specimens that contain hemolyzed, lipemic or icteric plasma when tested with PL1 and DL3 reagents on the Panther. Overall, the results from this study demonstrated that specimens containing potentially interfering substances do not affect the specificity or sensitivity of the Procleix® Ultrio Elite Assay.

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

To evaluate the specificity and sensitivity of the Procleix® Ultrio Elite Assay in specimens collected in various anticoagulants and serum, blood was collected in 11 different collection tubes:

- ACD-A (Acid Citrate Dextrose),
- K2-EDTA (ethylenediaminetetraacetic acid)
- K3-EDTA
- PPT (Plasma Preparation Tube, K2-EDTA Plasma Preparation Tube)
- Sodium citrate, (NaC)
- CPD (citrate phosphate dextrose),
- CP2D
- CPDA-1,
- Lithium heparin (LiH),
- Greiner plasma tube (Greiner, K2-EDTA Sep Tube)
- Serum collection tube (b) (4)

(b) (4) unique donor samples were collected in each of the 11 anticoagulant types and tested on the Panther System divided among the 2 lots of reagents to determine specificity. A set of (b) (4) unique donor samples collected in each of the 11 anticoagulant types were also spiked with HIV-1, HIV-2, HCV, or HBV and tested to evaluate sensitivity.

Testing of specimens collected in different anticoagulants demonstrated 100% (275/275) specificity for the Ultrio Elite Assay and was not different between the 11 anticoagulant tube types using both reagent lots.

No effect of blood collection tube type (various anticoagulants and serum) was observed in the sensitivity or specificity of the Procleix® Ultrio Elite Assay on the Panther system. No differences in assay performance were observed between the lots of reagents tested. The ten anticoagulant types and serum evaluated in this study represent a comprehensive sampling of tube types used for the collection of blood donations worldwide.

13. Specificity and Sensitivity of the Ultrio Elite Assay in Cadaveric Specimens

To evaluate specificity and sensitivity of the Procleix® Ultrio Elite Assay in cadaveric specimens, blood was collected from cadaveric donors from 0 to 24-hours post-mortem. To determine the specificity, a set of 50 cadaveric specimens (25 unique cadaveric serum specimens and 25 unique cadaveric plasma specimens) was tested with the Procleix® Ultrio Elite Assay, the Procleix® Ultrio Elite dHIV, Ultrio Elite dHCV, and Ultrio Elite dHBV Assays. To determine assay sensitivity, a set of 50 cadaveric specimens (25 unique cadaveric serum specimens and 25 unique cadaveric plasma specimens) was used for each analyte. Fifty cadaveric specimens spiked with (b) (4) c/mL HIV-1, 50 spiked with (b) (4) c/mL HIV-2, 50 spiked with (b) (4) c/mL HCV and 50 spiked with (b) (4) mL dHBV were all tested with the Ultrio Elite Assay and the discriminatory assay corresponding to the spiked analyte. The same number of normal serum and plasma specimens, spiked and unspiked, was tested as normal controls.

Tables 15-18 show that there were no differences in specificity and sensitivity of the Procleix® Ultrio Elite, dHIV, dHCV and dHBV Assays when comparing results of cadaveric specimens to control specimens. The results from testing the cadaveric plasma and serum specimens demonstrate that the Procleix® Ultrio Elite, dHIV, dHCV and dHBV specificity and sensitivity are not affected by cadaveric specimens. All observed inhibition or interference properly resulted in test invalidation by the internal control, and all specimens exhibiting inhibition or interference were successfully retested with valid results.

Table 15: Combined Summary of Specificity- Plasma

Condition	Assay	Mean S/CO	Specificity	95% CI of Specificity	# Tested
Cadaveric	Ultrio Elite	0.04	100.0%	86.3% -100.00%	25
	dHIV	0.06	100.0%	86.3% -100.0%	25
	dHCV	0.00	100.0%	86.3% -100.0%	25
	dHBV	0.00	100.0%	86.3% -100.0%	25
Control	Ultrio Elite	0.06	100.0%	86.3% -100.0%	25
	dHIV	0.07	100.0%	86.3% -100.0%	25
	dHCV	0.00	100.0%	86.3% -100.0%	25
	dHBV	0.00	100.0%	86.3% -100.0%	25

Table 16: Combined Summary of Specificity - Serum

Condition	Assay	Mean S/CO	Specificity	95% CI of Specificity	# Tested
Cadaveric	Ultrio Elite	0.04	100.0%	86.3% -100.0%	25
	dHIV	0.05	100.0%	86.3% -100.0%	25
	dHCV	0.00	100.0%	86.3% -100.0%	25
	dHBV	0.00	100.0%	86.3% -100.0%	25
Control	Ultrio Elite	0.05	100.0%	86.3% -100.0%	25
	dHIV	0.06	100.0%	92.9% -100.0%	50*
	dHCV	0.00	100.0%	86.3% -100.0%	25
	dHBV	0.00	100.0%	86.3% -100.0%	25

*N=50 due to inclusion of all specimens used in sensitivity build

Table 17: Combined Summary of Sensitivity –Plasma

Condition	Analyte	Assay	Mean S/CO	Sensitivity	95% CI of Sensitivity	# Tested
Cadaveric	HIV-1	Ultrio Elite	9.47	100.0%	86.3% -100.0%	25
		dHIV	17.29	100.0%	86.3% -100.0%	25
Control		Ultrio Elite	12.07	100.0%	86.3% -100.0%	25
		dHIV	20.23	100.0%	86.3% -100.0%	25
Cadaveric	HIV-2	Ultrio Elite	6.10	100.0%	86.3% -100.0%	25
		dHIV	10.39	100.0%	86.3% -100.0%	25
Control		Ultrio Elite	6.80	100.00%	86.3% -100.0%	25
		dHIV	11.71	100.0%	86.3% -100.0%	25
Cadaveric	HCV	Ultrio Elite	7.92	100.0%	86.3% -100.0%	25
		dHCV	21.43	100.0%	86.3% -100.0%	25
Control		Ultrio Elite	9.05	100.0%	86.3% -100.0%	25
		dHCV	23.86	100.0%	86.3% -100.0%	25
Cadaveric	HBV	Ultrio Elite	14.39	100.0%	86.3% -100.0%	25
		dHBV	23.78	100.0%	86.3% -100.0%	25
Control		Ultrio Elite	13.89	100.0%	86.3% -100.0%	25
		dHBV	24.43	100.0%	86.3% -100.0%	25

Table 18: Combined Summary of Sensitivity –Serum

Condition	Analyte	Assay	Mean S/CO	Sensitivity	95% CI of Sensitivity	# Tested
Cadaveric	HIV-1	Ultrio Elite	10.07	100.0%	86.3% -100.0%	25
		dHIV	17.29	100.0%	86.3% -100.0%	25
Control		Ultrio Elite	12.20	100.0%	86.3% -100.0%	25
		dHIV	19.41	100.0%	86.3% -100.0%	25
Cadaveric	HIV-2	Ultrio Elite	5.57	100.0%	86.3% -100.0%	25
		dHIV	9.70	100.0%	86.3% -100.0%	25
Control		Ultrio Elite	6.74	100.00%	86.3% -100.0%	25
		dHIV	11.85	100.0%	86.3% -100.0%	25
Cadaveric	HCV	Ultrio Elite	7.42	100.0%	86.3% -100.0%	25
		dHCV	21.68	100.0%	86.3% -100.0%	25
Control		Ultrio Elite	8.94	100.0%	86.3% -100.0%	25
		dHCV	23.54	100.0%	86.3% -100.0%	25
Cadaveric	HBV	Ultrio Elite	13.09	100.0%	86.3% -100.0%	25
		dHBV	21.85	100.0%	86.3% -100.0%	25
Control		Ultrio Elite	13.76	100.0%	86.3% -100.0%	25
		dHBV	24.37	100.0%	86.3% -100.0%	25

VI. CLINICAL STUDIES

A clinical trial was conducted to determine the performance characteristics of the Procleix® Ultrio Elite Assay in human plasma and serum on the Procleix® Panther System. Five studies were conducted to support the intended use of the Procleix® Ultrio Elite Assay on the Procleix® Panther System.

- Clinical Specificity
- Clinical Sensitivity (Known Positive Samples)
- Reproducibility
- HIV-1, HCV and HBV High Risk US Population
- HIV-2 High Risk Endemic Area Population

1. Clinical Specificity Study

A prospective multicenter clinical trial was conducted in the US. Three (3) blood testing sites obtained donations from affiliated US blood collection centers; 2 sites tested samples from donors of whole blood and blood components and 1 site tested samples from source plasma donors. Samples were linked to donor information to allow for donor identification, deferral, and follow-up for donors with reactive results.

Plasma from donors of whole blood and blood components was tested in 16-sample pools or individually. Plasma from source plasma donors was tested in 96-sample pools. Procleix® Ultrio Elite Assay results were compared to results observed with FDA-licensed HIV-1, HCV, and HBV assays to estimate clinical specificity (with 2-sided 95% CI). Specificity was calculated separately for individual donor samples (never pooled), 16-sample pools, and 96-sample pools.

Specificity in 16-sample pools was 100% (10,467/10,467, 95% CI: >99.9% to 100%). Specificity in individual donor samples was 100% (11,939/11,939, 95% CI: >99.9% to 100%). Specificity in 96-sample pools from source plasma donations was >99.9% (2800/2801, 95% CI: 99.8% to >99.9%) (Table 19).

Specificity estimates were similar across reagent lots.

Review Issue:

One (1) 16-sample pool tested at (b) (4) had a false negative Procleix® Ultrio Elite assay result. The 16-sample pool was Procleix® Ultrio Elite assay nonreactive and Procleix® Ultrio Plus assay reactive. One individual donor sample in the pool was Procleix® Ultrio Plus assay reactive, HBV discriminated, and had a reactive HCV serologic test result. The sample was dHBV assay reactive, and dHIV and dHCV assay nonreactive. Volume from this donation was tested with the HCV alternate NAT and had <15 IU/mL detected; the volume was insufficient for testing with the alternate HBV NAT.

Four (4) 96-sample pools had false negative Procleix® Ultrio Elite assay results when compared to the comparator assay. Grifols explained these results in the responses to FDA Information Request dated July 7, 2017 and stated that viral loads for these four specimens were close to the LOD of the corresponding analyte and the specificity of the assay was within the expected range (>99.9%).

The review committee found Grifols' s response acceptable and the issue therefore is resolved.

Table 19: Clinical Specificity of the Procleix® Ultrio Elite Assay

	N	TN	FN	TP	FP	Specificity % (95% CI)[1]
Whole Blood Neat	11941	11939	0	2	0	100 (99.9 -100)
Whole Blood 16- Sample Pool	10546	10467	1	78	0	100 (99.9 – 100)
Source Plasma 96-Sample Pool	2925	2800	4	120	1	>99.9 (99.8 – 99.9)

[1]Score C.I., FN =False Negative, FP=False Positive, TN =True Negative, TP=True Positive

2. Clinical Sensitivity Study

The Clinical Sensitivity (Known-Positive samples) Study estimated the clinical sensitivity of the Procleix® Ultrio Elite Assay and Procleix® Ultrio Elite discriminatory assays on the Procleix® Panther System in known HIV-1, HIV-2, HCV, and HBV positive plasma and serum samples. Testing was performed by three external sites using three investigational reagent kit master lots. Neat and 1:16 diluted samples made from qualified positive samples were included for testing.

All results were provided to the sponsor. The sponsor performed statistical primary analyses to calculate sensitivity estimates (with corresponding 2-sided 95% Score confidence intervals [CIs]) for samples with the following characteristics:

- ≥100 HIV-1 RNA copies/mL
- ≥30 HCV RNA IU/mL
- ≥6.25 HBV DNA IU/mL.
- ≥100 HIV-2 RNA copies/mL.

As shown in Table 20 all the samples tested in this study were reactive by the Ultrio Elite assay both neat and 1:16 dilution. The sensitivity of the assay for HIV-1, HCV and HBV was 100 % when tested neat. The sensitivity of the assay when tested at a 1:16 dilution were as follows:

- HIV-1 samples 100% (931/931, 95% CI: 99.6% to 100%).
- HCV samples 100% (985/985, 95% CI: 99.6% to 100%).
- HBV samples 99.1% (428/432, 95% CI: 97.7% to 99.8%)

Sensitivity estimates were similar across sites and reagent lots.

Table 20: Clinical Sensitivity Known –Positive Sample Study (Primary Analysis)

Sample	Neat*				1:16 Dilution*			
	N	TP	FN	Sensitivity (95% CI)	N	TP	FN	Sensitivity (95% CI)
HIV-1	931	931	0	100 (99.6-100)	764	764	0	100 (99.6-100)
HCV	985	985	0	100 (99.6-100)	959	959	0	100 (99.6-100)
HBV	503	503	0	100 (99.3-100)	432	428	4	99.1 (97.7-99.8)
Co-infected					27	27	0	100 (87.2-100)
All	2419	2419	0	100 (99.8-100)	2182	2178	4	99.8 (99.5-99.9)

*Samples with viral loads ≥100 copies/mL for HIV-1, ≥30 IU/mL for HCV, and ≥6.25 IU/mL for HBV included
CI = confidence interval; TP: true positive; FN: false negative

In addition, Sensitivity of the dHIV assay in neat samples containing HIV-1 or HIV-2, (combined plasma and serum samples) was 100% (929/929, 95% CI: 99.6% to 100%) for HIV-1 samples and 100% (26/26, 95% CI: 86.8% to 100%) for HIV-2 samples.
Sensitivity of the dHCV assay in neat samples containing HCV, combined plasma and serum samples, was 100% (980/980, 95% CI: 99.6% to 100%).
Sensitivity of the dHBV assay in neat samples containing HBV, combined plasma and serum samples, was 100% (503/503, 95% CI: 99.3% to 100%).
For the HIV-2 NAT reactive samples tested neat, sensitivity of the Procleix® Ultrio Elite assay was 100% (26/26, 95% CI: 87.1% to 100%). Sensitivity of the Procleix® Ultrio Elite assay in HIV-2 NAT reactive samples prepared 1:16 diluted was 100% (6/6).

Review Issue:

Grifols conducted the secondary analysis to estimate sensitivity of the Procleix® Ultrio Elite assay or discriminatory assay results relative to known-positive status for all qualified known positive samples, regardless of the viral load. In the midcycle review, a few issues were raised regarding the specimens with viral loads greater than the claimed LOD for each analyte but less than the Lower Limit of the Quantitation (LLOQ). In the responses to FDA Information Request dated July 6, 2017, Grifols explained that testing results with the specimens whose viral loads were less than the LLOQ would be included in the secondary analysis and it was for information only. For

some specimens, the testing results were included in the primary analysis for neat but excluded from the primary analysis when tested 1:16 dilution. Grifols also indicated that the secondary analysis would not be presented in the package insert.
The review committee found Grifols' s response acceptable and the issue therefore is resolved.

3. Reproducibility Study

The Clinical Reproducibility Study was conducted to estimate the reproducibility and repeatability of the Procleix® Ultrio Elite Assay and the dHIV, dHCV, and dHBV assays on the Procleix® Panther System. Testing was performed by three external sites. A 13-member reproducibility panel containing 1 negative panel member and 12 panel members containing HIV-1, HIV-2, HCV or HBV were tested using at least 3 investigational reagent kit master lots. At each site, 2 operators each performed 2 runs per day with each assay over at least 9 days (days did not need to be consecutive and only 1 operator performed Procleix® Ultrio Elite Assay Grifols testing each day) to obtain a total of 36 valid runs per assay over at least 18 days of testing at each site. One Procleix® Panther system was used to perform testing at each site. Each run contained 2 replicates of each reproducibility panel member (26 samples per run). Three reagent kit lots were used equally by each operator. Agreement with expected positive (reactive) or negative (nonreactive) results was calculated with 2-sided 95% confidence interval [CI]) for each panel member by site, by reagent kit lot, by operator at each site, and overall. For each panel member, results were reported using descriptive statistics of the signal to cutoff ratio (S/CO), including mean, standard deviation (SD), and coefficient of variation (CV). Variability was calculated using the random effect linear model for the following sources of variation: 1) within runs, 2) between runs, 3) between operators, 4) between sites/instruments, 5) between reagent kit lots, and 6) between days.
The total variability was determined from these 6 sources. These analyses were performed separately for each assay.

All panel members were tested with the Procleix® Ultrio Elite Assay, which detects HIV-1, HIV-2, HCV, and HBV, but does not discriminate between them. As shown in Table 21, agreement values were ≥95.3% in the negative panel members, and in the moderate positive and low positive panel members for HIV-1, HIV-2, HCV, and HBV. Eleven (11/446, 2.5%) low positive panel members (Panel Members C and L) had discordant results. High negative panel members for HIV-1, HIV-2, HCV, and HBV had negative agreement values of 56.9% (123/216; 95% CI: 50.3% to 63.4%), 38.4% (83/216; 95% CI: 32.2% to 45.1%), 46.3% (100/216; 95% CI: 39.8% to 53.0%), and 56.9% (123/216; 95% CI: 50.3% to 63.4%), respectively. This was expected as the panel members were spiked with low concentrations of each analyte (below the 95% LOD of the assay). 435 (435/446, 97.5%) high negative panel members (Panel Members B, E, H, and K) had discordant results.

Table 21: Reproducibility Study: Overall Agreement of Procleix® Ultrio Elite Assay with Expected Results

Panel Member	Description	Expected Result	Agreed/Tested	Agreement % (95% CI)
A	Negative	Nonreactive	214/214	100 (98.2-100)
B	HIV-1 High Negative	Nonreactive	123/216	56.9 (50.3-63.4)
C	HIV-1 Low Positive	Reactive	215/216	99.5 (97.4-99.9)
D	HIV-1 Moderate Positive	Reactive	216/216	100 (98.3-100)
E	HIV-2 High Negative	Nonreactive	83/216	38.4 (32.2-45.1)
F	HIV-2 Low Positive	Reactive	216/216	100 (98.3-100)
G	HIV-2 Moderate Positive	Reactive	216/216	100 (98.3-100)
H	HCV High Negative	Nonreactive	100/216	46.3 (39.8-53.0)
I	HCV Low Positive	Reactive	216/216	100 (98.3-100)
J	HCV Moderate Positive	Reactive	216/216	100 (98.3-100)
K	HBV High Negative	Nonreactive	123/216	56.9 (50-63.4)
L	HBV Low Positive	Reactive	205/215	95.4 (91.7-97.5)
M	HBV Moderate Positive	Reactive	216/216	100 (98.3-100)

4. HIV-1, HCV, and HBV High Risk Population Study

Subjects at high risk of acquiring HIV-1, HCV or HBV infection were tested to evaluate the ability of the Procleix® Ultrio Elite Assay, dHIV, dHCV and dHBV assays to detect HIV-1, HCV and/or HBV in plasma samples. Testing was performed in-house by Grifols in San Diego, CA. A total of 520 prospectively collected plasma specimens from men and women at high risk of HIV-1, HCV, and/or HBV infection but with unknown infection status were tested individually using three reagent master lots.

There were 520 samples processed in valid Procleix® Ultrio Elite, dHIV, dHCV, and dHBV assay runs: all (100%, 520/520) had final valid results for each assay.

Of the 520 samples with valid Procleix® Ultrio Elite Assay results, one sample was withdrawn because it did not meet the study inclusion criteria. In addition, one sample was excluded from the sensitivity analysis for the Procleix® Ultrio Elite Assay because the infected status for HIV-1, HCV, and HBV could not be determined. Table 22 and Table 23 show the sensitivity of the Procleix® Ultrio Elite, dHIV, dHCV, and dHBV assays and the sensitivity of the Procleix® Ultrio Elite assay system.

Review Issue:

There were four samples with false negative Procleix® Ultrio Elite Assay or one false negative dHCV assay results. The review committee raised this issue and Grifols responded to an FDA Information Request dated July 6, 2017, by stating that the additional testing for these samples indicated they contained low levels of HCV RNA. The review committee found Grifols' s response acceptable and the issue therefore is resolved.

Table 22: Clinical Sensitivity US High Risk Study: Clinical Sensitivity of the Procleix® Ultrio Elite, dHIV, dHCV, and dHBV Assays

Assay	Analyte	# Tested	TN	FP	TP	FN
Ultrio Elite	HIV-1, HCV, and/or HBV	518	417	8	89	4
dHIV	HIV-1	519	513	0	6	0
dHCV	HCV	519	420	10	88	1
dHBV	HBV	519	515	2	2	0

Table 23: The US High Risk Study: Procleix® Ultrio Elite Assay System by Virus

Analyte	# Tested	TN	FP	TP	FN
HIV-1	519	513	0	6	0
HCV	519	427	4	87	1
HBV	519	515	2	2	0

*Clopper-Pearson exact CI

5. HIV-2 Endemic Area High Risk Population Study

The HIV-2 Endemic High Risk Study was conducted to evaluate the ability of the Procleix® Ultrio Elite Assay and the dHIV assay to detect HIV-2 in plasma samples from subjects at high risk of HIV-2 infection. Testing was performed in-house by Grifols in San Diego, CA, using three reagent master lots. Samples (from subjects residing in Côte d'Ivoire with unknown infection status) were tested individually. Samples were tested with the investigational Procleix® Ultrio Elite assay and the dHIV assay on the Procleix® Panther system; samples with reactive Procleix® Ultrio Elite assay results were tested with the dHCV and dHBV assays, volume permitting. Three (3) Procleix® Ultrio Elite assay reagent kit master lots were used, with samples split approximately equally among the lots.

All results were provided to the sponsor. The sponsor performed statistical analyses. Sensitivity of the Procleix® Ultrio Elite assay and dHIV assay (with 2-sided 95% Clopper-Pearson Exact confidence intervals [CIs]) were calculated in HIV-2 seropositive samples and in HIV-2 NAT reactive samples.

All final test results were valid for the Procleix® Ultrio Elite Assay (n=520), dHIV (n=520), dHCV (n=133), and dHBV (n=133) assays. Of the 520 samples tested, 10 were withdrawn for not meeting the inclusion criteria and were not included in the sensitivity analyses.

As shown in Table 24, of the 9 HIV-2 seropositive samples, 5 were Procleix® Ultrio Elite assay reactive and HIV discriminated (sensitivity = 55.6%, 5/9). Of the 4 samples with FN Procleix® Ultrio Elite assay system results, all 4 had no or very low viral loads: 2 did not have reactive HIV-2 NAT results and 2 had viral loads below the 95% LOD. In

comparison, 2 of the 4 HIV-2 seropositive samples tested had HIV-2 NAT reactive results (sensitivity = 50.0%, 2/4).

The study results indicate that the Procleix® Ultrio Elite assay sensitivity is similar to the validated HIV-2 NAT, and that the assay is validated for the intended use.

Table 24: Clinical Sensitivity HIV-2 High Risk Study: Clinical Sensitivity of the Procleix® Ultrio Elite Assay for HIV-2 Detection

	# Tested	TP	FN
Modified CDC Algorithm Ultrio Elite System	9	5	4
Validated HIV-2 NAT Ultrio Elite Assay	4	2	2
Validated HIV-2 NAT dHIV Assay	4	2	2

VII: ADVISORY COMMITTEE MEETING

An advisory committee meeting was not needed. **OTHER RELEVANT REGULATORY ISSUES:**

Bio Monitoring Inspection:

Two clinical sites were selected for inspection based on previous inspection history, sample types tested, and protocols conducted. The following four protocols were evaluated:

B10241-UEPS-CSP-01 Clinical Specificity of the Procleix® Ultrio Elite Assay Using the Procleix® Panther System in US Whole Blood and Source Plasma Donors

B10241-UEPS-CSP-04 Clinical Sensitivity of the Procleix® Ultrio Elite Assay and Discriminatory Assays in US Subjects at High Risk for Human Immunodeficiency Virus Type 1, Hepatitis C Virus, and Hepatitis B Virus Infection

B10241-UEPS-CSP-05 Clinical Sensitivity Evaluation of the Procleix® Ultrio Elite Assay System Using the Procleix® Panther System for Detection of HIV-2 in Samples from Subjects Residing in an HIV-2 Endemic Area Who Are at High Risk of HIV-2 Infection

B10241-UEPS-CSP-06 Clinical Sensitivity Evaluation of the Procleix® Ultrio Elite Assay Using the Procleix Panther® System in Known Positive Plasma and Serum Samples (Note: Due to contamination, Protocol 03 was repeated as 06.)

Bioresearch Monitoring Inspections of two clinical investigators did not reveal substantive problems that impact the data submitted in the application.

Study Site	Location	Protocols	Form FDA 483 Issued?	Inspection Status
Hologic	San Diego, CA	B10241-UEPS-CSP-04 B10241-UEPS-CSP-05	No	No Action Indicated
Biomat	San Marcos, TX	B10241-UEPS-CSP-01 B10241-UEPS-CSP-06	No	No Action Indicated

IX: LABELING

This BLA was originally submitted by Hologic, Inc. in November 2016. Grifols Diagnostic Solutions, Inc., acquired Hologic donor screening products on February 1, 2017, including the Procleix® Ultrio Elite Assay. Grifols submitted labeling updates for the Procleix® Ultrio Elite Assay to reflect the transfer of ownership of the Procleix® Ultrio Elite Assay original BLA from Hologic, Inc., to Grifols Diagnostic Solutions, Inc. The Advertising and Promotional Labeling Branch (APLB) reviewed the Instructions for Use (IFU) and carton/container labels from a promotional and comprehension perspective and found them acceptable.

X. RECOMMENDATIONS and RISK/BENEFIT ASSESSMENT

During the mid-cycle review of this BLA, the review committee identified deficiencies in the original submission. These issues were communicated to the sponsor as Information Requests. All issues were resolved in the responses to the FDA Information Requests.

Risk/Benefit assessment:

The Procleix® Ultrio Elite Assay has very high sensitivity and specificity for the detection of HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA in plasma/serum specimens. The Limits of Detection for these different analytes for the Procleix® Ultrio Elite Assay are equivalent to or better than those for the currently licensed Procleix® Ultrio Plus. In addition, the Procleix® Ultrio Elite Assay utilizes two sets of HIV-1 primers to target two distinct genomic regions (i.e., gag and LTR) to increase the detection rate for HIV-1 positive specimens that may contain mutations in the primer/probe binding sequences of the LTR region. It is estimated that such LTR

mutations occur in approximately 1.7% of HIV-1 NAT-positive/antibody-negative donations; the estimated frequency of such a mutation is approximately 1 in 121 million blood donations in the U. S.

Compared to the Procleix® Tigris system, the Panther system is a compact instrument and therefore it is easy to access.

The benefit/risk analysis has demonstrated that the benefit of the Procleix® Ultrio Elite Assay outweighs any risk to the blood donor and the safety and availability of the nation's blood supply.

Final Review and Recommendations:

The Review Committee has reviewed the original submission and all related materials Grifols provided as amendments to the BLA. All the review issues have been resolved. The Committee recommends licensure of the Procleix® Ultrio Elite Assay.