EBV Quant Assay (Panther Fusion™)

For *in vitro* diagnostic use

For US export only

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General Information

Intended Use

The Panther Fusion™ EBV Quant assay is a fully automated real-time PCR (RT-PCR) *in vitro* nucleic acid amplification test for the quantitation of human Epstein–Barr virus (EBV) DNA in human EDTA plasma and whole blood specimens.

The Panther Fusion EBV Quant assay is intended for use to aid in the diagnosis and to aid in the management of solid-organ transplant patients and hematopoietic stem cell transplant patients.

The Panther Fusion EBV Quant assay is not intended for use as a screening assay for the presence of EBV in blood or blood products. This assay is designed for use on the Panther Fusion system.

Summary and Explanation of the Test

EBV is an ubiquitous, linear double stranded DNA virus of 172kb that belongs to the herpesvirus family. There are two main EBV genotypes, Type 1 and 2, distinguished by the differences in the EBNA-2 gene.

Following a primary infection, EBV enters the circulating B lymphocyte, and remains in a latent state thereafter. It is estimated that 90% of the population worldwide is infected with EBV.[1] In immunocompetent individuals, EBV infection can be asymptomatic during childhood. However, the EBV infection could lead to infectious mononucleosis[2] in adults and is associated with different types of cancers: lymphomas, leukemias, epithelial malignancies, and gastric cancer.[3]

In immunocompromised individuals, such as transplant recipients and individuals infected with human immunodeficiency virus (HIV), the reactivation of EBV may result in malignant lymphoproliferation and is an important cause of morbidity and mortality. The majority of these EBV-associated tumors, known as “post-transplant lymphoproliferative disease” (PTLD), often occur within the first year following transplantation.[3]

Quantitative nucleic acid amplification testing from whole blood or plasma specimens is the preferred method for monitoring of EBV infection and disease in transplant recipients because it is rapid, sensitive, convenient, and non-invasive. Recent guidelines recommend a weekly monitoring of EBV viral load to support decisions to start anti-EBV therapy and to monitor response to therapy.[4]

In general, higher viral load values are correlated with increased risk for EBV-related disease.[5] Thus, quantitation of EBV DNA in conjunction with clinical presentation and other laboratory markers is crucial in the management of patients with EBV infection.

Principles of the Procedure

The Panther Fusion system fully automates specimen processing, including cell lysis, nucleic acid capture, amplification, and detection for the Panther Fusion EBV Quant assay. The Panther Fusion EBV Quant assay targets the highly conserved EBNA-1 gene to ensure an accurate quantification of EBV DNA. The assay is standardized to the 1st WHO international standard (NIBSC code: 09/260) for EBV.[6]

Sample processing and nucleic acid capture: An internal control (IC-B) is added automatically to each specimen via the working Fusion Capture Reagent-B (wFCR-B) to monitor for interference during specimen processing, amplification, and detection caused by reagent failure or inhibitory substances. Specimens are first added to the Fusion Capture Reagent-B (FCR-B) and Fusion Enhancer Reagent-B (FER-B) to release nucleic acid for hybridization to magnetic particles. The capture particles are then separated from residual specimen matrix in a magnetic field by a series of wash steps with a mild detergent. The captured nucleic acid is then eluted from the magnetic particles with a reagent of low ionic strength (Panther Fusion Elution Buffer).

Note: *The Panther Fusion system adds the IC-B to the FCR-B. After the IC-B is added to the FCR-B, it is referred to as wFCR-B.*

PCR amplification and fluorescence detection: Lyophilized single unit dose PCR master mix is reconstituted with the Panther Fusion Reconstitution Buffer I and combined with the eluted nucleic acid into a reaction tube. Panther Fusion Oil reagent is added to prevent evaporation during the PCR reaction. PCR-based target amplification subsequently occurs with target-specific forward and reverse primers generating a fluorescence signal.

The Panther Fusion system provides a Ct value proportional to the EBV concentration in the test samples. The sample concentration is determined by the Panther Fusion system software using the EBV Ct values for each reaction and comparing them to the calibration curve. EBV results are reported in IU/mL and $\log_{10}$ IU/mL for both whole blood and plasma specimens. When the Whole Blood Conversion Factor is selected on the Panther Fusion software, a dilution factor of 4 is automatically applied to EBV viral load results to account for the dilution step during whole blood specimen processing.

The targets and the channels used for their detection on the Panther Fusion system are summarized in the table below:

Target	Gene Targeted	Instrument Channel
EBV	EBNA-1	HEX
Internal Control	Not Applicable	Quasar 705

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. Carefully read the entire package insert and the *Panther/Panther Fusion System Operator's Manual* prior to performing this assay.

D. The Panther Fusion Enhancer Reagent-B (FER-B) is corrosive, harmful if swallowed, and causes severe skin burns and eye damage.

E. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

F. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only

personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[7]

G. Use routine laboratory precautions. Do not pipet by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

H. Use only supplied or specified disposable laboratory ware.

I. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.

J. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

K. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

L. Avoid cross-contamination during the specimen handling steps. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

M. Do not use the reagents, calibrators, or controls after the expiration date. Do not use the Aptima™ Whole Blood Diluent tube after its expiration date.

N. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements* and *Panther Fusion System Test Procedure* for more information.

O. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther Fusion system verifies reagent levels.

P. Avoid microbial and nuclease contamination of reagents.

Q. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

R. Do not use the assay cartridge if the storage pouch has lost its seal or if the assay cartridge foil is not intact. Contact Hologic Technical Support if either occurs.

S. Do not use the fluid packs if the foil seal is not intact. Contact Hologic Technical Support if this occurs.

T. Handle the assay cartridges with care. Do not drop or invert assay cartridges. Avoid prolonged exposure to ambient light.

U. Some reagents of this kit are labeled with risk and safety symbols.

Note: *Hazard communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.*

EU Hazard Information	
	Panther Fusion EBV Quant Assay Cartridge ***ALPHA-CYCLODEXTRIN 20-25%*** — H412 - Harmful to aquatic life with long lasting effects P273 - Avoid release to the environment P280 - Wear eye protection/ face protection
	**Panther Fusion Oil** ***POLYDIMETHYLSILOXANE 100%*** **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation
 	**Panther Fusion Enhancer Reagent-B (FER-B)** ***LITHIUM HYDROXIDE MONOHYDRATE 5-10%*** **DANGER** H302 - Harmful if swallowed H314 - Causes severe skin burns and eye damage P260 - Do not breathe dust/fume/gas/mist/vapours/spray P280 - Wear protective gloves/protective clothing/eye protection/face protection P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P310 - Immediately call a POISON CENTER or doctor/physician

Reagent Storage and Handling Requirements

A. The following table provides storage and handling requirements for this assay.

Reagent	Unopened Storage	On Board/ Open Stability[1]	Opened Storage
Panther Fusion EBV Quant Assay Cartridge	2°C to 8°C	60 days	2°C to 8°C[2]
Panther Fusion Capture Reagent-B (FCR-B)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Enhancer Reagent-B (FER-B)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-B (IC-B)	2°C to 8°C	(In wFCR-B)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Reconstitution Buffer I	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion EBV Quant Calibrators (1-5)	-15°C to -35°C	Single use vial	Not applicable-single use
Panther Fusion EBV–BKV Quant High Positive Control	-15°C to -35°C	Single use vial	Not applicable-single use
Panther Fusion EBV–BKV Quant Low Positive Control	-15°C to -35°C	Single use vial	Not applicable-single use
Panther Fusion Transplant Negative Control (III)	-15°C to -35°C	Single use vial	Not applicable-single use

When reagents are removed from the Panther Fusion system, return them immediately to their appropriate storage temperatures.

[1] On board stability starts at the time the reagent is placed on the Panther Fusion system for the Panther Fusion EBV Quant assay cartridge, FCR-B, FER-B, and IC-B. On board stability for the Panther Fusion Reconstitution Buffer I, Panther Fusion Elution Buffer, and Panther Fusion Oil Reagent starts when the reagent pack is first used.

[2] If removed from the Panther Fusion system, store the assay cartridge in an air-tight container with desiccant at the recommended storage temperature.

B. Working Panther Fusion Capture Reagent-B (wFCR-B) and Panther Fusion Enhancer Reagent-B (FER-B) are stable for 60 days when capped and stored at 15ºC to 30ºC. Do not refrigerate.

C. Discard any unused reagents that have surpassed their stability.

D. Avoid cross-contamination during reagent handling and storage.

E. **Do not freeze reagents.**

F. **Do not re-freeze controls or calibrators.**

Specimen Collection, Processing, and Storage

Specimens – Clinical material collected from a patient and placed in an appropriate transport system. For the Panther Fusion EBV Quant assay, this includes whole blood specimens collected in tubes containing EDTA anticoagulants or plasma preparation tubes (PPTs).

Samples – Represents a more generic term to describe any material for testing on the Panther Fusion system including specimens, processed specimens transferred into a Whole Blood Diluent tube, calibrators, and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

Note: *Only plastic secondary tubes are recommended for sample storage.*

A. Specimen Collection

Whole blood specimens collected in the following glass or plastic tubes may be used to prepare plasma:

- Tubes containing EDTA anticoagulants
- Plasma preparation tubes (PPTs)

B. Specimen Processing

1. Plasma: Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Plasma can be prepared from either EDTA or PPT primary tubes. Separate the plasma from the pelleted red blood cells following the manufacturer's instructions for the tube used. Plasma can be tested on the Panther Fusion system in a primary tube or transferred to a secondary tube such as an Aptima Specimen Aliquot Tube (SAT).

   To ensure adequate sample volume, refer to the following table:

   Table 1: Minimum Sample Volume

Tube (Size and Type)	Minimum Volume for 1 Replicate
Aptima Sample Aliquot Tube (SAT)	0.6 mL
12x75 mm	0.9 mL
13x100 mm	0.9 mL
13x100 mm with Gel	0.7 mL
16x100 mm with Gel	1.1 mL

   If not tested immediately, plasma can be stored in accordance with the specifications in *Specimen Storage Conditions*. If transferred to a secondary tube, plasma may be frozen at -20°C or -70°C. Do not freeze plasma specimens in EDTA primary collection tubes.

2. Whole blood must be processed using pre-filled Whole Blood Diluent tubes before being tested on the Panther Fusion system. For more information, see *Whole Blood Specimen Handling*.

C. Specimen Storage Conditions

Specimens can be stored under one of the following conditions:

1. Plasma Stability
   - Unprocessed specimens are stable for 24 hours at 2°C to 30°C after centrifugation.
   - Unprocessed specimens are stable for 5 days at 2°C to 8°C after centrifugation.
   - Unprocessed and processed specimens are stable for 60 days at -20°C or -70°C.
2. Whole Blood Stability
   - Unprocessed specimens are stable for 36 hours at 2°C to 30°C.
   - Unprocessed specimens are stable for 5 days at 2°C to 8°C.
   - Unprocessed and processed specimens are stable for 60 days at -20°C or -70°C.

Samples Onboard the Panther Fusion System

Plasma and processed whole blood samples may be left on the Panther Fusion system uncapped for up to 8 hours. Samples may be removed from the Panther Fusion system and tested as long as the total time onboard does not exceed 8 hours prior to the pipetting of the sample by the Panther Fusion system.

Specimen Transport

Maintain specimen storage conditions during transport as described under *Specimen Collection, Processing, and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther Fusion System

The Panther Fusion system is an integrated nucleic acid testing system that fully automates all steps necessary to perform various Panther Fusion assays from sample processing through amplification, detection, and data reduction.

Reagents and Materials Provided

Assay Packaging

Components	Part No.	Storage
Panther Fusion EBV Quant Assay Calibrators PCAL 1 qEBV, 3 per box PCAL 2 qEBV, 3 per box PCAL 3 qEBV, 3 per box PCAL 4 qEBV, 3 per box PCAL 5 qEBV, 3 per box	PRD-07159	-15°C to -35°C
Panther Fusion EBV–BKV Quant Assay Controls HPC High Positive Control tube, 5 per box LPC Low Positive Control tube, 5 per box NC III Transplant Negative Control tube, 5 per box	PRD-07158	-15°C to -35°C
Panther Fusion EBV Quant Assay Cartridge 96 Tests Panther Fusion qEBV assay cartridge, 12 tests, 8 per box	PRD-07157	2°C to 8°C
Panther Fusion Internal Control-B 960 Tests Panther Fusion Internal Control-B tube, 4 per box	PRD-06234	2°C to 8°C
Panther Fusion Extraction Reagent-B 960 Tests Panther Fusion Capture Reagent-B bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-B bottle, 240 tests, 4 per box	PRD-06232	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion Reconstitution Buffer I 1920 Tests Panther Fusion Reconstitution Buffer I, 960 Tests, 2 per box	PRD-04333	15°C to 30°C
Panther Fusion Oil Reagent 1920 Tests Panther Fusion Oil Reagent, 960 tests, 2 per box	PRD-04335	15°C to 30°C

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther System	303095
Panther Fusion Module	PRD-04173
Panther Fusion System	PRD-04172
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL, filtered, liquid-sensing, conductive, and disposable: *Not all products are available in all regions. Contact your representative for region-specific information.*	901121 (10612513 Tecan) 903031(10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Panther Fusion Tube Trays, 1008 Tests, 18 trays per box	PRD-04000
Whole Blood Diluent tubes (for processing whole blood specimens only)	PRD-06783 (100 pre-filled tubes per bag)
Replacement Hologic Solid Caps (single-use tube cap)	PRD-06720 (100 caps per bag)
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable powderless gloves	—
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Primary collection tubes (EDTA and PPT) options: 13 mm x 100 mm 12 mm x 75 mm 16 mm x 100 mm	—
Centrifuge	—
Vortex mixer	—

*Needed only for Panther Aptima TMA assays.

Optional Materials

Material	Cat. No.
Secondary tube options: 12 mm x 75 mm 13 mm x 100 mm 16 mm x 100 mm	 — — —
Aptima Specimen Aliquot Tubes (SATs) (100 pack)	503762
Transport tube cap (100 pack) *cap for SAT*	504415
Aptima Specimen Diluent	PRD-03003
Aptima Specimen Diluent Kit *contains Aptima Specimen Diluent, 100 SATs and 100 caps*	PRD-03503
Transfer pipets	—
Tube rocker	—

Panther Fusion System Test Procedure

Note: *Refer to the Panther/Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (Step A.1).
3. Clean any pipettors. Use the cleaning procedure described above (Step A.1).

B. Calibrators and Controls Preparation

Allow the calibrators and controls to reach 15°C to 30°C prior to processing as follows:

1. Remove the calibrators and controls from storage (-15°C to -35°C) and place at 15°C to 30°C. Throughout the thawing process, gently invert each tube to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Option. Calibrator and control tubes may be placed on a tube rocker to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Note: *Avoid creating excessive foam when inverting the calibrators and controls. Foam compromises the level-sensing by the Panther Fusion system.*
2. When the tube contents have thawed, dry the outside of the tube with a clean, dry disposable wipe.
3. To prevent contamination, do not open the tubes at this time.

C. Reagent Preparation

1. Remove the bottles of IC-B, FCR-B, and FER-B from storage.
2. Mix FCR-B until the beads are fully suspended. Avoid creating foam during this step.
3. Open the bottles of IC-B, FCR-B, and FER-B, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion system.
4. Place the IC-B, FCR-B, and FER-B bottles in the appropriate positions on the TCR carousel.
5. Close the TCR door.

Note: *The Panther Fusion system adds the IC-B to the FCR-B. After the IC-B is added to the FCR-B, it is referred to as wFCR-B (working FCR-B). If the wFCR-B and FER-B are removed from the system, use new caps and immediately store according to the proper storage conditions.*

D. Specimen Handling

Note: *Prepare specimens per the instructions in the Specimen Collection, Processing, and Storage section before loading specimens onto the Panther Fusion system.*

Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

E. Plasma Specimen Handling

1. Ensure that processed specimens in primary tubes or undiluted specimens in secondary tubes are stored properly per *Specimen Collection, Processing, and Storage*.
2. Ensure frozen specimens are thoroughly thawed. Vortex the thawed specimens for 3 to 5 seconds to mix thoroughly.
3. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther Fusion System* for additional onboard information.
4. Ensure each primary or secondary tube contains adequate specimen. Refer to Table 1 for minimum sample volume for 1 replicate.
5. Just prior to loading specimens into a Sample Rack, centrifuge each specimen at 1000 to 3000*g* for 10 minutes. Do not remove caps at this step.

   See Step G.2 below, for information about loading the rack and removing the caps.

F. Whole Blood Specimen Handling

1. Ensure that specimens in primary tubes are stored properly per *Specimen Collection, Processing, and Storage*.
2. Ensure frozen specimens are thoroughly thawed. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther Fusion System* for additional onboard information.
3. Gently invert whole blood tubes at least 3 times, or mix gently on a rocker, until blood is homogeneous.
4. Before loading on the system, perform the following procedure on each specimen.
   a. Blood in the primary tubes should be mixed thoroughly by inversion and the sample should be immediately transferred into the tube containing whole blood diluent.
   b. Add 500 µL whole blood specimen to the pre-filled Whole Blood Diluent tube.

c. Replace the cap and vortex the sample for at least 5 seconds.

See Step G.2 below, for information about loading the rack and removing the caps.

G. System Preparation

1. For instructions on setting up the Panther Fusion system including loading samples, reagents, assay cartridges and universal fluids, refer to the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes.*
2. Load samples into the Sample Rack. Perform the following steps for each sample tube (specimen, and, when necessary, calibrators, and controls):
   a. Loosen one sample tube cap, but do not remove it yet.

      Note: *Be especially careful to avoid contamination by the spread of aerosols. Gently loosen caps on samples.*

   b. Load the sample tube into the Sample Rack.
   c. Repeat Steps 2.a and 2.b for each remaining sample.
   d. After the samples have been loaded into the Sample Rack, remove and discard each sample tube cap in one Sample Rack. To avoid contamination, do not pass a cap over any other Sample Racks or sample tubes.
   e. If necessary, use a new, disposable transfer pipet to remove any bubbles or foam. Bubbles in the tube compromise the level-sensing by the Panther Fusion system.
   f. When the last cap has been removed, load the Sample Rack into the Sample Bay.

      Note: *If running other assays and sample types at the same time, secure the Sample Retainer prior to loading the Sample Rack into the Sample Bay.*

   g. Repeat Steps 2.a to 2.f for the next Sample Rack.

H. System Preparation: Applying the Whole Blood Specimen Conversion Factor

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual.*
2. Load specimen rack.
3. Apply Whole Blood Conversion Factor to assay test orders for whole blood specimens.

   Note: *Whole Blood Conversion Factor may be applied to an entire rack or a single test order.*

   To apply the Whole Blood Conversion Factor to an entire rack of whole blood specimens:

   a. From the *Sample Rack Bay* screen, double-click the loaded rack of interest. The *Sample Rack Loading* screen appears for the selected rack.
   b. Select **Dilute All**.

The *Dilution Factor* window appears (Figure 1).

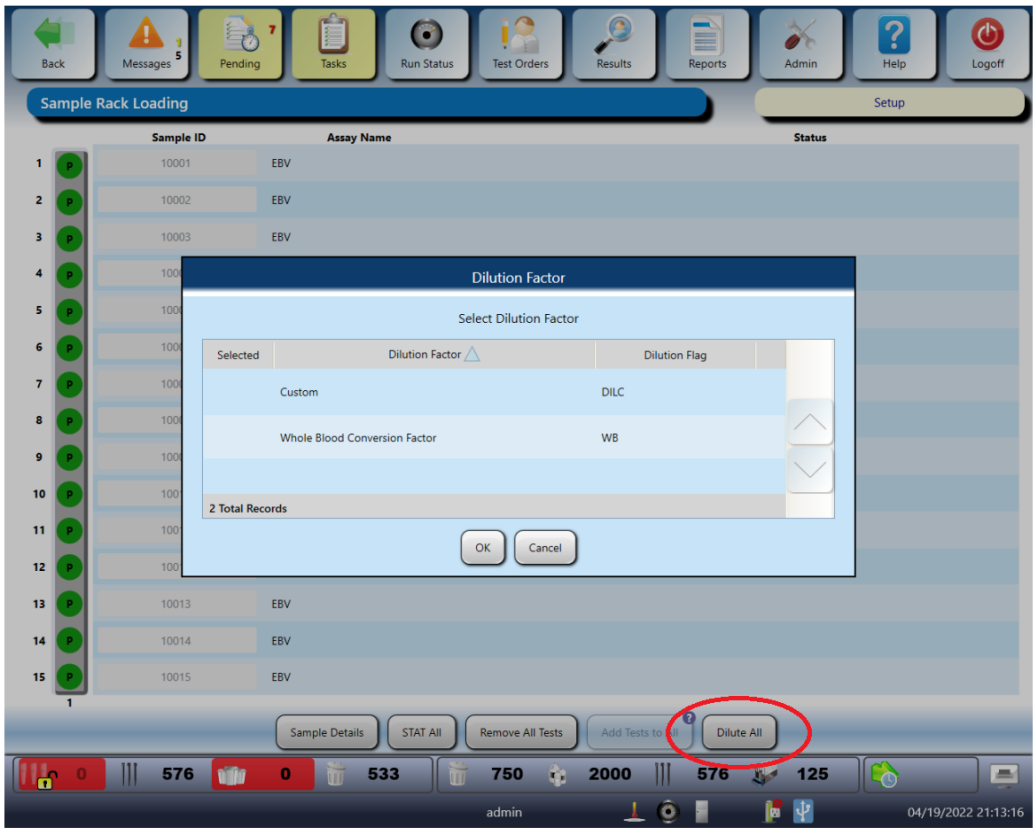

Figure 1. The Dilution Factor Window in the Sample Rack Loading Screen (Example)

c. Select **Whole Blood Conversion Factor**.

d. Select **OK.**

A *Set Dilution Factor for Rack* window appears.

e. Select **Yes** to apply the Whole Blood Conversion Factor flag to the entire rack of whole blood specimens.

To apply the Whole Blood Conversion Factor to a single test order (see Figure 2):

a. From the *Sample Rack Bay* screen, double-click the loaded rack with the specimen(s) of interest.

The *Sample Rack Loading* screen appears for the selected sample rack.

b. From the *Sample Rack Loading* screen, double-click the specimen of interest.

The *Sample Details* screen appears with the current test orders for the selected specimen.

c. Select the test order of interest from the *Test Orders* panel.

d. Select **Apply Dilution**.

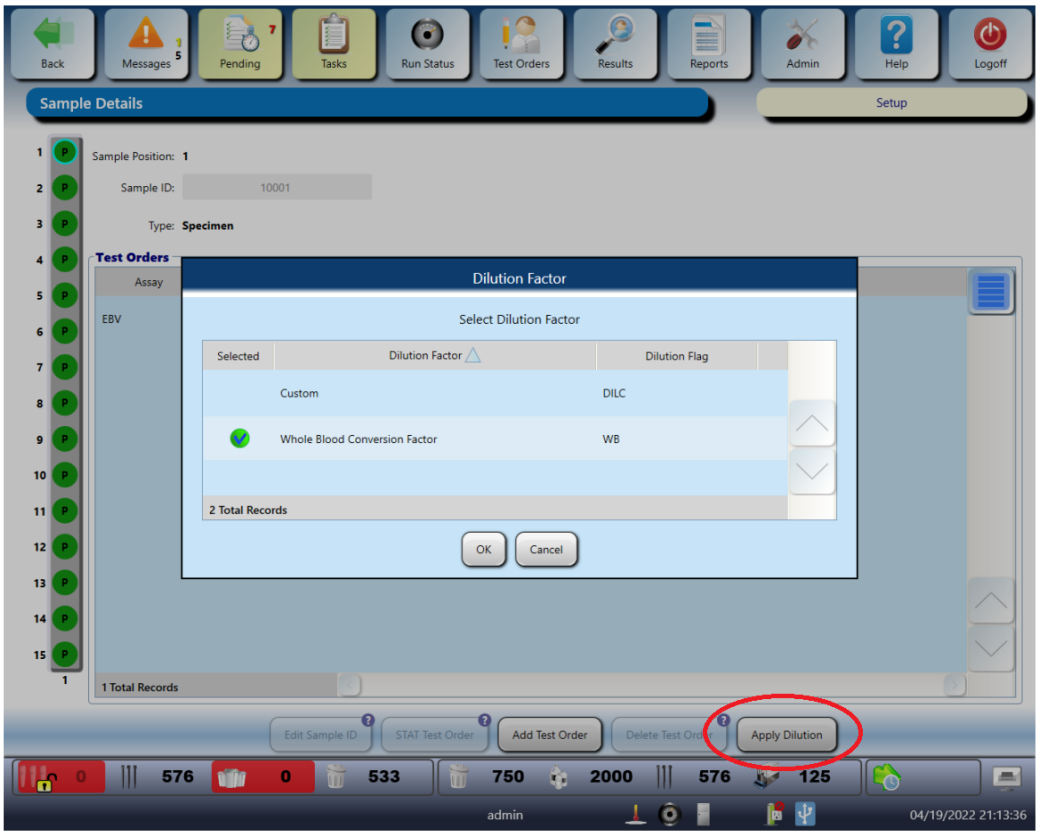

Figure 2. The Dilution Factor Window in the Sample Details Screen (Example)

e. Select **Whole Blood Conversion Factor**.

f. Select **OK** to apply the Whole Blood Conversion Factor flag to all selected test orders.

4. If necessary, the Whole Blood Factor can be removed from test orders prior to the start of processing.

To delete the Whole Blood Conversion Factor from an entire rack:

1. From the *Sample Rack Bay* screen, double-click the loaded rack of interest.

   The *Sample Rack Loading* screen appears for the selected rack.

2. Select **Dilute All**.
3. From the *Dilution Factor* window, de-select **Whole Blood Conversion Factor**.
4. Select **OK.**

   A *Set Dilution Factor for Rack* window appears.

5. Select **Yes** to delete the Whole Blood Conversion Factor from an entire rack.

To delete the Whole Blood Conversion Factor assay test orders:

1. From the *Sample Rack Bay* screen, double-click the loaded rack with the specimen(s) of interest.

   The *Sample Rack Loading* screen appears for the selected sample rack.

2. From the *Sample Rack Loading* screen, double-click the specimen of interest.

   The *Sample Details* screen appears with the current test orders for the selected specimen.
3. Select the test order of interest from the *Test Orders* panel.
4. Select **Apply Dilution**.
5. From the *Dilution Factor* window, de-select **Whole Blood Conversion Factor**.
6. Select **OK** to delete the Whole Blood Conversion Factor from the test order.

Procedural Notes

A. Calibrators and Controls

1. The qEBV calibrators (5 tubes), the EBV–BKV low positive control (LPC), the EBV–BKV high positive control (HPC), and the Transplant negative control (NC III) tubes can be loaded in any position in the Sample Rack and in any Sample Bay lane on the Panther Fusion system. Calibrator and control pipetting will begin when EBV specimens have been loaded onto the system. Specimen pipetting will begin when one of the following two conditions has been met:
   a. The calibrators and controls are currently being processed by the system.
   b. Valid results for the calibrators and controls are registered on the system.
2. After the calibrator and control tubes have been pipetted and are being processed for the Panther Fusion EBV Quant assay, specimens can be tested. Calibration results are valid for 60 days and control results are valid for up to 30 days (frequency configured by an administrator) **unless:**
   a. The calibrator results are invalid.
   b. The control results are invalid.
   c. The operator requests to run new controls/calibrators in Panther Fusion system software.
3. A calibration is required for each new assay cartridge lot that is loaded onto the Panther Fusion system prior to using it for specimen processing.
4. Each calibrator and each control tube can be used once.

Quality Control

Assay Calibration

To generate valid results, assay calibration must be completed. The five positive calibrators are run in triplicate each time a new assay cartridge lot is loaded on the Panther Fusion system. Once established, the assay calibration is valid for up to 60 days. Software on the Panther Fusion system alerts the operator when calibration is required.

During processing, the Panther Fusion software automatically verifies the validity of the calibration curve. If the calibration fails the validity checks, the Panther Fusion system automatically invalidates any affected samples, and will require a new set of assay calibrators to be run prior to pipetting any additional samples.

By default, the assay will process samples as undiluted plasma. To process whole blood samples, the Whole Blood Conversion Factor dilution must be selected from the instrument user interface.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the NC III (transplant negative control), the LPC (low positive control), and the HPC (high positive control) must be tested each time a new lot of assay cartridges is loaded on the Panther Fusion system or when the current set of valid controls for an active cartridge lot has expired.

The Panther Fusion system is configured to require assay controls run at an administrator-specified interval of up to 30 days. Software on the Panther Fusion system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther Fusion system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther Fusion system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther Fusion system and a new set of assay controls will be required prior to pipetting any new samples.

If any of the assay controls fails the validity checks, the Panther Fusion system automatically invalidates the affected samples, and a new set of assay controls will be required prior to pipetting any additional samples.

Internal Control

An internal control is added to each sample during the extraction process. During processing, the internal control acceptance criteria are automatically verified by the Panther Fusion system software. Detection of the internal control is not required for samples that are positive for EBV. The internal control must be detected in all samples that are negative for EBV; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther Fusion system software is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/Panther Fusion System Operator's Manual*.

Interpretation of Results

The Panther Fusion system automatically determines the concentration of EBV DNA for specimens and controls by comparing the results to a calibration curve. EBV DNA concentrations are reported in IU/mL and $\log_{10}$ IU/mL. The interpretation of results is provided in Table 2 and Table 3.

Table 2: Plasma Result Interpretation

Reported EBV Quant Assay Results		
IU/mL	**$\log_{10}$ Value**	**Interpretation**
Not Detected	Not Detected	EBV DNA not detected.
< 120 detected	< 2.08	EBV DNA is detected but at a level below the lower limit of quantification (LLoQ).
120 to 1.50E09	2.08 to 9.18	EBV DNA concentration is within the quantitative range between LLoQ to ULoQ IU/mL.
> 1.50E09	> 9.18	EBV DNA concentration is above the upper limit of quantification (ULoQ).
Invalid[a]	Invalid[a]	There was an error in the generation of the result. Specimen should be retested.

[a] Invalid results are displayed in blue-colored font.

Table 3: Whole Blood Result Interpretation

Reported EBV Quant Assay Results		
IU/mL	**$\log_{10}$ Value**	**Interpretation**
Not Detected	Not Detected	EBV DNA not detected.
< 350 detected	< 2.54	EBV DNA is detected but at a level below the lower limit of quantification (LLoQ).
350 to 6.0E09	2.54 to 9.78	EBV DNA concentration is within the quantitative range between LLoQ to ULoQ IU/mL.
> 6.0E09	> 9.78	EBV DNA concentration is above the upper limit of quantification (ULoQ).
Invalid[a]	Invalid[a]	There was an error in the generation of the result. Specimen should be retested.

[a] Invalid results are displayed in blue-colored font.

Limitations

A. Use of this assay is limited to personnel who are trained in this procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. Though rare, mutations within the highly conserved regions of the viral genome covered by the primers and/or probes in the Panther Fusion EBV Quant assay may result in under quantification of or failure to detect the virus.

E. Negative results do not preclude EBV infections and should not be used as sole basis for treatment or other management decisions.

F. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

Performance

Limit of Detection Using the 1st WHO International Standard

The limit of detection (LoD) of the assay is defined as the concentration of EBV DNA that is detected at 95% or greater probability according to CLSI EP17-A2.[8]

Limit of Detection Using WHO Standards in Plasma

The LoD was determined by testing panels of the 1st WHO International Standard (NIBSC code 09/260) for EBV diluted in EBV-negative human plasma. Twenty (20) replicates of each dilution were tested with each of three reagent lots for a total of 60 replicates per dilution. Probit analysis was performed to generate the predicted detection limits. The LoD values shown in Table 4 are the results from the reagent lot with the highest predicted detection limit. The LoD for the Panther Fusion EBV Quant assay using the 1st WHO International Standard is 54.1 IU/mL for plasma.

Table 4: Limit of Detection for Plasma Using the 1st WHO International Standard for EBV

Predicted Detection Limit	Concentration (IU/mL)
10%	1.5
20%	2.4
30%	3.6
40%	5.1
50%	7.2
60%	10.2
70%	14.8
80%	22.4
90%	38.0
95%	54.1

Limit of Detection Using WHO Standards in Whole Blood

The LoD was determined by testing panels of the 1st WHO International Standard for EBV diluted in EBV-negative whole blood. Twenty (20) replicates of each dilution were tested with each of three reagent lots for a total of 60 replicates per dilution. Probit analysis was performed to generate the predicted detection limits. The LoD values shown in Table 5 are the results from the reagent lot with the highest predicted detection limit. The LoD for the Panther Fusion EBV Quant assay using the 1st WHO International Standard is 200.9 IU/mL for whole blood.

Table 5: Limit of Detection for Whole Blood Using the 1st WHO International Standard for EBV

Predicted Detection Limit	Concentration (IU/mL)
10%	4.9
20%	6.7
30%	9.1
40%	12.6
50%	17.8
60%	26.1
70%	40.2
80%	67.0
90%	129.6
95%	200.9

Linear Range

Linear Range in Plasma

The linear range was established by testing panels of EBV diluted in EBV-negative human plasma according to CLSI EP06-A.[9] Panels ranged in concentration from 1.98 log IU/mL to 9.26 log IU/mL. The Panther Fusion EBV Quant assay demonstrated linearity across the range tested. The upper limit of quantitation (ULoQ) of the assay is 9.18 log IU/mL as shown in Figure 3.

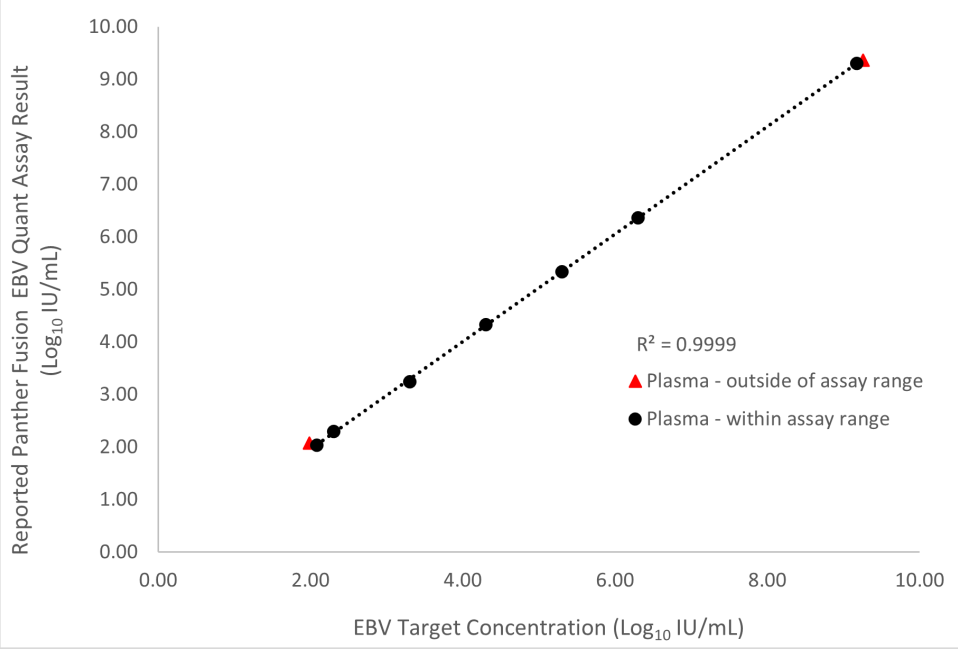

Figure 3. Linearity in Plasma

Linear Range in Whole Blood

The linear range was established by testing panels of EBV diluted in EBV-negative human whole blood according to CLSI EP06-A.[9] Panels ranged in concentration from 2.45 log IU/mL to 9.86 log IU/mL. The Panther Fusion EBV Quant assay demonstrated linearity across the range tested. The upper limit of quantitation (ULoQ) of the assay is 9.78 log IU/mL as shown in Figure 4.

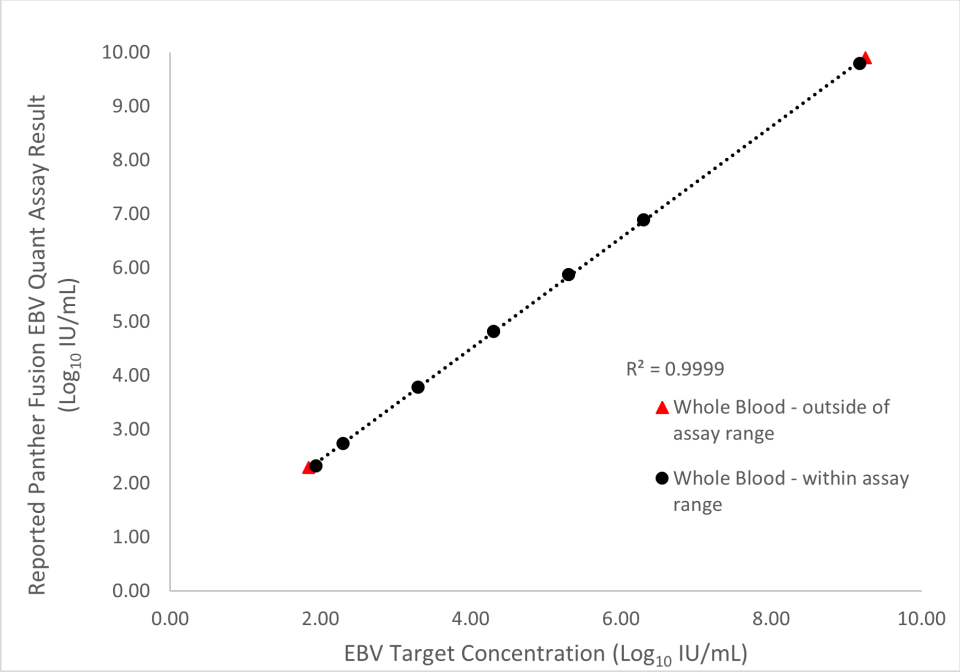

Figure 4. Linearity in Whole Blood

Lower Limit of Quantitation Using the 1st WHO International Standard

The lower limit of quantitation (LLoQ) is defined as the lowest concentration at which EBV is reliably quantitated, according to CLSI EP17-A2.[8] Total error was estimated using the Westgard Model: Total Error (TE) = |bias| + 2 SD. To ensure precision and accuracy of the measurements, the Total Error of the Panther Fusion EBV Quant assay was set at 1.2 log IU/mL, with a bias to the truth and a SD that must be ≤ 0.5 log IU/mL and ≤ 0.35 log IU/mL, respectively.

Lower Limit of Quantitation Using the WHO Standard in Plasma

The LLoQ was determined by testing panels of the 1st WHO International Standard (NIBSC code 09/260) for EBV diluted in EBV-negative human plasma. Twenty (20) replicates of each dilution were tested with each of three reagent lots for a total of 60 replicates per dilution. The LLoQ results for the three reagent lots are shown in Table 6. The LLoQ generated with the 1st WHO International Standard for EBV in plasma is 120 IU/mL (2.08 log IU/mL).

Table 6: Determination of LLoQ Using the 1st WHO International Standard for EBV Diluted in Plasma

Reagent Lot	N	N Detected	Target Concentration (log IU/mL)	EBV Quant Assay (log IU/mL)	SD (log IU/mL)	|Bias| (log IU/mL)	Calculated TE (log IU/mL
1	20	20	1.93	2.06	0.21	0.2	0.6
	20	20	2.08	2.33	0.21	0.3	0.7
	20	20	2.18	2.45	0.13	0.3	0.5
	20	20	2.26	2.44	0.15	0.2	0.5
2	20	20	1.93	1.61	0.30	0.3	0.9
	20	20	2.08	1.79	0.23	0.3	0.8
	20	20	2.18	2.00	0.18	0.2	0.6
	20	20	2.26	2.09	0.17	0.2	0.5
3	20	20	1.93	1.75	0.20	0.2	0.6
	20	20	2.08	1.88	0.25	0.2	0.7
	20	20	2.18	2.09	0.12	0.1	0.4
	20	20	2.26	2.04	0.15	0.2	0.5

SD=standard deviation ≤ 0.35 (log IU/mL).

|Bias|=bias to the truth ≤ 0.5 (log IU/mL).

The dilution corresponding to the LLoQ concentration and tested on each reagent lot is highlighted in gray.

Lower Limit of Quantitation Using the WHO Standard in Whole Blood

The LLoQ was determined by testing panels of the 1st WHO International Standard (NIBSC code 09/260) for EBV diluted in EBV-negative human whole blood. Twenty (20) replicates of each dilution were tested with each of three reagent lots for a total of 60 replicates per dilution. The LLoQ results for the three reagent lots are shown in Table 7. The LLoQ generated with the 1st WHO International Standard for EBV in whole blood is 350 IU/mL (2.54 log IU/mL).

Table 7: Determination of LLoQ Using the 1st WHO International Standard for EBV Diluted in Whole Blood

Reagent Lot	N	N Detected	Target Concentration (log IU/mL)	EBV Quant Assay (log IU/mL)	SD (log IU/mL)	|Bias| (log IU/mL)	Calculated TE (log IU/mL
1	20	18	2.40	2.48	0.30	0.3	0.9
	20	20	2.48	2.40	0.33	0.3	0.9
	20	20	2.54	2.50	0.30	0.2	0.8
	20	20	2.62	2.51	0.34	0.3	1.0
2	20	19	2.40	1.94	0.39	0.5	1.2
	20	20	2.48	1.94	0.36	0.5	1.3
	20	20	2.54	2.06	0.26	0.5	1.0
	20	19	2.62	2.16	0.19	0.5	0.9
3	20	20	2.40	1.84	0.48	0.60	1.5
	20	19	2.48	1.90	0.32	0.60	1.2
	20	20	2.54	2.12	0.26	0.40	0.9
	20	20	2.62	2.25	0.29	0.40	1.0

SD=standard deviation ≤ 0.35 (log IU/mL).
|Bias|=bias to the truth ≤ 0.5 (log IU/mL).
The dilution corresponding to the LLoQ concentration and tested on each reagent lot is highlighted in gray.

Confirmation of the Lower Limit of Quantitation Across EBV Genotypes

Lower Limit of Quantitation Across Genotypes in Plasma

The LLoQ established using the WHO standard was assessed by testing EBV genotypes 1 (Raji, Akata and B95-8) and 2 (AG876, P3H1 and Jijoye) spiked at 3X the LLoQ in EBV-negative human plasma. Three replicates of each panel member were tested with one reagent lot. The results are shown in Table 8.

Table 8: Confirmation of LLoQ Across Genotypes in Plasma

Isolate (Genotype)	N	N Detected	Target Concentration (log IU/mL)	EBV Quant Assay (log IU/mL)	SD (log IU/mL)	|Bias| (log IU/mL)
Raji (genotype 1)	3	3	2.56	2.84	0.12	0.3
Akata (genotype 1)	3	3	2.56	2.95	0.11	0.4
B95-8 (genotype 1)	3	3	2.56	2.59	0.08	0.1
AG876 (genotype 2)	3	3	2.56	2.72	0.23	0.2
P3H1 (genotype 2)	3	3	2.56	2.91	0.07	0.4
Jijoye (genotype 2)	3	3	2.56	2.75	0.16	0.2

SD=standard deviation.

Lower Limit of Quantitation Across Genotypes in Whole Blood

The LLoQ established using the WHO standard was assessed by testing EBV genotypes 1 (isolates Raji, Akata and B95-8) and genotype 2 (isolates AG876, P3H1 and Jijoye) spiked at 3X the LLoQ in EBV-negative human whole blood. Three replicates of each panel member were tested with one reagent lot. The results are shown in Table 9.

Table 9: Confirmation of LLoQ Across Genotypes in Whole Blood

Isolate (Genotype)	N	N Detected	Target Concentration (log IU/mL)	EBV Quant Assay (log IU/mL)	SD (log IU/mL)	|Bias| (log IU/mL)
Raji (genotype 1)	3	3	3.02	3.12	0.06	0.1
Akata (genotype 1)	3	3	3.02	2.95	0.05	0.1
B95-8 (genotype 1)	3	3	3.02	3.09	0.11	0.1
AG876 (genotype 2)	3	3	3.02	3.14	0.08	0.1
P3H1 (genotype 2)	3	3	3.02	3.45	0.10	0.4
Jijoye (genotype 2)	3	3	3.02	3.01	0.24	0.2

SD=standard deviation.

Traceability to the 1st WHO International Standard

A series of secondary standards with known concentrations were used throughout product development and product manufacturing to establish traceability to the WHO standard. The EBV 1st WHO standard was diluted and tested along with the secondary standards, as well as assay controls, and calibrators used in the Panther Fusion EBV Quant assay to evaluate traceability according to CLSI EP32-R.[10] The secondary standards ranged in concentration from 2.30 to 6.30 $\log_{10}$ IU/mL.

Traceability to the WHO Standard Using Plasma

The concentrations tested for the EBV 1st WHO standard were between 2.26 and 4.70 $\log_{10}$ IU/mL. The WHO plasma panels, secondary standards, assay controls, and assay calibrators recovered as expected across the linear range of the assay, as can be seen from Figure 5.

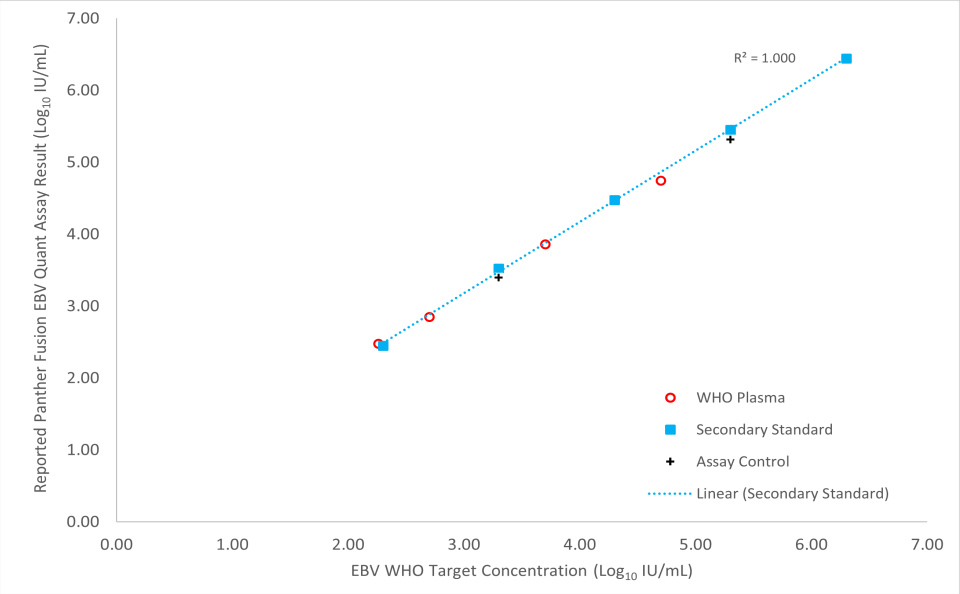

Figure 5. Traceability Between the EBV 1st WHO Standard Target Concentrations and Reported Concentrations in the Panther Fusion EBV Quant Assay (WHO Standard Diluted in Plasma)

Traceability to the WHO Standard Using Whole Blood

The concentrations tested for the EBV 1st WHO standard in whole blood were between 2.72 and 5.30 $\log_{10}$ IU/mL. The WHO whole blood panels, secondary standards, assay controls, and assay calibrators recovered as expected across the linear range of the assay, as can be seen from Figure 6.

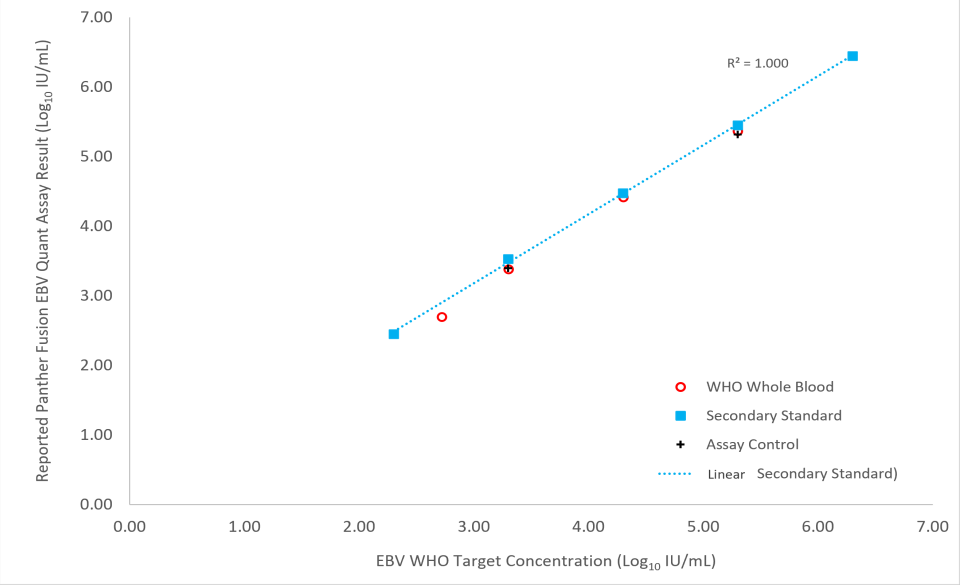

Figure 6. Traceability Between the EBV 1st WHO Standard Target Concentrations and Reported Concentrations in the Panther Fusion EBV Quant Assay (WHO Standard Diluted in Whole Blood)

Within Lab Precision

To assess within lab precision, a 3-member positive panel was made by diluting EBV DNA into EBV-negative whole blood. The positive panel and a negative whole blood sample were tested by 2 operators using 3 reagent lots on 3 Panther Fusion systems over 6 non-consecutive test days. Each operator performed 2 runs per day and each panel member was tested in triplicate in each run. The study was designed and analyzed following the recommendations of CLSI EP-05-A3.[11]

Table 10 shows the reproducibility of assay results (in log IU/mL) for the positive panel between instruments, operators, cartridge lots, runs, days, within runs, and overall. Total variability was primarily due to inter-lot variability. All replicates of the negative sample were negative.

Table 10: Reproducibility of the Panther Fusion EBV Quant Assay in Whole Blood

N	Mean Concentration (log IU/mL)	Inter-Lot	Inter-Instrument	Inter-Operator	Inter-Day	Inter-Run	Intra-Run	Total
		SD	SD	SD	SD	SD	SD	SD
52	2.96	0.17	0.04	0.05	0.06	0.12	0.13	0.20
52	4.00	0.15	0.02	0.04	0.04	0.10	0.06	0.15
52	5.03	0.15	0.01	0.05	0.03	0.13	0.04	0.13

SD=standard deviation.

Out of N=54, two (2) replicates of each panel were invalid and not retested due to insufficient volume.

Potentially Interfering Substances

The susceptibility of the Panther Fusion EBV Quant assay to interference by elevated levels of endogenous substances, anticoagulants, and drugs commonly prescribed to transplant patients was evaluated in EBV-negative matrices in the presence or absence of 2.56 log IU/mL and 3.02 log IU/mL of EBV in plasma and whole blood, respectively. The test concentrations for each of the interfering substances were selected based on available literature references and guidance provided by CLSI EP07[12] and EP37.[13]

No interference in the accuracy of quantification of the assay was observed in plasma or in whole blood samples in the presence of potential interfering endogenous substances listed in Table 11.

Table 11: Endogenous Substances

Potential Interfering Substance	Number of Replicates	Tested Concentration
Albumin	3	375 mg/dL
Conjugated bilirubin	3	40 mg/dL
Hemoglobin	3	1000 mg/dL
Heparin	3	0.66 mg/dL
Human Genomic DNA	3	0.2 mg/mL
Triglycerides	3	3.45 mg/dL
Unconjugated bilirubin	3	0.40 mg/dL

No interference in the accuracy of quantification of the assay was observed in the presence of the exogenous substances listed in Table 12.

Table 12: Exogenous Substances

Potential Interfering Substance	Number of Replicates	Tested Concentration
Acyclovir	3	6.6 mg/dL
Azathioprine	3	0.258 mg/dL
Cefotetan	3	71.1 mg/dL
Cidofovir	3	12.4 mg/dL
Clavulanate potassium	3	1.47 mg/mL
Cyclosporine	3	0.180 mg/dL
EDTA	3	0.099 mg/dL
Everolimus	3	0.0183 mg/dL
Fluconazole	3	2.55 mg/dL
Foscarnet	3	108 mg/dL
Ganciclovir	3	3.96 mg/dL
Letermovir	3	3.9 mg/dL
Mycophenolate mofetil	3	18.1 mg/dL
Mycophenolic acid	3	18.1 mg/dL
Piperacillin	3	110 mg/dL
Prednisone	3	0.0099 mg/dL
Sirolimus	3	0.0213 mg/dL
Sodium citrate	3	3200 mg/dL
Sulfamethoxazole	3	35.7 mg/dL
Tacrolimus	3	0.0144 mg/dL
Tazobactam sodium	3	10.2 mg/dL
Tenofovir disoproxil fumarate	3	0.0978 mg/dL
Ticarcillin disodium	3	151 mg/dL
Trimethoprim	3	4.2 mg/dL
Valacyclovir	3	3.83 mg/dL
Valganciclovir	3	4.83 mg/dL
Vancomycin	3	12 mg/dL

Analytical Specificity

Potential cross-reactivity to the pathogens listed in Table 13 was evaluated in EBV-negative matrices in the presence or absence of 2.56 log IU/mL and 3.02 log IU/mL of EBV in plasma and whole blood, respectively. Pathogens were tested at the highest concentration available. No cross-reactivity or interference in the accuracy of quantification was observed.

Table 13: Pathogens Tested for Analytical Specificity

Microorganism/Pathogen	Concentration	Microorganism/Pathogen	Concentration
ADV-4	1.00E+04 $TCID_{50}$/mL	Human Parainfluenza virus	1.00E+05 IU/mL
Aspergillus niger	1.00E+06 CFU/mL	Influenza A	1.00E+05 IU/mL
BKV	5.00E+06 cp/mL	*Klebsiella pneumoniae*	1.00E+06 cp/mL
Candida albicans	1.00E+06 CFU/mL	*Listeria monocytogenes*	1.00E+06 CFU/mL
Chlamydia trachomatis	1.00E+06 IFU/mL	*Mycoplasma pneumoniae*	1.00E+06 CCU/mL
Clostridium perfringens	1.00E+06 cp/mL	*Mycobacterium intracellulare*	1.00E+06 cp/mL
CMV	1.00E+05 cp/mL	*Neisseria gonorrhoeae*	1.00E+06 CFU/mL
Corynebacterium diphtheriae	1.00E+06 CFU/mL	*Propionibacterium acnes*	1.00E+06 CFU/mL
Cryptococcus neoformans	1.00E+06 CFU/mL	Rhinovirus	1.00E+06 cp/mL
Enterococcus faecalis	1.00E+06 CFU/mL	*Salmonella enterica*	1.00E+06 CFU/mL
Escherichia coli	1.00E+06 CFU/mL	*Staphylococcus aureus*	1.00E+06 CFU/mL
HBV	1.00E+05 IU/mL	*Staphylococcus epidermidis*	1.00E+06 CFU/mL
HCV	1.00E+04 IU/mL	*Streptococcus agalactiae*	1.00E+06 CFU/mL
HIV-1	1.00E+05 IU/mL	*Streptococcus pneumoniae*	1.00E+06 CFU/mL
HPV-18 (HeLa cells infected)	1.00E+05 cells/mL	*Streptococcus pyogenes*	1.00E+06 CFU/mL
Human Herpes Virus 6	1.00E+05 cp/mL	*Trichomonas vaginalis*	1.00E+05 Trophozoites/mL
Human Herpes Virus 7	1.00E+03 $TCID_{50}$/mL	Varicella Zoster Virus	1.00E+05 cp/mL
Human Herpes Virus 8	1.00E+05 $TCID_{50}$/mL	*Staphylococcus saprophyticus*	1.00E+06 CFU/mL
Human Metapneumovirus	1.00E+03 IU/mL	Zika virus	1.00E+05 $TCID_{50}$/mL

CCU/mL=colony changing units/mL.
CFU/mL=colony forming units per mL.
cp/mL=copies per mL.
IFU/mL=inclusion forming units per mL.
IU/mL=International units per mL.
$TCID_{50}$/mL=tissue culture infectious dose units per mL.

Method Correlation

This study was designed in accordance with CLSI EP09c.[14]

Plasma Method Correlation

The performance of Panther Fusion EBV Quant assay was assessed against a comparator assay by testing retrospectively collected specimens and contrived specimens covering the entire linear range. A total of 121 specimens within the linear range common to both assays were used for the Deming regression as shown in Figure 7.

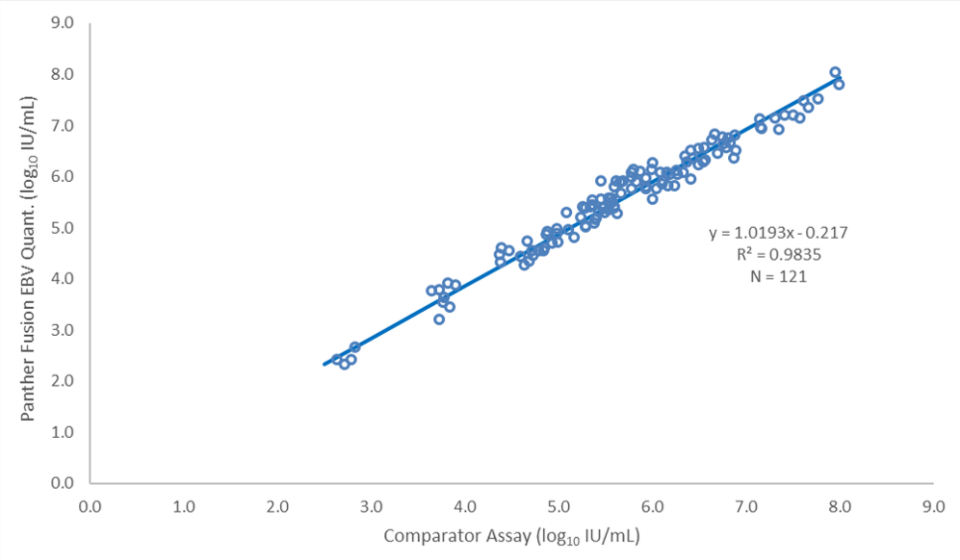

Figure 7. Correlation Between EBV Viral Load in the Panther Fusion EBV Quant Assay and a Comparator Assay on Testing Plasma Samples

Whole Blood Method Correlation

The performance of the Panther Fusion EBV Quant assay was assessed against a comparator assay by testing retrospectively collected specimens and contrived specimens covering the entire linear range. A total of 147 specimens within the linear range common to both assays were used for the Deming regression as shown in Figure 8.

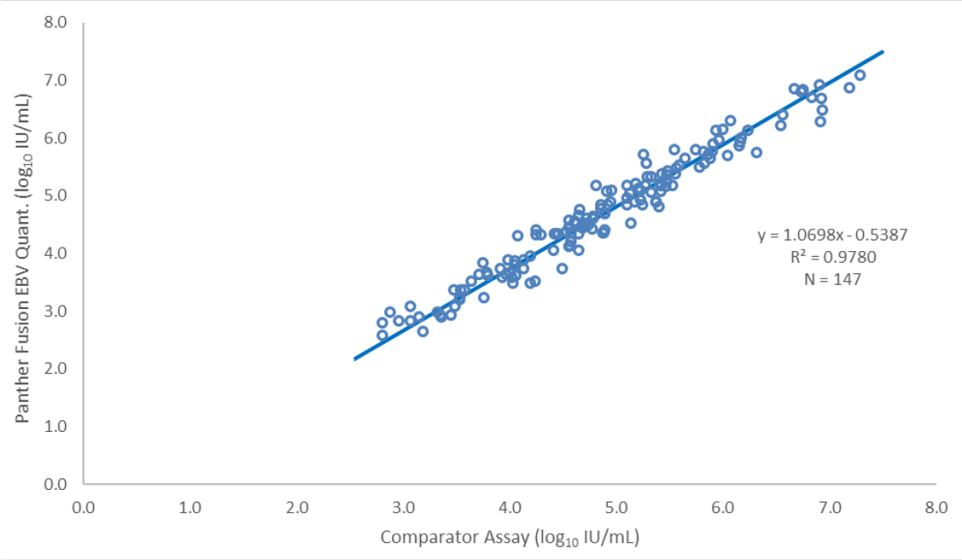

Figure 8. Correlation Between EBV Viral Load in the Panther Fusion EBV Quant Assay and a Comparator Assay on Testing Whole Blood Samples

Carryover/Cross-contamination

Carryover was assessed using high-titer EBV-spiked STM samples (1.5E+09 IU/mL) interspersed between EBV-negative samples in a checkerboard pattern. Testing was carried out over 5 runs. The overall carryover rate was 0.67% (1/150).

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AW-26019-001 Rev. 001
2022-05