# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY
ASSAY AND INSTRUMENT

## I Background Information:

### A 510(k) Number

K243489

### B Applicant

Abbott Molecular Inc.

### C Proprietary and Established Names

Alinity m EBV

### D Regulatory Information

| Product Code(s) | Classification | Regulation Section | Panel |
|---|---|---|---|
| QLX | Class II | 21 CFR 866.3183 - Quantitative Viral Nucleic Acid Test For Transplant Patient Management | MI - Microbiology |

## II Submission/Device Overview:

### A Purpose for Submission:

To obtain 510k clearance for the Alinity m EBV in vitro polymerase chain reaction (PCR) assay for the quantitation of Epstein-Barr Virus (EBV) DNA in human EDTA plasma on the automated Alinity m System. This assay is identical to the previous assay K212778 with the exception of the DNA polymerase. A new DNA polymerase designated MomentaTaq is being validated for this assay.

### B Measurand:

EBV DNA

### C Type of Test:

Quantitative polymerase chain reaction (qPCR)

## III Intended Use/Indications for Use:

### A Intended Use(s):

Alinity m EBV is an in vitro polymerase chain reaction (PCR) assay for the quantitation of Epstein-Barr Virus (EBV) DNA in human EDTA plasma on the automated Alinity m System.

Alinity m EBV is intended for use as an aid in the management of EBV in transplant patients. In patients undergoing assessment of EBV, serial DNA measurements can be used to indicate the need for potential treatment changes and to assess viral response to treatment. The results from Alinity m EBV must be interpreted within the context of all relevant clinical and laboratory findings.

Alinity m EBV is not intended to be used as a screening test for donors of blood, blood products, or human cells, tissues, and cellular and tissue-based products (HCT/Ps) for EBV.

### B Indication(s) for Use:

NA

### C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

**Special Instrument Requirements:**

Alinity m system

## IV Device/System Characteristics:

### A Device Description:

Alinity m EBV is an in vitro polymerase chain reaction (PCR) assay for the quantitation of EBV DNA in human plasma.
The steps of the Alinity m EBV assay consist of sample preparation, real-time PCR assembly, amplification/detection, result calculation, and reporting. All stages of the Alinity m EBV procedure are executed automatically by the Alinity m System. No intermediate processing or transfer steps are performed by the user. The Alinity m System is designed to be a random-access analyzer that can perform the Alinity m EBV assay in parallel with other Alinity m assays on the same instrument.

### B Principle of Operation:

EBV DNA from specimens is extracted automatically on-board in the Alinity m System using the Alinity m Sample Prep Kit 2, Alinity m Lysis Solution, and Alinity m Diluent Solution. The Alinity m System employs magnetic microparticle technology to facilitate nucleic acid capture, wash and elution. The resulting purified nucleic acids are then combined with the liquid unit-dose Alinity m EBV activation reagent and lyophilized unit-dose Alinity m EBV amplification/detection reagents and transferred into a reaction vessel. Alinity m Vapor Barrier Solution is then added to the reaction vessel which is then transferred to an amplification/detection unit for PCR amplification and real-time fluorescence detection of EBV targets.

An EBV calibration curve is required for the quantitation of EBV targets. Two levels of calibrators are processed through sample preparation and real-time PCR to generate the calibration curve. The concentration of EBV DNA in specimens and controls is then calculated from the stored calibration curve.

Assay controls are tested at or above an established minimum frequency to help ensure that instrument and reagent performance remain satisfactory. During each control event, a negative control, a low-positive control, and a high-positive control are processed through sample preparation and real-time PCR procedures that are identical to those used for specimens.

At the beginning of the Alinity m EBV sample preparation process, a lyophilized unit-dose of Internal Control (containing pumpkin hydroxypyruvate reductase cDNA sequences) on the AMP Tray is rehydrated by the Alinity m System and delivered into each sample preparation reaction. The Internal Control is then processed through the entire sample preparation and real-time PCR procedure along with the specimens, calibrators, and controls to demonstrate proper sample processing and assay validity.

The Alinity m EBV amplification and detection reagents include primers and probes that amplify and detect dual targets in the EBV genome. Amplification and detection of the two EBV targets ensures sensitive detection of the viral genome even at low levels.

The process of data reduction extracts relevant information from the real-time amplification curves that allows for determining a qualitative or quantitative response for the PCR reaction. The Alinity m System's data analysis software provides two basic types of analysis for each assay reaction.

• CT (threshold cycle) method: CT is defined as the cycle number (CN) at which the PCR fluorescence signal reaches a fixed detection threshold above the baseline fluorescence level.
• MaxRatio (maximum ratio) method: The maxRatio method is Abbott's proprietary algorithm to produce a maxRatio value that is related to PCR efficiency. The maxRatio value is used to differentiate positive from negative reactions.
The cycle number corresponding to the peak maxRatio value (MR) is identified as FCN (Fractional Cycle Number). CT and FCN are inversely proportional to the Log of the target concentration present in the sample prior to the amplification.
The Alinity m System automatically calculates and reports assay results.

Interpretation of Results

Undiluted Specimens

The Alinity m System will report a Result and an Interpretation for each specimen. If applicable, message codes or flags will also be displayed.

Diluted Specimens

For specimens tested with dilution procedure, the Alinity m System reports a Result, an Interpretation (if applicable), and a DIL flag indicating that the specimen has been diluted. The quantitative results represent the EBV DNA concentration in the specimen prior to dilution.
For diluted specimens with analyte concentration below the detection limit, no result is reported, and a message code (9827) is displayed. These specimens cannot be interpreted as target not detected and may be retested with a new neat specimen or a newly prepared dilution (refer to Specimen Dilution Procedure Scheme).

Note: The lower limit of quantitation (LLoQ) of Alinity m EBV is 20 IU/mL (1.30 Log IU/mL) for specimens tested without dilution. Therefore, the lowest EBV DNA concentration that can be reported for a specimen that is tested diluted is 50 IU/mL (1.70 Log IU/mL).

The upper limit of quantitation (ULoQ) of Alinity m EBV is 200,000,000 IU/mL (8.30 Log IU/mL) for specimens tested without dilution. Therefore, the EBV DNA concentration of a specimen that is tested diluted and returns a result of > ULOQ is > 500,000,000 IU/mL (8.70 Log IU/mL).

| **Alinity m System Reported** | | |
|---|---|---|
| **Result** | **Interpretation** | **Interpretation Additional Information** |
| Not Detected | EBV DNA not detected | |
| < LLoQ | EBV DNA detected but not quantified | EBV DNA concentration is below the Lower Limit of Quantitation (LLoQ) of the assay. |
| LLoQ to ≤ ULoQ | EBV DNA detected and quantified | EBV DNA concentration is within the linear range of the assay (≥ LLoQ to ≤ ULoQ). |
| > ULoQ | EBV DNA detected | EBV DNA concentration is above the Upper Limit of Quantitation (ULoQ) of the assay. |

For a table of device characteristics, see "Comparison of Technology to Predicate Devices" below.

## C Instrument Description Information:

1. Instrument Name:
   Alinity m system

2. Specimen Identification:
   EBV DNA in human EDTA plasma

3. Specimen Sampling and Handling:
   Alinity m Sample Prep Kit 2 (List No. 09N12-001)

4. Calibration:
   The Alinity m EBV assay formulated with MomentaTaq DNA Polymerase (subject device) utilizes the same labeling as the predicate device (K212778) for the CAL Kit and its components.

5. Quality Control:
   The Alinity m EBV assay formulated with MomentaTaq DNA Polymerase (subject device) utilizes the same labeling as the predicate device (K212778) for the CTRL Kit and its components.

## V Substantial Equivalence Information:

### A Predicate Device Name(s):
Alinity m EBV AMP Kit (List No. 09N43-095), Alinity m EBV CTRL Kit (List No. 09N43-085), Alinity m EBV CAL Kit (List No. 09N43-075)

### B Predicate 510(k) Number(s):
K212778

### C Comparison with Predicate(s):

| **Device & Predicate Device(s):** | **K2434897** | **K212778** |
|---|---|---|
| Device Trade Name | **Alinity m EBV** | **Alinity m EBV** |
| **General Device Characteristic Similarities** | | |
| **Assay Type** | Same | Quantitative |
| **Specimen Types** | Same | EDTA Plasma |
| **Intended Use** | Same | Alinity m EBV is an in vitro polymerase chain reaction (PCR) assay for the quantitation of Epstein-Barr Virus (EBV) DNA in human EDTA plasma on the automated Alinity m System.<br>Alinity m EBV is intended for use as an aid in the management of EBV in transplant patients. In In patients undergoing monitoring of EBV, serial DNA measurements can be used to indicate the need for potential treatment changes and to assess viral response to treatment.<br>The results from Alinity m EBV must be interpreted within the context of all relevant clinical and laboratory findings. Alinity m EBV is not intended to be used as a screening test for donors of blood, blood products, or human cells, tissues, and cellular and tissue-based products (HCT/Ps) for EBV. |
| **Assay Targets** | Same | 2 highly conserved regions of the EBV genome (*Gp350* and |

| | | |
|---|---|---|
| | | EBNA1) |
| | | |
| **General Device Characteristic Differences** | | |
| DNA Polymerase | MomentaTaq DNA Polymerase | KAPA2G DNA Polymerase |
| | | |

## VI Standards/Guidance Documents Referenced:

Special Control (89 FR 77448), labeling requirements under 21 CFR 809.10(b), the information for special controls pertaining to cytomegalovirus (CMV) quantitative nucleic acid test intended for transplant patient management. Found in Attachment 003a_Guidance and Special Controls.
CLSI EP05-A3: Clinical Laboratory Standards Institute. *Evaluation of the Precision of Quantitative Measurement Procedures; Approved Guideline* – Third Edition. CLSI Guideline EP05-A3, CLSI: Wayne, PA, 2014.
CLSI EP35: Clinical Laboratory Standards Institute. *Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures; Approved Guideline* – First Edition. CLSI Guideline EP35. CLSI: Wayne, PA: 2019.
CLSI EP09c: Clinical and Laboratory Standards Institute. *Measurement Procedure Comparison and Bias Estimation Using Patient Sample* – Third Edition. CLSI Guideline EP09c. Wayne, PA: CLSI; 2018.
CLSI EP06: Clinical Laboratory Standards Institute. *Evaluation of Linearity of Quantitative Measurement Procedures; Approved Guideline* – Second Edition. CLSI Guideline EP06, CLSI: Wayne, PA, 2020.
CLSI EP17-A2: Clinical Laboratory Standards Institute. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline* – Second Edition. CLSI Guideline EP17-A2, CLSI: Wayne, PA, 2012.

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

1. Precision/Reproducibility:

**Precision:**

Precision was evaluated in plasma by testing 8 panel members with EBV concentrations ranging from 20 to $2 \times 10^8$ IU/mL (1.30 to 8.30 Log IU/mL). Panel members were prepared by diluting EBV-positive clinical specimen, EBV cultured virus, or plasmid DNA in human plasma. EBV plasmid DNA was only used for panel members with high target concentrations (> 6 Log IU/mL). The quantitation of the panel members is traceable to the International Standard for Epstein Barr virus (EBV) (NIBSC code 09/260).

The study was conducted using 3 lots of Alinity m EBV AMP TRAY 1 manufactured with MomentaTaq DNA Polymerase enzyme, 1 lot of Alinity m EBV ACT TRAY 2,

3 lots of Alinity m EBV CTRL Kit reagents, 1 lot of Alinity m EBV CAL Kit reagents, 1 lot of Alinity m Sample Prep Kit 2 reagents, 1 lot of Alinity m Lysis Solution, 1 lot of Alinity m Diluent Solution, and 1 lot of Alinity m Vapor Barrier Solution on 1 Alinity m System by 3 operators (each of the 3 operators used 1 lot of Alinity m EBV AMP Kit).
Three replicates of each panel member were run twice each day for 15 days for each lot/operator combination. The first and second runs of testing of each panel member were separated by a minimum of 2 hours.

Precision was evaluated by performing the analysis based on CLSI EP05-A3.[1]

The precision study results are shown in Table 1

**Table 1** Alinity m EBV (formulation using the alternative DNA Polymerase) Precision Analysis – All Reagent Lots

| **Panel Member** | **N** | **Mean Conc. (Log IU/mL)** | **Within-Run** | | **Between-Run** | | **Between-Day** | | **Within-Laboratory[a]** | | **Between-Lot** | | **Total[b]** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| 01 | 270 | 1.35 | 0.24 | 18.0 | 0.11 | 8.2 | 0.00 | 0.0 | 0.27 | 19.8 | 0.00 | 0.0 | 0.27 | 19.8 |
| 02 | 270 | 1.69 | 0.19 | 11.1 | 0.00 | 0.0 | 0.06 | 3.3 | 0.20 | 11.6 | 0.00 | 0.0 | 0.20 | 11.6 |
| 03 | 270 | 2.78 | 0.08 | 2.9 | 0.00 | 0.0 | 0.02 | 0.7 | 0.08 | 3.0 | 0.00 | 0.0 | 0.08 | 3.0 |
| 04 | 270 | 3.99 | 0.07 | 1.6 | 0.02 | 0.4 | 0.00 | 0.0 | 0.07 | 1.7 | 0.00 | 0.0 | 0.07 | 1.7 |
| 05 | 270 | 5.07 | 0.05 | 1.0 | 0.03 | 0.6 | 0.01 | 0.2 | 0.06 | 1.2 | 0.01 | 0.1 | 0.06 | 1.2 |
| 06 | 270 | 6.10 | 0.05 | 0.9 | 0.03 | 0.5 | 0.00 | 0.0 | 0.06 | 1.0 | 0.01 | 0.1 | 0.06 | 1.0 |
| 07 | 270 | 7.03 | 0.06 | 0.9 | 0.02 | 0.3 | 0.01 | 0.1 | 0.07 | 1.0 | 0.01 | 0.1 | 0.07 | 1.0 |
| 08 | 270 | 8.39 | 0.17 | 2.0 | 0.08 | 1.0 | 0.00 | 0.0 | 0.19 | 2.3 | 0.00 | 0.0 | 0.19 | 2.3 |

[a] Within-Laboratory includes Within-Run, Between-Run, and Between-Day Components.

[b] Total includes Within-Run, Between-Run, Between-Day, and Between-Lot Components

**Reproducibility:**

The reproducibility study utilized a single lot (one lot) of Alinity m assay reagents (AMP Kit manufactured with the alternative enzyme) across 3 external testing sites. Additionally, a single lot (one lot) of calibrators and controls was used across 3 external testing sites. One Alinity m System was used at each site for the Reproducibility Study, and a single lot of Alinity m Sample Prep Kit 2 as well as Alinity m System Solutions (Alinity m Lysis Solution, Alinity m Diluent Solution, Alinity m Vapor Barrier Solution) was used across the 3 sites. At each site, testing occurred on 5 non-consecutive days with 2 runs per day where each panel was tested in replicates of 4.

Reproducibility testing was performed using 9-member panels prepared in EDTA plasma. The reproducibility panel spanned the analytical measuring interval and was inclusive of a negative panel member.

The 9-member panel consisting of 4 replicates of each panel member were tested in each run.

The positive panel members were prepared by spiking clinical specimen, cultured virus, or plasmid DNA into normal EBV-negative EDTA plasma to achieve concentrations of 20 to $2 \times 10^8$ IU/mL (1.30 to 8.30 Log IU/mL). The negative panel member was

prepared from a pool of normal EDTA plasma units. The panel target concentrations and panel stock source are shown in Table 2.

**Table 2** Plasma Reproducibility Panels

| Specimen Type | Panel Member | Panel Stock Source | Target Concentration (Log IU/mL) |
|---|---|---|---|
| EDTA Plasma | 1 | Plasmid DNA | 8.3 |
| | 2 | Plasmid DNA | 7 |
| | 3 | Cultured virus | 6 |
| | 4 | Cultured virus | 5 |
| | 5 | Cultured virus | 4 |
| | 6 | Clinical Specimen | 3 |
| | 7 | Clinical Specimen | 1.78 |
| | 8 | Clinical Specimen | 1.3 |
| | 9 | N/A | EBV Negative |

Results of the reproducibility study are found in Table 3.

In addition, the total SD and total %CV for each panel member in this study using MomentaTaq Polymerase were compared with the total SD and total %CV from the reproducibility study of the Alinity m EBV AMP Kit. The comparison was performed to assess statistical equivalence based on CLSI EP35,[2] Section 4.1.6.4: "Comparison of Measurement Procedure Precision with Candidate and Primary Specimen Type. The results are presented in Table 4.

**Table 3.** Alinity m EBV (formulation using alternative DNA Polymerase) Reproducibility for Positive Panel Members

| | | | Within-Run Component | | Between-Run Component | | Between-Day Component | | Within-Laboratory [a] | | Between-Site Component | | Total [b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Panel | N | Mean Conc. (Log IU/mL) | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 118 | 7.88 | 0.06 | 0.7 | 0.01 | 0.1 | 0.00 | 0.0 | 0.06 | 0.7 | 0.00 | 0.0 | 0.06 | 0.7 |
| 2 | 116 | 6.82 | 0.04 | 0.6 | 0.00 | 0.0 | 0.02 | 0.3 | 0.04 | 0.6 | 0.01 | 0.2 | 0.04 | 0.6 |
| 3 | 120 | 6.00 | 0.05 | 0.8 | 0.02 | 0.4 | 0.01 | 0.2 | 0.05 | 0.9 | 0.03 | 0.5 | 0.06 | 1.0 |
| 4 | 118 | 5.02 | 0.04 | 0.8 | 0.02 | 0.3 | 0.02 | 0.4 | 0.05 | 0.9 | 0.04 | 0.8 | 0.06 | 1.2 |
| 5 | 120 | 4.00 | 0.05 | 1.2 | 0.00 | 0.1 | 0.01 | 0.3 | 0.05 | 1.2 | 0.04 | 1.1 | 0.06 | 1.6 |
| 6 | 118 | 3.09 | 0.07 | 2.4 | 0.01 | 0.2 | 0.00 | 0.0 | 0.07 | 2.4 | 0.03 | 1.1 | 0.08 | 2.6 |
| 7 | 117 | 1.74 | 0.19 | 10.9 | 0.00 | 0.0 | 0.00 | 0.0 | 0.19 | 10.9 | 0.04 | 2.4 | 0.19 | 11.1 |
| 8 | 113 | 1.39 | 0.29 | 21.2 | 0.04 | 2.5 | 0.00 | 0.0 | 0.30 | 21.3 | 0.00 | 0.0 | 0.30 | 21.3 |

[a] Within-Laboratory includes Within-Run, Between-Run, and Between-Day Variance Components.
[b] Total (Overall) includes Within-Run, Between-Run, Between-Day, and Between-Site Variance Components.

**Evaluation of Total Precision for New Enzyme EBV vs. On-Market EBV**

**Table 4.** Equivalence Evaluation of Total SD and Total %CV for EBV New Enzyme vs. On-Market EBV

| | Mean Concentration | | Total SD | | Total %CV | | Ratios and 95% CI | | |
|---|---|---|---|---|---|---|---|---|---|
| Panel | New Mean (Log IU/mL) | Original Mean (Log IU/mL) | New SD | Original SD | New %CV | Original %CV | SD | %CV | Clinically acceptable? |
| 1 | 7.88 | 8.40 | 0.06 | 0.19 | 0.7 | 2.3 | 0.30 (0.07, 1.35) | 0.32 (0.07, 1.44) | Yes |
| 2 | 6.82 | 7.04 | 0.04 | 0.14 | 0.6 | 2.0 | 0.32 (0.07, 1.35) | 0.33 (0.08, 1.39) | Yes |
| 3 | 6.00 | 5.76 | 0.06 | 0.10 | 1.0 | 1.8 | 0.58 (0.21, 1.56) | 0.55 (0.20, 1.49) | Yes |
| 4 | 5.02 | 5.04 | 0.06 | 0.12 | 1.2 | 2.4 | 0.49 (0.19, 1.26) | 0.49 (0.19, 1.26) | Yes |
| 5 | 4.00 | 4.01 | 0.06 | 0.09 | 1.6 | 2.2 | 0.72 (0.36, 1.44) | 0.73 (0.36, 1.45) | Yes |
| 6 | 3.09 | 3.07 | 0.08 | 0.09 | 2.6 | 2.8 | 0.94 (0.64, 1.37) | 0.93 (0.63, 1.36) | Yes |
| 7 | 1.74 | 1.64 | 0.19 | 0.23 | 11.1 | 14.0 | 0.84 (0.56, 1.26) | 0.80 (0.53, 1.20) | Yes |
| 8 | 1.39 | 1.20 | 0.30 | 0.30 | 21.3 | 25.1 | 0.98 (0.80, 1.21) | 0.85 (0.69, 1.04) | Yes |

Note: New Mean, New SD, and New %CV data originate from EBV New Enzyme Reproducibility

2. Linearity:

Linearity was evaluated by testing 16 panel members that spanned the intended measuring range of the assay (50 to 200,000,000 IU/mL), including a panel member below the expected Lower Limit of Quantification (LLoQ) at 15 IU/mL, and a Panel member exceeding the expected Upper Limit of Quantification (ULoQ) at 250,000,000 IU/mL. Panels with different specimen types were designed to have at least a 2 Log IU/mL overlap with adjacent sample types. Panel member target concentrations and total replicates tested are listed in Table 5

**Table 5.** Panel Members for Linearity

| Panel Member | Targeted EBV Concentration | | Source |
|---|---|---|---|
| | IU/mL | Log IU/mL | |
| 01 | 250,000,000 | 8.40 | Plasmid DNA |
| 02 | 10,000,000 | 7.00 | Plasmid DNA |
| 03 | 1,000,000 | 6.00 | Plasmid DNA |
| 04 | 130,000 | 5.11 | Plasmid DNA |
| 05 | 100,000 | 5.00 | Cultured virus |

| | | | |
|---|---|---|---|
| 06 | 20,000 | 4.30 | Plasmid DNA |
| 07 | 10,000 | 4.00 | Cultured virus |
| 08 | 5,000 | 3.70 | Plasmid DNA |
| 09 | 2,500 | 3.40 | Clinical specimen |
| 10 | 1,000 | 3.00 | Plasmid DNA |
| 11 | 500 | 2.70 | Cultured virus |
| 12 | 200 | 2.30 | Clinical specimen |
| 13 | 50 | 1.70 | Clinical specimen |
| 14 | 25 | 1.40 | Plasmid DNA |
| 15 | 20 | 1.30 | Cultured virus |
| 16 | 15 | 1.18 | Clinical specimen |

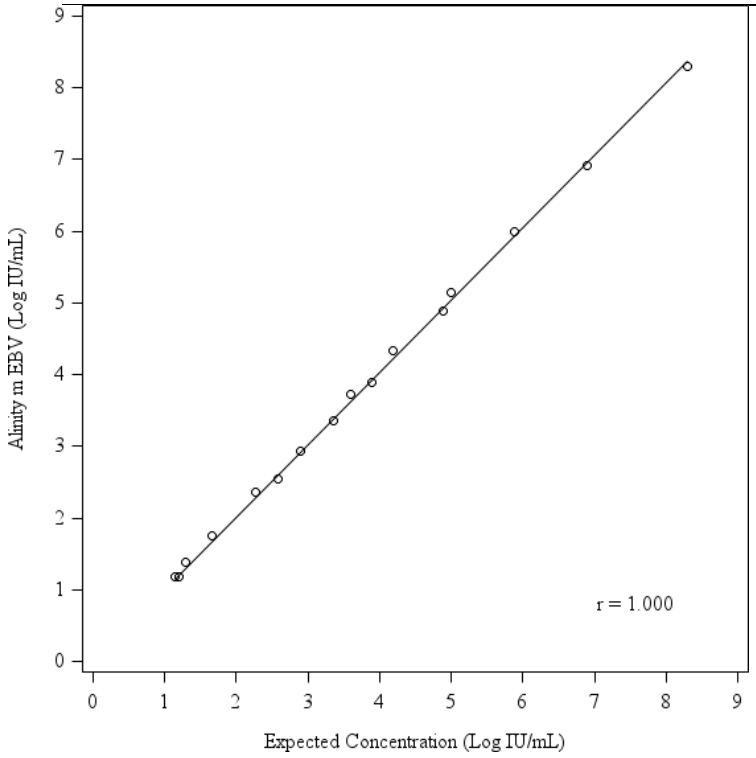
The study was conducted using 1 lot of Alinity m EBV AMP TRAY 1 (9N43P0101IUO) and Alinity m ACT Tray 2, Alinity m EBV CTRL Kit reagents, Alinity m EBV CAL Kit reagents, Alinity m Sample Prep Kit 2 reagents, Alinity m Lysis Solution, Alinity m Vapor Barrier Solution, Alinity m Diluent Solution, and 1 Alinity m System.

Linearity was evaluated based on the recommendation in CLSI EP06, *Evaluation of Linearity of Quantitative Measurement Procedures; Approved Guideline* – Second Edition.

The deviation (ADL) in all panel members tested was within the requirement of ±0.25 Log IU/mL, and the results thus demonstrate linearity from the range from the lowest panel member tested (15 IU/mL) to the highest panel member tested (targeted at 250,000,000 IU/mL).

Weight least squares (WLS) regression analysis was performed and the regression plot of Alinity m EBV assay (linearity range is 15 IU/mL to 250,000,000 IU/mL) is presented in Figure 1.

**Figure 1.** Linearity Weighted Lease Squares Regression Plot




| | | |
|---|---|---|
| Sample Size (n) | | 382 |
| Correlation Coefficient (r) | | 1.000 |
| Slope | | 1.01 |
| 95% CI for Slope | | (1.00, 1.02) |
| Expected Concentration (Log IU/mL) | Min: 1.14 | Min: 8.29 |
| Alinity m EBV (Log IU/mL) | Max: 1.18 | Max: 8.29 |

The data from this study demonstrated a linear range of 15 IU/mL to 250,000,000 IU/mL in plasma for the Alinity m EBV assay manufactured with MomentaTaq DNA Polymerase.

3. Analytical Specificity/Interference:
   Refer to K212778.

4. Assay Reportable Range:
   Refer to K212778. The linear range is 15 IU/mL to 250,000,000 IU/mL in plasma for the Alinity m EBV assay manufactured with MomentaTaq DNA Polymerase. Therefore, the claimed reportable range of 50 IU/mL to 200,000,000 IU/mL for Alinity m EBV is equivalent to the original assay in K212778.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
   Traceability to international standard material:
   The Alinity m EBV Assay (K212778) was standardized against the International Standard for Epstein-Barr virus for Nucleic Acid Amplification Techniques (NIBSC code: 09/260). Since the only change from K212778 to K243489 was the DNA polymerase it was not necessary to repeat these studies.

   Reagent stability:
   The data supports the following storage conditions for the Alinity m EBV assay with MomentaTaq DNA Polymerase:

**Table 6. Alinity m EBV kit stability**

| Stability study | Expiration |
|---|---|
| Reagent On-board | 34 days |
| Reagent Shelf-life | 15 months |

6. Detection Limit:
   A study was conducted to verify the limit of detection (LoD) of the Alinity m EBV assay for plasma samples using AMP TRAY 1 manufactured with MomentaTaq DNA Polymerase enzyme as an alternative to KAPA2G DNA Polymerase (Part No. 11-200303).
   The study design used 3 reagent lots of Alinity m EBV AMP TRAY 1 manufactured with MomentaTaq, 1 instrument system, 3 days, 3 operators, a minimum of 5 valid replicates for each concentration level and each dilution per day. An EBV WHO Standard viral stock (NIBSC code 09/260) was diluted with EBV negative plasma for preparing the 4 panel levels, including 2 samples at LLoQ (50 IU/mL), 2 samples at LoD (20 IU/mL), and 2 samples higher and lower than LoD claim.

   The data demonstrated that Alinity m EBV assay with MomentaTaq had an overall detection rate of 97.2% at the claimed LoD of 20 IU/mL (at LoD) with all lots combined. The results are presented in Table 7.

**Table 7.** EBV Sensitivity Percent Detection Rates

| Panel | Target Concentration (IU/mL) | AMP Kit Lot | Total No. of Replicates | No. of Replicates Detected | Detection Rate (%) | 95% Score Confidence Interval (%) |
|---|---|---|---|---|---|---|
| 1 | 15 | 1 | 48 | 45 | 93.8 | (83.2, 97.9) |
| | | 2 | 48 | 46 | 95.8 | (86.0, 98.8) |
| | | 3 | 47 | 45 | 95.7 | (85.8, 98.8) |
| | | All Combined | 143 | 136 | 95.1 | (90.2, 97.6) |
| 2 | 20 | 1 | 47 | 47 | 100.0 | (92.4, 100.0) |
| | | 2 | 48 | 46 | 95.8 | (86.0, 98.8) |
| | | 3 | 47 | 45 | 95.7 | (85.8, 98.8) |
| | | All Combined | 142 | 138 | 97.2 | (93.0, 98.9) |
| 3 | 50 | 1 | 48 | 48 | 100.0 | (92.6, 100.0) |
| | | 2 | 48 | 48 | 100.0 | (92.6, 100.0) |
| | | 3 | 47 | 47 | 100.0 | (92.4, 100.0) |
| | | All Combined | 143 | 143 | 100.0 | (97.4, 100.0) |
| 4 | 100 | 1 | 48 | 48 | 100.0 | (92.6, 100.0) |
| | | 2 | 47 | 47 | 100.0 | (92.4, 100.0) |
| | | 3 | 47 | 47 | 100.0 | (92.4, 100.0) |
| | | All Combined | 142 | 142 | 100.0 | (97.4, 100.0) |

7. Lower Limit of Quantitation (LLoQ)::
LLoQ is defined as the lowest concentration at which E|BV DNA is reliably quantitated within an acceptable total error. The LLoQ was evaluated based on the recommendation in CLSI EP17-A2. The study to verify the LLoQ analyzed data from the limit of detection study, above. EBV panel members at the LLoQ claim concentration of 50 IU/mL (1.70 Log IU/mL), as well as panel members higher and lower than the LoD claim concentration were analyzed.

Analysis of the LoD results for LLoQ confirmation are presented in Table 8.

**Table 8.** Alinity m EBV TAE and TE (Overall)

| Study | Panel | Target (IU/mL) | N | Target (Log IU/mL) | Mean (Log IU/mL) | Bias (Log IU/mL) | SD | TAE | TE | Acceptance Criteria |
|---|---|---|---|---|---|---|---|---|---|---|
| Sensitivity | 1 | 15 | 136 | 1.18 | 0.96 | -0.22 | 0.32 | 0.86 | 0.91 | N/A [a] |
| | 2 | 20 | 138 | 1.30 | 1.07 | -0.23 | 0.33 | 0.89 | 0.93 | N/A |
| | 3 | 50 | 143 | 1.70 | 1.52 | -0.18 | 0.20 | 0.59 | 0.57 | Met |
| | 4 | 100 | 142 | 2.00 | 1.86 | -0.14 | 0.17 | 0.48 | 0.47 | Met |

[a] N/A = Not Applicable

Panel members with the target concentration at 50 IU/mL (the assay's LLoQ) had a TAE and TE less than or equal to 1.00 Log IU/mL for all lots combined. The Lower

Limit of Quantitation (LLoQ) for the Alinity m EBV assay was verified to be 50 IU/ml, i.e., equivalent to K212778.

8. Accuracy (Instrument):
   Not applicable

9. Carry-Over:
   Carry-over was established for K212778 and since there was not change to any reagent other than the DNA polymerase it was not necessary to repeat this study for K243489.

## B Comparison Studies:

1. Method Comparison with Predicate Device:
   The objective of the Alinity m EBV clinical specimen testing was to demonstrate that the alternative enzyme formulation does not impact the ability of the assay to quantify Epstein-Barr Virus (EBV) in human EDTA plasma and therefore does not impact previously established clinical utility of the assay.

   The Alinity m EBV clinical specimen testing was conducted at one testing site (internal testing site). The study used retrospectively collected clinical specimens. The specimens were obtained from hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients. Samples were selected such that EBV levels in the samples span the measuring range of the Alinity m EBV assay.
   To ensure coverage of the measuring range, both neat clinical specimens and samples made with unique individual high-titer clinical specimens spiked into unique individual negative specimens were included.

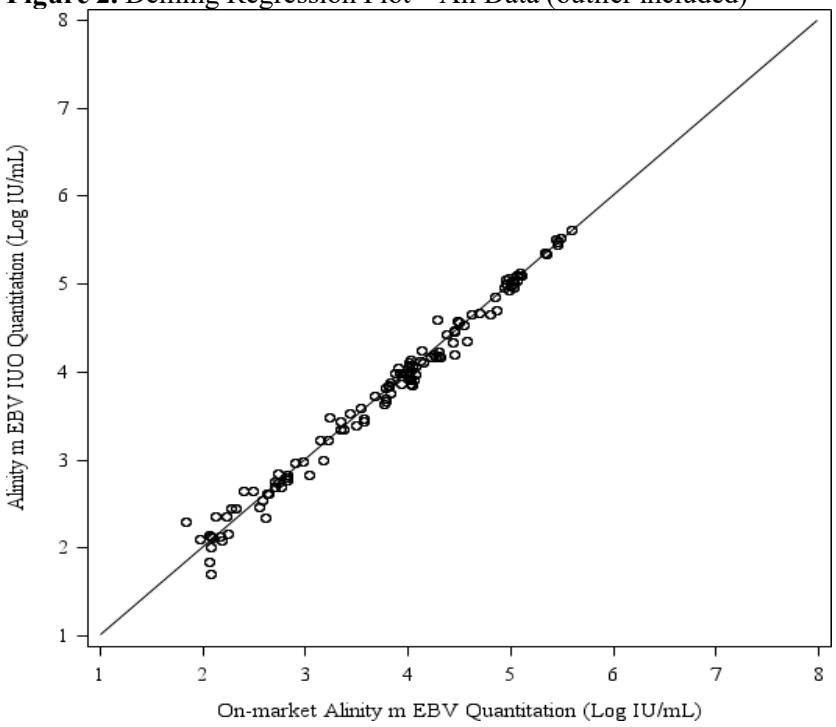
   All samples were tested with both the on-market Alinity m EBV assay with the KAPA2G enzyme and the Alinity m EBV assay with the alternative enzyme formulation. The results of the Alinity m EBV assay was compared to the on-market Alinity m EBV assay results.

   Out of 148 included valid results, 124 results from 148 specimens were used in Method Comparison Analysis. Of the 124 specimens, 96 were neat clinical specimens and 28 were spiked clinical samples. 24 results were outside the measuring range of either the comparator on-market and/or the Alinity m EBV candidate assay and were not used in the method comparison analysis:

   - 18 of the 24 results had the investigational testing result less than LLoQ (1.70 Log IU/mL) or not detected. Of note, 14 of the 18 results also had the comparator on-market results less than LLoQ (1.70 Log IU/mL) or not detected.
     6 of the 24 results had the comparator testing result less than LLoQ (1.70 log IU/mL) or not detected.

   Deming Regression / Scatter Plots with all Data (outlier included)
   The Deming regression included a total of 124 samples with results that fell within the analytical measuring interval of the Alinity m EBV assay.
   The correlation coefficient was 0.993, the slope was 1.00 (95% CI: 0.97, 1.02), and the intercept was 0.01 Log IU/mL (95% CI: –0.10, 0.11). The scatter plots of the Alinity m candidate assay results versus the on-market Alinity m assay results were generated along with the regression line as seen in **Figure 2**.

**Figure 2.** Deming Regression Plot – All Data (outlier included)




| | | |
|---|---|---|
| Sample Size (n) | | 124 |
| Correlation Coefficient (r) | | 0.993 |
| Slope | | 1 |
| 95% CI for Slope | | (0.97, 1.02) |
| Intercept | | 0.01 |
| 95% CI for Intercept | | (–0.10, 0.11) |
| On-Market Alinity m EBV | Min: 1.84 | Max: 5.59 |
| Alinity m EBV IUO | Min: 1.70 | Max: 5.61 |

Systematic Difference was calculated for selected viral load levels (1.7, 3.0, 4.0, 5.0 Log IU/mL) along with the two-sided 95% confidence intervals (**Table 9**).

**Table 9.** Summary for Systematic Difference at Selected Viral Load Levels (with all data)

| **Target Viral Load Level (per comparator assay) (Log IU/mL)** | **Systematic Difference (Log IU/mL)** | **95% CI (Log IU/mL)** |
|---|---|---|
| 1.70 (LLoQ) | 0.02 | (–0.05, 0.09) |
| 3.00 | 0.01 | (–0.03, 0.05) |
| 4.00 | 0.01 | (–0.01, 0.03) |
| 5.00 | 0.00 | (–0.02, 0.03) |

## Conclusion

The mean bias relative to the comparator assay is less than 0.25 Log IU/mL within the quantitation range:

- The mean paired difference (bias) was –0.01 Log IU/mL with all data (outlier included)

The statistical analysis of the clinical study has demonstrated the comparable performance of the Alinity m EBV assay with the alternative DNA polymerase enzyme formulation to the on-market Alinity m EBV assay.

2. Matrix Comparison:
   Not applicable

**C Clinical Studies:**

1. Clinical Sensitivity:
   Not applicable

2. Clinical Specificity:
   Not applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not applicable

**D Clinical Cut-Off:**
Not applicable

**E Expected Values/Reference Range:**
Not applicable

**F Other Supportive Instrument Performance Characteristics Data:**
Not applicable

## VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

## IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.