EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
cobas EBV
DECISION SUMMARY

A. De Novo Number

DEN200015

B. Purpose for Submission

De Novo request for evaluation of automatic class III designation for the cobas EBV test.

C. Measurand

EBV DNA

D. Type of Test

Quantitative Polymerase Chain Reaction (PCR)

E. Applicant

Roche Molecular Systems, Inc.

F. Proprietary and Established Names

cobas EBV

G. Regulatory Information

1. Regulation section

21 CFR 866.3183

2. Classification

Class II

3. Product code(s):

QLX

4. Panel

Microbiology (83)

H. Indications For Use

1. Indications for use:

   cobas EBV is an in vitro nucleic acid amplification test for the quantitation of Epstein-Barr virus (EBV) DNA in human EDTA plasma on the cobas 6800/8800 Systems.

   cobas EBV is intended for use as an aid in the management of EBV in transplant patients. In patients undergoing monitoring of EBV, serial DNA measurements can be used to indicate the need for potential treatment changes and to assess response to treatment.

   The results from cobas EBV are intended to be read and analyzed by a qualified licensed healthcare professional in conjunction with clinical signs and symptoms and relevant laboratory findings. Negative test results do not preclude EBV infection or EBV disease. Test results must not be the sole basis for patient management decisions.

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

   Special conditions for use statement(s)

   For prescription use only.

   For *in vitro* diagnostic use only.

2. Special instrument requirements

   The test is run on the cobas 6800/8800 instrument systems.

I. Device Description

cobas EBV is a quantitative test performed on the cobas 6800 System and cobas 8800 System. cobas EBV enables the detection of EBV DNA in plasma specimens. The cobas EBV assay is a dual target assay, with both targets using the same dye. The DNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. cobas EBV enables the detection and quantitation of EBV DNA in EDTA plasma from solid organ transplant patients (SOT) and from hematopoietic stem cell transplant (HSCT) patients. The test is intended for use as an aid in the management of SOT patients and HSCT patients.

The cobas EBV consists of:

- Proteinase Solution
- DNA Quantitation Standard (DNA QS)
- Elution Buffer

- Master Mix Reagent 1
- EBV Master Mix Reagent 2

The EBV viral load is quantified against a non-EBV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample preparation. The DNA-QS also functions as an internal control for sample preparation and the PCR amplification process.

In addition, the test utilizes the following separately packed and sold control materials:

1. cobas EBV Positive Control Kit:
   - EBV Low Positive Control (EBV L(+)C)
   - EBV High Positive Control (EBV H(+)C)

   The positive control contains phage packaged EBV DNA in normal human plasma and serves as a control for the cobas EBV test.

2. cobas Negative Control Kit:
   - cobas Buffer Negative Control (BUF (-) C)

Testing with the cobas EBV test requires the following materials that are not provided:
- cobas OMNI Reagents: Including the following reagents used for specimen processing, PCR and detection:
- cobas EBV Assay Specific Analysis Package (ASAP) software

The cobas EBV test uses sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection, all steps are fully automated by the cobas 6800/8800 platform.

Instrumentation and Software
The cobas 6800/8800 platform consists of two instrument versions: the cobas 6800 System, and the cobas 8800 System. Each system is comprised of a cobas 6800 or cobas 8800 instrument, system software, Assay Specific Analysis Packages (ASAP), and a sample source unit, which can be connected to a conveyor system for automated transport of samples to and from the system. The test kits consist of assay-specific reagents and omni reagents (or common reagents) which can be used with any of the cobas assays, and on either the cobas 6800 or the cobas 8800 system.

In addition, the cobas omni (common) reagents and consumables, such as the P-plates, racks, AD-plates, waste bags, pipette tips, and secondary tubes, can be used with any of the cobas assays, and can be used for both the cobas 6800 and the cobas 8800 systems.

J. Standard/Guidance Document Referenced

EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition

EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.

EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition

EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline —Second Edition

K. Test Principle

The cobas EBV test is a quantitative PCR test performed on the fully automated cobas 6800/ 8800 Systems that detects and quantifies EBV DNA from (EDTA) plasma specimens of transplant patients as follows:

Target Selection
Selective amplification of EBV target nucleic acid from the sample is achieved by the use of specific forward and reverse primers which are selected to amplify highly-conserved regions of the EBV DNA EBNA-1 and BMRF-2 gene. Specific probes for each amplicon are used to detect and quantify the EBV targets. Selective amplification of DNA-QS is achieved by the use of DNA-QS specific forward and reverse primers, selected to have no homology with the EBV genome, detected through a DNA-QS specific probe.

Sample Preparation (Nucleic Acid Extraction and Purification)
Nucleic acid from patient samples and external controls are extracted upon addition of a DNA Quantitation standard (DNA-QS). The DNA-QS molecules are extracted simultaneously with the samples/controls serving as an extraction control. Viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid, along with the added DNA-QS binds to magnetic glass particles. Unbound substances and impurities are removed with subsequent wash reagent steps and purified nucleic acid is then eluted from the magnetic glass particles with elution buffer.

Nucleic Acid Amplification and Target Detection
The cobas EBV master mix contains detection probes which are specific for the two EBV target sequences and the DNA-QS nucleic acid, respectively. The two EBV specific detection probes are labeled with the same fluorescent dye while the DNA-QS detection probe is labeled with a second fluorescent dye both acting as reporter dyes. Each probe also has a second dye which acts as a quencher that suppresses the fluorescent signals of the intact probes when they are not bound to their respective target sequence. Target bound probes however, emit fluorescence of the two reporter dyes. This fluorescence is measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the EBV targets and the DNA-QS amplification products generated by a thermostable DNA polymerase enzyme.

EBV DNA Quantitation
During the extension phase of the PCR process fluorescence readings are processed to generate Ct values for the EBV DNA target and the EBV QS DNA. The lot-specific

calibration constants provided with the cobas EBV test are used to calculate the titer value for the specimens and controls based on both the EBV DNA target and the EBV QS DNA Ct values. EBV viral load results are reported in International Units/mL (IU/mL).

L. Performance Characteristics

1. Analytical performance

a. Precision

The Precision was assessed for the predominant genotype (EBV Genotype 1) with seven panel members ranging from (b) (4) . Panel members were prepared by spiking a high titer EBV lambda phagemid into EBV negative EDTA plasma.

Precision was calculated on results generated over twelve days using three kit lots and three cobas 6800 systems by three operators. Per test day, two (2) runs were performed containing three (3) within-run replicates per panel member. The study design accounts for a total of 72 replicates per panel member.

For data analysis only samples with titers above the Lower Limit of Quantification (LLoQ), (i.e., (b) (4)), were used. Precision was determined according to the CLSI guideline EP05-A3 as a multivariance analysis accounting for reagent lots, operators/instruments, days, runs and within-run replicates.

Table 1: Precision - Standard Deviation

Nominal Concentration	Assigned concentration (IU/mL)	Standard Deviation			
		Lot 1	Lot 2	Lot 3	All Lots
5.00E+07	5.40E+07	0.03	0.04	0.04	0.04
1.00E+06	1.08E+06	0.02	0.03	0.02	0.02
1.00E+05	1.08E+05	0.02	0.02	0.03	0.02
1.00E+04	1.08E+04	0.04	0.02	0.03	0.03
1.00E+03	1.08E+03	0.05	0.05	0.05	0.05
1.00E+02	1.08E+02	0.17	0.18	0.15	0.17
6.00E+01	6.48E+01	0.17	0.17	0.13	0.16

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

The results for cobas EBV Precision are shown in **Table 1**. The Variance Component Analysis demonstrated the contribution of components of variance to the total precision

variance (**Table 2**). Overall, the total Precision as SD of log_{10} titer is comparable across all kits.

Table 2: Lognormal Percent Coefficient of Variation (% CV) *

Panel Member	Assigned Concentration (IU/mL)		N	Instrument/ Operator	Kit Lot	Day	Run	Within Run	Total
	Titer (IU/mL)	Log10 Titer (IU/mL)		%CV	%CV	%CV	%CV	%CV	%CV
PM01	1.08E+06	7.73	72	5	1	2	2	8	10
PM02	1.08E+05	6.03	72	1	4	1	3	4	7
PM03	5.94E+03	5.03	72	2	2	3	2	5	7
PM04	1.08E+04	4.03	72	2	1	3	3	7	8
PM05	1.08E+03	3.03	72	4	1	4	4	10	12
PM06	1.08E+02	2.03	72	3	5	8	14	42	43
PM07	6.48E+01	1.81	68	7	3	6	12	39	40

* Titer data are considered to be log-normally distributed and the %CV values are analyzed as Lognormal CV (%) = sqrt (10^ [SD^2 * ln (10)] - 1) * 100%

b. ***Reproducibility***

Reproducibility of cobas EBV was evaluated in EDTA plasma across three Reagent Lots, three Test Sites, three Instruments (two cobas 6800 Systems and one cobas 8800 System). Two runs were performed per operator per day (1 run = 1 batch; 1 batch = 1 panel + 3 controls) over five days per reagent lot and each run had three replicates per panel member. The total number of tests (not including controls) was as follows: 3 lots × 3 sites × 5 days/lot × 2 runs × 3 replicates/concentration = 270 test results/concentration.

Test panel members were prepared from EBV-VCA IgG sero-negative and RNA negative EDTA plasma spiked with EBV genotype 1 genomic material in the form of EBV cell culture supernatant. EBV phagemid was used for preparing the high-positive panel member ($5x10^7$ IU/mL) due to the lack of adequate volumes of high concentration sample. Test panel members had the following concentrations: Negative, 105 IU/mL, $5x10^3$ IU/mL, $5x10^4$ IU/mL, $5x10^5$ IU/mL, and $5x10^7$ IU/mL. Two invalid results were excluded. The results are summarized in **Table 3** below:

Table 3: Reproducibility Study

EBV DNA Concentration (log_{10} IU/mL)		Number of Tests[b]	Percent of Total Variance [Log-Normal CV (%)]					Total Precision	
Expected	Observed Mean[a]		Lot	Site	Day/ Operator	Batch	Within -Batch	SD[c]	Log-Normal CV (%)[d]
2.02	2.09	270	11% (11.97)	2% (5.30)	0% (0.00)	3% (6.34)	84% (34.25)	0.158	37.56
3.70	3.68	270	43% (10.07)	15% (5.92)	0% (0.00)	16% (6.23)	26% (7.81)	0.067	15.43
4.70	4.68	270	39% (8.54)	10% (4.24)	0% (0.00)	24% (6.63)	28% (7.18)	0.059	13.70
5.70	5.50	268	7% (11.39)	58% (34.36)	0% (0.00)	21% (20.18)	15% (17.08)	0.191	46.16
7.70	7.76	270	27% (8.63)	15% (6.52)	0% (0.88)	13% (6.01)	45% (11.26)	0.073	16.83

[a] Calculated using SAS MIXED procedure.
[b] Number of valid tests with detectable DNA level.
[c] Calculated using the total variability from the SAS MIXED procedure.
[d] Lognormal CV (%) = sqrt (10^ [SD^2 * ln (10)] - 1) * 100
Note: The table only includes results with detectable DNA level. CV (%) = percent coefficient of variation; SD = standard deviation.

Analysis of variance and a mixed model that included lot, site, day/operator, batch and within-batch (random error) as random effects was performed. The variance contribution of each component to the total variance was estimated. The range of the total lognormal coefficient of variation, among positive panel members, was from 13.7% to 46.16%. The largest total lognormal coefficient of variation was observed in the expected $5x10^5$-panel member and most of that variability (58% of the total variance) was attributed to site. The largest total lognormal coefficient of variation observed in the lowest panel member (37.56%) was explained by the within-batch component.

*c. **Linearity***

Linearity of the cobas EBV with Genotype 1

Linearity of the cobas EBV test was evaluated for the predominant EBV genotype (GT 1) in EDTA-plasma using a 17-member test panel. Eleven panel members were generated using an EBV phagemid DNA and covered the entire linear range. Six panel members were generated using an EBV GT1-positive clinical specimen. Due to the lack of sufficient volume of high positive EBV-positive transplant patient samples the clinical sample panel was designed to cover the range from 13.6 IU/mL to 4 log IU/mL (i.e., only the low and intermediate part of the measuring range) to overlap with the phagemid-based higher concentration panel members.

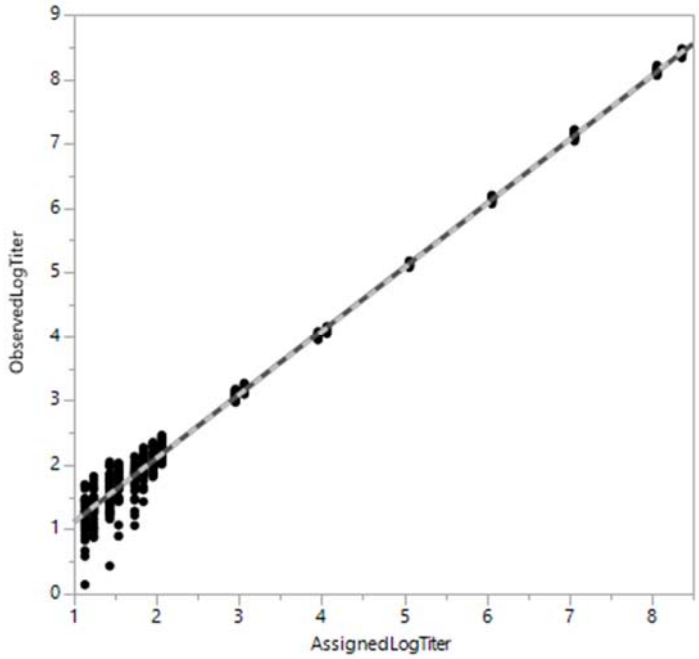
Panel member concentrations spanned the range of 1.5E+01 IU/mL to 2.00E+08 IU/mL (nominal concentration). Each panel member was tested in 36 replicates across three lots of cobas EBV test reagents (12 replicates/lot) and the results of the study are presented in **Table 4** and **Figure** 1 below. The Linearity panel was tested across three instruments by three operators. Resulting data were analyzed to identify the linear range according to CLSI guideline EP6-A and the best fitting polynomial regression fit.

Table 4: cobas EBV Linearity with EBV Genotype 1

Lot	Equation 1st Order $Y=b_0+b_1x$	Equation 2nd Order $Y=b_0+b_1x+b_2x^2$	Maximum Difference (log_{10} IU/mL)
Clinical Sample			
1	(b) (4)		
2	(b) (4)		N/A
3	(b) (4)		N/A
Phagemid			
1*	(b) (4)		
2	(b) (4)		N/A
3	(b) (4)		N/A
Clinical Sample and Phagemid Combined			
1	(b) (4)		N/A
2	(b) (4)		N/A
3	(b) (4)		N/A
ALL	(b) (4)		

b_0=Intercept; b_1=slope; all coefficients are provided in log_{10} IU/mL; best fitting model is bolded
N/A=not applicable; if linearity is described best by the 1st order model, there is no deviation to be shown between the 1st order model and any higher order model
* For this lot the 3rd order was the best fitting model, however, the difference to the 1st order regression model was so minor that it not shown here.

Figure 1: Linearity Across Both Panel Types (all lots)



Except for Lot 1 (clinical sample), the 1st order model is the best fitting model for all lots and all panel members (clinical sample and phagemid) when analyzed separately. However, for all panel member and lots combined the 2nd order model (b2) is significant at a 5% level. Therefore, the 2nd order polynomial was chosen as the best-fitting regression model for the analysis. However, the absolute difference between the 1st order and the better fitting 2nd order regression is minimal (i.e., equal or less than ± 0.01 $\log_{10}$ IU/mL). Across the linear range, the accuracy of the test was within ± 0.15 $\log_{10}$ IU/mL (Mean Square Error).

Based on the LLoQ (35 IU/mL) and the determined linear range, the claimed linear measurement range of the cobas EBV test is from 35 IU/mL (LLoQ) to 1.0E+08 IU/mL (ULoQ).

Verification of Linearity of cobas EBV with Genotype 2

Linearity was assessed with a cell culture derived EBV Genotype 2 panel (EBV strain Jiyoye) spanning the expected linear range from the 1 x 10^8 IU/mL (at or near expected ULoQ) to 30 IU/mL (at or near expected LLoQ). The following 8 panel members were prepared in EBV negative EDTA-plasma: 30 IU/mL, 150 IU/mL, 3, 4, 5, 6, 7, and 8 log IU/mL. Twelve replicates across three test-specific reagent lots were tested per concentration level (i.e., 4 replicates per kit lot). Data were analyzed according to CLSI guideline EP6-A and the 3rd order polynomial regression was the best fitting modes using a 5% significance level. The following equations were obtained.

1st order: $y = 0.2124 + 0.9713x$
2nd order: $y = 0.2800 + 0.9346x + 0.0039x^2$
3rd order: $y = 0.7323 + 0.5420x + 0.0980x^2 + -0.0066x^3$

The differences in log_{10} values calculated from the 1st order and the 3rd order regression were minor, ranging from -0.06 log IU/mL to +0.08 log IU/mL. Linearity for EBV genotype 2 is shown in
Figure 2. Across the linear range, the accuracy of the test was within ± 0.12 log_{10} IU/mL (Mean Square Error).

Figure 2: Verification of Linearity for EBV Genotype 2

Figure 1 Regression plot of EBV Genotype 2 – including outliers

d. ***Traceability, Stability, Expected values (controls, calibrators, or methods)***

i) Traceability

Several standards and controls were used during development of the cobas EBV test to provide traceability to the WHO EBV Standard [1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques (NIBSC 09/260)]. The standards used during development of the test include the WHO EBV Standard, the Roche Molecular Systems (RMS) EBV Secondary Standard, and the RMS EBV Calibration Panel.

Traceability of the calibration panel and the RMS EBV Secondary Standard to the 1st EBV WHO Standard was verified as shown in **Figure 3.**

The concentration range tested for the EBV WHO Standard was from (b) (4) to (b) (4), the RMS EBV Secondary Standard was tested at (b) (4), and the RMS EBV Calibration Panel was tested from (b) (4).

Figure 3: Traceability to the 1st WHO International Standard for EBV (NIBSC 09/260) using cobas EBV

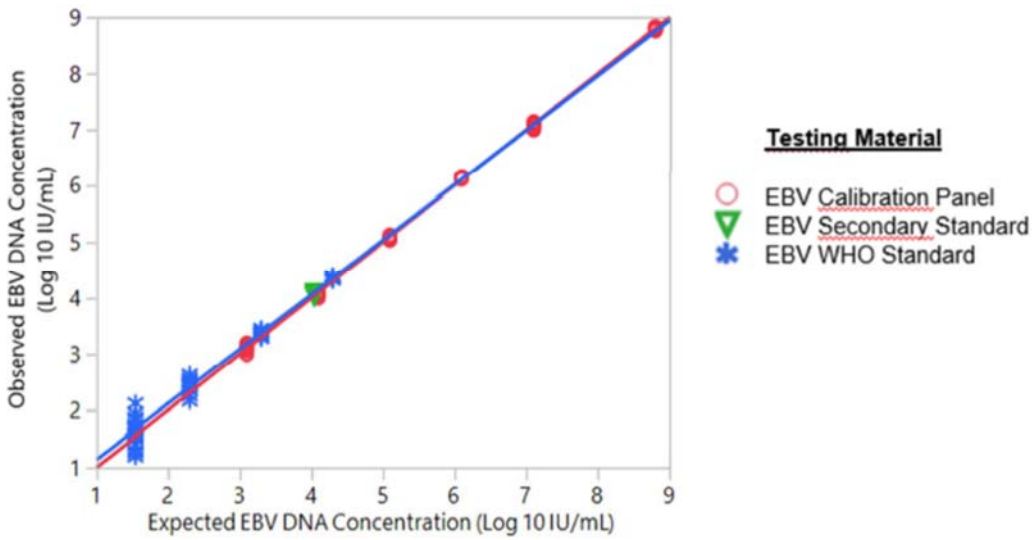


Figure 3 shows the bivariate fit of observed EBV DNA concentration (log_{10} IU/mL) by expected EBV DNA concentration (log_{10} IU/mL). Observed quantitation values are similar to the expected values with deviation of not more than 0.15 log_{10} IU/mL. All materials demonstrated co-linear dilution performance across the linear range of cobas EBV (Figure 2) The maximum deviation was observed at 200 IU/mL (approximately 6x LLoQ). The following linear regression equations were obtained:

EBV Calibration Panel: $y = 1.000x - 0.002$; $R^2 = 1.000$
EBV 1st WHO Standard: $y = 0.975x + 0.159$; $R^2 = 0.983$

Based on these results, the calibration and standardization process of cobas EBV provides quantitation values for the cobas EBV calibration panel and the RMS EBV Secondary Standard provide traceability to the 1st WHO international Standard for EBV.

ii) Expected values

To monitor the assay performance, reagent performance, and procedural errors, positive and negative external controls must be run in accordance with the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

External controls are provided separately from the cobas EBV test kit in the cobas EBV Positive Control Kit and the cobas Negative Control. The cobas EBV Positive Control includes an EBV High Positive control, and a Low Positive control containing EBV phagemid-DNA diluted into negative human plasma. The cobas Negative Control includes Tris buffer. The cobas Negative Control, the EBV Low Positive Control, and the EBV High Positive Control must be included in each run. The validity of the results for the controls as well as for the DNA-QS is determined by the assay specific analysis software package used by the cobas 6800/8800 instrument. The amount of EBV DNA for EBV high and low positive controls must fall within their acceptable titer ranges.

The failure rates of the kit controls and samples were assessed by evaluating outcomes of the nine analytical studies (LoD, LoD verification for Genotype, Linearity, Linearity verification for Genotype, Precision, Cross contamination, Lot interchangeability, Accuracy, and LoD in Plasma vs Buffer). Results demonstrated an overall invalid rate of 0% for QS and RMC and a sample failure rate of 0.02%.

iii) ***Stability***

iii.a. Clinical Specimen Stability

The clinical specimen stability of EBV viral target in whole blood specimens collected in EDTA-plasma preparation tubes and/or plasma samples after various storage conditions with or without freeze-thawing cycles was evaluated using cobas EBV for use on the cobas 6800/8800 Systems.

Freshly drawn whole blood from ten unique individual EBV-negative donors collected in EDTA-plasma tubes (five donor samples collected in PPT and five in lavender top tubes) were spiked with EBV to a concentration of 150 IU/mL (approximately 5xLLoQ). The reference time point (T0) was processed directly after spiking of the target by separating the blood into EDTA-plasma; all other samples were stored at indicated conditions until further processing. All samples used for this study in each PPT/ lavender top tube type were tested unspiked at time point 0 (T0).

All EBV-positive samples tested generated positive results for EBV and the mean $\log_{10}$ titers including the two-sided 95 confidence interval of each of the tested time points/conditions and tube types was within ±0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective reference condition (T0 = reference).

The results support the following storage conditions for whole blood collected in BD Vacutainer PPT or BD Vacutainer EDTA tubes (lavender top) and the respective separated plasma:

- Whole blood collected in EDTA-plasma tubes (lavender top and PPT) may be stored or transported for up to 24 hours at 2°C to 25°C before further processing and plasma separation.
- Afterwards, whole blood samples should be centrifuged, and the resulting plasma samples are additionally stable for:
  - 24 hours at 2°C to 30°C in primary or secondary tubes, followed by
  - up to 6 days at 2°C to 8°C in primary or secondary tubes, or
  - up to 6 months at -15°C to -80°C in secondary tubes.
- Plasma samples are stable in secondary tubes for up to four freeze/thaw cycles when frozen between -15°C to -80°C.

iii.b. Open kit and On-board Stability

The data submitted support the claim that, once opened, the cobas EBV 192T test-specific reagent cassettes are stable for up to 90 days at 2–8°C (Open Kit Stability) and remain stable for up to 40 hours at 37°C (On-Board Stability). Furthermore, 192T test-specific cassettes once opened are re-usable for up to 40 runs.

iii.c. Reagent Stability

Three lots of the cobas EBV were tested to demonstrate stability of the test-specific reagents of cobas EBV and cobas EBV Control Kit when stored at stressed temperature conditions (accelerated stability) and at the targeted storage temperature of 2°C to 8°C (real-time stability).

Real-time stability: The data submitted supports a shelf-life stability claim of 12 months when stored at 2–8°C.

e. ***Detection limit***

i) Limit of Detection (LoD) using the 1st WHO International Standard for EBV

The LoD of the cobas EBV test for the 1st WHO EBV Standard (Genotype 1) was determined by analysis of serial dilutions of the Standard diluted into a pooled EDTA-plasma derived from EBV IgG/IgM negative individuals following the recommendations in CLSI Guideline EP17-A2. Panels of six concentration levels plus a blank were tested with three lots of cobas EBV test reagents and three instruments with multiple runs and operators over a period of three days. Each dilution was determined in (b) (4) replicates per lot and day (n=(b) (4) total replicates per day). The results from testing the WHO EBV Standard in EDTA plasma as well as the calculated LoD values are shown in **Table 5**. The LoD values in **Table 5** were determined by Probit analysis and by 95% hit rate.

Table 5: LoD with EBV DNA 1st WHO International Standard in EDTA Plasma

Kit Lot	Nominal Concentration (IU/mL)	Number of Positive Replicates	Number of Valid Replicates	Hit Rate [%]	LoD by Probit [95% CI]
Lot 1	(b) (4)				

Kit Lot	Nominal Concentration (IU/mL)	Number of Positive Replicates	Number of Valid Replicates	Hit Rate [%]	LoD by Probit [95% CI]
Lot 2	(b) (4)				
Lot 3					
All lots combined					

When determined by Probit analysis, the different lots have similar LoD for all tested lots; the highest LoD of 18.8 IU/mL was obtained with lot 1, which is only slightly lower than the LoD determined by 95% hit rate. The LoD by 95% hit rate was (b) (4) and was the same for all tested lots. The claimed LoD value is (b) (4) determined by the least sensitive kit lot and this concentration was used in studies for confirmation of the LoD.

ii) Limit of Detection (LoD) Confirmation with EBV Genotype 2

The Limit of Detection (18.8 IU/mL) was verified for the cobas EBV test with EBV genotype 2 following the CLSI Guideline EP17-A2. EBV cell culture supernatants for genotype 2 strain Jiyoye (GT2-J) were diluted to three different concentration levels in EBV negative EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of cobas EBV reagents across three days of testing.

The results are shown in **Table 6** and verify that a hit rate of 95% or higher was observed above 18.8 IU/mL for EBV genotype 2. Thus, the observed hit rates verify the LoD for EBV Genotype 2 at 18.8 IU/mL.

Table 6: Verification of the LOD for EBV Genotype 2

Lot	Nominal Concentration (IU/mL)	Number of Positive Replicates	Number of Valid Replicates	Hit Rate [%]	LOD by Hit Rate
Lot 1	(b) (4)				
Lot 2					
Lot 3					
All Lots					

iii) Limit of Detection (LoD) in Plasma vs Buffer

This study evaluated whether the Limit of Detection in Generic Specimen Diluent (GSD) is equivalent to the LoD in EDTA-plasma in the cobas EBV assay, so that GSD can be used as a negative control for cobas EBV. Three independent dilution series (0.5x, 1.0x, and 1.5x LoD of cobas EBV) were prepared on three consecutive days using EBV WHO International Standard in GSD and tested.

The results (**Table 7**) demonstrated that the hit rate was 98.4% and 92.1% at 1.5xLoD and 1.0xLoD, respectively. The study demonstrated a comparable LoD performance of cobas EBV in Plasma and GSD.

Table 7: Hit Rates for all Dilution Series combined

Conc. Level	Conc. IU/mL	Hit Rate %	Two-sided 95% CI
(b) (4)			

f. ***Lower Limit of Quantitation (LLoQ)***

LLoQ using the 1st WHO International Standard for EBV

The LLoQ for cobas EBV was calculated using data generated from the Limit of Detection study using EBV WHO International Standard. The LLoQ was determined, per CLSI document EP17-A2, as the lowest titer within the linear range with a hit rate of at least 95% and at which the total analytical error (TAE) meets both of the following two criteria:

- The TAE, when calculated as (b) (4), and

– The TAE has to be such that the standard deviation for the difference between two measurements calculated as (b) (4)

The LLoQ was determined for each kit lot; all calculations are based on non-rounded values but the results shown in here are rounded.

Table 8: LLoQ - TAE and Difference between Measurements

Lot	Nominal Concentration (IU/mL)	Log_{10} titer Nominal	Mean log_{10} titer Observed	SD (log_{10})	Absolute Bias	TAE ([Bias] + 2SD)	Difference Between Measurements (SD)
1	20	1.30	1.34	0.38	0.04	0.80	1.07
	35	1.54	1.60	0.25	0.06	0.56	0.71
	50	1.70	1.74	0.22	0.04	0.49	0.63
2	20	1.30	1.29	0.37	0.01	0.75	1.05
	35	1.54	1.58	0.27	0.04	0.58	0.76
	50	1.70	1.77	0.23	0.07	0.53	0.65
3	20	1.30	1.33	0.31	0.03	0.65	0.88
	35	1.54	1.58	0.32	0.04	0.67	0.89
	50	1.70	1.76	0.21	0.06	0.48	0.60
All lots	20	1.30	1.32	0.35	0.02	0.73	1.00
	35	1.54	1.59	0.28	0.05	0.60	0.79
	50	1.70	1.76	0.22	0.06	0.50	0.63

The LLoQ was determined to be 35 IU/mL for lots 1 and 2 and 20 IU/mL for lot 3, calculated based on the calculation of the Total Analytical Error (TAE) and the difference between two measurements. The LLoQ for the cobas EBV test is 35 IU/mL.

g. ***Analytical specificity***

i) Cross reactivity

For potential cross reactants 35 microorganisms, including 17 viral isolates, 15 bacterial strains and three fungal isolates were used and divided into seven pools with 4 to 5 microorganisms per cross reactant pool and HCV as single interferent. Potential cross reactants in EBV-seronegative EDTA plasma were tested in the absence and presence of EBV DNA at a concentration of 5xLLoQ. Potential cross reactants were tested at (b) (4)

Results are shown in **Table 9**. For EBV-negative samples the negativity rate was determined. For EBV-positive samples the positivity rate was determined together with the correct

quantitation of EBV DNA by computing the Mean concentration detected across the replicates, the SD, and the difference between the control condition (no cross reactant) and the test condition containing the potential cross reactant organism. The mean $\log_{10}$ titer of each of the positive EBV samples containing potentially cross-reacting organisms was within $\pm$ 0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 9: Cross Reactivity

Pool	Organisms	Test Concentration	No EBV	EBV			
			Negativity Rate	Positivity Rate	Mean [Log_{10}]	SD [Log_{10}]	Mean Difference in log_{10} Titer
1	HSV 1	(b) (4)					
	HSV 2						
	HSV 6						
	HSV 7						
	HSV 8						
2	Adenovirus Type 5						
	Candida albicans						
	Chlamydia trachomatis						
	Clostridium perfringens						
	CMV						
3	Enterococcus faecalis						
	Escherichia coli						
	HBV						
	HIV-1						
	HIV-2						
4	Klebsiella pneumoniae						
	Listeria monocytogenes						
	Mycobacterium avium						
	Mycoplasma pneumoniae						
	Neisseria gonorrhoeae						

5	Parvovirus B19	(b) (4)
	Propionibacterium acnes	
	Salmonella enterica	
	Simian Virus 40	
	Staphylococcus aureus	
6	Staphylococcus epidermis	
	Streptococcus pyogenes	
	Streptococcus pneumoniae	
	VZV	
	Aspergillus niger	
7	Cryptococcus neoformans	
	Human Papilloma Virus (HPV)	
	JC Virus	
	BK Virus	
	Hepatitis C Virus (HCV)	
	Control (EBV negative)	
	Control (EBV positive) – for Pools 1-6 and HCV	
	Control (EBV positive) – for Pool 7	

*cp/mL= copies /mL

ii) Endogenous Interference

The effect of potentially interfering endogenous substances on the sensitivity/quantitation of cobas EBV was determined by testing 20 individual clinical EBV-seronegative samples spiked with selected endogenous substances and EBV target at 150 IU/mL (5xLLoQ). The negative sample spiked solely with EBV target was used as a Positive Spike Control (PSC). To analyze specificity, the same 20 individual clinical negative samples were individually spiked with potentially interfering endogenous substances and tested in the absence of EBV target DNA. The un-spiked samples were used as Negative Spiked Controls (NSC). Interferent concentrations were used as recommended by the CLSI guideline EP7-A2. Human DNA levels were tested at 2mg/mL. Control conditions were tested with one replicate per specimen, and test conditions were tested with 3 replicates per specimen. Results are summarized in **Table 10**.

Table 10: Endogenous Interference

Interferent	**C[1]**	**No EBV**	**EBV [150 IU/mL]**				
		Negativity Rate	**Positivity Rate**	**Mean Ct**	**Mean** [Log 10]	**SD** [Log 10]	**Mean Difference** in log10 Titer
Control	(b) (4)						
NaOH[2]							
Albumin							
Bilirubin (conj.)							
Bilirubin (unconj.)							
Human DNA							
Hemoglobin							
Triglycerides							

[1]C= Test Concentration; [2] solvent control; [3] 0.2 g/L = 342 µmol/L

EBV-negative samples with endogenous interferents all produced valid negative results (target not detected) in the presence of endogenous interferents.

For EBV-positive samples with endogenous interferents the mean $\log_{10}$ titer of each of the positive EBV samples containing endogenous interferents was within ± 0.05 $\log_{10}$ of the mean $\log_{10}$ titer of the spike control.

iii) Exogenous Interference

The effect of potentially interfering exogenous substances on the sensitivity/quantitation of cobas EBV was determined by testing 10 individual clinical EBV-negative samples spiked with pools of 24 commercially available drugs at three times the plasma peak level per CLSI EP7-A2. The same samples were also tested in the presence of EBV target at 150 IU/mL (5x LLoQ). The negative sample spiked solely with EBV target was used as a Positive Spike Control (PSC). The un-spiked samples were used as Negative Spiked Controls (NSC). Conditions were tested with 3 replicates per specimen. The following drugs were tested, and the results are summarized in **Table 11** below.

- Pool 1: Azathioprine, Sulfamethoxzole, Trimethoprim, Cefotan, Cidofovir
- Pool 2: Foscarnet, Piperacillin, Tazobactam, Prednisone, Vancomycin
- Pool 3: Cyclosporine, Everolimus, Fluconazole, Ganciclovir
- Pool 4: Mycophenolate mofetil, Mycophenolic acid, Valganciclovir
- Pool 5: Sirolimus, Tacrolimus
- Pool 6: Letermovir, Micafungin, Acyclovir Clavulanate potassium
- Pool 7: Ticarcillin disodium

Table 11: Exogenous Interference

Pool	No EBV	EBV [150 IU/mL]				
	Negativity Rate	Positivity Rate	Mean Ct	Mean [Log 10]	SD [Log 10]	Mean Difference in log10 Titer
Pool 1[1]	10/10 100%	30/30 100%	34.5	2.22	0.18	-0.18
Pool 2[1]	10/10 100%	30/30 100%	34.7	2.19	0.15	-0.21
Pool 3[2]	10/10 100%	30/30 100%	34.7	2.23	0.18	-0.17
Pool 4[2]	10/10 100%	30/30 100%	34.7	2.23	0.15	-0.17
Pool 5[3]	10/10 100%	30/30 100%	34.6	2.24	0.19	-0.16
Pool 6[1]	10/10 100%	30/30 100%	34.7	2.20	0.16	-0.20
Pool 7[1]	10/10 100%	30/30 100%	34.6	2.21	0.17	-0.19
PBS SC	10/10 100%	30/30 100%	34.4	2.30	0.19	-0.10
DMSO SC	10/10 100%	30/30 100%	34.5	2.26	0.15	-0.13
Ethanol SC	10/10 100%	30/30 100%	34.4	2.37	0.23	-0.03

Negative Control	10/10 100%	-	N/A	N/A	N/A	N/A
Positive Control	-	30/30 100%	34.2	2.4	0.22	N/A

The superscripts in Pools 1-7 indicate the solvent that was used for constituting the interferents (i.e., 1 = PBS; 2 = DMSO; and 3 = Ethanol); SC = solvent control.

h. Cross Contamination

The cross-contamination rate for cobas EBV was determined by testing 240 replicates of an EBV-negative matrix sample and 225 replicates of a high titer EBV sample at approximately 2.00E+07 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 240 replicates of the negative sample were negative, resulting in a cross-contamination rate of 0% (upper one-sided 95% confidence interval: 1.24%).

i. Assay cut-off

Not applicable

2. Comparison studies

a. Method comparison with predicate device

Not applicable

b. Matrix comparison

Not applicable

3. Clinical studies

Concordance of cobas EBV with a Comparator EBV Test

The clinical performance of cobas EBV was compared to a validated well-established comparator nucleic acid test(comparator EBV) by measuring EBV DNA levels in longitudinal clinical samples (neat and diluted) of EBV-infected and non-infected patients. Contrived EDTA plasma samples spiked with cultured EBV virus were used to cover the linear range.

The comparator EBV is well described, currently used in clinical practice at a major transplant center in the United States, is traceable to the WHO standard and its use is acceptable. Due to different methods of measuring EBV viral load at each institution, EBV viral load quantitation may vary between laboratories and hence should not be compared to make clinical management decisions.

A total of 464 samples (439 neat or diluted clinical samples of 72 transplant subjects and 25 contrived samples) were valid on both assays and evaluable for the clinical concordance

analysis. Results presented in **Table 12** demonstrate a column percent agreement between the cobas EBV and comparator EBV ranging from between 82.5% to 100% depending on the analyte concentration in the samples. DNA sequencing on representative samples from subjects with results consistently offset by more than 1 $\log_{10}$ IU/mL DNA level did not reveal any sequence mismatches for any primer or probe targets for the cobas EBV assay.

Table 12: Concordance analysis between cobas EBV and the comparator EBV DNA level results for all samples

	Comparator EBV ($\log_{10}$ IU/mL)						
cobas® EBV ($\log_{10}$ IU/mL)	**Target Not Detected**	**<LLoQ (<2)**	**2 to <2.6**	**2.6 to <3.2**	**3.2 to 3.8**	**> 3.8**	**Total**
Target Not Detected	95	17	17	0	0	0	129
< LLoQ (< 2)	39	46	75	11	0	0	171
2 to < 2.6	1	2	16	37	6	0	62
2.6 to < 3.2	1	0	5	15	30	1	52
3.2 to 3.8	0	0	0	0	9	11	20
> 3.8	0	0	0	0	1	29	30
Total	136	65	113	63	46	41	464
Column Agreement (%)	(134/136) 98.5%	(65/65) 100%	(96/113) 85.0%	(52/63) 82.5%	(40/46) 87.0%	(40/41) 97.6%	
(95% Score CI)[a]	(94.8, 99.6%)	(94.4%, 100%)	(77.2%, 90.4%)	(71.4%, 90.0%)	(74.3%, 93.9%)	(87.4%, 99.6%)	

Note: LLoQ = lower limit of quantitation of comparator EBV (100 IU/mL).
Standard Deviation of comparator EBV estimated at 0.3 $\log_{10}$ IU/mL (comparator EBV analytical precision study).
Paired samples evaluable for clinical concordance analysis were included in this table.
[a] Assumed independence between all samples; CI = Confidence Interval by Score Method.

Discordant results were defined as those that are more than one box away from the diagonal (indicated by shading). For Target Not Detected by comparator EBV Column Agreement the cobas EBV Target Not Detected and < LLoQ (< 2) cells were combined. The rationale for adding the adjacent < LLoQ and TND cells for the TND column is that the difference between a TND and < LLoQ is not clinically meaningful and that these are analytically at the lower end of the measuring range, which may be impacted by random error.

Forty one of the 43 comparator EBV negative samples collected for the estimation of the Negative Percent Agreement (NPA) with the cobas EBV were negative by cobas EBV; therefore, the NPA was 95.4% (95% Exact CI: 84.2%–99.4%). The two comparator EBV negative samples were positive (<LLoQ) by cobas EBV and were seropositive for EBV VCA IgG and EBNA-1 IgG by supplemental serology testing. Concordance between cobas EBV and the comparator EBV was also evaluated using different clinical thresholds as in **Table 13**.

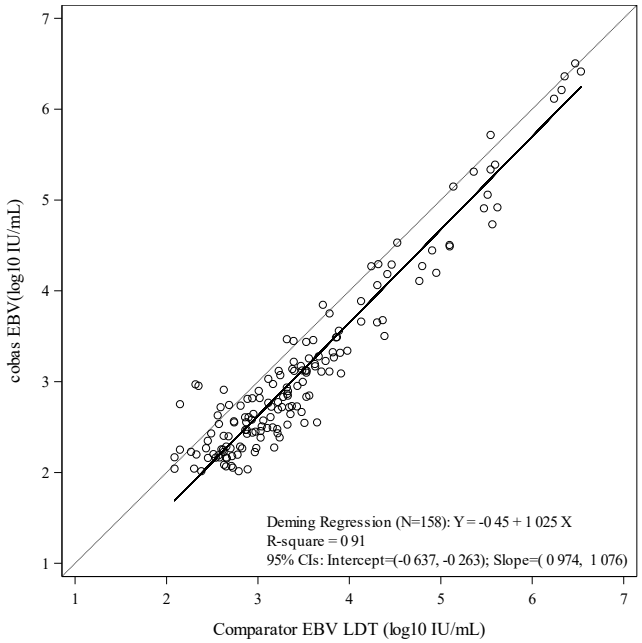
Table 13: Concordance summary of cobas EBV and comparator EBV using different thresholds

	Percent Agreement < Threshold (n/N) 95% CI	Percent Agreement ≥ Threshold (n/N) 95% CI
Target Not Detected	98.5% (134/136) (94.8, 99.6%)	89.6% (294/328) (85.9%, 92.5%)
LLoQ (2.0 Log_{10} IU/mL)	98.0% (197/201) (95.0%, 99.2%)	60.8% (160/263) (54.8%, 66.5%)
3.0 Log_{10} IU/mL	100.0% (363/363) (99.0%, 100.0%)	64.4% (65/101) (54.6%, 73.0%)
4.0 Log_{10} IU/mL	100.0% (431/431) (99.1%, 100.0%)	84.8% (28/ 33) (69.1%, 93.3%)

From all samples tested with cobas EBV that were EBV-positive with the comparator EBV there were a total of 158 (139 neat or diluted clinical samples of 28 transplant subjects and 19 contrived samples) that were evaluable for the correlation analysis at the three testing sites.

Analysis revealed no bias between neat clinical and contrived samples between the sites. Similarly, for the neat clinical and diluted samples the corresponding column percent agreements were poolable based on their 95% CIs, except for the "Target Not Detected" category where the 95%CI of column percent agreement did not overlap between neat clinical samples [90.6% (79.7%–95.9%)] and diluted clinical samples [57.3% (46.5%–67.5%)].

Figure 4: Correlation between cobas EBV and comparator EBV for all samples: Deming linear regression plot of DNA levels (log_{10} IU/mL)



Additional bias plot analysis of DNA level differences indicated a systematic difference between both assays that is constant across the overlapping linear range. The 95% CI of the intercept of the fitted line in the bias plots was (-0.456 to -0.104), which is within ±0.6 log_{10} IU/mL (± 2 times analytical precision standard deviation of comparator EBV).

External Controls

During the conduct of the clinical trial protocols, external control testing was performed according to the Instructions for Use.

c. Clinical specificity

Enrollment Inclusion/Exclusion Criteria

Study Demographics

d. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off**:**

Not applicable. Clinical thresholds for anti-EBV treatment are institution specific where they should follow professional guidelines (if available)

5. Expected values/Reference range:

   Not applicable

M. Instrument Name

cobas 6800/8800

N. System Descriptions

1. Modes of Operation
   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?
   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?
   Yes ___X_____ or No _____

2. Software
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes ___X_____ or No _____

   The device does not contain any software or instrument components.

3. Specimen Identification

4. Specimen Sampling and Handling

   See Instructions for Use.

5. Calibration

6. Quality Control

P. Labeling

The labeling supports the decision to grant the De Novo request for this device.

Q. Identified Risks to Health and Mitigation Measures

Identified Risk	Mitigation Measures
Risk of false results	Certain warnings, limitations, results interpretation information, and explanation of procedures in labeling. Certain device descriptions and specifications, analytical studies, clinical studies, and risk analysis in design verification and validation.
Failure to correctly interpret test results	Certain warnings, limitations, results interpretation information, and explanation of procedures in labeling.
Failure to correctly operate the device	Certain warnings, limitations, results interpretation information, and explanation of procedures in labeling.

R. Benefit/Risk Analysis:

Summary of the Assessment of Benefit

The benefits of the assay are the quantitation of Epstein-Barr virus (EBV) DNA in plasma samples from transplant patients. The assay will be used to monitor and treat patients after transplant for post-transplant lymphoproliferative disorders (PTLD) that are associated with EBV. PTLD are considered among the most serious and fatal complications of transplantation. Known sequelae of PTLD include continued symptoms and increased morbidity and mortality due to organ dysfunction. Monitoring and treatment of PTLD can result in improved patient outcomes by mitigating the sequelae of untreated disease, namely by preserving transplanted organs and eradicating PTLD, thus decreasing morbidity and mortality in these patients.

An additional benefit of the device is standardization of EBV viral load measurement in transplant patients as there are currently no FDA-approved alternatives marketed in the United States. Standardization of EBV viral load measurement will benefit patients by facilitating medical research about the clinical significance of specific viral load measurements with the device.

Summary of the Assessment of Risk

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results and failure to correctly operate the instrument.

Risks of incorrectly high test results are improper patient management, including imaging, biopsy, and/or implementation of treatment for PTLD with its associated side effects and risks such as rejection of a transplanted organ or graft. Risks of incorrectly low test results are improper patient management, including missing the opportunity to mitigate the known sequelae of PTLD, such as continued symptoms and increased morbidity and mortality due to organ dysfunction, preserving transplanted organs, and eradicating PTLD.

While the performance of the device in the clinical and analytical studies suggests that patients will benefit from the assay, expected and acceptable sources of uncertainty are the wide confidence intervals around point estimates during subgroup analysis. Another source of uncertainty of the benefits of the assay are that the physiological or clinically meaningful range of the diagnostic output are unknown. The special controls, including the interpretation of results and the limiting statements in device labeling will help to ensure that errors will be uncommon and will facilitate accurate assay implementation and interpretation of results.

A third source of uncertainty of the benefits of the assay is an imperfect comparator as there is currently no gold standard to measure EBV DNA levels. This uncertainty is acceptable given that the comparator in the clinical study is laboratory-developed test that is currently used in clinical practice at a major transplant center in the United States and is traceable to the World Health Organization (WHO) standard

Summary of the Assessment of Benefits-Risks:
The clinical benefits outweigh the risks for the proposed assay when considering the mitigations of the risks provided in the special controls as well as general controls. The special controls, including performance and total product life cycle commitments, an explanation of procedures, and the warnings, limiting statements, and results interpretation information in device labeling will help to ensure that errors will be rare and will facilitate accurate assay implementation and interpretation of results. The device's performance observed in the clinical study suggests that errors will be infrequent and that the assay will provide substantial benefits to patients, along with other clinical information, to measure EBV DNA to monitor and manage PTLD in appropriate populations.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

S. Conclusion

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:
Product Code(s): QLX
Device Type: Quantitative Viral Nucleic Acid Test for Transplant Patient Management
Class: II (special controls)
Regulation: 21 CFR 866.3183