Rx Only

cobas® EBV

Quantitative nucleic acid test
for use on the cobas® 5800/6800/8800 Systems

For in vitro diagnostic use

cobas® EBV	P/N: 09040943190
For use on the cobas® 5800 System	
cobas® EBV/BKV Control Kit	P/N: 09040951190
cobas® Buffer Negative Control Kit	P/N: 09051953190
For use on the cobas® 6800/8800 Systems	
cobas® EBV/BKV Control Kit	P/N: 09040951190 or P/N: 08688214190
cobas® Buffer Negative Control Kit	P/N: 09051953190 or P/N: 07002238190

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Intended 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**® 5800/6800/8800 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).

Summary and explanation of the test

Background

Transplant recipients are at increased risk for many viral and bacterial infections that are more likely to cause severe adverse health outcomes in the transplant patient population compared to the general healthy population. This increased risk is partly attributable to diminished immune system function conferred by the immunosuppressive medications that transplant patients receive in order to reduce their likelihood of graft rejection.[1,2]

EBV is a member of the herpes virus family. It is a double stranded, enveloped deoxyribonucleic acid (DNA) virus (~172kb). Two main EBV genotypes, type 1 and type 2, have been defined by the differences in the EBNA-2 gene. EBV infections in humans are quite common; more than 90% of adults are infected, and latent infection persists for life. EBV causes infectious mononucleosis in a subset of newly infected adolescents and adults, and is associated with several types of cancer, including nasopharyngeal carcinoma, Burkitt lymphoma, and Hodgkin lymphoma. EBV can be the cause of lymphoproliferative disorders in persons with congenital or acquired immunodeficiency, including transplant recipients and patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS).[3]

In transplant recipients, EBV can cause disease either through reactivation of latent virus from memory B cells, or through a new primary infection, especially in EBV-negative transplant recipients who receive grafts from EBV-positive donors.[3] For these patients, the most severe form of EBV-related disease is post-transplant lymphoproliferative disorder (PTLD), which results from uncontrolled proliferation of lymphocytes, typically B cells.[4] Overall, > 70% of PTLD cases among transplant recipients are linked to EBV infection. The highest risk for PTLD occurs during the first year after transplant, and >90% of PTLD cases that occur during this period are linked to EBV. Up to 20% of PTLD cases that occur after the first year post-transplant are EBV-negative.[4,5]

Risk factors for early-onset PTLD include serostatus at transplantation (EBV sero-negative for solid organ transplant, EBV sero-positive for hematologic stem cell transplant), younger age, exposure to lymphocyte-depleting antibodies, and type of organ transplanted.[5,6]

Early identification of primary EBV infections and DNA level monitoring can support prompt therapeutic intervention to prevent progression to EBV-related disease. Guidelines recommend regular EBV monitoring using quantitative nucleic

acid tests (NATs), especially in transplant recipients who are in a high risk serostatus group.[4,5] While the exact medically relevant viral DNA threshold is still a subject of debate due to inter-assay variability, the critical threshold concept appears valid and has been reported in natural history studies showing that higher EBV DNA levels correlate with increased risk for the development of EBV disease and PTLD.[4,5,7] Both plasma and whole blood sample types have been used for EBV testing, but evidence suggests that plasma is more specific for detection of PTLD.[4,5,7,8]

Common therapeutic interventions to reduce EBV DNA levels and prevent onset of PTLD include reduction of immunosuppression medication doses and treatment with B cell depleting antibodies.[7] Pre-emptive therapy to reduce EBV DNA levels is successful in most patients, although up to 20% of patients may still develop PTLD, especially those who are more than one year post-transplant.[7]

Most laboratory tests for EBV quantitation are not standardized, which has resulted in inter-laboratory and inter-assay variability in DNA level results and precludes comparison of DNA levels generated from different laboratories and tests.[7] To address this problem, the WHO has created an International Standard for EBV quantitation, allowing standardized tests to report in IU/mL.[9] Formal assessment of the reproducibility and validity of EBV DNA levels is critical to ensure consistent results across laboratories in order to improve the clinical management of patients at increased risk for developing EBV-related diseases and PTLD.

Rationale for NAT testing

EBV serologies of donor and recipient are determined before transplantation to help determine a transplant patient's risk of EBV-related complications, but serology is not sufficiently sensitive or precise for monitoring patients after transplantation. EBV culture methods are slow and have poor predictive value in this setting. Direct detection of EBV DNA by real-time PCR potentially offers a wide dynamic range, precision, and high sensitivity and specificity.

Explanation of the test

cobas® EBV is a quantitative test that is run on the **cobas**® 5800/6800/8800 Systems. **cobas**® EBV enables the detection and quantitation of EBV DNA in EDTA plasma of infected patients. The DNA level is quantified against a non-EBV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample processing. The DNA-QS also functions to monitor for the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Principles of the procedure

cobas® EBV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 5800 System is designed as one integrated instrument. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 5800 or **cobas**® 6800/8800 System software which assigns test results for all tests as either target not detected, EBV DNA detected < LLoQ (lower limit of quantitation), EBV DNA detected > ULoQ (upper limit of quantitation), or a value in the linear range LLoQ < x < ULoQ. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid from patient samples and added lambda DNA-QS molecules is simultaneously extracted. In summary, viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the sample is achieved by the use of a dual target virus specific approach from highly-conserved regions of the EBV located in the EBV EBNA-1 gene and the EBV BMRF gene. Selective amplification of DNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the EBV genome. A thermostable DNA polymerase enzyme is used for amplification. The target and DNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[10-12] Any contaminating amplicon from previous PCR runs is eliminated by the AmpErase enzyme, which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® EBV master mix contains two detection probes specific for EBV target sequences and one for the DNA-QS. The probes are labeled with target-specific fluorescent reporter dyes allowing simultaneous detection of EBV target and DNA-QS in two different target channels.[13,14] The fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probe to the specific single-stranded DNA templates results in cleavage by the 5'-to-3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and DNA-QS.

Reagents and materials

cobas® EBV reagents and controls

The materials provided for **cobas®** EBV can be found in Table 1. Materials required, but not provided can be found in Table 2 through Table 4, Table 8 and Table 9.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

Table 1 cobas® EBV

(EBV)

Store at 2-8°C

192 test cassette (P/N 09040943190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol EUH210: Safety data sheets available on request. EUH208: Contains Subtilisin from Bacillus subtilis. May produce an allergic reaction.	22.3 mL
DNA Quantitation Standard (DNA QS)	Tris buffer, < 0.05% EDTA, < 0.001% non-EBV DNA construct containing non-EBV primer binding and a unique probe region (non-infectious DNA), 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
EBV Master Mix Reagent 2 (EBV MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream EBV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for EBV and the EBV Quantitation Standard, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 **cobas**® EBV/BKV Control Kit

(EBV/BKV CTL)

Store at 2–8°C

For use on the **cobas**® 5800 System (P/N 09040951190)

For use on the **cobas**® 6800/8800 Systems (P/N 08688214190 or P/N 09040951190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
EBV/BKV Low Positive Control (EBV/BKV L(+)C)	< 0.001% synthetic (plasmid) EBV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, EBV DNA not detectable by PCR methods. The mean concentration of EBV DNA is approximately 1600 fold lower than the mean concentration of EBV DNA in the EBV/BKV H(+)C. 0.1% ProClin® 300 preservative**	4 mL (8 x 0.5 mL)	  **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
EBV/BKV High Positive Control (EBV/BKV H(+)C)	< 0.001% synthetic (plasmid) EBV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, EBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	4 mL (8 x 0.5 mL)	  **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance or mixture

Table 3 **cobas**® Buffer Negative Control Kit

(BUF (-) C)

Store at 2-8°C

For use on the **cobas**® 5800 System (P/N 09051953190)

For use on the **cobas**® 6800/8800 Systems (P/N 07002238190 or P/N 09051953190)

Kit components	Reagent ingredients	Quantity per kit
cobas® Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas® omni reagents for sample preparation

Table 4 **cobas® omni** reagents for sample preparation

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning*
cobas® omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas® omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas® omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate**, 5% (w/v) polydocanol**, 2% (w/v) dithiothreitol**, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411 Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas® omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance or mixture

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 5800 or **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® EBV	2–8°C
cobas® EBV/BKV Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas® **omni** Lysis Reagent	2–8°C
cobas® **omni** MGP Reagent	2–8°C
cobas® **omni** Specimen Diluent	2–8°C
cobas® **omni** Wash Reagent	15–30°C

Reagent handling requirements for the cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The system allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 5800 System.

Table 6 Reagent expiry conditions enforced by the **cobas**® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® EBV	Date not passed	90 days from first usage	Max 40 runs	Max 36 days**
cobas® EBV/BKV Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas® Buffer Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas® **omni** Lysis Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** MGP Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Specimen Diluent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Wash Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable

* Single use reagents.

Time is measured from the first time that reagent is loaded onto the **cobas® 5800 System.

Reagent handling requirements for the cobas® 6800/8800 Systems

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 7 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® EBV	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® EBV/BKV Control Kit	Date not passed	Not applicable*	Not applicable	Max 8 hours
cobas® Buffer Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 10 hours
cobas® **omni** Lysis Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** MGP Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Specimen Diluent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Wash Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable

* Single use reagents

** Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

Additional materials required for the cobas® 5800 System

Table 8 Materials and consumables for use on the **cobas®** 5800 System

Material	P/N
cobas® omni Processing Plate 24	08413975001
cobas® omni Amplification Plate 24	08499853001
cobas® omni Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1mL	04639642001
Tip CORE TIPS with Filter, 300µL	07345607001
cobas® omni Liquid Waste Container	07094388001
cobas® omni Lysis Reagent	06997538190
cobas® omni MGP Reagent	06997546190
cobas® omni Specimen Diluent	06997511190
cobas® omni Wash Reagent	06997503190
Solid Waste Bag or Solid Waste Bag With Insert	07435967001 or 08030073001
cobas® omni Secondary Tubes 13x75 (optional)*	06438776001

*Contact your local Roche representative for a detailed order list for sample racks.

Additional materials required for the cobas® 6800/8800 Systems

Table 9 Materials and consumables for use on the **cobas**® 6800/8800 Systems

Material	P/N
cobas® **omni** Processing Plate	05534917001
cobas® **omni** Amplification Plate	05534941001
cobas® **omni** Pipette Tips	05534925001
cobas® **omni** Liquid Waste Container	07094388001
cobas® **omni** Lysis Reagent	06997538190
cobas® **omni** MGP Reagent	06997546190
cobas® **omni** Specimen Diluent	06997511190
cobas® **omni** Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer Solid Waste Update	07435967001 and 07094361001 or 08030073001 and 08387281001
cobas® **omni** Secondary Tubes 13x75 (optional)*	06438776001

*Contact your local Roche representative for a detailed order list for sample racks.

Instrumentation and software required

The **cobas**® 5800 software and **cobas**® EBV analysis package for the **cobas**® 5800 System must be installed on the **cobas**® 5800 instrument. The Data Manager software and PC for the **cobas**® 5800 System will be provided with the system.

The **cobas**® 6800/8800 software and **cobas**® EBV analysis package must be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 10 Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
cobas® 6800 System (Option Moveable)	06379672001
cobas® 6800 System (Fix)	05524245001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001

Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for additional information.
Note: Contact your local Roche representative for a detailed order list for primary and secondary sample tubes, sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- **cobas®** EBV has not been evaluated for use as a screening test for the presence of EBV in blood or blood products or human cells, tissues, and cellular and tissue-based products (HCT/Ps).
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[15,16] Only personnel proficient in handling infectious materials and the use of **cobas®** EBV and **cobas®** 5800/6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water or follow appropriate site procedures.
- **cobas®** EBV/BKV Control Kit contains plasma derived from human blood. The source material has been tested by PCR methods and showed acceptable traces of low levels of EBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- Since > 90% of adults are chronic EBV carriers who may shed up to 10^8 EBV IU/mL in their saliva, and given the high sensitivity of the assay, it is important to implement adequate contamination control measures in laboratories.[17]

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas® omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.

- **cobas**® EBV, **cobas**® **omni** MGP Reagent, and **cobas**® **omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas**® **omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® EBV kits, EBV/BKV Low Positive Control (EBV/BKV L(+)C), EBV/BKV High Positive Control (EBV/BKV H(+)C), **cobas**® Buffer Negative Control (BUF (-) C) and **cobas**® **omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800 or **cobas**® 6800/8800 instruments, follow the instructions in the **cobas**® Systems – User Assistance and/or User Guides to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Store all samples at specified temperatures.

Sample stability is affected by elevated temperatures.

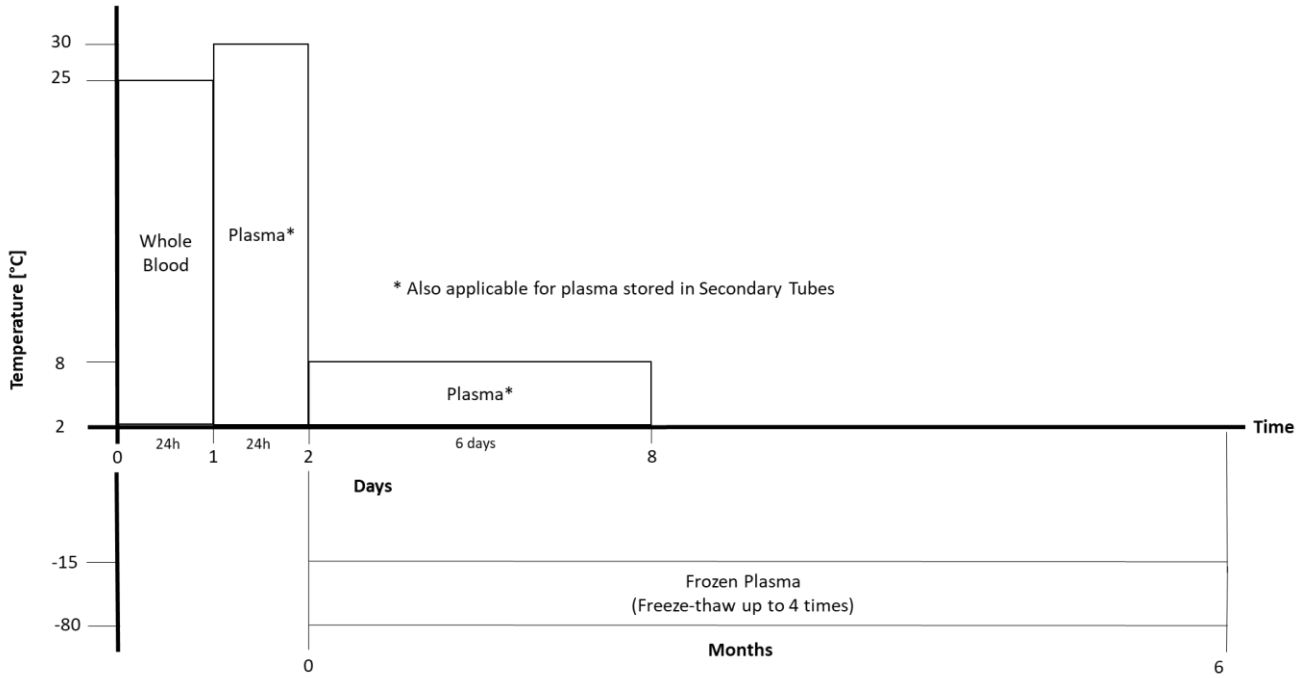
If using frozen samples in secondary tubes, place the samples at room temperature (15-30ºC) until completely thawed and then briefly mix (e.g. vortex for 3-5 seconds) and centrifuge to collect all sample volume at the bottom of the tube.

After centrifugation, if there is potential that cells have re-suspended into the plasma consider re-centrifugation before processing on the instrument.

Samples

- Whole blood should be collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions. Refer to Figure 1.
- Whole blood collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 24 hours at 2-25°C prior to plasma preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation plasma samples may be stored for 24 hours at 2-30°C in primary or secondary tubes, followed by:
    - Storage in primary or secondary tubes for up to 6 days at 2-8°C.
    - Storage in secondary tubes for up to 6 months at -15°C to -80°C.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at -15°C to -80°C.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Figure 1 Sample storage conditions



Instructions for use

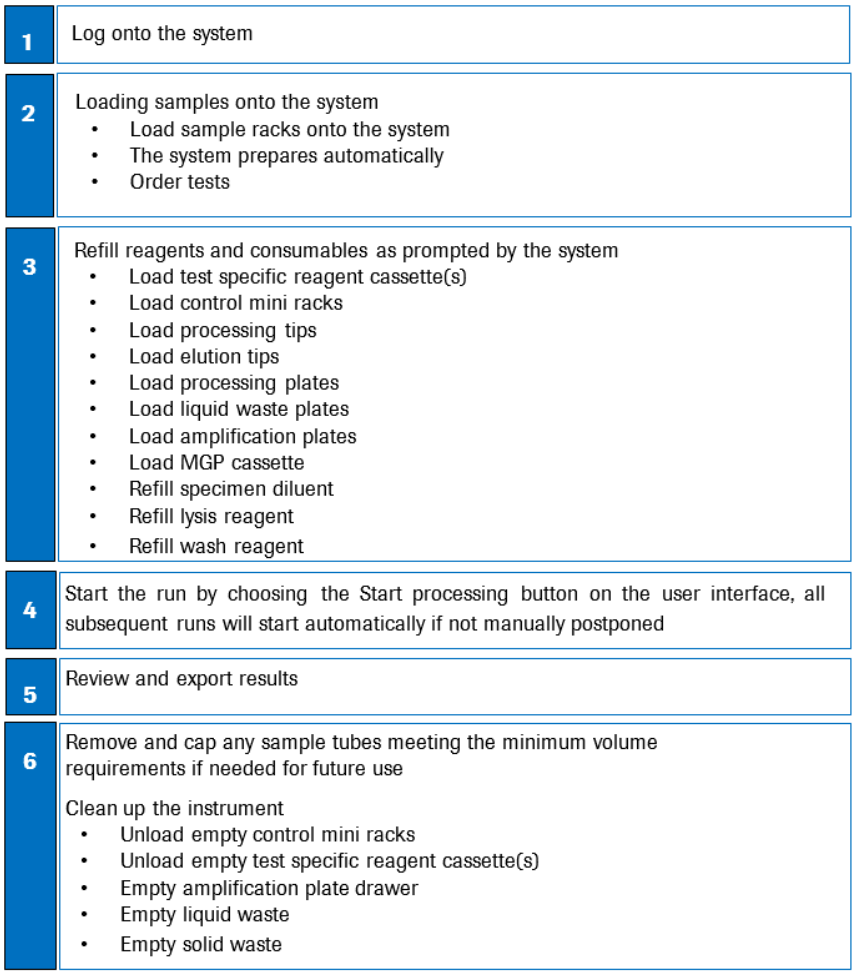
Procedural notes

- Do not use **cobas**® EBV reagents, **cobas**® EBV/BKV Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas**® **omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for proper maintenance of instruments.

Running cobas® EBV on the cobas® 5800 System

cobas® EBV can be run with a minimum sample volume of 350 µL of which 200 µL is processed when using **cobas**® **omni** secondary tubes. The test procedure is described in detail in the **cobas**® 5800 Systems User Assistance and/or User Guide. Figure 2 below summarizes the procedure.

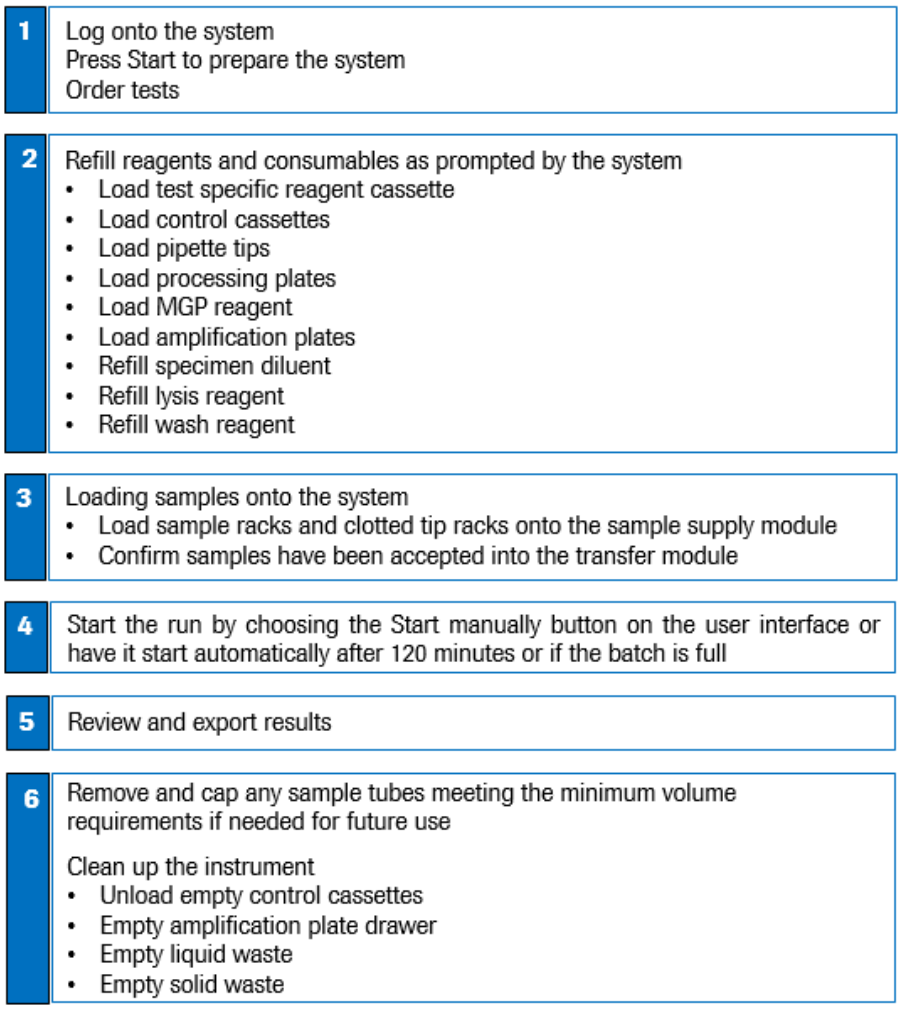
Figure 2 **cobas**® EBV procedure on the **cobas**® 5800 System



Running cobas® EBV on the cobas® 6800/8800 Systems

cobas® EBV can be run with a minimum sample volume of 350 µL of which 200 µL is processed when using **cobas**® **omni** secondary tubes. The test procedure is described in detail in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide. Figure 3 below summarizes the procedure.

Figure 3 **cobas**® EBV procedure on the **cobas**® 6800/8800 Systems



Results

The **cobas**® 5800/6800/8800 Systems automatically determine the EBV DNA concentration for the samples and controls. The EBV DNA concentration is expressed in International Units per milliliter (IU/mL).

Quality control and validity of results on the cobas® 5800 System

- One negative control [(-) Ctrl] and two positive controls, a low positive control [EBV/BKV L (+) C] and a high positive control [EBV/BKV H (+) C] are processed at least every 72 hours and with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 software and/or report, check for flags and their associated results to ensure the result validity.

Invalidation of results is performed automatically by the **cobas**® 5800 software based on negative or positive control failures.

NOTE: The **cobas**® 5800 System will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Control results on the cobas® 5800 System

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all Targets of the control are reported valid. Controls are marked with "Invalid" in the column "Control result" if all or one Target of the control are reported invalid.
- Controls marked with "Invalid" show a flag in the "Flags" column. More information on why the control is reported invalid including flag information is shown in the detail view
- If one of the controls is invalid, repeat testing of all controls and all associated samples is required.

Quality control and validity of results on the cobas® 6800/8800 Systems

- One negative control [(–) Ctrl] and two positive controls, a low positive control [EBV/BKV L (+) C] and a high positive control [EBV/BKV H (+) C] is processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: EBV/BKV L (+) C, EBV/BKV H (+) C. The negative control result is displayed as (–) Ctrl and the low and high positive controls are displayed as EBV/BKV L (+) C and EBV/BKV H (+) C.

Invalidation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control failures.

Control flags on the cobas® 6800/8800 Systems

Table 11 Control flags for negative and positive controls

Negative Control	**Flag**	**Result**	**Interpretation**
(–) Ctrl	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the negative control is not negative.
Positive Control	**Flag**	**Result**	**Interpretation**
EBV/BKV L (+) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.
EBV/BKV H (+) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

If the control batch is invalid, repeat testing of all samples of affected batch

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**® 5800 and **cobas**® 6800/8800 System software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.

Table 12 Target results for individual target result interpretation

Results	**Interpretation**
Target Not Detected	EBV DNA not detected. Report results as “EBV not detected”.
< Titer Min[a]	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as “EBV detected, less than (Titer Min)”. Titer min = 35.0 IU/mL
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max. Report results as “(Titer) of EBV detected”.
> Titer Max[b]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as “EBV detected, greater than (Titer Max)”. Titer max = 1.0E+08 IU/mL

[a] Sample results < Titer min (Target Detected < LLoQ) should be interpreted with the context of other clinical data and should not be the sole basis for treatment decisions.

[b] Sample result > Titer Max refers to EBV positive samples detected with titers above the upper limit of quantitation (ULoQ). If a quantitative result is desired, the original sample should be diluted with EBV-negative human EDTA plasma and the test should be repeated. Multiply the reported result by the dilution factor.

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 software in the "Results" app.

For a valid control batch, check each individual sample for flags in the **cobas**® 5800 software and/or report. The result interpretation should be as follows:

- Samples associated with a valid control batch are shown as 'Valid' in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if all Control Target Results reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
  - Q05D : Result validation failure because of an invalid positive control
  - Q06D :Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as show in Table 14 above.
- If one or more sample targets are marked with "Invalid" the **cobas**® 5800 software shows a flag in the "Flags"column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Interpretation of results on the cobas® 6800/8800 Systems

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 Systems software and/or report. The result interpretation should be as follows:

- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values for individual sample target result should be interpreted as shown in Table 12 above.

Procedural limitations

- Recommendations regarding monitoring EBV viral load post-transplant and medically relevant EBV DNA thresholds vary among transplant type and transplant institutions.
- While elevated EBV viral load may suggest post-transplant lymphoproliferative disorders (PTLD), the diagnosis of PTLD is made based on histological evaluation of tissue biopsy. PTLD may be present without detectable EBV viral load, and an increase in EBV viral load is not necessarily diagnostic of PTLD.
- **cobas**® EBV test results should be interpreted in the context of other clinical data and should not be the sole basis for treatment decisions.
- **cobas**® EBV has been evaluated only for use in combination with the **cobas**® EBV/BKV Control Kit, **cobas**® Buffer Negative Control Kit, **cobas**® **omni** MGP Reagent, **cobas**® **omni** Lysis Reagent, **cobas**® **omni** Specimen Diluent, and **cobas**® **omni** Wash Reagent for use on the **cobas**® 5800/6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been validated only for use with EDTA plasma. Testing of other sample types with **cobas**® EBV may result in inaccurate results. Plasma DNA level measurements are not directly comparable to those of other sample types.

- Quantitation of EBV DNA may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.As with any molecular test, mutations within the target regions of **cobas**® EBV could affect primer and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
- Due to the potential for variability in EBV DNA measurements across different EBV assays, it is recommended that the same device be used for the serial quantitation of EBV DNA when managing individual patients.
- **cobas**® EBV is not intended for use as a screening test for the presence of EBV in blood or blood products or human cells, tissues, and cellular and tissue-based products (HCT/Ps).

Non-clinical performance evaluation

Key performance characteristics performed on the cobas® 6800/8800 Systems

Limit of Detection (LoD)

The limit of detection (LoD) of **cobas**® EBV was determined by analysis of serial dilutions of the WHO International Standard (genotype 1) and verified for genotype 2. The overall concentration for which 95% hit rate is expected by PROBIT is 18.8 IU/mL for EDTA plasma.

WHO International Standard

The limit of detection of **cobas**® EBV was determined by analysis of serial dilutions of the 1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques (1st EBV WHO International Standard) obtained from NIBSC (NIBSC 09/260), in EBV-negative human EDTA plasma. Panels of six concentration levels plus a blank were tested over three lots of **cobas**® EBV reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma are shown in Table 13 through Table 15. The study demonstrates that with the least sensitive lot, the concentration for which 95% hit rate is expected by PROBIT is 18.8 IU/mL with a 95% confidence range of 14.5 to 27.5 IU/mL in EDTA plasma. The lowest concentration with a hit rate ≥ 95% is 20.0 IU/mL in EDTA plasma.

Table 13 EBV DNA WHO International Standard Limit of Detection in EDTA plasma, Lot 1

Input titer concentration (EBV DNA IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
50.0	63	63	100.0
35.0	63	62	98.4
20.0	63	61	96.8
10.0	63	53	84.1
5.0	63	37	58.7
2.5	63	26	41.3
0.0	63	0	0.0

LoD by PROBIT at 95% hit rate: 18.8 IU/mL, 95% confidence range: 14.5 – 27.5 IU/mL

Table 14 EBV DNA WHO International Standard Limit of Detection in EDTA plasma, Lot 2

Input titer concentration (EBV DNA IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
50.0	63	63	100.0
35.0	63	63	100.0
20.0	63	63	100.0
10.0	63	58	92.1
5.0	63	35	55.6
2.5	63	20	31.8
0.0	63	0	0.0

LoD by PROBIT at 95% hit rate: 12.4 IU/mL, 95% confidence range: 10.0 – 17.0 IU/mL

Table 15 EBV DNA WHO International Standard Limit of Detection in EDTA plasma, Lot 3

Input titer concentration (EBV DNA IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
50.0	63	63	100.0
35.0	63	63	100.0
20.0	63	62	98.4
10.0	63	48	76.2
5.0	63	38	60.3
2.5	63	26	41.3
0.0	63	0	0.0

LoD by PROBIT at 95% hit rate: 18.6 IU/mL, 95% confidence range: 14.4 – 27.1 IU/mL

Limit of Detection for genotype 2

EBV cell culture supernatant for genotype 2 was 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.

The combined results from three lots shown in Table 16 verify that – consistent with an LoD of 18.8 IU/mL – **cobas**® EBV detected EBV DNA for genotype 2 at a concentration of 18.8 IU/mL with a ≥ 95% hit rate.

Table 16 EBV DNA genotype 2 verification of Limit of Detection in EDTA plasma

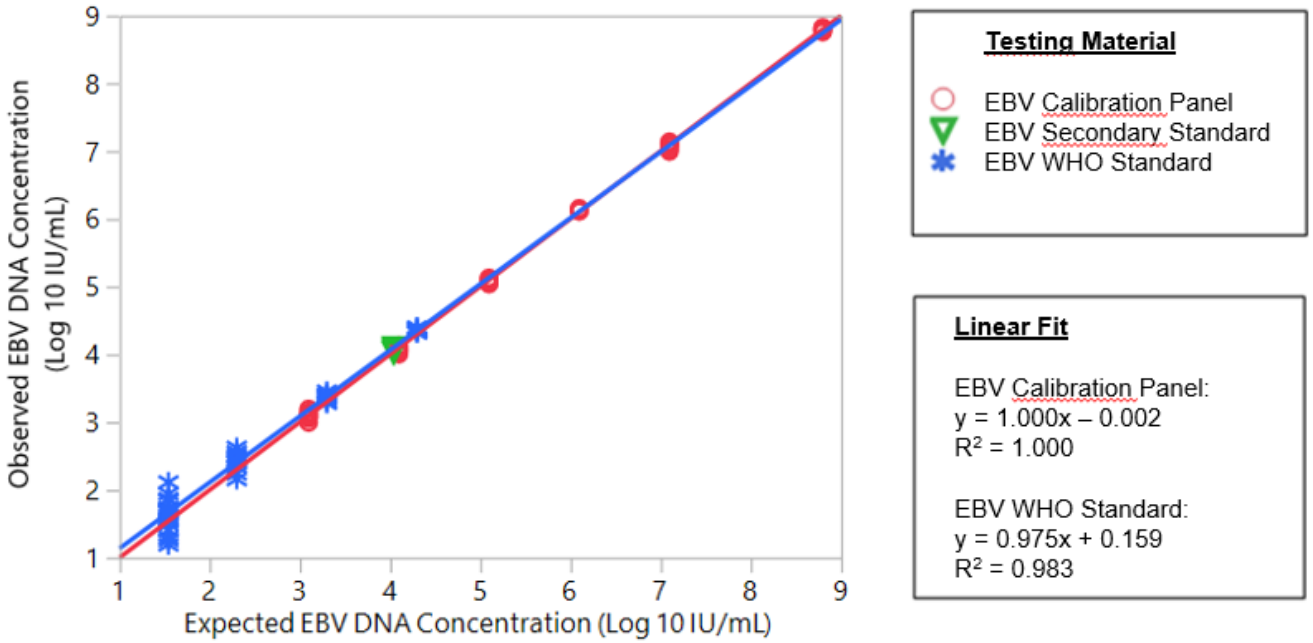
Genotype	Test concentration	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100
GT 2	9.4 IU/mL	63	58	92.1%
GT 2	18.8 IU/mL	63	62	98.4%
GT 2	28.2 IU/mL	63	63	100.0%

Traceability to the 1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques (NAT)-based assays

Several standards and controls have been used during development of this test to provide traceability to the WHO standard [the 1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques (NIBSC 09/260)[18]]. The standards used during development of the test include the EBV WHO Standard, the RMS EBV Secondary Standard, and the RMS EBV Calibration Panel. The Standards and the Calibration Panel were tested. The concentration range tested for the EBV WHO Standard was from 3.50E+01 IU/mL to 2.00E+04 IU/mL (1.54-4.30 $\log_{10}$ IU/mL), the RMS EBV Secondary Standard was tested at 1.10E+04 IU/mL (4.04 $\log_{10}$ IU/mL), and the RMS EBV Calibration Panel was tested from 1.00E+03 IU/mL to 5.00E+08 IU/mL (3.00-8.70 $\log_{10}$ IU/mL).

The calibration and standardization process of **cobas**→ EBV provides quantitation values for the calibration panel, the RMS EBV Secondary Standard, and the EBV WHO Standard that are similar to the expected values with deviation of not more than 0.15 $\log_{10}$ IU/mL (Figure 3). The maximum deviation was obtained at 200 IU/mL (approximately 6-fold the test LLoQ).

Figure 3 Traceability to WHO International Standard [bivariate fit of observed EBV DNA concentration ($\log_{10}$ IU/mL) by expected EBV DNA concentration ($\log_{10}$ IU/mL)] using **cobas**® EBV



Linear range

Linearity of **cobas**® EBV was evaluated using a dilution series consisting of 17 panel members with EBV genotype 1 DNA spanning the assay linear range. A high titer lambda DNA stock was used to prepare 11 panel members spanning the entire linear range. A clinical specimen was used to prepare 6 panel members covering the intermediate and lower levels of the linear range.

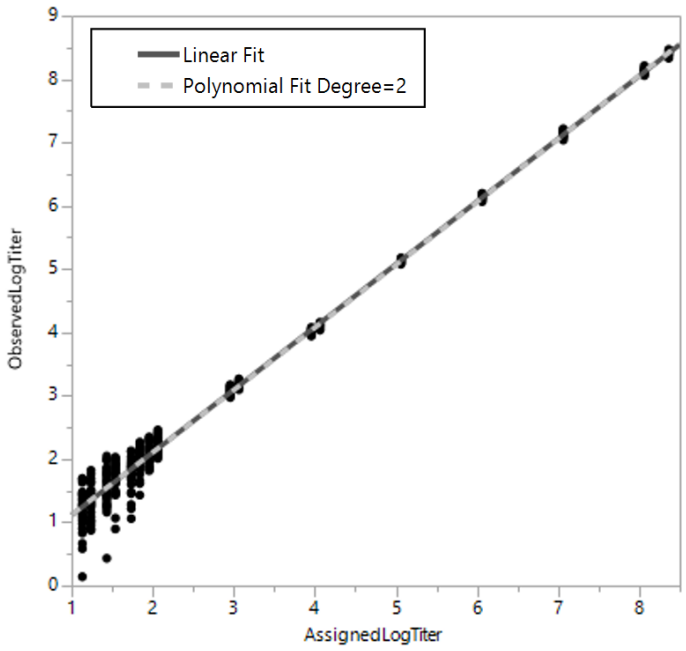
Each panel member was tested in 36 replicates across three lots of **cobas**® EBV reagents and the results of the study are presented in Figure 4.

cobas® EBV was demonstrated to be linear from 1.40E+01 IU/mL to 2.30E+08 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less or equal than ± 0.1 $\log_{10}$ in human EDTA plasma (see Figure 4). Across the linear range, the accuracy of the test was within ± 0.2 $\log_{10}$.

The lower limit of quantitation (LLoQ) is 35.0 IU/mL, calculated based on a goal for acceptable total analytical error (TAE) of ≤ 1.0 $\log_{10}$, where TAE = |bias| + 2 standard deviations in alignment with the CLSI EP-17A2 guideline, and TAE = SQUARE ROOT(2) x 2 standard deviations based on the "difference between 2 measurements" approach.

Based on the LLoQ and the determined linear range, as well as the medical value the linear measurement range of the test was set to 35.0-1.0E+08 IU/mL. The results of calculation and claimed LLoQ are shown in Table 18.

Figure 4 Linear range determination in EDTA plasma



Linearity for genotype 2

The dilution series used in the verification of genotypes linearity study of **cobas**® EBV consisted of eight panel members spanning the linear range of the assay. Testing was conducted with three lots of **cobas**® EBV reagent, 12 replicates per level were tested in EDTA plasma. The results of the study are presented in Table 17.

The linearity within the linear range of **cobas**® EBV was verified for genotype 2. The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.3 $\log_{10}$.

Table 17 Linearity verification on genotype 2

EBV genotype	Linear regression	Better fitting higher order model regression	Maximum difference between linear regression and the better fitting higher order model ($\log_{10}$ IU/mL)
GT 2	$y = 0.9713x + 0.2124$	$y = 0.0066x^3 + 0.0980x^2 + 0.5420x + 0.7323$	0.08

Lower Limit of Quantitation

The analysis for LLoQ was performed with data obtained from the LoD study at concentration levels of 20.0 IU/mL, 35.0 IU/mL and 50.0 IU/mL. The LLoQ is the lowest titer within the linear range that is not lower than the LoD and meets the acceptance criterion for the Total Analytical Error (|Bias| + 2x SD) (TAE). The TAE criterion is ≤ 1$\log_{10}$.

The results of calculation and claimed LLoQ are shown in Table 18, the lower limit of quantitation (LLoQ) is 35.0 IU/mL.

Table 18 Lower Limit of Quantitation (LLoQ) of **cobas**® EBV using the 1st WHO International Standard for Epstein-Barr Virus (EBV) (NIBSC 09/260)

Lot	Nominal concentration (IU/mL)	$\log_{10}$ titer nominal	Mean $\log_{10}$ titer observed	SD ($\log_{10}$)	Absolute Bias	TAE (|Bias| + 2x SD)	Difference between Measurements in SD (= SQRT(2) x 2x SD)
1	20.0	1.30	1.34	0.38	0.04	0.80	1.07
1	35.0	1.54	1.60	0.25	0.06	0.56	0.71
1	50.0	1.70	1.74	0.22	0.04	0.49	0.63
2	20.0	1.30	1.29	0.37	0.01	0.75	1.05
2	35.0	1.54	1.58	0.27	0.04	0.58	0.76
2	50.0	1.70	1.77	0.23	0.07	0.53	0.65
3	20.0	1.30	1.33	0.31	0.03	0.65	0.88
3	35.0	1.54	1.58	0.32	0.04	0.67	0.89
3	50.0	1.70	1.76	0.21	0.06	0.48	0.60
3 lots combined	20.0	1.30	1.32	0.35	0.02	0.73	1.00
3 lots combined	35.0	1.54	1.59	0.28	0.05	0.60	0.79
3 lots combined	50.0	1.70	1.76	0.22	0.06	0.50	0.63

Precision – within laboratory

Precision of **cobas**® EBV was determined by analysis of serial dilutions of high titer EBV DNA (genotype 1) in EBV-negative EDTA plasma. Seven dilution levels were tested in 72 replicates for each level across three lots of **cobas**® EBV reagents using three instruments and three operators over 12 days. Each sample was carried through the entire **cobas**® EBV procedure on fully automated **cobas**® 6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. The results are shown in Table 19. The results of the variance component estimation are shown in Table 20.

cobas® EBV showed high precision for three lots of reagents tested across a concentration range of 6.48E+01 IU/mL to 5.40+07 IU/mL.

Table 19 Within-laboratory precision of **cobas**® EBV*

Nominal concentration [IU/mL]	Assigned concentration [IU/mL]	EDTA plasma Lot 1 SD	EDTA plasma Lot 2 SD	EDTA plasma Lot 3 SD	EDTA plasma All lots Pooled SD
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.

Table 20 Lognormal Percent Coefficient of Variation (%CV) of **cobas**® EBV by positive panel and contributing components of variance*

Nominal concentration Titer (IU/mL)	Nominal concentration Log_{10} titer (IU/mL)	Assigned concentration Titer (IU/mL)	Assigned concentration Log_{10} titer (IU/mL)	N	Instrument/ Operator %CV	Lot %CV	Day %CV	Run %CV	Within Run %CV	Total %CV
5.00E+07	7.70	5.40E+07	7.73	72	5%	1%	2%	2%	8%	10%
1.00E+06	6.00	1.08E+06	6.03	72	1%	4%	1%	3%	4%	7%
1.00E+05	5.00	1.08E+05	5.03	72	2%	2%	3%	2%	5%	7%
1.00E+04	4.00	1.08E+04	4.03	72	2%	1%	3%	3%	7%	8%
1.00E+03	3.00	1.08E+03	3.03	72	4%	1%	4%	4%	10%	12%
1.00E+02	2.00	1.08E+02	2.03	72	3%	5%	8%	14%	42%	43%
6.00E+01	1.78	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%

Analytical specificity

The analytical specificity of **cobas**® EBV was evaluated by testing a panel of microorganisms at a concentration of 1.00E+06 units/mL (cells/mL, CFU/mL, IFU/mL, CCU/mL) for bacteria and yeast and between 3.00E+05 and 1.00E+06 units/mL (IU/mL, copies/mL, cells/mL, $TCID_{50}$/mL) for viruses. Microorganisms were diluted into EBV DNA negative human EDTA plasma as well as human EDTA plasma containing (150 IU/mL) EBV DNA. The specific organisms tested are listed in Table 21. Each sample was tested in replicates of three. None of the non-EBV pathogens interfered with test performance at the concentrations tested. Negative results were obtained with **cobas**® EBV for all microorganism samples without EBV target and positive results were obtained for all of the microorganism samples with EBV target. Furthermore, the mean log_{10} titer of each of the positive EBV samples containing potentially cross-reacting organisms was within ± 0.5 log_{10} of the mean log_{10} titer of the respective positive spike control.

Table 21 Microorganisms tested for cross-reactivity

Viruses	Bacteria	Yeast
Adenovirus Type 5	*Propionibacterium acnes*	*Aspergillus niger*
BK Polyomavirus	*Staphylococcus aureus*	*Candida albicans*
Cytomegalovirus	*Chlamydia trachomatis*	*Cryptococcus neoformans*
Hepatitis B Virus	*Clostridium perfringens*	-
Hepatitis C Virus	*Enterococcus faecalis*	-
Herpes Simplex VirusType 1	*Escherichia coli*	-
Herpes Simplex VirusType 2	*Klebsiella pneumoniae*	-
Human Herpes Virus Type-6	*Listeria monocytogenes*	-
Human Herpes Virus Type-7	*Mycobacterium avium*	-
Human Herpes Virus Type-8	*Neisseria gonorrhoeae*	-
Human Immunodeficiency Virus-1	*Staphylococcus epidermidis*	-
Human Immunodeficiency Virus-2	*Streptococcus pyogenes*	-
Human Papillomavirus	*Mycoplasma pneumoniae*	-
JC Virus	*Salmonella enterica*	-
Parvovirus B19 Simian Virus 40	*Streptococcus pneumoniae*	-
Varicella-Zoster Virus	-	-

Interfering substances

Elevated levels of triglycerides (37 mmol/L), conjugated bilirubin (0.2 g/L), unconjugated bilirubin (0.2 g/L), albumin (60 g/L), hemoglobin (2 g/L) and human DNA (2 mg/L) in samples were tested in the presence (150 IU/mL) and absence of EBV DNA. The tested endogenous interferences were shown not to interfere with the test performance of **cobas**® EBV.

In addition, drug compounds listed in Table 22 were tested at three times the C_{max} in presence (150 IU/mL) and absence of EBV DNA.

All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with **cobas**® EBV for all samples without EBV target and positive results were obtained on all of the samples with EBV target. Furthermore, the mean log_{10} titer of each of the positive EBV samples containing potentially interfering substances was within ± 0.5 log_{10} of the mean log_{10} titer of the respective positive spike control.

Table 22 Drug compounds tested for interference with the quantitation of EBV DNA by **cobas®** EBV

Class of drug	Generic drug name	Test concentration
Antimicrobial	Cefotetan	711 µg/mL
Antimicrobial	Clavulanate potassium	3 µg/mL
Antimicrobial	Fluconazole	20.1 µg/mL*
Antimicrobial	Piperacillin	894 µg/mL*
Antimicrobial	Tazobactam sodium	112 µg/mL
Antimicrobial	Sulfamethoxazole	204 µg/mL
Antimicrobial	Ticarcillin disodium	972 µg/mL
Antimicrobial	Trimethoprim	41 µg/mL
Antimicrobial	Vancomycin	189 µg/mL
Antimicrobial	Micafungin	49.2 µg/mL*
Compounds for Treatment of Herpes Viruses	Ganciclovir	27 µg/mL
Compounds for Treatment of Herpes Viruses	Valganciclovir	16.8 µg/mL
Compounds for Treatment of Herpes Viruses	Acyclovir	16.8 µg/mL*
Compounds for Treatment of Herpes Viruses	Cidofovir	60 µg/mL
Compounds for Treatment of Herpes Viruses	Foscarnet	1869 µmol/L
Compounds for Treatment of Herpes Viruses	Letermovir	39 µg/mL
Immune suppressant	Azathioprine	3 µg/mL
Immune suppressant	Cyclosporine	5.4 µg/mL
Immune suppressant	Everolimus	12 µg/mL
Immune suppressant	Mycophenolate mofetil	75 µg/mL
Immune suppressant	Prednisone	36 µg/mL
Immune suppressant	Sirolimus	0.045 µg/mL
Immune suppressant	Tacrolimus	0.21 µg/mL
Immune suppressant	Mycophenolic acid	111 µg/mL

*Drug compounds were tested at concentrations lower than three times the C_{max}

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%).

Clinical performance evaluation performed on the cobas® 6800/8800 Systems

Reproducibility of cobas® EBV

The reproducibility of **cobas®** EBV was evaluated across factors (reagent lot, test site, batch and testing days) that could affect reported results in routine clinical testing. The evaluation was conducted at 3 testing sites, using 3 reagent lots, of a positive and a negative sample panel with a total number 270 tests (not including controls). The panels were made from EDTA plasma that was EBV VCA IgG negative and were tested for EBV with a plasma NAT release protocol, and spiked with an EBV WHO international standard, EBV cell culture supernatant or lambda phagemid with EBV DNA. Two operators at each site tested each reagent lot for 5 days. Two runs (1 run = 1 batch; 1 batch = 1 panel + 3 controls) per operator were performed each day and 3 replicates of each panel member were performed for each run. The evaluation results are summarized in Table 23.

Table 23 Attributable percentage of total variance (%TV), total precision Standard Deviation (SD), and lognormal CV(%) of EBV DNA concentration (log_{10} IU/mL) by positive panel member

Expected EBV DNA Concentration (log_{10} IU/mL)	Observed Mean[a] EBV DNA Concentration (log_{10} IU/mL)	Number of Tests[b]	Lot %TV[c] (CV%)[d]	Site %TV[c] (CV%)[d]	Day/ Operator %TV[c] (CV%)[d]	Batch %TV[c] (CV%)[d]	Within -Batch %TV[c] (CV%)[d]	Total Precision SD[e]	Total Precision 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] %TV = Percent contribution to Total Variance.

[d] CV% = Lognormal percent coefficient of variation = sqrt(10^[SD^2 * ln(10)] - 1) * 100.

[e] Calculated using the total variability from the SAS MIXED procedure.

Note: The table only includes results with detectable DNA level. SD = standard deviation. CV = coefficient of variation; EBV = Epstein Barr Virus.

cobas® EBV showed acceptable clinical reproducibility on the respective comparative concentration. In addition, the system detected 100% of the 3 x LLoQ samples. The **cobas®** 6800 and **cobas®** 8800 Systems share a modular design and they showed equivalency when using the **cobas®** EBV. All of the estimated 95% confidence limits (CLs) for the difference between 2 measurements from the same subject were within ± 0.53 log_{10} IU/mL, indicating that the assay can assess changes in EBV DNA levels that are thought to be clinically significant.

Of the 270 valid tests for the negative panel members performed on the **cobas®** 6800/8800 Systems, 14 samples (5.19%) showed detection of < LLoQ positivity. The results were not associated with a particular instrument/site or reagent lot. Heminested PCR and DNA sequencing confirmed the presence of EBV DNA in these samples.

Performance of cobas® EBV

The clinical performance of **cobas**® EBV was further evaluated at three testing sites by measuring EBV DNA levels in clinical samples (neat and diluted) of EBV infected and non-infected patients and contrived EDTA plasma samples spiked with cultured EBV virus, compared with a well-established laboratory developed nucleic acid test (LDT)(comparator EBV LDT).

From all samples tested with **cobas**® EBV and the comparator EBV test, there were a total of 464 samples (439 neat or diluted clinical samples of 72 transplant subjects and 25 contrived samples) that were valid on both assays and evaluable for the clinical concordance analysis.

Table 24 Concordance analysis between **cobas**® EBV and the comparator LDT on EBV DNA level results for all samples

cobas® **EBV (log_{10} IU/mL)**	**Comparator EBV LDT (log_{10} IU/mL) Target Not Detected**	**Comparator EBV LDT (log_{10} IU/mL) < LLoQ (< 2)**	**Comparator EBV LDT (log_{10} IU/mL) 2 to < 2.6**	**Comparator EBV LDT (log_{10} IU/mL) 2.6 to < 3.2**	**Comparator EBV LDT (log_{10} IU/mL) 3.2 to 3.8**	**Comparator EBV LDT (log_{10} IU/mL) > 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 LDT (100 IU/mL).

Standard Deviation of comparator EBV LDT estimated at 0.3 log_{10} IU/mL (EBV LDT analytical precision study).

Paired samples evaluable for clinical concordance analysis were included in this table.

[a] Assumed independence between all samples.

CI = Confidence Interval.

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.

Discordant results were defined as those that are more than one box away from the diagonal (indicated by shading). For Target Not Detected by LDT Column Agreement the **cobas**® EBVTarget 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.

Out of the 43 comparator EBV LDT negative samples collected for the estimation of the Negative Percent Agreement (NPA) with the **cobas**® EBV, 41 samples were negative by **cobas**® EBV therefore the NPA was 95.4% with the 95% Exact CI of 84.2% to 99.4%. The two comparator EBV LDT 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 LDT was also evaluated using different clinical thresholds.

Table 25 Summary of concordance of **cobas**® EBV and comparator EBV LDT using different thresholds for all samples

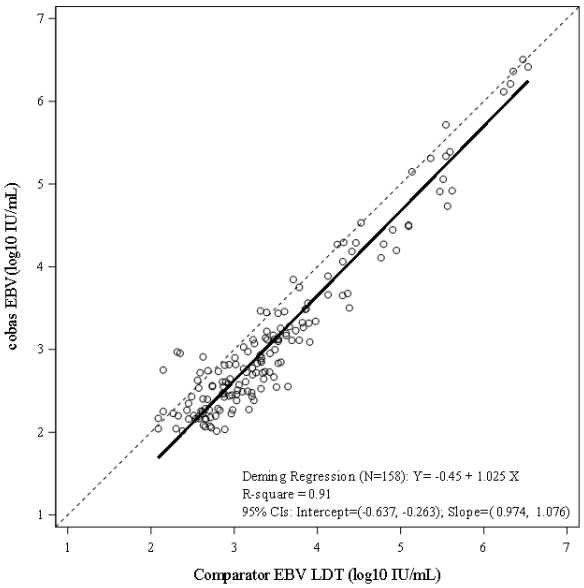
-	**Percent Agreement < Threshold 95% CI[b] (n/N)**	**Percent Agreement ≥ Threshold 95% CI[b] (n/N)**
Target Not Detected	98.5% (134/136) (94.8%, 99.6%)	89.6% (294/328) (85.9%, 92.5%)
LLoQ[a] (2.0 Log_{10} IU/mL)	98.0% (197/201) (95.0%, 99.2%)	60.8% (160/263) (54.8%, 66.5%)
3.0 Log_{10} IU/mL	100.0% (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%)

[a] LLoQ = lower limit of quantitation of comparator EBV LDT (100 IU/mL).

[b] CI = Confidence Interval

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

Figure 5 Correlation between **cobas**® EBV and comparator EBV LDT 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 LDT). Furthermore, the mean bias was estimated at -0.364 log_{10} IU/mL and the systematic difference between both assays was -0.352 log_{10} IU/mL and -0.376 log_{10} IU/mL for samples with DNA levels at 3 and 4 log_{10} IU/mL, respectively.

System equivalency / system comparison

System equivalency of the **cobas**® 5800, **cobas**® 6800 and **cobas**® 8800 Systems was demonstrated via performance studies. The results presented in the Instructions for Use support equivalent performance for all systems.

Additional information

Key test features

Sample type	EDTA plasma
Minimum amount of sample required	350 µL*
Sample processing volume	200 µL
Analytical sensitivity	18.8 IU/mL (two-sided 95% confidence interval: 14.5 IU/mL – 27.5 IU/mL)
Linear range	35.0 IU/mL to 1E+08 IU/mL
Genotypes detected	EBV Genotypes 1 and 2

*Dead volume of 150 µL identified for the **cobas® omni** Secondary tubes. Other tubes used for testing may have different dead volume and require more or less minimum volume. Contact your local Roche service representative for further information.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 26 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and distributors

Table 27 Manufacturer and distributors

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Made in USA

Distributed by Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Trademarks and patents

See https://diagnostics.roche.com/us/en/about-us/patents

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References

1. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplant recipients: a global perspective. Preface. *Bone Marrow Transplant*. 2009;44:453-5. PMID: 19861977.

2. Green M. Introduction: Infections in solid organ transplantation. *Am J Transplant*. 2013;13 Suppl 4:3-8. PMID: 23464993.

3. Cohen JI. Epstein-Barr virus infection. *N Engl J Med*. 2000;343:481-92. PMID: 10944566.

4. Styczynski J, van der Velden W, Fox CP, et al. Management of Epstein-Barr Virus infections and post-transplant lymphoproliferative disorders in patients after allogeneic hematopoietic stem cell transplantation: Sixth European Conference on Infections in Leukemia (ECIL-6) guidelines. *Haematologica*. 2016;101:803-11. PMID: 27365460.

5. Allen UD, Preiksaitis JK. Epstein-Barr virus and posttransplant lymphoproliferative disorder in solid organ transplantation. *Am J Transplant*. 2013;13 Suppl 4:107-20. PMID: 23465004.

6. San-Juan R, Comoli P, Caillard S, et al. Epstein-Barr virus-related post-transplant lymphoproliferative disorder in solid organ transplant recipients. *Clin Microbiol Infect*. 2014;20 Suppl 7:109-18. PMID: 24475976.

7. Nijland ML, Kersten MJ, Pals ST, Bemelman FJ, Ten Berge IJ. Epstein-Barr virus-positive posttransplant lymphoproliferative disease after solid organ transplantation: Pathogenesis, clinical manifestations, diagnosis, and management. *Transplant Direct*. 2016;2:e48. PMID: 27500242.

8. Tsai DE, Douglas L, Andreadis C, et al. EBV PCR in the diagnosis and monitoring of posttransplant lymphoproliferative disorder: Results of a two-arm prospective trial. *Am J Transplant*. 2008;8:1016-24. PMID: 18312608.

9. Fryer JF, Heath AB, Wilkinson DE, Minor PD. A collaborative study to establish the 1st WHO International Standard for Epstein-Barr virus for nucleic acid amplification techniques. *Biologicals*. 2016;44:423-33. PMID: 27461128.

10. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene*. 1990;93:125-8. PMID: 2227421.

11. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. *Nature*. 1995;373:487-93. PMID: 7845459.

12. Mol CD, Arvai AS, Slupphaug G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase: Structural basis for specificity and catalysis. *Cell*. 1995;80:869-78. PMID: 7697717.

13. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. *Biotechnology (N Y)*. 1992;10:413-7. PMID: 1368485.

14. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. *Genome Res*. 1996;6:986-94. PMID: 8908518.

15. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.

16. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline, 4th ed. CLSI Document M29-A4. Wayne, PA: CLSI, 2014.

17. Hadinoto V, Shapiro M, Sun CC, Thorley-Lawson DA. The dynamics of EBV shedding implicate a central role for epithelial cells in amplifying viral output. *PLoS Pathog*. 2009;5:e1000496. PMID: 19578433.

18. National Institute for Biological Standards and Control. 1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques (NIBSC 09/260) version 4.0: Hertfordshire, England;NIBSC, 2014.

Document revision

Document Revision Information	
Doc Rev. 1.0 11/2022	First Publishing.
Doc Rev. 2.0 05/2023	Added footnote and column to **Table 22**, specifying drug test concentration. Return minimum sample volume to original level in **Instructions for use** and **Key test features** section. Updated **cobas**® branding. Minor wording corrections. Please contact your local Roche Representative if you have any questions.