

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: 08688214190 or
P/N: 09040951190

cobas[®] Buffer Negative Control Kit

P/N: 07002238190 or
P/N: 09051953190

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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 diagnosis and 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 viral response to treatment.

The results from **cobas® EBV** must be interpreted within the context of all relevant clinical and laboratory findings.

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 over the lifetime. 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 EBV-negative recipient serostatus at transplantation, 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 EBV-negative in high risk transplant patients.^{4,5} While the exact medically relevant viral 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 viral load 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. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to EBV WHO International Standard. Each Amplification/Detection kit lot is calibrated traceable to EBV WHO International Standard.

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 deoxythymidine 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, and Table 8 through 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

cobas® 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**cobas® EBV/BKV Control Kit**

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. 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 dust/fume/gas/mist/vapours/spray. 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-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
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 dust/fume/gas/mist/vapours/spray. 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-4-isothiazolin-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**cobas® Buffer Negative Control Kit**

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 + H332: Harmful if swallowed or if inhaled. 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. 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 requirements

Reagents shall be stored and will be handled as specified in Table 5, Table 6 and Table 7.

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

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, for 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.
- 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 10⁸ EBV copies/mL in their saliva, and given the high sensitivity of the assay, it is important to implement adequate contamination control measures in laboratories.¹⁷
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

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** test kits, **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** instrument, follow the instructions in the **cobas® 5800** or **cobas® 6800/8800** Systems – User Assistance and/or User Guide 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.

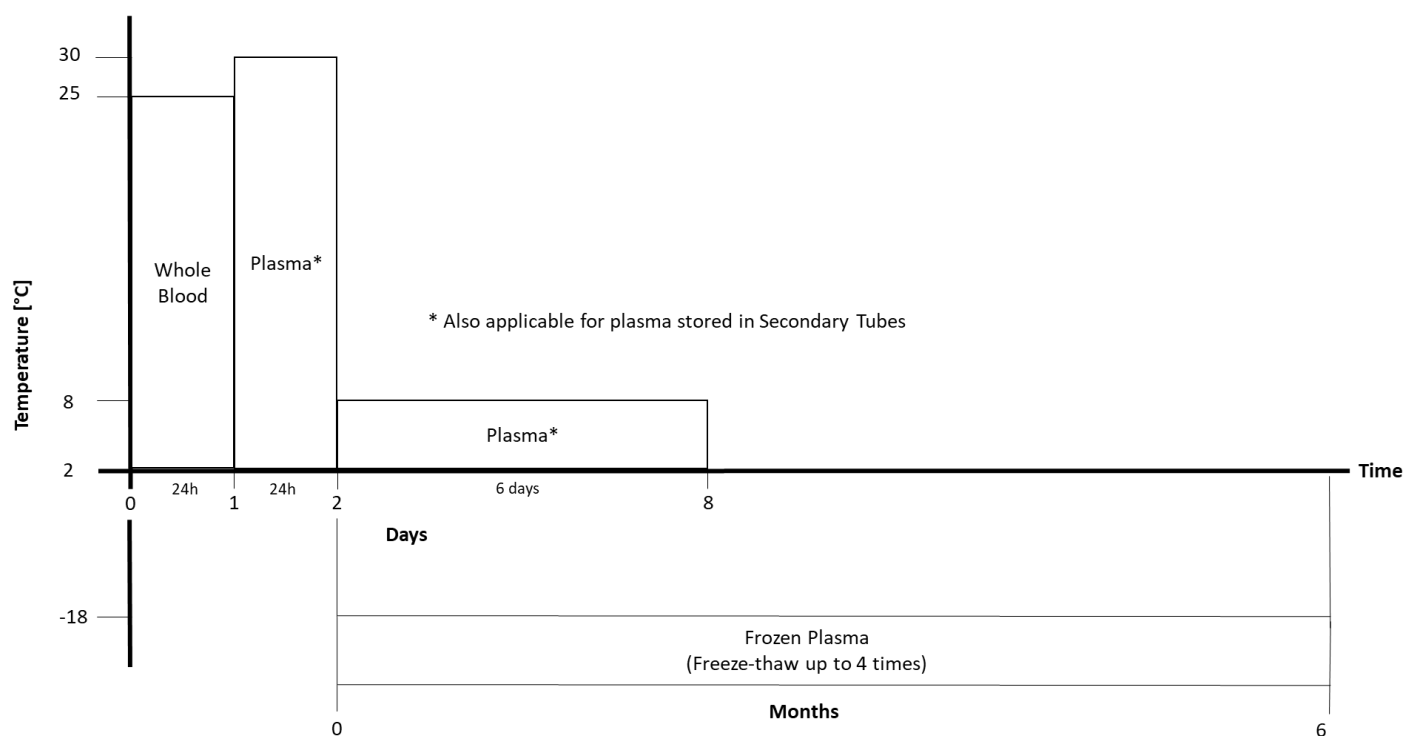
Sample stability is affected by elevated temperatures.

If using frozen samples in secondary tubes, place the samples at room temperature (15–30°C) 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 ≤ -18°C.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.

Figure 1 Sample storage conditions

- 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.

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. 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 test procedure on the cobas® 5800 System

1	Log onto the system
2	Loading samples onto the system • Load sample racks onto the system • The system prepares automatically • Order tests
3	Refill reagents and consumables as prompted by the system • Load test specific reagent cassette(s) • Load control mini racks • Load processing tips • Load elution tips • Load processing plates • Load liquid waste plates • Load amplification plates • Load MGP cassette • Refill specimen diluent • Refill lysis reagent • Refill wash reagent
4	Start the run by choosing the Start processing button on the user interface, all subsequent runs will start automatically if not manually postponed
5	Review and export results
6	Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use Clean up the instrument • Unload empty control mini racks • Unload empty test specific reagent cassette(s) • Empty amplification plate drawer • Empty liquid waste • Empty solid waste

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

cobas® EBV can be run with a minimum sample volume of 350 µL of which is processed 200 µL. 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

- 1** Log onto the system
Press Start to prepare the system
Order tests
- 2** Refill reagents and consumables as prompted by the system
 - Load test specific reagent cassette
 - Load control cassettes
 - Load pipette tips
 - Load processing plates
 - Load MGP reagent
 - Load amplification plates
 - Refill specimen diluent
 - Refill lysis reagent
 - Refill wash reagent
- 3** Loading samples onto the system
 - Load sample racks and clotted tip racks onto the sample supply module
 - Confirm samples have been accepted into the transfer module
- 4** Start the run by choosing the Start manually button on the user interface or have it start automatically after 120 minutes or if the batch is full
- 5** Review and export results
- 6** Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use

Clean up the instrument
 - Unload empty control cassettes
 - Empty amplification plate drawer
 - Empty liquid waste
 - Empty solid waste

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** Systems software and/or reports. 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 shown in Table 11 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

- **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 viral load 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.
- **cobas® EBV** is not intended for use as a screening test for the presence of EBV in blood or blood products.

Non-clinical performance evaluation

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

Limit of Detection (LoD)

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 $\geq$ 95% is 20.0 IU/mL in EDTA plasma.

Table 13 Limit of detection in EDTA plasma, Lot 1

Input titer concentration (EBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
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 Limit of detection in EDTA plasma, Lot 2

Input titer concentration (EBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
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 Limit of detection in EDTA plasma, Lot 3

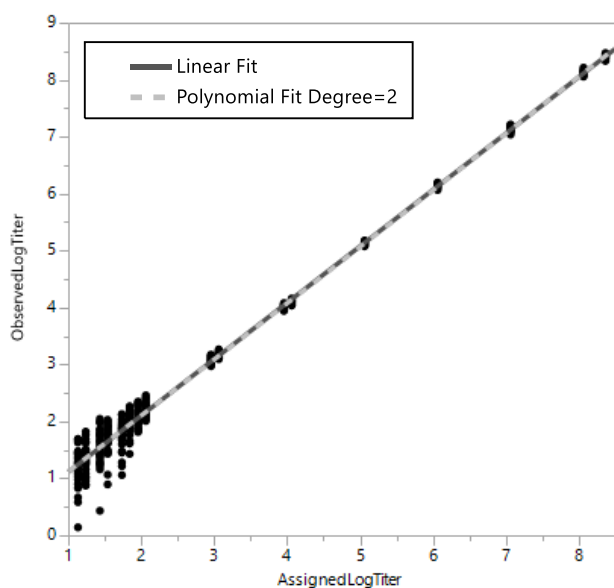
Input titer concentration (EBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
50.0	63	63	100.0
35.0	63	63	100.0
20.0	63	62	98.4
10.0	63	48	76.2
5.0	63	38	60.3
2.5	63	26	41.3
0.0	63	0	0.0
LoD by PROBIT at 95% hit rate	18.6 IU/mL 95% confidence range: 14.4 – 27.1 IU/mL		

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 $\pm 0.1 \log_{10}$ in human EDTA plasma (see Figure 4). Across the linear range, the accuracy of the test was within $\pm 0.2 \log_{10}$.

Figure 4 Linear range determination in EDTA plasma

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. Six 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 16.

cobas® EBV showed high precision for three lots of reagents tested across a concentration range of 1.08E+02 IU/mL to 5.40+07 IU/mL.

Table 16 Within-laboratory precision of cobas® EBV

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

Genotype verification

The performance of cobas® EBV on EBV genotype 2 was evaluated by:

- Verification of the limit of detection
- Verification of the linear range

Verification of limit of detection for genotype 2

EBV DNA 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, multiple runs, days, operators, and instruments. The results verify that cobas® EBV detected EBV DNA for genotype 2 at a concentration of 18.8 IU/mL with a hit rate of $\geq 95\%$.

Verification of linear range 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 linear range of cobas® EBV was verified for genotype 2.

Specificity

The specificity of cobas® EBV was determined by analyzing EBV-negative EDTA plasma samples from individual donors. One hundred and one individual EDTA plasma samples were tested with three lots of cobas® EBV reagents. All samples were tested negative for EBV DNA. In the test panel the specificity of cobas® EBV was 100% (lower one-sided 95% confidence interval 97.08%).

Analytical specificity

The analytical specificity of cobas® EBV was evaluated by diluting a panel of microorganisms to 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, TCID₅₀/mL) for viruses with EBV DNA positive and EBV DNA negative EDTA plasma. The specific organisms tested are listed in Table 17. Each panel member was evaluated with cobas® EBV. None of the non-EBV pathogens were shown to interfere with test performance.

Table 17 Microorganisms tested for cross-reactivity

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

Analytical specificity – interfering substances

Elevated levels of triglycerides (33.0 g/L), conjugated bilirubin (0.2 g/L), unconjugated bilirubin (0.2 g/L), albumin (60.0 g/L), hemoglobin (2.0 g/L) and human DNA (2 mg/L) in samples were tested in the presence 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 18 were tested at three times the C_{max} in presence and absence of EBV DNA.

All potentially interfering substances have been shown to not interfere with the test performance.

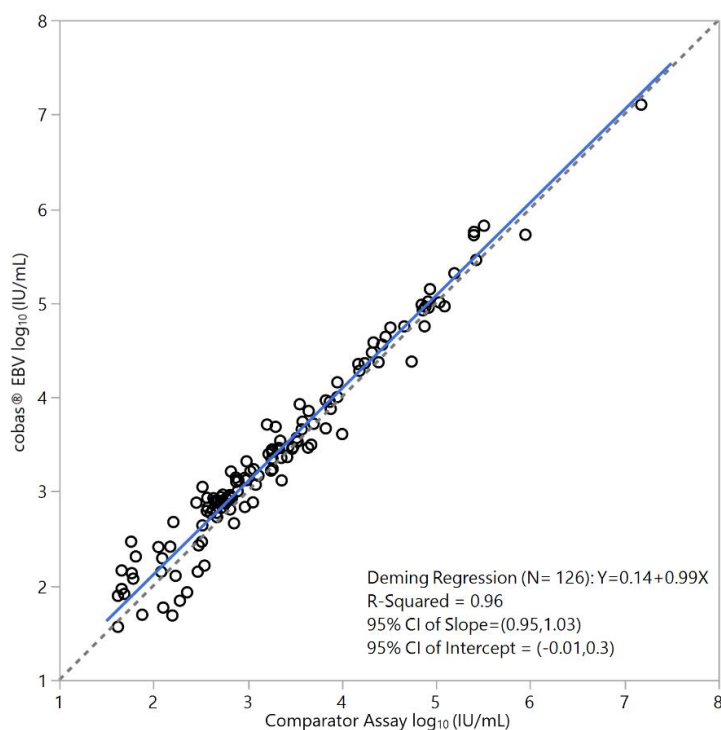
Table 18 Drug compounds tested for interference with the quantitation of EBV DNA by cobas® EBV

Class of drug	Generic drug name	
Antimicrobial	Cefotetan	Sulfamethoxazole
	Clavulanate potassium	Ticarcillin disodium
	Fluconazole	Trimethoprim
	Piperacillin	Vancomycin
	Tazobactam sodium	Micafungin
Compounds for Treatment of Herpes Viruses	Ganciclovir	Cidofovir
	Valganciclovir	Foscarnet
	Acyclovir	Letermovir
Immune suppressant	Azathioprine	Prednisone
	Cyclosporine	Sirolimus
	Everolimus	Tacrolimus
	Mycophenolate mofetil	Mycophenolic acid

Method correlation

The performance of cobas® EBV was assessed against a comparator assay by analyzing EDTA plasma specimens from EBV-infected patients. EDTA plasma specimens, within the quantitation range of both tests, were tested as single replicates. Deming regression analysis was performed.

The Deming regression results are shown in Figure 5.

Figure 5 Regression analysis of cobas® EBV versus comparator assay

Whole system failure

The whole system failure rate for **cobas**® EBV was determined by testing 100 replicates of EDTA plasma spiked with an EBV positive clinical specimen. These samples were tested at a concentration of 3 x LoD.

The results of this study determined that all replicates were valid and positive for the EBV target, resulting in a whole system failure rate of 0% (upper one-sided 95% confidence interval 2.95%).

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 19.

Table 19 Attributable percentage of total variance (%TV), total precision Standard Deviation (SD), and lognormal CV(%) of EBV DNA concentration (log₁₀ 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 = $\sqrt{10^{[SD^2 \times \ln(10)]} - 1} \times 100$.

^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 $\pm 0.53 \log_{10}$ 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 20 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 log₁₀ 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 log₁₀ 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® 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.

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

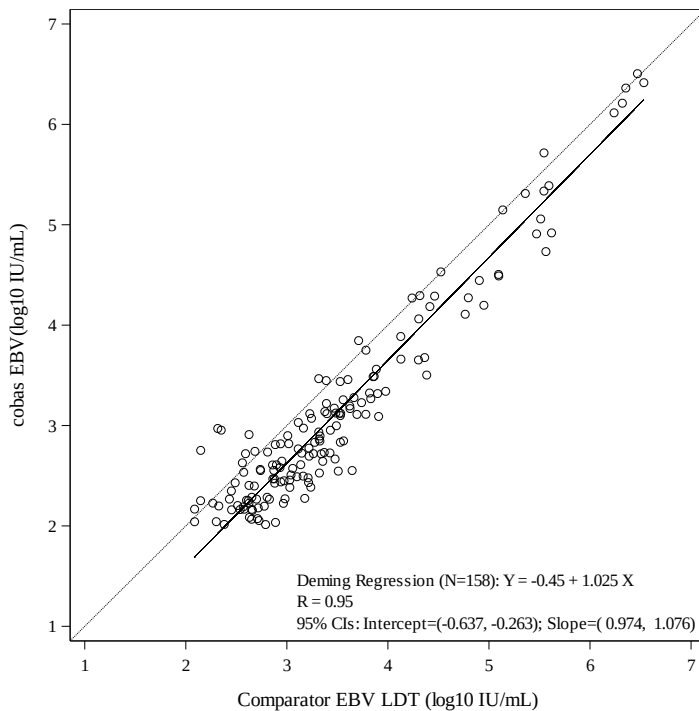
	Percent Agreement < Threshold 95% CI ^b (n/N)	Percent Agreement ≥ Threshold 95% CI ^b (n/N)
Target Not Detected	98.5% (134/136) (94.8%, 99.6%)	89.6% (294/328) (85.9%, 92.5%)
LLoQ ^a (2.0 Log ₁₀ IU/mL)	98.0% (197/201) (95.0%, 99.2%)	60.8% (160/263) (54.8%, 66.5%)
3.0 Log ₁₀ IU/mL	100.0% (363/363) (99.0%, 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 6 Correlation between **cobas**® EBV and comparator EBV LDT for all samples: Deming linear regression plot of DNA levels (log₁₀ 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 $\pm 0.6 \log_{10}$ 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

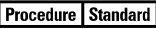
Sample type	EDTA plasma
Minimum amount of sample required	350 µL*
Sample processing volume	200 µL
Analytical sensitivity	18.8 IU/mL
Linear range	35.0 IU/mL to 1E+08 IU/mL
Specificity	100%
Genotypes detected	EBV Genotypes 1 and 2

*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 22 Symbols used in labeling for Roche PCR diagnostics products

 Age/DOB	Age or Date of Birth		Device not for near-patient testing		QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
	Ancillary Software		Device not for self-testing		
	Assigned Range (copies/mL)		Distributor (Note: The applicable country/region may be designated beneath the symbol)		Serial number
	Assigned Range (IU/mL)		Do not re-use		Site
	Authorized representative in the European Community		Female		Standard Procedure
	Barcode Data Sheet		For IVD performance evaluation only		Sterilized using ethylene oxide
	Batch code		Global Trade Item Number		Store in dark
	Biological risks		Importer		Temperature limit
	Catalogue number		In vitro diagnostic medical device		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		Lower Limit of Assigned Range		This way up
	Collect date		Male		Ultrasensitive Procedure
	Consult instructions for use		Manufacturer		Unique Device Identifier
	Contains sufficient for <n> tests		Negative control		Upper Limit of Assigned Range
	Content of kit		Non-sterile		Urine Fill Line
	Control		Patient Name		US Only: Federal law restricts this device to sale by or on the order of a physician.
	Date of manufacture		Patient number		Use-by date
	Device for near-patient testing		Peel here		
	Device for self-testing		Positive control		
			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 importer

Table 23 Manufacturer and importer



Roche Molecular Systems, Inc.
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Branchburg, NJ 08876 USA
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Made in USA



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68305 Mannheim, Germany

Trademarks and patents

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

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Document revision

Document Revision Information	
Doc Rev. 2.0 09/2022	Updated front page and table 2 and 3 with additional P/N for the control kits. Updated Trademarks and patents section, including the link. Please contact your local Roche Representative if you have any questions.
Doc Rev. 3.0 05/2023	Return minimum sample volume to original level in Instructions for use and Key test features section. Updated competent authority statement. Updated cobas® branding. Minor wording corrections. Please contact your local Roche Representative if you have any questions.

The summary of safety and performance report can be found using the following link: <https://ec.europa.eu/tools/eudamed>.