Elecsys EBV IgM

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REF		Σ	SYSTEM
08355274190	08355274500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
EBVIGM	10163

Please note

For the serological determination of the EBV infection stage, the Elecsys EBV IgM assay should only be used in combination with the Elecsys EBV VCA IgG assay and the Elecsys EBV EBNA IgG assay.

Intended use

Immunoassay for the in vitro qualitative detection of IgM antibodies to Epstein-Barr virus (EBV) in human serum and plasma. The test is intended for use as an aid in the diagnosis of an infectious mononucleosis and the determination of the EBV infection stage.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Epstein-Barr virus, also known as human herpesvirus 4 (HHV4) is one of the 8 known human herpes viruses, infecting about 90 % of the world population already at young age and generally causing little complications. The majority of these infections are either asymptomatic or manifest with only minor unspecific symptoms.[1] The most common EBV-linked disease is the symptomatic acute primary infection called infectious mononucleosis (IM), mainly affecting adolescents and young adults. IM is characterized by the triad of fever, pharyngitis and cervical lymphadenopathy, and is generally a self-limiting disease with supportive therapy as the mainstay of treatment.[2] Yet, early and accurate diagnosis is valuable as EBV is highly communicable, and in rare cases complications may develop, posing serious health risks.[1] Following the lytic replication during primary infection, EBV remains latent for life, mainly in B-cells.[3] EBV infection has been associated to various autoimmune diseases as well as several distinct malignant diseases including both lymphomas and carcinomas.[4] Immunosuppression can result in post-transplant lymphoproliferative disorder (PTLD), a frequently fatal disorder of uncontrolled B-cell proliferation.[5] EBV is mainly transmitted by saliva, but sexual transmission, and transmission via solid-organ and hematopoietic-stem-cell transplantation has been reported.[6]

Various viral, bacterial, and parasitic diseases can cause mononucleosis-like symptoms, especially in early infection.[7] A combination of biomarkers is commonly used for differential diagnosis, to rule out other infections or conditions with similar symptoms, such as acute HIV or CMV infection or toxoplasmosis. EBV serology is also used for the determination of the immune status of transplant donors and recipients assessing the risk of a patient to develop PTLD, that can be caused by a reactivation or a new EBV infection in the previously EBV naïve patient.[8,9,10,11]

Serologic tests specific for EBV are routinely used to confirm the diagnosis of an acute EBV infection, as clinical signs and symptoms are not very sensitive or specific.[2] 3 different biomarkers are routinely used in combination to determine the stage of EBV infection: IgM antibodies to EBV antigens, IgG antibodies to EBV viral capsid antigens (VCA), and IgG antibodies to EBV nuclear antigen-1 (EBNA-1).[12,13] Anti-EBV IgM and anti-EBV VCA IgG antibodies are typically detectable at the clinical onset of illness. IgM may remain positive until 2 to 6 months after primary infection, and VCA IgG antibodies typically show lifelong persistence. EBNA-1 IgG antibodies usually appear within 6-12 weeks after primary infection and persist lifelong. Therefore, the presence of IgM and VCA IgG antibodies, and the absence of EBNA-1 IgG, in combination with the typical clinical presentation are indicative for acute infection. The absence of IgM antibodies and presence of VCA IgG and EBNA-1 IgG antibodies are indicative for past infection and a state of latency.[12,13] For EBV-monitoring in cancer, transplantation, HIV/AIDS and autoimmune syndromes, specific rules may apply, that differ per disease condition.[12,14,15]

Test principle

µ-Capture principle. Total duration of assay: 18 minutes.

- 1st incubation: 6 µL of sample are automatically prediluted 1:20 with Diluent Universal. Biotinylated monoclonal anti-h IgM-specific antibody fragments are added.
- 2nd incubation: EBV-specific recombinant antigens labeled with a ruthenium complex[a)] and streptavidin-coated microparticles are added. Anti-EBV IgM antibodies present in the sample react with the ruthenium-labeled EBV-specific recombinant antigens. The complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack (M, R1, R2) is labeled as EBVIGM.

M	Streptavidin-coated microparticles, 1 bottle, 14.1 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	Anti-h-IgM-Ab~biotin, 1 bottle, 19.7 mL: Biotinylated monoclonal anti-h-IgM antibody fragments (mouse) > 500 µg/L; MES[b)] buffer 50 mmol/L, pH 6.5; preservative.
R2	EBV-Ag~$Ru(bpy)_3^{2+}$, 1 bottle, 19.7 mL: EBV-specific antigens (recombinant, E. coli) labeled with ruthenium complex > 50 µg/L; MES buffer 50 mmol/L, pH 5.8; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

EBVIGM Cal1	Negative calibrator 1 (lyophilized), 1 bottle for 1.0 mL: Human serum, non-reactive for EBV IgM; buffer; preservative.
EBVIGM Cal2	Positive calibrator 2 (lyophilized), 1 bottle for 1.0 mL: Human serum, reactive for EBV IgM; buffer; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P261	Avoid breathing dust.
P273	Avoid release to the environment.
P280	Wear protective gloves.

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Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious.

All products derived from human blood (EBVIGM Cal1, EBVIGM Cal2) are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing anti-EBV IgM (EBVIGM Cal2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[16,17]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents (M, R1, R2) in the kit are ready-for-use and are supplied in **cobas e** packs.

Calibrators:

Carefully dissolve the contents of 1 bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation.

Transfer the reconstituted calibrators into the supplied empty labeled snap-cap bottles.

Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the freshly reconstituted calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C or -20 °C (± 5 °C) for later use.

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the **cobas e** pack:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Stability of the calibrators:	
unopened at 2-8 °C	up to the stated expiration date
reconstituted at 2-8 °C	4 weeks
reconstituted at -20 °C (± 5 °C)	16 weeks (freeze only once)
on the analyzers at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Absolute deviation of negative samples ± 0.25 COI (cutoff index) from serum value; reactive samples: recovery within 75-125 % of serum value.

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 3 months at -20 °C (± 5 °C). The samples may be frozen 3 times.

The sample types listed were tested with a selection of sample collection tubes or systems that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube/collection system manufacturer.

Specimens should not be subsequently altered with additives (e.g. biocides, anti-oxidants or substances that could possibly change the pH or ionic strength of the sample) in order to avoid erroneous findings.

Pooled samples and other artificial material may have different effects on different assays and thus may lead to discrepant findings.

Centrifuge samples containing precipitates and thawed samples before performing the assay.

Do not use heat-inactivated samples.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

The performance of the Elecsys EBV IgM assay has not been established with cadaveric samples or body fluids other than serum and plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 4 bottle labels
- 2 empty labeled snap-cap bottles

Materials required (but not provided)

- REF 08355428190, PreciControl EBV IgM/VCA IgG, 6 x 2.0 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer
- Distilled or deionized water

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

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Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibrators:

Place the reconstituted calibrators in the sample zone.

Read in all the information necessary for calibrating the assay.

Calibration

No international standard is available for EBV IgM.

Calibration frequency: Calibration must be performed once per reagent lot using EBVIGM Cal1, EBVIGM Cal2 and fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl EBV IgM/VCA IgG.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of EBVIGM Cal1 and EBVIGM Cal2. The result of a sample is given either as reactive, borderline or non-reactive as well as in the form of a cutoff index (COI; signal sample/cutoff).

Interpretation of the results

Numeric result	Result message	Interpretation
COI < 0.6	Non-reactive	Negative for EBV-specific IgM
COI ≥ 0.6 to < 1.0	Borderline	Indeterminate for EBV-specific IgM[c)]
COI ≥ 1.0	Reactive	Positive for EBV-specific IgM

c) It is recommended to interpret this result in conjunction with the results obtained with the Elecsys EBV VCA IgG assay and Elecsys EBV EBNA IgG assay (see table below on determination of EBV infection stage).

The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present in the sample.

The EBV IgM results in a given specimen, as determined by assays from different manufacturers, can vary due to differences in methods.

For the serological determination of the EBV infection stage, the Elecsys EBV IgM assay should only be used in combination with the Elecsys EBV VCA IgG assay and the Elecsys EBV EBNA IgG assay.

The following result interpretation table is proposed to determine the EBV infection stage when using Elecsys EBV assays, based on what has been described in the literature.[12,18,19]

Result message of the Elecsys assay			Corresponds to the EBV infection stage
EBV IgM	**EBV VCA IgG**	**EBV EBNA IgG**	
non-reactive	non-reactive	non-reactive	**Seronegative**
non-reactive	*borderline*	non-reactive	
borderline	non-reactive	non-reactive	**Presumed early phase of EBV infection***
reactive	non-reactive	non-reactive	
borderline	*borderline*	non-reactive	**Acute phase of EBV infection**
reactive	*borderline*	non-reactive	
reactive	**reactive**	non-reactive	
borderline	**reactive**	non-reactive	
reactive	**reactive**	**reactive**	**Presumed transient phase of EBV infection* #**
reactive	*borderline*	**reactive**	
borderline	**reactive**	**reactive**	**Past EBV infection**
borderline	*borderline*	**reactive**	
non-reactive	**reactive**	**reactive**	
non-reactive	*borderline*	**reactive**	
non-reactive	**reactive**	non-reactive	**Isolated VCA IgG reactivity***
non-reactive	non-reactive	**reactive**	**Isolated EBNA IgG reactivity* #**

For infection stages marked with an asterisk (*) and any other combination of result messages that is not listed in the table above, the EBV infection stage is considered indeterminate. Additional and/or follow-up testing is recommended in those cases.[12]

Automated assessment of the EBV infection stage based on the Elecsys EBV IgM, Elecsys EBV VCA IgG and Elecsys EBV EBNA IgG assays can be performed including result dependent sequential testing strategies (see section "**cobas e** flows").

"Presumed transient phase of infection" and "Isolated EBNA IgG reactivity" are not applicable in the EBVSER E ("EBNA first") **cobas e** flow but will be interpreted as "past infection", as no additional testing is performed following a reactive EBV EBNA IgG result.

The individual immune response following EBV infection varies considerably[12] and might give different results with assays from different manufacturers. Results of assays from different manufacturers should not be used interchangeably.

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1130 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Human serum albumin	≤ 7 g/dL

Criterion: For samples with a COI ≥ 1.0, the deviation is ≤ 20 %. For samples with a COI < 1.0, the deviation is ≤ 0.2 COI.

A non-reactive EBV IgM test result in combination with a reactive EBV VCA IgG result and a non-reactive EBV EBNA IgG result, does not completely rule out the possibility of an acute infection with EBV. Individuals at the early stage of acute infection may not exhibit detectable amounts of EBV IgM antibodies. In some of these individuals a reactive result with the Elecsys EBV VCA IgG assay together with a non-reactive result with the Elecsys EBNA IgG assay may be found and may indicate an acute phase of EBV infection. Additional testing is recommended.

A reactive EBV IgM test result in combination with a non-reactive EBV VCA IgG result and a non-reactive EBV EBNA IgG result is not sufficient to prove an acute phase of EBV infection. Additional testing is recommended.

A reactive EBV IgM test result in combination with a reactive EBV VCA IgG result and a reactive EBV EBNA IgG result is not sufficient to prove a

transient phase of EBV infection. In single cases elevated IgM antibody levels may persist even for years after initial infection. Additional testing is recommended.

As with many μ-capture assays, an interference with unspecific IgM is observed. Increasing amounts of unspecific IgM may lead to a decrease in the recovery of positive samples with the Elecsys EBV IgM assay.

Polyclonal B-cell activation during acute EBV infection, can lead to the production of unspecific IgM antibodies.[20,21]

Sera from patients with primary CMV infections, primary parvovirus B19 infections or primary Toxoplasma infections can demonstrate positive results in the Elecsys EBV IgM assay. These potential interferences are known for EBV IgM assays.[22,23,24]

The Elecsys EBV IgM assay is a qualitative assay. The numeric result is not indicative of a specific stage of an EBV infection and should not be compared to the numeric result of EBV IgM assays of other manufacturers.

No false negative results due to a high-dose hook effect were found with the Elecsys EBV IgM assay but occurrence of high-dose hook effect cannot be completely excluded.

In HIV patients, in patients undergoing immunosuppressive therapy, or in patients with other disorders leading to immune suppression different interpretations of the serological profile might apply.[12,15]

Pharmaceutical substances
In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For clarification of the EBV infection stage the combination of the 3 Elecsys tests should always be performed and results should be assessed in conjunction with the patient's medical history, clinical symptoms and other findings.

Dilution

Use Diluent Universal for automatic sample predilution.

cobas e flows

cobas e flows are procedures programmed into the system to enable a fully automated sequence of measurements and the calculation of assay combinations to perform decision algorithms.

The following **cobas e** flows are available to characterize the EBV infection stage by combination of Elecsys EBV serologic test results:

cobas e flows	Function
EBVSEROL A	The **cobas e** flow "EBV Serology all assays" performs a Elecsys EBV IgM, Elecsys EBV VCA IgG and Elecsys EBV EBNA IgG test in parallel on the same sample and automatically interprets the EBV infection stage according to the table shown in section "interpretation of results".
EBVSEROL E	The **cobas e** flow "EBV Serology EBNA IgG first" initially performs an Elecsys EBV EBNA IgG test on the sample. If the EBNA IgG result is reactive, no further testings are performed and the serologic EBV infection stage "past infection" is reported together with the test subresult. If the EBNA IgG result is non-reactive, Elecsys EBV IgM and Elecsys EBV VCA IgG testings are automatically performed in parallel in a second step. The serologic EBV infection stage is interpreted based on the test results and reported together with the individual test subresults. Please note that the serologic interpretation "presumed transient phase of infection" (see interpretation table) is not applicable in this **cobas e** flow but will be interpreted as "past infection", as no additional testing is performed following a reactive EBV EBNA IgG result.

The individual numeric assay results in COI and their qualitative interpretation are listed in the **cobas e** flow subresults.

Please note that the **cobas e** flow main result does not differentiate isolated EBV VCA IgG reactivity and isolated EBV EBNA IgG reactivity, but results are interpreted with the generic main result message "isolated IgG reactivity". Please refer to the subresults for reactivity details.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HSP[d)], negative	0.197	0.002	1.2	0.023	11.4
HSP, near cutoff / negative	0.943	0.012	1.3	0.023	2.4
HSP, near cutoff / positive	1.08	0.017	1.6	0.023	2.2
HSP, positive	1.32	0.013	1.0	0.028	2.1
HSP, high positive	10.3	0.120	1.2	0.364	3.5
PC[e)] EBV IgM/VCA IgG 1	0.254	0.003	1.3	0.021	8.2
PC EBV IgM/VCA IgG 2	2.63	0.022	0.8	0.070	2.7

d) HSP = human specimen (serum/plasma)

e) PC = PreciControl

Analytical specificity

Potentially cross-reacting samples (characterized positive for potentially cross-reacting analytes with commercially available assays) were tested with the Elecsys EBV IgM assay. In case reactive Elecsys EBV IgM assay results were obtained, 2 comparator assays were performed in addition to evaluate concordance. 169 samples were tested internally, another 49 samples were tested externally as a part of the multicenter evaluation study.

		Elecsys EBV IgM assay result		
Containing potentially cross-reacting analytes	**N**	**Non-reactive**	**Borderline**	**Reactive**
CMV IgM	33**	22	5	6
HSV-1 IgG	13	12	1	0
VZV IgG	10	10	0	0
Parvovirus B19 IgM	35**	32	1	2
Toxoplasma IgM	18	15	2	1
Rubella IgM	12	12	0	0
HIV (total antibodies)	10	8	2	0
HAV (total antibodies)	10	9	1	0
HAV IgM	10	10	0	0
HBV (reactive for HBsAg and HBeAg)	10	10	0	0
HEV IgM	3	3	0	0
HAMA	10	10	0	0
ANA	12	11	1	0
SLE/dsDNA	10	9	1	0

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		Elecsys EBV IgM assay result		
Containing potentially cross-reacting analytes	**N**	**Non-reactive**	**Borderline**	**Reactive**
Rheumatoid factor	15	15	0	0

** 6 CMV IgM samples and 1 parvovirus B19 IgM sample were excluded, as they were found concordant reactive with 3 EBV IgM assays (Elecsys and both comparators). Cross-reactivity with Toxoplasma IgM, CMV IgM and parvovirus B19 IgM cannot be excluded.

Reactivity may be related to cross-reactions with IgM to other viruses or to reappearance of EBV IgM as a consequence of unspecific polyclonal B-cell activation induced by other pathogens.[20,21,22,23,24]

Relative specificity and relative sensitivity

Relative sensitivity and specificity were assessed on a total of 1734 specimens (1068 specimens with request for EBV testing from daily laboratory routine, 467 presumed acute specimens and 199 presumed seronegative specimens). All specimens were tested with the Elecsys EBV IgM assay, the Elecsys EBV VCA IgG assay and the Elecsys EBV EBNA IgG assay (referred to as Elecsys EBV assay panel) as well as with 2 different comparator EBV assay panels. The EBV infection stage was determined with the Elecsys EBV assay panel (according to the table in the section “Interpretation of the results”), and with the comparator panels according to their respective instructions for use. The ultimately assigned stage of EBV infection of a specimen was defined by the concordant EBV infection stage of at least 2 out of the 3 EBV assay panels (majority approach). In case each of the 3 EBV assay panels suggested a different EBV infection stage for a sample, no EBV infection stage could be defined and the sample was excluded from the performance calculations shown below.

Relative sensitivity

414 specimens with the assigned stage “acute phase of an EBV infection” (n = 397) or “confirmed early phase of EBV infection” (if at least 1 manufacturer showed a reactive VCA IgG result; n = 17) were included in the calculation. The results are shown in the table below.

		Elecsys EBV IgM assay result			95 % confidence interval	
Sample cohort	N	Concordant to stage	Discordant to stage	**Relative sensitivity**	Lower limit	Upper limit
Acute phase and confirmed early phase of EBV infection	414	407	7	**98.31 %**	96.55 %	99.18 %

Relative specificity

1174 specimens with the assigned stage “seronegative for EBV” (n = 318) or “past EBV infection” (n = 856) were included in the calculation. The results are shown in the table below.

		Elecsys EBV IgM assay result			95 % confidence interval	
Sample cohort	N	Concordant to stage	Discordant to stage	**Relative specificity**	Lower limit	Upper limit
Seronegative for EBV	318	314	4	**98.74 %**	96.81 %	99.51 %
Past EBV infection	856	830	26	**96.96 %**	95.59 %	97.92 %
Combined	1174	1144	30	**97.44 %**	96.38 %	98.20 %

Determination of EBV infection stage

The EBV infection stage was determined with the Elecsys EBV assay panel (according to the table in the section “Interpretation of the results”), and with the 2 comparator EBV assay panels according to their respective instructions for use.

Using the majority approach, an EBV infection stage was assigned to each specimen. The table below shows the number of specimens classified into each EBV infection stage with the Elecsys EBV assay panel in relation to the majority approach.

EBV infection stage	**Number of samples per EBV infection stage as classified with the Elecsys EBV assay panel in relation to the majority approach**	**% of concordantly classified samples by the Elecsys EBV assay panel**
Seronegative	310/318	97.48 %
Acute infection	369/397	92.95 %
Past infection	826/856	96.50 %
Total	**1505/1571**	**95.80 %**

In addition, 145 specimens were classified according to the majority approach into one of the “indeterminate EBV infection stages” (presumed early phase, transient phase, isolated VCA IgG, or Isolated EBNA IgG), and in 18 specimens no majority approach was possible (3 different EBV infection stages found by the 3 different EBV assay panels).

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE

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