EBV VCA IgM Reagent Kit

Read Highlighted Changes: Revised March 2020.

REF 09P2222
REF 09P2232

Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

NAME

Alinity i EBV VCA IgM Reagent Kit (also referred to as EBV VCA M)

INTENDED USE

The Alinity i EBV VCA IgM assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgM antibodies to Epstein-Barr Virus (EBV) Viral Capsid Antigen (VCA) in human serum and plasma on the Alinity i analyzer.

The Alinity i EBV VCA IgM assay is to be used as an aid in the diagnosis of infectious mononucleosis (IM) and an aid in determining the stage of EBV infection.

SUMMARY AND EXPLANATION OF THE TEST

EBV, also called human herpes virus 4 (HHV-4), is one of the most common viruses in humans. EBV is a lymphotropic, enveloped double-stranded DNA virus. It belongs to the herpesviridae family, subfamily gamma herpes viruses. In adults, who are older than 25 years, the seroprevalence is > 95%.[1]

The virus is mainly transmitted by saliva; however, sexual transmission, transmission by transplantation or blood products containing lymphocytes has been shown.[2, 3] EBV is the causative agent of infectious mononucleosis (IM) and is also associated with Burkitt's lymphoma and nasopharyngeal carcinoma.

During the lytic cycle, the pathogen replicates in B cells and epithelial cells of the salivary glands and oral mucosa and is secreted via saliva. Upon resolution of primary infection, EBV remains latent in B lymphocytes. Reactivations occur frequently in life, but are usually not clinically relevant in immuno-competent hosts. After primary infection, the virus is secreted lifelong intermittently via saliva.

EBV infections in childhood are often asymptomatic, whereas they lead to IM in 35-50% of adolescents. The incubation period ranges from 4-6 weeks.

Diagnosis of IM may be suspected from the triad of fever, pharyngitis and lymphadenopathy together with hematologic findings. Serologic tests are used for staging of the infection, to differentiate EBV infection from other infections, e.g. with cytomegalovirus, *Toxoplasma gondii*, hepatitis A virus, HIV with similar clinical symptoms [4] and to determine the immune status in transplantation donors and recipients.

For infection stage determination, tests for detection of IgM and IgG antibodies to EBV Viral Capsid Antigen (VCA) and IgG antibodies to Epstein-Barr Nuclear Antigen-1 (EBNA-1) are commonly used.[5]

VCA IgG and VCA IgM antibodies in the absence of EBNA-1 IgG antibodies are typically found in patients with acute primary infections. In contrast, past infections are characterized by the presence of VCA IgG and EBNA-1 IgG antibodies in the absence of VCA IgM antibodies. In some cases VCA IgM antibodies persist longer - up to the period when EBNA-1 IgG antibodies are already produced. Serology may be further complicated by the fact that some individuals do not produce VCA IgM antibodies during primary infection and the fact that some individuals lack EBNA-1 IgG antibodies (either the individuals are EBNA-1 nonresponders or the individuals may have lost the EBNA-1 IgG antibodies under circumstances such as immunosuppression) even some months and sometimes years after the primary infection.[6] In these cases further diagnostic approaches are required.

For reliable infection stage determination the EBV VCA IgM, EBV VCA IgG and EBV EBNA-1 IgG assays should be evaluated in parallel, as displayed in the table below. Specimens classified as transient infection, early phase acute primary infection, isolated VCA IgG, isolated EBNA-1 IgG or that show VCA IgM and EBNA-1 IgG reactivity in the absence of VCA IgG reactivity are considered unresolved and may require a follow up sample and / or further testing.

EBV VCA IgM	EBV VCA IgG	EBV EBNA-1 IgG	May indicate…/ Testing recommendation
-	-	-	**Seronegative (no infection)**
+	-	-	Early phase acute primary infection*
+	+	-	**Acute Primary Infection**
+	+	+	Transient Infection*
-	+	+	**Past Infection**
-	+	-	Isolated VCA IgG*
-	-	+	Isolated EBNA-1 IgG*

- nonreactive

+ reactive

* obtain and test new sample 1-2 weeks after initial sample

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the qualitative detection of IgM antibodies to EBV VCA in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Diluted sample, VCA antigen coated paramagnetic microparticles, and assay diluent are combined and incubated. The anti-EBV VCA IgM present in the sample binds to the VCA antigen coated microparticles. The mixture is washed. Anti-human IgM acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of anti-EBV VCA IgM in the sample and the RLUs detected by the system optics.

The presence or absence of anti-EBV VCA IgM in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.

For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity i EBV VCA IgM Reagent Kit 09P22

NOTE: Some kit sizes are not available in all countries. Please contact your local distributor.

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	09P2222	09P2232
Tests per cartridge	100	500
Number of cartridges per kit	2	2
Tests per kit	200	1000
MICROPARTICLES	6.6 mL	27.0 mL
CONJUGATE	6.1 mL	26.5 mL
ASSAY DILUENT	10.4 mL	47.1 mL

MICROPARTICLES EBV VCA antigen coated microparticles in TRIS buffer with protein (bovine) stabilizers and detergent. Minimum concentration: 0.08% solids. Preservatives: ProClin 950 and sodium azide.

CONJUGATE Murine acridinium-labeled anti-human IgM conjugate in MES buffer with protein (bovine) stabilizers and detergent. Minimum concentration: 25 ng/mL. Preservative: ProClin 300.

ASSAY DILUENT Acetate buffer with detergent. Preservative: ProClin 300. Note: Assay Diluent is for the suppression of rheumatoid factor interference.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[7-10]

The following warnings and precautions apply to: MICROPARTICLES	
WARNING	Contains methylisothiazolone and sodium azide
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
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 in accordance with local regulations.

The following warnings and precautions apply to: CONJUGATE	
WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
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 in accordance with local regulations.

The following warnings and precautions apply to: ASSAY DILUENT	
WARNING	Contains methylisothiazolones and Polyethylene glycol octylphenyl ether (Triton X-405).
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H412	Harmful to aquatic life with long lasting effects.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P280	Wear protective gloves / protective clothing / eye protection.
P264	Wash hands thoroughly after handling.
P272	Contaminated work clothing should not be allowed out of the workplace.
P273	Avoid release to the environment.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 8.

Reagent Handling

- Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.
  - Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.
Onboard	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, refer to the Alinity ci-series Operations Manual, Section 5.

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when a calibration error occurs or a control value is out of the specified range. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The Alinity i EBV VCA IgM assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the Alinity ci-series Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity ci-series Operations Manual, Section 5.

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator
Plasma	Dipotassium EDTA
	Tripotassium EDTA
	Lithium heparin
	Lithium heparin (plasma separator)
	Sodium heparin
	Sodium citrate

- Performance has not been established for the use of cadaveric specimens or the use of bodily fluids other than human serum/plasma.
- Liquid anticoagulants may have a dilution effect resulting in lower S/CO values for individual specimens.
- The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Do not use:
  - heat-inactivated specimens
  - pooled specimens
  - grossly hemolyzed specimens
  - specimens with obvious microbial contamination
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge at a minimum of 100 000 g-minutes.
- Examples of acceptable time and force ranges that meet this criterion are listed in the table below.

  Centrifugation time using alternate RCF values can be calculated using the following formula:

$$\text{Minimum Centrifugation time (minutes)} = \frac{\text{100 000 g-minutes}}{\text{RCF}}$$

Recentrifugation Time (Minutes)	RCF (x g)	g-Minutes
10	10 000	100 000
20	5000	100 000
40	2500	100 000

$RCF = 1.12 \times r_{max}\ (rpm/1000)^2$

RCF -	The relative centrifugal force generated during centrifugation.
rpm -	The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).
Centrifugation Time -	The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.
r_{max} -	Radius of the rotor in millimeters. NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (r_{max}) should be manually measured in millimeters and the RCF calculated.
g-minutes -	The unit of measure for the product of RCF ($\times$ g) and centrifugation time (minutes).

- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/ Plasma	15 to 30°C	3 days	Specimens may be stored on or off the clot or red blood cells.
	2 to 8°C	14 days	Specimens may be stored on or off the clot or red blood cells.

If testing will be delayed more than the recommended storage time, remove serum or plasma from the clot, red blood cells, or separator gel and store frozen (-20°C or colder).

Avoid more than 3 freeze/thaw cycles.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

PROCEDURE

Materials Provided

09P22 Alinity i EBV VCA IgM Reagent Kit

Materials Required but not Provided

- Alinity i EBV VCA IgM assay file
- 09P2201 Alinity i EBV VCA IgM Calibrator
- 09P2210 Alinity i EBV VCA IgM Controls or other control material
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, refer to the Alinity ci-series Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Maximum number of replicates sampled from the same sample cup: 10
  - Priority:
    - Sample volume for first test: 75 µL
    - Sample volume for each additional test from same sample cup: 25 µL
  - ≤ 3 hours on the reagent and sample manager:
    - Sample volume for first test: 150 µL
    - Sample volume for each additional test from same sample cup: 25 µL
  - > 3 hours on the reagent and sample manager:
    - Replace with a fresh aliquot of sample.
- Refer to the Alinity i EBV VCA IgM calibrator package insert and/or Alinity i EBV VCA IgM control package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples cannot be diluted for the Alinity i EBV VCA IgM assay.

Calibration

For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.

Each assay control must be tested to evaluate the assay calibration.

Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.
- Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.
  - If statistically-based quality control limits are not available, then the calibration should not exceed a 30-day limit for recalibration frequency.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

The recommended control requirement for the Alinity i EBV VCA IgM assay is that a single sample of each control level be tested once every 24 hours each day of use.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.[11]

- If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.
- If quality control results do not meet the acceptance criteria defined by your laboratory, sample results may be suspect. Follow the established quality control procedures for your laboratory. Recalibration may be necessary. For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[12]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity i analyzer calculates results for the Alinity i EBV VCA IgM assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.

Cutoff RLU = Calibrator 1 Mean RLU x 2

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

The cutoff is 1.00 S/CO.

S/CO	Interpretation
< 0.50	Nonreactive
0.50 to < 1.00	Grayzone
≥ 1.00	Reactive

Specimens with Alinity i EBV VCA IgM grayzone results might contain low levels of VCA IgM antibodies. It is recommended to test these specimens with Alinity i EBV VCA IgG and Alinity i EBV EBNA-1 IgG and/or obtain and test a new bleed after 1-2 weeks. To determine infection stage, refer to the table in Determination of EBV Infection Stage in the SPECIFIC PERFORMANCE CHARACTERISTICS section of this insert.

For details on configuring the Alinity i analyzer to use grayzone interpretations, refer to the Alinity ci-series Operations Manual, Section 2.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- If the Alinity i EBV VCA IgM results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For infection stage determination, results of the Alinity i EBV VCA IgM assay should be used in conjunction with the results of the Alinity i EBV VCA IgG and the Alinity i EBV EBNA-1 IgG assays.
- For diagnostic purposes, results should be used in conjunction with other data; e.g. symptoms, results of other tests, clinical impressions, etc.
- Cross reactivity to other potentially interfering disease states, especially other herpesviruses[13], e.g. CMV and HHV-6, may be observed. For testing results, refer to the table in Unrelated Medical Conditions in the SPECIFIC PERFORMANCE CHARACTERISTICS section of this insert.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[14, 15] Specimens containing HAMA may produce anomalous values when tested with assay kits (such as Alinity i EBV VCA IgM) that employ mouse monoclonal antibodies.[15]

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.[16] Testing was conducted using 1 lot of the Alinity i EBV VCA IgM Reagent Kit, 1 lot of the Alinity i EBV VCA IgM Calibrator, and 1 lot of the Alinity i EBV VCA IgM Controls and 1 instrument. Two controls and 2 recalcified human plasma panels (high nonreactive and low reactive) were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

		Mean	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	n	(S/CO)	SD	%CV	SD	%CV
Negative Control	117	0.10	0.003	2.9	0.003	3.0
Positive Control	119	2.80	0.050	1.8	0.067	2.4
High Nonreactive Panel	119	0.76	0.015	1.9	0.021	2.7
Low Reactive Panel	120	1.13	0.022	2.0	0.028	2.5

[a] Includes within-run, between-run, and between-day variability.

Percent Agreement

A study was performed to determine the percent agreement between the Alinity i EBV VCA IgM assay and the ARCHITECT EBV VCA IgM assay. Reactive and nonreactive EBV specimens were assayed across 3 lots of the Alinity i EBV VCA IgM Reagent Kit, 1 lot of the Alinity i EBV VCA IgM Calibrator, and 1 lot of the Alinity i EBV VCA IgM Controls and 1 instrument. The specimens were also tested on 1 ARCHITECT i2000SR instrument using 1 lot each of the ARCHITECT EBV VCA IgM Reagent Kit, ARCHITECT EBV VCA IgM Calibrator, and ARCHITECT EBV VCA IgM Controls. The percent agreement between the Alinity i EBV VCA IgM assay and the ARCHITECT EBV VCA IgM assay for reactive and nonreactive specimens is presented in the table below.

Eighteen grayzone specimens on either the Alinity i EBV VCA IgM assay and/or the ARCHITECT EBV VCA IgM assay were excluded from the evaluation. Sixteen of these specimens had a grayzone interpretation on both assays. The grayzone rate for both, the Alinity i EBV VCA IgM assay and the ARCHITECT EBV VCA IgM assay, was 2.61% (17/651).

	ARCHITECT EBV VCA IgM	
Alinity i EBV VCA IgM	**Reactive**	**Nonreactive**
Reactive	169	0
Nonreactive	0	464

Positive % agreement = 100.00% (169/169); 95% Confidence Interval = 97.84% to 100.00%

Negative % agreement = 100.00% (464/464); 95% Confidence Interval = 99.21% to 100.00%

Relative Specificity and Relative Sensitivity

This study was performed on the ARCHITECT i System.

Relative specificity and relative sensitivity were assessed on a total of 1495 specimens (250 presumed acute infection, 250 presumed seronegative, 995 random diagnostic). Random diagnostic specimens were mainly selected based on an incoming request for EBV IgM. Discrepant results between ARCHITECT EBV VCA IgM and the comparator assay were confirmatory tested using 2 commercially available immunoblots and by immunofluorescence testing (IFT) for EBV-VCA-IgM and EBV-VCA-IgG avidity. Out of the 1495 specimens a total of 1153 specimens were classified as VCA IgM negative and 294 specimens as VCA IgM positive in acute infection. Twenty-five specimens were classified to be VCA IgM positive in non-acute infection and were not included in the calculation of sensitivity. Additional 23 specimens were excluded from the calculation of relative sensitivity and relative specificity as inconclusive confirmation results had been obtained.

Grayzone specimens in either the ARCHITECT or the comparator assay were excluded from the relative sensitivity and relative specificity calculations. The ARCHITECT EBV VCA IgM assay showed an overall grayzone rate of 4.62% to 4.82% across the 3 lots tested. The comparator assay exhibited a grayzone rate of 6.76%.

Relative Specificity

This study was performed on the ARCHITECT i System.

Relative specificity after discordant resolution testing ranged from 99.62% to 99.90% for 3 ARCHITECT EBV VCA IgM lots evaluated. The comparator assay showed specificities of 98.86% to 99.05% on the same population tested. Data from this study are summarized in the table below.

		ARCHITECT EBV VCA IgM			Comparator EBV VCA IgM		
n	**Number of GZ[a] Samples Excluded**	**Lot**	**Relative Specificity [%]**	**95% CL[b] [%]**	**Lot**	**Relative Specificity [%]**	**95% CL[b] [%]**
1053	100	Lot 1	99.81 (1051/1053)	99.32 - 99.98	Lot A	98.86 (1041/1053)	98.02 - 99.41
1052	101	Lot 2	99.90 (1051/1052)	99.47 - 100.00	Lot A	99.05 (1042/1052)	98.26 - 99.54
1052	101	Lot 3	99.62 (1048/1052)	99.03 - 99.90	Lot A	98.86 (1040/1052)	98.02 - 99.41

[a] GZ = grayzone

[b] CL = confidence limit

Relative Sensitivity

This study was performed on the ARCHITECT i System.

Out of 294 specimens that were classified as VCA IgM positive in acute infection, 7 showed grayzone results in ARCHITECT EBV VCA IgM and/or the comparator assay. Resolved relative sensitivity on the remaining 287 specimens was 100% with a 95% confidence interval of 98.72% to 100.00% for 3 lots of the ARCHITECT EBV VCA IgM assay and 1 lot of the comparator assay.

Determination of EBV Infection Stage

The 3 Alinity i EBV assays (Alinity i EBV assay panel: Alinity i EBV VCA IgM, EBV VCA IgG, and EBV EBNA-1 IgG) should be evaluated in combination for EBV infection stage determination, to maximize sensitivity in the detection of acute primary EBV infection while maintaining high specificity for the detection of a past EBV infection (refer to table below).[a]

Alinity i EBV VCA IgM	**Alinity i EBV VCA IgG**	**Alinity i EBV EBNA-1 IgG**	**Infection Stage**
-	-	- or GZ	Seronegative (no infection)
+ or GZ	-	- or GZ	Early phase acute primary infection
+ or GZ	**+ or GZ**	- or GZ	Acute primary infection
+ (or GZ)[b]	**+ or GZ**	+	Transient infection
-	**+ or GZ**	+	Past infection
-	**+ or GZ**	- or GZ	Isolated VCA IgG
-	-	+	Isolated EBNA-1 IgG

[a] Combined VCA IgM and EBNA-1 IgG reactivity in the absence of VCA IgG reactivity is not covered in the table as they are not expected per the serological course of infection and are considered unresolved.

[b] Specimens with an Alinity i EBV VCA IgM grayzone result that are reactive for Alinity i EBV EBNA-1 IgG are recommended to be classified as past infection.

This study was performed on the ARCHITECT i System.

The EBV infection stage was determined with the ARCHITECT EBV assay panel, by classifying infection stage according to the table above. The infection stage determination results for the ARCHITECT EBV assay panel and the comparator EBV assay panel were compared to the infection staging results obtained per the final interpretation (i.e. after resolution and confirmation testing using IFT, immunoblot and further immunoassay results). The evaluation was based on 1463 specimens. Thirty-two specimens were excluded from evaluation since inconclusive confirmation results had been obtained for at least 1 of the 3 markers. Of 288 specimens classified per final interpretation as early phase or acute primary infection, 283 (98.26%) were correctly identified by the ARCHITECT EBV and comparator EBV assay panel. Of 790 specimens classified per final interpretation as past infection, no specimens were wrongly classified as seronegative or primary infection, and 773 (97.85%) were classified as past infection by the ARCHITECT EBV assay panel versus 745 (94.30%) by the comparator EBV assay panel. Of 320 specimens classified per final interpretation as seronegative, 298 (93.13%) were categorized as seronegative by the ARCHITECT EBV panel versus 287 (89.69%) by the comparator EBV panel. The overall agreement of infection stage determination based on final interpretation assay results compared to the infection stage determination per the ARCHITECT EBV assay panel was 95.01% (see table below for more detail) versus 92.28% determined per the comparator EBV assay panel.

EBV Infection Stage per ARCHITECT Assay Panel	EBV Infection Stage per Final Interpretation							
	Seronegative	Early Phase Acute Primary Infection[a]	Acute Primary Infection	Transient Infection[a]	Past Infection	Isolated VCA IgG[a]	Isolated EBNA-1 IgG[a]	Total
Seronegative	298	2	1	0	0	0	0	301
Early phase acute primary infection[a]	13	18	24	0	0	0	0	55
Acute primary infection	0	0	241	1	0	3	0	245
Transient infection[a]	0	0	1[b]	24	6	0	0	31
Past infection	0	0	0	1	773	0	0	774
Isolated VCA IgG[a]	9	0	1	0	11	34	0	55
Isolated EBNA-1 IgG[a]	0	0	0	0	0	0	2	2
Total	320	20	268	26	790	37	2	1463
Agreement 95.01%								

[a] Infection stage is considered unresolved and may require a follow up sample and / or further testing.

[b] Specimen was ARCHITECT EBV EBNA-1 IgG reactive, negative in 2 EBNA-1 IgG confirmation tests, but showed grayzone VCA IgG avidity.

Interference

These studies were performed on the ARCHITECT i System.

Potentially Interfering Endogenous Substances

No interference was observed between experimental controls and nonreactive or reactive specimens tested with the following potentially interfering substances.

Potentially Interfering Substance	Interferent Level
Unconjugated Bilirubin	≤ 20 mg/dL
Conjugated Bilirubin	≤ 20 mg/dL
Triglycerides	≤ 3000 mg/dL
Protein	≤ 12 g/dL
Hemoglobin	≤ 500 mg/dL

Unrelated Medical Conditions

Studies were performed to evaluate the impact of unrelated medical conditions on the ARCHITECT EBV VCA IgM assay in comparison to a comparator assay; refer to table below. Specimens with grayzone results on either assay were not taken into account for relative agreement calculation.

Category	n	ARCHITECT EBV VCA IgM Nonreactive	ARCHITECT EBV VCA IgM Reactive	ARCHITECT EBV VCA IgM Grayzone	Relative Agreement between ARCHITECT EBV VCA IgM and a Comparator Assay [%]
Cytomegalovirus IgM	10	6	4	0	88[a] (7/8)
Human anti-mouse antibodies (HAMA)	10	10	0	0	100 (10/10)
Hepatitis A Virus IgM	10	6	4	0	100 (9/9)
HBc IgM	10	10	0	0	100 (10/10)
HBsAg	10	8	1	1	100 (7/7)
Human Herpesvirus-6 IgM	10	7	1	2	100 (8/8)
Herpes Simplex Virus IgM	10	7	1	2	100 (7/7)
Parvovirus B19 IgM	10	8	1	1	88[a] (7/8)
Rubella IgM	10	8	0	2	88[a] (7/8)
Toxoplasma gondii IgM	10	10	0	0	100 (10/10)
Varicella Zoster Virus IgM	10	9	1	0	100 (10/10)
Anti-HCV	10	10	0	0	100 (10/10)
Anti-HIV	20	19	0	1	100 (19/19)
Anti-dsDNA autoantibodies	10	10	0	0	100 (10/10)
Antinuclear antibodies (ANA)	10	10	0	0	100 (10/10)
Influenza vaccine recipients	10	10	0	0	100 (10/10)
Patients with elevated IgM	10	10	0	0	100 (10/10)
Patients with monoclonal IgM	10	10	0	0	100 (10/10)
Patients with streptococcal infection	10	9	1	0	100 (10/10)
Pregnant women (1st trimester)	10	8	2	0	89[b] (8/9)
Pregnant women (2nd trimester)	10	10	0	0	90[a] (9/10)
Pregnant women (3rd trimester)	10	8	1	1	100 (7/7)
Rheumatoid factor (RF)	10	9	0	1	100 (9/9)
Specimens from leukemia patients	10	9	0	1	100 (9/9)
Specimens from lymphoma patients	10	10	0	0	100 (10/10)

[a] One reactive ARCHITECT EBV VCA IgM result from a patient with CMV IgM was confirmed as positive by 2 commercially available EBV IgM immunoblots.

Three nonreactive ARCHITECT EBV VCA IgM results from 1 patient with Parvovirus B19 IgM, 1 patient with Rubella IgM, and 1 specimen from a pregnant woman (2nd trimester) were confirmed as negative by 2 commercially available EBV IgM immunoblots.

[b] One specimen from a pregnant woman (1st trimester) with a reactive result on ARCHITECT EBV VCA IgM was confirmed as negative by 2 commercially available EBV IgM immunoblots.

BIBLIOGRAPHY

1. Rickinson AB, Kieff E. Epstein-Barr virus. In: Knipe PM, Howley DE, Griffin MA, et al., editors. *Fields Virology*, 4th ed. Philadelphia, PA 2001;2575-2627.
2. Crawford DH, Swerdlow AJ, Higgins C, et al. Sexual history and Epstein-Barr virus infection. *J Infect Dis* 2002;186:731-736.
3. Schooley RT. Epstein-Barr virus (infectious mononucleosis). In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Benett's Principles and Practice of Infectious Diseases*, vol. 2, 4th ed. New York, NY: Churchill Livingstone; 1995:1364-1377.
4. Berth M, Bosmans E. Comparison of three automated immunoassay methods for the measurement of Epstein-Barr virus-specific immunoglobulin M. *Clin Vaccine Immunol* 2010;17:559-563.
5. De Ory F, Guisasola ME, Sanz JC, et al. Evaluation of four commercial systems for the diagnosis of Epstein-Barr virus primary infections. *Clin Vaccine Immunol* 2011;18:444-448.
6. Hess RD. Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. *J Clin Microbiol* 2004;42(8):3381-3387.
7. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
8. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
9. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
10. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.
11. Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition.* CLSI Document C24-A3. Wayne, PA: CLSI; 2006.
12. Westgard JO. *Basic QC Practices.* 3rd ed. Madison, WI: Westgard Quality Corporation; 2010.
13. Balachandran N, Oba DE, and Hutt-Fletcher LM. Antigenic cross-reactions among herpes simplex virus types 1 and 2, Epstein-Barr virus, and cytomegalovirus. *J Virol* 1987;61:1125-1135.
14. Primus FJ, Kelley EA, Hansen HJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-264.
15. Schroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45(2):879-885.
16. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.* CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.

Note for number formatting:

- A space is used as thousands separator (example: 10 000 specimens).
- A period is used to separate the integer part from the fractional part of a number written in decimal form (example: 3.12%).

Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ASSAY DILUENT	Assay Diluent
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
INVERSIONS PERFORMED	Inversions Performed
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRODUCT OF GERMANY	Product of Germany
REF	List Number
SN	Serial number

Abbott GmbH
Max-Planck-Ring 2
65205 Wiesbaden
Germany
+49-6122-580

CE

Customer Service: Contact your local representative or find country-specific contact information on www.abbottdiagnostics.com

Revised March 2020.