ARCHITECT

SYSTEM

en

EBV VCA IgG

REF 3P65

B3P650

G3-3887/R01

EBV VCA IgG

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

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	REAGENT LOT	Reagent Lot
IVD	*In Vitro* Diagnostic Medical Device	CONTROL NO.	Control Number
LOT	Lot Number	REACTION VESSELS	Reaction Vessels
	Expiration Date	SAMPLE CUPS	Sample Cups
		SEPTUM	Septum
2°C 8°C	Store at 2-8°C	REPLACEMENT CAPS	Replacement Caps
	Consult instructions for use	CONTAINS: AZIDE	Contains sodium azide. Contact with acids liberates very toxic gas.
SN	Serial Number	WARNING: SENSITIZER	Warning: May cause an allergic reaction
		GTIN	Global Trade Item Number
	Manufacturer	PRODUCT OF GERMANY	Product of Germany

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

NAME

ARCHITECT EBV VCA IgG

INTENDED USE

The ARCHITECT EBV VCA IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG antibodies to Epstein-Barr Virus (EBV) Viral Capsid Antigen (VCA) in human serum and plasma. The assay is intended 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 TEST

Epstein-Barr virus (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 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	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

The ARCHITECT EBV VCA IgG assay is a two-step immunoassay for the qualitative detection of IgG antibodies to EBV VCA in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

In the first step, sample, assay diluent, and VCA antigen coated paramagnetic microparticles are combined. Anti-EBV VCA IgG present in the sample binds to VCA antigen coated microparticles. After washing, anti-human IgG acridinium-labeled conjugate is added to create a reaction mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti-EBV VCA IgG in the sample and the RLUs detected by the ARCHITECT *i* System optics.

The presence or absence of anti-EBV VCA IgG in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active calibration curve. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the sample is considered reactive for anti-EBV VCA IgG.

REAGENTS

Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT EBV VCA IgG Reagent Kit (3P65)

- **MICROPARTICLES** 1 Bottle (6.6 mL per 100-test bottle/27.0 mL per 500-test bottle) EBV VCA antigen coated microparticles in TRIS buffer with protein (bovine) stabilizers and detergent. Minimum concentration: 0.06% solids. Preservatives: sodium azide and ProClin 950.
- **CONJUGATE** 1 Bottle (5.9 mL per 100-test bottle/26.3 mL per 500-test bottle) recombinant murine monoclonal acridinium-labeled anti-human IgG conjugate in MES buffer with protein (bovine) stabilizers and detergent. Minimum concentration: 40 ng/mL. Preservatives: ProClin 300 and ProClin 950.
- **ASSAY DILUENT** 1 Bottle (10.0 mL per 100-test bottle/50.9 mL per 500-test bottle) assay diluent containing TRIS buffer and detergent. Preservatives: sodium azide and ProClin 950.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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.[7] Biosafety Level 2[8] or other appropriate biosafety practices[9,10] should be used for materials that contain or are suspected of containing infectious agents.

The following warnings and precautions apply to these components:

- Microparticles
- Assay Diluent

WARNING:	Contain methylisothiazolone and sodium azide.
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist/vapours/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.
P363	Wash contaminated clothing before reuse.
This material and its container must be disposed of in a safe way.	

The following warnings and precautions apply to this component:

- Conjugate

WARNING:	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist/vapours/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.
P363	Wash contaminated clothing before reuse.
This material and its container must be disposed of in a safe way.	

- 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 ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT EBV VCA IgG Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - **When handling conjugate vials, change gloves that have contacted human plasma/sera, since introduction of human IgG will result in a neutralized conjugate.**
  - Once a septum has been placed on the reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT EBV VCA IgG Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT EBV VCA IgG Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT EBV VCA IgG assay file must be installed on the ARCHITECT *i* System from an ARCHITECT *i* System Assay CD-ROM prior to performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used. The specimen collection tubes listed below were verified for use with the ARCHITECT EBV VCA IgG assay. Other specimen collection tubes have not been tested with this assay.

Human serum	Serum
	Serum separator tubes
Human plasma	Dipotassium EDTA
	Tripotassium EDTA
	Lithium heparin
	Sodium heparin
	Sodium citrate
	Plasma separator tubes (lithium heparin)

- Liquid anticoagulants may have a dilution effect resulting in lower S/CO values for individual patient specimens.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed (> 500 mg/dL hemoglobin)
  - obvious microbial contamination
  - cadaver specimens or any other body fluids
- 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.

- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Prepare frozen specimens as follows:
  - Frozen specimens must be completely thawed before mixing.
  - Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous. If samples are not mixed thoroughly, inconsistent results may be obtained.
  - Centrifuge mixed specimens as described below.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at a Relative Centrifugal Force (RCF) of ≥ 10000 x g for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter,
  - they were frozen and thawed.

  Transfer clarified specimen to a sample cup or secondary tube for testing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.

Storage

- Specimens may be stored on or off the clot or red blood cells for up to 3 days at 15-30°C or 14 days refrigerated at 2-8°C.
- 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.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped ambient, on wet ice, or dry ice. Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 3P65 ARCHITECT EBV VCA IgG Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT EBV VCA IgG assay file, may be obtained from the following:
  - ARCHITECT *i* System e-Assay CD-ROM at www.abbottdiagnostics.com
  - ARCHITECT *i* System Assay CD-ROM
- 3P65-01 ARCHITECT EBV VCA IgG Calibrator
- 3P65-10 ARCHITECT EBV VCA IgG Controls or other control material
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional)

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT EBV VCA IgG Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue inverting the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions on placing septums on bottles refer to the **Handling Precautions** section of this package insert.
- Load the ARCHITECT EBV VCA IgG Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary assay reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
  - Priority: 75 µL for the first ARCHITECT EBV VCA IgG test plus 25 µL for each additional ARCHITECT EBV VCA IgG test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first ARCHITECT EBV VCA IgG test plus 25 µL for each additional ARCHITECT EBV VCA IgG test from the sample cup.
  - > 3 hours on board: additional sample volume is required. For information on sample evaporation and volumes, refer to the ARCHITECT System Operations Manual, Section 5.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient sample is present.
- Prepare calibrator and controls.
  - Mix ARCHITECT EBV VCA IgG Calibrator and Controls by gentle inversion before use.
  - To obtain the recommended volume requirements for the ARCHITECT EBV VCA IgG Calibrator and Controls, hold the bottles **vertically** and dispense 4 drops of the calibrator or 4 drops of each control into each respective sample cup.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

Specimens cannot be diluted for the ARCHITECT EBV VCA IgG assay.

Calibration

- To perform an ARCHITECT EBV VCA IgG calibration, test the calibrator in replicates of three. A single sample of each ARCHITECT EBV VCA IgG control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the controls package insert. The calibrator should be priority loaded.
- Once an ARCHITECT EBV VCA IgG 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.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT EBV VCA IgG assay is that a single sample of each control be tested once every 24 hours each day of use. If your quality control procedures in your laboratory require more frequent use of controls to verify test results, follow those procedures.

The ARCHITECT EBV VCA IgG control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and samples must be retested. Recalibration may be indicated.

RESULTS

Calculation

The ARCHITECT *i* System calculates the ARCHITECT EBV VCA IgG Calibrator mean chemiluminescent signal from three calibrator replicates and stores the result.

The ARCHITECT *i* System calculates the result for the ARCHITECT EBV VCA IgG assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control. The result unit for the ARCHITECT EBV VCA IgG assay is S/CO.

- S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

S/CO	Instrument Interpretation
< 0.75	Nonreactive
0.75 to < 1.00	Grayzone
≥ 1.00	Reactive

- Specimens with ARCHITECT EBV VCA IgG grayzone results might contain low levels of VCA IgG antibodies. It is recommended to test these specimens with ARCHITECT EBV VCA IgM and ARCHITECT EBV EBNA-1 IgG and/or obtain and test a new bleed after 1-2 weeks. Refer to Table 5, for infection stage determination.

For details on configuring the ARCHITECT *i* System to use grayzone interpretations, refer to the ARCHITECT System 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 ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- If the ARCHITECT EBV VCA IgG results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For infection stage determination, results of the ARCHITECT EBV VCA IgG assay should be used in conjunction with the results of the ARCHITECT EBV VCA IgM and the ARCHITECT 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.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[11] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[12,13] Specimens containing HAMA may produce anomalous values when tested with assay kits (such as ARCHITECT EBV VCA IgG) that employ mouse monoclonal antibodies.[13]

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT EBV VCA IgG assay is designed to have a within laboratory total imprecision of less than or equal to an SD of 0.10 S/CO for high nonreactive samples (0.60-0.99 S/CO) and less than or equal to 10% CV for low reactive samples (1.00-4.00 S/CO).

A 20-day precision study was performed for the ARCHITECT EBV VCA IgG assay based on guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS), Protocol EP5-A2[14]. Testing was conducted internally using three ARCHITECT EBV VCA IgG assay reagent lots on two ARCHITECT *i*2000SR instruments, and using one ARCHITECT EBV VCA IgG assay reagent lot on one *i*1000SR instrument. Each reagent lot was tested with one control lot. For the *i*2000SR instrument two calibrator lots were used for instrument calibration, for the *i*1000SR instrument one calibrator lot.

A panel consisting of negative control, positive control and two human plasma panels (high nonreactive and low reactive) were assayed in replicates of three, at two separate times per day for a total of 20 days.

Data from this study are summarized in Table 1*.

Table 1
20-day CLSI Precision Study

ARCHITECT *i*2000SR

		Mean	Within-Run		Within Laboratory (Total)**	
Sample	n	S/CO	SD	%CV	SD	%CV
Negative Control	720	0.02	0.002	8.7	0.002	10.0
High Nonreactive Panel	716	0.74	0.017	2.3	0.022	3.0
Low Reactive Panel	717	1.37	0.028	2.1	0.038	2.8
Positive Control	720	2.89	0.063	2.2	0.086	3.0

ARCHITECT *i*1000SR

		Mean	Within-Run		Within Laboratory (Total)**	
Sample	n	S/CO	SD	%CV	SD	%CV
Negative Control	120	0.03	0.003	11.1	0.004	13.5
High Nonreactive Panel	120	0.74	0.017	2.3	0.022	2.9
Low Reactive Panel	120	1.36	0.029	2.1	0.034	2.5
Positive Control	120	2.87	0.070	2.4	0.074	2.6

* Representative data; results in individual laboratories may vary from these data.

** Total is an accumulation of within run, between run and between day.

A 5-day precision study was performed internally and at one external site each on one ARCHITECT *i*2000SR instrument for the ARCHITECT EBV VCA IgG assay based on guidance from CLSI document EP15-A2[15]. Testing was conducted once per day using three ARCHITECT EBV VCA IgG assay reagent lots. In total three calibrator lots were used per site for instrument calibration.

A panel consisting of three lots of negative control, three lots of positive control and two human plasma panels (high nonreactive and low reactive) were assayed in replicates of four.

Data from this study are summarized in Table 2*.

Table 2
5-day CLSI Precision Study
ARCHITECT *i*2000SR

		Mean	Within Run		Within Laboratory (Total)**	
Sample	n	S/CO	SD	%CV	SD	%CV
Negative Control	360	0.02	0.001	5.1	0.001	5.3
High Nonreactive Panel	120	0.76	0.013	1.7	0.015	2.0
Low Reactive Panel	120	1.40	0.028	2.0	0.032	2.3
Positive Control	360	2.90	0.054	1.8	0.068	2.3

* Representative data; results in individual laboratories may vary from these data.

** Total is an accumulation of within run, between run and between day.

Relative Specificity and Relative Sensitivity

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 IgG and the comparator assay were confirmatory tested by immunofluorescence testing (IFT) for EBV-VCA-IgG avidity. Out of the 1495 specimens, a total of 1132 specimens were classified as VCA IgG positive and 357 specimens as VCA IgG negative. Six specimens showed an inconclusive confirmation result and were therefore not included in the calculation of relative sensitivity or relative specificity.

Grayzone specimens were excluded from the relative sensitivity and relative specificity calculations. For the ARCHITECT EBV VCA IgG assay the overall grayzone rate ranged from 1.20% to 1.81% across the three lots tested.

Relative Specificity

Relative specificity after discordant resolution testing for the overall sample categories ranged from 97.47% to 97.75% for three ARCHITECT EBV VCA IgG lots evaluated. The comparator assay exhibited a specificity of 97.19% on the same population tested. Data for one lot of the ARCHITECT EBV VCA IgG assay and the comparator assay are summarized in Table 3*.

Table 3
Relative Specificity

			ARCHITECT EBV VCA IgG		Comparator EBV VCA IgG	
	n	Number of GZ[a] Samples Excluded	Relative Specificity [%]	95% CL[b] [%]	Relative Specificity [%]	95% CL[b] [%]
Overall Sample Categories	356	1	97.47 (347 / 356)	95.26 - 98.84	97.19 (346 / 356)	94.89 - 98.64
Presumed Seronegatives	242	1	98.35 (238 / 242)	95.82 - 99.55	96.28 (233 / 242)	93.06 - 98.29

* Representative data; results in individual laboratories may vary from these data.

[a] GZ = grayzone

[b] CL = confidence limit

Relative Sensitivity

A total of 1132 specimens were classified as VCA IgG positive. Grayzone specimens were excluded from the relative sensitivity calculation, so that the calculation was based on 1108 specimens minimally. Data from this study are summarized in Table 4*.

Table 4
Relative Sensitivity

		ARCHITECT EBV VCA IgG			Comparator EBV VCA IgG	
n	Lot	Relative Sensitivity [%]	95% CL[a] [%]	Lot	Relative Sensitivity [%]	95% CL[a] [%]
1117	Lot 1	96.06 (1073 / 1117)	94.75 - 97.12	Lot A	96.60 (1079 / 1117)	95.36 - 97.58
1108	Lot 2	97.65 (1082 / 1108)	96.58 - 98.46	Lot A	96.66 (1071 / 1108)	95.43 - 97.64
1111	Lot 3	96.76 (1075 / 1111)	95.54 - 97.72	Lot A	96.58 (1073 / 1111)	95.34 - 97.57

* Representative data; results in individual laboratories may vary from these data.

[a] CL = confidence limit

Determination of EBV Infection Stage using the ARCHITECT EBV assays

The three ARCHITECT EBV assays (ARCHITECT EBV assay panel: ARCHITECT 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 5).

Table 5[a]

Determination of EBV Infection Stage using the ARCHITECT EBV Assay Panel

ARCHITECT EBV VCA IgM	ARCHITECT EBV VCA IgG	ARCHITECT 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 Table 5 as they are not expected per the serological course of infection and are considered unresolved.

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

The EBV infection stage was determined with the ARCHITECT EBV assay panel, by classifying infection stage according to Table 5. 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 one of the three 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 6 for more detail) versus 92.28% determined per the comparator EBV assay panel.

Table 6[a]

EBV Infection Stage using the ARCHITECT EBV Assay Panel vs. Final Interpretation

	EBV infection stage per final interpretation							
EBV infection stage per ARCHITECT assay panel	**Seronegative**	**Early phase acute primary infection[c]**	**Acute primary infection**	**Transient infection[c]**	**Past infection**	**Isolated VCA IgG[c]**	**Isolated EBNA-1 IgG[c]**	**Total**
Seronegative	298	2	1	0	0	0	0	301
Early phase acute primary infection[c]	13	18	24	0	0	0	0	55
Acute primary infection	0	0	241	1	0	3	0	245
Transient infection[c]	0	0	1[b]	24	6	0	0	31
Past infection	0	0	0	1	773	0	0	774
Isolated VCA IgG[c]	9	0	1	0	11	34	0	55
Isolated EBNA-1 IgG[c]	0	0	0	0	0	0	2	2
Total	320	20	268	26	790	37	2	1463
Agreement 95.01%								

[a] Representative data; results in individual laboratories may vary from these data.

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

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

Interference

No interference was observed between experimental controls and nonreactive or reactive specimens tested with elevated levels of unconjugated bilirubin/conjugated bilirubin (20 mg/dL), triglycerides (3000 mg/dL), protein (12 g/dL), or hemoglobin (500 mg/dL).

Potentially Cross-Reacting Disease States

Studies were performed to evaluate the impact of other disease states on the ARCHITECT EBV VCA IgG assay in comparison to a comparator assay, refer to Table 7*. No grayzone results were observed for ARCHITECT EBV VCA IgG.

Table 7

Potentially Cross-Reacting Disease States

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

* Representative data; results in individual laboratories may vary from these data.

** One specimen from a patient infected with *Toxoplasma gondii* with a reactive result on ARCHITECT EBV VCA IgG was confirmed to be positive by immunofluorescence testing.

One specimen containing elevated IgG with a reactive result on ARCHITECT EBV VCA IgG was grayzone by immunofluorescence testing.

*** One leukemia patient specimen with a reactive result on ARCHITECT EBV VCA IgG was negative by immunofluorescence testing. The specimen showed a positive VCA IgG band on two commercially available EBV IgG immunoblots.

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