ImmunoWELL™

EBV VCA IgM TEST

$C\mkern-3mu\epsilon$

Product No. 3260

IVD **For *In Vitro* Diagnostic Use**

INTENDED USE

ImmunoWELL VCA IgM Test is an ELISA method for the qualitative detection of IgM antibody to Epstein-Barr Virus viral capsid antigen (VCA) in human serum. When the VCA IgM test is used in conjunction with other testing such as the EBV nuclear antigen (EBNA-1), VCA IgG, and EBV early antigen tests and/or heterophile tests, the results can serve as an aid in the diagnosis of infectious mononucleosis (IM).

SUMMARY AND EXPLANATION

Except for primary EBV infections, laboratory evaluation of all other EBV–associated conditions is not straightforward.[1,2] The cultivation of EBV is seldom practical because it takes too long (up to 4 weeks) and requires freshly fractionated cord blood lymphocytes. Antigen or nucleic acid detection, very useful for demonstrating the presence of EBV, usually requires biopsies and special reagents

ASSAY PRINCIPLE

The ImmunoWELL Test utilizes an EIA microtiter plate technique for the detection of antibodies. Serum from which IgG antibodies have been removed is added to antigen coated microtiter wells and allowed to react. After removal of unbound antibodies, horseradish peroxidase-conjugated antihuman IgM antibodies are allowed to react with bound antibodies. The bound peroxidase reacts with tetramethylbenzidine (TMB), the chromogenic substrate, developing a color. Finally, the substrate reaction is stopped and the optical density is read with a microwell spectrophotometer.

REAGENTS

Reaction Wells coated with purified EBV glycoprotein (gp125, purified by affinity chromatography)

Specimen Diluent - antihuman IgG in 0.01M phosphate buffered saline (PBS, pH 6.2-7.6), carrier protein and <0.1% NaN3

VCA IgM Calibrators (3) - human anti–VCA prediluted, ready for use in 0.01M PBS, carrier protein and <0.1% NaN3

VCA IgM Positive Control - human anti–VCA serum containing <0.1% NaN[3]

VCA Negative Control - nonreactive human serum containing <0.1% NaN3

Wash Buffer Concentrate consisting of a 20X concentrate of 0.01 M PBS (pH 6.2-7.6) and 0.05% Tween

Conjugate - peroxidase-conjugated goat antihuman IgM in PBS (pH 6.2-7.6) and carrier protein containing preservatives

Substrate - tetramethylbenzidine (TMB).

Stop Solution - 0.5 N Hydrochloric acid

Warnings and Precautions

For *In Vitro* Diagnostic Use: ImmunoWELL reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoWELL Test reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use any kits beyond the stated expiration date. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since this may result in poor assay performance.

Some reagents contain sodium azide that may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Warning - Potential Biohazardous Material: Human sera used in the preparation of this product were tested using FDA approved procedures and found non-reactive for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, and hepatitis C virus. Because no test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting disease.

Reconstitution and Storage

Kit is stored at 2-8°C. Assuming good laboratory practices are used, opened reagents remain stable as indicated by the expiration date.

Reaction wells are removed from the foil pouch and unused wells are resealed in the pouch using the integral zip-lock. Unused wells must be used within six weeks of opening the pouch.

Wash Buffer (pH 6.2-7.6) is prepared by adding the contents of the Wash Buffer Concentrate (20X) bottle into 1 liter of distilled/deionized water. After reconstitution, the 1X solution is stored at 2-8°C. Discard when visibly turbid.

Note: In some instances the Wash Buffer Concentrate (20X) may develop crystals upon storage at 2-8°C. It is important that these crystals are completely redissolved before dilution of the Concentrate. This can be accomplished by warming the Concentrate to 37°C in a water bath with occasional mixing.

SPECIMEN COLLECTION AND HANDLING

ImmunoWELL Test is performed on serum. The test requires 10 µL of serum. Lipemic or hemolyzed serum has not been shown an acceptable specimen.

Store samples at room temperature for no longer than eight hours. If the assay will not be completed within eight hours, refrigerate the sample at 2–10°C. If the assay or shipment of the samples will not be completed within 48 hours, freeze at –20°C.

PROCEDURE

Materials Provided

Microtiter Wells in Carrier	Specimen Diluent
Calibrators	Positive Control
Negative Control	Wash Buffer Concentrate (20X)
Conjugate	Substrate
Stop Solution	Package Insert

Materials Required But Not Provided

Distilled or deionized water	Pipets
Microwell washer	Test tubes
Microwell spectrophotometer (450 nm)	

Performance Considerations

Reproducibility in the assay is largely dependent upon the consistency with which the microwells are washed. Carefully follow the recommended washing sequence as outlined in the assay procedure.

Positive and Negative Control Sera (Undiluted) are used to assure test performance.

Calibrators (prediluted) are used to construct a standard curve.

Substrate Blank - All reagents, except serum, are added to the substrate blank well. This blank well is intended to baseline (zero) the microwell spectrophotometer.

Assay Procedure

1. Allow all components including diluted Wash Buffer to warm to room temperature (22-27°C).

2. Determine the total number of specimens to be run. Include one blank and duplicates of calibrators (or calibrator if using the normalizing calculation) and controls in each run.

3. For each control and specimen, pipet 10 µL serum into a clean tube containing 1 mL Specimen Diluent and mix (1:100 dilution).

CAUTION: Calibrators are prediluted. Do not dilute further.

4. Determine the total number of wells to be run including blank, calibrators, controls, and specimens. Well strips can be broken to the exact number needed to conserve reagent wells. Strips need to be completed with used wells to facilitate washing procedures.

5. Add 100 µL of Specimen Diluent into the first well as a substrate blank.

6. Pipet 100 µL of the prediluted calibrators and diluted controls and specimens (step 3) into each assigned well.

7. Incubate at room temperature (22–27°C) for 60±2 minutes.

8. Aspirate the samples out of the wells.

9. Wash the wells three times by completely filling the wells with Wash Buffer (see Reconstitution and Storage) and aspirating the wells completely after washes.

10. Pipet 100 µL Conjugate into all wells.

11. Incubate the wells at room temperature (22–27°C) for 30±2 minutes.

12. Aspirate the conjugate out of the wells.

13. Wash the wells three times as described in step 9.

14. Pipet 100 µL of Substrate into each well.

15. Incubate at room temperature (22–27°C) for 30±2 minutes.

16. Add 100 µL of Stop Solution to each well.

17. Inspect the outside bottom surface of the microwells for the presence of condensation, dried buffer salts or wash solution that might interfere with the spectrophotometric reading. Carefully clean the well bottoms with a soft tissue.

18. Using the substrate blank to zero the spectrophotometer, read the optical density of each well at 450 nm within 30 minutes of completion of step 16.

Quality Control

GenBio provides positive and negative controls with defined ranges provided in a Supplement included in the kit. The positive control value is approximately five standard deviations (absorbance) above the upper cutoff and the negative control value is less than 0.15 absorbance units. Interpretations should not be made unless the control results fall within these limits.

NCCLS C24-A should be consulted for guidance on appropriate quality control practices. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION

Procedure for Calculating Activity of Specimen

ImmunoWELL calibrators are assigned values of antibody concentration (U/mL) which may vary by lot number. Please verify that the lot numbers on the vials match the lot numbers on the Package Insert Supplement to assure the proper values are used in the calculation.

Depending on the number of calibrators run, activity of the specimen may be calculated in one of two ways:

Construct a point-to-point standard curve using the absorbance values you observe and their corresponding assigned values. Use this curve to calculate antibody concentration of controls and specimens.

OR

Calculate activity of the specimen by normalizing to the Mid Calibrator according to the following:

$$V_S = A_S \times V_{MC}/A_{MC}$$

Where:

V_S = Value of the specimen (U/mL)

A_S = Absorbance of the specimen

V_{MC} = Assigned Value of the Mid Calibrator (U/mL) given in the Supplement

A_{MC} = Mean absorbance of the Mid Calibrator obtained in the assay

The interpretive ranges are:

	Units/mL	Interpretation
Negative	<400	Specific Antibody not detected
Equivocal	400-500	Report as negative or retest. If retested, the second result is considered final. If the repeat test is also equivocal, report as equivocal.
Positive	>500	Specific antibody detected

The cutoff level is defined relative to the mean of negative sera. Clinical interpretation requires knowledge of the patient's condition, other EBV serological results and other laboratory results.[1,2] The magnitude of a measured result above the cutoff is not indicative of the total amount of antibody present.

Negative results do not rule out the diagnosis of infectious mononucleosis. The specimen may have been drawn before appearance of detectable antibodies. Negative results in suspected early IM should be retested in 4-5 weeks.

Although EBV serodiagnosis requires measurement of more than one analyte, universal agreement of a serological profile does not exist. One profile criterion[1,2] is shown in Table 1.

Table 1: Interpretation Criteria

Interpretation	VCA IgG	EBNA IgG	VCA IgM	Heterophile
Past Infection	+*	+	-	-
Recent Infection	+	+	+ or -	+ or -
Current/Acute	+	-	+ or -	+ or -
No Past Infection	-	-	-	-

* A few samples are VCA IgG negative and EBNA IgG positive. These are classified as from either past or recent infections.

LIMITATIONS

No cross reactivity testing has been performed with this assay. Anti-nuclear antibody positive sera have not been tested for cross reactivity. Absorbent binds at least 15 mg/mL human IgG and is used to prevent interference due to rheumatoid factor or competing IgG in patient specimens. However, testing demonstrated removal of RF factor at a maximum of 67%, therefore presence of these agents in a high concentration could affect results of this assay. VCA IgM performance characteristics have been established for mononucleosis but not for other diseases (e.g., nasopharyngeal carcinoma, Burkitt's Lymphoma, and lymphoproliferative disorders). Performance characteristics of samples from immunocompromised patients have not been established. The assay performance characteristics have not been established for matrices other than serum.

A diagnosis should not be made on the basis of anti-VCA IgM results alone. Test results for anti-VCA IgM should be interpreted in conjunction with results of antibody tests for other EBV specific antigens: VCA IgG, EBNA IgG, and/or EA IgG.

EBV serodiagnosis is made by the profile analysis of at least two or more EBV antibody responses. Caution should be used if interpreting a profile using more than one manufacturer's kits or different assay methods.

EXPECTED RESULTS

Prevalence may vary depending on geographical location, age, socioeconomic status, race, type of test employed, and other epidemiological and clinical criteria used to select patients[3].

The prevalence of antibody to VCA or EBNA IgG varies by geographic region. In developing and tropical areas, most children have been infected by age 6 years. It is only when a significant percentage of the population reaches ages 15-25 before exposure to and infections with EBV that infectious mononucleosis emerges as an important clinical entity. This delay in exposure is largely limited to nations with high economic and hygienic levels and to middle and upper socioeconomic classes in any country. A typical antibody profile is illustrated in Figure 1.

Figure 1
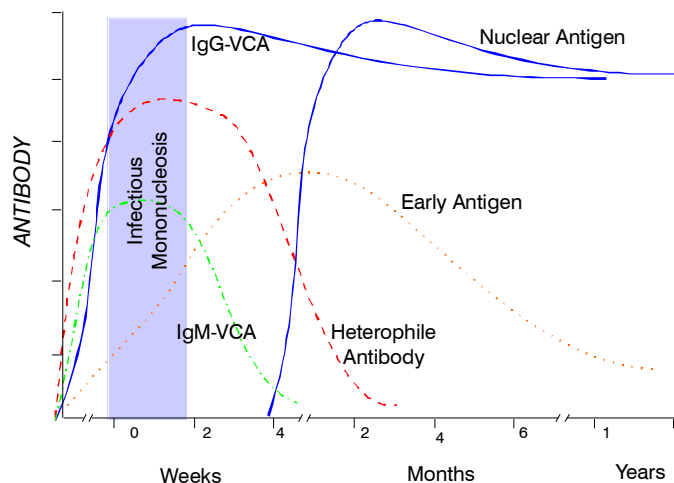
During the acute phase of IM, IgG and IgM responses to viral capsid antigen complex (VCA) are rapid and occur almost simultaneously. Due to the variable incubation period and intensity of symptoms, by the time patients consult their physicians, nearly all have reached peak titers of IgG; 10–15% of the IM patients have no detectable VCA-IgM by the time of the first serum collection. In general, both IgG and IgM become detectable within 2–3 weeks of onset and peak at 4–6 weeks. VCA-IgM disappears rapidly thereafter, while IgG wanes slightly and then varies little for life. VCA-IgM antibodies are reliably detectable only during primary infections and not during reactivation of latent infection.

The time course and the strength of the EBNA IgG response often can yield valuable information as to the patient's underlying problems. EBNA antibodies are absent during the acute phase. Their gradual appearance begins during the first to second month after onset, persisting for life. Hence, VCA-IgG antibodies found with low titer or no anti-EBNA indicates acute IM phase serum, whereas VCA-IgG antibodies in the presence of peak titer anti-EBNA indicates a later infection. Anti-EBNA-1 appears later than do other EBNA components, increasing to peak titers 6 to 12 months after infection. (GenBio EBNA IgG test measures only EBNA-1.) In patients with inherited or acquired immunodeficiencies, response to EBNA can be very late, protracted and weak. In seropositive patients with acquired immunodeficiencies, anti-EBNA titers are related to the degree of T–cell dysfunction.

Early Antigen (EA) antibodies are usually transient after primary infection. By the sixth month after onset, anti-EA wanes and may reappear with EBV reactivations.

The unit activity of forty-eight sera collected from asymptomatic blood donors and thirty-three samples from subjects identified as EBV mononucleosis positive (see Performance Characteristics) are shown in Figure 2. These data show that ImmunoWELL VCA IgM activity in asymptomatic blood donors (Normals) is low and the activity in the positives is high. It should be noted that slightly greater than half of the EBV mononucleosis cases are aged 12-19 years.
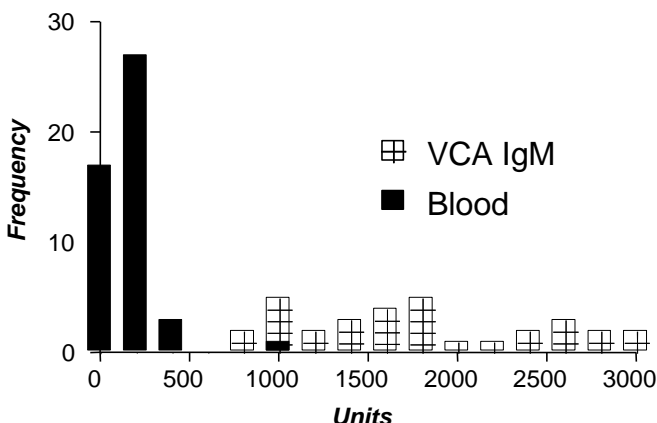

Figure 2: Unit Activity

The above information is not meant to predict how this product will perform in various populations. The incidence of VCA IgM positive samples is usually much less than shown above and is typically more like the population tested in the national reference laboratory (see Table 2). However, the incidence of U.S. blood donor results may be representative if the demographics in the tested population are similar to a blood donor population.

PERFORMANCE CHARACTERISTICS

Ninety-three sera samples submitted to a national reference laboratory and received from throughout the U.S. for EBV serological studies were evaluated. These samples were tested using IFA at the reference laboratory and later tested after being stored frozen at GenBio using an alternate commercial microtiter EIA and ImmunoWELL. Comparison testing using the ImmunoWELL VCA IgM assay and an alternate EIA kit was only tested at GenBio and is shown in Table 2. Comparison between IFA tested at the reference laboratory and ImmunoWELL tested at GenBio is shown in Table 3. Relative assay specificity, compared to EIA is 100% (96-100%) and relative specificity to IFA is 97.8% (92-99.7%). The relative sensitivity for EIA is 99.5% (28-100%) and the relative sensitivity to IFA is 66.7% (9-99.2%). The percent agreement is 98.9% and 96.8% for EIA and IFA respectively. The confidence intervals are calculated using the exact method.

Table 2: Relative Comparison to Commercial EIA

		Alternate EIA	
		Negative	**Positive**
ImmunoWELL	Negative	88	1
	Positive	0	4

Table 3: Relative Comparison to IFA

		IFA	
		Negative	**Positive**
ImmunoWELL	Negative	88	1
	Positive	2	2

Note: Please be advised that "relative" refers to the comparison of this assay's results to that of a similar assay. No judgment can be made as to either assay's accuracy to predict disease.

Due to the low prevalence of mononucleosis in the above population, sera from thirty-three EBV mononucleosis cases, serologically defined by a reference laboratory, were tested. All were ImmunoWELL VCA IgM positive (100% agreement).

Precision Data

Precision was determined by testing ten samples as duplicates within each run and testing these twice each day for twenty days runs. The study was conducted at GenBio (Site A) and an outside site (Site B). The results are shown in Table 4.

Table 4: Assay Precision

		Site A		Site B	
Units/mL	**Type**	**Within Run (%CV)**	**Between Run (%CV)**	**Within Run (%CV)**	**Between Run (%CV)**
1516	High	3	7	3	8
1296	High	3	7	5	8
1163	High	4	8	3	10
794	Moderate	5	9	5	12
738	Moderate	4	7	7	16
562	Moderate	7	11	8	18
361	Low	6	10	10	22
341	Low	9	13	14	22
98	Negative	24	28	31	49
28	Negative	157	257	74	75

BIBLIOGRAPHY

1. Lennette, ET. Epstein-Barr Virus (EBV). In Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections, 7th Ed (EH Lennette, DA Lennette, ET Lennette) American Public Health Association, Washington D.C., 1995.
2. Lennette, ET. Epstein-Barr Virus. In Manual of Clinical Microbiology, 6th Ed. (PR Murray, EJ Baron, MA Pfaller, FC. Tenover, RH Yolken). ASM Press, Washington D.C., 1995.
3. Evans, AS and JC Niederman. Epstein-Barr Virus. In Viral Infections of Humans, Epidemiology and Control, 2nd Ed. (AS Evans) Plenum Medical Book Company, New York, New York, 1982.

QUICK REFERENCE PROCEDURE
ImmunoWELL VCA IgM

- Prepare Wash Buffer from Wash Concentrate.

- Dilute each control and specimen 1:100 in Specimen Diluent.

- Add 100 μL of Specimen Diluent into the first well as a substrate blank.

- Pipet 100 μL of the prediluted calibrators, and diluted controls and specimens into coated microwells and incubate 60 minutes at room temperature.

- Aspirate microwells and wash microwells three times with Wash Buffer.

- Pipette 100 μL of Conjugate into microwells and incubate 30 minutes at room temperature.

- Aspirate microwells and wash microwells three times with Wash Buffer.

- Pipet 100 μL of Substrate into microwells and incubate 30 minutes at room temperature.

- Pipet 100 μL Stop Solution into microwells and read results at 450 nm.

To place an order for ImmunoWELL products, contact your local distributor or call GenBio directly for the distributor nearest you and for additional product information.

For assistance, please call toll-free 800-288-4368.

GenBio

15222-A Avenue of Science
San Diego, CA 92128

| EC | REP |

EMERGO EUROPE
Molenstraat 15
2513 BH, The Hague
The Netherlands