ImmunoWELL ™

CE

EBV EA(D) IgG TEST

Product No. 3240

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

INTENDED USE

ImmunoWELL EA(D) IgG Test is an ELISA method for the qualitative detection of IgG antibody to Epstein-Barr Virus Early Antigen (Diffuse) in human serum. When used in conjunction with other EBV serologies, results are used to determine immunological response to EBV infection and may aid in determining the stage of convalescent Epstein-Barr virus (EBV) infection.

SUMMARY AND EXPLANATION

Infectious mononucleosis (IM) is characterized by malaise, fever, hepatosplenomegaly, lymphadenopathy, and abdominal discomfort. Clinical manifestations similar to IM can also be induced by a number of other pathogenic infectious agents including Cytomegalovirus, *Toxoplasma gondii*, various hepatitis viruses, Human Immunodeficiency Virus (HIV), and others.

Except for primary EBV infections, laboratory evaluation of all other EBV–associated conditions is not straightforward. The cultivation of EBV is seldom practical because it takes too long (up to 4 weeks) and requires freshly fractionated cord blood lymphocytes. Serology is useful in three circumstances: (1) to diagnose IM, (2) to confirm the clinical diagnosis of nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma (BL), and (3) to monitor the efficacy of treatment for these two tumors. Antigen or nucleic acid detection, very useful for demonstrating the presence of EBV, usually require biopsies and special reagents.

Interpretations of EBV serologies, unlike standard viral serologies on paired sera, are based on the differential profiles of antibodies in a single serum against multiple antigens. There is wide variation in the peak titers of each antibody and the time to develop a full spectrum of antibodies among patients. Many asymptomatic individuals maintain high, unchanging titers. This variability, together with the false-positive and -negative problems associated with IgM detection, precludes the use of simple IgG/IgM testing for the diagnosis of IM.

Traditionally, anti-complement immunofluorescence assays (ACIF) utilizing cells expressing EBV-associated nuclear antigen are often used to detect antibodies to EBNA antigens.

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. Hence, anti-EA in the presence of peak titers of anti-EBNA are compatible with reactivations rather than primary infections.

ASSAY PRINCIPLE

The ImmunoWELL Test utilizes an EIA microtiter plate technique for the detection of antibodies. Serum is added to antigen coated microtiter wells and allowed to react. After removal of unbound antibodies, horseradish peroxidase-conjugated antihuman IgG 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 micro-well spectrophotometer.

REAGENTS

Reaction Wells coated with EBV Early Antigen (recombinant protein prepared in E. coli and purified by affinity column chromatography)

Specimen Diluent - 0.01 M phosphate buffered saline (PBS, pH 6.2-7.6) and carrier protein and <0.1% NaN_3

EA Calibrators (3) - human anti-EA prediluted, ready for use in Specimen Diluent

EA Positive Control - human anti-EA serum containing <0.1% NaN_3

EA Negative Control - nonreactive human serum containing <0.1% NaN_3

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

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

Substrate - Contains 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°. 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.

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 which 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. 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.

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	<500	Specific Antibody not detected
Equivocal	500-1000	Retest. The second result is considered final. If the repeat test is also equivocal, report as equivocal.
Positive	>1000	Report as positive

Fifty-nine sera collected from asymptomatic blood donors were used to establish cutoff levels. Two sera reporting values over 6000 units/ml were excluded from calculations. The lower level (500 units/mL) cutoff is the upper 95% confidence interval and the upper cutoff level is one standard deviation unit above the mean, normal value. 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.

LIMITATIONS

1. The values obtained in the assay are intended to be an aid to diagnosis only. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures.
2. Results obtained from immunocompromised individuals should be interpreted with caution.
3. The performance characteristics have not been established for any matrices other than serum.
4. There is a possibility of assay cross-reactivity with specimens containing high amounts of anti-*E. coli* antibody.
5. The prevalence of the analyte will affect the assay's predictive value.
6. The performance characteristics have not been established for patients with nasopharyngeal carcinoma, Burkitt's lymphoma, other EBV associated lymphadenopathies, and other EBV associated diseases other than EBV related mononucleosis.
7. Since EA(D) IgG antibodies are present in normal convalescent sera, a single result cannot be used for diagnosis. Accurate interpretation of EBV infection is based on the results of VCA IgG, VCA IgM, EBNA IgG, EA(D) IgG and heterophile antibody.

EXPECTED RESULTS

Two hundred sera submitted for EBV serological testing, including anti-EA(D) testing, and approximately fifty sera collected from asymptomatic blood donors by a blood bank were tested in the ImmunoWELL EA(D) IgG Test and these results are shown in Figure 1.

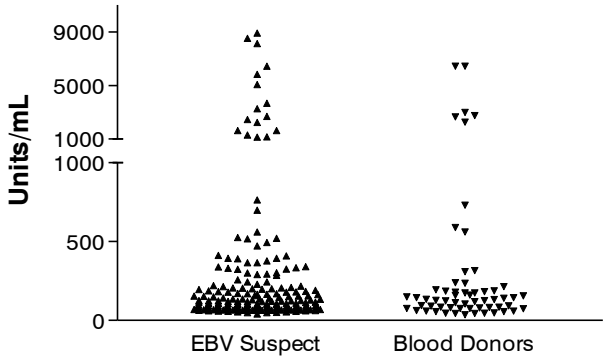

Figure 1: Anti-EA(D) Distribution of EBV Suspect Subjects and Normal Sera from Asymptomatic Blood Donors

PERFORMANCE CHARACTERISTICS

Two hundred sera submitted for EBV serological testing, including anti-EA(D) testing, were evaluated in two laboratories. Site A and GenBio also tested all samples using ImmunoWELL VCA IgG, VCA IgM and EBNA IgG. GenBio also tested the samples using ImmunoDOT Mono G and Mono M test kits. ImmunoDOT detects VCA IgG, VCA IgM and EBNA IgG, but also detects heterophile antibodies using a purified bovine red cell extraction.

EBV diagnostic profile interpretation was made following criteria listed in Table 1. If the profiles determined at GenBio and Site A agreed, it was directly assigned. Initially there were twelve (6%) discrepant profile results. In all discrepant cases, the ImmunoDOT profile result did agree with one site's interpretation, resolving the discrepancy. The EBV serological profile distribution is shown in Table 2.

Table 1: Profile Interpretation Criteria

Profile	VCA IgM	VCA IgG	EBNA IgG
Current or Acute Infection	Positive	Positive	Negative
Current, Late or Recent	Positive	Positive	Positive
No Past Infection or Negative	Negative	Negative	Negative
Past Infection	Negative	Positive	Positive

Table 2: EBV Serological Profile Distribution of the Population

Profile	Number	Percentage
Current (Acute) Infection	17	8.5%
Current, Late (Recent) Infection	2	1.0%
No Past Infection or Negative	31	15.5%
Past Infection	150	75%

It is generally recognized that anti-EA does not occur in subjects with no past infection. This information can be summarized by the following rules:

1. Patients form anti-EA(D) during acute, primary infection.
2. Anti-EA(D) presence in subjects with past infections (anti-VCA IgG positive, EBNA IgG positive, VCA IgM negative, and heterophile negative) indicates recurrent infection
3. Anti-EA(D) should not occur in seronegative subjects.

Based on these criteria, samples from current or acute infected subjects are expected to be anti-EA(D) positive. Serum from people with current-late (recent) infection is expected to be anti-EA(D) negative. Sera from subjects with no past Infection should be anti-EA(D) negative, and those with past EBV infection and no recurrent infectivity should also be anti-EA(D) negative. ImmunoWELL EA(D) performance data, using these criteria, is summarized in Table 3 and Table 4. For comparison, the performance characteristics for these same 200 sera using another commercially available anti-EA(D) microtiter test kit are shown in Table 5. In all cases, equivocal results are excluded from calculations.

Table 3: Site A Performance Characteristics

	Percentage	Range†
Relative Sensitivity	31 (5/16)	11-59%
Relative Specificity (Seronegative)	100 (31/31)	89 100%
Relative Specificity (Past Infection)	92 (1336/144)	87-96%

† 95% Confidence Interval, based on exact method

Table 4: Site B Performance Characteristics

	Percentage	Range†
Relative Sensitivity	43 (6/14)	18–71%
Relative Specificity (Seronegative)	100(31/31)	89–100%
Relative Specificity (Past Infection)	85 (114/134)	78–91%

† 95% Confidence Interval, based on exact method

Table 5: Predicate Device Performance Characteristics

	Percentage	Range†
Relative Sensitivity	44 (7/16)	20–70%
Relative Specificity (Seronegative)	87 (26/30)	69–96%
Relative Specificity (Past Infection)	72 (97/134)	64–80%

† 95% Confidence Interval, based on exact method

Note: Please be advised that "relative" refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with the disease presence or absence. No judgment can be made on the comparison assay's accuracy to predict disease.

Cross-reactivity Study

Three sera each (total of 12 sera) containing high IgG concentrations of anti-varicella zoster, anti-cytomegalovirus, anti-herpes simplex type 1 and anti-herpes simplex type 2 were tested in the assay. All were anti-EA(D) negative. In addition, several possible interfering substances (hemoglobin, bilirubin, triglycerides, or cholesterol) were tested by adding a measured amount of the substance into a negative serum and testing for analyte using the ImmunoWELL kit . The interfering substance low level equals the amount normally found in blood serum. The mid level is twice the normal concentration and the high level is four times the amount normally seen in serum. Six negative sera were evaluated. No interference was observed.

Precision Study

The precision study was conducted at three sites: Site A, Site B and at GenBio. Seven samples were tested. Samples were aliquoted so that each site received three sets of twenty-one samples. The twenty-one samples represented triplicates. The sites performed the test three times. These results are summarized in Table 6.

Table 6: Assay Precision

	Within Run		Between Runs within Laboratory		Between Laboratories	
Mean†	Standard Deviation†	%CV	Standard Deviation†	%CV	Standard Deviation†	%CV
65	5	7%	3	4%	19	28%
61	5	8%	4	6%	17	28%
194	10	5%	10	5%	29	15%
257	18	7%	15	6%	32	12%
837	78	9%	86	10%	189	23%
1211	188	16%	181	15%	329	27%
1908	176	9%	141	7%	380	20%

† The Average and Standard Deviation values are expressed in Units/mL

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.

QUICK REFERENCE PROCEDURE

***ImmunoWELL* EA IgG**

- 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 min at room temperature.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Pipet 100 µL of Conjugate into microwells and incubate 30 min at room temperature.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Pipet 100 µL of Substrate into microwells and incubate 30 min 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.

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