IMMUNOWELL™
EBNA IGG TEST

CE

Product No. 3270

IVD For *In Vitro* Diagnostic Use

INTENDED USE

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

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.

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

ASSAY PRINCIPLE

The ImmunoWELL Test uses 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 microwell spectrophotometer.

REAGENTS

Reaction Wells coated with recombinant EBNA-1 (baculovirus origin, purified by affinity chromatography)
Specimen Diluent - 0.01M phosphate buffered saline (PBS, pH 6.2-7.6) and carrier protein and <0.1% NaN_3
EBNA Calibrators (3) - human anti-EBNA prediluted, ready for use in Specimen Diluent
EBNA Positive Control - human anti-EBNA serum containing <0.1% NaN_3
EBNA 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 - 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.

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. It may be harmful if enough is ingested (more than supplied in kit). On disposal of liquids, flush with a large volume of water to prevent azide build-up (3). This dilution is not subject to GHS, US HCS and EU Regulation 2008/1272/EC labeling requirements.

The safety data sheet (SDS) is available at support.genbio.com or upon request.

⚠️ **Human source material**. Material used in the preparation of this product has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV), and antibodies to human immunodeficiency virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease (4). Follow recommended Universal Precautions for bloodborne pathogens as defined by OSHA (5), Biosafety Level 2 guidelines from the current CDC/NIH Biosafety

in Microbiological and Biomedical Laboratories (6), WHO Laboratory Biosafety Manual (7), and/or local, regional and national regulations.

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.

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.

MATERIALS PROVIDED

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

MATERIALS REQUIRED BUT NOT PROVIDED

Distilled or deionized water	Test tubes
Microwell washer	Pipets
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 indicated in Table 2 below. The positive control value is approximately five standard deviations (absorbance) above the upper cutoff and the negative control value is less than 0.30 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

Activity of the specimen may be calculated in one of two ways:

1. 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.
2. 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)
A_{MC} = Mean absorbance of the Mid Calibrator obtained in the assay

Table 1: Interpretation

	Units/mL	Interpretation
Negative	<200	Specific Antibody not detected
Equivocal	200-300	Report as negative or retest. If retested, the second result is considered final. If the repeat test is also equivocal, report as equivocal.
Positive	>300	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.

Table 2: Calibration Values

	Values	Units
High Calibrator Assigned Value	1698	Units/mL
Mid Calibrator Assigned Value	728	Units/mL
Low Calibrator Assigned Value	91	Units/mL
Mid Calibrator Low Limit	0.29	Absorbance
Positive Control Expected Range	400-1000	Units/mL
Negative Control Expected Range	<200	Units/mL

Results should not be interpreted if calibrator absorbance is below the low limit.

LIMITATIONS

No cross reactivity testing has been performed with this assay. EBNA IgG 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.

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.

This assay detects antibody to EBNA-1 antigen and no other EBNA antigens. Patients with severe immunocompromised conditions may show negative results for EBNA antibodies even if antibodies to VCA are present. An anti-EBNA response may fail to develop in patients who have an immunodeficiency disease or are immunosuppressed.

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 (8).

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
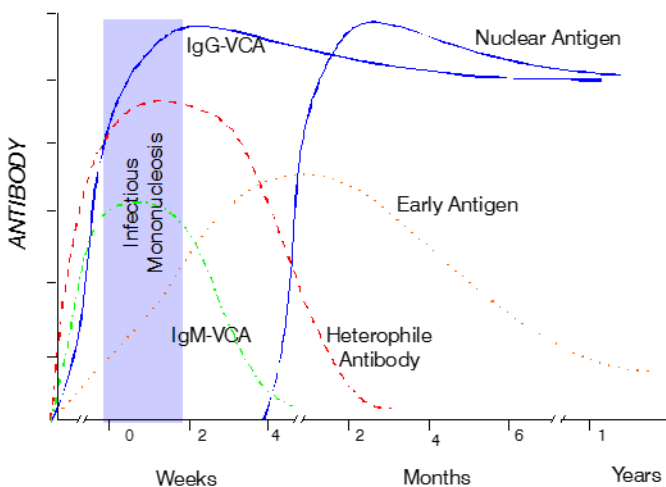
During the acute phase of IM, IgG and IgM responses to viral capsid antigen complex (VCA) are rapid and occur almost simultaneously. Due to the variable incubation period and intensity of symptoms, by the time patients consult their physicians, nearly all have reached peak titers of IgG; 10–15% of the 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. (GenBio ImmunoWELL EBNA is somewhat more sensitive detecting anti-EBNA1 than some IFA tests.) 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 activity of Site B samples (see Performance Characteristics) is shown in Figure 2. The left axis details the frequency of specimen activities; and the right axis (and curve), the cumulative frequency expressed as a percent of the total. It should be noted that approximately 20% of the samples are below 200 U/mL and 60% of the samples are above 2000 U/mL.

Figure 2: Expected Activity Distribution (U/mL)
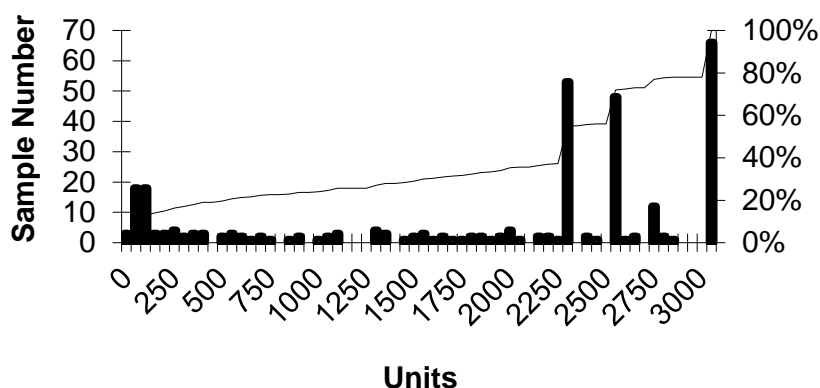
The above information is not meant to predict how this product will perform in all populations, but does represent the expected results seen in a large U.S. reference laboratory.

PERFORMANCE CHARACTERISTICS

Studies were conducted using samples prospectively submitted to two U.S. reference laboratories for EBV serology studies. Ninety-nine samples collected from throughout the U.S. (Site A) and 317 samples collected primarily from the northern California region (Site B) were initially received. Ninety-four specimens at Site A and 287 sera at site B contained sufficient volume for evaluation by all tests. Site A samples sent frozen to GenBio were tested using commercial EIA and ImmunoWELL (VCA IgG, EBNA-1 IgG, and VCA IgM) kits. Site B tested all samples using immunofluorescence (IFA) VCA IgG and anticomplement immunofluorescence (ACIF) EBNA. Suspect recent and current infections were tested for VCA IgM using IFA. Site B samples were sent frozen to GenBio for comparative testing with ImmunoWELL kits.

Although EBV serodiagnosis requires measurement of more than one analyte, universal agreement on a serological profile does not exist. One profile criterion[1,2] is shown in Table 3.

Table 3: 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.

Using these criteria, comparison between an alternate EIA kit (Site A) and IFA/ACIF (Site B) are shown in Table 4 and Table 5.

Table 4: Site A Profile

ImmunoWELL	Alternate EIA		
	Past/Recent	Current	No Past Infection
Past/Recent	65	8	0
Current	0	7	0
No Past Infection	5	1	8

Table 5: Site B Profile

ImmunoWELL	IFA/ACIF		
	Past/Recent	Current	No Past Infection
Past/Recent	247	3	1
Current	6	3	3
No Past Infection	0	0	24

Assuming "No Past Infection" results represent negative reactions, the relative performances between ImmunoWELL and the other methods are shown in Table 6.

Table 6: Relative Performance

Site/Method	Agreement	Relative Sensitivity	Confidence Interval†	Relative Specificity	Confidence Interval†
Site A (EIA)	88.9%	93% (80/86)	85-97%	100% (8/8)	63-100%
Site B (IFA)	97.2%	100% (259/259)	99-100%	86% (24/28)	67-96%

† The confidence interval (95%) is calculated by the 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 disease presence or absence. No judgment can be made on the comparison assay's accuracy to predict disease.

Subject ages at site A were not available, but most subject ages were acquired by site B. The age range and infection classification are shown in Table 7.

Table 7: Infection Classification by Age

Age Range (years)	Current Infection	No Past Infection	Past Infection
Less than 8	2 (11%)	6 (32%)	11 (58%)
8-18	5 (12%)	13 (31%)	24 (58%)
18-25	1 (4%)	1 (4%)	24 (92%)
25-45	2 (2%)	3 (3%)	105 (95%)
Greater than 45	1 (1%)	0	78 (99%)

PRECISION DATA

Precision was determined by testing ten samples as duplicates within each run and testing these twice each day for twenty days. The study was conducted at GenBio (Site A) and one outside site (Site B). The results are shown in Table 8.

Table 8: Assay Precision

Units/mL	Type	Site A Within Run (%CV)	Site A Between Run (%CV)	Site B Within Run (%CV)	Site B Between Run (%CV)
2232	High	5	8	2	6
1890	High	5	8	2	8
1636	High	4	9	5	14
1115	Moderate	5	12	6	21
973	Moderate	6	11	8	22
460	Low	8	11	9	27
321	Low	5	10	8	28
268	Negative	6	12	9	26
76	Negative	15	40	12	33
67	Negative	19	54	19	52

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QUICK REFERENCE PROCEDURE

IMMUNOWELL EBNA 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 minutes 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.

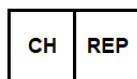For assistance, please call toll-free 800-288-4368.

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