AccuDiag™ Epstein-Barr Virus Early Antigen IgG (EBV EA IgG) ELISA Kit

REF 1415-P1

IVD See External Label 2-8°C 96 Tests

EBV EA IgG ELISA	
Principle	Indirect ELISA
Detection	Qualitative
Sample	10 µL serum/plasma
Incubation Time	60 minutes
Sensitivity	100%
Specificity	97%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. AccuDiag™ Epstein-Barr Virus Early Antigen Diffuse (EA) IgG Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the qualtitative determination of IgG antibodies in human serum to EA antigen. The Diagnostic Automation Inc. EA IgG assay may be used in conjunction with other Epstein-Barr serologies (VCA IgG, VCA IgM, EBNA-1 IgG, EBNA-1 IgM and heterophile) as an aid in the diagnosis of infectious mononucleosis.

For *in vitro* diagnostic use. High complexity test.

SIGNIFICANCE AND SUMMARY

Epstein-Barr virus (EBV) is a common human pathogen, affecting 80% of adults in the US. Since the discovery of Epstein-Barr virus in 1964, EBV has been etiologically implicated in an increasing number of human diseases, such as infectious mononucleosis, Burkitt's lymphoma, and nasopharyngeal carcinoma.[1] EBV has also been associated with B cell lymphomas in immunosuppressed individuals, including both transplant patients and patients with AIDS. EBV is classified as a member of the herpesvirus family based upon its characteristic morphology.[2,3] All herpesviruses share the ability to establish a latent infection in their hosts. Although primary infection with EBV during childhood is usually asymptomatic, nearly one-half to two-thirds of primary infections with the virus in older adolescents and young adults result in overt clinical disease such as infectious mononucleosis (IM).[1] Infectious mononucleosis is an acute, self-limited lymphoproliferative disease caused by EBV. WheFincuban primary infection is delayed until young adulthood and adolescence, however, there is about a 50% chance that it will occur with the classic clinical manifestations associated with IM.[4,5]

Infection by EBV results in the production of antibodies to four distinct antigenic complexes. These include: EBV-induced Nuclear Antigen (EBNA), EBV-induced Early Antigen (EA), Viral Capsid Antigen (VCA), and EBV-induced Membrane Antigen (MA). The EA complex is divided into two components which include EA-D (diffuse component) and EA R (restricted component).[6]

The EA complex results from early transcription before the synthesis of DNA. The mRNA involved with transcription encodes enzymes and polymerases that are needed for the synthesis of viral nucleic acid. This non-structured product then enters the replication cycle and produces complete virions. IgG antibodies to the early antigen complex can be found in early or late stages of Infectious Mononucleosis.[7]

The Diagnostic Automation Inc. EA IgG kit is an Enzyme-Linked Immunosorbent Assay to be used as an aid in the diagnosis of infectious mononucleosis.

ASSAY PRINCIPLE

Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO_4, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.[12,13,14]

SPECIMEN COLLECTION & PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the Diagnostic Automation Inc. ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed,

nonlipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.[16] Early separation from the clot prevents hemolysis of serum.

3. Store serum between 2° and 8°C if testing will take place within two days. If specimens are to be kept for longer periods, store at -20°C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).[16]

REAGENTS

Materials provided with the kit

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. Recombinant EA antigen (the carboxy-terminal of EA genome representing ~200 codons) coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)
2. Serum Diluent Type I: Ready for use. Contains ProClin® (0.1%) as a preservative, (96T: one bottle, 30 mL)
3. Calibrator: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T: one vial, 0.4 mL)*
4. High Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The High Positive Control is utilized to control the upper dynamic range of the assay. (96T: one vial, 0.4 mL) *
5. Low Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Low Positive Control is utilized to control the range near the cutoff of the assay. (96T: one vial, 0.4 mL)*
6. Negative Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label.The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 mL)*
7. Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgG, containing ProClin® (0.1%) and gentamicin as preservatives. (96T: one bottle, 16 mL)
8. Chromogen/Substrate Solution Type I: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 mL)
9. Wash Buffer Type I (20X concentrate): Dilute 1 part concentrate +19 parts deionized or distilled water. Contains TBS, Tween-20 and ProClin® (0.1%) as a preservative, (96T: one bottle, 50 mL)
10. Stop Solution: Ready to use, contains a 1N H_2SO_4 solution. (96T: one bottle, 15 mL)

Note: serum vials may contain excess volume.

Materials required but not provided

- Wash bottle, automated or semi-automated microwell plate washing system.
- Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
- One liter graduated cylinder.
- Paper towels.
- Test tube for serum dilution.
- Reagent reservoirs for multichannel pipettes.
- Pipette tips.
- Distilled or deionized water (dH20), CAP (College of American Pathology) Type 1 or equivalent.[19,20]
- Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 minutes).
- Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH20).
- Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware

REAGENT PREPARATION

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21 to 25°C). Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H_2O. Mix well.

ASSAY PROCEDURE

1. Place the desired number of strips into a microwell frame. Allow six (6) Control/Calibrator determinations (one Negative Control, three Calibrators, one High Positive Control and one Low Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient #2
1B	NC	2B	Patient #3
1C	Cal	2C	Patient #4
1D	Cal	2D	Patient #5
1E	Cal	2E	Patient #6
1F	HPC	2F	Patient #7
1G	LPC	2G	Patient #8
1H	Patient #1	2H	Patient #9

RB = Reagent Blank – Well without serum addition run with all reagents. Utilized to blank reader.
NC = Negative Control
Cal = Calibrator
HPC = High Positive Control
LPC = Low Positive Control

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µL + 200 µL in Serum Diluent. Mix well. (For manual dilutions it is suggested to dispense

the Serum Diluent into the test tube first and then add the patient serum.)

3. To individual wells, add 100 µL of the appropriate diluted Calibrator, Controls and patient sera. Add 100 µL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21° to 25°C) for 25 minutes +/- 5 minutes.
5. Aspirate or shake out liquid from all wells. If using semi automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

IMPORTANT NOTE: Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semiautomated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of up to five (5) washes may be necessary with automated equipment. Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21° to 25°C) for 25 minutes +/- 5 minutes.
8. Repeat wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21° to 25°C) for 10-15 minutes.
11. Stop reaction by addition of 100 µL of Stop Solution (1N H_2SO_4) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is > 0.150 the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

INTERPRETATION

CALCULATIONS

1. Mean Calibrator O.D. (Optical Density) - Calculate the mean value for the Calibrator from the three Calibrator determinations. If any of the three Cutoff Calibrator Values differ by more than 15% from the mean, discard that value and calculate the average of the two remaining values.
2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by Diagnostic Automation Inc. for each lot of kits. The Correction Factor is printed on the Calibrator vial.
3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.
4. ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cutoff Calibrator Value determined in Step 3.

Example:

O.D's obtained for Calibrator	= 0.38, 0.42
Mean O.D for Calibrator	= 0.40
Correction Factor	= 0.50
Cutoff Calibrator Value	= 0.50 x 0.40 = 0.20
O.D. obtained for patient sera	= 0.60
ISR Value	= 0.60/0.20 = 3.00

ANALYSIS

1. The patients' ISR (Immune Status Ratio) values are interpreted as follows:

ISR	Results	Interpretation
≤ 0.90	Negative	No detectable IgG antibody to EBV EA by the ELISA test.
0.91 – 1.09	Equivocal	Samples should be retested. See Number (2) below.
≥ 1.10	Positive	Indicates presence of detectable IgG antiboy to EBV EA by the ELISA test.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.
3. To determine the cutoff of the assay, fifteen seronegative (VCA G, VCA M, EBNA G negative) sera were assayed by the EBV EA IgG Test. The mean and standard deviation of the optical density readings for the sera were 0.326 and 0.158, respectively. The positive threshold for the assay was determined by adding the mean and 2.5 standard deviations (0.326 + 2.5 (0.158) = 0.72). A positive sera was titrated to give a constant ratio of the threshold value to obtain a Cutoff Calibrator sera. On all subsequent assays this sera was run and the assay was calibrated by multiplying the O.D. value for the Cutoff Calibrator by the ratio to the cutoff to obtain the cutoff O.D. This value was then divided into the O.D. for the patient sera to obtain an immune status ratio (ISR). By definition the cutoff ISR is equal to 1.00. To account for inherent variation in immunoassay values of 0.91-1.09 were considered equivocal. Therefore values ≤ 0.90 are considered negative and values ≥ 1.10 are considered positive.
4. The following is a recommended method for reporting the results obtained; "The following results were obtained with the Diagnostic Automation Inc. EBV EA IgG ELISA. Values obtained with different methods may not be used interchangeably. The magnitude of the reported IgG level cannot be correlated to an endpoint titer."
5. Four distinctive EBV antibodies are used to provide a comprehensive picture of EBV infection: these are IgM-viral capsid antibody, IgG - viral capsid antibody, IgG- antibody to early antigen, and EBV nuclear antibody (EBNA). Accurate interpretation of EBV infection is based on the results from all these antibodies, and usually should not rely on single test result for a diagnosis (refer to the Expected Values section).

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:

1. Calibrator and Controls must be run with each test run.
2. Reagent blank (when read against air blank) must be < 0.150 Absorbance (A) at 450 nm.

3. Negative Control must be ≤ 0.250 A at 450 nm (when read against reagent blank).
4. Each Calibrator must be ≥ 0.250 A at 450 nm (when read against reagent blank).
5. High Positive Control must be ≥ 0.500 A at 450 nm (when read against reagent blank).
6. The ISR (Immune Status Ratio) Values for the High Positive, Low Positive and Negative Controls should be in their respective ranges printed on the vials. If the Control values are not within their respective ranges, the test should be considered invalid and should be repeated.
7. Additional Controls may be tested according to guidelines, or requirements of local, state, and/or federal regulations or accrediting organizations.
8. Refer to NCCLS C24-A for guidance on appropriate QC practices.[18]
9. If above criteria are not met upon repeat testing, contact Diagnostic Automation Inc. Technical Services.

EXPECTED RANGES OF VALUES

ACUTE PHASE

VCA IgG antibodies are rising. VCA IgM and heterophile antibodies are normally present. EBNA-1 IgG antibodies are normally absent or at very low levels. EBV EA IgG antibodies are starting to increase.

LATE ACUTE TRANSITIONAL PHASE

VCA IgG antibodies persists and VCA IgM and heterophile antibodies usually decline. EBNA-1 IgG antibodies begin to increase. EBV EA IgG antibodies usually persists.

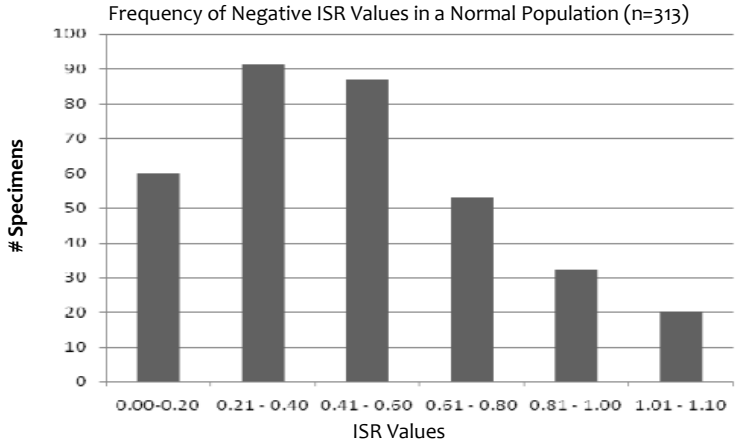
CONVALESCENT PHASE

VCA IgM and heterophile antibodies drop to negative or very low. VCA IgG and EBNA-1 IgG antibodies persist usually for life. EBV EA IgG antibodies are usually transient but they may persist for life.

PREVALENCE

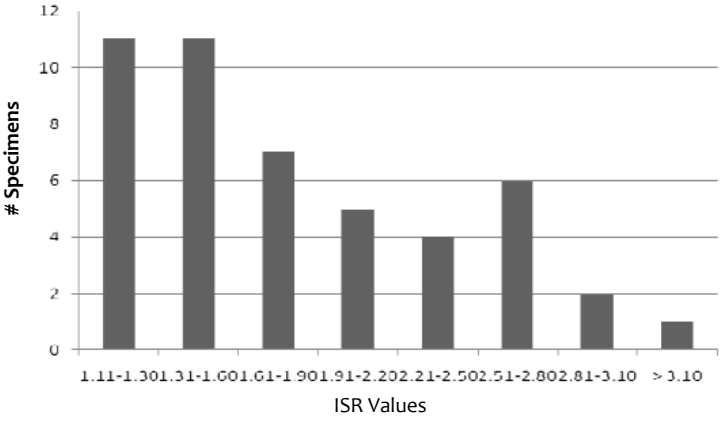
1. A group of 360 sera from a normal population from various ages, genders and geographical areas of the U.S. were tested on the EBV EA IgG assay. The positive rate for the Diagnostic Automation EBV EA IgG ELISA assay was found to be 13.1% and the equivocal rate was 5.0%. The distribution of negative ISR values from this study is presented in the Chart 1 below.

Chart 1
Frequency of Negative ISR Values in a Normal Population (n=313)



2. **The distribution of positive ISR values in the normal population for the EBV EA IgG ELISA assay is illustrated in Chart 2 below.**

Chart 2
Frequency of Positive ISR Values in a Normal Population (n=47)



PERFORMANCE CHARACTERISTICS

Sensitivity and specificity based on serum characterization

One hundred and ninety-three selected serum were tested at a clinical lab. The serum from the study were characterized as seronegative (no serological evidence of past or present EBV infection), early acute (VCA IgM and heterophile antibody present, EBNA IgG absent), late acute or transitional (VCA IgM, EBNA IgG and heterophile antibody present, approximately 4-12 weeks post infection), or seropositive (presence of VCA IgG antibodies and EBNA IgG, no evidence of VCA IgM or heterophile antibody, indicative of past infection). The sensitivity, specificity and agreement of the assay was determined based on this characterization. It was assumed that the EBV EA IgG response should be negative for seronegative, early acute, and convalescent serum, and positive for late acute or transitional serum. The results are summarized in Table 1 and Table 1A.

Table 1

		Early Acute VCA IgM + EBNA IgG – Heterophile +	Late Acute VCA IgM + EBNA IgG – Heterophile +	Seropositive VCA IgG + EBNA IgG + VCA IgM – Heterophile –	Seronegative VCA IgG + EBNA IgG + VCA IgM – Heterophile –
Diagnostic Automation Inc. EBV EA IgG	Positive	1	9	26	0
	Equivocal	1	0	3	1
	Negative	32	0	106	14
	Total	34	9	135	15

Table 1A Summary of Relative Sensitivity & Specificity Data

	*Results***	*Results as Percentage*	*95% Confidence Intervals****
Relative Sensitivity (Late Acute)	9/9	100%	67.9% - 100%*****
Relative Specificity (Seronegative)	14/14*	100%	79.1% - 100%*****
Relative Specificity (Early Acute)	32/33*	97.0%	91.0% - 100%
Relative Sensitivity (Seropositive)	26/132*	19.7%	12.8% - 26.6%
Relative Specificity (Seropositive)	106/132*	80.3%	73.4% - 87.2%
Relative Agreement	161/188*	85.6%	80.5% - 90.8%

* **Equivocal Results were not included in the calculations.**
** **Equivocal results were not retested. They were reported as equivocal.**
*** **The 95% confidence intervals were calculated using the normal method.**

****** The Seronegative 95% confidence interval was calculated assuming one false positive.**

Seven different sera were assayed ten times each on three different assays at three different sites to determine the precision of the assay. The data from this study is presented in Table 2.

Table 2
Diagnostic Automation Inc. EBV EA IgG ELISA
Inter-Site Precision Data
(n=90)

Serum #	X	S.D.	C.V.
1	1.43	0.126	8.83%
2	1.29	0.100	7.73%
3	2.14	0.128	5.96%
4	2.12	0.124	5.85%
5	0.96	0.085	8.84%
6	0.31	0.035	11.35%
7	0.24	0.040	16.34%
HPC (n=9)	2.86	0.062	2.18%
LPC (n=9)	2.22	0.074	3.32%
Cal (n=27)	1.79	0.082	4.59%
NC (n=9)	0.01	0.012	154.52%

Serum #5 was the only serum to change status. It was equivocal 66 times, negative 21 times, and positive 3 times.
X = Mean ISR
SD = Standard Deviation
CV = coefficient of Variation
The methods in NCCLS EP5 were utilized for precision parameters.[17]

CROSS-REACTIVITY
Serum containing IgG antibody detectable by ELISA to Herpes Simplex Virus I & II, Cytomegalovirus, and Varicella Zoster Virus were assayed. The data summarized in Table 3 indicates that antibodies to these Herpes Viruses do not cross-react with the Diagnostic Automation Inc. EA IgG ELISA kit.

Table 3
EA Cross-Reactive Sera

Serum	EA IgG	Alternate Assay	
1	0.26	3.59	(VZV IgG)
2	0.83	7.58	(VZV IgG)
3	0.45	2.17	(VZV IgG)
4	0.40	2.77	(VZV IgG)
5	0.74	3.95	(CMV IgG)
6	0.54	2.44	(CMV IgG)
7	0.31	1.57	(CMV IgG)
8	0.37	3.45	(HSV 1 IgG)
9	0.53	3.51	(HSV 1 IgG)
10	0.56	3.70	(HSV 1 IgG)
11	0.38	3.57	(HSV 2 IgG)
12	0.64	3.58	(HSV 2 IgG)

Sera ≥ 1.10 were considered positive.
Sera ≤ 0.90 were considered negative.

LIMITATIONS OF THE ASSAY

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
2. The values obtained from this 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.
3. Results obtained from immunocompromised individuals should be interpreted with caution.
4. Icteric, lipemic, hemolyzed or heat inactivated sera may cause erroneous results and should be avoided.
5. Kit procedures or practices outside those in this package insert may yield questionable results.
6. The performance characteristics have not been established for any matrices other than sera.
7. There is a possibility of assay cross-reactivity with specimens containing anti-E.coli antibody.
8. The prevalence of the analyte will affect the assay's predictive value.
9. 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.
10. Since EBV EA 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 from VCA IgG, VCA IgM, EBNA IgG, EBV EA IgG and heterophile antibody.

STORAGE CONDITIONS

1. Store unopened kit between 2° and 8°C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2° and 8°C. Unused strips must be immediately resealed in a sealable bag with desiccant and returned to storage between 2° and 8°C.
3. Store HRP Conjugate between 2° and 8°C.
4. Store the Calibrator, Positive Control, and Negative Control between 2° and 8°C.
5. Store Serum Diluent Type I and 20X Wash Buffer Type I between 2° and 8°C.
6. Store the Chromogen/Substrate Solution Type I between 2° and 8°C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (diluted) Wash Buffer Type I at room temperature (21° to 25°C) for up to 5 days, or up to one week between 2° and 8°C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination.

PRECAUTIONS

1. **For *in vitro* diagnostic use.**
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.

3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.15
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type I, and Serum Diluent Type I. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21° to 25°C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may occur**.
7. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. **Cross-contamination of reagents and/or samples could cause false results**.
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. **Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents**.
13. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing ProClin®, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.
15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with water.
17. Caution: Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. The concentrations of anti-EA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

The safety data sheet is available upon request.

WARNING
Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300R, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
H317: May cause an allergic skin reaction.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P302 + **P352**: IF ON SKIN: Wash with plenty of soap and water.
P333 + **P313**: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.
WARNING
Serum Diluent and Controls contain < 0.1% sodium azide.
H302: Harmful if swallowed
P264: Wash thoroughly with plenty of soap and water after handling
P270: Do not eat, drink or smoke when using this product
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
P330: If swallowed, rinse mouth
P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

REFERENCES

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MANUFACTURER AND BRAND DETAILS

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Date Adopted	**2023-09**
Brand Name	**AccuDiag™**
REF 1415-P1	**AccuDiag™ - Epstein-Barr Virus EA IgG (EBV EA IgG) ELISA**
Revision Date: 2015-03-30	