Trinity Biotech

Captia™ EBNA-1 IgG

REF	2325800	96 Tests
REF	2325801	480 Tests

Pour d'autres langues | Para outras línguas
Für andere Sprachen | Για τις άλλες λώσσες
Para otras lenguas | För andra språk
Per le altre lingue | For andre språk
Dla innych języków | Pro jiné jazyky

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INTENDED USE

The Trinity Biotech Captia™ Epstein-Barr Virus Nuclear Antigen-1 (EBNA-1) IgG Enzyme- Linked Immunosorbent Assay (ELISA) is intended for the qualitative and semi-quantitative determination of IgG antibody in human serum to EBNA-1 recombinant antgen. The Trinity Biotech EBNA-1 IgG assay may be used in conjunction with other Epstein-Barr serologies (VCA IgG, VCA IgM, EA (R&D), and heterophile) as an aid in the diagnosis of infectious mononucleosis. **For *in vitro* diagnostic use. High complexity test.**

INTRODUCTION

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.[4] 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 lympho-proliferative disease caused by EBV. When 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.[5,6]

Infection of the target cells leads to two forms of viral cycles: 1) latent, nonproductive and 2) productive, replicative infections.[7] In both cycles, one of the earliest antigens expressed is lymphocyte-detected membrane antigen, a cell-surface antigen recognized by T-cells. It has been well established that most individuals exposed to EBV develop a heterophile antibody response. Expression of EBNA-1 either follows or parallels membrane antigen at 12 to 24 hours post infection. EBNA-1 is found as nonstructural, intranuclear antigen(s), present in all EBV-transformed cell lines as in tumors from Burkitt's and nasopharyngeal carcinoma patients. In the fully productive, replicative cycle, the synthesis of antigen follows EBNA-1. The viral capsid antigen complex (VCA) appears late in the replicative cycle.

It has recently become apparent that EBNA-1 is probably not a single antigenic moiety, but a multicomponent antigen complex, on the basis of reactivities of sera from different classes of patients. The major component EBNA-1 has been purified and sequenced in its entirety.[7]

Antibody levels of EBNA-1 IgG, are diagnostic in determining acute and convalescent stages in IM. IgG antibodies to EBNA-1 are rarely present in acute IM and rise during convalescence. They will rise to a plateau level in three months to a year and will normally persist for life.[8,9]

The Trinity Biotech EBNA-1 IgG kit utilizes the ELISA technology where a purified recombinant EBNA-1 antigen is bound to the wells of a microplate. A peroxidase coupled anti-human IgG conjugate is used as the detection system.

PRINCIPLE OF THE ASSAY

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 antihuman 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.[10,11,12,13] The sensitivity, specificity, and reproducibility of ELISAs can be comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radio-immunoassay.[14,15,16]

KIT PRESENTATION

MATERIALS SUPPLIED

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

1. Recombinant EBNA-1 antigen (the carboxy-terminal of EBNA-1 genome representing ~200 codons) coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with a desiccant. (96T: one plate; 480T: five plates)
2. Serum Diluent Type I: Ready for use. Contains ProClin® (0.1%) as a preservative. (96T: one bottle, 30 mL, 480T: two bottles, 60 mL each)
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; 480T: one vial, 0.8 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; 480T: one vial, 0.8 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; 480T: one vial, 0.8 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; 480T: one vial, 0.8 mL) *
7. Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat antihuman IgG, containing ProClin® (0.1%) and gentamicin as preservatives. (96T: one bottle, 16 mL; 480T: five bottles, 16 mL each)
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; 480T: five bottles, 15 mL each)
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; 480T: one bottle, 250 mL)
10. Stop Solution: Ready to use, contains a 1N H_2SO_4 solution. (96T: one bottle, 15 mL; 480T: five bottles, 15 mL each)

*** Note: serum vials may contain excess volume.**

The following components are not kit lot # dependent and may be used interchangeably within the Trinity Biotech ELISA IgG assays: Serum Diluent Type I, Chromogen/Substrate Solution Type I, Wash Buffer Type I, and Stop Solution. Please check that the appropriate Trinity Biotech Reagent Type (Type I, Type II, etc.) is used for the assay.

ADDITIONAL REQUIREMENTS

- 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 (dH_20), CAP (College of American Pathology) Type 1 or equivalent.[22,23]
- Timer capable of measuring to an accuracy of +/- 1 second (0 to 60 minutes).
- Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH_20).
- 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.

STORAGE AND STABILITY

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, High Positive Control, Low 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 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. [17]
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, 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-EBNA-1 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 300®, 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.

SPECIMEN COLLECTION AND STORAGE

1. Handle all blood and serum as if capable of transmitting infectious agents.[17]
2. Optimal performance of the Trinity Biotech ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, non-lipemic, nonicteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.[20] 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. If paired sera are to be collected, acute samples should be collected as soon as possible after the onset of symptoms. The second sample should be collected 14 to 21 days after the acute specimen was collected. Both samples must be run in duplicate on the same plate to test for a significant rise. If the first specimen is obtained too late during the course of the infection, a significant rise may not be detectable.
5. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).[20]

METHODS FOR USE

PREPARATION FOR THE ASSAY

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_20. Mix well.

ASSAY PROCEDURE

Note: To evaluate paired sera, both serum samples must be tested in duplicate and run in the same plate. It is recommended that the serum pairs be run in adjacent wells.

1. Place the desired number of strips into a microwell frame. Allow six (6) Control/ Calibrator determinations (one Negative Control, three Calibrators and one each High Positive and Low Positive Controls) 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 (Acute 1)
1F	HPC	2F	Patient #6 (Acute 2)
1G	LPC	2G	Patient #6 (Convalescent 1)
1H	Patient #1	2H	Patient #6 (Convalescent 2)

RB = Reagent Blank - Well without serum addition run with all reagents. Utilized to blank reader.
NC = Negative Control
Ca = 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 semi-automated 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.

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 vial labels. 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. [21]
9. If above criteria are not met upon repeat testing, contact Trinity Biotech Technical Services.

INTERPRETATION

CALCULATIONS

1. Mean Calibrator O.D. (Optical Density) - Calculate the mean O.D. value for the Calibrator from the three Calibrator determinations. If any of the three Calibrators 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 Trinity Biotech 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, 0.40, 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

5. The maximum linearity of the assay is an ISR of 5.40, therefore ISR values of > 5.40 should be reported as greater than 5.40.

ANALYSIS

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

ISR	Results	Interpretation
≤ 0.90	Negative	No detectable IgG antibody to EBNA-1 by the ELISA test.
0.91-1.09	Equivocal	Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken
≥ 1.10	Positive	Indicates presence of detectable IgG antibody to EBNA-1.

2. To determine the cutoff of the assay, thirty-seven (37) negative sera were assayed by the Trinity Biotech EBNA-1 IgG test. The negativity and positivity of specimens used to determine the cutoff for the assay were determined by another ELISA method. The mean and standard deviation of the optical density readings for the sera were 0.0262 and 0.0306, respectively. The positive threshold for the assay was determined by adding the mean and four standard deviations (0.0262 + 4(0.0306) = 0.15). A positive serum was titrated to give a constant ratio of the threshold value to obtain a Calibrator serum. On all subsequent assays this serum was run and the assay was calibrated by multiplying the O.D. Value for the Calibrator by the ratio to the cutoff to obtain the Cutoff Calibrator Value. 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.
3. The following is a recommended method for reporting the results obtained; "The following results were obtained with the Trinity Biotech EBNA-1 IgG ELISA test. Values obtained with different methods may not be used interchangeably. The magnitude of the reported IgG level cannot be correlated to an endpoint titer". When a single specimen is assayed the magnitude of the measured result of the cutoff is not indicative of the total amount of antibody present.
4. 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 results for a diagnosis.
5. The performance characteristics for this product have been established using one calibrator. If a linear dose response curve with the assay is desired, the customer should establish a minimum of two additional calibrators.
6. To evaluate paired sera for significant change in antibody level, both samples must be tested in duplicate in the same assay. The mean ISR of both (acute and convalescent) must be greater than 1.00 to evaluate the paired sera for a significant rise in antibody level.
7. Additional Quality Control for paired sera (see NOTE under General Procedure). As a check for acceptable reproducibility of both the acute sera (tested in duplicate) and the convalescent sera (tested in duplicate), the following criteria must be met for valid results:

$$\frac{\text{Acute 1 ISR}}{\text{Acute 2 ISR}} = 0.8 \text{ to } 1.2 \qquad \frac{\text{Convalescent 1 ISR}}{\text{Convalescent 2 ISR}} = 0.8 \text{ to } 1.2$$

8. Compare the ISR of the pairs by calculating as follows:

$$\frac{\text{Mean ISR (convalescent sample)} - \text{Mean ISR (acute sample)}}{\text{Mean ISR (acute sample)}} \times 100 = \%\text{Rise in ISR level}$$

% Rise in ISR	Interpretation
< 46.0%	No significant change in antibody level. No evidence of recent infection. If active disease is still suspected, a third sample should be collected and tested in the same assay as the first sample to look for significant rise in antibody level.
≥ 46.0%	Statistically significant change in antibody level detected. This is indicative of acquiringan active primary EBV infection in the past 2 to 6 months.

9. When evaluating paired serum, it should be determined if samples with high absorbance values are within linearity specifications of the spectrophotometer. For reportable results, the acute serum must be ≤ 3.70, due to the maximum linearity of the assay. Read the Operator's Manual or contact the instrument's manufacturer to obtain the established linearity specifications of your spectrophotometer.

EXPECTED VALUES

ACUTE PHASE

VCA IgG and VCA IgM antibodies are normally present. EBNA-1 IgG antibodies are normally absent or at very low levels.

TRANSITIONAL PHASE

VCA IgG antibodies persist and VCA IgM antibodies usually decline. EBNA-1 IgG antibodies begin to increase.

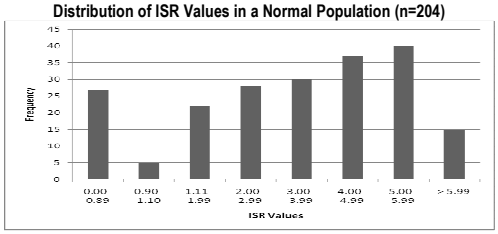
CONVALESCENT PHASE

VCA IgM drop to negative or very low. VCA IgG and EBNA-1 IgG antibodies persist usually for life. In the US, about 50% of the population seroconverts before age 5. Another wave of seroconversion occurs midway through the second decade of life. By adulthood, 90-95% of most populations will have EBNA-1 antibodies. [3]

PREVALENCE

A group of 204 sera from a healthy population in the northeast portion of the U.S.were tested on the Trinity Biotech EBNA-1 IgG assay. The sera were randomized for gender, age, and race. The distribution of ISR Values from this study is presented in the following chart. In this study, 86.4% of the population were positive in the assay.

Distribution of ISR Values in a Normal Population (n=204)



LIMITATIONS OF USE

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. This kit is designed to measure IgG antibody in patient samples. Positive results in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the fetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
3. The performance characteristics have not been established for any matrices other than serum.
4. 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.
5. Results from children should be reviewed with caution.[18]
6. Individuals with chronic active Epstein-Barr virus infection may not produce an antibody response to EBNA-1.[19]
7. Results obtained from immunocompromised individuals should be interpreted with caution.
8. The maximum linearity of this assay is 5.40 ISR.
9. There is a possibility of assay cross-reactivity with specimens containing anti-*E.coli* antibody.
10. 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.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

Three different sites compared the Trinity Biotech EBNA-1 IgG ELISA kit relative to a commercially available ELISA test kit. Two of the sites were R&D laboratories at commercial companies located in Maryland and New York.The other site was a large commercial laboratory located in Pennsylvania. The results of the studies are compiled and summarized in Tables 1 and 1A. None of the performance characteristics were established with specimens from patients having nasopharyngeal carcinoma or Burkitt's lymphoma.

Table 1
Relative Sensitivity and Specificity of the Trinity Biotech EBNA-1 IgG ELISA kit

		Trinity Biotech EBNA-1 IgG ELISA Kit			
		Positive	Equivocal	Negative	
		≥ 1.10	0.91–1.09	≤ .90	Total
Alternate EBNA IgG ELISA	Positive	270	5	6	281
	Equivocal	0	0	1	1
	Negative	0	1	74	75
	Total	270	6	81	357

Table 1A
Summary of Relative Sensitivity & Specificity Data

	Results*	Results as Percentage	95% confidence intervals**
Relative Sensitivity	270/276	97.8%	96.1%-99.6%
Relative Specificity	74/74	100%	96.0%-100% ***
Relative Agreement	344/350	98.3%	96.9%-99.7%

* Equivocal results were not included in the calculations.

** The 95% confidence intervals were calculated using the normal method.

*** The 95% confidence interval for relative specificity was calculated assuming one false positive.

SENSITIVITY AND SPECIFICITY BASED ON SERUM CHARACTERIZATION

The serum from the first study site were characterized as seronegative (no serological evidence of past or present EBV infection), acute (VCA IgM present), or seropositive (presence of VCA IgG antibodies, no evidence of VCA IgM, indicative of past infection). The sensitivity, specificity and accuracy of the assay was determined based on this characterization. It was assumed that the EBNA-1 IgG response should be negative for seronegative and acute serum, and positive for seropositive serum. The results are summarized in Tables 2 and 2A.

Table 2

		Seropositive VCA IgG + VCA IgM -	Acute VCA IgM +	Seronegative VCA IgG – VCA IgM -	Total
Trinity Biotech	Positive	89	1	1	91
EBNA-1 IgG	Negative	6	23	30	59
ELISA	Total	95	24	31	150

Table 2A
Summary of Relative Sensitivity & Specificity Data Based On Serum Characterization

	Results*	Results as Percentage	95% confidence intervals**
Relative Sensitivity	89/95	93.7%	88.7%-98.7%
Relative Specificity	53/55	96.4%	91.3%-100%
Relative Agreement	142/150	94.7%	91.0%-98.3%

*Eight Equivocal results were not included in the calculations.
**The 95% confidence intervals were calculated using the normal method.

PRECISION

Four different sera (High Positive, Mid Positive, Low Positive, and Negative) were assayed at three different sites to determine the precision of the assay. Each sera was tested three times each, twice a day for twenty days at each of the three study sites. The inter-site coefficent of variation (CV) for each serum is presented in Table 3.

Table 3
Inter Site Precision
n = 360

	Mean	Std Dev	CV%
High Positive	4.95	0.381	7.70%
Mid Positive	2.89	0.229	7.92%
Low Positive	1.41	0.152	10.78%
Negative	0.01	0.022	not applicable
Calibrator (n=240) High Positive	4.17	0.113	2.71%
Control (n=120)	6.90	0.419	6.07%

LINEARITY

The data in Table 4 illustrate the Trinity Biotech EBNA-1 IgG ISR Values for serially two fold diluted sera. The ISR Values are compared to log2 of dilution by standard linear regression. The data indicates that the antibody can be semi-quantitated by using a single serum dilution. The detection of a significant antibody increase may be made only by an evaluation of paired specimens, acute and convalescent.

Table 4
EBNA-1 IgG Linearity

Serum #	Neat	1:2	1:4	1:8	1:16	1:32	1:64	Slope	Y Intercept	r
1	8.60	5.96	3.50	1.96	0.87			1.95	-1.66	0.985
2	11.4	9.30	6.90	4.10	2.50	1.30	0.42	1.91	-2.49	0.985
3	6.90	4.90	2.70	1.50	0.69			1.58	-1.41	0.983
4	8.70	6.50	4.07	2.32	1.20	0.55		1.67	-1.95	0.979
5	9.40	7.17	4.80	3.20	1.50	0.70		1.77	-1.75	0.989

CROSS-REACTIVITY

Serum containing IgG antibody detectable by ELISA to Herpes Simplex Virus 1 & 2, Cytomegalovirus, and Varicella-Zoster Virus were assayed. The data summarized in Table 5 indicate that antibodies to these Herpesviruses do not cross-react with the Trinity Biotech EBNA-1 IgG ELISA kit.

Table 5
Cross-Reactivity

Serum	Trinity Biotech EBNA-1 IgG	HSV1 IgG	HSV2 IgG	CMV IgG	VZV IgG
1	0.67 -	2.43 +	0.60 -	1.69 +	2.28 +
2	0.36 -	0.32 -	0.08 -	0.18 -	3.17 +
3	0.73 -	3.67 +	0.86 -	1.11 +	2.45 +
4	0.29 -	0.30 -	0.39 -	0.18 -	3.21 +
5	0.34 -	6.18 +	5.98 +	0.18 -	2.14 +

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

EVALUATION OF PAIRED SERA

To validate the sensitivity of paired sera, 20 high positive sera were serially two fold diluted and run on the Trinity Biotech EBNA-1 IgG test. From these dilutions, there were 68 pairs that had a four fold dilution where the acute sera had a value of less than 3.70. All 68 pairs demonstrated a > 46% rise in ISR Value, showing a significant rise in antibody. Therefore, the paired sera demonstrated 100% sensitivity in being able to detect a four fold increase in antibody level when the acute sera has a value of < 3.70.

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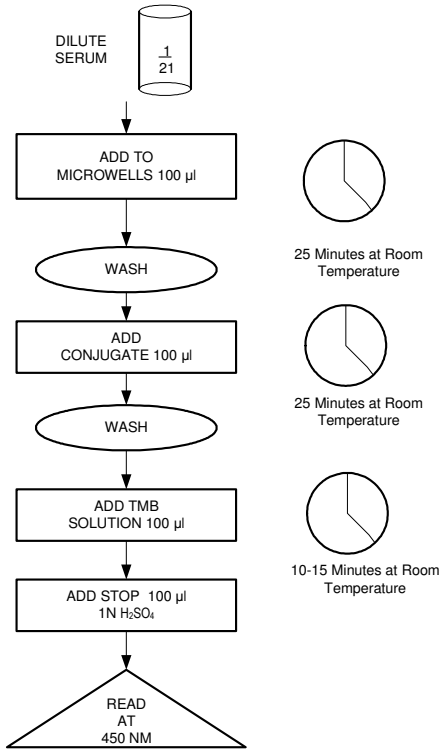
CAPTIA™ EBNA-1 IGG SUMMARY OF ASSAY PROCEDURE



The safety data sheet is available upon request.

WARNING

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300®, 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.

ORDERING INFORMATION

KIT		Captia™ EBNA1 IgG Test Kit
Catalog No.	Item	Quantity
2325800	Captia™ EBNA1 IgG Test Kit	96 Tests
2325801	Captia™ EBNA1 IgG Test Kit	480 Tests

Manufactured

EC REP
Authorized Representative

Consult accompanying documents

REF
Product Number

LOT
Lot

Use by

Caution, consult accompanying documents

Store at 2-8ºC

Store at 2-30ºC

CONTROL + +
High Pos or Positive Control

CONTROL +
Low Pos or Cut-Off Control

CONTROL -
Negative Control

CAL
Calibrator

C.F.
Coefficient Factor

RNG
Range

STD
Standard

IVD
For *In Vitro* Diagnostic use

or
Warning

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14701
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Tel. 353 1 2769800
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