Trinity Biotech

Captia™ EBNA-1 IgM

REF	2325860	96 Tests
REF	2325861	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- (EBNA-1) IgM Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the qualitative determination of IgM antibodies in human serum to EBNA-1 recombinant antigen. The Trinity Biotech anti-EBNA-1 IgM assay may be used in conjunction with other Epstein-Barr serologies (VCA IgG, VCA IgM, EA-D IgG, EBNA-1 IgG and heterophile) as an aid in the diagnosis of infectious mononucleosis in adult populations. **For *in vitro* diagnostic use. High comlexity 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. EBV is classified as a member of the herpesvirus family based upon its characteristic morphology.[2] All herpesviruses share the ability to establish a latent infection in their hosts.[3] 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. 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.[4,5]

Infection of the target cells leads to two forms of viral cycles: 1) latent, nonproductive and 2) productive, replicative infections.[6] 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 either follows or parallels membrane antigen at 12 to 24 hours post infection. EBNA 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. The viral capsid antigen complex (VCA) appears late in the replicative cycle.

It has recently become apparent that EBNA 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 (6). Antibody levels of EBNA-1 IgM and EBNA-1 IgG, when measured concurrently, are diagnostic in determining acute and convalescent stages in IM.[1,7,8]

The Trinity Biotech EBNA-1 IgM kit utilizes the ELISA technology in which a purified EBNA-1 recombinant antigen is bound to the wells of a microplate. Serum pretreatment solution is used to remove the interferences potentially caused by IgG and IgM-RF. A peroxidase coupled antihuman IgM 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 anti-human IgM globulin 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 yellow color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.[9,10,11,12] The sensitivity, specificity, and reproducibility of ELISAs can be comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radioimmunoassays.[13,14,15]

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 (full length clone ~83K from Sf-9/Baculovirus cells) coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate; 480T: five plates)
2. Serum Diluent Plus: Ready for use. Contains goat/sheep antihuman IgG for serum absorption to remove competing IgG, with protein stabilizers and ProClin® (0.1%) as a preservative. (96T: two bottles, 45 mL each, 480T: two bottles, 225 mL each)
3. Cutoff Calibrator (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 Cutoff Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions.The source material for the Cutoff Calibrator is different than the Controls. (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 IgM, 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 with the Trinity Biotech ELISA IgM assays: Chromogen/Substrate Solution Type I, Wash Buffer Type I and Stop Solution. The Serum Diluent Plus is specific for the EBV IgM kits. Do not interchange the Serum Diluent Plus with other IgM kits. 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 – 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 Cutoff Calibrator, High Positive Control, Low Positive Control and Negative Control between 2° and 8° C.
5. Store Serum Diluent Plus and 20X Wash Buffer between 2° and 8°C.
6. Store the Chromogen/Substrate TMB 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 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 Cutoff 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 (16).
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 and Wash Buffer. Serum Diluent Plus supplied with EBV IgM kits can be used only with other EBV IgM kits. 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 Cutoff 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 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.
2. Optimal performance of the kit depends upon the use of fresh serum samples (clear, non-hemolyzed, non-lipemic, 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 Pro-cedures for the Handling and Processing of Blood Specimens, H18-A. 1990).[16]

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.

SERUM TREATMENT

Solid phase immunoassays for the detection of virus-specific IgM are known to be sensitive to interfering factors. This kit overcomes interferences by treating samples prior to running the assay. The goat/sheep anti-human IgG in the Serum Diluent Plus diminishes competing virus specific IgG, which would be responsible for false negative reactions. False positives are similarly minimized by removing the IgG, thus neutralizing the bound rheumatoid factor in the samples.

ASSAY PROCEDURE

1. Place the desired number of strips into a microwell frame. Allow six (6) Control/Cutoff Calibrator determinations (one Negative Control, three Cutoff 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/Cutoff Calibrator configurations. 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 al reagents. Used to blank reader.
NC = Negative Control
Cal = Cutoff Calibrator
HPC = High Positive Control
LPC = Low Positive Control

2. Dilute test sera, Cutoff Calibrator and Control sera 1:81 (e.g., 10 µL + 800 µL) in Serum Diluent Plus. (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 diluted patient sera, Cutoff Calibrator and Control sera. Add 100 µL of Serum Diluent Plus to the reagent blank well. Check software and reader requirements of
the correct reagent blank well configuration.
4. Incubate each well at room temperature (21° to 25° C) for **30 minutes +/- 2 minutes.**
5. Aspirate or shake out liquid from all wells. 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 washes) for semiautomated equipment or four times (for a total of five 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 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 the 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 30 minutes +/- 2 minutes.
8. Repeat Wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate solution (TMB) solution to each well, including reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21° to 25° C) for 15 minutes +/- 2 minutes.
11. Stop reaction by addition of 100 µL of Stop Solution (1N H2SO4) following the same order Chromogen/Substate addition, including reagent blank well. Tap the plate gently along the outsides to mix contents of the wells. The plate may be held up to one (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. 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. Cutoff 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.[20]
9. If above criteria are not met upon repeat testing, contact Trinity Biotech Technical Services.

INTERPRETATION

CALCULATIONS

1. Mean Cutoff Calibrator O.D. (Optical Density) - Calculate the mean O.D. value for the Cutoff 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 Cutoff 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

ANALYSIS

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

ISR Value	Results	Interpretation
≤ 0.90	Negative	No detectable EBNA-1 IgM antibody by the ELISA test.
0.91-1.09	Equivocal	Samples should be retested. See number 2 below.
≥ 1.10	Positive	Significant level of detectable EBNA-1 IgM antibody. Indicative of current or recent infection.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method, e.g., immunofluorescence assay (IFA).
3. To determine the cutoff of the assay, fifty-seven normal sera were assayed by the Trinity Biotech EBNA –1 IgM ELISA test. The mean and standard deviation of the optical density readings for the sera were 0.206 and 0.182, respectively. The positive threshold for the assay was determined by adding the mean and 2.5 deviations (0.206 + 2.5(0..182) = .67). 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 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 are 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 Trinity Biotech EBNA-1 IgM ELISA. Values obtained with different methods may not be used interchangeably. The magnitude of the reported IgM 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 (VCA), IgG – viral capsid antibody (VCA), IgG- antibody to early antigen (EA), 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.

EXPECTED VALUES

ACUTE PHASE:

EBNA-1 IgM increases rapidly in EARLY acute phase and is detectable before or concurrently with VCA IgG and IgM and heterophile antibodies. EBNA-1 IgM decreases during the LATE phase along with VCA IgM, but VCA IgG persists.

TRANSITIONAL PHASE:

EBNA-1 IgM has decreased to low and approximately similar levels as EBNA-1 IgG which is beginning to increase. VCA IgG persists.

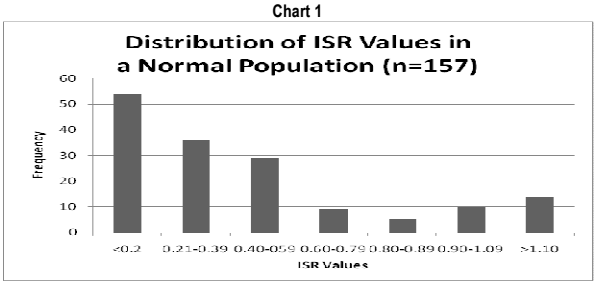
CONVALESCENT PHASE:

EBNA-1 IgM very low level to negative with EBNA-1 IgG increasing to high levels.
Note: EBNA-1 IgM has occasionally been detected into the convalescent phase.

PREVALENCE

A group of 157 sera from a normal population from various ages, genders and geographical areas of the U.S. were tested on the Trinity Biotech EBNA-1 IgM ELISA assay. The positive rate for the Trinity Biotech EBNA-1 IgM ELISA assay was found to be 7.0% and the equivocal rate was 6.4%. The prevalence found in the literature is 1-2% in apparently healthy blood donors'.[19] The distribution of ISR values from this study is presented in Chart 1 below:

Chart 1



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. The results of ELISA immunoassays performed on serum from immunosupressed patients must be interpreted with caution.
3. 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.
4. This device is not intended for the determination of immune status. It is intended for the determination of immune response to indicate primary infection or virus reactivation.
5. The absence of detectable IgM antibody does not rule out the possibility of recent or current infection. If EBV infection is still suspected, obtain a second specimen 5-7 days later and repeat the testing. Often, however, at the time of presentation, IgM antibodies are in decreasing concentrations.
6. Specific IgG may compete with the IgM for sites and may result in a false negative. Conversely, rheumatoid factor in the presence of specific IgG may result in a false positive reaction. The goat/sheep anti-human IgG in the Serum Diluent Plus diminishes competing specific IgG and minimizes rheumatoid factor interference in samples. The Serum Diluent Plus is formulated to remove IgG from patient serum expressing IgG different concentrations. At the final sample dilution of 1:81, the in the Serum Diluent Plus was demonstrated to remove >99% of IgG at concentrations of 340 mg/dL, and 2250 mg/dL. This corresponds to a value in excess of the high end and low end of the acceptable normal range of IgG (~700-1500 mg/dL). Samples <340 mg/dL and >2250 mg/dL should be interpreted with caution.
7. Heterotypic (false positive) IgM responses to EBV may occur in patients infected with CMV and also in patients infected with HSV1.
8. Some antinuclear antibodies have been found to cause a false positive reaction on some ELISA tests.
9. Results of this test should be interpreted by the physician in the light of other clinical findings and diagnostic procedures.
10. A negative test for EBV EBNA-1 (IgM) does not exclude current EBV infection. The sample may have been collected before development of demonstrable antibody or after antibody still detectable.
11. Results from children should be reviewed with caution.[17]
12. Icteric, lipemic, hemolyzed, or heat inactivated sera may cause erroneous results and should be avoided.
13. Kit procedures or practices outside those in this package insert may yield questionable results.
14. The performance characteristics have not been established for any matrices other than sera.
15. The prevalence of the analyte will affect the assay's predictive value.
16. 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.
17. Since EBNA-1 IgM antibodies can be present in normal convalescent sera, a single result can not be used for diagnosis. Accurate interpretation of EBV infection is based on the results from VCA IgG, VCA IgM, EBNA IgG, EA-D IgG and heterophile antibody.
18. Specimens close to the cutoff of the assay may become equivocal after serum absorption.
19. The performance characteristics for this assay have not been established for pediatric specimens.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY BASED ON SERUM CHARACTERIZATION

One hundred and sixty-six selected frozen retrospective sera were tested at a clinical lab in the mid-Atlantic area of the US. The sera from the study were characterized as seronegative (no serological evidence of past or present EBV infection), acute (VCA IgM and heterophile antibody present, EBNA IgG absent), 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 were determined based on this characterization. It was assumed that the EBNA-1 IgM response should be negative for seronegative and convalescent serum, and positive for acute serum. The results are summarized in Table 1 and Table 1A.

Table 1
Sensitivity and Specificity Based On Serum Characterization

		Acute VCA IgM + EBNA IgG – Heterophile +	Seropositive VCA IgG + EBNA IgG + VCA IgM - Heterophile -	Seronegative VCA IgG - EBNA IgG - VCA IgM - Heterophile -
	Positive	19	5	0
Trinity Biotech	Equivocal	2	8	0
EBNA-1 IgM	Negative	18	86	28
	Total	39	99	28

Table 1A
Summary of Relative Sensitivity & Specificity Data

	Results**	Results as Percentage	95% confidence intervals***
Relative Sensitivity (Acute)	19/37*	51.4%	34.9%-67.8%
Relative Specificity (Seronegative)	28/28	100.0%	89.4%-100%****
Relative Specificity (Seropositive)	86/91	94.5%	89.7%-99.3%
Relative Agreement	133/156	85.3%	79.6%-90.9%

*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.

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.

PRECISION

The Trinity Biotech EBNA-1 IgM ELISA was evaluated for precision by testing six sera six times each at site one and ten times each at the second site on three different days. The results are summarized in the table below.

Inter Site Precision (n=48)

Serum#	X	S.D.	C.V.
1	1.87	0.161	8.60%
2	1.56	0.166	10.63%
3	2.38	0.156	6.57%
4	1.30	0.132	10.18%
5	0.47	0.050	10.52%
6	0.19	0.051	26.36%
HPC*	3.28	0.146	4.45%
CAL**	2.22	0.144	6.48%
NC*	0.01	0.012	181.66%

X = Mean ISR Value
S.D. = Standard Deviation
C.V. = Coefficient of Variation
* HPC and NC - n=6
** CAL - n=18

CROSS-REACTIVITY

Sera containing IgM antibody detectable by ELISA to Herpes Simplex Virus I & II, Cytomegalovirus, and Varicella-Zoster Virus were assayed. Sera containing rheumatoid factor (RF) were also assayed. The data summarized below indicate that antibodies to Varicella and sera containing RF do not cross-react with the Trinity Biotech EBNA-1 IgM ELISA kit. There may be some cross-reactivity to Herpes Simplex Virus and to Cytomegalovirus.

Specificity	EBNA-1 IgM		Alternate Assay	
RF +	0.15	-	1.87	+
RF +	0.09	-	1.82	+
RF +	0.01	-	1.73	+
RF +	0.10	-	1.80	+
RF +	0.10	-	1.85	+
VZV M +	0.44	-	3.28	+
VZV M +	0.17	-	5.46	+
VZV M +	0.10	-	4.98	+
VZV M +	0.29	-	2.34	+
VZV M +	0.20	-	2.18	+
HSV 1 M +	2.53	+	2.53	+
HSV 1 M +	0.27	-	1.65	+
HSV 1 M +	0.05	-	1.34	+
HSV 1 M +	0.08	-	1.32	+
HSV 2 M +	0.53	-	1.76	+
HSV 2 M +	0.08	-	1.60	+
HSV 2 M +	0.03	-	2.09	+
HSV 2 M +	0.62	-	1.96	+
CMV M +	1.57	+	1.23	+
CMV M +	0.98	E	1.92	+
CMV M +	2.00	+	3.83	+
CMV M +	0.38	-	1.32	+

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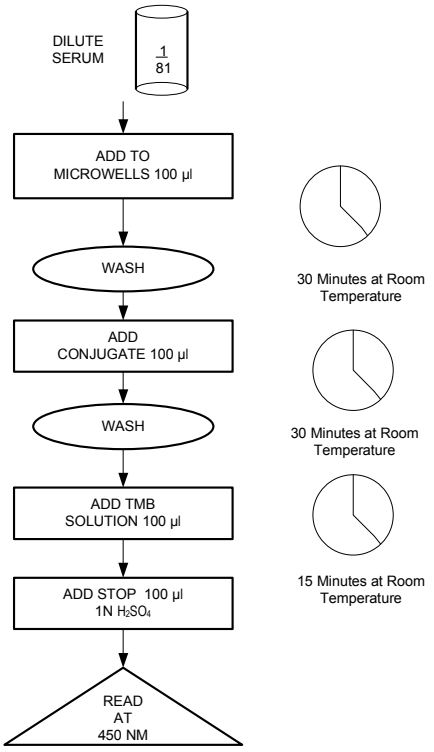
CAPTIA™ EBNA - 1 IGM SUMMARY OF ASSAY PROCEDURE



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™ EBNA-1 IgM Test Kit
Catalog No.	Item	Quantity
2325860	Captia™ EBNA-1 IgM Test Kit	96 Tests
2325861	Captia™ EBNA-1 IgM Test Kit	480 Tests

Manufactured

CONTROL + +
High Pos or Positive Control

EC REP
Authorized Representative

CONTROL +
Low Pos or Cut-Off Control

Consult accompanying documents

CONTROL -
Negative Control

REF
Product Number

CAL
Calibrator

LOT
Lot

C.F.
Coefficient Factor

Use by

RNG
Range

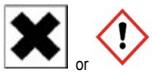
Caution, consult accompanying documents

STD
Standard

Store at 2-8ºC

IVD
For *In Vitro* Diagnostic use

Store at 2-30ºC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