Antibody levels of EBNAS1 IgG, are diagnostic in determining acute and convalescent stages in classic clinical manifestations associated with IM. Disease such as infectious mononucleosis (IM).

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. 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). Infectious mononucleosis is an acute, self-limited, non-productive, replicative infections.

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 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. The specific activity to the antigen present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N HSO₄, 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 microplate reader.

**MATERIALS SUPPLIED**

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

1. Recombiant EBNA-1 antigen (the carboxy-terminal of EBNA-1 genome representing ~200 codons) coated microplate assay: 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. Calibrators: 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 negative range 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 anti human 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 sensitivity, specificity, and reproducibility of ELISAs can be compared to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radio-immunossay.

**KIT PRESENTATION**

For the following components, the expiration date of the kit. Refer to the package label for the expiration date.

- 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. Unused microassay plates must be stored between 2° and 8°C. Unused strips must be immediately resealed in a sealable bag with dessicant 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 TX (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.

**STORAGE AND STABILITY**

- If not constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to the 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. Use only clean, dry glassware.

*Note: All reagents must be at room temperature (21° to 25 °C) before running assay. Remove 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 antigens 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.

The Trinity Biotech Reagent Type (Type I, Type II, etc.) is used for the assay.
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.

P270: 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/container in accordance to local, regional, national and international regulations.

**WARNING**
Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed.

P334: 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 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 Trinity Biotech ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, non-epicrnic, nonicteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.
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 heat-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.

**METHODS FOR USE**

**PREPARATION FOR THE ASSAY**

1. All reagents must be removed from refrigerator 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 H2O. 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 (8) 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:**

<table>
<thead>
<tr>
<th>Plate</th>
<th>Sample</th>
<th>Location</th>
<th>Description</th>
<th>Location</th>
<th>Rate</th>
<th>Sample</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>RB</td>
<td>2A</td>
<td>Patient #2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1B</td>
<td>NC</td>
<td>2B</td>
<td>Patient #3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1C</td>
<td>Cal</td>
<td>2C</td>
<td>Patient #4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1D</td>
<td>Cal</td>
<td>2D</td>
<td>Patient #5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E</td>
<td>Cal</td>
<td>2E</td>
<td>Patient #6 (Acute 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1F</td>
<td>LPC</td>
<td>2F</td>
<td>Patient #6 (Acute 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1G</td>
<td>LPC</td>
<td>2G</td>
<td>Patient #6 (Convalescent 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**RB** = Reagent Blank - Well without serum addition run with all reagents. Utilized to blank readings.

**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 10X 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 towel 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 HSO4) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outside 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 instrumental equipment 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 batch 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 according organizations.
8. Refer to NCCLS C24-A for guidance on appropriate QC practices.
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.
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:

<table>
<thead>
<tr>
<th>ISR Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.90</td>
<td>Negative</td>
</tr>
<tr>
<td>0.91-1.09</td>
<td>Equivocal</td>
</tr>
<tr>
<td>≥ 1.10</td>
<td>Positive</td>
</tr>
</tbody>
</table>

2. To determine the cutoff of the assay, thirty-seven (37) negative sera were assayed by the Trinity Biotech EBNA-1 IgG ELISA 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 0.0006, respectively. The positive threshold for the assay was determined by adding the mean and one standard deviation (0.0262 + 0.0006) = 0.0368. 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 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 titre. 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 IgG 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 accuracy of 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:

   - Acute 1 ISR = 0.8 to 1.2
   - Convalescent 1 ISR = 0.8 to 1.2
   - Acute 2 ISR
   - Convalescent 2 ISR

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

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

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 ≤ 0.370, 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.

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.

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.

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.

6. Individuals with chronic active Epstein-Barr virus infection may not produce an antibody response to EBNA-1.

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 lymphoproliferations, 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

<table>
<thead>
<tr>
<th>Relative Sensitivity and Specificity of the Trinity Biotech EBNA-1 IgG ELISA kit</th>
<th>Trinity Biotech EBNA-1 IgG ELISA Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>270</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>342</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternate</td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>96.9%</td>
</tr>
<tr>
<td>Alternate</td>
<td>Equivocal</td>
</tr>
<tr>
<td>0.91-1.09</td>
<td>96.9%</td>
</tr>
<tr>
<td>Alternate</td>
<td>Negative</td>
</tr>
<tr>
<td>&lt; 0.90</td>
<td>96.9%</td>
</tr>
</tbody>
</table>

** Equivocal results were not included in the calculations.

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

Table 1A

<table>
<thead>
<tr>
<th>Summary of Relative Sensitivity &amp; Specificity Data</th>
<th>Results as</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Sensitivity</td>
<td>96.9%</td>
</tr>
<tr>
<td>Relative Specificity</td>
<td>96.9%</td>
</tr>
<tr>
<td>Relative Agreement</td>
<td>96.9%</td>
</tr>
</tbody>
</table>

Sensitivity and specificity based on serum characterization

The serum from the first site study was 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.
demonstrated 100% sensitivity in being able to detect a four fold increase in antibody level when 46% rise in ISR Value, showing a significant rise in antibody. Therefore, the paired sera four fold dilution where the acute sera had a value of less than 3.70. All 68 pairs demonstrated a >

run on the Trinity Biotech EBNAS1 IgG test. From these dilutions, there were 68 pairs that had a

Twice a day for twenty days at each of the three study sites. The interSite coefficient of variation different sites to determine the precision of the assay. Each sera was tested three times each, Page 4 of 5 – EN 5800-29 Rev M

To validate the sensitivity of paired sera, 20 high positive sera were serially two fold diluted and

Sera ≤ 0.90 were considered negative.

Sera ≥ 1.10 were considered positive

Serum containing IgG antibody detectable by ELISA to Herpes Simplex Virus 1 & 2,

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 coefficient of variation (CV) for each serum is presented in Table 3.

The data in Table 4 illustrate the Trinity Biotech EBNAS1 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.

The data in Table 4 indicate that antibodies to these Herpesviruses do not cross-react with the Trinity Biotech EBNAS1 IgG ELISA kit.

Cross-Reactivity

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 EBNAS1 IgG test. From these dilutions, there were 65 pairs that had a four fold dilution where the acute sera had a value of less than 3.70. All 68 pairs demonstrated a >

REFERENCES


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 coefficient of variation (CV) for each serum is presented in Table 3.

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REFERENCES


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.
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 in accordance to local, regional, national and international regulations.

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>KIT</th>
<th>Captia™ EBNA1 IgG Test Kit</th>
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Trinity Biotech USA
Jamestown, NY
14701
Tel. 1 800-325-3424
Fax: 716-488-1990

Trinity Biotech plc
Bray Co. Wicklow, Ireland
Tel. 353 1 2768800
Fax 353 1 2769888
www.trinitybiotech.com

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