Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

EBV-EA-D IgM

Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-650 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of samples in Sample Diluent. Mix well.
2. Add 100 µl of diluted samples into the antigen wells. Reserve one well for reagent blank (100 µl of Sample Diluent).
3. Incubate at room temperature (18-30° C) for 30 ± 5 min.
4. Discard contents of the wells. Wash the wells 3 times with Wash Solution.
5. Add 100 µl of Conjugate to each well.
6. Incubate at room temperature (18-30° C) for 30 ± 5 min.
7. Wash the wells as in #4 above.
8. Add 100 µl Substrate Solution to each well.
9. Incubate at room temperature for 30 ± 5 min.
10. Add 100 µl Stop Solution to each well.
11. Read the absorbances at 450/600-630 nm against the reagent blank.

INTENDED USE

For the qualitative determination of IgM antibodies to Epstein-Barr Virus (recombinant) Early Antigen Diffuse (EBV-EA-D IgM) in human serum by indirect enzyme immunoassay. The *Is*-EBV-EA-D IgM Test Kit should be used in combination with other Epstein-Barr serologies, Viral Capsid Antigen (VCA) IgG and IgM, Epstein-Barr Nuclear Antigen-1 (EBNA-1) IgG and IgM, Early Antigen-Diffuse (EA-D) IgG and heterophile antibody as an aid in the diagnosis of infectious mononucleosis (IM).

SUMMARY AND EXPLANATION

Epstein-Barr virus (EBV) is a member of the *herpesvirus* family that infects human lymphocytes.[1,2] It is known to cause infectious mononucleosis (IM) and is transmitted primarily by saliva. EBV has been found in oropharyngeal secretions of healthy, asymptomatic, adults and is a possible source of infection for susceptible individuals.[1,2,3] As with other herpesviruses, EBV causes a persistent latent infection with intermittent reactivations. EBV infection is usually asymptomatic in infants and young children. In adolescents and young adults infection usually results in IM.[1,3,4,5,6] Diagnosis is generally based on the characteristic symptoms of sore throat, lymphadenopathy, fever, splenomegaly, and possibly the presence of heterophile antibodies.[1,6] Because not all symptoms may be present and, since other infectious agents such as *Toxoplasma gondii* and cytomegalovirus may cause similar symptoms, serological detection of circulating antibodies is an important step in the diagnosis of EBV infection.[1,6]

Humoral response to primary EBV infection appears to be quite rapid. Antibodies to EBV are made to various viral proteins, with specific antibodies correlating to disease state. In acute infection IgM and then IgG antibodies are sequentially made to early antigen-diffuse (EA-D), viral capsid antigen (VCA), and nuclear antigen. Current or recent infection is marked by the presence of IgM antibodies to EA-D, VCA, and EBNA. IgG antibodies to EA-D and VCA are normally present in current infection, while IgG antibodies to EBNA are absent. Post EBV infection is indicated by sustaining IgG antibody to VCA and EBNA and the absence of IgM antibodies.[1,3,7,8] Thus, the monitoring of EBV antibody patterns may assist in the diagnosis of EBV infection since individual levels of specific antibodies may not necessarily be indicative of disease, but can be of diagnostic importance when monitored as a profile.

The indirect fluorescent antibody (IFA) assays for detecting antibodies to EBV have been largely replaced by enzyme-linked immunosorbent assays (ELISA or EIA) which are easier to perform, easier to interpret, and amenable to automation.

The Immunosimplicity® *Is*-EBV-EA-D IgM Test Kit is an EIA procedure intended for the qualitative detection of EA-D IgM antibodies.

PRINCIPLE OF THE PROCEDURE

Recombinant EA-D antigen is bound to microwells. Diluted patient sera, Cut-Off Calibrator and controls are placed in the microwells and incubated. Anti-EA-D IgM antibodies, if present, will bind to the antigen forming antigen-antibody complexes. Residual sample is eliminated by aspirating and washing. Conjugate (horseradish peroxidase-labeled anti-human IgM) is added and will bind to these complexes. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm (reference 600-630 nm) and is a measurement of IgM antibodies to EA-D present in the sample.

REAGENTS

Each Is-EBV-EA-D IgM Test Kit contains reagents for 96 tests.

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded pink, coated with purified recombinant (*E. coli* as vector) EA-D antigen (a 28kd protein from the c-terminal half of EA-D).
Cut-Off Calibrator	One vial with blue cap containing 0.25 ml of human serum or defibrinated plasma, weakly reactive for EA-D IgM antibodies, 0.1% sodium azide. The Cut-Off Calibrator is used to determine the cut-off of the assay.
Low Positive Control	One vial with white cap containing 0.25 ml of human serum or defibrinated plasma, 0.1% sodium azide. Assigned range printed on label. The positive control is used to control the low range of the assay.
Negative Control	One vial with black cap containing 0.25 ml of non-reactive human serum or defibrinated plasma. Assigned range printed on the label. The Negative Control is used to control the negative range of the assay and to control the removal of IgG antibodies. Note that the Cut-Off Calibrator and controls are prepared from different serum lots.
Sample **C** Diluent	One bottle with green cap containing 60 ml Phosphate buffer with goat anti-human IgG and protein stabilizers. Contains Proclin® 300, 15 ppm active ingredient. Color-coded green.
Wash **T** Concentrate (20X)	Two bottles with clear caps containing 50 ml of Tris buffer with detergent and Proclin® 300, 15 ppm active ingredient. Each bottle is sufficient to make 1050 ml of wash solution.
Conjugate	One bottle with red cap containing 25 ml goat anti-human immunoglobulin M labeled with horseradish peroxidase. Also includes protein stabilizers and preservatives. Color-coded pink.
Substrate **HRP**	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' Tetramethylbenzidine).
Stop **N** Solution	One bottle with white cap containing 30 ml of 1 N Sulfuric Acid. **CAUTION:** Acids are corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water. See Warnings & Precautions section.

Store these reagents at 2 to 8° C.

OTHER MATERIALS REQUIRED

Manual Users:

Wash bottle or automated microplate washer.
Pipettors capable of dispensing appropriate volumes.
Timer.
One liter graduated cylinder.
One liter wash solution reservoir.
Deionized or distilled water.
Absorbent toweling.
Tubes or microwell plate for sample dilution.
Reader capable of reading absorbance at 450 nm, reference at 600-630 nm. (Performance characteristics have not been established for single wavelength readers.)

Plus Automated EIA Processor Users:

One liter graduated cylinder.
Deionized or distilled water.
Pre-dilution cups, strips or plates.
ProbeClean™ Concentrate, or tip washing detergent solution, if applicable.

WARNINGS & PRECAUTIONS

REAGENTS: For *in vitro* Diagnostic Use.

1. Handle samples, Calibrator, controls and the materials that contact them as potential biohazards. Each donor unit in the Calibrator and controls has been found negative for Hepatitis B surface antigen and HIV-I antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, Hepatitis B virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1993.
2. Never pipette by mouth.
3. Avoid contact with open skin and mucous membranes.
4. Certain of the test reagents contain Proclin® 300 as a preservative. When disposing of reagents containing Proclin® 300, flush drains with copious amounts of water to dilute the active components below active levels.
5. Reagents containing Sodium Azide:
   (a) **CAUTION:** Some reagents in this kit contain Sodium Azide as preservative. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts", in the Manual Guide – Safety Management No. CDC-22, issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

   European Communities Hazardous Substance Risk Phrases (Regulation (EC) No 1272/2008)

   H300 –Fatal if swallowed.
   H310 – Fatal if contact with skin.
   EUH032 – Contact with acids liberates very toxic gas.
   H410 – Very toxic to aquatic life with long lasting effect.
   P264 – Wash all exposed external body areas thoroughly after handling.
   P302+P352 – IF ON SKIN: Wash with plenty of water and soap.
   P301+P310/P330 – IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.
   P270 – Do not eat, drink or smoke when using this product.
   P501 – Dispose of contents/container as hazardous waste.
   P391 – Collect spillage.
   P273 – Avoid release to the environment. Refer to special instructions/ Safety Data Sheet.
   (b) Sodium Azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.
6. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.

ADDITIONAL PRECAUTIONS

1. Do not interchange reagents from different reagent lots except for Sample **C** Diluent, Wash **T** Concentrate, Substrate **HRP** and Stop **N** Solution.
2. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
3. Store unused reagents at 2 to 8°C.
4. Incubations above or below the recommended temperatures or times may give erroneous results.
5. The EIA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times, and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
6. Antigen coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
7. *(Manual Procedure Only)* The washing procedure is very important and requires special attention. (Please refer to the Procedure section.)

   NOTE: *Improperly washed wells may give erroneous results.*
8. The reported concentration of anti-EA-D IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

SPECIMEN COLLECTION

Whole blood should be collected by accepted medical techniques. Separated serum should remain at 22°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated (2-8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -20°C. Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen sera to room temperature slowly and mix gently, avoiding foam formation. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used. The CLSI, formerly NCCLS, provides recommendations for collecting and storing blood specimens, (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18A3).

CAUTION: *Serum samples must not be heat-inactivated prior to use.*

PROCEDURE

Allow all test components and patient samples to warm to room temperature before use. Invert reagent bottles gently several times before use. Return promptly to the refrigerator after use.

Prepare Wash Solution by adding 50 ml of Wash Concentrate (20X) to one liter of deionized or distilled H_2O.

MANUAL USERS

1. Prepare 1:101 dilutions of the Cut-Off Calibrator (in triplicate), controls and patient samples in Sample Diluent. (e.g., by addition of 2 µl sample to 200 µl Sample Diluent or 5 µl sample to 500 µl Sample Diluent).
2. Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of Calibrator, controls and diluted patient samples, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.

   NOTE: Include one well, which contains 100 µl of Sample Diluent as a reagent blank. This will ultimately be used to "zero" the photometer before reading test results.
3. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.
4. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling. Wash the wells by rinsing 3 times with at least 300 µl of Wash Solution. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer's instructions.
5. Place 100 µl of Conjugate into each well, avoiding bubble formation.
6. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.
7. Wash the wells as described in Step 4 above.
8. Place 100 µl of Substrate into each well, avoiding bubble formation.
9. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.
10. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
11. Read the absorbance of each well at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 60 minutes of adding Stop Solution.

AUTOMATED EIA PROCESSOR USERS:

When using an Automated EIA Processor, refer to the Operator's Manual for the test setup and procedures.

NOTE: *Automated EIA Processor users must validate their equipment to demonstrate that the results obtained are equivalent to those obtained using manual assay.*

QUALITY CONTROL

1. The Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank must be < 0.25.
3. The absorbance of the Cut-Off Calibrator must be > 0.10.
4. The Positive and Negative Controls must be within their assigned ranges.

If any of these criteria are not met, the results are invalid and the test should be repeated.

NOTE: Additional controls may be tested according to guidelines or requirements of local, state or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to CLSI, formerly NCCLS, document C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions.

RESULTS

1. Calculation

Calculate the mean absorbance of the Cut-Off Calibrator. Note: When calculating the mean absorbance value for the Cut-Off Calibrator exclude any absorbance value that deviates by more than 15% from the mean of the three absorbance values. Use the mean of the remaining two replicates in calculations. Exclusion of more than one of the three absorbance values invalidates the run.

Determine the Index Value for each patient specimen or control using the following formula:

$$\frac{\text{Absorbance of Sample}}{\text{Mean Absorbance of Cut-Off Calibrator}} = \text{Index Value}$$

An Automated EIA Processor (e.g. MAGO® Plus Automated EIA Processor) will calculate results using the above formula and will print them automatically.

Example: Absorbance values obtained for Cut-Off Calibrator: 0.276,0.288, 0.258 (after subtraction of blank)

Mean Absorbance of Cut-Off Calibrator = 0.274

Sample Absorbance = 1.150

Index Value = 1.150 / 0.274 = 4.2

2. Interpretation

Index Value	*Interpretation*
< 0.90	No detectable EA-D IgM antibody; result does not exclude EBV infection. An additional sample should be tested within 4-6 weeks if early infection is suspected. Other EBV serology tests are necessary to rule out acute infection.
0.90 – 1.09	Equivocal for antibodies to EA-D. Sample can be retested, tested by another method or a new sample can be tested.
≥ 1.10	EA-D IgM antibody detected. Other EBV serology tests are necessary for confirmation of acute EBV-associated infectious mononucleosis.

3. Reporting Results

When the Index Value is reported for a single specimen the following statement should be included: "The following results were obtained with the *Is*-EBV-EA-D IgM Test Kit. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present. The magnitude of the reported IgM level cannot be correlated to an endpoint titer".

CUT-OFF ESTABLISHMENT

The *Is*-EBV-EA-D IgM Test Kit cut-off value was established to optimally differentiate those individuals with, from those without, immunological experience to EBV-EA-D. The optimal cut-off was determined by statistical analysis of the results of 32 sera shown to be negative for EA-D IgM antibodies in the *Is*-EBV-EA-D IgM Test Kit as well as other methods. The mean and standard deviation of the absorbance values for these sera were 0.142 and 0.070 respectively. The cut-off was determined as being equal to the mean plus 3 standard deviations, 0.142 + (3 x 0.070) = 0.35. The Cut-Off Calibrator has been titrated to equal this result. Therefore, the mean value of the Cut-Off Calibrator will be equal to the cut-off for the assay. To account for the inherent variations in EIA methods, an equivocal range of ± 10% has been included.

LIMITATIONS

1. The results obtained with the *Is*-EBV-EA-D IgM Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
2. Assay performance characteristics have not been established for visual result determination.
3. The test should be performed on serum. The use of whole blood or plasma has not been established.
4. There is a possibility of assay cross-reactivity with specimens containing anti-*E.coli* antibody.
5. The performance characteristics have not been established for patients with nasopharyngeal carcinoma, Burkitt's lymphoma, other EBV-associated lymphadenopathies, and EBV-associated diseases other than EBV-related mononucleosis.
6. A single result cannot be used for diagnosis. Accurate interpretation of EBV infection is based on the results from EA-D IgG, EA-D IgM, EBNA IgG, EBNA IgM, VCA IgG, VCA IgM, and heterophile antibody testing.
7. Screening of the general population should not be performed. The positive predictive value depends on the likelihood of Epstein Barr Virus being present. Testing should only be performed when clinical symptoms are present or exposure is suspected.
8. Results from immunosuppressed patients should be interpreted with caution.
9. The performance characteristics of the *Is*-EBV-EA-D IgM Test Kit with automated equipment other than the MAGO® Plus Automated EIA Processor have not been established.
10. Since rheumatoid factor (RF) binds to IgG in immunocomplexes, false positive results may arise in sera with RF and specific IgG. False negatives may arise due to specific IgG competing with the specific IgM. The goat anti-human IgG in the sample diluent diminishes RF interference and minimizes competing specific IgG in the samples. The sample diluent removes >95% of the IgG at levels of 1400 mg/dl. Samples with IgG levels >1400 mg/dl should be interpreted with caution.
11. Performance characteristics have not been established for diagnosing adolescents with infectious mononucleosis.

EXPECTED VALUES

The prevalence of EBV-EA-D IgM antibodies in the normal population can vary depending on a number of factors such as age, geographical location, socio-economic status, race and testing method used. It is estimated that in the Western Hemisphere approximately 50% of the population is exposed to the virus before 5 years of age and by adulthood 90% of the population demonstrates antibody to the virus.

In the present studies sera from 197 normal individuals of various ages and genders from different geographic locations were evaluated in the *Is*-EBV-EA-D IgM Test Kit. One hundred sixty-five samples (83.8%) were negative for antibodies to EA-D IgM, fourteen samples (7.1%) were positive and eighteen samples (9.1%) were equivocal for EA-D IgM antibodies. The age distribution and prevalence for this population is shown in Table 1. Histograms showing the distribution of Index Values for the positive and negative populations are shown in Figures 1 and 2.

TABLE 1

	Number of Donors	Prevalence
Total Number	197	7.1%
Geographic locations: Various	197	7.1%
Age		
4 - 10	18	16.7%
11 - 20	26	19.2%
21 – 40	49	6.1%
41 - 60	57	1.8%
61 - 80	34	2.9%
81 - 91	13	7.7%

FIGURE 1
***Is*-EBV-EA-D IgM Positive Population**

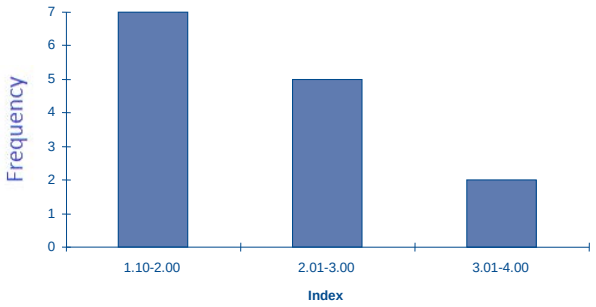
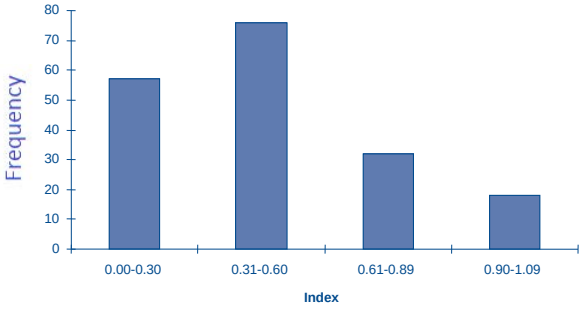


FIGURE 2
***Is*-EBV-EA-D IgM Negative Population**



PERFORMANCE CHARACTERISTICS

A. Clinical Sensitivity and Specificity Using Characterized Sera

Frozen retrospective sera from one hundred and seventy-six patients were characterized using commercially available kits for VCA IgM, VCA IgG, EBNA IgG and heterophile antibodies. Based on the results of this testing, the patient sera were characterized as follows:

* 102 sera were characterized as convalescent (past infection). These were positive for VCA IgG and/or EBNA IgG antibodies and negative for VCA IgM and heterophile antibody.
* 32 sera were characterized as seronegative. These were negative for VCA IgG, VCA IgM, EBNA IgG and heterophile antibody.
* 42 sera were characterized as having a current (recent) infection. These were positive for VCA IgM and/or heterophile antibody and were negative for EBNA IgG.

All 176 sera were then tested by an independent clinical commercial laboratory using the *Is*-EBV-EA-D IgM Test Kit. The results obtained are shown in Table 2:

TABLE 2
EBV Serological Status

Is-EBV-EA-D IgM	*Convalescent*	*Current Infection*	*Seronegative*
POSITIVE	**5**	**17**	**1**
NEGATIVE	**93**	**20**	**29**
*** EQUIVOCAL**	**4**	**5**	**2**

			95% CI
Relative Specificity (Convalescent)	93/98	= 94.9%	88.5-98.3
Relative Sensitivity (Current Infection)	17/37	= 45.9%	29.5-63.1
Relative Specificity (Seronegative)	29/30	= 96.7%	82.8-99.9
Overall Agreement	139/165	= 84.2%	78.7-89.8

* Equivocal results were excluded from calculations.

NOTE: Please be advised that "relative" refers to the comparison of the 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's accuracy to predict disease. Since the above studies were performed on a pre-selected, retrospective population, no calculations for the assay's positive and negative predictive value may be done or inferred.

B. Precision

To determine the precision of the *Is*-EBV-EA-D IgM Test Kit, four positive and two negative sera were assayed ten times each in three different runs at three different sites. The 3 sites include: the manufacturer, a research and development laboratory, and a clinical commercial laboratory. The intra- and interassay precision obtained at each site is shown in Tables 3, 4 and 5. The Inter-Site precision is shown in Table 6.

TABLE 3
Site #1- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY RUN 1 MEAN INDEX	CV%	INTRA-ASSAY RUN 2 MEAN INDEX	CV%	INTRA-ASSAY RUN 3 MEAN INDEX	CV%	INTERASSAY MEAN INDEX	CV%	
A (POS)	6.59	5.42	6.37	4.53	6.25	5.17	6.40	5.33	
B (POS)	1.86	12.60	1.79	10.84	1.95	6.18	1.87	10.46	
C (POS)	2.90	10.05	2.67	7.31	2.61	9.00	2.73	9.77	
D (POS)	1.37	8.92	1.38	12.45	1.41	12.81	1.39	11.20	
E (NEG)	0.14	44.84	0.15	39.58	0.16	46.08	0.15	42.74	
F (NEG)	0.44	15.23	0.45	39.42	0.32	35.23	0.40	34.16	
						CAL	0.97	10.49	n = 9
						PC	1.65	12.75	n = 3
						NC	0.29	38.40	n = 3

TABLE 4
Site #2- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY RUN 1 MEAN INDEX	CV%	INTRA-ASSAY RUN 2 MEAN INDEX	CV%	INTRA-ASSAY RUN 3 MEAN INDEX	CV%	INTERASSAY MEAN INDEX	CV%	
A (POS)	6.328	6.78	5.514	4.34	6.055	3.85	5.966	7.68	
B (POS)	1.813	9.32	1.580	10.07	1.722	6.65	1.705	10.21	
C (POS)	2.465	5.70	2.376	17.21	2.521	6.15	2.454	10.72	
D (POS)	1.261	4.89	1.118	6.07	1.219	3.78	1.199	6.97	
E (NEG)	0.144	28.01	0.155	16.49	0.150	14.21	0.149	19.71	
F (NEG)	0.289	15.47	0.341	8.87	0.308	10.00	0.313	13.11	
						CAL	1.001	4.69	n = 18
						PC	1.564	12.03	n = 12
						NC	0.303	4.72	n = 12

TABLE 5
Site #3- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY RUN 1 MEAN INDEX	CV%	INTRA-ASSAY RUN 2 MEAN INDEX	CV%	INTRA-ASSAY RUN 3 MEAN INDEX	CV%	INTERASSAY MEAN INDEX	CV%	
A (POS)	6.35	11.01	6.53	12.63	6.05	11.29	6.31	11.74	
B (POS)	1.98	9.08	1.90	10.74	1.91	11.87	1.93	10.36	
C (POS)	2.63	16.02	2.59	15.59	2.52	17.40	2.58	15.86	
D (POS)	1.55	20.22	1.49	26.10	1.42	14.80	1.49	20.67	
E (NEG)	0.13	15.27	0.12	19.99	0.13	23.17	0.13	19.85	
F (NEG)	0.29	15.16	0.24	15.18	0.36	10.88	0.29	21.96	
						CAL	1.00	11.59	n = 9
						PC	1.42	6.45	n = 3
						NC	0.30	5.77	n = 3

TABLE 6
Inter-Site Precision

SERUM (n = 90)	INTER-SITE MEAN INDEX	CV%
A (POS)	6.23	9.10
B (POS)	1.83	11.51
C (POS)	2.58	12.97
D (POS)	1.36	17.32
E (NEG)	0.14	31.41
F (NEG)	0.34	30.28
CAL (n = 36)	0.99	8.21
LPC (n = 18)	1.55	11.87
NC (n = 18)	0.30	13.42

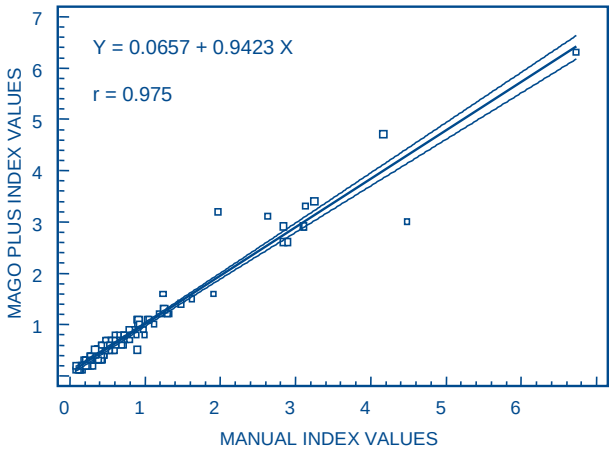
C. Specificity with Potentially Cross-Reactive Sera

Thirteen sera, reactive (positive) for IgM antibodies to varicella zoster, cytomegalovirus and herpes simplex virus were tested in the *Is*-EBV-EA-D IgM Test Kit. 4/4 anti-VZV IgM positive sera were non-reactive for anti-EA-D IgM; 3/5 anti-CMV IgM positive sera were non-reactive for anti-EA-D IgM and 4/4 anti-HSV IgM positive sera were non-reactive for anti-EA-M IgM. This suggests that some cross-reactivity should be expected with the *Is*-EA-D IgM Test Kit from these analytes.

D. Correlation of Manual and MAGO Plus Results

The *Is*-EBV-EA-D IgM Test Kit has been developed for automated as well as manual use. To demonstrate the equivalence of the manual and MAGO Plus procedures, the results of 128 serum samples tested by both methods were plotted. A scattergram and regression line of the results obtained with 95% confidence intervals is shown in Figure 3. The data indicate good correlation with a Pearson Correlation Coefficient of 0.975.

FIGURE 3
Manual and MAGO Plus Result Correlation



E. MAGO Plus Precision

The precision of the assay when performed on the MAGO® Plus Automated EIA Processor was determined by assaying six sera ten times each in three different runs. Table 7 shows the intra-and interassay precision obtained using the MAGO Plus.

TABLE 7
Site #2- Intra-Assay and Interassay Precision - MAGO Plus

SERUM	INTRA-ASSAY MEAN INDEX	RUN 1 CV%	INTRA-ASSAY MEAN INDEX	RUN 2 CV%	INTRA-ASSAY MEAN INDEX	RUN 3 CV%	INTERASSAY MEAN INDEX	CV%	
A (POS)	6.1	3.76	5.8	4.29	5.8	3.98	5.9	4.35	
B (POS)	1.8	6.38	1.7	7.27	1.8	8.12	1.8	7.44	
C (POS)	2.7	5.51	2.5	7.23	2.6	7.49	2.6	6.97	
D (POS)	1.4	16.10	1.3	7.77	1.3	8.34	1.3	11.76	
E (NEG)	0.1	37.16	0.1	28.75	0.1	0.00	0.1	30.51	
F (NEG)	0.3	23.80	0.3	20.45	0.3	15.06	0.3	20.86	
						CAL	1.0	2.80	n = 12
						PC	1.7	3.50	n = 4
						NC	0.4	0.00	n = 4

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