BIO-RAD

BioPlex® 2200 System
EBV IgG

Instructions For Use

REF 665-1250

Σ 100

IVD

CE

Federal law restricts this device to sale by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

This IFU is effective beginning with Lot # 963349 of the EBV IgG Reagent Pack and above, and BioPlex 2200 Software Version 2.0 and above.

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Multiple Language CD-ROM

This Instructions For Use (IFU) document is provided in Adobe PDF form on a multiple language CD-ROM in the following languages: English, German, French, Italian, Spanish, Portuguese, Swedish, Danish, and Czech.

REF		LOT		Σ		
Catalog Number	WARNING	Lot Number	Manufactured by	Number of Tests	Temperature Limit	Use by (YYYY-MM-DD)
Katalognummer	ACHTUNG	Chargen-Nr.	Hergestellt von	Anzahl der Tests	Temperaturgrenze	Verwendbar bis (JJJJ-MM-TT)
Numéro de catalogue	ATTENTION	Numéro de lot	Fabriqué par	Nombre de tests	Limite de température	Date de péremption (AAAA-MM-JJ)
Numero di catalogo	ATTENZIONE	Numero di lotto	Fabbricato da	Numero di analisi	Limite di temperatura	Data di scadenza (AAAA-MM-GG)
Número de catálogo	ATENCIÓN	Número de lote	Fabricado por	Número de pruebas	Límite de temperatura	Usar hasta el (AAAA-MM-DD)
Número de catálogo	ATENÇÃO	Número de lote	Fabricado por	Número de testes	Limite de temperatura	Utilizar até (AAAA-MM-DD)
Katalognummer	VARNING	Batchnummer	Tillverkare	Antal tester	Temperaturgränser	Använd före (ÅÅÅÅ-MM-DD)
Katalognr	ADVARSEL	Lotnummer	Fremstillet af	Antal tests	Temperaturgrænse	Anvend før (ÅÅÅÅ-MM-DD)
Katalogové číslo	VAROVÁNÍ	Číslo šarže	Výrobce	Počet stanovení	Teplotní rozmezí od do	Datum exspirace (RRRR-MM-DD)

IVD		CE	EC REP
For In Vitro Diagnostic Use	Consult Instructions for Use	European Conformity	Authorized Representative in the European Community
In-vitro-Diagnostikum	Gebrauchsanweisung beachten	CE-Konformitätskennzeichnung	Bevollmächtigter in der Europäischen Union
Utilisation comme test de diagnostic in vitro	Consulter la notice d'emploi	Conformité aux normes européennes	Représentant agréé dans la Communauté Européenne
Per uso diagnostico in vitro	Consultare le istruzioni per l'uso	Conformità europea	Mandatario autorizzato per l'Unione Europea
Para uso en diagnóstico in vitro	Consulte las instrucciones de uso	Conformidad europea	Representante autorizado en la Comunidad Europea
Para uso em diagnóstico in vitro	Consulte as instruções de utilização	Conformidade com as normas europeias	Representante autorizado na Comunidade Europeia
För in vitro-diagnostiskt bruk	Läs bruksanvisningen	Europeisk överensstämmelse	Auktoriserad representant i Europeiska gemenskapen
Til in vitro-diagnostisk brug	Benyt brugsanvisninger	Europæisk overensstemmelse	Repræsentant i det Europæiske Fællesskab
Pro diagnostiku in vitro	Viz návod k použití	Evropská shoda	Zplnomocněný zástupce

EBV IgG PACK	BEAD	DIL	CONJ	CONT	VER
EBV IgG Reagent Pack	Bead Set	Sample Diluent	Conjugate	Contains	Version
EBV IgG Reagenzienpack	Bead-Set	Probenverdünnungsmittel	Konjugat	Enthält	Version
EBV IgG Cartouche de réactifs	Réactif contenant les billes	Diluant échantillon	Conjugué	Contient	Version
EBV IgG Cartuccia reagenti	Set di microsfere	Diluente per campione	Coniugato	Contiene	Versione
EBV IgG Paquete de reactivos	Conjunto de micropartículas	Diluyente para muestras	Conjugado	Contiene	Versión
EBV IgG Kit de reagentes	Conjunto de esferas	Diluente de amostras	Conjugado	Contém	Versão
EBV IgG reagenskit	Mikrosfär-set	Provdiluent	Konjugat	Innehåller	Version
EBV IgG reagenspakke	Perlesæt	Prøvefortynder	Konjugat	Indeholder	Version
EBV IgG reagenční sada	Souprava částic	Ředící roztok pro vzorky	Konjugát	Obsah	Verze

Caution, consult accompanying documents
Achtung, Begleitdokumente beachten
Attention, consulter la documentation jointe
Attenzione, consultare la documentazione allegata
Precaución, consulte los documentos que acompañan al instrumento
Atenção, consultar os documentos inclusos
Försiktighet: se medföljande dokument
Forsigtig, der henvises til den vedlagte dokumentation
Pozor, obraťte se průvodních dokladů

Table of Contents

INTENDED USE

The BioPlex® 2200 EBV IgG kit is a multiplex flow immunoassay intended for the qualitative detection of IgG antibodies to three (3) separate EBV antigens; Epstein-Barr Virus Nuclear Antigen-1 (EBV NA-1), Viral Capsid Antigen (EBV VCA), and Early Antigen diffuse (EBV EA-D) in human serum. The test system can be used in conjunction with the BioPlex 2200 EBV IgM kit as an aid in the laboratory diagnosis of infectious mononucleosis (IM).

The EBV IgG kit is intended for use with the Bio-Rad BioPlex 2200 System.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, or infants. Assay performance characteristics have not been established for the diagnosis of nasopharyngeal carcinoma, Burkitt's lymphoma, and other EBV-associated lymphomas.

SUMMARY AND EXPLANATION

Epstein-Barr Virus (EBV) is a member of the herpesvirus family. Primary infection with EBV usually results in infectious mononucleosis (IM), an acute, self-limited lymphoproliferative disease. Approximately 80 - 90% of the human population is infected by EBV.[1-2]

Infection by EBV results in the production of antibodies to four different distinct antigenic complexes: EBV induced Nuclear Antigen (NA), Early Antigen (EA), Viral Capsid Antigen (VCA), and Membrane Antigen. The EA complex is divided into two components which include *diffused* and *restricted*. Because of the complex relationship that exists between the EBV/host reaction and clinical manifestation, tracking of EBV antibody patterns may assist in diagnosis of EBV infection. Individual levels of specific antibodies are not necessarily indicative of disease state but can be of diagnostic significance when tracked as an antibody response profile. Antibody response profiles for the different EBV antigens demonstrate a characteristic pattern for silent primary or persistent latent EBV infection.[3-4]

EBV NA-1:	IgG antibodies to NA-1 are rarely present in acute IM and normally rise during convalescence. A rise may be indicative of progression from early to later stages of convalescence. The presence of antibodies in otherwise healthy individuals usually indicates past exposure. Antibody levels will rise to a plateau level in three months to a year and will normally persist for life.
EBV VCA:	IgG antibodies to VCA are normally present in acute and convalescent IM. A rise is indicative of an acute stage of infection. The presence of antibodies in otherwise healthy individuals usually indicates immunological exposure either as silent primary infection or past exposure. Antibody levels tend to rise and peak after 3 - 4 weeks, then decline and usually persist for life.
EBV EA-D:	IgG antibodies to EA-D are frequently present in acute IM and generally absent in convalescence. A rise may indicate acute infection, reactivation, or chronic infection. The presence of antibodies in otherwise healthy individuals usually indicates reactivation, especially when paired with rising NA-1 levels. Antibody levels tend to rise and peak after 3 - 4 weeks, then decline and usually dissipate after 6 months.

Currently, the heterophile antibody test is the most common procedure for the diagnosis of acute IM. It uses mononucleosis antigens to detect IM heterophile antibodies. However, up to 20% of patients with primary EBV infection do not develop heterophile antibodies. With the introduction of EBV VCA IgM testing, the detection of acute IM has been enhanced. However, EBV VCA IgM may not be detectable in all acute IM patients, thus testing for both heterophile antibody and anti-VCA IgM increases the likelihood of detecting acute primary infection.[5-8]

Bio-Rad's EBV IgG kit uses a group of antigens that detect three (3) analytes simultaneously. Beads are individually coated with individual antigens, so that the presence of each antibody can be individually determined.

PRINCIPLE OF THE PROCEDURE

The EBV IgG kit uses multiplex flow immunoassay, a methodology that greatly resembles traditional EIA, but permits simultaneous detection and identification of many antibodies in a single tube.[9-10]

Three (3) different populations of beads are coated with *E. coli* derived recombinant proteins, EBV NA-1 (28kD and 45kD), EBV VCA p18 (40kD), and EBV EA-D (28kD) associated with infectious mononucleosis.[11-12] The BioPlex 2200 System combines an aliquot of patient sample, sample diluent, and bead reagent into a reaction vessel. The mixture is incubated at 37°C. After a wash cycle, anti-human IgG antibody, conjugated to phycoerythrin (PE), is added to the dyed beads and this mixture is incubated at 37°C. The excess conjugate is removed in another wash cycle, and the beads are re-suspended in wash buffer. The bead mixture then passes through the detector. The identity of the dyed beads is determined by the fluorescence of the dyes, and the amount of antibody captured by the antigen is determined by the fluorescence of the attached PE. Raw data is calculated in relative fluorescence intensity (RFI).

Three additional dyed beads, an Internal Standard Bead (ISB), a Serum Verification Bead (SVB) and a Reagent Blank Bead (RBB) are present in each reaction mixture to verify detector response, the addition of serum to the reaction vessel and the absence of significant non-specific binding in serum. Refer to the BioPlex 2200 System Operation Manual for more information.

The instrument is calibrated using a set of seven (7) distinct calibrator vials, supplied separately by Bio-Rad Laboratories. A combination of four (4) vials representing four (4) different antibody concentrations are used for calibration. The result for each of these antibodies is expressed as an antibody index (AI).

KIT COMPONENTS

EBV IgG (REF 665-1250). The reagent pack contains supplies sufficient for 100 tests.

Vial	Description
Bead Set BEAD	One (1) 10 mL vial, containing 3 different populations of dyed beads coated with affinity-purified *E. coli* derived recombinant proteins to EBV NA-1 (28kD and 45kD), EBV VCA p18 (40kD), EBV EA-D (28kD); an Internal Standard (ISB), a Serum Verification (SVB), and a Reagent Blank (RBB); with Glycerol and protein stabilizers (bovine) in a MOPS (3-[N-Morpholino] propanesulfonic acid) buffer. ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) as preservatives.
Conjugate CONJ	One (1) 5 mL vial, containing murine monoclonal anti-human IgG/phycoerythrin conjugate, and murine monoclonal anti-human FXIII/phycoerythrin conjugate, with protein stabilizers (bovine) in a phosphate buffer. ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) as preservatives.
Sample Diluent DIL	One (1) 10 mL vial, containing protein stabilizers (bovine and murine) in a triethanolamine buffer. ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) as preservatives.

ADDITIONAL REQUIRED ITEMS, AVAILABLE FROM BIO-RAD

REF	Description
663-1200	BioPlex 2200 EBV IgG Calibrator Set: Seven (7) 500 µL vials, containing antibodies to EBV NA-1, EBV VCA, and EBV EA-D, in a human serum matrix made from defibrinated plasma. ProClin 300 (≤ 0.3%) as a preservative for all calibrators.
663-1230	BioPlex 2200 EBV IgG Control Set: Two (2) 1.5 mL vials of Positive Control containing antibodies to EBV NA-1, EBV VCA, and EBV EA-D, in a human serum matrix made from defibrinated plasma; and two (2) 1.5 mL vials of Negative Control in a human serum matrix made from defibrinated plasma. ProClin 300 (≤ 0.3%) as a preservative for all controls.
660-0817	BioPlex 2200 Sheath Fluid: Two (2) 4 L bottle containing Phosphate Buffered Saline (PBS). ProClin 300 (0.03%) and sodium azide (< 0.1%) as preservatives.
660-0818	BioPlex 2200 Wash Solution: One (1) 10 L bottle containing Phosphate Buffered Saline (PBS) and Tween 20. ProClin 300 (0.03%) and sodium azide (< 0.1%) as preservatives.
660-0000	BioPlex 2200 Instrument and Software.

PRECAUTIONS/WARNINGS

1. **For In Vitro Diagnostic (IVD) Use.**
2. For professional use only.

WARNING
H317*

Contains ≤ 0.3% ProClin 300*

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.

3. Each unit of human serum used in the manufacture of the BioPlex 2200 EBV IgG kit (including calibrator and control sets) was tested by FDA accepted methods and found non-reactive for Hepatitis B surface antigen (HBsAg), antibody to HIV-1, HIV-2 and Hepatitis C (HCV). No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice (GLP), all human source material should be considered potentially infectious for Hepatitis B (HBV), Hepatitis C (HCV), HIV-1, HIV-2, and all other infectious agents; therefore, handle the BioPlex 2200 EBV IgG kit (including reagent packs, calibrators and control sets) with the same precautions used with patient specimens. It is recommended that these reagents and human specimens be handled in accordance with the *Biosafety in Microbiological and Biomedical Laboratories*[14]*, WHO Laboratory Biosafety Manual*[15], Biosafety Level 2 or other appropriate biosafety practices[16] for materials which contain or are suspected of containing infectious agents.
4. Consider any materials of human origin as infectious and handle them using typical biosafety procedures and Universal Precautions according to 29 CFR 1910.1030[13] and in accordance with local, regional and national regulations.
5. Do not smoke, eat, or drink in areas where patient samples and kit reagents are handled.
6. Do not pipette by mouth.
7. Wear personal protective equipment while handling all reagents and samples and while operating the BioPlex 2200 System.
8. Dispose of all wastes in accordance with applicable national, and/or local regulations.
9. Waste material containing patient samples or biological products should be considered biohazardous when disposing or treating.
10. Chemical reagents should be handled in accordance with Good Laboratory Practices.
11. Refer to the kit and additional required component Safety Data Sheets (SDS) for more safety information and warnings about chemical and biological hazards. The Safety Data Sheets are available at bio-rad.com and on request.
12. Clean up all spills immediately and thoroughly. Decontaminate the area for any spills involving biohazardous materials with an effective disinfectant. Dispose of all contaminated materials appropriately.
13. Do not use tests beyond their expiration date. The date is printed on all boxes.
14. Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents.
15. Adherence to the protocol specified herein is necessary to ensure proper performance of this product. If aberrant results are obtained, contact Bio-Rad Technical Service.
16. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
17. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[17]

SPECIMEN COLLECTION AND HANDLING

Specimen Collection Precaution

Consider any materials of human origin as infectious and handle them using typical biosafety procedures.

Specimen Type

Serum is the recommended sample type. Avoid hemolysis.

Specimen Storage

Sera may be stored under refrigeration (2-8°C) for up to 7 days. For longer storage of samples, keep at -20°C or colder.

Specimen Preparation

Thoroughly mix thawed specimens; it is also recommended to centrifuge thawed specimens to remove gross particulate matter. Avoid multiple freeze/thaw cycles (up to 3 cycles is acceptable).

Specimen Shipping

All specimens and other samples of human origin must be shipped in accordance with national and international transportation regulations. Do not exceed the storage time and temperature limitations listed above.

PREPARATION AND STORAGE OF REAGENTS

- **Do not freeze the EBV IgG kit.**
- Reagents in the EBV IgG kit are ready to use. After initial use, the reagents are stable for 30 days or until the date of expiration when stored unopened and on the instrument or refrigerated at 2-8ºC.
- Do not use reagents beyond expiration dates.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Store all reagents at the labeled temperature and do not use past their expiration dates.

Do not use any reagents which have any indications of discoloration, cloudiness or precipitation. Do not use any reagents that show any signs of leakage.

PROCEDURE

In order to obtain reliable and consistent results, strictly adhere to the instructions in this Instructions for Use. Do not modify the handling and storage conditions for kit reagents or patient samples.

Operating instructions, including calibration, quality control, and maintenance for the BioPlex 2200 System are further described in the BioPlex 2200 System Operation Manual. Prior to using the EBV IgG kit, ensure that the BioPlex 2200 System is powered on, loaded with reagent packs and bulk solutions, and that all required maintenance has been performed. Please refer to the BioPlex 2200 System Operation Manual for more information regarding these activities.

Any lots of the BioPlex 2200 System Sheath Fluid and BioPlex 2200 System Wash Solution can be interchanged.

A) Calibration

The EBV IgG Calibrator Set should be loaded and assayed at minimum in duplicate every 14 days or with each new Reagent Pack lot. A point-to-point curve fit using four calibrators is used to calculate results. Refer to the BioPlex 2200 System Operation Manual for more information.

B) Quality Control

At the beginning of each day that the EBV IgG Control Set is to be used, load and process the EBV IgG control set as indicated in the BioPlex 2200 System Operation Manual. The EBV IgG Control Set should be run at least once per day, and with each new Reagent Pack lot.

Note: The Negative and Positive Controls of the EBV IgG Control Set are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff.

Lot specific values for the positive control are loaded into the BioPlex 2200 System database via the provided media or by manual input. After identifying the control via the barcoded vial, the BioPlex 2200 System compares the control results to the expected lot specific control values stored in the BioPlex 2200 System database.

The EBV IgG Control Set includes a negative control as well as a multi-analyte positive control in a human serum matrix made from defibrinated plasma, containing antibodies present for analytes within the EBV IgG kit. The positive control is manufactured to give

positive results, with values above the cut-off for each specific bead. The negative control is manufactured to give negative results, with values below the cut-off for each specific bead. The negative control must have a negative result, and the positive control must have a positive result.

Failure to obtain the appropriate values for controls will invalidate the assay and indicates procedural error, improper sample handling or deterioration of reagents. Additional controls may be tested in accordance with local, state and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

IMPORTANT QC TROUBLESHOOTING: At low frequency, reagent packs may exhibit falsely low signals for certain analytes and generate QC errors. The following troubleshooting steps should be followed when observing the noted QC behavior:

1. **QC Warning - LOW for only some analytes:**
   Repeat QC testing. If the QC Warning repeats, remove the pack with the flagged QC results and **do not use**. Please call Bio-Rad Technical Support to report the suspected low signal pack. Run QC with a new reagent pack. If QC results are within the acceptable range on the new reagent pack, discard the affected reagent pack with the QC Warning - Low results and **do not report patient test results** from that reagent pack. If the QC Warning repeats on the new reagent pack, please call Bio-Rad Technical Support for assistance with troubleshooting. Re-test any samples that were tested using the affected reagent pack. If multiple packs for a particular BioPlex 2200 assay are on-board the instrument, the reagent pack (kit) serial number associated with the QC Warning - low results can be determined by viewing the Control Result dialog for the corresponding QC Event.
2. **QC Warning - HIGH for only some analytes:**
   Re-calibrate the reagent pack with the QC Warning - high and re-run QC. Verify that QC results are within the acceptable range for all analytes before reporting out patient results. Any samples tested using the pack with the QC Warning - high must be re-tested. Patient samples run on the affected pack are valid due to the fact that a calibration occurred with that pack.

Note: Certain analytes in the EBV IgG positive control must be diluted to achieve the lot specific values which are loaded into the BioPlex 2200 System database. The BioPlex 2200 System automatically dilutes these analytes and performs the operation using an additional reaction vessel. If a control result is out of its specified range, any test results generated since the last acceptable control results must be evaluated to determine if test results may have been adversely affected. Adversely affected results are invalid, and these samples must be retested.

C) Load/Process Samples

Load samples into the racks provided with the BioPlex 2200 System as indicated in the BioPlex 2200 System Operation Manual. Sample processing on the BioPlex 2200 System is fully automated. Refer to the BioPlex 2200 System Operation Manual for appropriate software setup.

D) Traceability to Reference Material

There are no known certified reference materials available for any of the analytes in the EBV IgG kit.

GUIDELINES FOR INTERPRETATION OF RESULTS

Calculation

All calculations necessary to interpret the results are performed automatically by the BioPlex 2200 System Software.

Data Analysis

The results for each of the antibodies are expressed as an antibody index (AI). Results of ≤0.8 AI are negative, 0.9 and 1.0 AI are equivocal, and ≥1.1 AI are reported as positive.

Antibody Index (AI)	Result	Interpretation
≤ 0.8 AI	Negative	A result of ≤ 0.8 AI for any of the three EBV markers indicates no detectable IgG antibody to that particular marker and should be reported as non-reactive for IgG antibody to that marker. If all three markers are negative and exposure to Epstein-Barr virus is suspected, a second sample should be collected and tested no less than one to two weeks later.
0.9 AI, 1.0 AI	Equivocal	Specimens with results in the equivocal range (0.9 to 1.0 AI) for any of the three markers should be tested by an alternate serologic procedure. Alternatively, a second freshly collected sample should be obtained and tested.
≥ 1.1 AI	Positive	A result of ≥ 1.1 AI for any of the three EBV markers indicates that the specimen is positive for IgG antibody to that marker. A positive test result presumes a current or past infection with EBV, and should be reported as reactive for IgG antibody to the marker(s). Other EBV serology assays such as the EBV VCA IgM should be performed to confirm serological status, active acute, past or indeterminate infection for EBV-associated infectious mononucleosis.

For specimens that are equivocal, the patient sample can be re-collected for additional testing. In conjunction with these results, the immune status of patients should be evaluated based on their clinical status, related risk factors, and other diagnostic test results.

Selection of Desired Results

The laboratory may elect to run any of the antibodies requested individually, or any group of antibodies may be requested for a specific sample. The laboratory may pre-define the test groupings by using the software screen entitled “Test Group Setup,” allowing the operator to request “customized” groupings. For example, EBV NA-1 and EBV VCA may be analyzed together.

“Add-On” test(s) for immediate reporting of results for additional individual antibodies, or a user-defined test grouping containing antibodies, not previously requested, can also be performed. If the selected antibody has been previously requested, the sample will be treated as a repeated request. Refer to the BioPlex 2200 System Operation Manual for more information.

Typical Antibody Response Characterization

The following table demonstrates a generally accepted algorithm for classifying patients into an EBV status via serologic profiles. EBV status can be applied to any patient based on results of standard tests. In acute IM, both EBV IgM and EBV IgG antibodies to viral capsid antigen (VCA) rise rapidly. EBV VCA IgM antibody disappears over about four weeks. Heterophile antibody, which is of the IgM class, appears only during acute infection and fades rapidly over about four weeks. EBV EA-D IgG antibody shows a transient rise during acute infection, and becomes undetectable after 3 - 6 months. EBV NA-1 IgG antibody usually appears 3 months after initial infection and typically remains for life, as well as EBV VCA IgG.[4, 8, 18-21]

Table A. Serological Status

EBV Serological Status	EBV NA-1 IgG	EBV VCA IgG	EBV EA-D IgG	EBV VCA IgM	Heterophile Antibody
Primary Acute	Neg (-)	Pos (+)	Pos (+)	Pos (+)	Neg (-)
	Neg (-)	Neg (-)	Pos (+)	Pos (+)	Pos (+)
	Neg (-)	Pos (+)	Neg (-)	Pos (+)	Pos (+)
	Neg (-)	Neg (-)	Neg (-)	Pos (+)	Pos (+)
	Neg (-)	Neg (-)	Neg (-)	Pos (+)	Neg (-)
	Neg (-)	Neg (-)	Pos (+)	Pos (+)	Neg (-)
	Neg (-)	Pos (+)	Pos (+)	Pos (+)	Pos (+)
	Neg (-)	Pos (+)	Pos (+)	Neg (-)	Pos (+)
Late Acute	Neg (-)	Pos (+)	Neg (-)	Pos (+)	Neg (-)
	Pos (+)	Pos (+)	Pos (+)	Pos (+)	Pos (+)
	Pos (+)	Pos (+)	Pos (+)	Pos (+)	Neg (-)
	Pos (+)	Pos (+)	Neg (-)	Pos (+)	Pos (+)
	Pos (+)	Pos (+)	Pos (+)	Neg (-)	Neg (-)
	Pos (+)	Pos (+)	Neg (-)	Pos (+)	Neg (-)
Recovering	Neg (-)	Pos (+)	Pos (+)	Neg (-)	Neg (-)
Previous Infection	Neg (-)	Pos (+)	Neg (-)	Neg (-)	Neg (-)
	Pos (+)	Pos (+)	Neg (-)	Neg (-)	Neg (-)
Susceptible	Neg (-)	Neg (-)	Neg (-)	Neg (-)	Neg (-)

Notes: For the purposes of serological characterization, equivocal results were considered negative.
Any serological pattern not identified in Table A should be considered inconclusive.

LIMITATIONS OF THE PROCEDURE

- The EBV IgG kit is not, in and of itself, diagnostic for infectious mononucleosis, and should be considered in conjunction with other laboratory test results, such as the BioPlex 2200 EBV IgM kit, and the clinical presentation of the patient. Only a physician should interpret results.
- The performance characteristics have not been established with nasopharyngeal carcinoma, Burkitt's lymphoma, other EBV associated lymphadenopathies, and other EBV associated diseases other than EBV related mononucleosis.
- Results obtained from immunocompromised individuals and neonates should be interpreted with caution.
- The performance characteristics have not been established for any matrices other than serum.
- There is a possibility of assay cross-reactivity with specimens containing anti-*E.coli* antibody.
- Immune complexes or other immunoglobulin aggregates present in patient samples may cause increased non-specific binding and produce false positive results. However, the EBV IgG kit incorporates a Reagent Blank Bead (RBB) with each test that verifies the absence of significant non-specific binding in serum.
- Antibody index (AI) values reflect qualitative changes in antibody concentration that cannot be directly associated with clinical condition or disease state.
- Testing should not be performed as a screening procedure for the general population. The predictive value of a positive or negative result depends on the prevalence of analyte in a given patient population. Testing should only be done when clinical evidence suggests the diagnosis of EBV-associated infectious mononucleosis.

EXPECTED VALUES

Prevalence

Expected values for the EBV IgG kit are presented by age and gender in the following tables for serum samples from unselected hospitalized pediatric and adult patients (N=303) and patients for which an EBV test was ordered (N=620). A total of 621 serum samples from patients for which an EBV test was ordered were tested. One (1) sample from the patients for which an EBV test was ordered population was excluded due to RBB analysis error messages during BioPlex 2200 EBV IgG testing. For all analytes, results of ≤0.8 AI are negative, 0.9 and 1.0 AI are equivocal, and ≥1.1 AI are reported as positive.

Table B. Hospitalized Patient Samples: EBV NA-1 IgG

Age	Gender	BioPlex 2200 EBV NA-1 IgG						Total
		Positive		Equivocal		Negative		
		N	%	N	%	N	%	N
< 5 years of age	F	11	41%	0	0%	16	59%	27
	M	6	30%	0	0%	14	70%	20
5-12 years of age	F	13	59%	0	0%	9	41%	22
	M	15	44%	0	0%	19	56%	34
13-20 years of age	F	28	80%	0	0%	7	20%	35
	M	10	67%	0	0%	5	33%	15
21-30 years of age	F	5	83%	0	0%	1	17%	6
	M	1	50%	0	0%	1	50%	2
31-40 years of age	F	10	100%	0	0%	0	0%	10
	M	11	100%	0	0%	0	0%	11
41-50 years of age	F	13	100%	0	0%	0	0%	13
	M	7	100%	0	0%	0	0%	7
51-60 years of age	F	22	96%	0	0%	1	4%	23
	M	18	95%	0	0%	1	5%	19
61-70 years of age	F	11	100%	0	0%	0	0%	11
	M	12	100%	0	0%	0	0%	12
71-80 years of age	F	11	100%	0	0%	0	0%	11
	M	6	100%	0	0%	0	0%	6
81-90 years of age	F	11	100%	0	0%	0	0%	11
	M	5	83%	0	0%	1	17%	6
91-100 years of age	F	0	0%	0	0%	0	0%	0
	M	2	100%	0	0%	0	0%	2
Total		228	75%	0	0%	75	25%	303

Table C. Hospitalized Patient Samples: EBV VCA IgG

Age	Gender	BioPlex 2200 EBV VCA IgG						Total
		Positive		Equivocal		Negative		
		N	%	N	%	N	%	N
< 5 years of age	F	11	41%	0	0%	16	59%	27
	M	5	25%	0	0%	15	75%	20
5-12 years of age	F	14	64%	0	0%	8	36%	22
	M	15	44%	0	0%	19	56%	34
13-20 years of age	F	29	83%	0	0%	6	17%	35
	M	9	60%	0	0%	6	40%	15
21-30 years of age	F	6	100%	0	0%	0	0%	6
	M	1	50%	0	0%	1	50%	2
31-40 years of age	F	10	100%	0	0%	0	0%	10
	M	11	100%	0	0%	0	0%	11
41-50 years of age	F	13	100%	0	0%	0	0%	13
	M	7	100%	0	0%	0	0%	7
51-60 years of age	F	22	96%	0	0%	1	4%	23
	M	18	95%	0	0%	1	5%	19
61-70 years of age	F	10	91%	1	9%	0	0%	11
	M	11	92%	1	8%	0	0%	12
71-80 years of age	F	10	91%	1	9%	0	0%	11
	M	6	100%	0	0%	0	0%	6
81-90 years of age	F	11	100%	0	0%	0	0%	11
	M	5	83%	0	0%	1	17%	6
91-100 years of age	F	0	0%	0	0%	0	0%	0
	M	2	100%	0	0%	0	0%	2
Total		226	75%	3	1%	74	24%	303

Table D. Hospitalized Patient Samples: EBV EA-D IgG

Age	Gender	BioPlex 2200 EA-D IgG						Total
		Positive		Equivocal		Negative		
		N	%	N	%	N	%	N
< 5 years of age	F	2	7%	0	0%	25	93%	27
	M	1	5%	1	5%	18	90%	20
5-12 years of age	F	4	18%	0	0%	18	82%	22
	M	5	15%	0	0%	29	85%	34
13-20 years of age	F	9	26%	2	6%	24	69%	35
	M	3	20%	3	20%	9	60%	15
21-30 years of age	F	2	33%	0	0%	4	67%	6
	M	1	50%	1	50%	0	0%	2
31-40 years of age	F	4	40%	2	20%	4	40%	10
	M	6	55%	0	0%	5	45%	11
41-50 years of age	F	5	38%	0	0%	8	62%	13
	M	2	29%	0	0%	5	71%	7
51-60 years of age	F	8	35%	5	22%	10	43%	23
	M	10	53%	1	5%	8	42%	19
61-70 years of age	F	3	27%	0	0%	8	73%	11
	M	5	42%	1	8%	6	50%	12
71-80 years of age	F	4	36%	2	18%	5	45%	11
	M	1	17%	0	0%	5	83%	6
81-90 years of age	F	7	64%	1	9%	3	27%	11
	M	5	83%	0	0%	1	17%	6
91-100 years of age	F	0	0%	0	0%	0	0%	0
	M	1	50%	0	0%	1	50%	2
Total		88	29%	19	6%	196	65%	303

Table E. Samples from Patients for which an EBV Test was Ordered: EBV NA-1 IgG

Age	Gender	BioPlex 2200 EBV NA-1 IgG						Total
		Positive		Equivocal		Negative		
		N	%	N	%	N	%	N
< 5 years of age	F	6	20%	0	0%	24	80%	30
	M	9	26%	0	0%	25	74%	34
5-12 years of age	F	22	35%	0	0%	40	65%	62
	M	24	39%	0	0%	38	61%	62
13-20 years of age	F	46	59%	1	1%	31	40%	78
	M	19	49%	0	0%	20	51%	39
21-30 years of age	F	42	91%	0	0%	4	9%	46
	M	25	76%	0	0%	8	24%	33
31-40 years of age	F	50	96%	0	0%	2	4%	52
	M	22	92%	0	0%	2	8%	24
41-50 years of age	F	33	100%	0	0%	0	0%	33
	M	30	97%	0	0%	1	3%	31
51-60 years of age	F	26	96%	0	0%	1	4%	27
	M	21	81%	0	0%	5	19%	26
61-70 years of age	F	11	85%	0	0%	2	15%	13
	M	19	90%	0	0%	2	10%	21
71-80 years of age	F	2	100%	0	0%	0	0%	2
	M	3	100%	0	0%	0	0%	3
81-90 years of age	F	2	100%	0	0%	0	0%	2
	M	2	100%	0	0%	0	0%	2
91-100 years of age	F	0	0%	0	0%	0	0%	0
	M	0	0%	0	0%	0	0%	0
Total		414	67%	1	0%	205	33%	620

Table F. Samples from Patients for which an EBV Test was Ordered: EBV VCA IgG

Age	Gender	BioPlex 2200 EBV VCA IgG						Total
		Positive		Equivocal		Negative		
		N	%	N	%	N	%	N
< 5 years of age	F	6	20%	0	0%	24	80%	30
	M	11	32%	0	0%	23	68%	34
5-12 years of age	F	21	34%	0	0%	41	66%	62
	M	28	45%	0	0%	34	55%	62
13-20 years of age	F	47	60%	0	0%	31	40%	78
	M	20	51%	0	0%	19	49%	39
21-30 years of age	F	43	93%	1	2%	2	4%	46
	M	26	79%	0	0%	7	21%	33
31-40 years of age	F	52	100%	0	0%	0	0%	52
	M	22	92%	0	0%	2	8%	24
41-50 years of age	F	32	97%	0	0%	1	3%	33
	M	31	100%	0	0%	0	0%	31
51-60 years of age	F	27	100%	0	0%	0	0%	27
	M	24	92%	0	0%	2	8%	26
61-70 years of age	F	12	92%	0	0%	1	8%	13
	M	18	86%	0	0%	3	14%	21
71-80 years of age	F	2	100%	0	0%	0	0%	2
	M	3	100%	0	0%	0	0%	3
81-90 years of age	F	2	100%	0	0%	0	0%	2
	M	1	50%	0	0%	1	50%	2
91-100 years of age	F	0	0%	0	0%	0	0%	0
	M	0	0%	0	0%	0	0%	0
Total		428	69%	1	0%	191	31%	620

Table G. Samples from Patients for which an EBV Test was Ordered: EBV EA-D IgG

Age	Gender	BioPlex 2200 EA-D IgG						Total
		Positive		Equivocal		Negative		
		N	%	N	%	N	%	N
< 5 years of age	F	3	10%	3	10%	24	80%	30
	M	6	18%	2	6%	26	76%	34
5-12 years of age	F	7	11%	3	5%	52	84%	62
	M	6	10%	4	6%	52	84%	62
13-20 years of age	F	16	21%	8	10%	54	69%	78
	M	12	31%	2	5%	25	64%	39
21-30 years of age	F	16	35%	2	4%	28	61%	46
	M	9	27%	3	9%	21	64%	33
31-40 years of age	F	15	29%	5	10%	32	62%	52
	M	7	29%	1	4%	16	67%	24
41-50 years of age	F	10	30%	2	6%	21	64%	33
	M	4	13%	1	3%	26	84%	31
51-60 years of age	F	13	48%	3	11%	11	41%	27
	M	10	38%	1	4%	15	58%	26
61-70 years of age	F	6	46%	1	8%	6	46%	13
	M	5	24%	3	14%	13	62%	21
71-80 years of age	F	0	0%	1	50%	1	50%	2
	M	1	33%	0	0%	2	67%	3
81-90 years of age	F	0	0%	0	0%	2	100%	2
	M	1	50%	0	0%	1	50%	2
91-100 years of age	F	0	0%	0	0%	0	0%	0
	M	0	0%	0	0%	0	0%	0
Total		147	24%	45	7%	428	69%	620

PERFORMANCE CHARACTERISTICS

Comparative Testing

Comparison of BioPlex 2200 EBV IgG kit and Microplate EIA

Performance of the BioPlex 2200 EBV IgG kit was tested against corresponding commercially available microplate EIAs. A total of 621 banked serum samples from patients for which an EBV test was ordered were tested at 3 U.S. clinical testing sites. The BioPlex 2200 EBV IgG kit was run in conjunction with the BioPlex 2200 EBV IgM kit to allow for a complete antibody response profile. The characterization by antibody response was not compared with clinical data regarding presence, absence or status of disease. Two (2) samples were excluded due to RBB analysis error messages during BioPlex 2200 EBV IgM testing. One (1) sample was excluded due to RBB analysis error messages during BioPlex 2200 EBV IgG testing. Using Table A as a guideline, results were analyzed by BioPlex 2200 EBV IgG analytes and corresponding EBV IgG reference assays according to serological characterization based on reference assay results. For the purpose of percent agreement calculations, BioPlex 2200 EBV IgG equivocal results were assigned to the opposite clinical interpretation than that of the corresponding reference assay result. Likewise, the reference IgG assay equivocal results were assigned to the opposite clinical interpretation than that of the corresponding BioPlex 2200 EBV IgG result. Results from all sites are shown and summarized in Tables H-M.

Table H. BioPlex 2200 EBV NA-1 IgG vs. EIA: Comparison by Serological Pattern Characterization

EBV Serological Status	Reference EBV NA-1 IgG Interpretation									Total
	Positive			Equivocal			Negative			
	BioPlex 2200 EBV NA-1 IgG			BioPlex 2200 EBV NA-1 IgG			BioPlex 2200 EBV NA-1 IgG			
	Pos	Eqv	Neg	Pos	Eqv	Neg	Pos	Eqv	Neg	
	N	N	N	N	N	N	N	N	N	N
Primary Acute	0	0	0	0	0	0	0	0	31	31
Late Acute	104	0	4	0	0	0	0	0	2	110
Recovering	0	0	0	0	0	0	0	0	4	4
Previous Infection	285	1	4	0	0	0	4	0	11	305
Susceptible	0	0	0	0	0	1	1	0	125	127
Inconclusive	20	0	9	0	0	0	0	0	12	41
Overall	409	1	17	0	0	1	5	0	185	618

Table I. BioPlex 2200 EBV NA-1 IgG vs. EIA: Percent Agreement & Confidence Intervals by Serological Pattern Characterization

EBV Serological Status	Positive Agreement		95% CI	Negative Agreement		95% CI
Primary Acute	(0/0)	N/A*	N/A*	(31/31)	100%	89.0 -100%
Late Acute	(104/108)	96.3%	90.9 - 98.6%	(2/2)	100%	34.2 - 100%
Recovering	(0/0)	N/A*	N/A*	(4/4)	100%	51.0 -100%
Previous Infection	(285/290)	98.3%	96.0 - 99.3%	(11/15)	73.3%	48.0 - 98.1%
Susceptible	(0/1)	0.0%	N/A*	(125/126)	99.2%	95.6 - 99.9%
Inconclusive	(20/29)	69.0%	50.8 - 82.7%	(12/12)	100%	75.8 - 100%
Overall	(409/428)	95.6%	93.2 - 97.1%	(185/190)	97.4%	94.0 - 98.9%

**In cases where agreement resulted in a numerator of zero (0), 95% confidence interval could not be calculated; in cases where agreement resulted in (0/0) samples, percent agreement and 95% confidence interval could not be calculated.*

Table J. BioPlex 2200 EBV VCA IgG vs. EIA: Comparison by Serological Pattern Characterization

EBV Serological Status	Reference EBV VCA IgG Interpretation									Total
	Positive			Equivocal			Negative			
	BioPlex 2200 EBV VCA IgG			BioPlex 2200 EBV VCA IgG			BioPlex 2200 EBV VCA IgG			
	Pos	Eqv	Neg	Pos	Eqv	Neg	Pos	Eqv	Neg	
	N	N	N	N	N	N	N	N	N	N
Primary Acute	4	0	3	0	0	1	0	0	23	31
Late Acute	106	0	4	0	0	0	0	0	0	110
Recovering	4	0	0	0	0	0	0	0	0	4
Previous Infection	296	1	8	0	0	0	0	0	0	305
Susceptible	0	0	0	0	0	0	0	0	127	127
Inconclusive	16	0	0	0	0	0	1	0	24	41
Overall	426	1	15	0	0	1	1	0	174	618

Table K. BioPlex 2200 EBV VCA IgG vs. EIA: Percent Agreement & Confidence Intervals by Serological Pattern Characterization

EBV Serological Status	Positive Agreement		95% CI	Negative Agreement		95% CI
Primary Acute	(4/8)	50.0%	21.5 - 78.5%	(23/23)	100%	85.7 - 100%
Late Acute	(106/110)	96.4%	91.0 - 98.6%	(0/0)	N/A*	N/A*
Recovering	(4/4)	100%	51.0 - 100%	(0/0)	N/A*	N/A*
Previous Infection	(296/305)	97.0%	94.5 - 98.4%	(0/0)	N/A*	N/A*
Susceptible	(0/0)	N/A*	N/A*	(127/127)	100%	97.1 - 100%
Inconclusive	(16/16)	100%	80.6 - 100%	(24/25)	96.0%	80.5 - 99.3%
Overall	(426/443)	96.2%	93.9 - 97.6%	(174/175)	99.4%	96.8 - 99.9%

**In cases where agreement resulted in (0/0) samples, percent agreement and 95% confidence interval could not be calculated.*

Table L. BioPlex 2200 EBV EA-D IgG vs. EIA: Comparison by Serological Pattern Characterization

EBV Serological Status	Reference EBV EA-D IgG Interpretation									Total
	Positive			Equivocal			Negative			
	BioPlex 2200 EBV EA-D IgG			BioPlex 2200 EBV EA-D IgG			BioPlex 2200 EBV EA-D IgG			
	Pos	Eqv	Neg	Pos	Eqv	Neg	Pos	Eqv	Neg	
	N	N	N	N	N	N	N	N	N	N
Primary Acute	18	1	2	0	0	0	2	1	7	31
Late Acute	72	1	3	4	0	0	6	3	21	110
Recovering	4	0	0	0	0	0	0	0	0	4
Previous Infection	0	0	0	5	3	3	23	22	249	305
Susceptible	0	0	0	0	1	1	0	9	116	127
Inconclusive	10	2	1	0	0	0	2	2	24	41
Overall	104	4	6	9	4	4	33	37	417	618

Table M. BioPlex 2200 EBV EA-D IgG vs. EIA: Percent Agreement & Confidence Intervals by Serological Pattern Characterization

EBV Serological Status	Positive Agreement		95% CI	Negative Agreement		95% CI
Primary Acute	(18/21)	85.7%	65.4 - 95.0%	(7/10)	70.0%	39.7 - 89.2%
Late Acute	(72/76)	94.7%	87.2 - 97.9%	(21/34)	61.8%	45.0 - 76.1%
Recovering	(4/4)	100%	51.0 - 100%	(0/0)	N/A*	N/A*
Previous Infection	(0/3)	0.0%	N/A*	(249/299)	83.3%	78.6 - 87.1%
Susceptible	(0/1)	0.0%	N/A*	(116/125)	92.8%	86.9 - 96.2%
Inconclusive	(10/13)	76.9%	49.7 - 91.8%	(24/28)	85.7%	68.5 - 94.3%
Overall	(104/118)	88.1%	81.1 - 92.8%	(417/496)	84.1%	80.6 - 87.0%

**In cases where agreement resulted in a numerator of zero (0), 95% confidence interval could not be calculated; in cases where agreement resulted in (0/0) samples, percent agreement and 95% confidence interval could not be calculated.*

Comparison of Characterization EBV Serological Status

Using Table A as a guideline, samples characterized into serological status associated with EBV disease, using the commercially available microplate EIA and agglutination tests, were compared with characterizations using BioPlex 2200 EBV IgG and IgM kits. The EBV IgG kit was run in conjunction with the EBV IgM kit to allow for a complete antibody response profile. The characterization by antibody response was not compared with clinical data regarding presence, absence or status of disease. Results from 618 serum samples tested at 3 U.S. clinical testing sites are shown in Table N.

Table N. Comparison of EBV Serological Status

EBV Serological status		BioPlex 2200 EBV IgG & IgM Profile								
		Primary Acute	Late Acute	Recovering	Previous Infection	Susceptible	Inconclusive	Total	% Serological Agreement	95% Confidence Interval
Commercially Available Assays	Primary Acute	30	0	0	0	0	1	31	96.8%	83.8 - 99.4%
	Late Acute	5	90	1	13	0	1	110	81.8%	73.6 - 87.9%
	Recovering	1	0	3	0	0	0	4	75.0%	30.0 - 95.4%
	Previous Infection	0	31	2	263	4	5	305	86.2%	81.9 - 89.7%
	Susceptible	4	0	0	0	122	1	127	96.1%	91.1 - 98.3%
	Inconclusive	6	10	0	7	11	7	41	17.1%	8.5 - 31.3%
	Overall	46	131	6	283	137	15	618	83.3%	80.2 - 86.1%

Note: Calculations are performed for unshaded areas only.

Comparison of Acute and Non-Acute EBV Serological Status

The results obtained from the summarized information provided in Table N were further classified into two groups; Acute Infection and Non-Acute Infection. Acute Infection includes Primary Acute and Late Acute. Non-Acute Infection includes samples characterized as Susceptible, Recovering and Previous Infection as defined in Table A. Inconclusive includes any samples whose patterns of antibody reactivity are not consistent with any category listed in Table A. Results are summarized in Table O.

Table O. Acute vs. Non-Acute

EBV Serological status		BioPlex 2200 EBV IgG & IgM Profile					
		Acute	Non-Acute	Inconclusive	Total	% Serological Agreement	95% Confidence Interval
Commercially Available Assays	Acute	125	14	2	141	88.7%	82.4 - 92.9%
	Non-Acute	36	394	6	436	90.4%	87.2 - 92.8%
	Inconclusive	16	18	7	41	17.1%	8.5 - 31.3%
	Overall	177	426	15	618	85.1%	82.1 – 87.7%

Note: Calculations are performed for unshaded areas only.

Reproducibility Studies

A reproducibility panel, consisting of nine (9) panel members was prepared by Bio-Rad Laboratories. Two (2) of the nine (9) panel members had high levels of EBV NA-1 and EBV VCA; two (2) of the nine (9) panel members had high levels of EBV EA-D; two (2) of the nine (9) panel members had antibody levels near the cutoff for EBV NA-1 and EBV VCA; two (2) of the nine (9) panel members had antibody levels near the cutoff for EBV EA-D. All were prepared from positive patient samples. One (1) of the nine (9) panel members was negative for all three (3) analytes contained in the BioPlex 2200 EBV IgG kit. In addition, three (3) lots of the EBV IgG Control Set [1 positive control (antibody positive) and a negative control (antibody negative)] were also tested.

Reproducibility testing was performed at each of three (3) US testing facilities on a total of three (3) lots of the EBV IgG kit, three (3) lots of the EBV IgG Calibrator Set and three (3) lots of the EBV IgG Control Set. Each testing facility evaluated reproducibility using one (1) kit lot of EBV IgG with matched calibrators and controls. The panels were provided to each of the testing sites. Each of the nine (9) panel members and positive and negative controls was tested in quadruple (x4) on each day for three (3) days at each of three (3) US testing facilities using one (1) lot of EBV IgG reagent pack, one (1) lot of EBV IgG Calibrator Set and one (1) lot of EBV IgG Control Set (4 times x 3 days x 3 sites = 36 replicates per panel member and controls). The data were analyzed for intra-assay and inter-assay reproducibility according to the principles described in the Clinical Laboratory Standards Institute guidance EP5-A2, revised November 2004 and ISO/TR 22971:2205. The standard deviation (SD) and percent coefficient of variation (%CV) were calculated. Positive results can be found in Tables P - R.

Table P. Reproducibility; BioPlex 2200 EBV NA-1 IgG

EBV NA-1 IgG Panel Members	Sample N	Grand Mean AI	Within-Run		Between-Day		Between-Run		Between-Site*		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	36	4.2	0.1	3.2%	0.0	0.0%	0.1	2.9%	0.3	7.4%	0.4	8.5%
High Positive 2	36	4.3	0.1	3.4%	0.1	2.5%	0.1	2.8%	0.3	7.5%	0.4	9.0%
Low Positive 1	36	1.5	0.1	4.8%	0.0	0.0%	0.1	5.7%	0.2	11.6%	0.2	13.8%
Low Positive 2	36	2.1	0.1	3.2%	0.0	1.1%	0.1	3.7%	0.2	9.4%	0.2	10.7%
Positive Control	36	2.9	0.1	1.9%	0.0	1.1%	0.1	2.4%	0.5	17.2%	0.5	17.5%

**Between site variance includes between lot variance.*

Table Q. Reproducibility; BioPlex 2200 EBV VCA IgG

EBV VCA IgG Panel Members	Sample N	Grand Mean AI	Within-Run		Between-Day		Between-Run		Between-Site*		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	36	3.3	0.1	3.5%	0.0	0.0%	0.1	3.3%	0.4	12.7%	0.4	13.5%
High Positive 2	36	3.2	0.1	3.4%	0.0	0.0%	0.1	3.7%	0.2	7.3%	0.3	8.9%
Low Positive 1	36	1.5	0.1	5.0%	0.0	0.0%	0.1	5.5%	0.2	16.2%	0.3	17.8%
Low Positive 2	36	1.3	0.1	5.8%	0.0	0.0%	0.1	5.0%	0.1	7.6%	0.1	10.8%
Positive Control	36	2.3	0.1	2.8%	0.0	0.0%	0.1	2.7%	0.1	5.6%	0.2	6.9%

**Between site variance includes between lot variance.*

Table R. Reproducibility; BioPlex 2200 EBV EA-D IgG

EBV EA-D IgG Panel Members	Sample N	Grand Mean AI	Within-Run		Between-Day		Between-Run		Between-Site*		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	36	4.1	0.2	4.4%	0.0	0.8%	0.0	0.0%	0.4	8.7%	0.4	9.8%
High Positive 2	36	4.0	0.1	3.7%	0.0	0.7%	0.1	3.5%	0.2	6.2%	0.3	8.1%
Low Positive 1	36	2.3	0.2	8.8%	0.1	2.8%	0.0	0.0%	0.1	5.7%	0.2	10.9%
Low Positive 2	36	2.2	0.1	4.6%	0.0	0.0%	0.1	3.5%	0.1	4.2%	0.2	7.2%
Positive Control	36	3.0	0.1	3.1%	0.0	0.0%	0.1	2.5%	0.6	18.8%	0.6	19.2%

**Between site variance includes between lot variance.*

Precision Studies

A precision panel, consisting of six (6) panel members was prepared by Bio-Rad Laboratories. Two (2) of the six (6) panel members had high levels of the antibodies contained in the BioPlex 2200 EBV IgG kit (EBV NA-1 IgG, EBV VCA IgG, and EBV EA-D IgG) and two (2) of the six (6) panel members had antibody levels near the cutoff, both prepared from positive patient samples. Two (2) of the six (6) panel members were negative (one high negative and one low negative) for both of the analytes.

Precision testing was performed at Bio-Rad Laboratories on one lot of the EBV IgG kit, one lot of the EBV IgG Calibrator Set and one lot of the EBV IgG Control Set. Each of the six (6) panel members was tested in duplicate (x2) on two (2) runs per day for ten (10) days using one (1) lot of EBV IgG kit, one (1) lot of EBV IgG Calibrator Set and one (1) lot of EBV IgG Control Set (2 times x 2 runs x 10 days = 40 replicates per panel member). The data were analyzed for intra-assay and inter-assay precision according to the principles described in the Clinical Laboratory Standards Institute guidance EP5-A2, revised November 2004 and ISO/TR 22971:2205. The standard deviation (SD) and percent coefficient of variation (%CV) were calculated. Results can be found in Tables S - U.

Table S. Precision Results; BioPlex 2200 EBV NA-1 IgG

EBV NA-1 IgG Panel Members	Sample N*	AI Mean	Within-Run		Between-Day		Between-Run		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	42	4.6	0.2	4.2%	0.3	6.3%	0.3	5.9%	0.4	9.6%
High Positive 2	42	4.6	0.2	4.0%	0.2	5.4%	0.4	8.6%	0.5	10.9%
Low Positive 1	42	1.9	0.1	5.5%	0.0	0.0%	0.2	11.9%	0.2	13.1%
Low Positive 2	42	2.2	0.1	5.0%	0.0	0.0%	0.2	10.4%	0.3	11.5%
High Negative	43	0.7	0.1	7.1%	0.0	3.8%	0.1	12.1%	0.1	14.5%
Low Negative	44	0.0	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.0	0.0%

**Additional samples were run.*

Table T. Precision Results; BioPlex 2200 EBV VCA IgG

EBV VCA IgG Panel Members	Sample N*	AI Mean	Within-Run		Between-Day		Between-Run		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	42	3.5	0.1	3.9%	0.1	3.9%	0.2	6.8%	0.3	8.7%
High Positive 2	42	3.4	0.2	5.0%	0.1	3.7%	0.3	9.1%	0.4	11.0%
Low Positive 1	42	1.6	0.1	8.4%	0.0	2.6%	0.1	8.1%	0.2	11.9%
Low Positive 2	42	1.3	0.1	7.4%	0.1	4.1%	0.1	10.2%	0.2	13.3%
High Negative	43	0.6	0.1	10.8%	0.0	0.0%	0.1	11.4%	0.1	15.7%
Low Negative	44	0.2	0.0	16.3%	0.0	6.8%	0.0	5.1%	0.0	18.4%

**Additional samples were run.*

Table U. Precision Results; BioPlex 2200 EBV EA-D IgG

EBV EA-D IgG Panel Members	Sample N*	AI Mean	Within-Run		Between-Day		Between-Run		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	42	4.3	0.2	4.6%	0.0	0.0%	0.3	6.9%	0.4	8.3%
High Positive 2	42	4.2	0.3	6.0%	0.3	6.3%	0.3	7.2%	0.5	11.2%
Low Positive 1	42	2.3	0.2	10.3%	0.1	4.5%	0.2	9.8%	0.3	14.9%
Low Positive 2	42	2.3	0.2	7.0%	0.2	6.8%	0.2	9.1%	0.3	13.4%
High Negative	43	0.7	0.1	13.4%	0.0	0.0%	0.1	12.9%	0.1	18.6%
Low Negative	44	0.2	0.1	29.8%	0.0	5.3%	0.0	0.0%	0.1	30.3%

**Additional samples were run.*

Cross-Reactivity

A cross-reactivity study was performed to determine if samples from various disease states and other potentially interfering factors interfere with test results when tested with the BioPlex 2200 EBV IgG kit. A panel of ten (10) specimens* positive for each cross reactant were evaluated for possible cross reactivity with the BioPlex 2200 EBV IgG kit for each of the three EBV IgG antibody assays. Due to the high prevalence of EBV IgG antibodies in the normal population, the test specimens were also evaluated on corresponding commercially available microplate EIAs. Most of the samples evaluated were high positive for each disease state. The majority of all samples that elicited a positive result were also confirmed positive by the corresponding commercially available microplate EIA, indicating reactivity to EBV IgG antibodies rather than cross reactivity with a potentially interfering factor. Results can be found in Table V.

**Due to limited availability of samples, only four E. coli specimens were evaluated.*

Table V. Cross-Reactivity

Cross Reactives	N	Method	BioPlex 2200 EBV IgG		
			EBV NA-1 IgG	EBV VCA IgG	EBV EA-D IgG
ANA	10	BioPlex 2200	9	10	7
		EIA	9	10	7
		Discrepants	0	0	0
Rheumatoid Factor	10	BioPlex 2200	10	10	1
		EIA	10	10	1*
		Discrepants	0	0	0
Toxo IgG	10	BioPlex 2200	9	9	2
		EIA	9	9*	2*
		Discrepants	0	0	0
Rubella IgG	10	BioPlex 2200	10	10	2
		EIA	10	10	1*
		Discrepants	0	0	1
CMV IgG	10	BioPlex 2200	10	10	2
		EIA	10	10	2**
		Discrepants	0	0	1
VZV IgG	10	BioPlex 2200	8	8	1
		EIA	9	8	1
		Discrepants	1	0	0
HSV-1 IgG	10	BioPlex 2200	10	10	2
		EIA	10	10	3
		Discrepants	0	0	1
HSV-2 IgG	10	BioPlex 2200	10	10	3
		EIA	10	10	4
		Discrepants	0	0	1
HIV	10	BioPlex 2200	10	9	1
		EIA	10	10	2**
		Discrepants	0	1	1
E. coli	4	BioPlex 2200	4	4	0
		EIA	4	4	0*
		Discrepants	0	0	0
Pregnant women	10	BioPlex 2200	9	9	3
		EIA	9	10	3*
		Discrepants	0	1	0

**One Equivocal Sample; **Two Equivocal Samples*

Interfering Substances

Testing for interfering substances was conducted according to NCCLS Protocol EP7-A (Vol. 22, No. 27). No significant interference was observed in any of the substances tested. The following substances, listed in Table W, were tested (N=10) at maximum levels on one reagent lot.

Table W. Interfering Substances

Substance	Concentration
Hemoglobin	≤ 500 mg/dL
Bilirubin (unconjugated)	≤ 20 mg/dL
Bilirubin (conjugated)	≤ 20 mg/dL
Triglycerides	≤ 3000 mg/dL
Protein (total)	≤ 12 g/dL
Cholesterol	≤ 500 mg/dL
Red Blood Cells	≤ 0.4% Concentrate
Gamma-globulin	≤ 2.5 g/dL
Ascorbic Acid	≤ 3 mg/dL

REFERENCES

1. S Tsuchiya. Diagnosis of Epstein-Barr virus-associated diseases. *Crit Rev Oncol/Hematol* 2002; 44: 227-38.
2. M Papesch and R Watkins. Epstein-Barr virus infectious mononucleosis. *Clin Otolaryngol* 2001; 26: 3-8.
3. G Henle, W Henle and CA Horwitz. Antibodies to Epstein-Barr Virus-Associated Nuclear Antigen in Infectious Mononucleosis. *J Infec Dis* 1974; 130(3): 231-9.
4. TD Rea, RL Ashley, JE Russo and DS Buchwald. A Systematic Study of Epstein-Barr Virus Serologic Assays Following Acute Infection. *Am J Clin Pathol* 2002; 117: 156-61.
5. AS Evans, JC Niederman, LC Cenabre, B West and VA Richards. A prospective Evaluation of Heterophile and Epstein-Barr Virus-Specific IgM Antibody Tests in Clinical and Subclinical Infectious Mononucleosis: Specificity and Sensitivity of the Tests and Persistence of Antibody. *J Infec Dis* 1975; 132(5): 546-54.
6. CV Sumaya and Y Ench. Epstein-Barr Virus Infectious Mononucleosis in Children. II. Heterophile Antibody and Viral-Specific Responses. *Pediatrics* 1985; 75(6): 1011-9.
7. F Elgh and M Linderholm. Evaluation of six commercially available kits using purified heterophile antigen for the rapid diagnosis of infectious mononucleosis compared with Epstein-Barr virus-specific serology. *Clin Diagn Virol* 1996; 7: 17-21.
8. BC Gartner, JM Fischinger, K Roemer, M Mak, B Fleurent and N Mueller-Lantzsch. Evaluation of a recombinant line blot for diagnosis of Epstein-Barr Virus compared with ELISA, using immunofluorescence as reference method. *Journal of Virological Methods* 2001; 93: 89-96.
9. A Zlatkins, KY Lee, CF Poole and G Holzer. Capillary column gas chromatographic profile analysis of volatile compounds in sera of normal and virus-infected patients. *J Chromatogr* 1979; 163(2): 125-33.
10. DGA Baetens and LML Van Renterghem. Coupled Particle Light Scattering: A New Technique for Serodiagnosis of Epstein-Barr Virus Infection. *J Med Virol* 2001; 64: 519-25.
11. WMJ Van Grunsven, EC Van Heerde, HJW De Haard, WJM Spaan and JM Middeldorp. Gene Mapping and Expression of Two Immunodominant Epstein-Barr Virus Capsid Proteins. *J Virol* 1993; 7: 3908-16.
12. G Milman, AL Scott, MS Cho, SC Hartman, DK Ades, GS Hayward, PF Ki, JT August and SD Hayward. Carboxyl-Terminal Domain of the Epstein-Barr Virus Nuclear Antigen is Highly Immunogenic in Man. *Proc Natl Acad Sci* 1985; 82(18): 6300-4.
13. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.
14. US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: US Government Printing Office; January 2007.
15. World Health Organization. Laboratory Biosafety Manual. 3rd ed. Geneva: World Health Organization; 2004.
16. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.
17. 1976. Current Intelligence Bulletin 13: Explosive Azide Hazard. In: *NIOSH Current Intelligence Reprints - Bulletins 1-18 (1975-1977)*. DHEW(NIOSH) Publication 78-127, NTIS PB83-105-080.
18. Z Debyser, M Reynders, P Goubau and J Desmyter. Comparative evaluation of the three ELISA techniques and an indirect immunofluorescence assay for the serological diagnosis of Epstein-Barr virus infection. *Clin Diagn Virol* 1997; 8: 71-81.
19. YW Tang, JA Helgason, D Arlo and TF Smith. Detection of Epstein-Barr virus-specific antibodies by an automated enzyme immunoassay: performance evaluation and cost analysis. *Diagnostic Microbiology and Infectious Disease* 1998; 31: 594-554.
20. G Bauer. The rational basis for efficient Epstein-Barr Virus (EBV) serology. *Clinical Laboratory* 1995; 41: 623-34.
21. P Robertson, S Beynon, R Whybin, C Brennan, U Vollmer-Conna, I Hickie and A Lloyd. Measurement of EBV-IgG anti-VCA avidity aids in the early and reliable diagnosis of primary EBV infection. *Journal of Medical Virology* 2003; 70: 617-23.

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