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SUBJECT **NEW PRODUCT**



We announce the launching of a new member of the bioelisa family:

bioelisa EBV-EBNA IgG 96T
Code 3000-1248

This kit is intended for the serological diagnosis of Epstein-Barr virus, the ethiological agent of Infectious Mononucleosis.

Among the different EBV specific markers, EBNA or Epstein Barr Nuclear Antigens is the Marker of the convalescence and past infections.

This assay complete the other two bioelisa products for EBV diagnosis:

bioelisa EBV-VCA IgG 96T Code 3000-1243

bioelisa EBV-VCA IgM 96T Code 3000-1244

Enclosed you will find product information that we hope will be helpful for introducing the assay.

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Epstein-Barr Virus

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General information on Epstein-Barr virus:



Epstein-Barr virus (EBV) was first discovered in 1964 as the cause of infectious mononucleosis (IM). This disorder is usually an acute, benign and self-limiting lymphoproliferative condition, which tends to be sub-clinical in children and a mild condition in adults. EBV is also the cause of nasopharyngeal carcinoma and neoplasms of the thymus, parotid gland and larynx. EBV also causes Burkitt's lymphoma, a malignant tumour of the lymphoid tissue that occurs primarily in African children.

Epstein-Barr virus is a gamma-herpesvirus (see Figure 1). EBV has a very limited host range and tissue tropism: human B-lymphocytes and epithelial cells of the oropharynx and nasopharynx.

EBV is widely disseminated. It is estimated that 95% of the world population is exposed to the virus, making it the most widespread virus known to man.

EBV appears to be transmitted primarily by close contact with infectious oral-pharyngeal secretions. However, the virus has reportedly been transmitted by blood transfusion and transplacental routes. Under ordinary conditions, transmission of the virus by transfusion or transplacental exposure is unlikely. In addition, EBV-associated post-transplantation lymphoproliferative disease develops in 1% to 10% of organ transplant recipients.

The frequency of seronegative patients is nearly 100% in early infancy but lowers with age, more or less rapidly, depending on socio-economic conditions, to less than 10% in young adults. Following primary exposure, a person is considered to be immune and generally no longer susceptible to reinfections.

In Western society primary exposure to EBV occurs in two waves. Approximately half of the population is exposed to the virus before the age of 5. A second wave of seroconversion occurs during late adolescence, between 15 and 24 years of age. More than 90% of EBV-infected individuals intermittently shed the virus for life even when totally asymptomatic. Children can acquire the virus at an early stage by sharing contaminated drinking glasses, and generally develop sub-clinical disease. Saliva sharing between adolescents and young adults often occurs by kissing, hence its popular name of "kissing disease". In these individuals the disease may go unnoticed or be present in varying degrees of severity as infectious mononucleosis. Approximately 70% of the population of the United States have been infected by age 30.

Individuals at risk include those who lack antibodies to the virus. EBV is only a minor problem for immunocompetent persons, but can become a major one for immunologically compromised patients. Blood transfusion from an immune donor to a non-immune recipient may produce a primary infection in the recipient known as IM postperfusion syndrome. IM or IM-like illness following blood transfusion may often be the result of a concomitant Cytomegalovirus (another herpes virus) infection rather than EBV.

A low percentage of patients experience symptomatic reactivation. Reactivation of latent infection has been implicated in a persistent illness referred to as the EBV-associated fatigue syndrome.

Clinically apparent IM has an estimated frequency of 45/100,000 in adolescents. In immunosuppressed patients the incidence of EBV infection varies from 35 to 47%.

As occurs with other herpes viruses, there is a carrier state after primary infection.

EBV can also be associated with neoplasms. Transplant patients, AIDS patients and genetically immunodeficient individuals are at high risk for lymphoproliferative disorders initiated by EBV. These may appear as polyclonal and monoclonal B-cell lymphomas.

Epstein-Barr antigens

There are three major antigens of the EBV structure capable of producing a specific immunologic response in the patient:

Viral Capsid Antigen:

Viral Capsid Antigen is produced by infected B cells and can be found in the cytoplasm. Anti-VCA IgM is usually detectable early in the course of infection, but it is low in concentration and disappears within 2 to 4 months.

Anti-VCA IgG is usually detectable within 7-15 days following the onset of signs and symptoms. It peaks at 2 – 4 weeks after onset, declines slightly and persists for life.

If antibodies to viral capsid antigen go undetected, the patient is then susceptible to EBV infection.

Early Antigen:

Early antigen is a complex of two components, early antigen diffuse (EA-D), which is found in both the nucleus and the cytoplasm of the B infected cells, and early antigen restricted (EA-R), which is usually found as a mass only in the cytoplasm.

Anti-EA-R IgG is not usually found in young adults during the acute phase, but it is sometimes shown in the serum of very young children during the acute phase. Anti-EA-R IgG appears transiently in the late convalescence phase. In general, anti-EA-D and anti-EA-R IgG are not consistent indicators of the disease stage.

Epstein-Barr Nuclear Antigen

Epstein-Barr Nuclear Antigen (EBNA) is found in the nucleus of all EBV-infected cells. Although the synthesis of nuclear antigens precedes early antigen synthesis during the infection of B cells, the EBNA does not become available for antibody stimulation until after the incubation period of infectious mononucleosis, when activated T lymphocytes destroy the EBV genome-carrying B cells. As a result, antibodies to nuclear antigen are absent or barely detectable during acute IM.

Anti-EBNA IgG does not appear until a patient has entered the convalescent stage. EBNA antibodies are almost always present in sera containing IgG antibodies to VCA of EBV unless the patient is in the early acute phase of IM. Patients with severe immunologic defects or immunosuppressive disease may not have EBNA antibodies, even if antibodies to VCA are present.

Diagnosis of Epstein-Barr virus infection

Diagnosis of EBV can be divided into non-specific and specific methods.

Heterophile antibodies

The immune system of infected patients produces heterophile antibodies, mostly IgM. These antibodies do not react directly to EBV antigens but appear even slightly earlier than the specific IgM antibodies to EBV. The heterophile antibody that appears in the EBV infection also has the capacity to react to a glycoprotein present in the surface of sheep erythrocytes erithrocitary antigen. This antigen was discovered by Paul Bunnell in 1932.

Heterophile antibody assays are very useful as screening tests since they detect very early primary infection. **biokit** has a well-known rapid test based on the Paul Bunnell antigen, **Monolates** (Code number 3000-1001) and a new rapid test based on slide haemagglutination, **Color-mono** (Code number 3000-1005).

Elisa tests

Enzyme immunoassay tests based on purified proteins recombinant antigens or synthetic peptides. There are specific tests for all EBV antigens for IgG or IgM detection. The Elisa test provides the best combination of sensitivity, specificity and user-friendliness.

Immunofluorescence assays

These are regarded as a reference method for EBV diagnosis. The assays are also based on recombinant antigens or synthetic peptides. They use microscope slides as a support for the solid phase and conjugate anti-IgG or IgM labelled with fluorescent marker.

These methods require very skilled professionals for reading and interpreting results.

Immunoblot

Enzyme immunoassay tests based on recombinant antigens which use a nitro-cellulose strip as a support for the solid phase. The strip incorporates all the antigens separately. Thus it enables the assay to evaluate the presence of specific antibodies samples to these antigens in the patient.

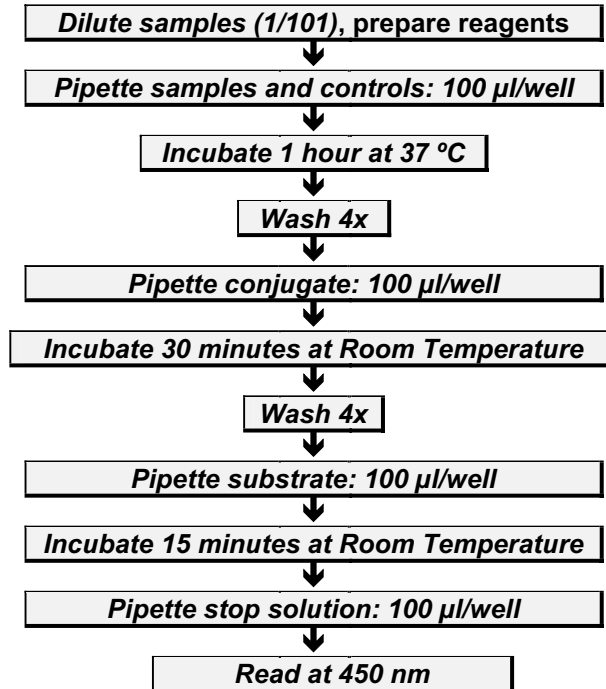
bioelisa EBV VCA IgG

bioelisa EBV EBNA IgG is an indirect enzyme immunoassay for the detection of IgG class antibodies to EBV Nuclear Antigen in human serum or plasma. Specific EBNA IgG antibodies present in serum will bind to microtiter wells coated with purified proteins corresponding to EBNA antigens.

Following a wash step, peroxidase-labelled rabbit anti-human IgG is added, which binds to the human EBV EBNA IgG present. The whole complex is then detected by the addition of substrate (TMB), which turns blue in the presence of peroxidase. A stable yellow end product is obtained by the addition of a stopping reagent.

Solid phase	Microtiter wells coated with highly purified proteins corresponding to Epstein-Barr Virus nuclear antigen. Breakable wells.
Conjugate	Rabbit polyclonal antibodies to human IgG labelled with peroxidase. Red dye. Ready to use.
Controls	Negative, Low Positive and Positive. Yellow dye. Ready to use
Substrate	TMB with substrate buffer. Ready to use
Sample dilution	1/100. Manually in a tube: 10 µl sample in 1,000 µl dilution buffer. Yellow dye.
Wash solution	PBS buffer with detergent concentrated 20x.
Stop solution	Sulphuric acid 1N.
Cut-off value	Mean Low Positive Control

bioelisa EBV EBNA IgG Procedural Flow Chart



Features and Benefits

Features		Benefits
Breakable wells		- Allow the use of the exact number of wells needed
Highly purified EBNA antigens		- Allow maximum sensitivity without affecting specificity.
<ul style="list-style-type: none"> - Controls - Sample diluent - Conjugate - Substrate 	ready to use	- User-friendly kits
Control Cut-Off		<ul style="list-style-type: none"> - Adapts cut-off value to the day to day run conditions - Improves sensitivity - Improves specificity
Colour-coded reagents		- User-friendly kits
High sensitivity		- > 95%
High specificity		- > 89%

Performance

Internal evaluations

Comparison with the biotest EBNA EIA assay

EPSTEIN-BARR VIRUS (EBNA) Novatec

IgG - ELISA

Lot: BK1

Exp: 2005-05

PANEL EBV (VCA) IgG

	EBNA IgG biokit			EBNA IgG BIOTEST		VCA IgG Biokit	
	OD	OD/COV	RES.	OD/CO	RES.	OD/CO	RES.
Blank	(7)						
HPC	652	2.26	+				
LPC (COV)	289	1.00	+/-				
NC	42	0.15	-				
pccG 1	2635	9.12	+	17.28	+	7.20	+
pccG 2	1373	4.75	+	9.91	+	10.60	+
pccG 3	1357	4.70	+	15.68	+	11.50	+
pccG 5	1050	3.63	+	14.35	+	6.20	+
pccG 6	1328	4.60	+	16.32	+	9.60	+
pccG 7	2339	8.09	+	17.63	+	6.70	+
pccG 8	778	2.69	+	9.12	+	5.90	+
pccG 9	1369	4.74	+	10.19	+	7.30	+
pccG 10	1993	6.90	+	13.76	+	10.80	+
pccG 11	1043	3.61	+	11.65	+	10.90	+
pccG 12	968	3.35	+	2.26	+	8.30	+
pccG 13	2269	7.85	+	17.75	+	5.40	+
pccG 14	2122	7.34	+	15.81	+	5.80	+
pccG 15	1589	5.50	+	14.08	+	7.40	+
pccG 16	1063	3.68	+	13.06	+	2.30	+
pccG 17	553	1.91	+	0.95	+/-	8.10	+
pccG 18	561	1.94	+	0.06	-	3.50	+
pccG 19	2047	7.08	+	15.03	+	11.20	+
pccG 20	1627	5.63	+	14.09	+	11.50	+
pccG 21	968	3.35	+	3.34	+	8.20	+
pccG 22	1701	5.89	+	16.13	+	8.40	+
pccG 23	943	3.26	+	12.21	+	10.80	+
pccG 24	1899	6.57	+	16.61	+	10.20	+
pccG 25	1481	5.12	+	17.72	+	10.30	+
pccG 26	1032	3.57	+	7.88	+	8.80	+
pccG 27	1157	4.00	+	1.71	+	4.20	+
pccG 28	2216	7.67	+	15.64	+	8.50	+
pccG 29	2426	8.39	+	15.43	+	8.90	+
pccG 30	799	2.76	+	3.94	+	11.30	+
pccG 31	1198	4.15	+	1.29	+	5.50	+
pccG 33	2158	7.47	+	14.72	+	10.60	+
pccG 34	1545	5.35	+	10.88	+	7.50	+
pccG 43	957	3.31	+	ND		0.80	-
pccG 44	1393	4.82	+	ND		0.80	-

Results

Correlation with Biotest (33/34): 97.05%.

Only one sample positive by Biokit, Negative by Biotest

Diagnostic performance evaluation Epstein-Barr Virus (EBNA) IgG

Introduction

The Evaluation of performance was performed in two comparisons to EBV (EBNA) ELISA of the company DiaSorin. The intention was to determine the efficiency of the assay to differentiate between positive and negative clinical samples.

Materials

bioelisa EBV EBNA IgG

LOT: 003 and 007

DiaSorin EBV (EBNA) IgG

LOT: 3160160B and 3160200D

Results

Total number of samples: 45

biokit	DiaSorin
36 positive sera	34 positive sera
7 negative sera	11 negative sera
2 GZ sera	

The results are shown below:

		Diasorin	
		Pos	Neg
biokit	Pos	34	2
	Neg	0	9

Specificity: (7/9): 82 %

Sensitivity: (34/34): 100 %

Agreement: 95.6 %

Second assay

Total number of samples: 40

biokit	DiaSorin
31 positive sera	32 positive sera
9 negative sera	8 negative sera

		Diasorin	
		Pos	Neg
biokit	Pos	31	0
	Neg	1	8

Specificity: (8/8): 100 %

Sensitivity: (31/32): 96.9 %

Agreement: 97.5 %

For both tests together the following results arise:

Specificity: 91 %

Sensitivity: 98.3 %

Agreement: 96.6 %

bioelisa EBV-EBNA IgG

ELISA test for the detection of IgG antibodies to Nuclear Antigen of Epstein Barr virus in human serum

Summary

The Epstein-Barr (EBV) virus was named after its two discoverers. It is the etiological agent of Infectious Mononucleosis (IM) and has been associated to Burkitt's lymphoma and nasopharyngeal carcinoma. EBV belongs to the herpes virus family. Approximately 90% of adults universally are infected by EBV. After primary infection, the virus persists for life in the host in a latent state, which may be reactivated in immunocompromised patients such as HIV infection or organ transplant recipients. The virus infects some epithelial cells and mature B-lymphocytes. In the epithelial cells of the salivary glands, there is full replication and release of new virus.

Primary infection in childhood is generally sub-clinical and leads to a life-long carrier state with periodic virus shedding from the salivary glands. This acts as a source of infectious virus for spread by close contact, hence the popular name of "kissing disease".

When infection is delayed to adolescence or young adulthood, symptoms occur in 50% of cases and could be quite severe and debilitating.

For the diagnosis of primary and secondary EBV infections it is necessary to use tests for demonstrating the presence of specific EBV antibodies. In serodiagnosis of EBV is commonly used a screening test for heterophil antibodies which are associated with IM. If result is positive for heterophil antibodies or in case of children suspected of IM, presence of specific antibodies to EBV should be investigated. Viral capsid (VCA) of the EBV induces the earliest humoral response of the patient. IgG to VCA appears in the acute phase, peaks at 2-4 weeks after the onset, declines slightly, and persists for life. Epstein-Barr Nuclear Antigen (EBNA) is found in the nucleus of all EBV-infected cells. Although the synthesis of nuclear antigens precedes early antigen synthesis during the infection of B cells, the EBNA does not become available for antibody stimulation until after the incubation period of infectious mononucleosis, when activated T lymphocytes destroy the EBV genome-carrying B cells. As a result, antibodies to nuclear antigen are absent or barely detectable during acute IM. Anti-EBNA IgG does not appear until a patient has entered the convalescent stage. EBNA antibodies are almost always present in sera containing IgG antibodies to VCA of EBV unless the patient is in the early acute phase of IM.

Principle

bioelisa EBV-EBNA IgG is an immunoenzymatic method in which the wells of a microplate are coated with nuclear antigens of Epstein-Barr virus (EBNA). The sample to be analysed is incubated diluted in one of the microplate wells. IgG antibodies specific to EBNA present in the sample will bind to the solid-phase antigen. Subsequently, the wells are washed to remove residual test sample and antibodies to human IgG conjugated with the enzyme peroxidase are added. The conjugate will bind to the captured specific antibodies anti-EBNA on the well during the first incubation. After another washing to eliminate unbound material, a solution of enzyme substrate and chromogen is added. This solution will develop a blue colour if the sample contains anti-EBNA IgG antibodies. The blue colour changes to yellow after blocking the reaction with sulphuric acid. The intensity of colour is proportional to the anti-EBNA IgG concentration in the sample.

Components

1. **MCPL** MICROPLATE:
12 x 8 well strips coated with Epstein-Barr virus nuclear antigen. Individually separable wells.
2. **CONJ** CONJUGATE:
1 x 20 ml of rabbit anti-human IgG conjugated with peroxidase. Contains red dye and 0.2% Bronidox L. Ready to use.
3. **DIL** **SAMP** SAMPLE DILUENT:
1 x 100 ml of phosphate buffer containing yellow dye and 0.1% Kathon. Ready to use.

4. **WASH SOLN 20x** WASHING SOLUTION:
1 x 50 ml of concentrate phosphate buffer (20x) containing detergent. To be diluted 1/20 in distilled or deionised water before use. Contains 0.01% Kathon after dilution.
5. **SUBS TMB** SUBSTRATE-TMB:
1 x 15 ml of 3,3', 5,5'-Tetramethylbenzidine (TMB). Ready to use.
6. **CONTROL H** HIGH POSITIVE CONTROL:
1 x 2.0 ml of control solution for anti-EBNA. Contains yellow dye and 0.1% Kathon. Ready to use.
7. **CONTROL L** LOW POSITIVE CONTROL:
1 x 2.0 ml of low control solution for anti-EBNA. Contains yellow dye and 0.1% Kathon. Ready to use.
8. **CONTROL -** NEGATIVE CONTROL:
1 x 2.0 ml of negative control for anti- EBNA. Contains yellow dye and 0.1% Kathon. Ready to use.
9. **H₂SO₄ 1N** STOPPING SOLUTION:
1 x 12 ml of 1N sulphuric acid. Ready to use.
10. **SEALS** ADHESIVE SEALS:
To cover the microplate during incubations.
11. **BAG** RESEALABLE BAG:
For storage of unused strips.

Precautions

bioelisa EBV-EBNA IgG is intended for IN VITRO diagnostic use.

For professional use only.

WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL.

All human source material used in the preparation of this product was found to be negative for the presence of HIV-1/HIV-2 and HCV antibodies, as well as for the hepatitis B surface antigen, using a commercial licensed method. Nevertheless, because no test method can offer complete assurance of the absence of infectious agents, this product should be handled with caution:

- Avoid contact of reagents with the eyes and skin. If that occurs, wash thoroughly with water.
- Wear gloves.
- Do not pipette by mouth.
- Do not smoke.
- Dispose all used materials in a suitable biohazardous waste container. Remains of samples, controls, aspirated reagents and pipette tips should be collected in a container for this purpose and autoclaved 1 hour at 121°C or treated with 10% sodium hypochlorite (final concentration) for 30 min before disposal. (Remains containing acid must be neutralised prior addition of sodium hypochlorite).

Handling instructions:

- Adjust washer to the plate used (flat bottom) in order to wash properly.
- Do not mix reagents from different lots.
- Do not use reagents after expiration date.
- Extreme care should be taken to avoid microbial contamination and cross contamination of reagents.
- Use a new pipette tip for each specimen and each reagent.
- Soaps and/or oxidising agents remaining in containers used for the substrate-TMB solution can interfere with the reaction. If glass containers are used, they should be washed with 1N sulphuric or hydrochloric acid, rinsed well with distilled water and dried before use. We recommend using disposable plastic containers.

Storage and stability

The components will remain stable through the expiration date shown on the label if stored between 2-8°C. The bag containing the microplate should be brought to room temperature before opening to avoid

condensation in the wells. Once opened the bag, the remaining strips should be resealed in the plastic bag along with the silicagel and stored at 2-8°C. Once diluted, the washing solution is stable for four weeks if stored between 2-8°C. Store the substrate-TMB in the dark. The substrate-TMB solution should be colourless or have a slight blue tinge, discard if it becomes blue.

- Crystals may form when CONCENTRATE WASHING SOLUTION (20x) is stored at 2-8°C. These must be dissolved by warming at 37°C prior to use.

Required material not included

- Distilled or deionised water.
- Multichannel pipettes and micropipettes (10 µl, 100 µl, 1000 µl) and disposable tips.
- Incubator at 37°C ± 1°C.
- Tubes / microtubes for dilutions.
- Timer.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 630 nm is advisable.
- Manual or automated wash system.

Sample collection

Use fresh serum. Samples can be stored at 2-8°C for 3 days. For longer periods, samples should be frozen (-20°C). Avoid repeated freezing and thawing. Samples showing visible particulate matter should be clarified by centrifugation. Serum samples should not be heat inactivated, since that may cause incorrect results.

Automatic processing

Automated or semi-automated assay may be used with different instruments. It is very important to validate any automated system to demonstrate that results obtained for samples are equivalent to the ones obtained using manual assay. It is recommended that the user validate periodically the instrument. If there is any difficulty in the programming and setting of Biokit automatic processors, please contact your distributor.

PROCEDURE (See summary of protocol on the last page)

Previous operations

Allow all the reagents to reach room temperature (20-25°C) before running the assay.

Gently mix all liquid reagents before use.

Dilute the concentrate washing solution 1/20 with distilled or deionised water. For one plate, mix 25 ml of the concentrate solution with 475 ml of water. If less than a whole plate is used, prepare the proportional volume of solution.

Prepare 1/101 dilution of test specimens by adding, for example, 10 µl of serum to 1 ml of sample diluent. Mix well. DO NOT DILUTE CONTROLS AS THEY ARE READY TO USE.

NOTE: In manual assays it is advisable to perform the dilutions in microtubes and transfer to the microplate with a multichannel pipette.

Assay procedure

1. Use only the number of strips required for the test. Reserve 7 wells for blank and controls. Pipette 100 µl of each diluted sample to the designated wells. Transfer 100 µl of negative control to 2 wells, 100 µl of low positive control to 3 wells and 100 µl of high positive control to 2 wells. DO NOT DILUTE CONTROLS; THEY ARE READY TO USE. Leave a well empty for the substrate blank.
2. Cover the microplate with an adhesive seal, mix gently, and incubate for 1 hour at 37°C.
3. Remove and discard the adhesive seal. Aspirate the contents of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 3 more times. Ensure that each column of wells soaks for at least 15 seconds before the next aspiration cycle. After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.
4. Transfer 100 µl of conjugate to each well, except the one reserved for the substrate blank. Avoid bubbles upon addition.
5. Cover the plate with an adhesive seal and incubate for 30 minutes at room temperature (20-25°C).

6. Remove and discard the adhesive seal. Aspirate and wash the wells as in step 3.
7. Add 100 µl of substrate-TMB solution to each well, including the blank.
8. Incubate for 15 minutes at room temperature (20-25°C), protected from light.
9. Add 100 µl of stopping solution in the same sequence and time intervals as for the substrate-TMB.
10. Blank the reader at 450 nm with the blank well and read the absorbance of each well, within 30 minutes. It is recommended to read in bichromatic mode using a 620 - 630 nm reference filter.

Quality control

Results of an assay are valid if the following criteria are accomplished:

Substrate blank: absorbance value must be less than or equal to 0.100.

2. Negative control: absorbance less than 0.300 after subtracting the blank.
3. Low positive control: absorbance between 0.250 and 0.900 after subtracting the blank.
4. High positive control: absorbance greater than 1.2 times the low positive control.

Results

1. Calculate the mean absorbance value of the low positive control (LPCx). The cut-off value is:

$$\text{Cut-off} = \text{LPCx}$$

Divide the sample absorbance by the cut-off value.

- Positive: ratio absorbance/cut-off ≥ 1.1
 Negative: ratio absorbance/cut-off < 0.9
 Equivocal: ratio absorbance/cut-off $\geq 0.9 < 1.1$

To express the results in arbitrary units use the formula below:

$$\text{AU/ml} = \frac{\text{Sample absorbance}}{\text{Cut-off value}} \times 10$$

AU/ml	Interpretation
≥ 11	Positive
< 9	Negative
$\geq 9 < 11$	Equivocal

Interpretation of the results

Anti-EBNA IgG does not appear until a patient has entered the convalescent stage. EBNA antibodies are almost always present in sera containing IgG antibodies to VCA of EBV unless the patient is in the early acute phase of IM. In this situation IgG antibodies to EBNA are barely undetectable. Patients with severe immunologic defects or immunosuppressive disease may not have EBNA antibodies, even if antibodies to VCA are present. For the serological diagnosis of convalescence phase of infectious mononucleosis, especially in the equivocal results, paired serum samples taken at 2-4 weeks interval must be tested at the same time in adjacent wells. To confirm EBV EBNA a positive results should appear with the EBV VCA IgG marker. However, it is recommended to associate this procedure with the detection of specific IgG to EBV VCA antigen, e.g. with bioelisa EBV-VCA IgG REF 3000-1243.

Expected values

Epstein-Barr virus is worldwide disseminated and most of people become infected sometime in their lives. Infants become susceptible to EBV as soon as maternal antibody protection disappears. Infection of children usually causes no symptoms. Infection during adolescence or young adulthood causes infectious mononucleosis in 35% to 50% of the cases. About 90% of adults universally have detectable VCA and EBNA specific IgG antibodies.

Limitations of the procedure

As with other serological tests, the results obtained with bioelisa EBV-EBNA IgG serve only as an aid to diagnosis and the patients' clinical history should be taken into consideration.

Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.

In case of an equivocal result it is recommend repeating the test again 2-4 weeks later with a fresh sample. If the result in the second test is again equivocal, the sample should be considered negative.

As in all sensitive immunoassays, there is the possibility that non-repeatable positive results occur.

Performance characteristics

Sensitivity

The diagnostic sensitivity is defined as the probability of reporting positive in the presence of the specific analyte. It is higher than 95%.

Specificity

The diagnostic specificity is defined as the probability of reporting negative in the absence of the specific analyte. It is higher than 89%.

Precision

Intra-assay reproducibility:

The coefficient of variation obtained for the absorbance values (mean = 2.800) of a positive sample assayed in 10 replicates was 2.0%.

Inter-assay reproducibility:

The coefficient of variation obtained for the absorbance values (mean = 2.760) of a positive sample run in 3 assays was 2.4%.

Interferences

Interferences with haemolytic, lipaemic or icteric sera are not observed up to a concentration of 10 mg/ml haemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

bioelisa EBV-EBNA IgG

1x96 TESTS

REF 3000-1248

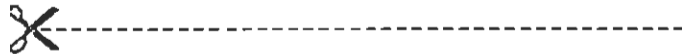
- 1 MCPL
- 1 x 20 ml CONJ
- 1 x 100 ml DIL SAMP
- 1 x 50 ml WASH SOLN 20x
- 1 x 15 ml SUBS TMB
- 1 x 2.0 ml CONTROL + H
- 1 x 2.0 ml CONTROL + L
- 1 x 2.0 ml CONTROL -
- 1 x 12 ml H₂SO₄ 1N
- 1 BAG
- 1 SEALS



IVD



CE



bioelisa EBV-EBNA IgG

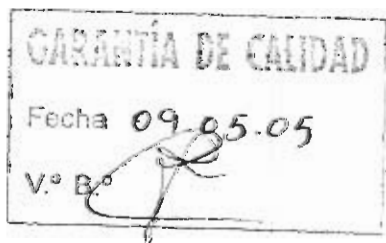
1x96 TESTS

REF 3000-1248

ELISA test for detection of IgG antibodies to nuclear antigen of Epstein-Barr virus (EBNA) in human serum / test de ELISA para la detección de anticuerpos IgG contra antígeno nuclear del virus Epstein-Barr en suero humano (EBNA) / ELISA-Test zur Bestimmung von IgG Antikörpern gegen das Epstein-Barr-Virus nuclear antigen (EBNA) in Humanserum / test d'ELISA pour la détection des anticorps IgG contre l'antigène nucléaire (EBNA) du virus d'Epstein-Barr en sérum humain / test ELISA per il rilevamento di anticorpi IgG contro l'antigene nucleare (EBNA) del virus di Epstein-Barr nel siero umano / teste de ELISA para a detecção de anticorpos IgG contra o antígeno nuclear (EBNA) do vírus Epstein-Barr em soro humano



LOT D-2005
2006-06-26



biokit

bioelisa EBV-EBNA IgG

MCPL

IVD

LOT D-2005
2006-06-26



biokit

bioelisa EBV-EBNA IgG

DIL SAMP

100 ml

IVD

LOT D-2005
2006-06-26 RTU



biokit

bioelisa EBV-EBNA IgG

CONJ

20 ml

IVD

LOT D-2005
2006-06-26

RTU



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SUBS TMB

15 ml

IVD

LOT D-2005
2006-06-26

RTU



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WASH SOLN 20x

50 ml

IVD

LOT D-2005
2006-06-26



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bioelisa EBV-EBNA IgG

CONTROL + H

2.0 ml

IVD

LOT D-2005
2006-06-26

RTU



biokit

bioelisa EBV-EBNA IgG

CONTROL + L

2.0 ml

IVD

LOT D-2005
2006-06-26

RTU



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bioelisa EBV-EBNA IgG

CONTROL -

2.0 ml

IVD


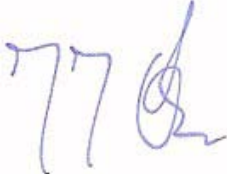

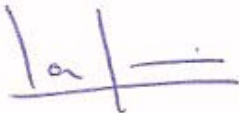
LOT D-2005
2006-06-26

RTU





**VALIDATION of the
bioelisa EBV-EBNA IgG assay
used in combination with the best 2000**

	Author	Reviewed	Reviewed	Approved
Name	Albert Royo	Joaquín Ortiz	Iná Camargo	Joan Guixer
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Signature				
Date	26-09-05	26-09-05	26-09-05	26-09-05

**VALIDATION of the bioelisa EBV-EBNA IgG assay used in combination
with the best 2000**

A. Introduction

The purpose of this validation is to demonstrate users and authorities, that automated performance of the **bioelisa EBV-EBNA IgG** assay used in combination with the best 2000 instrument, is equal to that of the performance achieved using manual techniques. Sensitivity, specificity and reproducibility intra-run data has been scrutinized.

Expected values: 100% correlation for sensitivity and specificity
 CV% ≤ 10 % reproducibility intra-run OD's
 CV% ≤ 15 % when compared to the automated-manual procedure in quantitative assays.

B. Materials and methods

1- Instruments

- Automated instrumentation:
Biokit best 2000; Revelation DSX version 5.15; DSX Automated Elisa System
Serial N°: 1DXA-0160 Dynex technologies Inc. 14340 Sullyfield Circle, Chantilly, VA 20151-1683 USA.
- Manual instrumentation:
Washer: Bioelisa Washer ELX 50; Incubator: Incubator 500 PH Electronica; Reader: Ultra Microplate reader ELX 808 Bio-Tek Instruments, Inc. (SN:1380009).

2- Diagnostic kit

- **bioelisa EBV-EBNA IgG**; Batch number: H-0405; Exp.date: 30 SEPT 06.

3- Samples

- Samples for sensitivity and specificity: 64 blood bank samples (07SEPT05) were tested. Among the 64 samples there were 3 negative samples.
- Samples for reproducibility intra-run: 24 replicates of the identified sample #5 with an OD around 1.000 were tested. These replicates were dispensed in 3 full strips.

4- Manual protocol: (see annex 2)

5- best 2000 protocol: (see annex 3)

C. Results: (Plates' results are described in annex 1)

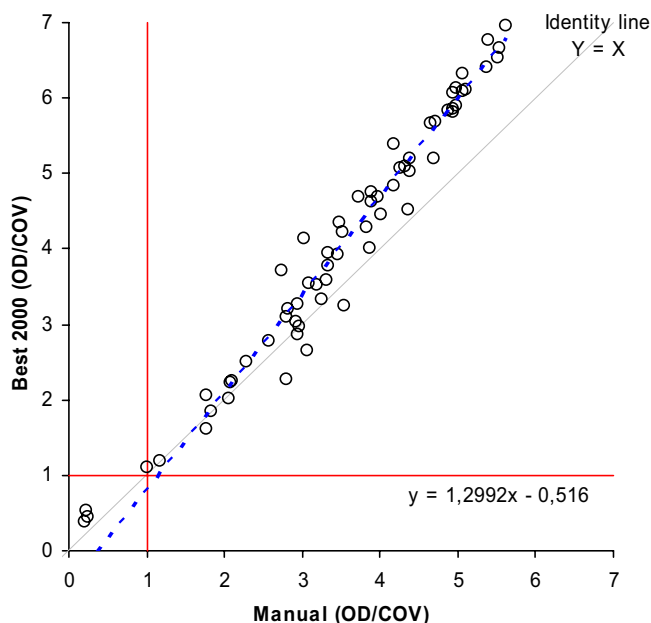
Manual/automated correlation: It has been tested 64 unselected blood donor samples. The chart below shows the normalized absorbances (OD/COV) for the manual technique compared to the best 2000 automated procedure.

No discrepancies between the two methods were detected.

100% correlation for both positive and negative samples was achieved.

VALIDATION of the bioelisa EBV-EBNA IgG assay used in combination with the best 2000

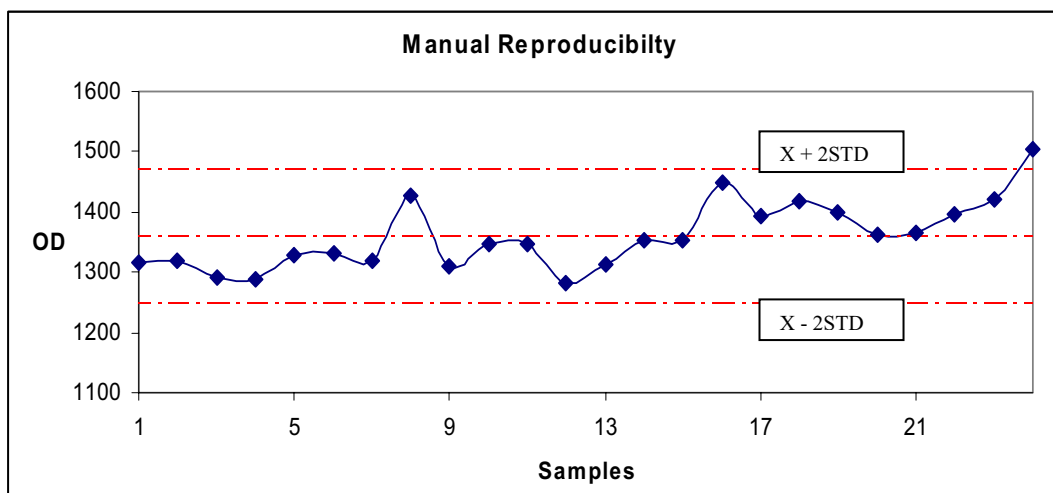
The correlation for this assay: r statistic: 0.982 (98.2%)



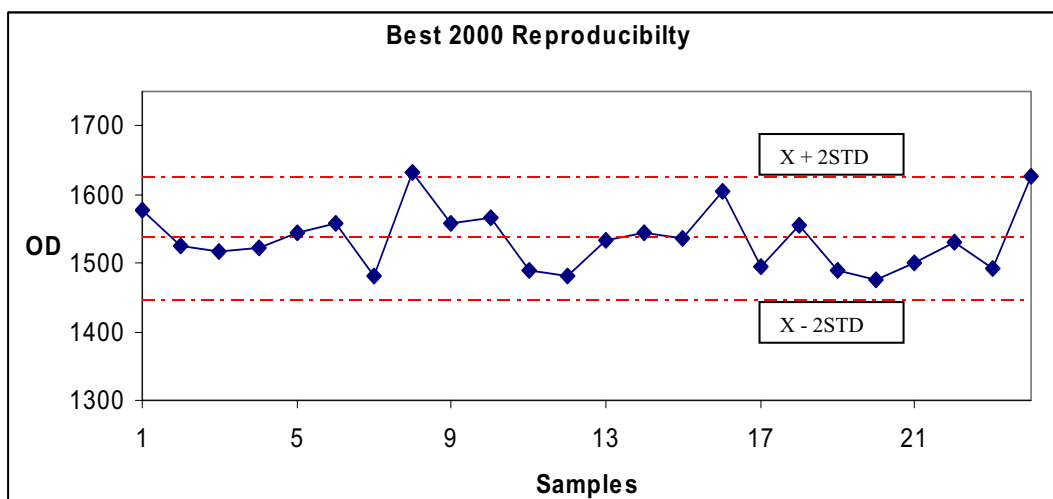
- Discontinuous line shows the regression line.
- Continuous lines show the cut-off position in ratio equal to 1.

- Reproducibility: 24 replicates of the same sample were run on the best 2000. Results detailed below.

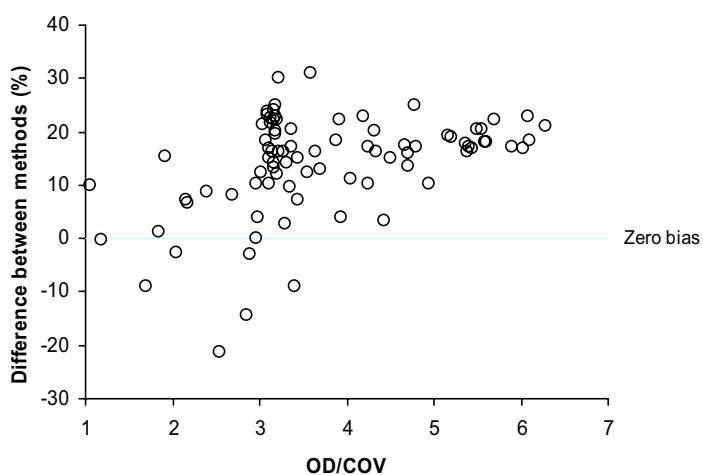
	Manual	Best 2000
n° samples	24	24
Mean OD	1359	1535
Min.	1281	1475
Max.	1503	1632
CV %	4.1%	2.9%



VALIDATION of the bioelisa EBV-EBNA IgG assay used in combination with the best 2000

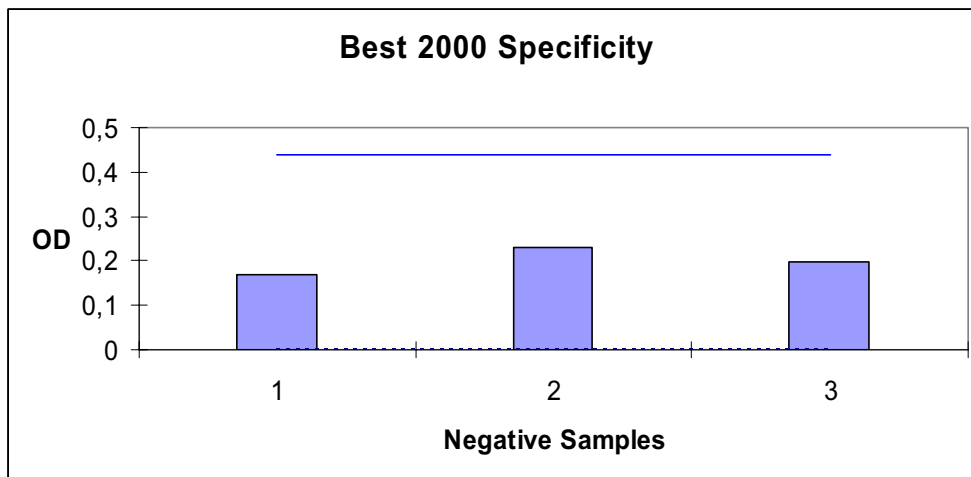
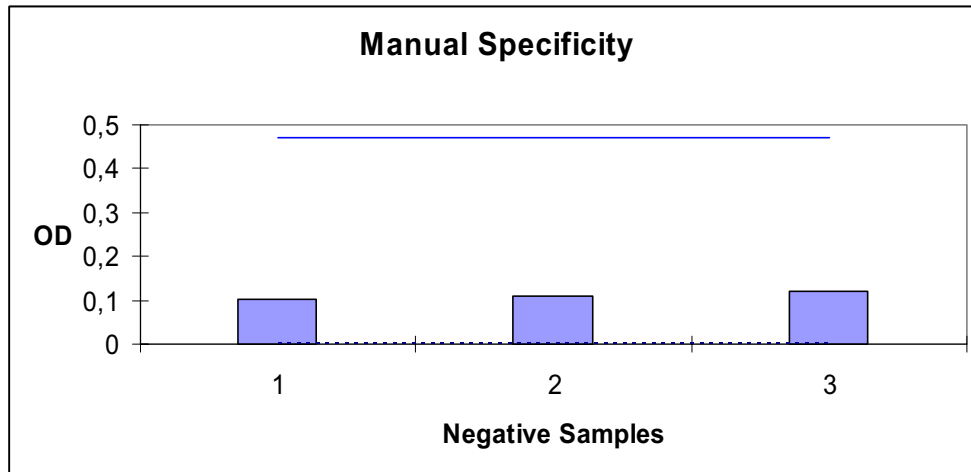


- Differences between methods, manual vs automated best 2000 (%): Each positive sample, expressed in normalised absorbances (OD/COV), including all positive results used for reproducibility, has been plotted against the Best 2000. The variation is expressed as a percentage.



VALIDATION of the bioelisa EBV-EBNA IgG assay used in combination with the best 2000

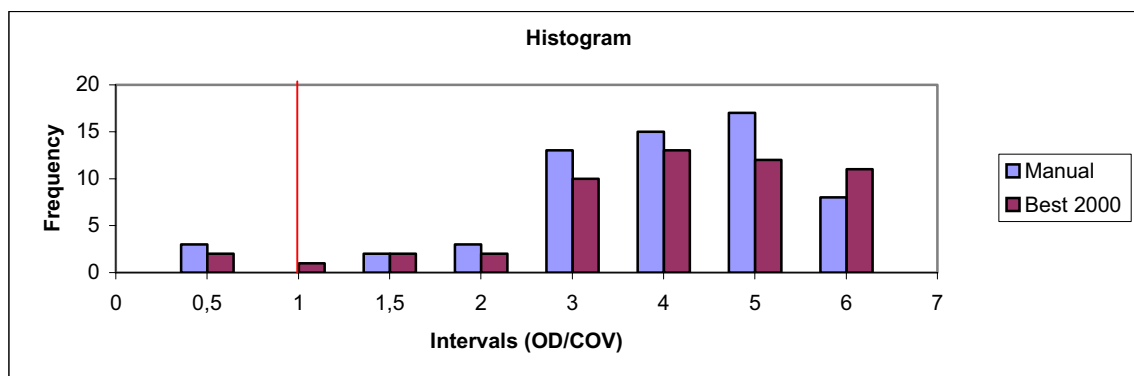
- Specificity: The chart show the OD's of the negative samples.



- Discontinuous line shows the COV optical density.
- Continuous line shows the NC optical density.

VALIDATION of the bioelisa EBV-EBNA IgG assay used in combination with the best 2000

- Population distribution: The chart shows the distribution of samples between the two methods as a histogram.



- Single line shows the cut-off position.

D. Conclusion:

bioelisa EBV-EBNA IgG assay, performed in combination with the best 2000 (protocol detailed in annex 3), produces comparable results to those obtained manually as reference.

Sensitivity, specificity and intra-run reproducibility results correspond to the expected values prepared for this evaluation

The user should utilize this validated protocol in the best 2000 to obtain reliable and consistent results.

VALIDATION of the bioelisa EBV-EBNA IgG assay used in combination with the best 2000

Annex 1:

Plates' results
 bioelisa EBV-EBNA IgG
 Lot: H-0405
 Exp.date: 30SEPT06
 Plate 1

	MANUAL			BEST 2000		
	OD	OD/CO	Res.	OD	OD/CO	Res.
Blank	16			15		
NC	0,0015	0,00		0,001	0,00	
LPC	472,3	1,00		440	1,00	
HPC	1991	4,22		2268,5	5,16	
Blood donors: 07-09-05						
1	2655	5,62	+	3060	6,95	+
2	1645	3,48	+	1916	4,35	+
3	2543	5,38	+	2978	6,77	+
4	1084	2,30	+	1102	2,50	+
5	1296	2,74	+	1633	3,71	+
6	1978	4,19	+	2367	5,38	+
7	1433	3,03	+	1823	4,14	+
8	102	0,22	-	170	0,39	-
9	833	1,76	+	905	2,06	+
10	2355	4,99	+	2697	6,13	+
11	1838	3,89	+	2094	4,76	+
12	1757	3,72	+	2059	4,68	+
13	2202	4,66	+	2488	5,65	+
14	1970	4,17	+	2132	4,85	+
15	1579	3,34	+	1734	3,94	+
16	1661	3,52	+	1860	4,23	+
17	1325	2,81	+	1367	3,11	+
18	1454	3,08	+	1563	3,55	+
19	1839	3,89	+	2032	4,62	+
20	2333	4,94	+	2667	6,06	+
21	981	2,08	+	983	2,23	+
22	2395	5,07	+	2678	6,09	+
23	2217	4,69	+	2287	5,20	+
24	559	1,18	+	520	1,18	+
25	474	1,00	+	488	1,11	+
26	2073	4,39	+	2209	5,02	+
27	1388	2,94	+	1256	2,85	+
28	865	1,83	+	816	1,85	+
29	1402	2,97	+	1308	2,97	+
30	1507	3,19	+	1547	3,52	+
31	976	2,07	+	885	2,01	+

**VALIDATION of the bioelisa EBV-EBNA IgG assay used in combination
with the best 2000**

	MANUAL			BEST 2000		
	OD	OD/CO	Res.	OD	OD/CO	Res.
32	1336	2,83	+	1408	3,20	+
33	1880	3,98	+	2060	4,68	+
34	1676	3,55	+	1428	3,25	+
35	110	0,23	-	229	0,52	-
36	2410	5,10	+	2689	6,11	+
37	1379	2,92	+	1338	3,04	+
38	2605	5,52	+	2871	6,53	+
39	2619	5,55	+	2932	6,66	+
40	1900	4,02	+	1960	4,45	+
41	1572	3,33	+	1657	3,77	+
42	2068	4,38	+	2291	5,21	+
43	1445	3,06	+	1164	2,65	+
44	837	1,77	+	712	1,62	+
45	1532	3,24	+	1466	3,33	+
46	1214	2,57	+	1227	2,79	+
47	2336	4,95	+	2561	5,82	+
48	1389	2,94	+	1435	3,26	+
49	2046	4,33	+	2236	5,08	+
50	2015	4,27	+	2234	5,08	+
51	1806	3,82	+	1883	4,28	+
52	1824	3,86	+	1768	4,02	+
53	2060	4,36	+	1986	4,51	+
54	1630	3,45	+	1727	3,93	+
55	119	0,25	-	199	0,45	-
56	2542	5,38	+	2816	6,40	+
57	1326	2,81	+	998	2,27	+
58	994	2,10	+	991	2,25	+
59	2307	4,88	+	2566	5,83	+
60	2335	4,94	+	2580	5,86	+
61	2349	4,97	+	2593	5,89	+
62	2389	5,06	+	2780	6,32	+
63	2225	4,71	+	2504	5,69	+
64	1569	3,32	+	1573	3,58	+
Reproducibility # 5						
Rep-1	1316	2,79	+	1576	3,58	+
Rep-2	1318	2,79	+	1526	3,47	+
Rep-3	1291	2,73	+	1517	3,45	+
Rep-4	1287	2,72	+	1523	3,46	+
Rep-5	1327	2,81	+	1545	3,51	+
Rep-6	1331	2,82	+	1558	3,54	+
Rep-7	1320	2,79	+	1480	3,36	+
Rep-8	1426	3,02	+	1632	3,71	+
Rep-9	1311	2,78	+	1557	3,54	+
Rep-10	1346	2,85	+	1567	3,56	+

VALIDATION of the bioelisa EBV-EBNA IgG assay used in combination with the best 2000

	MANUAL			BEST 2000		
	OD	OD/CO	Res.	OD	OD/CO	Res.
Rep-11	1347	2,85	+	1488	3,38	+
Rep-12	1281	2,71	+	1480	3,36	+
Rep-13	1313	2,78	+	1534	3,49	+
Rep-14	1354	2,87	+	1545	3,51	+
Rep-15	1352	2,86	+	1535	3,49	+
Rep-16	1450	3,07	+	1605	3,65	+
Rep-17	1393	2,95	+	1494	3,40	+
Rep-18	1417	3,00	+	1556	3,54	+
Rep-19	1399	2,96	+	1489	3,38	+
Rep-20	1362	2,88	+	1475	3,35	+
Rep-21	1366	2,89	+	1499	3,41	+
Rep-22	1397	2,96	+	1531	3,48	+
Rep-23	1420	3,01	+	1492	3,39	+
Rep-24	1503	3,18	+	1626	3,70	+
N	24			24		
MEAN	1359			1535		
STD	56,1			44,9		
CV%	4,1%			2,9%		

VALIDATION of the bioelisa EBV-EBNA IgG assay used in combination with the best 2000

Annex 2: Manual protocol

Assay procedure

1. Use only the number of strips required for the test. Reserve 7 wells for blank and controls. Pipette 100 µl of each diluted sample to the designated wells. Transfer 100 µl of negative control to 2 wells, 100 µl of low positive control to 3 wells and 100 µl of high positive control to 2 wells. DO NOT DILUTE CONTROLS; THEY ARE READY TO USE. Leave a well empty for the substrate blank.
2. Cover the microplate with an adhesive seal, **mix gently**, and incubate for 1 hour at 37°C.
3. Remove and discard the adhesive seal. Aspirate the contents of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 3 more times. Ensure that each column of wells soaks for at least 15 seconds before the next aspiration cycle. After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.
4. Transfer 100 µl of conjugate to each well, except the one reserved for the substrate blank. Avoid bubbles upon addition.
5. Cover the plate with an adhesive seal and incubate for 30 minutes at room temperature (20-25°C).
6. Remove and discard the adhesive seal. Aspirate and wash the wells as in step 3.
7. Add 100 µl of substrate-TMB solution to each well, including the blank.
8. Incubate for 15 minutes at room temperature (20-25°C), protected from light.
9. Add 100 µl of stopping solution in the same sequence and time intervals as for the substrate-TMB.
10. Blank the reader at 450 nm with the blank well and read the absorbance of each well, within 30 minutes. It is recommended to read in bichromatic mode using a 620 - 630 nm reference filter.

Quality control

Results of an assay are valid if the following criteria are accomplished:

1. Substrate blank: absorbance value must be less than or equal to 0.100.
2. Negative control: absorbance less than 0.300 after subtracting the blank.
3. Low positive control: absorbance between 0.250 and 0.900 after subtracting the blank.
4. High positive control: absorbance greater than 1.2 times the low positive control.

Results

1. Calculate the mean absorbance value of the low positive control (LPCx). The cut-off value is:

Cut-off = LPCx

2. Divide the sample absorbance by the cut-off value.

- Positive: ratio absorbance/cut-off ≥ 1.1
- Negative: ratio absorbance/cut-off < 0.9
- Equivocal: ratio absorbance/cut-off ≥ 0.9 < 1.1

To express the results in arbitrary units use the formula below:

$$\text{AU/ml} = \frac{\text{Sample absorbance}}{\text{Cut-off value}} \times 10$$

AU/ml	Interpretation
≥ 11	Positive
< 9	Negative
≥ 9 < 11	Equivocal

REVELATION DSX 5.15

Assay type : Endpoint
 Assay title : bioelisa EBV-EBNA IgG
 Password :
 Written by : biokit SA
 Prefix :
 Suffix :
 Report layout : Laboratory information
 : Header information
 : Lot specific data
 : Removed outliers
 : Edited wells
 : Calculation mode
 : Blank mode
 : Q.C. equations
 : Data matrix
 : Ratio
 : Threshold
 Header information : Filename, Plate ID, Assay title
 Footer : Date, Page, Q.C. summary

Pipette Samples/Standards/Controls

Plate dispense time is not time critical
 Prepare all deep wells first before transfer to microtiter plate

Pipette 100 ul of Sample to wells of type: Test (T)
 Preparation order: 1
 Tip to dispense into microtiter well must be clean
 Fluid into microtiter well must be a single shot dispense
 Pipette diluent first into deep wells
 Share deep well dilutions for replicates on this assay
 Deep well contents can be shared across multiple plates
 Dispense of sample into the deep well must be from a clean tip (single shot dispense)
 When mixing in the deep well the tip does not have to be clean
 Mixing in the deep well must occur immediately after the dispense of sample
 Dilute 10 ul of sample with 1000 ul of Diluent EBV EBNA IgG, using deep well plate, 3 mix cycles
 Dilution volume will be optimised with a minimum sample volume of 10 ul

Pipette 100 ul of NC EBC EBNA IgG to wells of type: NC1
 Preparation order: 2
 Fluid aspirate/dispense profile: 1 / 4
 Tip to dispense into microtiter well does not have to be clean
 Fluid into microtiter well can be from a multiple shot dispense

Pipette 100 ul of LPC EBV EBNA IgG to wells of type: PC1
 Preparation order: 3
 Fluid aspirate/dispense profile: 1 / 4
 Tip to dispense into microtiter well does not have to be clean
 Fluid into microtiter well can be from a multiple shot dispense

Pipette 100 ul of HPC EBV EBNA IgG to wells of type: PC2
 Preparation order: 4
 Fluid aspirate/dispense profile: 1 / 4
 Tip to dispense into microtiter well does not have to be clean
 Fluid into microtiter well can be from a multiple shot dispense

Incubate for 60 minutes at 37,0 C

Longest Time: 60 minutes
 Shake for 10 seconds at medium speed

Wash plate

Purge the washer with 4,00 mls of Bioelisa EBV-VCA IgG_IgM-EBNA IgG
 Perform a 4 cycle wash
 Soak in between cycles for 15 seconds
 For each strip perform the following operations:
 Dispense 350 uls of Bioelisa EBV-VCA IgG_IgM-EBNA IgG
 Do final aspirate cycle
 Clean the washer after use with 6,00 mls of DISTILLED WATER

Dispense 100 uls of Conjugate EBV EBNA IgG to wells B1-H12, aspirate profile 1, dispense profile 4**Incubate for 30 minutes at ambient temperature**

Longest Time: 30 minutes

Wash plate

Purge the washer with 4,00 mls of Bioelisa EBV-VCA IgG_IgM-EBNA IgG
 Perform a 4 cycle wash
 Soak in between cycles for 15 seconds
 For each strip perform the following operations:
 Dispense 350 uls of Bioelisa EBV-VCA IgG_IgM-EBNA IgG
 Do final aspirate cycle
 Clean the washer after use with 6,00 mls of DISTILLED WATER

Dispense 100 uls of TMB EBV EBNA IgG to wells A1-H12, aspirate profile 1, dispense profile 4

Dispense Fluid is time critical. Lifetime is 30. Prep Time is 30.

Incubate for 15 minutes at ambient temperature

Longest Time: 15 minutes

Dispense 100 uls of STOP SOLUTION to wells A1-H12, aspirate profile 1, dispense profile 4

Reader

Test wavelength : 450 nm
 Ref. wavelength : 620 nm
 Initial shake : 5 Seconds
 Start mode : Immediate
 Calculation mode : Endpoint
 Results format : OD

	1	2	3	4	5	6	7	8	9	10	11	12
A	B1s	T1s	T9s	T17s	T25s	T33s	T41s	T49s	T57s	T65s	T73s	T81s
B	NC1s	T2s	T10s	T18s	T26s	T34s	T42s	T50s	T58s	T66s	T74s	T82s
C	NC1s	T3s	T11s	T19s	T27s	T35s	T43s	T51s	T59s	T67s	T75s	T83s
D	PC1s	T4s	T12s	T20s	T28s	T36s	T44s	T52s	T60s	T68s	T76s	T84s
E	PC1s	T5s	T13s	T21s	T29s	T37s	T45s	T53s	T61s	T69s	T77s	T85s
F	PC1s	T6s	T14s	T22s	T30s	T38s	T46s	T54s	T62s	T70s	T78s	T86s
G	PC2s	T7s	T15s	T23s	T31s	T39s	T47s	T55s	T63s	T71s	T79s	T87s
H	PC2s	T8s	T16s	T24s	T32s	T40s	T48s	T56s	T64s	T72s	T80s	T88s

s indicates that a sample ID is required for this well location

Blank mode : Individual
 Q.C. equations : B1<=0.100 (Blank too high)
 : NC1<0.300 (NC too low)
 : 0.25<=PC1<=0.9 (PC1 out of range)
 : PC2>1.2*(PC1) (PC2 out of range)
 Full Q.C. Report : Yes
 Suppress results : No
 Lot specific check : No
 Output format : Matrix
 Matrix options : Calculated data, Sample ID
 Average replicates : No
 Mean : Arithmetic
 Area statistics : No
 Export to file : ASCII Text, Matrix, comma separated
 File options : Reading date, Reading time, Assay title,
 : Kit Lot Data, Reagent Lot Data, User name,
 : Area statistics, OD QC equations, Threshold Q.C.,
 : Threshold cutoffs, Curve fit Q.C., Sample IDs,
 : Position, Well Label, OD Results,
 : Threshold, Curve Fit, Ratio,
 : Spreadsheet

Threshold

- equation : (PC1)*0.9
 + equation : (PC1)*1.1
 No. of segments : 1
 - label : -
 0 label : ?
 + label : POS
 Histogram : No
 Q.C. equations :
 Full Q.C. Report : Yes
 Suppress results : No
 Lot specific check : No
 Output format : Matrix: 3 dps
 Average replicates : Yes
 Mean : Arithmetic

Ratio

Ratio equation : Sample/PC1
 Result units :
 Data conversion :
 Result units :
 Results flagging : If Result(Sample)>=1.1 then Flag="POS"
 : If Result(Sample)<0.9 then Flag="NEG"
 : If 1.1>Result(Sample)>=0.9 then Flag="???"
 Output format : Matrix: 3 dps
 : Table: 3 dps
 Table options : Sample ID, Location, Replicate, Mean, S.D., C.V.
 Table order : T, S, C, NC, PC, CO, PR, SC, AC, N, HS
 Average replicates : No
 Mean : Arithmetic

biokit protocol in the biomaster junior

bioelisa EBV-EBNA IgG

Protocol Heading

Protocol Name: bioelisa EBV EBNA IgG
 Version Number: 0
 Revision Number: 21
 Protocol info:
 Writing Date: 28/09/2005
 Author Name: biokit
 Software Version: 2.1

Pipetting

Name	Type	Repl.	Vol[ul]	Conjugate	Substrate	Stop
blk	BLANK	1	0	0	100	100
Negative	NEG	2	100	100	100	100
LPositive	LPOS	3	100	100	100	100
Positive	POS	2	100	100	100	100
Samples	SAMPLE	1	100	100	100	100

Predilution

Name	Vol[ul]	Dil[ul]	Ser. Vol[ul]	Ser. Dil[ul]
Samples	10	990		

Pipetting Tip

Stds/Samples	Reagents
Plastic	Plastic

Pipetting Mode

ImmunoSubtraction No

Procedure steps

Samples Predilution

Samples Pipetting

Standards Pipetting

Shaking	Speed	Time[sec]
	Fast	5

Incubation	Hours	Minutes	Temp.[°C]
	1	0	37

Washing	Priming	Vol.[ul]	Cycles	SoakTime	Flow	Method
	Short	350	4	30	Normal	By Rack

Reagent Pipetting	Conjugate	Hours	Minutes	Temp.[°C]
		0	30	25

Washing	Priming	Vol.[ul]	Cycles	SoakTime	Flow	Method
	Short	350	4	30	Normal	By Rack

Reagent Pipetting	Substrate	Hours	Minutes	Temp.[°C]
		0	15	25

Reagent Pipetting	Stop	Main Filter	Over Range	Double Beam	Mult.Fact.
		450	450	620	1.0

Results Interpretation

Assay Method	CutOff
Cut-off Formula	MEAN(LPOS)
Grey Zone %	10
Positiveness	Above

Validation Criteria

BLANK<=0.100
 NEG<=0.300
 MEAN(LPOS)>0.250
 MEAN(LPOS)<0.900
 MEAN(POS)>=1.2* MEAN(NEG)

