EPSTEIN-BARR EA VIRCLIA® IgG MONOTEST

For *in vitro* diagnostic use

VCM044: Indirect chemiluminescent immunoassay (CLIA) to test IgG antibodies against Epstein-Barr Early in human serum/plasma. 24 tests.

INTRODUCTION:

Epstein-Barr virus (EBV) is a member of the herpesvirus family and one of the most common human viruses. The virus is present worldwide, and most people become infected with EBV at some point during their lives. In general terms, as many as 95% of adults between 35 and 40 years of age have been infected. Infectious mononucleosis is the most common disease caused by EBV, leading to fever, cervical adenopathies, splenomegaly, and pharyngitis. Some cases can be caused by cytomegalovirus, Toxoplasma gondii, adenovirus, etc. EBV is also in the origin of proliferative syndromes in immunosuppressed patients, as well as EBV infection is associated with Burkitt´s lymphoma and nasopharyngeal carcinoma. Antibodies to several antigen complexes may be measured for detection of Epstein-Bar virus. These antigens are the viral capsid antigen (VCA), the early antigen (EA), and the EBV nuclear antigen (EBNA).

The presence of IgM antibodies to VCA and absence of antibodies to EBNA, are indicative of primary EBV infection. An increase or high IgG antibody titers to VCA, and a lack of antibody response to EBNA after at least 4 weeks of illness, strongly suggest primary infection also.

In addition, 80% of patients with active EBV infection produce antibodies to EA. The presence of antibodies to both VCA and EBNA is indicative of past infection (infections ocurred 4-6 months, or even years, earlier). Since 95% of adults have been infected with EBV at some point, most adults will show antibodies to EBV, from earlier infections. High or elevated antibody levels may be present for years and are not necessarily an indicator of recent infection.

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (**C**hemi**L**uminescent **I**mmuno**A**ssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use.

Serum dilution solution and conjugate are coloured to help in the performance of the technique.

Sample predilution is not necessary.

Reagents required for the run of the test are included in the monodose presentation.

KIT CONTENTS:

1 VIRCLIA® EPSTEIN-BARR EA MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:

Wells A, B, C: Reaction wells; wells coated with purified proteins of EA of Epstein-Barr virus. Contains inactivated antigen. Contains material of animal origin.

Well D: Conjugate: orange; containing anti-human IgG peroxidase conjugate dilution and Neolone and Bronidox as preservatives. Contains material of animal origin.

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers and Neolone and Bronidox as preservatives. Contains material of animal origin.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservative. Contains material of human origin. Contains material of animal origin.

Well G: Substrate component B: clear; containing peroxide.

Well H: Substrate component A: clear; containing luminol.

Store at 2-8°C and check expiration date.

Materials required but not supplied:

-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR).

-A CLIA automated processor.

-Precision micropipettes.

STORAGE REQUIREMENTS:

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day

STABILITY AND HANDLING OF REAGENTS:

Do not let the plate dry between washing and reagent addition.

Substrate component A is light sensitive. Avoid light exposure. Substrate solutions should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The device is intended for single use.
4. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
5. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
6. Wear personal protective equipment when handling samples. Wash hands properly after handling the samples. All

procedures must be carried out in accordance with the approved safety standards.
7. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
8. Never pipette by mouth.
9. Do not use in the event of damage to the package.
10. Do not use the kit after expiration date.
11. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
12. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
13. Keep containers for samples and reagents closed while they are not being handled.
14. Avoid using samples subjected to repeated freeze-thaw cycles.
15. Handle in aseptic conditions to avoid microbial contaminations.
16. Reagents in this kit could include substances of animal and/or human origin and/or inactivated antigen (refer to Kit Contents). Although materials of human origin have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, all material and patient specimens should be handled and dispose as potentially infectious using safety laboratory procedures. No present method can offer complete assurance that these or other infectious agents are absent. Dispose of unused reagents and waste in accordance with all applicable regulations.
17. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
18. Do not use this product in automated processors unless they have been previously validated for that purpose.
19. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

SPECIMEN COLLECTION AND HANDLING:
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples are to be refrigerated (2-8°C) upon collection or frozen (-20°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma. Samples should be inactivated at 56°C for 30 minutes before testing.

PRELIMINARY PREPARATION OF THE REAGENTS:
All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting. Once diluted, store at 2-8°C.

ASSAY PROCEDURE:
- **AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

- **MANUAL**

Contact the manufacturer for further information on the manual procedure.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:
Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit. The software of the instrument will validate the values obtained for the controls and display them in the results report.
Follow the Operator´s Manual of the Automated Processor. Results cannot be validated if the control values deviate from the expected values.

INTERPRETATION OF RESULTS:
Antibody index= (sample RLU /calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below <0.9 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit. In case of a positive result close to the threshold, a new sample should be required for seroconversion confirmation.

LIMITATIONS:
1. This kit is intended to be used with human serum/plasma.
2. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by direct diagnostic techniques.
3. This test will not indicate the site of infection. It is not intended to replace isolation.
4. Samples collected at the beginning of infection may not have detectable levels of antibodies. In these cases it is recommended to obtain a second sample between 14 and 21 days to be tested in parallel with the original sample, in order to determine a seroconversion.
5. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
6. A negative result in immunosuppressed patients does not always exclude the possibility of infection.

7. Lack of a detectable antibody level does not exclude the possibility of infection.
8. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
9. The performance of this test has not been evaluated for use in patients without clinical signs and symptoms of infection.
10. CMV infected patient may give false positive results in Epstein-Barr virus assays.
11. Other diseases (cytomegalovirus, toxoplasmosis, adenovirus and rubella) produce similar syndromes to infectious mononucleosis, and should also be tested in suspected cases of this syndrome.
12. Final result should be the consequence of the overall evaluation of the classical markers used for EBV serological diagnosis and not derives from a single assay result.
13. The performance characteristics have not been studied for patients with nasopharyngeal carcinoma, Burkitt´s lymphoma and other EBV associated diseases different from infectious mononucleosis.
14. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely in low prevalence scenarios.
15. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

- **SENSITIVITY AND SPECIFICITY:**

148 serum/plasma samples were assayed against a commercial IFA kit.
The results were as follows:

Samples No.	148	
Sensitivity (%)	89	
	95% CI	81-94
Specificity (%)	85	
	95% CI	79-90
PPV (%)	90	
NPV (%)	84	
LR+/LR-	-1.05/-1.03	

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

- **PRECISION:**

4 samples were assayed. 2 replicates of each one were analyzed in 2 different instruments for 20 days. Within-run precision, between-run precision, between-day precision and between-laboratory precision were determined.
The results were as follows:

Sample	Within-run precision % CV	Between-run precision % CV	Between-day precision % CV	Between-laboratory precision % CV
Calibrator	7.5	10.2	4.3	11.9
Positive sample	5.7	7.3	10.3	13.9
Negative control & Negative sample	No change in the interpretation	No change in the interpretation	No change in the interpretation	No change in the interpretation

CV: Coefficient of variation

- **INTERFERENCES:**

Interferences – ANA/RF:
10 samples known to be positive for antinuclear antibodies and rheumatoid factor were assayed. No interferences with antinuclear antibodies (4 samples tested) were found. No interferences with rheumatoid factor (6 samples tested) were found.

Interferences – Endogenous substances:
3 samples were tested with each interferent. Specifications were fulfilled in all cases. No interferences were found with haemolytic (8.5 g/L hemoglobin), icteric (6 g/L bilirubin), hyperlipemic (5.8 g/L cholesterol and 11 g/L tributyrin) or hyperproteic (60 g/L ɣ-globulin and 60 g/L albumin) samples.

Interferences – Anticoagulants:
3 samples were tested with each anticoagulant. Specifications were fulfilled in all cases. No interferences were found with heparin (30 UI/mL), citrate (0.13 mol/L) and EDTA (2 mg/mL).

- **CROSS REACTIONS**

29 samples known to be positive for other microorganisms (herpes simplex type 1, herpes simplex type 2, cytomegalovirus and varicella-zoster virus) were assayed.
No cross reactivity with cytomegalovirus (5 samples tested) was found. Cross reactivity with herpes simplex type 1 (3 out of 13 samples tested), herpes simplex type 2 (1 out of 3 samples tested) and varicella-zoster virus (2 out of 8 samples tested) was found.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-y°C
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

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