EPSTEIN-BARR VCA VIRCLIA® IgG MONOTEST

For *in vitro* diagnostic use

VCM027: Indirect chemiluminescent immunoassay (CLIA) to test IgG antibodies against VCA (Viral Capside Antigen) of Epstein-Barr virus (EBV) 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® VCA EPSTEIN-BARR IgG 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 VCA of Epstein-Barr virus.

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

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers and Neolone and Bronidox as preservatives.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservative.

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

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

Store at 2-8ºC and check expiration date.

Materials required but not supplied:

-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)
-Precision micropipettes 5 and 100 µl.
-Eight channel micropipette 100 µl.
-Adapted microplate washer.
-Thermostatized incubator/water bath.
-Microplate luminometer.
-Alternatively, a CLIA automated processor.

STORAGE REQUIREMENTS:

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

STORAGE OF REAGENTS ONCE OPENED:

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

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

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

Substrate component A is light sensitive. Avoid light exposure. Substrate solution 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. 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.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly

after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.
7. Serum dilution solution, reaction wells, conjugates and calibrator in this kit include substances of animal origin. Calibrator includes as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. Reaction wells are coated with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
8. Substrate solution may be irritant to eyes, respiratory system and skin. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available.
9. Do not use this product in automated processors unless they have been previously validated for that purpose.

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 samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

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 37ºC before diluting. Once diluted, store at 2-8ºC.

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**

1. Set incubator/water bath to 37±1ºC.
2. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
3. Remove the monodoses from the package and let them reach room temperature before use (approximately 20 minutes). Determine the numbers of monodoses to be employed (one for every sample to be tested).
4. Place the strips into a frame. With the aid of a clean pipette tip, puncture the foil of the well in position E (see the left side of the frame for reference), draw 100 µl of reagent (sample diluent) and dispense it into the white well in position B. Add 5 µl of sample into the white well in position B. Mix homogenously with the aid of a pipette. With the aid of a clean pipette tip, puncture the foil of the well in position F, draw 100 µl of reagent (calibrator) and dispense it into white well in position A. Draw 80 µl of reagent from well E with a new clean tip and add it into the white well in position C, then draw 20 µl of reagent from well F and add it into the white well in position C. Mix homogenously with the aid of a pipette.
5. Incubate at 37±1ºC for 20 minutes.
6. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
7. Immediately add 50 µl of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) into each one of wells A, B, C.
8. With the aid of a clean pipette tip, puncture the foil of the well in position D, draw 50 µl of reagent (conjugate) and dispense it into the white well in position A. Repeat this step for wells B and C.
9. Incubate at 37±1ºC for 20 minutes.
10. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
11. With the aid of a clean pipette tip, puncture the foil of the well in position H, draw 50 µl of reagent (substrate component A) and dispense it into the white well in position A. Repeat this step for wells B and C.
12. With the aid of a clean pipette tip, puncture the foil of the well in position G, draw 50 µl of reagent (substrate component B) and dispense it into the white well in position A. Repeat this step for wells B and C. Mix gently for 10 seconds with the aid of a microtiter plate shaker or by gently tapping on the sides of the plate.
13. Incubate at 37ºC for 5 minutes protected from light.
14. Measure relative luminescence units (RLU) in wells A, B, C with the help of a luminometer.

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.

RLU of the calibrator and the negative control must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	RLU
CALIBRATOR	2-7
NEGATIVE CONTROL	<2

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.

LIMITATIONS:
1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. 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.
8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
9. Sera from immunosuppressed patients may show false negative results.
10. Other diseases (cytomegalovirus, toxoplasmosis, adenovirus and rubella) produce similar syndromes to infectious mononucleosis, and should also be tested in suspected cases of this syndrome.
11. 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.
12. The performance characteristics have not been studied for patients with nasopharyngeal carcinoma, Burkitt´s lymphoma and other EBV associated diseases different from infectious mononucleosis.
13. 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.
14. 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:**

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

Samples No.	Sensitivity	Specificity
85	100%	100%

Indeterminate values were omitted from the final calculations.

- **INTRA-ASSAY PRECISION:**

3 sera were individually run 10 times each serum in a single automated assay in essentially unchanged conditions.
The results were as follows:

Serum	N	% C.V.
Sample +	10	6
CAL	10	4
CN	10	6

C.V. Coefficient of variation

- **INTER-ASSAY PRECISION:**

3 sera were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Serum	N	% C.V.
Sample +	10	5
CAL	10	7
CN	10	11

C.V. Coefficient of variation

- **CROSS REACTIVITY AND INTERFERENCES:**

11 samples known to be positive for other herpesvirus (herpes simplex type 1 and 2, cytomegalovirus, varicella-zoster) and members of the syndromic group (*Brucella melitensis*) were assayed. 3 samples known to be positive for antinuclear antibodies were assayed.
The negative results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

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

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