EPSTEIN-BARR VIRUS (VCA) IFA IgM

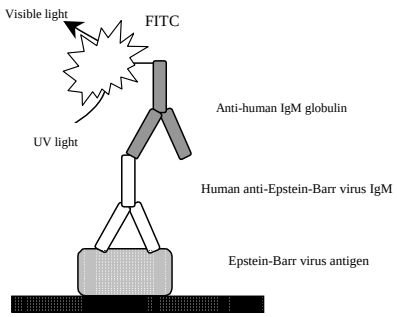
PVCAM10: Indirect immunofluorescent assay (IFA) kit for the diagnosis of Epstein-Barr virus IgM antibodies in human serum.

INTRODUCTION:

Antibodies in response to the viral capsid antigens (VCA) of Epstein-Barr (EBV) are found early in the infection. IFA is the most widespread technique due to its simplicity, precocity and the absence of cross-reactions. Infectious mononucleosis is the most common disease caused by EBV, leading to fever, cervical adenopathies, splenomegaly, and pharyngitis. Some of this cases can be produced 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.

PRINCIPLE OF THE TEST:

The IFA method is based upon the reaction of antibodies in the sample, tested with the antigen adsorbed on the slide surface. The specific antibodies present in the sample react with the antigen, and the immunoglobulins not binded to the antigen are removed in the washing step. In the next step, the antigen-antibody complexes react with the fluorescein-labeled anti-human globulin. It can be examined using an immunofluorescence microscope.



KIT FEATURES:

All reagents, except for the PBS, are supplied ready to use. All the reagents have a number assigned for an easy identification. In the Assay Procedure, the numbers of the reagents to be used in each step are indicated.

KIT CONTENTS:

1 VIRCELL EPSTEIN BARR VIRUS (VCA) SLIDE: 10 slides of 10 wells each, coated with formaldehyde treated and acetone fixed P3HR1 cells (ATCC HTB-62), of which only 10-20% express VCA antigens.
2 VIRCELL PBS: 1 vial of PBS pH 7.2 powder to reconstitute with 1 l of distilled water.
3 VIRCELL EBV IgM POSITIVE CONTROL: 200 µl of positive control serum, containing sodium azide.
4 VIRCELL EBV NEGATIVE CONTROL: 200 µl of negative control serum, containing sodium azide.
5M VIRCELL ANTI-HUMAN IgM FITC CONJUGATE: 2 vials with 1.1 ml of fluorescein-labeled anti-human IgM fluorescein conjugate in a phosphate buffer containing Evan´s blue, sodium azide and a protein stabilizer.
6 VIRCELL MOUNTING MEDIUM: 3 ml of mounting medium: buffered glycerol, containing sodium azide.
7 VIRCELL ANTI HUMAN IgG GLOBULIN (SORBENT): 2 vials with 1.5 ml of sorbent (goat anti-human IgG, containing sodium azide).

Store at 2-8ºC and check expiration date.

Materials required, but not supplied:
Adequate precision micropipettes.
Thermostatized incubator.
Distilled water.
24x60 mm coverslips.
Fluorescence microscope and suitable filters according to the manufacturer´s recommendations.
Humid chamber.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date printed on the label. Kits are stable through the end of the month indicated in the expiration date, when stored closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

REAGENT	STABILITY AND STORAGE
Reconstituted PBS	4 months at 2-8ºC, never beyond its expiration date
Rest of the components	Refer to package label for expiration date (at 2-8ºC)

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations. Use only the amount of sorbent, PBS, control serum and conjugate solutions required for the test. Do not return the excess solution into the bottles. After reconstitution, store PBS at 2-8ºC and do not use if turbidity appears.

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 differents kits or manufacturers. Only the PBS, sorbent, mounting medium solutions and slides are compatible with the equivalents from other VIRCELL IFA references and lots. The rest of the components are compatible with other kits when the lot is the same.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Do not use in the event of damage to the package.
5. Never pipette by mouth.
6. Sorbent, conjugates and controls in this kit include substances of animal origin. Controls include as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for HBsAg, Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. The wells are coated with inactivated Epstein-Barr virus antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that these or other infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
7. Conjugate, sorbent, mounting medium and controls contain sodium azide (concentration <0.1%). Avoid contact with acids and heavy metals.
8. Mounting medium contains glycerol. Avoid contact with acids and keep away from high temperatures.

9. Evan´s blue (concentration <0.1%) is a carcinogen. Avoid contact with skin or eyes. In case of contact with this solution, rinse thoroughly with water and seek medical attention.
10. Use only protocols described in this insert. Incubation times and temperatures other than specified may give erroneous results.
11. Cross-contamination of patient specimens on a slide can cause erroneus results. Take precautions to avoid it.
12. Microscope optics, light source condition and type will affect the fluorescence quality.
13. Do not leave the reagents at room temperature longer than absolutely necessary.
14. Each slide can be use only once. Do not break it, and do not reuse the wells not used.
15. The glass elements contained in kits could cause physical damage in the event of break. Handle with care.
16. Check that a visible precipitate appears after the addition of the sorbent to the sample.

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, to avoid immunoglobulin titer decrease, specially IgM. Do not use hyperlipemic or contaminated sera. Samples containing particles should be clarified by centrifugation.

PRELIMINARY PREPARATION OF THE REAGENTS:

Only the PBS must be prepared in advance. Add the contents of the vial 2 to 1 litre of distilled water. Shake it until the complete disolution. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:

1.-Bring all reagents to room temperature before use. Allow the slides to reach room temperature before opening.
2.-Prepare a 1/2 dilution of serum samples by adding 25 µl of sample to 25 µl of PBS 2. The control sera 3 and 4 should not be diluted.
3.-Treat diluted sera with anti-human IgG sorbent 7, by adding 5 µl of sera to 25 µl of sorbent and thoroughly mix. Control sera 3 y 4 must not be diluted nor sorbent treated. The treated sera should be centrifugated to remove the precipitate, which interfere with the test.
4.-Add 20 µl of sorbent-treated serum in every slide well 1. Do the same with positive 3 and negative 4 control.
5.-Place the slide in a humid chamber and incubate at 37ºC for 90 minutes.
6.-Rinse slide 1 briefly with a gentle stream of PBS 2 (avoid directing PBS at wells) and immerse for ten minutes in PBS. Dip wash slide briefly in distilled water.
7.-Allow the slide 1 to air dry.
8.-Add 20 µl of anti-human IgM FITC conjugate solution 5M to each well. (No dilution required).
9.-Incubate slide in a humid chamber for 30 minutes at 37ºC.
10.-Repeat steps 6 and 7.
11.-Add a small drop of mounting medium 6 to each well and carefully cover with a coverslip.
12.-Read the slide as soon as possible in a fluorescence microscope at 400x magnification. If this is not possible, store in the dark at 2-8ºC up no more than 24 hours, until observation.

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:

Positive and negative controls should be included into each test run. It allows the validation of the assay and kit.
The observed fluorescence pattern should be:
Positive control: Apple green peripheral fluorescence.
Negative control: Red cellular pattern.

INTERPRETATION OF RESULTS:

The reaction is positive when apple green peripheral fluorescence can be observed.
The reaction is negative when a red cellular pattern can be observed.
Occassionally, a serum may react giving a positive fluorescence with over 50% of the cell. This pattern corresponds to a non-evaluable inespecific reaction.
Results different from the specified in this insert should not be considered as positive.
IgG and IgM antibodies show a different behaviour during the primoinfections and reinfections. In a primoinfection IgG and IgM appear in almost all cases (IgM appears before than IgG). In reinfections IgM antibodies do not appear in all cases, therefore IgG detection is the only method useful to perform the diagnosis. High titers of IgG can exist in a lot of diseases during the whole patient life, while IgM, generally, only is measurable in sera during 2 or 3 months after the infection, and therefore is a suitable marker of recent infection.

LIMITATIONS:

1.-This kit is intended to be used with human serum.
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.
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 patients with autoimmune diseases may give a non-specific reaction over cells when using IFA. Those sera cannot be evaluated with this method.

PERFORMANCE

SENSITIVITY AND SPECIFICITY:

101 serum samples were assayed with EPSTEIN-BARR VIRUS (VCA) IFA IgM against another commercial available IFA KIT.
The results were as follows:

	SAMPLE NR	SENSITIVITY	SPECIFICITY
IgM	101	97.9%	100.0%

Sera with non-specific reactivity were excluded from final calculations.

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INTRA-ASSAY PRECISION:

3 sera (2 positive and 1 negative) were individually pipetted in groups of 5 in a single assay performed by the same operator in essentially unchanged conditions.
Titer shifts of no more than one dilution were observed.

INTER-ASSAY PRECISION:

3 sera (2 positive and 1 negative) were individually pipetted on 5 different conditions in which the operator or the test day were different.
Titer shifts of no more than one dilution were observed.

CROSS REACTIVITY AND INTERFERENCES:

9 samples known to be positive for other virus or microorganism of the taxonomic group (citomegalovirus, herpes simplex type 1 and 2, varicella-zoster) and syndromic group (*Brucella melitensis*), were assayed.
The negative results of the test demonstrate the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

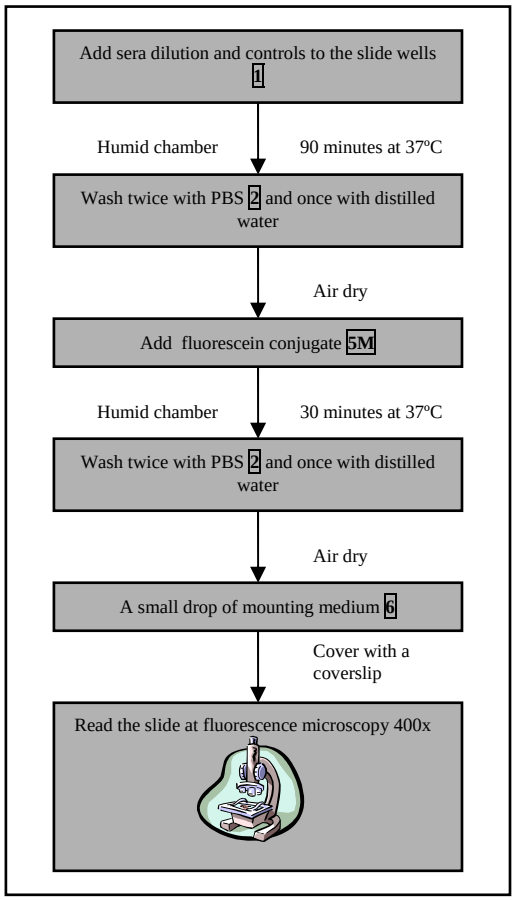
OTHER INTERFERENCE STUDIES:

An IFA assay was performed to 25 sera, known to be positive for rheumatoid factor, to determine IgG and IgM antibodies against 2 viral and 2 bacterial antigens. It also was performed an IFA assay for IgG and IgM testing to another 2 sera determined for each one antigen. For IgM testing the sera were treated with anti-IgG sorbent. The results showed the efficacy of the sorbent to avoid interferences in IgM testing caused by rheumatoid factor.
The recommended sorbent has been tested and found effective to prevent false negative results due to an excess of IgG antibodies.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
8ºC 2ºC	Store at 2-8ºC
Σ x	Contains sufficient for <X> tests
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS x	<X> wells

SUMMARY OF THE ASSAY PROCEDURE:



LITERATURE:

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