CYTOMEGALOVIRUS VIRCLIA® IgG AVIDITY MONOTEST

For *in vitro* diagnostic use

VCM038: Indirect chemiluminescent immunoassay (CLIA) to test avidity of IgG antibodies against cytomegalovirus in human serum/plasma. 12 tests.

INTRODUCTION:

Cytomegalovirus (CMV) is a member of the herpesvirus family and characteristically produces latent infection after primoinfection. It is an important agent of congenital infection and can produce life-threatening illness in transplant and AIDS patients. An active infection resulting from a primary or reactivated latent infection during pregnancy may be transmitted to the fetus or to the infant during birth. Children congenitally infected can have severe central nervous system damage. The risk of severe abnormalities is greatest when maternal infection occurs in the first trimester of pregnancy. A primary infection in adults may be asymptomatic or result in various syndromes, including mononucleosis, hepatitis or pneumonitis. The presence of active CMV infection can be detected by serological methods, but have to be confirmed by using viral isolation, antigen or nucleic acids detection. Although IgM detection is commonly associated to primary infections, IgM may persist long after an acute infection at above detection levels. IgG avidity testing can be of aid for the serological diagnosis of primary infections. Avidity reflects the aggregate strength with which polyclonal IgG binds to antigens measured by the effect that a chemical denaturant has onto that binding. The avidity of IgG is low early in infection, gradually maturing to high-avidity IgG approximately 22–24 weeks after 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 assayed in parallel in two strips. Unbound immunoglobulins are washed off. Upon addition of a urea solution to one of the strips, only high avidity antibodies remain bound to the antigen on the plate. The other strip receives normal washing solution during this step. 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. Avidity is expressed as an index calculated with the RLUs measured in both strips.

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.
Dissociating solution is divided in several vials.

KIT CONTENTS:

1 VIRCLIA® CYTOMEGALOVIRUS IgG AVIDITY A MONODOSE: Blue. 12 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:
Wells A, B, C: reaction wells; wells coated with CMV antigen, strain AD169.
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: Low avidity calibrator: clear; low avidity rabbit IgG dilution containing Neolone and Bronidox as preservative.
Well G: Substrate component B: clear; containing peroxide.
Well H: Substrate component A: clear; containing luminol.

2 VIRCLIA® CYTOMEGALOVIRUS IgG AVIDITY B MONODOSE: Black. 12 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:
Wells A, B, C: reaction wells; wells coated with CMV antigen, strain AD169.
Well D: Conjugate: orange; containing anti-human IgG peroxidase conjugate dilution and Neolone and Bronidox as preservatives.
Well E: Conjugate: orange; containing anti-rabbit IgG peroxidase conjugate dilution and Neolone and Bronidox as preservatives.
Well F: High avidity calibrator: clear; high avidity serum dilution containing Neolone and Bronidox as preservative.
Well G: Substrate component B: clear; containing peroxide.
Well H: Substrate component A: clear; containing luminol.

3 VIRCLIA® DISSOCIATING SOLUTION: 3 vials each with 3 ml of Tris-saline buffer containing urea, Tween-20 and Proclin.

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
Rest of reagents	Refer to package label for expiration date (at 2-8ºC)

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 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. 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. Do not use this product in automated processors unless they have been previously validated for that purpose.
9. This kit is not intended for CMV IgG determination so only samples already known as positive must be used for the assay.

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**

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:
1. Each monodose includes one low avidity calibrator (well A) and one high avidity calibrator (well C). It allows the validation of the assay and kit.

RLU and avidity of the calibrators must fall in the following ranges. Otherwise, the test is invalid and must be repeated.
Calculate avidity indexes for calibrators:

Avidity index= (calibrator RLU in monodose A /calibrator RLU in monodose B)

Control	RLU
HIGH AVIDITY CALIBRATOR	2-7
LOW AVIDITY CALIBRATOR	2-7

Control	AVIDITY
HIGH AVIDITY CALIBRATOR	> 0.50
LOW AVIDITY CALIBRATOR	<0.30

2. Calculate the antibody index of the sample:

Antibody index = (sample RLU in monodose B/high avidity calibrator RLU in monodose B)

If antibody index is lower than 1.1, avidity results cannot be interpreted since the anti-CMV IgG levels of the sample are below the detection limit of the technique.

3. If the sample RLU is > 40, repeat the test with a 1/100 dilution of the sample.

INTERPRETATION OF RESULTS:

Avidity index= (sample RLU in monodose A /sample RLU in monodose B)

Index	Interpretation
<0.4	Low avidity
0.4-0.5	Intermediate avidity
>0.5	High avidity

Avidity indexes lower than 0.40 are more in favor of recent primo-infection of less than 4 months. However, such results do not allow confirmation of this diagnosis with absolute certainty.

Avidity indexes between 0.40 and 0.50 do not enable to distinguish a recent infection from a former infection. For these samples, other markers and/or avidity determination methods or a new serum sample (collected 3 or 4 weeks later) should be used.

Avidity indexes higher or equal to 0.50 are more in favor of past-infection of more than 5 months. However, such results do not allow exclusion of a recent primo-infection of less than 4 months with absolute certainty.

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. The result of avidity testing does not constitute sufficient proof for the diagnosis of a recent infection by CMV.
6. Some individuals show persistence of low avidity antibodies in serum for months.
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.
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. A negative result in immunosuppressed patients does not always exclude the possibility of infection.
10. 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:**

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

Samples No.	Sensitivity	Specificity
98	94%	99%

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	4
*LAvC	10	9
**HAvC	10	6

C.V. Coefficient of variation
*LAvC. Low avidity calibrator
**HAvC. High avidity calibrator

- **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	6
*LAvC	10	13
**HAvC	10	10

C.V. Coefficient of variation
*LAvC. Low avidity calibrator
**HAvC. High avidity calibrator

- **CROSS REACTIVITY AND INTERFERENCES:**

10 samples known to be positive for other specimens of the herpesvirus group (herpes simplex virus, Epstein-Barr virus, varicella-zoster virus) and other members of the syndromic group (*Toxoplasma gondii*) were assayed*****. 2 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.

*****These results have been obtained with CYTOMEGALOVIRUS VIRCLIA® IgG MONOTEST kit, Ref. VCM021.

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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