COVID-19 VIRCLIA® IgG MONOTEST

For *in vitro* diagnostic use

VCM097: Indirect chemiluminescent immunoassay (CLIA) to test IgG antibodies against SARS-CoV-2 in human serum/plasma. 24 tests.

INTRODUCTION:

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a new pathogen that emerged in the Chinese province of Hubei in December 2019 and spread worldwide in the following months having been declared pandemic in March 2020. Coronaviruses are enveloped, positive-sense, and single-stranded RNA viruses. SARS-CoV-2 shows great genetic homology with SARS-CoV and other SARS-like bat coronaviruses. The disease has been named as COVID-19 and may manifest either as an asymptomatic infection, a mild upper respiratory tract infection or a severe viral pneumonia with respiratory failure and even death. COVID-19 outbreaks cause significant mortality and morbidity. The signs and symptoms at illness onset include fever, cough, fatigue, anorexia, shortness of breath, sputum production or myalgias. Age and several co-morbidities (diabetes, cardiovascular or respiratory chronic diseases) are strong risk factors for severe illness, complications, and death. Transmission occurs mostly from person-to-person via respiratory droplets among close contacts. Aerosol and fomite transmission are plausible.

Detection of the virus nucleic acid in samples from the upper and lower respiratory tract is the most reliable laboratory diagnosis. Viral RNA shedding is greatest at the time of symptom onset and declines over the course of infection. The detection of RNA during convalescence does not necessarily indicate the presence of viable infectious virus. The sample type and collection procedure as well as the method of extraction may impact the recovery of viral RNA and lead to false negative results. Early serological responses have been described with a mean time of 11 days after symptom onset. Several relevant applications have been pointed out for serological tests: as an aid in diagnosis of patients with several days of evolution, or in suspected cases with repeatedly negative RNA results; in epidemiological serosurveys to determine the precise rate of infection; in the identification of individuals who could serve as donors for plasma immunotherapy strategies; to determine the immune status of individuals, specially in healthcare workers in order to limit their risk of exposure or inadvertent spread of the virus. The spike protein and the nucleoprotein have been suggested as the main targets for the measurement of antibody responses.

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® COVID-19 IgG MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:

Wells A, B: reaction wells; wells coated with antigen of SARS-CoV-2. Contain inactivated antigen. Contain material of animal origin.

Well C: blank reaction well; well processed and blocked similarly to the reaction well except that it is not coated with antigen of SARS-CoV-2. 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 2-8ºC and check expiration date.

Materials required but not supplied:
-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR).
-A CLIA automated processor.
-Precision micropipettes.

STORAGE REQUIREMENTS:

Store at 2-8ºC. 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 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 origin and/or human 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 (2-8ºC) 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 samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma. Samples should be inactivated at 56ºC 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 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:

Each monodose includes one calibrator (well A). 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:

Each sample is assayed onto two reaction wells: one coated with antigen and one processed and blocked similarly to the reaction well except that it is not coated with antigen. The blank well is used to subtract possible unspecific backgrounds.

Antibody index= ((sample antigen RLU - sample blank RLU)/calibrator RLU)

Index	Interpretation
<1.4	Negative
1.4-1.6	Equivocal
>1.6	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.

Samples with indexes below 1.4 are considered as not having antibodies of the specificity and class measured by this kit.

Samples with indexes above 1.6 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. 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.
11. The performance results showed correspond to studies in a defined population sample. Small differences can be found with different populations.

PERFORMANCES:

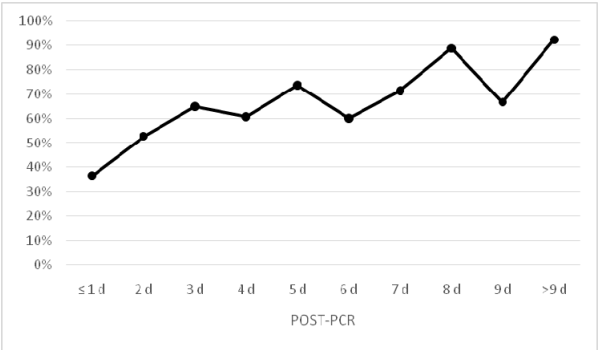
- **POSITIVE AND NEGATIVE PERCENTAGES**

867 positive and negative samples were assayed, from which 675 samples were collected from hospitalized patients at different days post-PCR+, and 192 negative pre-pandemic samples were selected from healthy donors. Positive and negative percentages of IgG were calculated:

Patients post-PCR+samples No.	675
Positive IgG (%)	56
Donors pre-pandemic samples No.	192
Negative IgG (%)	99

In addition, the evolution of the positivity percentage of IgG in a subset of 354 samples from hospitalized patients according to the time after the first PCR positive result was evaluated:

Time (days)	Samples (total)	IgG Positive	%IgG Positive
≤ 1 d	126	46	37
2 d	38	20	53
3 d	20	13	65
4 d	28	17	61
5 d	19	14	74
6 d	10	6	60
7 d	7	5	71
8 d	18	16	89
9 d	9	6	67
>9 d	79	73	92



- **WITHIN-RUN PRECISION:**

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

SAMPLE	%C.V.
Calibrator	15
Positive sample	10
Sample blank	10

C.V. Coefficient of variation

- **BETWEEN-RUN PRECISION:**

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

SAMPLE	%C.V.
Calibrator	16
Positive sample	11
Sample blank	27

C.V. 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 (5 samples tested) were found. No interferences with rheumatoid factor (5 samples tested) were found.

Interferences – Endogenous substances:

3 samples were tested with each interferent. Specifications were fulfilled in all cases. No interferences 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 were found

Interferences – Anticoagulants:

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

- **CROSS REACTIONS**

70 samples known to be positive for other microorganisms (parainfluenza 1 virus, parainfluenza 2 virus, parainfluenza 3 virus, influenza A virus, influenza B virus, adenovirus, *Mycoplasma pneumoniae, Chlamydophila pneumoniae*, *Coxiella burnetii, Legionella pneumophila* and respiratory syncytial virus) were assayed.

No cross reactivity with parainfluenza 1 virus (5 samples tested), parainfluenza 2 virus (1 sample tested), parainfluenza 3 (5 samples tested), influenza A virus (8 samples tested), influenza B virus (8 samples tested), adenovirus (8 samples

tested), *Chlamydophila pneumoniae* (8 samples tested), *Coxiella burnetii* (8 samples tested), *Legionella pneumophila* (4 samples tested) and respiratory syncytial virus (7 samples tested) was found. Cross reactivity with *Mycoplasma pneumoniae* (1 out of 8 samples tested) was found.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
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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