COVID-19 SPIKE QUANTITATIVE VIRCLIA® IgG MONOTEST

REF VCM100 Σ 24

CE For *in vitro* diagnostic use

INTENDED PURPOSE

Indirect chemiluminescent immunoassay (CLIA) to test IgG antibodies against SARS-CoV-2 Spike in human serum/plasma.
The test is a qualitative/quantitative and automated assay, intended to be used as an aid to diagnosis.

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, shortness of breath, fatigue, myalgias, headache, anosmia, sore throat, congestion or runny nose, nausea and diarrhea. Age and several co-morbidities (obesity, 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 as a follow-up of vaccinated individuals. Antibodies reacting with epitopes on the spike protein of the virus contribute the majority of the neutralizing activity of the immune response. Correlation between anti-spike antibody levels and neutralization titers have been found both in infected patients and in vaccinated individuals.
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 (ChemiLuminescent ImmunoAssay) tests with shorter incubation times while keeping or improving their sensitivity.

TEST PRINCIPLE

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.

MATERIALS PROVIDED

[1] VIRCLIA® COVID-19 SPIKE QUANTITATIVE 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. Contains inactivated antigen. Contains 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.
VIRCLIA® COVID-19 SPIKE QUANTITATIVE IgG 5-PL: Labels containing the values obtained for the different parameters during the generation of the master calibration curve at the manufacturer site. It contains specific labels for Vircell's automated systems, which are differentiated by a univocal symbol, as indicated in the section "Symbols used in labels".

Special materials required but not provided:
-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR).
-A CLIA automated processor.

STORAGE AND HANDLING CONDITIONS

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.

IN-USE STABILITY

VIRCLIA® MONODOSE: Once opened, use it in the same day.
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.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. 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.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. 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.
6. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
11. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
12. Keep containers for samples and reagents closed while they are not being handled.
13. Avoid using samples subjected to repeated freeze-thaw cycles.
14. Handle in aseptic conditions to avoid microbial contaminations.
15. Reagents in this kit could include substances of animal and/or human origin and/or inactivated antigen (refer to "Materials provided"). 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.
16. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the VIRCLIA® AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.

17. Do not use this product in automated processors unless they have been previously validated for that purpose.
18. 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.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

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.
Inactivation of samples at 56°C for 30 minutes is recommended before testing. Qualified healthcare staff can choose between inactivating or not inactivating the samples, according to their professional judgement. To this effect, please refer to the information included in the "Calculations and interpretation of results", "Limitations of use" and "Performance characteristics" sections.

PREPARATORY TREATMENT OF THE DEVICE

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

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.

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.

CALCULATIONS AND 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
<0.6	Negative
0.6-0.7	Equivocal
>0.7	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 0.6 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 0.7 are considered as having antibodies of the specificity and class measured by this kit.

In order to optimize assignment of signals to quantitative values, a master calibration curve can be used. For each lot, a 5-PL standard curve is established by Vircell from data generated by running samples containing different concentrations of the standard analyte in repeated independent runs. In order to compensate inter-run and inter-laboratory variations, indexes are used in the master calibration curve generation.
Determination of antibody concentrations (Conc) is carried out by the 5-parameter logistic (5 PL) model using the sample index according the following formula:

$$Conc = C\left(\left(\frac{A-D}{(SAMPLE\ RLU/CAL) - D}\right)^{\frac{1}{G}} - 1\right)^{\frac{1}{B}}$$

Where A, B, C, D and G define the exact shape of the curve:
A. Lower asymptote
B. Slope of the curve
C. Turning point
D. Upper asymptote
G. Parameter for Curve Asymmetry

These variables shown in an external label of the kit must be input onto the software of the instrument to get an automatic calculation if the concentration of the sample derived from this method is intended.
The IgG antibody activity is expressed in IU/mL and referenced to the WHO Reference Reagent for SARS-CoV-2 antibody, human serum NIBSC code: 20/136.

LIMITATIONS OF USE

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 been evaluated for use only in patients with clinical signs and symptoms of infection or in vaccinated individuals.
10. A calibration curve run in parallel together with the patient samples provides maximum accuracy and minimizes errors derived from interlaboratory or interassay variability.
11. If the master calibration curve is used, the parameters specific for the lot in use must be carefully input in the formula, otherwise the calculated concentrations will be erroneous.
12. It is recommended that laboratories carrying out quantitative calculations establish their own cutoff point after reviewing available literature and according to their own experience.
13. The master curve has been adjusted by Vircell for the corresponding automated systems. The parameters that define the curve are different in each system and, therefore, they are not interchangeable between them.
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 predicate devices in a defined population sample. Small differences can be found with different populations or different predicate devices.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

TEST 1 -Inactivated samples
Serum/plasma samples were assayed against a commercial ELISA kit.
The results were as follows:

Samples No.		176
Sensitivity (%)		96
	95% CI	90-98
Specificity (%)		100
	95% CI	98-100
PPV (%)		100
NPV (%)		97
LR+/LR-		-0.97 / -0.95

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

TEST 2 -Non-inactivated samples

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

Samples No.		100
Sensitivity (%)		100
	95% CI	92-100
Specificity (%)		100
	95% CI	93-100
PPV (%)		100
NPV (%)		100
LR+/LR-		-1.01 / -0.99

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

COMPARISON TO NEUTRALIZATION ASSAY

TEST 1

For calculation of the sensitivity, 85 serum samples of patients were assayed against a neutralization assay. For calculation of specificity, 100 pre-pandemic plasma samples were assayed.
The results were as follows:

Samples No.		185
Sensitivity (%)		99
	95% CI	92-100
Specificity (%)		100
	95% CI	96-100
PPV (%)		100
NPV (%)		99
LR+/LR-		-1.00 / -0.98

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

TEST 2

135 samples corresponding to 45 vaccinees (3 samples per each) were assayed against a neutralization assay. Agreement was measured through Cohen's kappa coefficient with a value of 0.951, indicating "almost perfect" agreement.

PRECISION

3 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	5.7	7.7	5.2	10.9
Positive sample	10.7	8.4	8.6	16.1
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 rheumatoid factor and antinuclear antibodies 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 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 γ-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 IU/mL), citrate (0.13 mol/L) and EDTA (2 mg/mL).

CROSS REACTIVITY

133 samples known to be positive for other microorganisms (parainfluenza virus, influenza A virus, influenza B virus, adenovirus, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Coxiella burnetii*, *Legionella pneumophila*, respiratory syncytial virus, Epstein-Barr VCA, Hepatitis A virus, Hepatitis B virus, cytomegalovirus and coronavirus no COVID-19) were assayed.
No cross reactivity with parainfluenza virus (11 samples tested), influenza A virus (5 samples tested), influenza B virus (7 samples tested), adenovirus (10 samples tested), *Mycoplasma pneumoniae* (10 samples tested), *Chlamydophila pneumoniae* (10 samples tested), *Coxiella burnetii* (10 samples tested), *Legionella pneumophila* (10 samples tested), respiratory syncytial virus (10 samples tested), Epstein-Barr VCA (10 samples tested), Hepatitis A virus (10 samples tested), Hepatitis B virus (10 samples tested), cytomegalovirus (10 sample tested) and coronavirus no COVID-19 (10 samples tested) was found.

ANALYTICAL SENSITIVITY / LIMITS OF DETECTION AND QUANTIFICATION (LoB, LoD, LoQ)

4 negative samples were run in triplicate with 2 different batches of the kit during 3 days. LoB, LoD and LoQ were calculated.
The results were as follows:
VIRCLIA® (TB)

LoB	1.68 UI/ml
LoD	2.77 UI/ml
LoQ	7.16 UI/ml

VIRCLIA® LOTUS

LoB	1.80 UI/ml
LoD	3.11 UI/ml
LoQ	8.36 UI/ml

TRUENESS / ACCURACY

This test is only performed when there is a certified reference material or a certified method of reference.
The reference material for this product is the WHO International Standard SARS-CoV-2 Antiserum (Human) 1st IS.
8 samples were run in triplicate in 3 different runs in, at least, 2 different automated systems. Bias was calculated and the results were as follows:
VIRCLIA® (TB)
Bias (trueness / accuracy) = 14.50 %
VIRCLIA® LOTUS
Bias (trueness / accuracy) = 16.50 %

MEASURING RANGE

Measuring range was stablished as: LoQ + highest internal calibrator.
The results were as follows:
VIRCLIA® (TB)
Measuring range: 1007.16 IU/ml
VIRCLIA® LOTUS
Measuring range: 1008.36 IU/ml

SYMBOLS USED IN LABELS

IVD	*In vitro* diagnostic medical device
	Use-by (expiry date)
	Store at x-y°C
	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number

Consult instructions for use

Parameters of specific Master curve for VIRCLIA® LOTUS automated system

Parameters of specific Master curve for VIRCLIA® (TB) automated system

Manufacturer

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