COVID-19 ELISA IgG

REF G1032 Σ 96

CE For *in vitro* diagnostic use

INTENDED PURPOSE

Indirect immunoenzyme assay to test IgG antibodies against SARS-CoV-2 in human serum/plasma.
The test is a qualitative and manual or alternatively 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, 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.

TEST PRINCIPLE

The ELISA 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 substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding the acid stopping solution.

KIT FEATURES

All reagents, except for the washing solution, are supplied ready to use.
Serum dilution solution and conjugate are coloured to help in the performance of the technique.
Break-apart individual wells are supplied, so that the same number of wells is consumed than the number of tests performed.

MATERIALS PROVIDED

[1] VIRCELL COVID-19 PLATE: 1 96-wells plate coated with antigen of SARS-CoV-2. Contains inactivated antigen. Contains material of animal origin.
[2] VIRCELL SERUM DILUENT: 25 ml of serum dilution solution: a blue coloured phosphate buffer containing protein stabilizers. Contains Neolone and Bronidox. Contains material of animal origin. Ready to use.
[3] VIRCELL IgG POSITIVE CONTROL: 1.5 ml of positive control serum. Contains Neolone and Bronidox. Contains material of human origin. Contains material of animal origin.
[4] VIRCELL IgG CUT OFF CONTROL: 1.5 ml of cut off control serum. Contains Neolone and Bronidox. Contains material of human origin. Contains material of animal origin.
[5] VIRCELL IgG NEGATIVE CONTROL: 1.5 ml of negative control serum. Contains Neolone and Bronidox. Contains material of human origin. Contains material of animal origin.
[6] VIRCELL IgG CONJUGATE: 2 x 7.5 ml of an orange-coloured anti-human IgG peroxidase conjugate dilution. Contains Neolone and Bronidox. Contains material of animal origin. Ready to use.
[7] VIRCELL TMB SUBSTRATE SOLUTION: 15 ml of substrate solution containing tetramethylbenzidine (TMB). Ready to use.
[8] VIRCELL STOP REAGENT: 15 ml of stopping solution: 0.5 M sulphuric acid.
[9] VIRCELL WASH BUFFER (20x): 50 ml of 20x washing solution: a phosphate buffer containing Tween®-20 and Proclin 300.

Special materials required but not provided:
-Precision micropipettes.
-ELISA plate washer.
-Thermostatized incubator/water bath.
-ELISA plate spectrophotometer with a 450 nm measuring filter and a 620 nm reference filter.
-Alternatively, an ELISA automated processor.
-Distilled water.

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

VIRCELL WASH BUFFER diluted (1x): 4 months at 2-8°C.
Rest of reagents: Refer to package label for expiration date (at 2-8°C).
Substrate solution is light sensitive. Avoid light exposure and discard if blue colour develops during storage. Substrate solution should not get in contact with oxidizers such as bleach solutions or metals. Make sure that no metal components come in contact with the substrate.
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 VIRCELL WASH BUFFER, VIRCELL TMB SUBSTRATE SOLUTION, VIRCELL STOP REAGENT and VIRCELL SERUM DILUENT are compatible with the equivalents in other VIRCELL ELISA references and lots.

17. Use only the amount of product required for the test. Do not return the excess solution into the vial.
18. During incubation times, an adequate sealing of the plates with the adhesive film included in the kit avoids the desiccation of the samples, and guarantees the repeatability of the results.
19. The kit contains Proclin 300 (refer to "Materials provided"). It may cause an allergic skin reaction. If on skin, wash with plenty of soap and water. For further information a Material Safety Data Sheet is available.
20. The kit contains sulfuric acid (0.5 M) (refer to "Materials provided"). Avoid contact with skin or eyes. If contact occurs, immediately flush the area with water. For further information a Material Safety Data Sheet is available.
21. Before incorporating this product onto an automatic processing system, we strongly recommend the performance of a pre-evaluation assay.
22. 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

Only the VIRCELL WASH BUFFER must be prepared in advance. Fill 50 ml of VIRCELL WASH BUFFER (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.

ASSAY PROCEDURE

1. Set incubator/water bath to 37±1°C.
2. Bring all reagents to room temperature before use (approximately 1 hour), without removing the plate from the bag.
3. Shake all components.
4. Remove the plate [1] from the package. Determine the numbers of wells to be employed counting in 4 wells for the controls: two for the cut off control and one each for the negative and positive control. Wells not required for the test should be returned to the pouch, which should then be sealed.
5. Prepare a 1/20 dilution of serum samples in tubes apart by adding 5 µl of sample to 95 µl of sample dilution solution [2] (1/20 dilution).
6. Add 80 µl of sample dilution solution [2] into all wells except in those assigned to controls. Add 20 µl of the 1/20 dilutions of serum samples, 100 µl of positive control [3], 100 µl of cut off control [4] (in duplicate) and 100 µl of negative control [5] into the corresponding wells.
7. Cover with a sealing sheet and incubate at 37±1°C for 45 minutes.
8. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution [9] per well. Drain off any remaining liquid.
9. Immediately add 100 µl of conjugate solution [6] into each well.
10. Cover with a sealing sheet and incubate at 37±1°C for 30 minutes.
11. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution [9] per well. Drain off any remaining liquid.
12. Immediately add 100 µl of substrate solution [7] into each well.
13. Incubate at room temperature for 20 minutes protected from light.
14. Add immediately 50 µl of stopping solution [8] into all wells.
15. Read with a spectrophotometer at 450/620 nm within 1 hour of stopping.

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, negative and cut off controls must be run with each test run. It allows the validation of the assay and kit.
Optical densities (OD) must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	OD
Cut off control	0.55 < OD < 1.50
Positive control	OD > 0.90
Negative control	OD < 0.50

CALCULATIONS AND INTERPRETATION OF RESULTS

Calculate the mean OD for cut off serum.

Antibody index=(sample OD/cut off serum mean OD) x 10

Index	Interpretation
<4	Negative
4-6	Equivocal
>6	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 4 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 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 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 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.

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.		264
Sensitivity (%)		99
	95% CI	94-100
Specificity (%)		99
	95% CI	97-100
PPV (%)		99
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 -Non-inactivated samples
Serum/plasma samples were assayed against a commercial ELISA kit.
The results were as follows:

Samples No.		262
Sensitivity (%)		99
	95% CI	94-100
Specificity (%)		99
	95% CI	97-100
PPV (%)		99
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

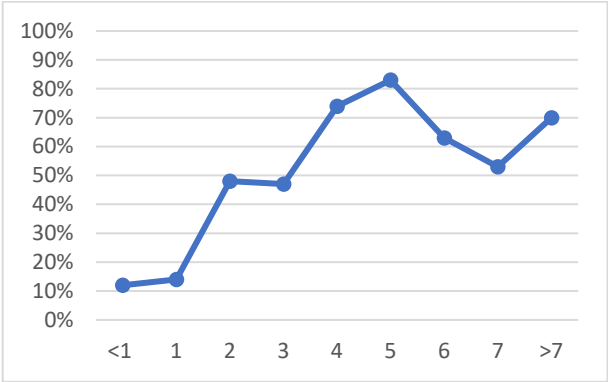
POSITIVE AND NEGATIVE PERCENTAGES

2181 positive and negative samples were assayed, from which 1193 samples were collected from hospitalized patients at different days post-PCR+, and 988 negative pre-pandemic samples were selected from healthy donors. Positive and negative percentages of IgG were calculated:

Patients post-PCR+samples No.	1193
Positive IgG (%)	58
Donors pre-pandemic samples No.	988
Negative IgG (%)	98

In addition, the evolution of the positivity percentage of IgG in a subset of 498 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	84	10	12
1	142	20	14
2	67	32	48
3	45	21	47
4	39	29	74
5	23	19	83
6	27	17	63
7	17	9	53
>7	54	38	70



PRECISION

4 samples were assayed. 2 replicates of each one were analyzed by 2 different operators 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
Cut off control	4.0	1.3	14.1	14.7
Positive control	6.3	2.8	12.7	14.4
Cut off sample	5.2	0.4	16.1	16.9
Negative control	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

39 samples known to be positive for antinuclear antibodies and rheumatoid factor were assayed. Interferences with antinuclear antibodies (1 out of 18 samples tested) were found. Interferences with rheumatoid factor (1 out of 21 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

150 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*, respiratory syncytial virus, Epstein-Barr VCA, Hepatitis A, Hepatitis B, cytomegalovirus and coronavirus no COVID-19) were assayed.
No cross reactivity with parainfluenza 1 virus (5 samples tested), parainfluenza 2 virus (1 sample tested), parainfluenza 3 virus (5 samples tested), influenza A virus (10 samples tested), influenza B virus (10 samples tested), adenovirus (10 samples tested), *Mycoplasma pneumoniae* (10 samples tested), *Chlamydophila pneumoniae* (10 samples tested), *Coxiella burnetii* (10 samples tested), *Legionella pneumophila* (3 samples tested), respiratory syncytial virus (9 samples tested), Epstein-Barr VCA (25 samples tested), Hepatitis A (10 samples tested), Hepatitis B (10 samples tested), cytomegalovirus (12 sample tested) and coronavirus no COVID-19 (10 samples tested) was found.

SYMBOLS USED IN LABELS

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

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