COVID-19 IgG
Confirmation

Strips-module Enzyme Immunoassay for the confirmation of IgG antibodies to COVID-19 major antigens in human serum and plasma

- for “in vitro” diagnostic use only –

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REF COV19CONF.CE
24 Tests

COVID-19 IgG Confirmation

A. INTENDED USE

Module-based Enzyme-Immuno-Assay (ELISA) for the confirmation of samples positive for IgG antibodies to COVID-19 in first screening. The test can be used in addition to identify the specificity of antibodies to the major immunodominant COVID-19 antigens.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first identified amid an outbreak of respiratory illness cases in Wuhan City, Hubei Province, China and has since then caused a global pandemic. SARS-CoV-2 is a positive-sense single-stranded RNA virus and belongs to the Betacoronavirus Genus, which also includes SARS CoV (2003) and MERS CoV (2012). Same as all other coronaviruses, the genome of SARS-CoV-2 (2019-nCoV) encodes the spike protein, the envelope protein, the membrane protein, and the nucleocapsid protein. Those who are infected with COVID-19 may have little to no symptoms. Symptoms of COVID-19 are similar to a cold or flu and may take up to 14 days to appear after exposure to SARS-CoV-2. Symptoms have included: fever, cough, difficulty breathing, pneumonia in both lungs. In severe cases, infection can lead to death. Current tests for SARS-CoV-2 look for genetic material of the virus in oral swabs, using the polymerase chain reaction (PCR). PCR only give a positive result when the virus is still present. The tests can't identify people who went through an infection, recovered, and cleared the virus from their bodies. Enzyme-linked immunosorbent assays (ELISAs) are more rapid serology tests that provide a readout of antigen-antibody interactions. Essentially, patient antibodies are "sandwiched" between the viral protein of interest and reporter antibodies, so that any active patient antibodies are detected.

C. PRINCIPLE OF THE TEST

The module, the product is based on, is composed of the following microplate strips:

Module

		Strips			
		1	**2**	**3**	**4**
Samples	A	Negative control	Spike glycoprot 1	Spike glycoprot 2	Nucleocapsid
	B				
	C				
	D				
	E				
	F				
	G				
	H				
Ag code		**N**	**S1**	**S2**	**C**

Where strips:
N = Negative internal control (BSA coated strip)
S1 = Coated with recombinant Spike glycoprotein 1
S2 = Coated with recombinant Spike glycoprotein 2
C = Coated with recombinant Nucleocapsid ''Core''

The dispensation of specimens and components is monitored by the DiaPro's **S**ample **A**ddition **M**onitoring **S**ystem ("**SAMS**") where the correct addition of the samples and the reagents of the assay is followed up visually by a step-wise change of colors.
Diluted samples (A, B, C etc.) are dispensed, in horizontal along the strips of the module, in all the wells from 1 to 4 (ex.: A1+A2+A3+A4 for sample A).

After washing out all the other components of the sample, in the 2nd incubation bound antibodies are detected by the addition of anti hIgG antibody, labeled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of IgG antibodies present in the sample. The presence of IgG in the sample may be determined semi-quantitatively by means of a cut-off value able to discriminate between negative and positive samples.

D. COMPONENTS

The kit contains reagents for 24 tests.
One complete module has to be used for testing 8 samples for a total of 3 runs.

1.Microplate: MICROPLATE
12 strips x 8 microwells coated with recombinant COVID-19 specific antigens. The plate contains 3 modules for a total of 24 tests. Plates are sealed into a bag with desiccant.

2.Negative Control CONTROL -
1x2.0ml/vial. Ready to use control. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% Proclin-300 as preservatives.
The control is yellow **color coded**.

Important Note: *The control is supplied in case the laboratory wants to carry out a Quality Control of the kit.*

3.Positive Control CONTROL +
1x1.2ml/vial. Ready to use control. It contains 1% goat serum proteins, inactivated human anti COVID-19 antibodies, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% Proclin 300 as preservatives.
The Positive Control is green **color coded**.

Important Note: *The control is supplied in case the laboratory wants to carry out a Quality Control of the kit.*

4.Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% Proclin-300.

5.Enzyme conjugate: CONJ
1x16ml/vial. Ready to use and **red colour coded**. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin-300 and 0.02% gentamicine sulphate as preservatives.

6.Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

7.Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. It contains 0.3 M H2SO4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8.Assay Diluent DILAS
1x8 ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.045% ProClin 300 for the pre-treatment of samples and controls in the plate, blocking interference. ***Note: The liquid changes color from light yellow to dark greenish blue when the diluted sample is added.***

9.Sample Diluent: DILSPE
1x50ml/bottle. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. To be used to dilute the sample.
Note: The diluent changes color from olive green to dark bluish green in the presence of sample.

10.Plate sealing foils n°2

11.Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (200ul and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minutes range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The **kit** has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.

2. All the **personnel** involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

3. Treat all **specimens** as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

4. The laboratory **environment** should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.

5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.

6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.

7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.

8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.

9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.

10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.

11. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.

12. Wastes produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

13. Accidental **spills** from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

14. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months.. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result. See also chapter S (Limitations).
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
7. As the Sample Diluent (DILSPE) contains a strong virus-inactivating substance, diluted samples may be stored at +2..8°C only for 48 hrs.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 months at +2..8°C.

Microplates:
Ready to use. When a strips-module only has to be used, take out the microplate from the bag, remove the other strips and put them back into the bag, closing then the zip.
The module can be used for testing 8 samples simultaneously.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use.

Sample Diluent:
Ready to use. Mix well on vortex before use.

Assay Diluent:
Ready to use. Mix well on vortex before use.

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

If this component has to be transferred, use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container.

Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention:
Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363).

Legenda:
Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The **ELISA incubator** has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   N° 5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances.
   If soaking is not possible add one more cycle of washing. An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The **ELISA microplate reader** has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; (d) repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
5. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
6. Turn on the ELISA reader at least 20 minutes before reading.
7. Check that the micropipettes are set to the required volume.
8. Check that all the other equipment is available and ready to use.
9. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Important Note: *The controls are used only in case the laboratory wants to carry out a Quality Control analysis of the kit. In case, do not dilute controls as they are ready to use.*
In such case, dispense 200 ul controls in the appropriate control wells, following the same dispensation scheme reported at page 6 for samples.

1. Dilute the sample 1:20 in a transparent dilution tube by adding 50 ul of sample to 1 ml of Sample Diluent (DILSPE). Mix on vortex. The whole solution turns from light green to dark bluish green upon addition of the sample.
2. Add 50 ul Assay Diluent (DILAS) into the sample wells.
3. Dispense horizontally 200 ul of diluted sample in each well of the module (e.g.: sample 1 in A1+A2+A3+A4).
   Check that the color of samples has turned to dark blue.
   Mix gently manually the plate avoiding any spills around.
4. Incubate the microplate for **45 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350ul/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well and cover with the sealer. Check that this pink/red colored component has been dispensed.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **45 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well. Then incubate the microplate at **room temperature (18-24°C) for 15 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown.
11. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction).
Blanking is not necessary.

Important notes:
1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*

2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Sample dilution Assay Diluent (DILAS) Diluted samples	50 ul + 1 ml DILSPE 50 ul/well 200 ul/well
1st incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 ul
2nd incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 ul
3rd incubation	**15 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate

	Module 1				Module 2				Module 3			
	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1				Sample 9				Sample 17			
B	Sample 2				Sample 10				Sample 18			
C	Sample 3				Sample 11				Sample 19			
D	Sample 4				Sample 12				Sample 20			
E	Sample 5				Sample 13				Sample 21			
F	Sample 6				Sample 14				Sample 22			
G	Sample 7				Sample 15				Sample 23			
H	Sample 8				Sample 16				Sample 24			

O. INTERNAL QUALITY CONTROL
See the table below:

Check	Requirements
Negative Control*	OD < 0.200 in all the wells module
Positive Control*	OD > 0.500 at least in the well coated with recombinant Nucleocapsid (C)
Wells "N"	OD < 0.250

Note * : *In case of a QC analysis.*

Should these values not be reached the assay is considered invalid.

P. CALCULATION OF THE CUT-OFF
The tests results are calculated by means of a cut-off value determined with the following formula on the OD450nm/620-630nm value of the **well N** of the specific module:

N + 0.250 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

Q. INTERPRETATION OF RESULTS
A sample is considered for a certain antibody:

Negative	S/Co < 1
Equivocal	1 ≤ S/Co < 1.2
Positive	S/Co ≥ 1.2

In case of an equivocal result repeat the test to assure no mistake has been done in the first run. If the sample is confirmed equivocal again, it is suggested to collect a new sample of serum/plasma from the patient after 4-5 days, repeat the first screening assay and in case of positivity repeat the confirmation assay.

Important notes:
1. Confirmation of a COVID-19 infection:
When the kit is used not only to confirm a positive sample in first screening but also to provide an indication of a COVID-19 infection, a Confidence Index of Infection (that defines its level of reliability) is reported in the table below:

Confidence Index of Infection	
Very high	Antibodies to all the **3 antigens**
High	Antibodies to Nucleocapsid (**Core) and Spike 1**
Medium	Antibodies to Nucleocapsid (**Core) only**

If such index is medium (only antibodies to Nucleocapsid "Core") it is suggested to follow-up the person by testing a second sample collected after 7-10 days from the first one.

2. Potential Neutralizing efficacy:
In order to provide an indication about the titer of IgG that would be interesting for a potential use as a hyper-immune plasma for immuno-therapy the following procedure is suggested:

a) *Dilute the plasma **1:40** by dispensing 20 µl sample + 800 µl DILSPE. Mix and then dilute further to **1:80** by mixing 300 µl of dilution 1:40 + 300 µl DILSPE and to **1:160** by mixing 300 µl of dilution 1:80 + 300 µl DILSPE.*
b) *Dispense 50 ul of DILAS in each sample wells.*
c) *Dispense 200 ul of each dilution and then proceed in testing as reported in the proper section.*
d) *Convalescent individuals showing a positive result (S/Co > 1.1) for the S1 well ideally with a dilution 1:160 or at the least with a dilution of 1:80 might be considered potential donors of hyper-immune plasma.*

It is recommend anyway fulfilling the national regulations and directives in force in the single countries on this specific matter.

***Important Note:** No international defined and approved directive has been released so far on the titer of IgG antibodies such to make an individual be considered (**a**) "protected" from a secondary infection or (**b**) to be identified as a potential donor of plasma for therapy.*

R. PERFORMANCES

The IgG Confirmatory assay by design and principle simply derived from the single DiaPro's ELISA tests for IgG to the mixture of Nucleocapsid ("Core") and Spikes antigens.
The antigens used in the Confirmatory/Typing kit come from a different source, by definition.

Diagnostic specificity:
The assay specificity, evaluated examining more than one hundred plasma collected before and after the outbreak of COVID-19, first tested negative for IgG on the specific DiaPro's ELISA, reached an overall value of almost 100%. No false positive reactions were observed.

Potential crossreactions with other respiratory infective agents were studied for the corresponding IgG ELISA on samples positive for antibodies to: PIV1-3, Influenza A and B, H.influenzae, hCoV 229E, hCoV OC43, hCoV HKU1, hCoV NL63, Rhinovirus, RSV, Adenovirus, M.pneumoniae and C.pneumoniae.
No crossreactions were observed.

Antibodies, commonly present in human sera and plasma, to unrelated infective agents were also studied for the corresponding IgG and IgM ELISA. Antibodies positive to CMV, EBV, HSV1&2, Toxoplasma, Rubella, H.pylori, Malaria sps, Coxsackie virus, Parvovirus B19 and HCV, HIV, Syphilis and HBsAg did not crossreacted.

No interferences were observed in pregnant women, abnormal levels of liver enzymes and other common organ-specific pathologies.

As seen for the corresponding IgG DiaPro's ELISA, past infections of SARS-CoV-1 (and MERS) may give a positive result due to the high level of genetic homology between the two viruses. Other Coronavirus strain my give a low response in view of similarity among different strains.
Well-known potentially interfering samples in EIA were studied on the corresponding ELISA kits. Results are reported in the table below:

Substances	Concentrations	Score
Haemoglobin	Up to 500 mg/dl	negative
Bilirubin	Up to 20 mg/dl	negative
Triglyceride (milky samples)	Up to 3000 mg/dl	negative
Serum proteins	Up to 15g/dl	negative
RF+	Up to 2500 U/ml (Cobas)	negative
Anti-E-Coli Ab+	Highly positive	negative

No false reactivity due to the method of analysis was found.

The same potential interfering samples were spiked with a specimen highly positive for IgG to COVID-19 Spike antigens. No false negative result was found assuring no interference of such substances in positive samples testing.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera were used to determine the value of specificity.
Frozen specimens were tested, as well, to check for interferences due to collection and storage. No interference was observed provided that the sample was clear and free of particles/aggregates.

Interferences were seen, as for the reference ELISA, when fibrin aggregates, visible particles and lipid layers were present in the sample, giving usually a false positive result. These samples have to be cleared by filtration on a 0.22 u filter (lipid layers) or centrifuged for 30 min at 4000 rpm (aggregates) before testing, or discarded as not suitable for testing.

Diagnostic Sensitivity
A study was carried out on samples collected from:
(***a***) a cohort of infected patients at end of infection with PCR negativity and clear clinical signs of full recovery, (***b***) normal population tested for epidemiologic studies and (***c***) healthcare operators in COVID-19 hospitals.
Such analysis conducted on 80 samples, previously tested positive for IgG in the corresponding ELISA, a correlated sensitivity of almost 100% was observed. No false negative result was in fact observed in the confirmation.

When samples, collected during the course of the infection at sequential dates from outset of first symptoms and PCR positivity, were tested with the Dia.Pro's Confirmatory Assay, IgG to Spike antigens came out quite later than antibodies to Nucleocapsid (Core) and kept on increasing in their S/Co values together with IgG to Nucleocapsid.

Precision:
Repeatability (intra-assay) was studied on 3 samples, one negative, one low positive and one high positive, examined in 16 replicates. Results showed values of CV in the range 4-20% depending on their OD450nm.
Reproducibility (inter-assay) was studied on the same 3 samples tested in 16 replicates for 3 times. The variability found of 4-20 % depending on the sample OD450nm did not lead in sample misclassification.

S. LIMITATIONS

As reported in the proper section highly lipemic ("milky") and haemolized ("red") samples may generate false positive reactions.
When testing frozen samples, in particular those that:
(a) were submitted to several cycles of freezing&thawing;
(b) were already "dirty" in origin when aliquoting;
(c) were aliquoted in a small volume, due to tendency to get jellified by evaporation;
(d) are composed of plasma because of their tendency to form aggregates of fibrin upon thawing;
(e) IgM samples that by nature tends to aggregate upon freezing&thawing and become "sticky",

some false positive reaction it's quite likely to come out.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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