abia SARS-CoV-2 IgG/IgM

REF DK.073.01.3
REF DK.073.05.3

IVD

CE

Note: Changes highlighted ★

abia

Intended use

abia SARS-CoV-2 IgG/IgM is an enzyme immunoassay for the qualitative detection of antibodies to SARS-CoV-2 that causes coronavirus infection COVID-19, in human serum or plasma.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture. For professional use only.

Clinical value

The disease COVID-19 is caused by coronavirus SARS-CoV-2 (2019-nCoV). This virus is the seventh coronavirus that has been proven to infect humans. It is the third coronavirus that has emerged in the past 2 decades, causing multinational outbreaks and carrying substantial morbidity and mortality. There are no specific clinical features of COVID-19 and symptoms are similar to those of other severe acute respiratory infections, such as MERS and SARS[1].

Four structural proteins are essential for the life cycle of COVID-19. Homotrimers of S proteins make up the spikes on the surface of virus particles and it is the key for the viral attachment to host receptor. The M protein has three transmembrane domains and it shapes the virions. The E protein plays a role in virus assembly and release. The N protein contains two domains, both of them can bind virus RNA[2].

Principle of the test

abia SARS-CoV-2 IgG/IgM is an indirect two-step immunoassay for the detection of antibodies to SARS-CoV-2 (COVID-19). The assay is based on microwells coated with recombinant antigens (rAg) of SARS-CoV-2 sequence. The conjugate contains a mix of peroxidase-labeled anti-human IgM and IgG antibodies.

Serum or plasma samples are added to the wells and if antibodies against SARS-CoV-2 are present in a sample, they form stable complexes with antigens immobilized on the wells.

Then the antigen-antibody complexes are identified by the addition of peroxidase-labeled anti-human IgM/IgG antibodies.

The unbound components are removed by washing. After the addition of the solution containing TMB, the wells with bound conjugate develop a blue color which is converted to yellow after the reaction has been stopped with sulphuric acid.

The color intensity is directly proportional to the concentration of SARS-CoV-2 antibodies in the specimen and can be read at 450 nm or 450 and 620–680 nm.

Kit contents

	S	XL	
SARS-CoV-2 Ag coated plate	1	5	polystyrene plate 12 × breakable 8-well strips coated with a mix of rAg of SARS-CoV-2
Conjugate (concentrated 21-fold)	1 × 0.8 ml	1 × 3.0 ml	mixture of peroxidase-labeled anti-human IgG/IgM Ab; transparent or slightly opalescent, colorless or pale yellow liquid; preservatives: ProClin 300 0.04 %, gentamicin sulfate 0.0009 %, 4-Dimethylaminoantipyrine 0.009 %
Conjugate diluent	1 × 12 ml	3 × 25 ml	transparent or slightly opalescent yellow liquid; preservatives: ProClin 300 0.1 %, phenol 0.01 %
Positive control (inactivated)	1 × 1.5 ml	1 × 1.5 ml	ready to use; mixture of heat inactivated negative human serum and humanized animal antibodies to recombinant SARS-CoV-2 antigens; transparent or slightly opalescent, red colored liquid; preservatives: ProClin 300 0.1 %
Negative control (inactivated)	1 × 2.0 ml	2 × 2.0 ml	ready to use; heat inactivated negative human serum with phosphate saline solution; transparent or slightly opalescent, green liquid; preservatives: ProClin 300 0.04 %, gentamicin sulfate 0.001 %, sodium azide 0.19 %
Sample diluent 1	1 × 12 ml	1 × 60 ml	ready to use; preliminary sample diluent; phosphate-saline solution; transparent or slightly opalescent, violet-blue colored liquid; preservatives: sodium azide 0.09 %
Sample diluent 2	1 × 12 ml	2 × 25 ml	ready to use; phosphate-saline solution with addition of casein bovine milk solution (0.96 %); transparent or slightly opalescent, pink colored liquid; sediment may form; preservatives: thimerosal 0.007 %, sodium azide 0.097 %
Washing solution (concentrated 25-fold)	1 × 50 ml	1 × 120 ml	phosphate saline buffer; colorless or pale yellow liquid; without preservatives
TMB (concentrated 11-fold)	1 × 2.5 ml	2 × 3.5 ml	solution containing TMB; transparent colorless liquid (coloration possible); without preservatives
Substrate buffer	1 × 25 ml	1 × 70 ml	citric acid (0.64%) solution (pH 4.1-4.3), containing H_2O_2; colorless liquid; preservatives: ProClin 300 0.04 %
Stopping reagent 0.2M H_2SO_4	1 × 25 ml	1 × 90 ml	ready to use; 0.20 mol/l sulphuric acid solution; colorless liquid; without preservatives
Protective film	2	10	
Plastic dish	2	-	
Plastic zip-lock bag	1	5	

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package.
The device is available in two different variants; DK.073.01.3 with 96 determinations (S) and DK.073.05.3 with 480 determinations (XL).

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- disposable plate for preliminary dilution
- microplate incubator set at 37.0 ± 1.0 °C
- automatic microplate washer
- microplate reader equipped with 450 nm or with 450 and 620–680 nm filters

Safety notes

- human origin material used in the preparation of the negative control and positive control has been tested and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2). The positive control was prepared using humanized animal antibodies to recombinant SARS-CoV-2 antigens
- as no known test method can offer complete assurance that infections agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke, or apply cosmetics in the laboratory
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use undiluted heparin/EDTA/citrate plasma or serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum or plasma as soon as possible to avoid any haemolysis
- do not use contaminated, hyperlipaemic or hyperhaemolysed specimens
- pooled specimens must not be used since the accuracy of test with such specimens has not been validated
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- freeze/thaw of the tested samples is not acceptable

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (18–24 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section “Washing procedure”
- for the description of test procedure with the automated analyzers see section “Automated analyzers”

Washing procedure

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- flow-through washing with a volume not less than 350 µl per well is used. When using a microplate washer for which this is not possible, ensure that the well is completely filled with a slight positive meniscus without overflow
- allow a soaking time of at least 40 seconds
- perform this procedure 4 times in total
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion **Stability:** 14 days at 18–24 ºC or 28 days at 2–8 ºC												
Washing solution (concentrated 25-fold), ml	1.5	3.0	4.5	6.0	7.5	9.0	10.5	12.0	13.5	15.0	16.5	20.0
Purified water, ml	36.0	72.0	108.0	144.0	180.0	216.0	252.0	288.0	324.0	360.0	396.0	480.0
Working solution of conjugate: mix the reagents thoroughly until diluted, avoid foaming **Note:** before use always mix the working solution by inverting, avoiding foaming **Stability:** 12 hours at 2–24 ºC in a dark place												
Conjugate (concentrated 21-fold), ml	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60
Conjugate diluent, ml	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
Substrate mixture: mix the reagents thoroughly until dilution **Note:** substrate mixture should be colorless! **Stability:** 10 hours at 2–24 ºC in a dark place												
TMB (concentrated 11-fold), ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2
Substrate buffer, ml	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0

Test procedure

abia SARS-CoV-2 IgG/IgM for the qualitative detection of antibodies to SARS-CoV-2 in human serum or plasma

1	Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant bag.
2	Add 90 µl of sample diluent 1 to preliminary plate (not provided). Add 10 µl of samples to be tested into appropriate wells in preliminary plate. Violet-blue color should change to blue-green when samples were added. Mix the contents of the wells by gentle pipetting. Dilution ratio is 1:10.
3	Add 100 µl of positive control in well A1. Add 100 µl of negative control in well B1, C1 and D1. Add 90 µl of sample diluent 2 in rest of the wells. Add 10 µl of prediluted samples to be tested from preliminary plate in the wells with sample diluent. Final dilution ratio is 1:100. Depending on system and the number of strips used, it is possible to modify the position of controls or the order of distribution. Mix the contents of the wells by gentle pipetting, then cover the plate with protective film.
4	Incubate in microplate incubator for 30 minutes at 37.0 ± 1.0 °C.
5	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 350 µl of working washing solution into each well and aspirate. Perform this procedure 4 times. Use double aspiration in the final step where possible.
6	Add 100 µl of working solution of conjugate into each well. Cover the plate with protective film.
7	Incubate in microplate incubator for 30 minutes at 37.0 ± 1.0 °C.
8	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 350 µl of working washing solution into each well and aspirate. Perform this procedure 4 times. Use double aspiration in the final step where possible.
9	Add 100 µl of substrate mixture to all the wells. Keep the plates in a dark place for 20 minutes at 18–24 °C.
10	Add 150 µl of stopping reagent into each well. Wait 1-2 minutes before reading.
11	Read the optical density at 450/620-680 using a plate reader. Reading the absorbance at 450 nm only is possible. Test results remain stable for reading within 20 minutes.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative.
For the instrumentation without established validated protocol follow section “Test procedure” and ensure all requirements described in section “Precautions” are followed.
All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of each negative control should be less than 0.150.
If one negative control does not respect this norm, disregard and recalculate the mean value using the two remaining values. Only one value may be eliminated by this way.
The absorbance (OD) of positive control should be greater than 0.600.

Calculation cut-off value

Mean OD value of the negative control = (OD value B1 + OD value C1 + OD value D1)/3
Cut-off = mean OD value of negative control + 0.300

Interpretation of the results

Non-reactive sample: sample OD value < cut-off - 20 %
Samples with absorbance values less than the cut-off - 20 % value are considered to be negative by the abia SARS-CoV-2 IgG/IgM test.

Reactive sample: sample OD value > cut-off + 20 %
Samples with absorbance values more than the cut-off +20 % value are considered to be positive by the abia SARS-CoV-2 IgG/IgM test.

If the OD of the tested sample exceeds or equal to the cut-off - 20 % value but less or equal than cut-off + 20 % this sample is in the “grey zone”. In this case it is necessary to test repeatedly the patient’s serum for antibodies to SARS-CoV-2 over time. It is advisable to test the sera samples simultaneously with the previous ones (“pair samples”), it will allow to assess specific antibody dynamics more accurately.

Performance characteristics

The performance of the abia SARS-CoV-2 IgG/IgM assay has been determined by testing samples from random blood donors, artificial positive samples containing anti-SARS-CoV-2, patients with confirmed COVID-19 infection and patients in other clinical categories.

Diagnostic sensitivity	Number of tested samples	Sensitivity, %
COVID-19 positive samples taken 17-29 days after symptoms onset	12	100.00
Artificial samples containing molecules of monoclonal/ polyclonal antibodies and Fc-fragments of human immunoglobulins	7	100.00
Positive COVID-19 samples taken before 1 week after symptoms onset	16	18.75
Positive COVID-19 samples taken1-2 weeks after symptoms onset	44	40.90
Positive COVID-19 samples taken 2-3 weeks after symptoms onset	13	76.90
Positive COVID-19 samples taken more than 3 weeks after symptoms onset	6	100.00

Interferences

Hemoglobin (up to 2.65 mg/ml), bilirubin (up to 0.293 mg/ml), triglycerides (up to 6.61 mg/ml) and rheumatoid factor (up to 77.4 IU/ml) have no influence on the assay results. No false positive results caused by antibodies to *E.coli*.

Diagnostic specificity	Number of tested samples	Specificity, %
Unselected blood donors	192	95.31
Pregnant women	15	93.33
Patients with infectious diseases	45	91.11
Patients with noncommunicable diseases	20	95.00
Artificial human samples to control cross reactivity	6	100.00
E.coli antibodies positive samples	8	100.00

Paired COVID-19 positive blood samples Acute stage vs. convalescent stage	1st bleeding (day 0), S/Co	2nd bleeding (day 5), S/Co
Sample 6	0.27	1.19
Sample 9	5.72	8.41
Sample 10	13.03	13.03
Sample 11	11.08	13.03
Sample 12	0.06	1.70
Sample 18	1.14	13.03

abia SARS-CoV-2 IgG/IgM demonstrate titer increase (S/Co) in all paired tested samples.

Precision

The repeatability within one plate, different plates and different lots were evaluated by testing 3 positive samples. The CV did not exceed 10 %.

Limitations of test

- the results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.
- the diagnosis of the COVID-19 infection can only be made in the presence of clinical manifestation and with a complex of laboratory tests (determination of an ANTI-SARS-CoV-2 increase, positive result of PCR).
- a negative result of the assay does not exclude the possibility of COVID-19 since the blood could be taken during the seronegative window period.

References

1. Arabi, Y. M., Murthy, S., & Webb, S. (2020). *COVID-19: a novel coronavirus and a novel challenge for critical care. Intensive care medicine*, 1-4.
2. Chen, Y., Liu, Q., & Guo, D. (2020). *Emerging coronaviruses: genome structure, replication, and pathogenesis.* Journal of medical virology, **92**(4), 418-423.
3. Flodgren, G. M. (2020). *Immunity after SARS-CoV-2 infection.* Rapid review 2020. Oslo: Norwegian Institute of Public Health.

Key to symbols used

	Manufacturer
IVD	For in vitro diagnostic use
REF	Catalogue number
LOT	Batch code
YYYY-MM-DD	Expiry date
2°C – 8°C	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
Σ n	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
★	Changes highlighted

Hazard and precautionary statements for certain kit components

Warning

Stopping reagent

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Warning

Conjugate (concentrated 21-fold), negative control

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of water.

Warning

Positive control, conjugate diluent

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Attention!
For complete precautionary statements and detailed information see safety data sheets (SDS).

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