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INSTRUCTION FOR USE
COVID-19 IgG ELISA

For the detection of IgG to SARS-CoV-2 coronavirus nucleocapsid antigen in human serum and plasma

in vitro diagnostic test

Only for professional *in vitro* diagnostic use

BACKGROUND INFORMATION

Early January 2020 a SARS-CoV-2 coronavirus which causes COVID-19 disease was identified as the infectious agent causing an outbreak of viral pneumonia in Wuhan, China in December 2019.
SARS-CoV-2 is an enveloped RNA virus of the coronavirus family (CoV) betacoronaviruse gen-era (Beta-CoV), a new strain that has not been previously identified in humans. Common signs of infection include respiratory symptoms, fever, cough and shortness of breath. In severe cases, causes pneumonia, severe acute respiratory syndrome, kidney failure and even death.
Currently, PCR-based viral RNA detection is almost the only way to confirm the diagnosis of SARS-CoV-2 infection in practice. Many cases that were strongly epidemiologically linked to SARS-CoV-2 exposure and with typical lung radiological findings remained RNA negative in their upper respiratory tract samples. As well one of the reasons for the false-negative results in PCR is the incorrect collection of respiratory swabs. Another issue for consideration is reporting of asymptomatically infected cases, or very mild cases of infection who are a large group of patients but not tested for viral RNA (which is impractical), therefore making the true rate of infection in the population unknown. In this regard, combination of PCR with serological tests should be used in COVID-19 diagnostics.
SARS-CoV-2 major structural proteins includes the spike (S, SP), membrane (M), envelope (E), and nucleocapsid (N, NP) proteins. The SP comprises of two subunits, the first one S1 contains the receptor-binding domain (RBD) which mediates the interaction with angiotensin-converting enzyme 2 (ACE2), which is a host cell cellular receptor. RBD is an important target for vaccine development, whereas NP, as the most immunogenic and very conservative antigen, can be used as a virus marker in serological tests, including ELISA.
According to recent data, in most patients with COVID-19, the seroconversion rate and the IgG and IgM levels (first at all to SP/RBD and NP) increased rapidly during the first 2-3 weeks from the onset. IgG persist for a long time, playing a protective role, whereas IgM levels drop after 4 weeks and disappear at the end of week 12. Antibody levels are higher after severe infection than after mild infections, so therefore serological tests should be sensitive not to miss persons with a mild infection.

INTENDED USE

COVID-19 IgG ELISA is an in vitro qualitative enzyme immunoassay for detection of IgG to SARS-CoV-2 nucleocapsid antigen human serum and plasma. The assay is for in vitro diagnostic use by a laboratory professional. The assay can be performed using standard ELISA equipment or automated open-type ELISA analyser using a validated protocol.

METHOD

COVID-19 IgG ELISA is an indirect ELISA. When human plasma/serum is placed into well anti-SARS-CoV-2 IgG are bond to the immobilized recombinant SARS-CoV-2 nucleocapsid antigen forming antigen-antibody complexes. Such complexes are further detected with HRP conjugated monoclonal anti-human IgG antibodies. Unbound components are removed by washing and TMB substrate is added into wells. The enzymatic reaction is stopped by the stop-reagent adding and the optical den-sity (OD) is read at 450/620 nm. The colour intensity is defined by amount of anti-SARS-CoV-2 IgG.

REAGENTS

Reagents	∑ 96
MCPL **Microplate** Stripplate coated with recombinant SARS-CoV-2 nucleocapsid antigen. Ready to use.	1 pcs
CON 11 **Conjugate concentrate (11x) – CC** Monoclonal anti-human IgG antibodies labelled with HRP. Preservative: 0.4% ProClin™300. Red slightly opalescent liquid.	1×1,5 ml
CONTROL + **Positive control – PC** Heat inactivated human serum reactive for anti-SARS-CoV-2 antibodies. Preservative: 0.4% ProClin™ 300. Light yellow slightly opalescent liquid. Ready to use.	1×0,5ml
CONTROL - **Negative control – NC** Heat inactivated human serum non receive for HBsAg, HIV-1 p24 antigen and antibodies to SARS-CoV-2, HIV-1/2 and HCV. Preservative: 0.2% ProClin™ 300 and 0,099% sodium azide. Light yellow slightly opalescent liquid. Ready to use.	1×1,2ml
WS 26X **Washing solution concentrate (26x) - WSC** Phosphate buffer with detergent. Colourless opalescent liquid, it`s allowed the segregation and crystalline sediment formation that dissolves by heating.	1×80 ml
SAMP-DIL **Specimen diluent – SD** Proteic buffered solution with detergent, blocking reagents, and preservative 0.4% ProClin™ 300. Violet opalescent liquid. Ready to use.	1×14 ml
CON-DIL **Conjugate diluent – CD** Proteic buffered solution with detergent, blocking reagents, and preservative 0.4% ProClin™ 300. Red opalescent liquid. Ready to use.	1×15 ml
TMB SUBS **TMB Substrate** Ready to use, one-step chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide. Ready to use.	1×12 ml
STOP **Stop-reagent** 0.5M sulphuric acid. Ready to use.	1×14 ml
SEAL **Plate sealer** Adhesive film	3 pcs
IFU **Instruction for use**	1 pcs

On customer request kit can additionally be supplied with vials for reagent preparation and pipette tips.

CONFIGURATIONS AVAILABLE

REF	∑	Description
30TI-CVG01	96 (including controls)	1 stripplate, 12 test runs: 12×8 wells

ADDITIONAL MATERIALS REQUIRED

- ELISA reader (with dual wavelength 450/620 nm);
- ELISA washer;
- incubator, 37±2°C;
- thermo shaker, 500 rpm, 37±2°C;
- automatic single- and multi-channel pipettes with disposable tips (e.g. 5-40, 50-300, 20-200, 200-1000 µl);
- timer;
- distilled or deionised water;
- disposable gloves;
- disposable V-shaped troughs;
- vials for reagents preparation (glass or plastic);
- beaker (1000 ml);
- absorbent paper;
- disinfectants;
- biohazard waste container for potentially infectious waste.

SAFETY PRECAUTIONS

- Assay can be only be performed in specially equipped ELISA laboratories by laboratory professional.
- The assay is for in vitro diagnostic use.
- ⚠ Human source material used for the Positive and Negative Controls preparation has been heat inactivated and tested negative for HBsAg, HIV-1 p24 antigen and antibodies to SARS-CoV-2 (except for the Positive Control), HIV-1/2 and HCV. Handle with human source materials as with potentially infectious since no test method can completely assure the absence of infectious agents.
- Do not pipette by mouth.
- Wear disposable gloves when performing assay and thoroughly wash hands when finished.
- Human source materials spills should be soaked and disinfected with an appropriate disinfectant.
- Spills containing acid should be neutralize with sodium bicarbonate.
- Dispose all used materials in biohazard waste container.
- Decontaminate potentially infectious wastes before disposal:
  a) either by immersion in appropriate disinfectant according to its Instruction of use, or
  b) by autoclaving at 124-128°C for 1 hour (under 0.15 MPa).
- Avoid contacts of reagents with skin and eyes. Wash affected area with water.

WARNING

⚠ CC, PC, NC, SD and CD contain ProClin™300 as preservative

Hazard statements
H317 - May cause an allergic skin reaction.
Precautionary statements
P261 - Avoid breathing vapours.
P272 - Contaminated work clothing should not be allowed out of the workplace.
P280 - Wear eye protection, protective gloves, protective clothing.
P302+P352 - IF ON SKIN: Wash with plenty of water.
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 - Take off contaminated clothing and wash it before reuse.
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.
- For additional information see Material Safety Data Sheet available on request.

DISPOSAL CONSIDERATION

• Dispose negative control and its packaging as potentially infectious materials.
• Dispose other kit reagents and their packaging using approved waste disposal service consider-ing national and local environmental protection requirements and waste disposal laws.
• Unless the legislation provides otherwise, non-contaminated packages may be treated like household waste or recycled.

SPECIMEN PREPARATION

Serum and plasma samples can be used in the assay, including those collected with an anticoagulant (K_2EDTA, K_3EDTA, sodium citrate, etc.) or a clot activator (with or without a separating gel).
Specimens can be stored at 2-8°C for no longer than 3 days, at –20°C for 3 months, at –70°C for 2 years. It is not allowed more than 3 freeze/thaw cycles.
Thawed specimens should be mixed to be homogeneous. To prevent fibrin precipitation, plasma specimens should be quickly thawed at 38-40°C.
Clarify specimens with visible precipitates by centrifugation at 1900 g (2500-3000 rpm) for 10 minutes.
Do not use specimens with sodium azide, haemolysis, hyperlipidemia or bacterial contamination.

REAGENT PREPARATION

Bring all reagents and specimens to room temperature (18-25°C) before use.
Generic reagents (interchangeable for COVID-19 IgG ELISA of different lots): WSC, TMB Substrate and Stop-reagent.

Microplate

Tear up the pouch with Microplate above the Ziploc. Return unused strips to the pouch and reseal it.

Washing solution

Intensively shake the vial content. If crystals are seen in the solution, dissolve them by heating at 35-37°C. Dilute **WSC** (26x) with distilled or deionised water (see the chart below).

Conjugate

Conjugate has to be prepared before use. Dilute CC (11x) with CD in a clean vial (see the chart below). Mix well avoiding foaming.

STORAGE AND STABILITY

Store the kit at 2-8°C. Do not freeze. The kit shelf life is 14 months.
Storage after dilution
Diluted Washing solution is stable for 1 month at 2-8°C or for 14 days at 18-25°C when stored in a clean vial tightly seal container.
Diluted Conjugate is stable for 20 hours at 2-8°C or for 8 hours at 18-25°C when stored in a clean vial tightly seal container in the dark.
Storage after initial opening
Once opened the reagents are stable until the expiry date of the kit when stored at 2-8°C in tightly sealed initial containers. Reagents may remain at room temperature for 8 hours during testing if kept in the dark.

REAGENT CONSUMPTION

Number of strips	Washing solution		Conjugate		TMB Substrate, ml	Stop-reagent, ml
	WSC, ml	Dist. water, ml	CC, µl	CD, ml		
1	6	150	100	1	1	1
2	12	300	200	2	2	2
3	18	450	300	3	3	3
4	24	600	400	4	4	4
5	30	750	500	5	5	5
6	36	900	600	6	6	6
7	42	1050	700	7	7	7
8	48	1200	800	8	8	8
9	54	1350	900	9	9	9
10	60	1500	1000	10	10	10
11	66	1650	1100	11	11	11
12	72	1800	1200	12	12	12

PROCEDURAL NOTES

Reliability of assay results depends on careful following the instruction for use. Do not deviate from the instruction for use.
- Do not use the kit beyond the stated expiry date.
- Do not mix the reagents from different kit lots except for ones listed in the section **Generic reagents**.
- Do not use the reagent with damaged package.
- When performing assay, the room temperature should be 18-25°C. Bring all reagents and specimens to room temperature (18-25°C) before use. Immediately after use return reagents to 2-8°C.
- Thoroughly mix the reagents during their preparation and assay performing.
- Use thoroughly cleaned and rinsed glassware or disposable vials for reagent preparation.
- Use a new tip for reagent and specimen pipetting.
- Immediately after use close the vials with reagents to avoid their contamination.
- Do not allow wells to become dry during assay procedure. Cover the plate with plate sealer or lid during incubation.
- Use calibrated equipment.
- When performing assay avoid direct sunlight, reactive fumes (sodium hypochlorite, acids, alkalis or aldehydes) or dust since the enzymatic reaction may be affected.
- Do not allow any metal element to come into contact with the conjugate or TMB substrate as enzymatic reaction is sensitive to metal ions.
- Do not use the same troughs for conjugate and TMB substrate.

WASH PROCEDURE

Perform washing as described below since insufficient washing can adversely affect the assay. Follow this procedure at each washing:
- aspirate the well contents;
- fill the wells with washing solution (350-400 µl per well) avoiding overflowing from one well to another;
- ensure that each well soaks approximately 40 seconds before the next aspiration;
- aspirate the well contents.

Ensure that no fluid is left in the wells and on the stripholder after the last aspiration. Tap out any residual washing solution onto absorbent paper.
Use automatic washer in Overflow regime. In case of the absence of washer or its faulty work a multi-channel pipette may be used.

ASSAY PERFORMING

Procedure 1 - without rotation, assay duration is 2 hours, sample volume is 30 µl
1. Fit the stripholder with required number of **strips**.
2. Wash the plate **once** with **washing solution** (according to section Wash procedure).
3. Add **70 µl** of the **SD** into wells.
4. Add **30 µl** of **controls** and **specimens** as follows (controls are added in the first wells in the least):
   -when 1-2 strips are used: **PC – 1** well, **NC – 2** wells;
   -when 3 and more strips are used: **PC - 2** wells, **NC - 3** wells.
   -the rest wells : **specimens**.

Carefully repipett the well content. During mixing the solution changes its colour that allows to check whether specimen is added into well.
5. Cover the plate with plate sealer and incubate at **37±2°C for 60 minutes**.
6. Wash the plate **4** times with **washing solution** (according to section Wash procedure).
7. Add **100 µl** of diluted **conjugate** into wells.
8. Cover the plate with plate sealer and incubate at **37±2°C for 30 minutes**.
9. Wash the plate **6** times with **washing solution** (according to section Wash procedure).
10. Add **100 µl** of the **TMB substrate** into wells.
11. Cover the plate with plate sealer and incubate at **18-25°C for 30 minutes** in the dark.
12. Stop the colour reaction by adding **100 µl** of **stop-reagent** with the same sequence and timing as for TMB substrate and read the plate at **450/620 nm** within 2-3 minutes.

Procedure 2 - with rotation, assay duration is 1 hour 30 minutes, sample volume is 30 µl
1. Fit the stripholder with required number of **strips**.
2. Wash the plate **once** with **washing solution** (according to section Wash procedure).
3. Add **70 µl** of the **SD** into wells.
4. Add **30 µl** of **controls** and **specimens** as follows (controls are added in the first wells in the least):
   -when 1-2 strips are used: **PC – 1** well, **NC – 2** wells;
   -when 3 and more strips are used: **PC - 2** wells, **NC - 3** wells.
   -the rest wells : **specimens**.

Carefully repipett the well content. During mixing the solution changes its colour that allows to check whether specimen is added into well.
5. Cover the plate with plate sealer and incubate at **500 rpm at 37±2°C for 50 minutes**.
6. Wash the plate **4** times with **washing solution** (according to section Wash procedure).
7. Add **100 µl** of diluted **conjugate** into wells.
8. Cover the plate with plate sealer and incubate at **500 rpm at 37±2°C for 20 minutes**.
9. Wash the plate **6** times with **washing solution** (according to section Wash procedure).
10. Add **100 µl** of the **TMB substrate** into wells.
11. Cover the plate with plate sealer and incubate at **500 rpm at 37±2°C for 20 minutes** in the dark.
12. Stop the colour reaction by adding **100 µl** of **stop-reagent** with the same sequence and timing as for TMB substrate and read the plate at **450/620 nm** within 2-3 minutes.

RESULTS

- Calculate the mean OD of the Negative Controls (NCm).
  Discard NC higher than 0.1 or exceeding more than twice NCm and recalculate NCm of the remaining controls*.
- Assay results are valid if NCm is not higher than 0.1 and PC is not lower than 0.6.

**NC and specimens below 0.00 (negative values) when calculating the Cut-off and analyzing the results is considered as zero*
- Calculate **Cut-off**:
  Cut-off = NCm + **0.2**
- Result is considered **negative** if specimen OD is below Cut-off.
- Result is considered **positive** if specimen OD is equal to or greater than Cut-off.
- Retest initially positive specimens in two or more wells:
  • specimens reactive in one or more wells are considered positive;
  • specimens nonreactive in two or more wells are considered negative.

All repeatedly reactive results should be confirmed in total Ab assay, for example, COVID-19 TOTAL Ig ELISA .

PERFORMANCE CHARACTERISTICS

Analytical Specificity
Cross-reactivity
COVID-19 IgG ELISA showed 100% specificity on samples with respiratory illnesses, antibodies to EBV and CMV. There is a possibility of falsepositive results when testing samples with HIV-1/2, HCV and HBsAg.
The data are summarized in the following table.

Category	n	COVID-19 IgG ELISA	
		Negatives	% Negative
Other respiratory illness	4	4	100%
EBNA IgG	20	20	100%
EBV IgG	16	16	100%
CMV IgG	9	9	100%
HIV-1/2	45	44	97.8%
HCV	11	10	90.9%
HBsAg	10	9	90%

Clinical Performance
Positive percent agreement (PPA)
PPA to RT-PCR was estimated on 114 serum specimens (n=114) from subjects who presented with COVID-19 symptoms. Specimens were confirmed in RT-PCR.

The results are given in the tables below.

Positive agreement by comparator

Comparator	n	COVID-19 IgG ELISA		
		Positive	Negative	PPA (95%CI*)
RT-PCR	114	82	32	71,93% (62,74% - 79,94%)

* Clopper-Pearson exact

Positive agreement by day post symptoms onset

Day post symptoms onset	n	Positive	Negative	PPA (95%CI)
≤ 8	16	2	14	12,5% (1,55% - 38,35%)
9 - 12	23	13	10	56,62% (34,49% - 76,81%)
13 - 17	28	20	8	71.43% (51.33% - 86.78%)
≥ 18	27	27	0	100 % (87.23% - 100%)

Negative percent agreement (NPA)

To estimate the NPA, 82 specimens taken prior to November 2019 (pre-COVID-19 outbreak) (n=82) and 540 presumed SARS-CoV-2 negative specimens (n=540) taken during COVID-19 outbreak in 2020 (including subjects who were exhibiting signs of respiratory illness) were tested in COVID-19 IgG ELISA.
The results are presented in the following table.

Category	n	COVID-19 IgG ELISA		
		Positive	Negative	Specificity / NPA
Pre-Covid-19 outbreak	82	4	78	Specificity 95.12% (87.98% - 98.66%)
During Covid-19 outbreak	540	1	539	NPA 99.81% (95% CI 98.97% - 100%)

The agreement of COVID-19 IgG ELISA results with the expected negative results for both categories (n=622) is 99.2% (95% CI 98.13% - 99.74%).

LIMITATIONS OF THE ASSAY

• Serological test result should not be used as the sole basis to diagnos or exclude a SARS-CoV-2 infection. Positive ELISA result should undergo confirmatory testing with certificated RT-PCR. Presence of symptoms and combination of test results from RT-PCR, antigen and antibody testing can provide a clearer picture on the status of a patient.
• Negative result does not exclude the COVID-19. The false-negatives could be in the early stages of infection during the seroconversion window (1-2 week from onset), when the antibodies are absent or their concentrations are below the assay sensitivity limit. In this case, in the presence of clinical symptoms, it is recommended to re-examine the patient`s follow-up sample in a few days.
• Pooled samples should not be tested. The manufacturer cannot guarantee the results reliability for pooled samples, as no such studies have been carried out.
• Potentially cross-reactants were evaluated and are represented in the PERFORMANCE CHARACTERISTICS section.
• Violations of samples handling and assay procedure specified in the Instruction for Use can also cause the erroneous test results.

SHIPPING

The kit should be shipped at 2-8°C. Do not freeze. It is allowed the transpor-tation at 9-25°C for 10 days.

REFERENCES

1. Juanjuan Zhao, Quan Yuan, Haiyan Wang, Wei Liu, Xuejiao Liao et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clinical Infectious Diseases, https://doi.org/10.1093/cid/ciaa344. Pub-lished: 28 March 2020
2. Evaluation of Nucleocapsid and Spike Protein-based ELISAs for 2 detecting antibodies against SARS-CoV-2. JCM Accepted Manuscript Posted Online 30 March 2020, J. Clin. Microbiol. doi:10.1128/JCM.00461-20
3. Trevor R.F. Smith, Ami Patel, Stephanie Ramos,et al. Rapid development of a synthetic DNA vaccine for COVID-19. DOI:10.21203/rs.3.rs-16261/v1
4. Xiaowei Li, Manman Geng, Yizhao Peng, Liesu Meng, Shemin Lu. Molecular immune pathogenesis and diagnosis of COVID-19. Journal of Pharmaceutical Analysis
5. Nisreen M.A. Okba, Marcel A. Müller, Wentao Li, et al. Severe Acute Respiratory Syndrome Coronavirus 2–Specific Antibody Responses in Coronavirus Disease 2019 Patients. Emerging Infectious Diseases, Volume 26, Number 7—July 2020, doi: 10.3201/eid2607.200841
6. Kelvin Kai-Wang To, MD, Owen Tak-Yin Tsang, FRCP, Wai-Shing Leung, FRCP, Anthony Raymond Tam, MRCP, Tak-Chiu Wu, FRCP, David Christopher Lung, FRCPath, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020; (published online March 23.) https://doi.org/10.1016/S1473-3099(20)30196-1
7. Shu-Yuan Xiao, Yingjie Wu, Huan Liu. Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring. Journal of medical virology. First published:07 February 2020. https://doi.org/10.1002/jmv.25702
8. Gang Li, M.D., Ph.D., Xuejuan Chen, Anlong Xu, Ph.D. Profile of Specific Antibodies to the SARS-Associated Coronavirus. The New England Journal of Medicine

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CE

Manufacturer | Attention, see instruction for use | REF Catalog number | LOT Lot number | Fragile; handle with care

Consult instruction for use | IVD *In vitro* diagnostic medical device | Σn Number of test | Storage temperature | Expiry date | This way up

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