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REF ODL150/10

COVID-19 IgG ELISA Kit

Qualitative Assay for COVID-19 IgG
Store at 2-8°C. DO NOT FREEZE.
For professional use only.

IVD CE

Σ 960

INTENDED USE

The test is a qualitative ELISA kit for the detection of IgG antibodies to SARS-CoV-2 in human venous serum, venous plasma (EDTA, lithium heparin, sodium citrate) or capillary plasma (lithium heparin). Capillary plasma is plasma obtained from capillary whole blood. The COVID-19 IgG test is an aid to identification of recent or prior infection of SARS-CoV-2 in symptomatic and asymptomatic individuals who may have gained an adaptive immune response. Test results should be considered in conjunction with other test results/clinical information and should not be used solely for diagnosis nor to exclude acute infection.

Note. Detection of antibodies may indicate immunity or suspected attenuation to subsequent re-infection. The length of time SARS-CoV-2 antibodies remain in the body post infection is unknown.

The COVID-19 IgG ELISA assay should not be used to diagnose acute SARS-CoV-2 infection, if acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

INTRODUCTION

Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) is the viral strain that causes coronavirus disease 2019 (COVID-19). Following infection, the immune system may generate antibodies to fight the infection. The test can detect immunoglobulin G (IgG) antibodies to SARS-CoV-2. Levels of IgG antibodies develop and decline at different rates within different individuals. The immunological response to viral infection can take several weeks and evidence suggests that antibodies to SARS-CoV-2 may take several days to appear. Serological testing of an individual before this window period may result in a misleading negative result.

Detection of IgG antibodies may indicate immunity or attenuation to subsequent re-infection for the individual and for seroprevalence studies to provide a measure of how many people have been exposed to SARS-CoV-2 (in the absence of other, e.g. antigen, test results).

PRINCIPLE OF THE TEST

The test is a serological, plate-based, enzyme linked immunosorbent assay (ELISA), for detecting and quantifying SARS-CoV-2 IgG in human venous serum, venous plasma (EDTA, lithium heparin, sodium citrate) or capillary plasma (lithium heparin). Capillary plasma is plasma obtained from capillary whole blood.

Diluted serum/plasma samples are incubated with SARS-CoV-2 antigens immobilised on microtitre wells. After washing away unbound serum/plasma components, anti-human IgG conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to indicate antibody binding. Addition of Stop Solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the cut-off control, positive control and samples are measured using a microplate reader at 450nm.

CONTENTS OF THE COVID-19 IgG ELISA KIT

Materials provided:

- MTP - 10 96-well microtitre plate: pre-coated with purified SARS-CoV-2 antigens (NP and S2 antigens), in a foil bag with desiccant
- DIL - 1 Sample Diluent: 150mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 120mL, (blue), concentrate (15×)
- WB - 6 Wash Buffer: 100mM Tris-buffered saline with detergent, pH 7.2, 170mL, concentrate (10×)
- CONJ - 1 Conjugate: Anti-human IgG conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 120mL, ready to use
- SUBS - 1 Substrate: aqueous solution of TMB and hydrogen peroxide, 120mL, ready to use
- STOP - 1 Stop Solution: 0.25M sulphuric acid, 120mL, ready to use
- CONTROL CO - 2 Cut-off Control: of 10mM Tris-buffered saline containing purified human IgG antibodies, 3mL, ready to use
- CONTROL + - 2 Positive Control: of 10mM Tris-buffered saline containing purified human IgG antibodies, 3mL, ready to use
- Instructions for use

Materials required, but not provided:

- Test tubes for dilution
- Graduated cylinder for preparing wash buffer and sample diluent
- Precision pipettes and disposable tips to deliver 5µL, 100µL, and 1mL
- Enzyme Immunoassay (EIA) microplate washer or multi-channel pipette or wash bottle
- Distilled or de-ionised water
- Absorbent paper
- EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

KIT STORAGE

On arrival, store the kit at 2-8°C. Do not use kits beyond their expiry date. Do not freeze any kit component.

QUALITY CONTROL

The expected OD values and the acceptance ranges for the Cut-off Control and Positive Control are given on the Quality Control Certificate included in the kit. The Positive Control is intended to monitor for substantial reagent failure. Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

SAMPLE COLLECTION AND TEST PROCEDURE

Human venous serum, venous plasma (EDTA, lithium heparin, sodium citrate) or capillary plasma (lithium heparin) samples can be used. Capillary plasma is plasma obtained from capillary whole blood. Serum and plasma samples should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Capillary blood collected into lithium heparin blood collection tubes can be stored for up to 7 days at 25°C prior to centrifugation and plasma collection. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Do not use grossly haemolysed, icteric or lipaemic samples. Do not freeze whole blood samples.

Preparing for the test

1. Allow the test kit to come to a temperature between 16-30°C before use.
2. Dilute the Sample Diluent **(Reagent 1 = DIL)** 1 in 15 in distilled water to make sufficient buffer for the assay run e.g. add 10mL Sample Diluent concentrate to 140mL water.
3. Dilute the Wash Buffer **(Reagent 2 = WB)** 1 in 10 in distilled water to make sufficient buffer for the assay run e.g. add 100mL Wash Buffer concentrate to 900mL water.
4. Ensure that the microtitre plate is correctly orientated.

Test procedure

5. Dilute samples 1 in 201 in diluted Sample Diluent (e.g. 5µL serum added to 1mL diluted Sample Diluent).
6. Dispense 100µL of the Sample Diluent, Cut-off Control, Positive Control, and the diluted samples into appropriate wells. It is recommended that all controls and samples are run in duplicate.
7. Incubate for **30 minutes** at a temperature between 16-30°C.
8. After 30 minutes, decant or aspirate the well contents and wash the wells 3 times with diluted Wash Buffer using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. Do not allow the wells to dry out.
9. Dispense 100µL of Conjugate (**Reagent 3 = CONJ**) into each well. Incubate the wells for **30 minutes** at a temperature between 16-30°C.
10. After 30 minutes, discard the well contents and carefully wash the wells 4 times with diluted Wash Buffer. Ensure that the wells are empty but do not allow to dry out.
11. Dispense 100µL of TMB Substrate (**Reagent 4 = SUBS**) into each well. Incubate the plate for **10 minutes** at temperature between 16-30°C.
12. Add 100µL of Stop Solution (**Reagent 5 = STOP**) to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.
13. Read the OD of each well at 450nm in a microplate reader within **10 minutes**. A 620nm filter may be used as a reference wavelength.

Manual Wash Procedure

Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with diluted Wash Buffer (this is equivalent to approximately 300µL per well). Empty by inversion and blot the wells on absorbent paper. Ensure that the wells are empty but do not allow the wells to dry out. Insufficient washing may cause high background signal.

INTERPRETATION OF RESULTS

14. Results are expressed by calculating a ratio using the following formula:

Sample OD / (Cut-off Control OD * 1.6) = Sample Ratio

Interpretation of sample ratios is as follows:

Ratio	Interpretation
< 0.8	**Negative**, i.e. the subject's sample does not contain IgG antibodies to SARS-CoV-2
≥ 1.1	**Positive**, i.e. the subject´s sample contain IgG antibodies to SARS-CoV-2
≥ 0.8 to < 1.1	**Indeterminate**, if an indeterminate result is obtained, the test should be repeated using a new sample.

LIMITATIONS

- For use with human venous serum, venous plasma (EDTA, lithium heparin, sodium citrate) or capillary plasma (lithium heparin) samples only. Capillary plasma is plasma obtained from capillary whole blood.
- For reliable results, please follow the instructions carefully.
- Test results should be used in conjunction with other clinical and patient information.

WARNINGS

- Read the instructions carefully before performing the test. Failure to follow the instructions may lead to inaccurate test results.
- Use of any other components except those supplied with the kit will invalidate the results.
- Do not use the kit beyond the expiry date.
- Do not use if any kit components are damaged.
- Do not use if the product has been exposed to excessive heat or humidity.
- Do not use grossly haemolysed, icteric or lipaemic samples. Do not freeze whole blood samples.

SAFETY AND HANDLING PRECAUTIONS

- **Safety Precautions**
  - i. Handle all samples as potentially infectious.
  - ii. Wear gloves and protective clothing while handling samples and running the test.
  - iii. Do not smoke, eat, or drink while handling samples or performing the test procedure.
  - iv. Apply standard biosafety precautions for handling and disposal of potentially infective material as per local legislation. Dispose of all packaging in a general waste bin.
  - v. Avoid splashing and aerosol formation.
  - vi. Clean up spills thoroughly using an appropriate disinfectant.
- **Handling Precautions**
  - i. Do not use kit components beyond the expiry date printed on the label. Always check expiry date prior to testing.
  - ii. Ensure a new disposable pipette tip is used for each sample and disposed of following standard biosafety precautions.
  - iii. Sample Diluent concentrate (DIL) contains <0.1% sodium azide as a preservative which may be hazardous to health if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

PERFORMANCE CHARACTERISTICS

Performance Evaluation Data

All performance evaluations were collected by hospital clinic sites within the UK.

A total of 644 positive samples (laboratory confirmed SARS-CoV-2 by RT-PCR, ranging from day 0 to day > 60) and 642 negative samples (from pre-COVID-19 outbreak) were used, with the following results:

	%	95% Confidence Intervals
Sensitivity (total SARS-CoV-2 positive samples ranging from day 0 - >60 post laboratory diagnosis with RT-PCR)	91.1% (587/644)	88.68% - 93.23%
Specificity (total SARS-CoV-2 negative samples from pre-COVID-19 outbreak)	98.6% (633/642)	97.36% - 99.36%

Sensitivity stratified by days post swab for RT-PCR

Days post laboratory diagnosis with RT-PCR	Sensitivity (%)	95% Confidence Intervals
0-6	82.4% (28/34)	65.47% - 93.24%
7-13	91.5% (75/82)	83.20% - 96.50%
14-20	94.3% (83/88)	87.24% - 98.13%
21-27	96.0% (48/50)	86.29% - 99.51%
21-60	92.5% (74/80)	84.39% - 97.20%
> 21	91.4% (74/81)	83.00% - 96.45%

Sensitivity stratified by onset of symptoms

Days post symptom onset	Sensitivity (%)	95% Confidence Intervals
0-6	87.5% (7/8)	47.35% - 99.68%
7-13	87.9% (29/33)	71.80% - 96.60%
14-20	94.3% (66/70)	86.01% - 98.42%
21-27	93.7% (59/63)	84.53% - 98.24%
21-60	91.8% (90/98)	84.55% - 96.41%
> 21	92.0% (92/100)	84.84% - 96.48%

Cross Reactivity

Cross reactivity: SARS-CoV-2 negative samples that tested positive for a range of other viruses and disease states.

Source	Organism/condition	Negative agreement (%)
Resp Virus PCR	Adenovirus	100% (8/8)
Resp Virus PCR	Bocavirus	100% (2/2)
Serology	*Bordetella pertussis*	100% (22/22)
Resp Virus PCR	Coronavirus 229	94.4% (17/18)
Resp Virus PCR	Coronavirus 43	100% (12/12)
Resp Virus PCR	Coronavirus 63	100% (5/5)
Serology	Dengue virus	100% (16/16)
Resp Virus PCR	*Enterovirus*	100% (7/7)
Serology	Epstein-Barr Virus	100% (38/38)
Culture	*Haemophilus influenzae*	100% (17/17)
Resp Virus PCR	Hong Kong Uni coronavirus	100% (4/4)
Resp Virus PCR	*Influenza A*	94.1% (16/17)
Resp Virus PCR	*Influenza B*	100% (2/2)
Resp Virus PCR	Influenza H1N1	100% (12/12)
Urinary antigen	*Legionella*	100% (4/4)
TB culture	*Mycobacterium tuberculosis*	83.3% (10/12)
Malaria blood film	Malaria	100% (8/8)
Resp Virus PCR	Metapneumovirus	100% (9/9)
Resp Virus PCR	*Mycoplasma*	100% (2/2)
Resp Virus PCR	Parainfluenza 1	100% (3/3)
Resp Virus PCR	Parainfluenza 2	100% (6/6)
Resp Virus PCR	Parainfluenza 3	100% (8/8)
Resp Virus PCR	Parainfluenza 4	100% (4/4)
Resp Virus PCR	Parechovirus	100% (2/2)
PCR	*Pneumocystis jirovecii*	100% (6/6)
Resp Virus PCR	Respiratory Syncytial Virus	100% (12/12)
Serology	Rheumatoid factor	100% (27/27)
Resp Virus PCR	Rhinovirus	100% (12/12)
Serology	Serum save	100% (14/14)
Serology	Systemic Lupus Erythematosus	94.1% (16/17)

Source	Organism/condition	Negative agreement (%)
Culture	*Streptococcus pneumoniae*	100% (15/15)
Culture	*Strep. pyogenes* (Beta-haemolytic Strep group A)	100% (13/13)
	TOTAL	**98.6% (349/354)**

Interfering Substances

No interference in the performance of COVID-19 IgG kit was evident when SARS-CoV-2 positive and negative samples were spiked with the following interferents: Haemolysate up to 1mg/mL, Bilirubin (conjugated) up to 0.15mg/mL and Rheumatoid Factor up to 10IU/mL.

SYMBOL LEGEND

The following symbols have been used within the labelling of this product.

CE	This product fulfils the requirements of Directive 98/79/EC on in vitro diagnostic medical devices.	IVD	*In vitro* diagnostic medical device
			Keep away from sunlight
LOT	Batch code		Manufacturer
REF	Catalogue number		Temperature limit
	Consult instructions for use		Use-by date
	Contains sufficient for "n" tests		

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