

# LD COVID-19 IgG Rapid Test

Rapid test for the qualitative detection of human IgG antibodies to SARS-CoV-2 in serum, plasma, or whole blood.

For professional in-vitro diagnostic use only.

Entirely manufactured in GERMANY

REF: COV19\_G\_10\_EN

Rev. 2.0 / 20200721

## INTENDED USE

The **LD COVID-19 IgG Rapid Test** is a visual test for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum, plasma, or whole blood within 15 min (latest 20 min). The test is for professional in vitro diagnostic use only and intended to aid in the diagnosis of SARS-CoV-2 infection and to complement direct pathogen detection. Serology can also be used to collect epidemiological data. The product is intended for use as an IVD but can also be used for research purposes.

## INTRODUCTION / FIELD OF APPLICATION

In December 2019, a novel zoonotic coronavirus SARS-CoV-2 was identified as an infectious agent that could cause an outbreak of viral pneumonia in human. Common signs of infection with the coronavirus include respiratory symptoms, breathing difficulties, fever, sore throat, stuffy nose and dry cough. In some severe cases, the infection can cause viral pneumonia, severe acute respiratory syndrome (SARS), as well kidney failure and finally death. To prevent an infection with the coronavirus it is recommended to avoid close contact with anyone showing symptoms of respiratory illness as well as standard hygienic methods like hand washing, as well covering mouth and nose. SARS-CoV-2 has structure proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike protein (S) is a glycoprotein, composed of two subunits (S1 and S2). It was found that the subunit S1 contains a receptor binding domain (RBD) which strongly interacts with human ACE2 receptor, causing infection of human respiratory cells.

As for other viral infections, immunoglobulin antibodies such as IgM and IgG may be present in the blood following infection with the SARS-CoV-2 in humans at different time and kinetics. IgM antibodies usually appear within 7-9 days post infection. IgG antibody is detectable after about 20 days post infection and is an important indicator of protection from the virus if the infected person is free of serious symptoms. The **LD COVID-19 IgG Rapid Test** is a detection tool which can be used for evaluation of IgG against SARS-CoV-2 Spike S1 in human serum, plasma, or whole blood. The **LD COVID-19 IgG Rapid Test** is an ASSURED test meaning it has the following characteristics: affordable, sensitive, specific, user-friendly, robust & rapid, delivered to users. **Please note: Ideally, two serum samples should be collected and tested for confirmation of a SARS-CoV-2 infection by antibody detection, which are taken in the first week after the onset of symptoms and at least 14-21 days later.**

## PRINCIPLE OF THE TEST

The test consists of one test strip, which is integrated in a test cassette. This test strip consists of a special human IgG antibody-binding protein, coupled to colored particles (conjugate), and a membrane with one test line and one control line. The test line contains SARS-CoV-2 Spike glycoprotein (S1), the control line consists of an antibody-binding protein. Test line and

control line in the result window are not visible before applying any samples.

After the sample (serum, plasma, or whole blood) is pipetted into the sample well (S) followed by the Diluent, the diluted sample passes through the conjugate and the antibodies in the sample bind to the conjugate. The antibody-conjugate complex migrates due to the capillary action to the site of the membrane where the SARS-CoV-2 Spike Glycoprotein (S1) is immobilized (test line). If IgG antibodies against S1 are present in the sample, they will bind to the test line.

Then one colored line appears in the **test zone ("T")**. The remaining complex migrates further across the membrane to the **control zone ("C")**. Again, a colored line appears, indicating that the test was performed correctly.

## SUPPLIED MATERIALS

Package sizes:

REF: **COV19\_G\_10\_EN (10 Tests)**: 10 test cassettes, 1 dropper bottle containing 3.5 mL of diluent and 10 disposable transfer pipettes.

## TEST COMPONENTS

- Diluent: 1 dropper bottle containing dilution buffer – 3.5 mL
- Test cassette: individually sealed in an aluminum bag with a single use pipet
- 1 Instructions for use
- 1 Quick reference guide

**Note:** Pictures may differ from the original.

## MATERIALS NEEDED BUT NOT SUPPLIED

- Containers for sample collection. We recommend using standard containers for blood collection
- Microliter pipettes and tips for 10-100 µL (optional for serum / plasma / whole blood)
- lancets (optional for whole blood)
- Stopwatch

## PREPARATION OF REAGENTS

All reagents are ready-to-use. No further preparation of reagents is necessary.

## STABILITY AND STORAGE CONDITIONS

Store the test at 2 - 30°C. Unopened kit components (aluminium bags and Diluent) are stable until the expiry date. The expiry date is printed on the labels of the aluminium bag, the Diluent and the outer packaging. Do not use if the aluminum bag is damaged. **DO NOT FREEZE** or expose to temperatures above 30°C.

**Aluminium pouch with test cassette:** Keep the test in unopened aluminium bag at 2 - 30°C.

**Opened aluminium bag:** Use test cassette within 1 hour!

**Diluent (dilution buffer):** Store the Diluent at 2 - 30°C. Unopened Diluent is stable until the expiry date. After first opening the Diluent is stable until the expiry date, if the bottle is tightly closed after every usage.

## WARNINGS AND PRECAUTIONS

- Read the instructions carefully before performing the test.
- In accordance with Good Laboratory Practice (GLP), all laboratory devices employed should be regularly checked for the accuracy and precision.
- For professional in-vitro diagnostics only!
- Use all reagents within the expiry period (printed on the labels).
- Do not use reagents from different kit lots or batch codes and avoid mixing of reagents of different kit lots or batch codes.
- Only for serum, plasma or whole blood. Do not use the test with other body fluids.
- Avoid contamination of the reagents. Do not use the same container for several samples! Use separate single-use pipets for each sample (included in the kit).
- Lipemic, hemolytic or bacterially contaminated samples should not be used. Avoid the use of turbid samples which may be contaminated with bacteria.
- Avoid repeated freezing and thawing of the samples because it could lead to denaturation of the antibodies.
- Do not ingest or swallow! Do not eat, drink and smoke in the laboratory! Do not work without wearing protective clothing (gloves, safety glasses and lab coat)! Avoid the contact of kit reagents with skin, eye or mucosa.
- All kit components should be considered as infectious agents. Decontaminate and dispose of residues of kit reagents and samples in accordance to local regulations, e.g. by autoclaving or using a disinfecting solution.
- Avoid touching of the membrane in the result window of the test device with your fingers (danger of contamination).
- Do not pipette samples and diluent directly onto the membrane in the result window of the test device.
- For single use only. The test is sensitive to moisture. Do not use if the outer packaging (aluminum bag) is damaged. After opening the aluminum bag, it must be used within 1 hour.
- All patients should be treated as potentially infectious. Observe established precautions against microbiological hazards throughout testing and follow standard procedures for proper disposal of specimens.
- Bring specimens to room temperature (preferably 15 - 30°C).
- Lipemic, hemolytic or bacterially contaminated samples must not be used.
- Avoid the use of turbid samples as it cannot be excluded that they are bacterially contaminated.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

## SAMPLE COLLECTION AND PREPARATION

The **LD COVID-19 IgG Rapid Test** is suitable for the detection of **IgG** antibodies to SARS-CoV-2 in serum, plasma, or whole blood. The test works best with fresh samples.

### Collection of whole blood from the vein:

Take the sample under standard laboratory conditions (aseptically, avoid haemolysis).

### Collection of whole blood from the fingertip:

- Disinfect your hands.
- Ask the patient to sit or to stretch himself out.
- Use disposable gloves.
- Disinfect a puncture site with a skin disinfectant.
- Wait for exposure and drying time of the disinfectant.
- Puncture the skin with a sterile lancet.
- Massage the hand towards the fingertip (Caution! Do not touch the puncture site! Avoid strong pressure!)
- Discard the first drop and gently massage the hand from wrist to fingers to cause the formation of a drop of blood.
- Keep the puncture site downward (horizontal or slightly inclined) and take the drop of blood with a single use capillary or pipet. Try to touch only the leaked blood and avoid air bubbles.

**Serum, plasma, or whole blood:** Separate as soon as possible from the red blood cells (e.g. by centrifugation). If the test cannot be performed immediately after the sampling, the samples can be stored for up to 2 days (48 hours) at 2 - 8°C. For longer storage, the whole blood must be centrifuged (separate serum or plasma from red blood cells). Serum and plasma can be stored at temperatures below -20°C. Frozen samples must be thawed prior to testing and well mixed. Avoid repeated freezing and thawing of samples!

## TEST PROCEDURE

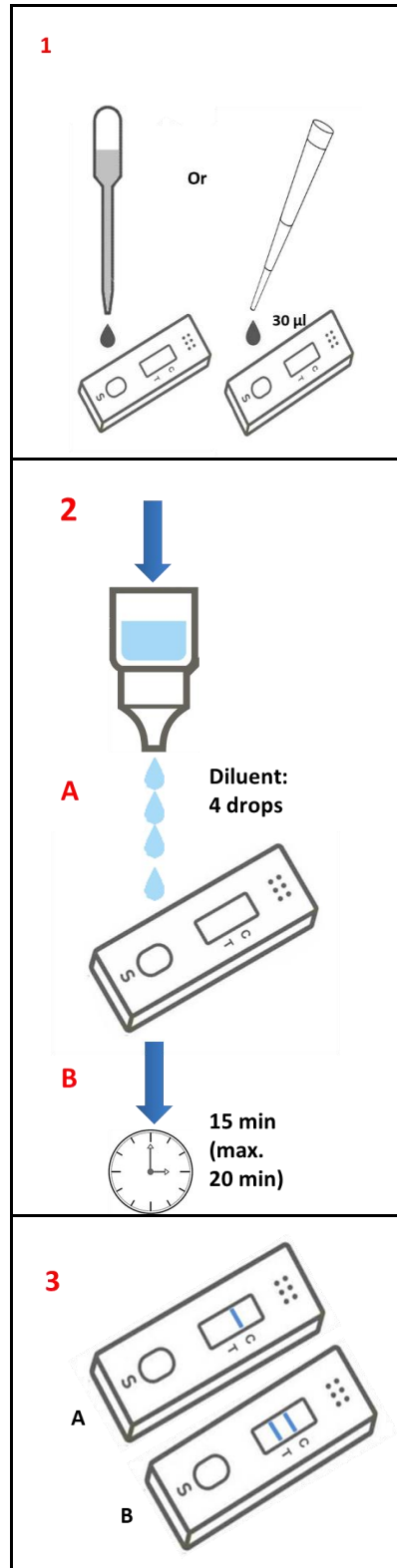
**Note:** After opening the aluminum bag, the test should be carried out quickly (less than 1 hour) as the test strip is sensitive to humidity. Test cassette, buffer and patient's samples should be brought to room temperature (preferably 15 - 30°C) prior to testing. Do not open pouches until ready to perform the assay.

1. Take the required number of test pouches from the packaging kit. Open the aluminum pouch and place the cassette /s on a clean, non-absorbent flat surface.
2. Label the test device with patient identification number.
3. For the Sample: Fill the disposable transfer pipette supplied by the kit with specimen and, by holding it vertically, dispense **one drop (~30 µl)** into sample well (S). Alternatively, you can use a microliter pipette, adjust the pipette to 30 µL (**Reference Guide 1**).
4. Immediately add 4 drops of diluent buffer into the sample well (S) on the cassette. Avoid dropping any solution in the observation window. (**Reference Guide 2A**).

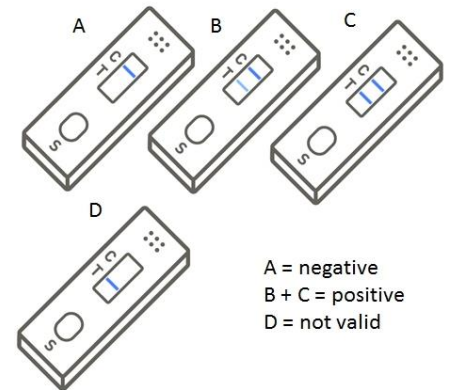
5. Adjust stopwatch to 15 min and start timing (**Reference Guide 2B**).
6. Wait until stopwatch shows that 15 min have elapsed and read the result (latest after 20 min).

Do not read results after more than 20 minutes (**Reference Guide 3**)

## REFERENCE GUIDE



## INTERPRETATION OF RESULTS



**Fig. 1:** Schematic diagram of possible test results for LD COVID-19 IgG Rapid Test: Negative result: only the control line appears (A); Positive result: two lines appear. Test band weaker or strong plus band at control zone (B) and (C). Invalid test: only the test line appears (D). The colour of the lines in the figure may differ from the actual line colour on the test.

**NEGATIVE:** Only one colored line appears in the control zone (control line "C", see Quick Reference Guide, and Fig. 1). In the test zone ("T") there should be no line visible.

**POSITIVE:** Two colored lines appear. One line should be visible in the control zone ("C") and other one line in the test zone ("T") (see Quick Reference Guide, and Fig. 1).

**The test lines "T" may be stronger or weaker than the control line "C".**

**DOUBTFUL:** Very weak shadow-like test line should be regarded as not clear. In this case it is recommended to take another sample from the same patient after 2-4 weeks and to measure it again using the **LD COVID-19 IgG Rapid Test** window

**INVALID:** No control line visible and/or background color affects readability of test results. Insufficient sample volume or incorrect handling of the test are the most likely reasons for a lack of control line and/or a formation of background color which affects the readability of control/test lines. Check again the instructions of sample preparation and test procedure and repeat the test with a new test device. If the problem persists, contact the manufacturer or your local distributor.

## QUALITY CONTROL

The **LD COVID-19 IgG Rapid Test** contains an internal control. A colored line in the control zone ("C") is considered as an internal procedural control. It confirms enough sample volume and correct test procedure. A clear background is an internal negative procedural control. If a background color appears in the result window and thereby the readability of the test results will be affected, the result may be invalid.

## PERFORMANCE CHARACTERISTICS

Reproducibility of measurements was validated by determination of Intra- and inter-assay variations, and inter-operator-variations. All measurements have shown that the test is highly reproducible regarding intra- an inter-assay as well as batch-to-batch and inter-operator variation. No significant variations were observed.

### Diagnostic sensitivity and specificity

The clinical sensitivity and specificity of the **LD COVID-19 IgG Rapid Test** (Whole Blood/Serum/Plasma) was determined by comparing the results with clinical PCR results (gold standard: RT-PC); the results showed that IgG Rapid Test (Whole Blood/Serum/Plasma) has a high sensitivity and specificity. 113 negative plasma or serum samples were measured by **LD COVID-19 IgG Rapid Test** (samples from patients with other lung diseases, from clinical laboratories or healthy individuals who have never been exposed to SARS-CoV-2 or from individuals were tested PCR negative). Furthermore, 39 positive samples from well-defined and validated COVID-19 patients (positive PCR) were tested. The results are presented in the table 1. Confidence intervals for sensitivity and specificity are "exact" Clopper-Pearson confidence intervals<sup>20</sup>. For the samples measured **sensitivity was 94.87% (CI = 82.68 to 99.37%) at a specificity of 97.35% (CI = 92.44 to 99.45%)**.

Table 1: **LD COVID-19 IgG Rapid Test** result: the rapid test results compared by results of Clinical Diagnosis (RT-PCR method).

Group	Number of specimens	Clinical sensitivity in % (95% Confidence interval)	Clinical specificity in % (95% Confidence interval)
Patients (RT-PCR positive) (TP/FN)	39 (37/2)	<b>94.87</b> (82.68 to 99.37)	<b>97.35</b> (92.44 to 99.45)
Negative (no history or contact with SARS-CoV2) (collected before December 3, 2019) (TN/FP) or RT-PCR negative	113 (110/3)		

As mentioned before and like all other viral infections, immunoglobulin antibodies such as IgM and IgG may be present in the blood following infection with the SARS-CoV-2 in humans at different time and kinetics<sup>21-27</sup>. Since all positive clinical samples were collected at different days and weeks after the COVID-19 symptoms; we applied additional analysis for measuring the clinical specificity and sensitivity for LD COVID-19 IgG Rapid Test (REF: COV19\_G\_10\_EN) + LD COVID-19 IgM Rapid Test (REF: COV19\_M\_10\_EN). The results of all positive samples with clinical evidence of COVID-19 were compared with all negative samples collected prior to the COVID outbreak [December 3, 2019] as they have never been in contact with SARS-CoV2.

In this analysis the rapid test results considered positive if IgM test cassette and/or IgG test cassette gave positive results. The combined results of **LD COVID-19 IgG Rapid Test** and **LD COVID-19 IgM Rapid Test** gave **sensitivity of 97.44% and a**

**specificity of 96.46%**. The results are summarized in table 2.

Table 2: The combined results of both **LD COVID-19 IgM Rapid Test + LD COVID-19 IgG Rapid Test** compared by results of Clinical Diagnosis (RT-PCR method).

Group	Number of specimens	Clinical sensitivity in % (95% Confidence interval)	Clinical specificity in % (95% Confidence interval)
Patients (RT-PCR positive) (TP/FN)	39 (38/1)	<b>97.44</b> (86.52% to 99.94)	<b>96.46</b> (91.18 to 99.03)
Negative (no history or contact with SARS-CoV2) (collected before December 3, 2019) (TN/FP) or RT-PCR negative	113 (109/4)		

## LIMITATIONS

### Follow the instructions of the test procedure and interpretation of results carefully!

Follow the instructions of the test procedure and interpretation of results carefully! Insufficient sample volume or incorrect handling of the test procedure are the most likely reasons for not reaching the required QC criteria of test performance (see section "Quality control of test").

A POSITIVE test result in combination with a proven survived disease suggests that a previous infection with SARS-CoV-2 is likely. A NEGATIVE result indicates that infection is unlikely. Note that questionable results require further confirmation. If the result is not clear, another sample should be taken from the same patient after 2-4 weeks and checked again.

The antibody determination does not replace the direct detection by PCR. It is important to note that a positive IgG result against SARS-CoV-2 indicates that an infection has taken place, but this does not necessarily mean that immunity (i.e. protection against infection) is assured. The long-term studies necessary for this statement cannot yet exist.

Cross-reactions of antibodies within the genus *Betacoronavirus* can occur.

It is recommended to consider the results of the test in combination with the clinical status of each patient, the results of other diagnostic tests and the epidemiological background information. If a patient sample has tested positive, further confirmatory tests should be performed (e.g. PCR, clinical symptoms). For a final diagnosis, include all available information on a given patient.

Likewise, a negative test result does not exclude a possible disease.

For meaningful serological results, we recommend to test two samples, the first from the acute phase (week 1 of the disease) and a second sample additionally from the convalescence phase (3 to 4 weeks after disease).

The test is designed for the detection of human IgG against SARS-CoV-2 in serum, whole blood, and plasma. For the detection of IgG against SARS-CoV-2 in body fluids other than human serum, whole

blood, and plasma the test has not been validated and may give false results.

Avoid bacterial contamination of the samples!

As with all diagnostic tests, a definitive clinical diagnosis should not be based solely on the results of a test but should only be made by the physician based on an evaluation of clinical and laboratory findings.

**Warning:** Samples from patients who have received monoclonal mouse antibody preparations for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such samples may show either falsely elevated or decreased values when tested with test kits such as SARS-CoV-2 IgG that use mouse monoclonal antibodies.

Heterophilic antibodies in human serum may react with reagent immunoglobulins and interfere with in vitro immunoassays. Patients routinely exposed to animals or animal serum products may be susceptible to this interference and abnormal values may occur.

Rheumatoid factor (RF) in human serum may react with reagent immunoglobulins and interfere with in vitro immunoassays.

### Interfering Substances:

Analytical specificity is determined by measuring potential interfering substances. Substances used for patient treatment, substances which may be ingested by the patient and substances encountered in specific specimens' types are considered.

Bilirubin	8 µg/mL
Cholesterol	5 mg/mL
Haemoglobin	1 mg/mL
Human Albumin	110 mg/mL
Ibuprofen	200 µg/mL

**Outcome:** no interference is observed for the substances tested.

To be on the safe side we recommend excluding haemolysed, lipemic and icteric samples from testing.

## LITERATURE

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