COVID-19-CHECK -1

Ref. 200081-4-2-3L (20 tests)/Ref. 200081-4-2-3L-10T (10 tests)

Immunochromatographic rapid test for the detection of IgM and IgG antibodies to SARS-CoV-2 virus in human whole blood, plasma or serum samples

I- INTENDED USE

The COVID-19-CHECK-1 test is to be used for the qualitative detection of the anti-SARS-CoV-2 IgM/IgG antibodies in serum, plasma or whole blood. It is intended to be used by medical healthcare professionals as a tool to assess the COVID-19 serological status of a person and cannot be directly used to diagnose a coronavirus 2019 infection (COVID-19).

II- PRINCIPLE

A new pneumonia-like disease outbreak appeared in China, Hubei province, in late 2019 (1). The agent of this disease was identified as a new zoonotic pan-Betacoronavirus variant, named SARS-CoV-2, which full-length genome sequence showed close relationship (96%) with the bat SARS-like coronavirus strain BatCov RaTG13 (2). The routes of transmission were identified as the contamination by droplets from the nose or mouth during close unprotected contact between the infector and healthy persons (1). Droplets from infected subjects can land on objects and other people can be affected by COVID-19 when being in contact with these objects.

By the end of February 2020, cases have been reported in 57 countries with rapid increase in Iran, South Korea and Italy (3).
Data collected on the disease show that the COVID-19 symptoms are not specific and that the disease signs can range from no symptoms to severe pneumonia and death in a few cases (1). Older patients (>65 years) with comorbidities and acute respiratory distress syndrome are at increased risk of death (4, 5). Patients having severe cardiovascular damage have also been reported with increased levels in cardiac troponin I and creatine kinase-MB when infected (6).
SARS-CoV-2 can be detected 1 or 2 days prior to symptom onset in the upper respiratory tract but viral shedding up to 24 days has also been reported (7). The virus can persist 7 to 12 days in moderate cases and up to 2 weeks in severe cases (1). The viral load in throat swab and sputum peaks approximately 5 or 6 days after symptom onset (8).

Antibodies to SARS-CoV-2 can be detected either 8 to 11days for IgM or 18 to 21 days for IgG (9) after having been infected by the virus. The antibodies specific to the SARS-CoV-2 are directed against the nucleocapsid protein and the spike protein antigens, mainly the S1 (10).

COVID-19-CHECK-1 is an immunochromatographic screening test for the detection of IgM and IgG antibodies directed to SARS-CoV-2. It can be used with plasma, serum or whole blood samples. The method relies on a mixture of recombinant SARS-CoV-2 antigens labelled with gold particles and IgM and/or IgG binding reagents coated on the membrane. The multiple epitope fusion SARS-CoV-2 proteins are produced synthetically according to the genetic information of the virus. This material is therefore 100% non-infectious.

As the sample flows through the absorbent pad, the anti-SARS-CoV-2 IgM or IgG antibodies, when present in the sample, bind to the recombinant antigens gold conjugate.
This complex migrates on the membrane and further binds to the IgM capture reagents (Test 1 Zone) and/or IgG capture reagents (Test 2 Zone) coated on the reaction zone producing distinct coloured bands.
In the absence of anti-SARS-CoV-2 antibodies, there is no line in the positive reaction zone. The liquid continues flowing through the membrane by capillarity and produces a pink-rose coloured band in the control zone (C), demonstrating that the reagents are functioning correctly.

III- COVID-19-CHECK-1 KIT COMPONENTS

Each kit contains the following components to perform 10 or 20 tests:

- COVID-19-CHECK-1 test units	10	20
- Diluent in a dropper bottle containing saline buffer, detergent and sodium azide (NaN_3 < 0.1%)	1.5mL	3 mL
- Instructions leaflet	1	1

Material required but not provided :
- Precision pipette or similar equipment to deliver 10 or 20 µL.

IV- STORAGE AND STABILITY

1- All COVID-19-CHECK-1 kit components should be stored between +4°C and +30°C in the sealed pouch.
2- **Do not freeze the test kit.**
3- COVID-19-CHECK-1 is stable until the expiry date stated on the package label.

V- PRECAUTIONS

1- This test is designed for *in vitro* diagnostic use and medical healthcare professional use only.
2- Read the instructions carefully before using the test.
3- Handle all specimens as if they contained infectious agents. When the assay procedure is completed, dispose of specimens carefully in accordance with your local guidelines or after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5 to 1% solution of sodium hypochlorite for one hour before disposal.
4- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples. Avoid any contact between hands and eyes or nose during specimen collection and testing.
5- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
6- Do not use beyond the expiry date stated on the package label.
7- Do not use a test from a damaged protective wrapper.

CE

VI- SPECIMEN COLLECTION AND PREPARATION

1- The test can be performed with serum, plasma (citrate, EDTA or heparin), or whole blood samples. **Do not use haemolysed samples**.
2- The specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid heamolysis).
3- It is possible to use Finger prick whole blood samples which should be tested immediately. Venous whole blood samples should be tested within 4 hours.
4- If not tested immediately, the plasma/serum sample should be stored in the refrigerator (+2°C to +8°C) for a maximum of 5 days. If testing is delayed more than 5 days (11), the specimen should be frozen (-20°C). The frozen specimen must be completely thawed, thoroughly mixed and brought to room temperature prior to testing. Avoid repeated freezing and thawing.
5- In case of cloudiness, high viscosity or presence of particulate matter into the serum specimen, it should be centrifuged before testing.
6- If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents.

Note: Independently of the severity of the disease, it is admitted, according to the current state of the art on COVID-19 (15), that the detection of IgM/IgG is optimal starting from 15 days after appearance of the symptoms. If the samples are collected too early, false negative results may be obtained due to insufficient level of antibodies.

VII- ASSAY PROCEDURE

1- Allow samples and COVID-19-CHECK-1 tests to come to room temperature prior to testing.
2- Remove the test unit from its protective wrapper (by tearing along the split) and place on a level surface.
3- Label the test unit with patient's name or identification number.

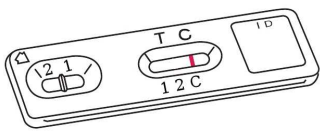
The sample well (▷) is split up into 2 sub-wells identified by mark 1 for the plasma, serum or whole blood samples (right side) and mark 2 for the diluent (left side).

4- Using a precision pipette, dispense 10 µL of serum or plasma into sample well **1**. If whole blood is used, dispense 20 µL into the sample well **1 and wait for the whole blood sample to be completely absorbed before adding diluent.**
5- Add 3 drops (100 µL) of diluent using the dropper bottle into the diluent well **2 with an interval of 2-3 seconds between each drop**.
6- Read results of the test between 10 to 15 minutes after adding the diluent.
DO NOT INTERPRET AFTER 15 MINUTES.

VIII- READING TEST RESULTS

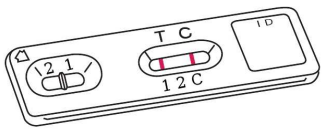
WARNING: Any visible line in the test area either strong or weak (even much weaker than the control line) should be interpreted as positive**.**

Negative :
One coloured band appears in the Control Zone (C). The sample does not contain detectable IgM nor IgG directed against SARS-CoV-2.

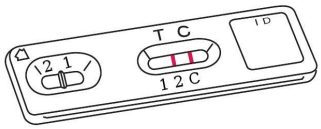


Positive :
Two or three coloured bands (T and C) appear.

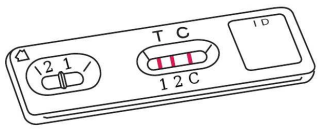
a) In addition to the coloured band in the control Zone (C), a coloured band appears in the T1 Zone. The sample contains IgM antibodies specific to the SARS-CoV-2 and indicates a primary/recent infection.



b) In addition to the coloured band in the Control Zone (C), a coloured band appears in the T2 Zone. The sample contains IgG antibodies specific to the SARS-CoV-2 and may indicate a secondary infection or late stage of the disease.



c) Three coloured bands appear in the window. The sample contains both IgM and IgG antibodies specific to the SARS-CoV-2 and may indicate an active or a secondary infection.



Inconclusive:
If there is no distinct coloured band visible in the Control Zone (C), the test is inconclusive. It is recommended, in this case, to repeat the test with a new device.

Clinical results interpretation:

COVID-19 clinical investigations in the laboratory follow two ways:
- Detection of the coronavirus itself using RT-PCR method (early stage of the disease).
- Assessment of the body's adaptive immune response to the presence of the virus (later stage of the disease).

The COVID-19-CHECK-1 test complements the RT-PCR to monitor the disease progression (13). To understand the clinical significance of the results obtained using COVID-19-CHECK-1 test, the following information must be considered.

Test results			Clinical interpretation
PCR	IgM	IgG	
+	-	-	Patient may be in the window period of infection.
+	+	-	Patient may be in the early stage of infection
+	+	+	Patient is in the active phase of infection.
+	-	+	Patient may be in the late or recurrent stage of infection
-	+	-	Patient may be in the early stage of infection. PCR may be false negative
-	-	+	Patient may have had a past infection, and has recovered
-	+	+	Patient may be in the recovery stage of an infection, or the PCR may be false negative

IX- PERFORMANCES CHARACTERISTICS

According to the French HAS recommendations, the diagnostic sensitivity should be assessed in comparison to a PCR method while the diagnostic specificity should be assessed using samples collected prior the onset of the COVID-19 epidemic (14).

The COVID-19-CHECK-1 test result is considered as being positive when either IgM or IgG or both are showing positive result.

a) Diagnostic sensitivity

Among the 140 serum samples and 12 whole blood samples tested by the PCR method, 105 serum samples and 12 whole blood samples were found positive. These samples were then tested with the COVID-19-CHECK-1 rapid test. The obtained results are summarized in the table 1 below.

		Reference method (PCR positive)		
		Serum	Whole blood	Total
COVID-19-CHECK-1	IgM+/IgG+	68	1	69
	IgM+/IgG-	1	0	1
	IgM-/IgG+	27	10	37
	IgM-/IgG-	9	1	10
	Total	105	12	117

Table 1

The diagnostic sensitivity is calculated as follows:

$\frac{(96+11)}{(105+12)} \times 100 = 91.45\%$ (CI 95% [84.24 – 95.94]*)

b) Diagnostic specificity

100 well documented serum samples collected prior to September 2019 have been used for this study. 71 serum samples were collected from healthy individuals and 29 samples were obtained from individuals with pathologies (tumor markers with high concentration levels).

Among the 100 serum samples, 97 of them showed negative results and 3 samples showed positive result when tested with the COVID-19-CHECK-1 test. The obtained results are summarized in the table 2 below.

COVID-19-CHECK-1	Negative	97
	IgM+/IgG+	2
	IgM+/IgG-	1
	IgM-/IgG+	0
	Total	100

Table 2

The diagnostic specificity is calculated as follows:

$\frac{97}{100} \times 100 = 97\ \%$ (CI 95% [91.06 – 99.61]*)

The overall agreement is calculated as follows:

$\frac{(107+97)}{(117+100)} \times 100 = 94\%$ (CI 95% [89.66 – 96.80]*)

**CI 95%: 95% Confidence interval.*

All the studies have been performed in France using well documented serum samples originating from Europe.

c) Cross-reactions and analytical specificity

20 serum samples each having very high levels of antibodies to either CMV (Cytomegalovirus), EBV (Epstein-Barr virus) or Dengue and 18 serum samples having very high levels of antibodies to Chikungunya have been assayed. Cross reaction occurred in 3 cases for CMV, 1 case each for EBV and Chikungunya while no cross-reaction occurred for Dengue.

Samples spiked with either anti-INFLUENZA A (H_1N_1), anti-INFLUENZA A (H_3N_2), anti-INFLUENZA B (Yamagata strain) or anti-RSV (respiratory syncytial virus) antibodies did not show any cross-reaction.

d) Interferences

The COVID-19-CHECK-1 test is not an antigen sandwich immunoassay using a pair of antibodies, i.e. an antibody immobilized on the solid phase and another antibody conjugated to colloidal gold, but is an immunocapture serological immunoassay for the detection of IgM and/or IgG antibodies reacting with the SARS-CoV-2 specific antigen (recombinant protein) coupled to colloidal gold. Therefore there is no possible interaction in between the different "active" components of the test.

1) HAMA

Different dilutions of HAMA (human anti-mouse antibodies) type 1 and type 2 high positive serum samples were tested in triplicates using the COVID-19-CHECK-1 rapid test. No cross-reaction was observed neither for the IgM nor IgG results.

2) RF

A serum sample having a RF (rheumatoid factor) concentration level of 4,000 IU/mL was tested in triplicate using the COVID-19-CHECK-1 rapid test. No cross reaction was observed neither for the IgM nor IgG results.

3) Anticoagulants

4 serum samples (1 negative and 3 positive) spiked with 40mg/mL of citrate, 14IU/mL of heparin and 2mg/mL of EDTA were tested each in four replicates using the COVID-19-CHECK-1 rapid test. Citrate, heparin and EDTA did not show any effect neither for IgM nor IgG when compared to the results obtained using the same samples without any anticoagulants.

4) Bilirubin and triglycerides

3 serum samples (1 negative and 2 positive) spiked with either bilirubin (0.3 g/L) or triglycerides (10 g/L) were tested using the COVID-19-CHECK-1 rapid test. The results show that bilirubin and triglycerides do not interfere up to concentrations of 0.3 g/L and 10 g/L respectively.

e) Matrix effect

10 positive and 10 negative serum samples spiked with red blood cells from negative whole blood samples have been tested using the COVID-19-CHECK-1 rapid test. No difference in results either for IgM or IgG was observed in between the serum and whole blood matrix.

f) Intra-lot repeatability

30 replicates each of 3 PCR assayed serum samples (1 negative sample, 2 IgM and/or IgG positive samples) have been performed. All obtained results are correct and are conforming to the expected results. The intra-lot repeatability is 100%.

g) Inter-lot reproducibility

3 negative whole blood samples, 3 negative serum samples and 5 positive serum samples were each assayed with 3 different lots of COVID-19-CHECK-1 devices. The negative and positive samples were correctly identified 100 % of time.

X- LIMITATIONS

1- The COVID-19-CHECK-1 procedure and the interpretation of results must be closely followed. This assay is designed for detecting antibodies against SARS-CoV-2 in human serum, plasma and whole blood. Any results derived from the assay of other body fluids and from assay of pooled serum, plasma or whole blood may not be interpreted correctly, based on the current criteria. So, other body fluids and pooled samples cannot be assayed using COVID-19-CHECK-1.
2- For asymptomatic patient or when signs are not clearly interpretable but a COVID-19 case is suspected, it is recommended to repeat the testing, in case of first negative result, 2 or 3 weeks later (11) or to perform a PCR test.
3- The immunological reaction of individuals will depend on the severity of the disease. Mild infections will lead to low levels of antibodies, i.e. weak test lines while severe infections will lead to high levels of antibodies, i.e. strong test lines.
4- As for any diagnostic procedure, the physician should evaluate the data obtained using this kit in the light of the other clinical information available.
5- COVID-19-CHECK-1 is a qualitative test that cannot be used to determine the anti-SARS-CoV-2 antibody concentration nor the rate of increase in antibody level.
6- An excess of biotin (vitamin B8) can lower the control line colour intensity or even lead to the control line disappearance if the biotin concentration level is very high.
7- SARS-CoV-2 infection in pregnant women may increase health risks to both mothers and infants during pregnancy (12).
8- In the early stage of the disease, IgM level could be below detectable levels.
9- The presence or the absence of antibodies cannot be used to determine the success or failure of a medical treatment.
10-Results in immunosuppressed patients should be interpreted with caution and need a medical advice.
11- If the test is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not preclude the possibility of SARS-CoV-2 infection.

XI- BIBLIOGRAPHY

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	Read the instructions before use	IVD	For *in vitro* diagnostic use
+30°C +4°C	Temperature limitations		Do not reuse
	Manufacturer		

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