NADAL® COVID-19 IgG/IgM Test (Ref. 243003N-25)

1. Intended Use

The NADAL® COVID-19 IgG/IgM Test is a lateral flow chromatographic immunoassay for the qualitative detection of anti-SARS-CoV-2 IgG and IgM in human whole blood, serum or plasma specimens of symptomatic patients (see section 12 'Limitations'). Note that in the early stages of infection (3 to 7 days after the onset of symptoms) anti-SARS-CoV-2 IgG and IgM may be below the detection limit of the test. This test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections. The test procedure is not automated and requires no special training or qualification. The NADAL® COVID-19 IgG/IgM Test is designed for professional use only.

2. Introduction and Clinical Significance

COVID-19 (Corona Virus Disease) is the infectious disease caused by the recently discovered coronavirus SARS-CoV-2. This new virus was unknown before the disease outbreak in Wuhan, China, in December 2019. The most common symptoms of COVID-19 are fever, dry cough, fatigue, sputum production, shortness of breath, sore throat and headache. Some patients may have myalgia, chills, nausea, nasal congestion and diarrhea. These symptoms begin gradually and are mild in most of the cases. Some people become infected but do not develop any symptoms and do not feel unwell. Most people (about 80%) recover from the disease without special treatment. Approximately one in six people who get infected with COVID-19 becomes seriously ill and develops difficulty breathing. Elderly people, and those with pre-existing conditions, such as high blood pressure, heart problems or diabetes, are more likely to develop serious illness. So far, about 2% of infected people have died.

COVID-19 is transmitted via respiratory droplets that are exhaled by infected people via coughing, sneezing or talking. These droplets can be inhaled or ingested directly by other people or can contaminate surfaces, which can then be infectious for several days. Most estimates of the incubation period for COVID-19 range from 1 to 14 days, during which people might already be infectious without showing disease symptoms.

3. Test Principle

The NADAL® COVID-19 IgG/IgM Test is a lateral flow chromatographic immunoassay for the qualitative detection of anti-SARS-CoV-2 IgG and IgM in human whole blood, serum or plasma specimens.

Anti-human IgM are pre-coated onto the test line region 'IgM' and anti-human IgG are pre-coated onto the test line region 'IgG' of the membrane. During testing, the specimen reacts with SARS-CoV-2 antigens which are conjugated to coloured particles. The mixture then migrates along the membrane chromatographically by capillary action and reacts with the anti-human IgM and anti-human IgG in the test line region 'IgM' and 'IgG' of the membrane. The presence of a red line in the test line region 'IgM' and/or 'IgG' indicates a positive result. The absence of a red line in the test line region 'IgM' and/or 'IgG'indicates a negative result.

The colour change from a blue line to a red one in the control line region 'C' serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

4. Reagents and Materials Supplied

- 25 NADAL® COVID-19 IgG/IgM Test cassettes*
- 25 disposable pipettes (5 µL)
- 1 buffer (3 mL)
- 1 package insert

*containing the preservative sodium azide: <0.02% (7.5 ng/test)

No hazard labelling is required according to Regulation (EC) Nº 1272/2008 CLP, Concentrations are below exemption threshold.

5. Additional Materials Required

- Specimen collection containers (appropriate for specimen material to be tested)
- Centrifuge (for serum or plasma specimens only)
- Alcohol pads
- Lancets (for fingerstick whole blood specimens only)
- Timer

6. Storage & Stability

Test kits should be stored at 2-30°C until the indicated expiry date. Test cassettes are stable until the expiry date printed on the foil pouches. Test cassettes must remain in the sealed foil pouches until use. Do not freeze the test kit. Do not use tests beyond the expiry date indicated on the packaging. Care should be taken to protect test kit components from contamination. Do not use test kit components if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to inaccurate results.

7. Warnings and Precautions

- For professional *in-vitro* diagnostic use only.
- Carefully read through the test procedure prior to testing.
- Do not use the test beyond the expiration date indicated on the packaging.
- Do not use test kit components if the primary packaging is damaged.
- Tests are for single use only.
- Do not add specimens to the reaction area (result area).
- In order to avoid contamination, do not touch the reaction area (result area).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Do not substitute or mix components from different test kits.
- Do not use the buffer if it is discoloured or turbid. Discolouration or turbidity may be a sign of microbial contamination.
- Do not eat, drink or smoke in the area where specimens and test kits are handled.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being assayed.
- Handle all specimens as if they contain infectious agents, Observe established precautions for microbiological risks throughout all procedures and standard guidelines for the appropriate disposal of specimens.

- The test kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled in accordance with usual safety precautions (e.g., do not ingest or inhale).
- Temperature can adversely affect test results.
- Failure to bring specimens and reagents to room temperature prior to testing may decrease assay sensitivity. Inaccurate or inappropriate specimen collection, storage or transport may yield false negative test results.
- Used testing materials should be disposed of according to local regulations.

8. Specimen Collection and Preparation

The NADAL® COVID-19 IgG/IgM Test can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.

To collect fingerstick whole blood specimens:

- Wash the patient's hand with soap and warm water or clean it with an alcohol pad. Allow it to dry.
- Massage the hand, without touching the puncture site, by rubbing along the hand towards the fingertip of the middle or ring finger.
- Puncture the skin with a sterile lancet, Wipe away the first drop of blood.
- Gently rub the hand from the wrist to the palm, and then to the finger to form a rounded drop of blood over the puncture site.

Fingerstick whole blood should be tested immediately.

Venipuncture whole blood specimens

Containers containing anticoagulants, such as EDTA, citrate, heparin or oxalate should be used for the preparation of venous whole blood or plasma specimens.

Testing should be performed immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods of time.

If the test is to be run within 3 days of specimen collection, whole blood collected by venipuncture should be stored at 2-8°C.

Do not freeze whole blood specimens.

Serum and plasma specimens

Separate serum or plasma from blood as soon as possible to avoid haemolysis, Use only clear, non-haemolysed specimens.

Testing should be performed immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods of time. Serum and plasma specimens can be stored at 2-8°C for up to 7 days. For long-term storage, specimens should be kept at -20°C.

Bring specimens to room temperature prior to testing. Frozen specimens should be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

If specimens are to be shipped, they should be packed in compliance with all applicable regulations for the transportation of etiologic agents.

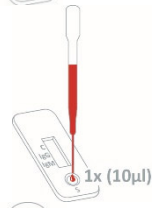
Icteric, lipemic, haemolysed, heat-treated and contaminated specimens may lead to inaccurate test results.

9. Test Procedure

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) prior to testing.

1. Remove the test cassette from the foil pouch and use it as soon as possible. The best results will be obtained if the test is performed immediately after opening the foil pouch. Label the test cassette with the patient or control identification.
2. Place the test cassette on a clean and level surface.
3. **a) For serum or plasma specimens:** Holding the pipette vertically, draw the specimen up to the first widening (approximately 5 µL) and add it to the specimen well (S) of the test cassette.

b) For venipuncture whole blood specimens: Holding the pipette vertically, add 1 drop (approximately 10 µL) of a whole blood specimen to the specimen well (S) of the test cassette.



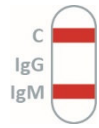
4. Holding the buffer bottle vertically, add 2 drops of buffer to the specimen well (S). **Avoid air bubbles forming.**

5. Start the timer.
6. Wait for the red line(s) to appear. Read the test result after exactly 15 minutes. Do not interpret the result after more than 15 minutes.

10. Result Interpretation

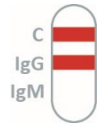
Positive for IgM

The blue line in the control line region 'C' turns red. Another red line develops in the test line region 'IgM'.



Positive for IgG

The blue line in the control line region 'C' turns red. Another red line develops in the test line region 'IgG'.



Positive for IgG and IgM

The blue line in the control line region 'C' turns red. A red line develops in the test line region 'IgM' and another one develops in the test line region 'IgG'.

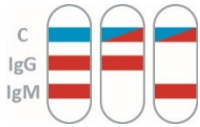
Note: The colour intensity in the test line region 'IgG' and 'IgM' may vary depending on the concentration of anti-SARS-CoV-2 antibodies in the specimen. Therefore, any shade of colour in the test line region 'IgG' or 'IgM' should be considered positive. Note that this is a qualitative test only and it cannot determine the analyte concentration in the specimen.

Negative

The blue line in the control line region 'C' turns red.No lines develop in the test line region 'IgM' and 'IgG'.

Invalid

Results from any test where the blue line in the control line region 'C' remains completely or partially blue at the specified reading time must be discarded. Please review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your distributor.



Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control line failure.

11. Quality Control

An internal procedural control is included in the test cassette:

The line turning from blue to red in the control line region 'C' is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Good laboratory practice (GLP) recommends the use of external control materials to ensure proper test kit performance.

12. Limitations

- The NADAL® COVID-19 IgG/IgM Test is for professional *in-vitro* diagnostic use only. It should be used for the qualitative detection of anti-SARS-CoV-2 antibodies in human whole blood, serum or plasma specimens only. Neither the quantitative value nor the rate of increase in the concentration of anti-SARS-CoV-2 antibodies can be determined with this qualitative test.
- The NADAL® COVID-19 IgG/IgM Test only detects the presence of anti-SARS-CoV-2 antibodies in specimens and should not be used as the sole criterion for a diagnosis of COVID-19.
- As with all diagnostic tests, all results should be interpreted in conjunction with other clinical information available to the physician.
- At the beginning of the disease, the concentration of anti-SARS-CoV-2 IgM may be below the detection limit of the test.
- The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
- Results from immunosuppressed patients should be interpreted with caution.
- A positive test result can also occur in case of negative PCR results because antibodies are still present in the blood after the illness and can be detected.
- If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of a SARS-CoV-2 infection.
- A high-dose hook effect may occur where the colour intensity of test lines decreases as the concentration of anti-SARS-CoV-2 IgM/IgG increases. If a high-dose hook effect is suspected, the dilution of specimens may increase the colour intensity of test lines.

13. Expected Values

SARS-COV-2 infection is characterised by the presence of detectable IgM 3-7 days after the onset of the first symptoms. Later in the course of infection, IgG may also be detected. Persistent IgG levels in specimens after SARS-COV-2 infections may cause positive test results even if the pathogen can no longer be detected by PCR.

14. Performance Characteristics

Clinical performance

Diagnostic sensitivity and specificity

The NADAL® COVID-19 IgG/IgM Test was evaluated using clinical specimens from patients with symptoms of pneumonia or respiratory infections in comparison with a PCR.

		PCR		
NADAL® COVID-19 IgG/IgM Test (IgM)		Positive	Negative	Total
	Positive	74	2	76
	Negative	5	225	230
	Total	79	227	306

Diagnostic sensitivity: 93.7% (86.0% - 97.3%)*

Diagnostic specificity: 99.1% (96.8% - 99.8%)*

Overall agreement: 97.7% (95.4% - 98.9%)*

*95% confidence interval

The NADAL® COVID-19 IgG/IgM Test was evaluated using clinical specimens from convalescent patients in comparison with a PCR.

		PCR		
NADAL® COVID-19 IgG/IgM Test (IgG)		Positive	Negative	Total
	Positive	82	3	85
	Negative	1	224	225
	Total	83	227	310

Diagnostic sensitivity: 98.8% (93.5% - 99.8%)*

Diagnostic specificity: 98.7% (96.2% - 99.5%)*

Overall agreement: 98.7% (96.7% - 99.5%)*

*95% confidence interval

Analytical performance

Detection limit

The detection limit of the NADAL® COVID-19 IgG/IgM Test is 3.4 ng/mL for anti-SARS-CoV-2 IgG and 210 ng/mL for anti-SARS-CoV-2 IgM.

Interfering substances

Solutions of the following potentially interfering substances were tested in 3 test series (without anti-SARS-CoV-2 antibodies, spiked with anti-SARS-CoV-2 IgM and spiked with anti-SARS-CoV-2 IgG) and showed no interference with the NADAL® COVID-19 IgG/IgM Test at the concentrations listed below.

Acetylsalicylic acid	**3.62 mmol/L**
Albumin	**50,000 mg/L**
Amoxicillin	**206 µmol/L**
Ascorbic acid	**342 µmol/L**
Bilirubin	**50 mg/L**
Caffeine	**308 µmol/L**
EDTA	**3.4 µmol/L**
Ethanol	**86.8 mmol/L**
Ethambutol	**58.7 µmol/L**
Fluconazole	**245 µmol/L**
Haemoglobin	**200,000 mg/L**
Heparin	**3000 U/L**
Ibuprofen	**2425 µmol/L**
Isoniazid	**292 µmol/L**
Loratadin	**0.78 µmol/L**
Nadolol	**3.88 µmol/L**
Naproxen	**2170 µmol/L**
Paroxetine	**3.04 µmol/L**
Potassium oxalate	**2 mg/mL**
Quinine	**148 µmol/L**
Rifampicin	**78.1 µmol/L**
Sodium citrate	**5 mg/mL**
Triglycerides	**5000 mg/L**

Cross-reactivity

ANA, anti-HSV-1 IgM, HAMA, anti-chikungunya virus, anti-HSV-2 IgM, anti-HBsAg, anti-*Chlamydia trachomatis*, anti-*rubella* virus IgM, Lyme borreliosis, anti-CMV IgM, syphilis, *P. falciparum*, anti-dengue virus, tuberculosis, *P. vivax*, anti-HAV IgM, yellow fever, RF (high titre), anti-HCV, anti-Zika virus, toxoplasmosis, anti-HEV IgM, Chagas disease, typhoid fever, anti-HIV and anti-EBV IgG positive specimens were tested using the NADAL® COVID-19 IgG/IgM Test. No cross-reactivity with the specimens was observed when tested using the NADAL® COVID-19 IgG/IgM Test.

Precision

Repeatability

Repeatability was established by testing 10 replicates of negative, anti-SARS-CoV-2 IgM strong and weak positive as well as anti-SARS-CoV-2 IgG strong and weak positive plasma specimens.

Reproducibility

Reproducibility was established by testing 3 replicates of negative, anti-SARS-CoV-2 IgM strong and weak positive as well as anti-SARS-CoV-2 IgG strong and weak positive plasma specimens with each of 3 independent lots of the NADAL® COVID-19 IgG/IgM Test.

The NADAL® COVID-19 IgG/IgM Test demonstrated acceptable repeatability and reproducibility. The negative and positive values were correctly identified >99% of the time.

15. References

1. World Health Organization (WHO), WHO Statement Regarding Cluster of Pneumonia Cases in Wuhan, China, Beijing: WHO; 9 Jan 2020,
2. World Health Organization (WHO), Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19), WHO; 28 Feb 2020
3. Weiss SR, Leibowitz JL, Coronavirus pathogenesis, Adv Virus Res 2011;81:85-164,
4. Cui J, Li F, Shi ZL, Origin and evolution of pathogenic coronaviruses, Nat Rev Microbiol 2019; 17:181-192,
5. Su S, Wong G, Shi W, et al, Epidemiology, genetic recombination, and pathogenesis of coronaviruses, TrendsMicrobiol 2016;24:490-502,

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