NADAL® COVID-19 Ag Test (Ref. 243103N-20)

1. Intended Use

The NADAL® COVID-19 Ag Test is a lateral flow chromatographic immunoassay for the qualitative detection of SARS-CoV-2 viral nucleoprotein antigens in human nasopharyngeal and oropharyngeal specimens (see section 12 'Limitations'). This test is intended for use as an aid in the diagnosis of infections with SARS-CoV-2. Note that the concentration of viral nucleoprotein antigens may vary in the course of the disease and might fall below the detection limit of the test. Possible infectiousness of test subjects cannot be ruled out based on negative test results. The test procedure is not automated and requires no special training or qualification. The NADAL® COVID-19 Ag 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. 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 diarrhoea. 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 Ag Test is a lateral flow chromatographic immunoassay for the qualitative detection of SARS-CoV-2 viral nucleoprotein antigens in human nasopharyngeal and oropharyngeal specimens.

Anti-SARS-CoV-2 antibodies are immobilised in the test line region (T) of the membrane. A specimen is added to an extraction tube containing buffer in order to release SARS-CoV-2 antigens. During the test, extracted antigens bind to anti-SARS-CoV-2 antibodies conjugated to coloured particles and precoated onto the sample pad of the test cassette. The mixture then migrates along the membrane chromatographically by capillary action and interacts with the reagents on the membrane. The complexes are then captured by anti-SARS-CoV-2 antibodies in the test line region (T). Excess coloured particles are captured in the control line region (C). The presence of a coloured line in the test line region (T) indicates a positive result. The absence of a coloured line in the test line region (T) indicates a negative result.

The formation of a coloured line 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

- 20 NADAL® COVID-19 Ag test cassettes*
- Additional material provided according to 93/42/EEC:
  Due to possible supply shortages of COVID-19 related accessory medical products, the swab manufacturer might change. Therefore, the supplied swabs are from one of the manufacturers listed below.
  a) 20 sterile swabs, CE0086
  Puritan Medical Products Company LLC
  31 School Street
  Guilford, Maine 04443-0149 USA (authorised EU representative EMERGO EUROPE, The Hague, The Netherlands)
  b) 20 sterile swabs, CE0197
  Jiangsu Changfeng Medical Industry Co., Ltd
  Touqiao Town, Guangling District, Yangzhou, Jiangsu 225109 China (authorised EU representative Llins Service & Consulting GmbH, Obere Seegasse 34/2, 69124 Heidelberg, Germany)
  c) 20 sterile swabs, CE0197
  CITOTEST LABWARE MANUFACTURING CO., LTD
  No.48, Xinxiu Road, Haimen, Jiangsu province (authorised EU representative WellKang Ltd, 16 Castle St, Dover, CT16 1PW, UK)
  d) 20 sterile swabs, Copan Floqswabs; CE 0123
  Copan Italia S.p.A.,
  Via Perotti 10, 25125 Brescia, Italy
- 20 extraction tubes incl. dropper caps
- 2 buffer bottles (7 mL each)**
- 1 reagent holder
- 1 package insert

*containing the preservative sodium azide: <0.1%

**Buffer containing the following preservatives: sodium azide: <0.1 mg/mL

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

5. Additional Materials Required

- 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 extraction tube 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.
- Further specimen processing and patient management should follow local COVID-19 guidelines and regulations.
- 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.
- Used testing materials should be disposed of according to local regulations.

8. Specimen Collection and Preparation

Oropharyngeal specimen:

- Gently insert a sterile swab into the pharynx and collect secretions by brushing the swab several times against the reddened posterior pharyngeal wall and both tonsillar pillars. Avoid touching the tongue, teeth and gums.

Nasopharyngeal specimen:

- Insert the swab into the nostril, parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx.
- Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions.
- Slowly remove the swab while rotating it. Specimens can be collected from both nostrils using the same swab, but it is not necessary to collect specimens from both sides if the tip is saturated with fluid from the first collection.

Note:

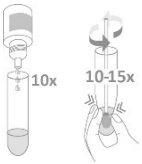
- Use only synthetic fibre swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit further testing.
- Swab specimens should be tested as soon as possible after collection. Use freshly collected specimens for best test performance.
- If not tested immediately, swab specimens can be stored at 2-8°C for 24 hours after collection.
- Viral transport media (VTM) without denaturing agents can be used during specimen storage for the subsequent antigen detection using the NADAL® COVID-19 Ag Test. In order to influence the sensitivity as little as possible, a low volume of VTM (e.g. 1 mL) is recommended.
- Do not use specimens that are obviously contaminated with blood, as it may interfere with the flow of specimens and lead to inaccurate test results.

9. Test Procedure

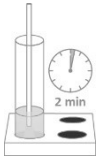
Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) prior to testing.

1. Place a clean extraction tube labeled with the patient or control identification into the designated area of the reagent holder.
2. Gently mix the buffer by carefully swiveling the bottle.

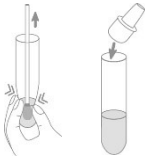
3. Holding the buffer bottle vertically and without touching the edge of the tube, add 10 drops to the extraction tube.
4. Insert the swab with the collected specimen into the tube. Swirl the swab and squeeze it 10-15 times by compressing the wall of the extraction tube against the swab to extract the antigens contained in the swab.



5. Let the solution stand for 2 minutes.
6. Remove the swab, pressing it firmly against the wall of the tube to release as much liquid as possible. Dispose of the swab in accordance with guidelines for the handling of infectious agents.



7. 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.

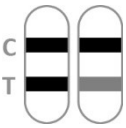


8. Place the test cassette on a clean and level surface.
9. Attach a dropper cap to the extraction tube, invert the tube and transfer 2 drops of the extracted solution to the specimen well (S) of the test cassette.

10. Start the timer.
11. Wait for the coloured 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:
Two coloured lines appear in the result area. One line appears in the control line region (C) and the other line appears in the test line region (T).



Note: The colour intensity in the test line region (T) may vary depending on the concentration of SARS-CoV-2 viral nucleoprotein antigens in the specimen. Any shade of colour in the test line region (T) should be considered a positive result. Note that this is a qualitative test only and it cannot determine the analyte concentration in the specimen.

Negative:
Only one coloured line appears in the control line region (C). No coloured line appears in the test line region (T).

Invalid
The control line (C) fails to appear. Results from any test which has not produced a control line 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:

A coloured line appearing 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 Ag Test is for professional *in-vitro* diagnostic use only. It should be used for the qualitative detection of SARS-CoV-2 viral nucleoprotein antigens in human nasopharyngeal and oropharyngeal specimens only. Neither the quantitative value nor the rate of increase/decrease in the concentration of SARS-CoV-2 viral nucleoprotein antigens can be determined with this qualitative test.
- The NADAL® COVID-19 Ag Test only detects the presence of SARS-CoV-2 viral nucleoprotein antigens in specimens and should not be used as the sole criterion for a diagnosis of COVID-19.
- Both viable and non-viable SARS-CoV-2 viruses can be detected using the NADAL® COVID-19 Ag Test.
- The sections 'Specimen Collection and Preparation' as well as 'Test Procedure' must be followed closely while testing. Failure to follow them may lead to inaccurate test results because the antigen concentration in the swab is highly dependent on the correct procedure.
- As with all diagnostic tests, all results should be interpreted in conjunction with other clinical information available to the physician.
- In the course of SARS-CoV-2 infection, the concentration of viral nucleoprotein antigens may fall below the detection limit of the test.
- 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 and should be confirmed via molecular assay.

13. Expected Values

SARS-CoV-2 viral particles are normally present in the respiratory tracts of COVID-19 patients. A positive test result can indicate an acute infection. Virus concentrations in nasopharyngeal and oropharyngeal swab specimens may vary in the course of the disease and might fall below the detection limit of rapid tests, even though patients are still showing symptoms. Conversely, the virus might continue be detectable over long periods of time even in convalescent patients. Possible infectiousness of test subjects cannot be ruled out based on negative test results.

14. Performance Characteristics

Clinical performance

Diagnostic sensitivity and specificity

The NADAL® COVID-19 Ag Test was evaluated with clinical specimens whose status was confirmed using RT-PCR (C_t range positive: 20-37). The sensitivity was calculated for the range from high to medium viral load (C_t 20-30) and from high to very low viral load (C_t 20-37). The results are presented in the following tables.

		RT-PCR, C_t 20-30		
		Positive	Negative	Total
NADAL® COVID-19 Ag Test	Positive	120	0	120
	Negative	3	161	164
	Total	123	161	284

Diagnostic sensitivity (C_t 20-30): 97.6% (93.1% - 99.2%)*

Overall agreement (C_t 20-30): 98.9% (96.9% - 99.6%)*

Diagnostic specificity: >99.9% (97.7% - 100%)*

*95% confidence interval

		RT-PCR, C_t 20-37		
		Positive	Negative	Total
NADAL® COVID-19 Ag Test	Positive	150	0	150
	Negative	37	161	198
	Total	187	161	348

Diagnostic sensitivity (C_t 20-37): 80.2% (73.9% - 85.3%)*

Overall agreement (C_t 20-37): 89.4% (85.7% - 92.2%)*

Diagnostic specificity: >99.9% (97.7% - 100%)*

*95% confidence interval

In order to show the strong dependence of the diagnostic sensitivity on the viral load, the following table demonstrates the sensitivity for different C_t value ranges of the reference PCR:

C_t range	Sensitivity
20 – 25	97.12%
20 – 30	97.56%
20 – 32	96.21%
20 – 35	85.71%
20 – 37	80.21%

Please note that C_t values may vary between different PCR systems at the same virus concentration.

Detection limit

The detection limit of the NADAL® COVID-19 Ag Test is $2 \times 10^{2.4}$ $TCID_{50}$/mL and was determined with a SARS-CoV-2 control with a known virus titre.

The detection limit of the NADAL® COVID-19 Ag Test is 0.4 ng/mL for recombinant SARS-CoV-2 nucleoprotein.

Interfering substances

The following substances, normally present in respiratory specimens or artificially introduced into the respiratory tract, were evaluated at the concentrations listed below and showed no interference with the NADAL® COVID-19 Ag Test.

Substance	Concen-tration	Substance	Concen-tration
3 OTC nasal sprays	10%	Guaiacol glyceryl ether	20 mg/mL
3 OTC mouthwashes	10%	Mucin	1%
3 OTC sore throat liquids	10%	Mupirocin	250 µg/mL
4-acetamido-phenol	10 mg/mL	Oxymetazoline	10 mg/mL
Acetylsalicylic acid	20 mg/mL	Phenylephrine	10 mg/mL
Albuterol	20 mg/mL	Phenylpro-panolamine	20 mg/mL
Chlorpheniramine	5 mg/mL	Relenza® (zanamivir)	20 mg/mL
Dexamethasone	5 mg/mL	Rimantadine	500 ng/mL
Dextromethor-phan	10 mg/mL	Tamiflu® (oseltamivir)	100 mg/mL
Diphenhydramine	5 mg/mL	Tobramycin	40 mg/mL
Doxylamine succinate	1 mg/mL	Triamcinolone	14 mg/mL
Flunisolide	3 mg/mL		

Cross-reactivity

Specimens spiked with the following pathogens were tested using the NADAL® COVID-19 Ag Test:

HCoV-HKU1, HCoV-OC43, HCoV-NL63, HCoV-229E, measles virus, *Streptococcus pneumoniae,* Epstein-Barr virus*, Bordetella parapertussis,* influenza A (H1N1) pdm09, influenza A (H3N2), influenza A (H5N1), influenza A (H7N9), influenza A (H7N7), influenza B Victoria lineage, Influenza B Yamagata lineage, *Haemophilus influenzae*, *Candida albicans*, *Mycobacterium tuberculosis,* respiratory syncytial virus, adenovirus, parainfluenza virus type 1, 2, 3, human metapneumovirus, rhinovirus, coxsackievirus type A16, norovirus, mumps virus, *Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus* group C, *Staphylococcus aureus.*

No cross-reactivity with the specimens was observed when tested using the NADAL® COVID-19 Ag Test.

Precision

Repeatability and reproducibility

Precision was established by testing 10 replicates of negative, low positive and high positive controls.

Reproducibility was established by testing triplicates of negative, low and high positive controls. Testing was performed by 3 operators using 3 independent NADAL® COVID-19 Ag test lots at 3 different sites on 5 separate days.

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

15. References

1. Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK – ninth update, 23 April 2020. Stockholm: ECDC; 2020.
2. Cui J, Li F, Shi ZL, Origin and evolution of pathogenic coronaviruses, Nat Rev Microbiol 2019; 17:181-192.
3. He, X., Lau, E.H.Y., Wu, P. et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 26, 672–675 (2020).
4. Su S, Wong G, Shi W, et al, Epidemiology, genetic recombination, and pathogenesis of coronaviruses, TrendsMicrobiol 2016;24:490-502.
5. Weiss SR, Leibowitz JL, Coronavirus pathogenesis, Adv Virus Res 2011; 81:85-164.

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