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Τμήμα: Αξιολόγησης Υγειονομικού Υλικού
Πληροφορίες: Δ.Δουδουνή
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Ημερ/νία Έκδοσης: 17-11-2020
Αρ. Πρωτ.: 121863/2020

Προς: PROGNOSIS BIOTECH A.E
ΦΑΡΣΑΛΩΝ 153,
41335, ΛΑΡΙΣΑ, ΕΛΛΑΔΑ

HELLENIC REPUBLIC
MINISTRY OF HEALTH
NATIONAL ORGANIZATION FOR MEDICINES
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EVALUATION DIVISION
Medical Devices Section
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Issue Date: 17-11-2020
Our Ref.: 121863/2020

To: PROGNOSIS BIOTECH S.A.
FARSALON 153,
41335, LARISA, GREECE

ΠΙΣΤΟΠΟΙΗΤΙΚΟ ΕΛΕΥΘΕΡΗΣ ΚΥΚΛΟΦΟΡΙΑΣ

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ΛΑΡΙΣΑ, ΕΛΛΑΔΑ

Rapid Test Ag 2019-nCoV (V1310, V1330)

που περιλαμβάνονται στην υπ΄αριθ. **114084, 121776/17-11-2020** Βεβαίωση Εγγραφής στο Μητρώο Κατασκευαστών In Vitro Διαγνωστικών Ιατροτεχνολογικών Προϊόντων του ΕΟΦ (ημερομηνία λήξης **25-05-2022**), κυκλοφορούν ελεύθερα στην Ελληνική αγορά και την αγορά της Ευρωπαϊκής Ένωσης, σύμφωνα με την Οδηγία 98/79/ΕΟΚ.

Το παρόν χορηγείται για εξαγωγικούς σκοπούς.

Η ΑΝΑΠΛΗΡΩΤΡΙΑ ΔΙΕΥΘΥΝΤΡΙΑ

Μ. ΟΡΦΑΝΟΥ

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LARISA, GREECE

Rapid Test Ag 2019-nCoV (V1310, V1330)

which are included in ref. no. **114084, 121776/17-11-2020** Registration of Manufacturers of In Vitro Diagnostic Medical Devices in the National Organization for Medicines of Greece (expiry date **25-05-2022**), are sold freely in the Greek and the European Union market, according to the 98/79/EEC Directive.

This certificate is issued for exports.

EVALUATION DIVISION ASSOCIATE DIRECTOR

M. ORFANOU

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Διεύθυνση Αξιολόγησης Προϊόντων
Τμήμα Αξιολόγησης Υγειονομικού Υλικού
Πληροφορίες: Δ. Δουδουνή
Τηλέφωνο: 213 2040 450

Χολαργός, 17/11/2020
Αρ. Πρωτ. : 114084, 121776

Προς
PROGNOSIS BIOTECH ΑΕ
ΦΑΡΣΑΛΩΝ 153, 41335
ΛΑΡΙΣΑ

ΒΕΒΑΙΩΣΗ ΣΥΜΠΛΗΡΩΜΑΤΙΚΗΣ ΕΓΓΡΑΦΗΣ

ΣΤΟ ΜΗΤΡΩΟ ΚΑΤΑΣΚΕΥΑΣΤΩΝ IN VITRO ΔΙΑΓΝΩΣΤΙΚΩΝ

ΙΑΤΡΟΤΕΧΝΟΛΟΓΙΚΩΝ ΠΡΟΪΟΝΤΩΝ

Έχοντας υπόψη:

1. Την Κοινή Υπουργική Απόφαση (ΚΥΑ) ΔΥ8δ/οικ.3607/892/ΦΕΚ1060Β/10-8-2001 "Εναρμόνιση της Ελληνικής Νομοθεσίας προς την Οδηγία 98/79/ΕΚ του Ευρωπαϊκού Κοινοβουλίου και του Συμβουλίου της 27ης Οκτωβρίου 1998 για τα in vitro διαγνωστικά ιατροτεχνολογικά προϊόντα"
2. Τις υπ' αριθ. 62701/27-11-2003 και 63222/1-12-2003 εγκυκλίους ΕΟΦ
3. Τον Κανονισμό (ΕΕ) 2017/746 του Ευρωπαϊκού Κοινοβουλίου και του Συμβουλίου της 5ης Απριλίου 2017 για τα in vitro διαγνωστικά ιατροτεχνολογικά προϊόντα και για την κατάργηση της οδηγίας 98/79/ΕΚ και της απόφασης 2010/227/ΕΕ της Επιτροπής
4. Τις Συμπληρωματικές Αιτήσεις της εταιρίας **PROGNOSIS BIOTECH ΑΝΩΝΥΜΗ ΕΤΑΙΡΙΑ** με αρ. πρωτ. **114084/09-11-2020** και **121776/17-11-2020,** τις Διορθωτικές Αιτήσεις με αρ. πρωτ. **121773/17-11-2020**, **121819/17-11-2020** και **121818/17-11-2020** για εγγραφή στο Μητρώο Κατασκευαστών Ι/Π και τα συνημμένα σ' αυτές απαιτούμενα δικαιολογητικά

Εγγράφεται η εταιρεία σας στο Μητρώο Κατασκευαστών Ιατροτεχνολογικών Προϊόντων του ΕΟΦ, σύμφωνα με τα οριζόμενα στο άρθρο 10 της σχετ. ΚΥΑ ΔΥ8δ/3607/892/2001, για τα προϊόντα που περιλαμβάνονται στη συνημμένη λίστα.

Ο κατασκευαστής ακολουθεί, προκειμένου να επιθέσει τη σήμανση CE, τη διαδικασία που αναφέρεται στο παράρτημα ΙΙΙ και συντάσσει τη Δήλωση Συμμόρφωσης CE που απαιτείται πριν να διαθέσει στο εμπόριο αυτά τα βοηθήματα.

Ο Κατασκευαστής πρέπει να φυλάσσει τη Δήλωση Συμμόρφωσης και την Τεχνική Τεκμηρίωση που αναφέρεται στο παράρτημα ΙΙΙ και να τα θέτει στη διάθεση του ΕΟΦ προς έλεγχο, για περίοδο πέντε ετών από την κατασκευή του τελευταίου προϊόντος. Όταν ο κατασκευαστής δεν είναι εγκατεστημένος στην Κοινότητα, η παραπάνω υποχρέωση βαρύνει τον εντολοδόχο του.

Η εγγραφή στο Μητρώο Κατασκευαστών In Vitro Διαγνωστικών Ιατροτεχνολογικών Προϊόντων, γίνεται σε εφαρμογή του άρθρου 10 της ΔΥ8δ/οικ.3607/892/ΦΕΚ1060Β/10-8-2001.

Η εγγραφή στο Μητρώο Κατασκευαστών In Vitro Διαγνωστικών Ιατροτεχνολογικών Προϊόντων βασίζεται στη Δήλωση Συμμόρφωσης που καταθέσατε και δεν αποτελεί κανενός είδους έγκριση της ποιότητας, ασφάλειας και αποτελεσματικότητας των προϊόντων.

Η εγγραφή σας ισχύει μέχρι **25/05/2022.**

Η ΑΝ. ΠΡΟΪΣΤΑΜΕΝΗ

Δ/ΝΣΗΣ ΑΞΙΟΛΟΓΗΣΗΣ ΠΡΟΪΟΝΤΩΝ

Μ.ΟΡΦΑΝΟΥ

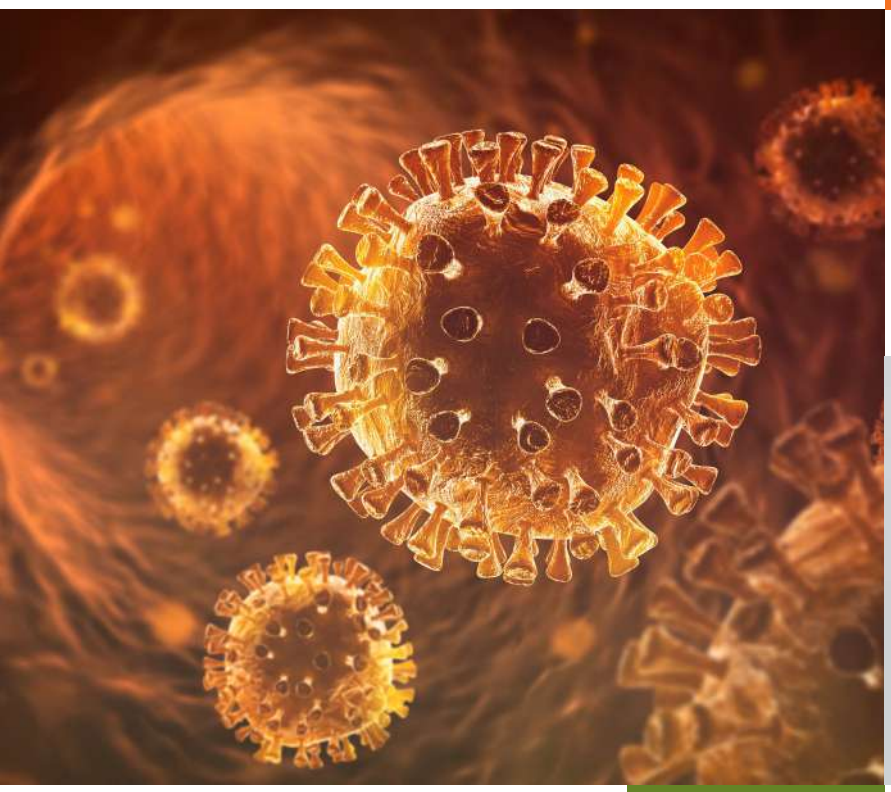
ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ ΥΠΟΥΡΓΕΙΟ ΥΓΕΙΑΣ ΕΘΝΙΚΟΣ ΟΡΓΑΝΙΣΜΟΣ ΦΑΡΜΑΚΩΝ

ΘΕΩΡΗΘΗΚΕ ΓΙΑ ΤΗΝ ΑΚΡΙΒΕΙ
Η ΑΝΑΠΛΗΡΩΤΡΙΑ ΠΡΟΪΣΤΑΜΕΝΗ
ΓΕΝΙΚΗΣ ΓΡΑΜΜΑΤΕΙΑΣ

ΧΑΡΑΛΑΜΠΙΑ ΜΑΡΟΥΔΑ

1. Rapid Test Ag 2019-nCoV 10 test (V1310)	**2830000636996**
Ονομασία (αγγλικά)	Rapid Test Ag 2019-nCoV 10 test
EDMA	(15 70 90 90) OTHER OTHER VIROLOGY - RT & POC
CND	(W0105099099) Other Other Virology - RT & POC
2. Rapid Test Ag 2019-nCoV 30 test (V1330)	**2830000637016**
Ονομασία (αγγλικά)	Rapid Test Ag 2019-nCoV 30 test
EDMA	(15 70 90 90) OTHER OTHER VIROLOGY - RT & POC
CND	(W0105099099) Other Other Virology - RT & POC

VALIDATION REPORT
Rapid Test Ag 2019-nCoV



PROGNOSIS
BIOTECH

Rapid Test Ag 2019-nCoV Test kit

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Rapid Test Ag-2019-nCoV

1. Introduction

A novel coronavirus (identified as 2019-nCoV) emerged in the Chinese province of Hubei (Wuhan) in December 2019, which has resulted in hundreds of thousands of confirmed human infections worldwide. Cases of severe illness and deaths have been reported. On February 11, 2020 the International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2. The median incubation time is estimated to be approximately 5 days with symptoms estimated to be present within 12 days of infection. The most common symptoms of COVID-19 (according to WHO), are similar to other viral respiratory diseases and include fever, dry cough and tiredness. The virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes. Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses with a nucleocapsid of helical symmetry and are composed of several proteins including the Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) proteins (Figure 1). Molecular and antigen testing are the only techniques capable of detecting the SARS-CoV-2 virus. Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid. Nucleocapsid protein is a highly immunogenic phosphoprotein also implicated in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N protein of coronavirus is chosen as a diagnostic tool.

1.1 Principle of the method

The Rapid Test Ag 2019-nCov is a qualitative, lateral flow immunoassay for the detection of Nucleocapsid protein (NP) in nasal or nasopharyngeal specimens. In this test, antibodies specific to the NP are coated on the test line region of the test card. During testing, the specimen reacts with the antibodies to NP that are coated onto particles. The mixture migrates up the membrane to react with the antibodies to NP on the membrane and generate one colored line in the test region. The presence of this colored line in the test region indicates a positive result. To serve as a procedural control, a colored line will always appear in the control region if the test has been performed properly.

1.2 Kit Characteristics

Refer to the instruction manual V1310/ V1330 VERSION 2021-06-02/rev.07.

1.3 Specimen collection

1.3.1 Nasal specimen collection

- Add Running Buffer till the line to the extraction tube from the dropper bottle (300ul).
- Tilt the patient's head back 70 degrees.
- Remove a sterile swab from the pouch and place it into one of the patient's nostrils while rotating (insert the swab less than one inch-2cm). Rotate the swab five times against the nasal wall then slowly remove from the nostril. (Figure 2)
- Using the same swab repeat the collection procedure with the second nostril.

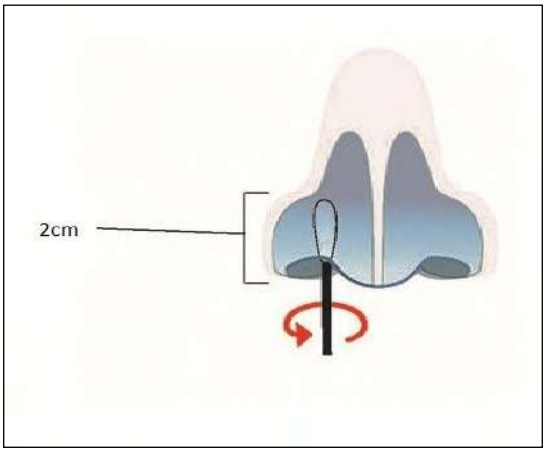


Figure 2. Nasal swab procedure

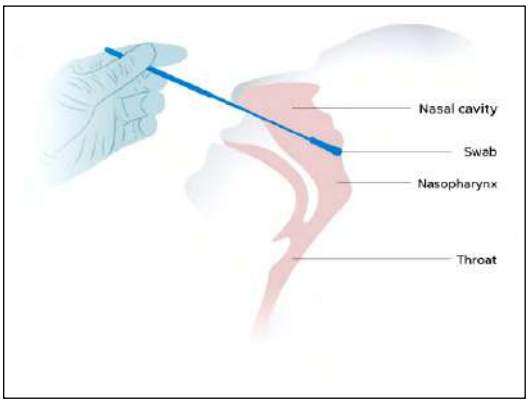


Figure 3. Nasopharyngeal swab procedure

1.3.2 Nasopharyngeal specimen collection

- Add Running Buffer till the line to the extraction tube from the dropper bottle (300ul).
- Tilt the patient's head back 70 degrees.
- Remove a sterile swab from the pouch and place it into one of the patient's nostrils. When it reaches the posterior nasopharynx rotate three to five times and then remove it slowly. (Figure 3)

1.4 Method procedure

1 Calculate the number of swabbing sticks and tubes needed, according to the number of samples to collect.

2 Mark the extraction tubes according to the specimens you intend to collect and add Running Buffer till the line to each one of them from the dropper bottle (300ul).

3 After the specimen collection (see Chapter 1.3), place the swab in the extraction tube, rotate the swab forcefully against the side of the tube for 1min. Best results are obtained when the specimen is vigorously extracted in the solution.

4 Remove the swab, squeezing the sides of the tube to extract as much liquid as possible.

5 Discard the swab.

6 Immerse the test stick following the direction shown by the arrows, so the uncovered area of the sticks gets soaked.

Note: In case the test stick gets inserted in the wrong direction (arrows pointing up) and gets wet at the top label area, it becomes useless and has to be replaced with a new test stick.

7 After 15 minutes, the test stick can be visually read and interpreted according to the corresponding figure.

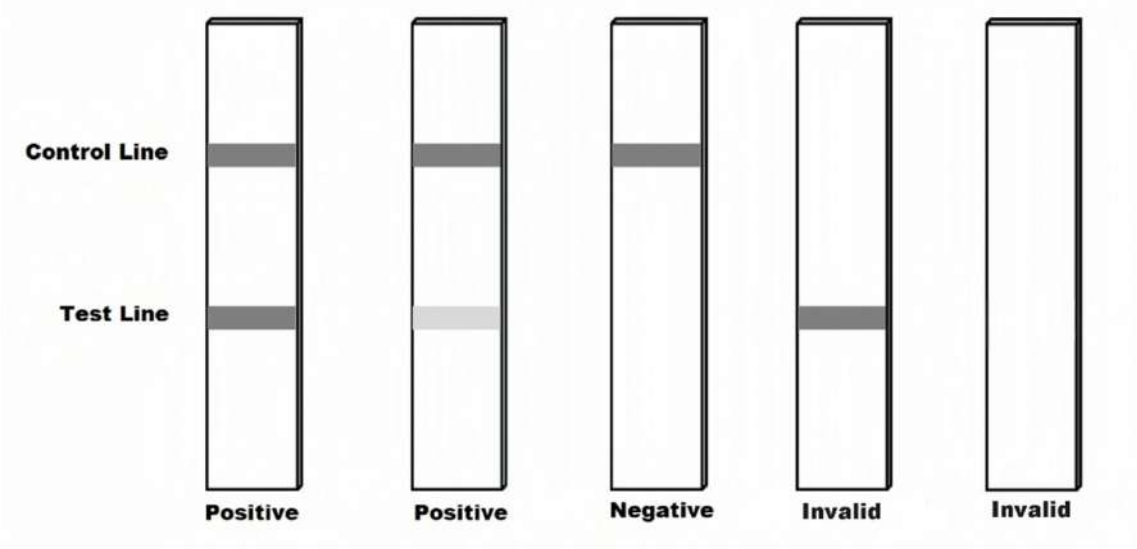
Note: The test result should not be read and interpreted after 15 minutes.

POSITIVE CONTROL: Immerse the stick directly into the positive control tube.

NEGATIVE CONTROL: Immerse the stick directly into the negative control tube.

1.5 Interpretation of results

Positive: Two visible colored bands appear at both Test (T) and Control (C) line. It indicates a positive result for the SARS-CoV-2 Nucleocapsid Protein in the specimen.
Negative: One visible colored band appears at Control line. It indicates that the concentration of the SARS-CoV-2 NP is zero or below the detection limit of the test.
Invalid: No colored band appears at Control line no matter whether it appears at Test line or not.



2. Immunoassay Specifications

2.1 General Specifications

The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
After the extraction specimens should be tested as soon as possible. Otherwise they can be stored at room temperature 20-25°C (68-77°F) for two hours. In this case use the cap to close the extraction tube without discarding the droplet.
The test should be used for the detection of SARS-CoV-2 antigen ONLY in nasal or nasopharyngeal swab specimens.
Failure to follow the guidelines for proper specimen collection, test procedure and interpretation of test results may adversely affect test performance and/or produce invalid result.
USE ONLY the sterile swabs that are provided in the kit for the specimen collection.
During specimen collection avoid contact with bleeding areas and excess of mucus of the nasopharynx as both of them may give a false positive result due to interference with the test performance.
Positive results indicate the presence of SARS-CoV-2 antigens but a diagnosis of an infection should only be made by a physician evaluating all clinical and laboratory findings and must be based in the correlation of the results with further clinical observations.
A negative test result may occur if the level of extracted antigen in a specimen is below the sensitivity of the test or if a poor quality specimen is obtained.
Positive test results do not rule out co-infection with other pathogens.
The Rapid Test Ag will indicate the presence of SARS-CoV-2 NP in the specimen from both viable and non-viable virus.

2.2 Cross-reactivity

In order to determine the cross reactivity of Rapid Test Ag, an evaluation was performed; no cross reactivity against organism, pathogens that could cause infections.

- Adenovirus
- Astrovirus
- Alpha coronavirus 229E
- Alpha coronavirus NL63
- Beta coronavirus OC43
- Beta coronavirus HKU1
- Escherichia Coli O157
- Human Transferrin
- Influenza A virus
- Influenza B virus
- Listeria monocytogenes
- Salmonella enteritidis
- Streptococcus pneumococcal
- Streptococcus pyogenes

Rapid Test Ag 2019-nCov could have some cross-reaction with SARS and very low with MERS.

2.3 Interference Data

The following substances showed no significant interference on the test results of Rapid Test Ag 2019n-CoV.

No	Interfering Substances	Final Test Concentration
1	Azithromycin	84 mg/ml
2	Amoxicillin	54 mg/L
3	Albuterol	0.05 mg/L
4	Acarbose	0.3 mg/L
5	Chlorpheniramine	0.8 mg/L
6	Chlorothiazide	27 mg/L
7	Rheumatoid factor	200 IU/ml
8	Triglycerides	1.5 mg/L
9	Hemoglobin	100 mg/L
10	Human Chorionic Gonadotropin Hormone (pregnancy)	10-fold dilution
11	Ibuprofen	219 mg/L
12	Xylometazoline (Otriven)	10%
13	Acetylsalicylic Acid	3 mg/ml
14	Mucin	0.5%

3. Validation

Determination of the Limit of Detection LOD

The lowest detectable concentration of an analyte in a method is known as LOD. In this case, we check the concentration of heat inactivated SARS-CoV-2 isolate USA-WA1/2020 in Rapid Test Ag 2019-nCov. The LOD is the level at which 95% of the replicates are characterized as positive. The results of 20 replicates of 6 dilutions with heat inactivate virus are shown at the table below.

LOD : 358.75 $TCID_{50}$/mL

3.1 Determination of the Limit of Detection LOD in the methodology

Concentration ($TCID_{50}$ /ml)	Positive Replicates	Visual Interpretation of results
1.15×10^7	20 / 20	Strong positive
1.15×10^6	20 / 20	Strong positive
1.15×10^5	20 / 20	Strong positive
1.15×10^4	20 / 20	Strong positive
5.75×10^3	20 / 20	Positive
2.87×10^3	20 / 20	Positive
1.435×10^3	20 / 20	Positive
717.5	20 / 20	Positive
358.75	20 / 20	Positive
179	3 / 20	Negative

Table 1 . LOD of Heat Inactivated Virus in liquid

3.2 High Dose Effect

No high dose hook effect was observed up to 1.15×10^7 $TCID_{50}$/mL of inactivated SARS-CoV-2 with the Rapid Test Ag 2019-nCoV.

3.3 Clinical performance characteristics

3.3.1 Nasal specimens

In order to determine the clinical performance of the Rapid Test Ag 2019-nCoV, 386 negative and 142 positive NASAL specimens confirmed with RT-PCR assay SARS-COV-2 R-GENE® Biomerieux, RNeasy Mini Kit Qiagen were tested. The results are presented at the table below.

Rapid Test Ag 2019-nCoV	Real-time RT PCR		
	Positive	*Negative*	*Total*
Positive	140	1	141
Negative	2	385	387
Total	142	386	528

	Mean Value	95% confidence interval
Sensitivity	98.59%	95.00% to 99.83%
Specificity	99.74%	98.57% to 99.99%
PPV	99.29%	95.18% to 99.90%
NPV	98.86%	97.54% to 99.58%

CT cycles	RT-PCR positive	Rapid Test Ag positive	Positive Agreement (95% CI)
15-20	53	53	100% (92.28% to 100.00%)
21-25	44	44	100% (91.96% to 99.99%)
26-30	27	27	100% (87.23% to 100.00%)
31-35	18	16	88.89% (65.29% to 98.62%)

Clinical Diagnostic Specificity: 99.74%

Clinical Diagnostic Sensitivity: 98.59%

3.3.2 Nasopharyngeal specimens

In order to determine the clinical performance of the Rapid Test Ag 2019-nCoV, 478 negative and 135 positive NASO-PHARYNGEAL specimens confirmed with RT-PCR assay SARS-COV-2 R-GENE® Biomerieux, RNeasy Mini Kit Qiagen) were tested. The results are presented at the table below.

Rapid Test Ag 2019-nCoV	Real-time RT PCR		
	Positive	*Negative*	*Total*
Positive	129	2	131
Negative	6	476	482
Total	135	478	613

	Mean Value	95% confidence interval
Sensitivity	95.56%	90.58% to 98.35%
Specificity	99.58%	98.50% to 99.95%
PPV	98.47%	94.18% to 99.61%
NPV	98.76%	97.32% to 99.43%

CT cycles	RT-PCR positive	Rapid Test Ag positive	Positive Agreement (95% CI)
15-20	48	48	100% (92.60% to 100.00%)
21-25	43	43	100% (91.78% to 100.00%)
26-30	23	23	100% (85.18% to 100.00%)
31-35	21	15	71.43% (47.82% to 88.72%)

Clinical Diagnostic Specificity: 99.58%

Clinical Diagnostic Sensitivity: 95.56%

4. Results and discussion

During the COVID-19 pandemic, the development of highly sensitive and rapid diagnostic devices has become increasingly important. Currently there are two types of diagnostic tests– molecular tests, such as RT-PCR tests, that detect the virus's genetic material, and antigen tests that detect specific proteins from the virus.

In contrast with the gold standard for COVID-19 diagnosis (RT-PCR), antigen tests can be used immediately, thus enabling the rapid detection of new infected individuals, their isolation and the implementation of confinement measures.

We developed a Rapid Test for the detection of the nucleocapsid protein of SARS-CoV-2 virus from nasal or naso-pharyngeal swabs that provides simple, rapid (15 minutes) and highly responsive detection of SARS-CoV-2 virus with excellent sensitivity and specificity.

The diagnostic value of the Rapid Test Ag-2019-nCov was determined in comparison to RT-PCR in 528 nasal swabs and 548 nasopharyngeal swabs collected from individual patients who were suspected of COVID-19. The Rapid Test Ag –2019-nCov showed 98.59% sensitivity, 99.74% specificity in nasal swabs and 95.56% of sensitivity, 99.56% of specificity in nasopharyngeal swabs.

Considering short turnaround times, user friendliness, low costs and opportunities for decentralized testing, this test can improve our efforts to control transmission of SARS-CoV-2.

- https://www.fda.gov/consumers/consumer-updates/coronavirus-disease-2019-testing-basics
- https://www.nature.com/articles/s41579-020-00461-z
- https://www.cdc.gov/flu/symptoms/flu-vs-covid19.htm
- Wu F, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265-269

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Farsalon 153 | 41335 Larissa, Greece

"DECLARATION OF CE CONFORMITY"

Manufacturer:	ProGnosis Biotech SA
Business address	Farsalon 153, 41335 Larissa, Greece
In Vitro Medical device designation	Rapid Test Ag 2019-nCoV,
Catalogue no	V1310/V1330,
GIVD code	15.70.90.90 (Other virology RT&POC)
Classification:	Other Device, Self-Declaration IVD MD
Conformity assessment route:	In vitro diagnostic medical device self-certification (not included in list A or B of Annex II of the Directive 98/79/EC), Annex III Applied (IVDD 98/79/EC)

We hereby declare under our sole responsibility that the above In Vitro medical device is manufactured according to certified ISO 13485:2016 Quality Management System and conforms with the essential requirements listed in the Annex I of the European in vitro Medical Device Directive 98/79/EC (IVD).

Authorized Signatory **13/11/2020**

Name, signature, company stamp

Date

HELLENIC REPUBLIC
MINISTRY OF HEALTH & SOCIAL SOLIDARITY
GENERAL UNIVERSITY HOSPITAL OF LARISSA
LABORATORY OF MICROBIOLOGY
HEAD: Professor Euthimia Petinaki

Date: 29/1/2021

Clinical evaluation of the performance of the Lateral Flow Assay [V1301 -V1310-V1330] Rapid Test Ag 2019-nCoV, from ProGnosis Biotech SA that directly detect antigens of SARS-CoV-2

To whom it may concern:

This letter serves as verification of the Clinical Evaluation Study that took place on the period of 2nd of December 2020 until the 29th January 2021 at the Laboratory of Microbiology of the General University Hospital of Larissa, which also serves as one of the official Laboratories for SARS-CoV-2 in Greece during the pandemic of COVID-19.

The purpose of this study was the Clinical Evaluation of the **Lateral Flow Assay Test [V1301-V1330] Rapid Test Ag 2019-nCoV** produced from **ProGnosis Biotech SA**, that directly detect antigens of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The samples used for the conduction of the Clinical Evaluation on the following report were positive and negative samples confirmed with RT-PCR at the **Laboratory of Microbiology of the General University Hospital of Larissa.** Both the negative and positive samples were assayed on **RT-PCR using SARS-COV-2 R-GENE® Biomerieux, RNeasy Mini Kit Qiagen & Vircell SARS-COV-2 REALTIME PCR KIT** and on the **Lateral Flow Assay using [V1301-V1330] Rapid Test Ag 2019-nCoV ProGnosis Biotech SA.**

ΕΦΗ ΠΕΤΕΙΝΑΚΗ
ΚΑΘΗΓΗΤΡΙΑ ΙΑΤΡΙΚΗΣ ΒΙΟΠΑΘΟΛΟΓΙΑΣ-
ΚΛΙΝΙΚΗΣ ΜΙΚΡΟΒΙΟΛΟΓΙΑΣ
ΔΙΕΥΘΥΝΤΡΙΑ ΕΡΓΑΣΤΗΡΙΟΥ ΜΙΚΡΟΒΙΟΛΟΓΙΑΣ
ΚΑΙ ΚΛΙΝΙΚΗΣ
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1. Protocol synopsis:

Title	Evaluation of lateral flow assay tests that directly detect antigens of SARS-CoV-2 and can be interpreted visually
Short title	COVID-19 Antigen **Rapid Diagnostic Test (RDT)** Evaluation
Use case of test	Rapid, point-of-care (POC) detection of active infection in adults with suspected COVID-19 infection.
Rationale and background	The aim of this study is to independently evaluate the performance of a novel, rapid, **point-of-care (POC)** lateral flow assay developed and produced by **ProGnosis Biotech SA**, for the direct detection of SARS-CoV-2 antigens (Ag) in comparison to the current gold standard(method) for testing, RT-PCR. **This protocol covers the approach to assess the performance of index RDTs:** • A retrospective clinical approach using respiratory swabs collected in assay-specific buffer from individuals confirmed or suspected COVID-19, as defined by national or WHO case definitions. • If rapid diagnostic tests (RDTs) that detect SARS-CoV-2 antigen are shown to have sufficient accuracy and sensitivity, then their use could facilitate rapid clinical decision making, as these tests are very simple to perform and the turnaround time for results is typically < 30 minutes
Primary objective(s)	[Clinical Evaluation] To determine the diagnostic accuracy of COVID-19 antigen RDT in patients using upper respiratory tract specimens compared to gold-standard method (RT-PCR).
Exploratory objective(s)	To assess the feasibility, of the index test (NP swabs and processing with RDT)
Study design	**Clinical Evaluation:** This is a **retrospective , performance evaluation** study of a SARS-CoV-2 Ag RDT. All index test results are compared to RT-PCR results, are for research use only, and will not be reported for patient care. Briefly, at least 100 COVID-19 PCR positive remnant, archived remnant swab samples and at least 200 COVID-19 PCR negative remnant, archived remnant swab samples should be assessed per test; operators will be blinded to sample reactivity.
Index Test	V1301/ V1302/ V1310/V1330 Rapid Test Ag 2019-nCov from Prognosis Biotech SA that detect antigens of the SARS-CoV-2 virus within 15 min
Reference test(s)	RT-PCR (lab validated, site-specific)
Study Samples	**Clinical Evaluation**: (retrospective) samples sources from de-identified, remnant swab specimens which have been collected from individuals suspected to have COVID19. All samples will have documented RT-PCR results. If possible, a range of samples across days from symptom onset and severity of symptoms should be included.

Sample size	A minimum of 50 COVID-19 RT-PCR positives (100 preferred); a minimum of 100 COVID-19 RT-PCR negatives (200 preferred)
Ethics	All clinical studies will be performed on samples in which individuals provided informed consent for additional or archived/remnant samples to be used for research purposes.

2. Product Info:

Manufacturer Name	Prognosis Biotech SA
Test name	Rapid Test Ag 2019-nCoV
Product Code(s)	V13
Pack size(s)	1 / 2 / 10 / 30 tests / kit
Contents of kit	Tests with desiccant in a pot, Buffer, Extraction tubes, positive control, negative control, sample collection swabs, quick reference guide, IFU
Equipment and consumables required, but not provided	PPE, Timer, Biohazard container
Product Storage (temperature range)	2-30°C
Shelf-life (months)	12 months
Manufacturing Site (country)	Greece

3. Study details:

Clinical Study Design:	Prospective diagnostic evaluation studies across multiple, independent sites to determine the accuracy of COVID-19 antigen RDTs, using consecutive enrolment. Interim analyses are performed at 25% and 50% enrolment, and the evaluation is stopped if tests do not meet 97% specificity. Presence of symptoms, date of symptom onset and hospitalization status is collected for all enrolled participants.
Index assays:	Novel lateral flow format tests that detect recombinant SARS-CoV-2 antigens.
Reference method:	Results of the index test are compared to the routine, diagnostic RTPCR result, which is used for clinical management
Clinical	**Sensitivity** was calculated as the proportion of true positive results detected by Rapid Test Ag 2019-nCoV among all positives by the reference method, and reported as a percentage.

Performance:	**Specificity** was calculated as the proportion of true negative specimens, identified as negative by Rapid Test Ag 2019-nCoV among all negatives by the reference method, and reported as a percentage. The 95% confidence intervals were calculated in order to assess the level of uncertainty introduced by sample size, using the Wilson's score method.

4. Evaluation Details:

Country of Collaborator	Greece
Location of clinical site(s) (city, town)	University Hospital of Larissa, Larissa, Thessaly, Greece
Health care level of site(s)	Emergency Department
Study period (date to date)	December 2020 -January 2021
Sample type, antigen test	Nasopharyngeal swab
Reference PCR Method	Genesig Primer design Coronavirus (COVID-19) CE IVD, Vircell SARS-COV-2 REALTIME PCR KIT
Sample type, PCR test	Nasopharyngeal / oral swab

5. Results:

Table 1

Rapid Test Ag 2019-nCoV	**Real-time RT PCR**		
	Positive	***Negative***	***Total***
Positive	105	260	370
Negative	4	1	
Total	109	261	

6. Estimations of Clinical Performance

- ***All Samples included Performance Characteristics***

Table 2

	Mean Value	95% confidence interval
Sensitivity	96.33%	90.87% to 98.99%
Specificity	99,62%	97.88% to 99.99%
Positive Predicted Value	99,06%	93.69% to 99.87%
Negative Predicted Value	96.49%	96.13% to 99.42%
Accuracy	98.65%	96.87% to 99.56%

Table 3

CT cycles	RT-PCR positive	Rapid Test Ag 2019-nCoV positive	Positive Agreement (95% CI)
<21	1	1	100.00% (54.07% to 100.00%)
22-25	8	8	100.00% (63.06% to 100.00%)
26-29	43	41	95.83% (85.75% to 99.49%)
30-33	52	52	100% (93.15% to 100.00%)
>34	3	1	33.33% (0.84% to 90.57%)

- Only samples up to 33 PCR cycle included Performance Characteristics.

Table 4

	Mean Value	95% confidence interval
Sensitivity	98.08%	93.23% to 99.77%
Specificity	99.62%	97.88% to 99.99%
Positive Predicted Value	99,03%	93.51% to 99.86%
Negative Predicted Value	99.24%	97.05% to 99.81%
Accuracy	99.18%	97.62% to 99.83%