Instruction for use
nPLEX SARS-CoV-2 Detection Kit

IVD

This test is being distributed in accordance with Section IV.C.2 of the FDA guidance, Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency

For prescription use only
For in vitro diagnostic use only

Catalog # TAGK01029

Distributed by: TAAG Genetics Corp.
3710 Illinois Avenue, Unit A, St. Charles, IL.
60174 United States

Manufactured by: TAAG Genetics S.A.
Río Refugio 9641 - Núcleo Empresarial ENEA,
Pudahuel - Santiago , Chile

Contents

1.- **Intended use**

The nPLEX SARS-CoV-2 Detection Kit is a test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal and nasal swabs) collected from individuals suspected of COVID-19. Testing is limited to laboratories certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA). 42 U.S.C. §263a to perform high complexity tests. The test has been validated but FDA's independent review of this validation is pending. Laboratories using the nPLEX SARS-CoV-2 kit should include the statement above in patient reports to healthcare providers.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in nasopharyngeal swab specimens during the acute phase of infection. Positive results are indicative of active infection. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities and their reports should mention that the test has been validated but FDA's independent review of this validation is pending

Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations. patient history. and epidemiological information.

The nPLEX SARS-CoV-2 Detection Kit is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays.

2.- Technical principles

1) Product Overview:

The nPLEX SARS-CoV-2 Detection Kit is a multiplex real-time reverse transcription polymerase chain reaction (rRT-PCR) test for detection of SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal and nasal swabs) collected from individuals suspected of COVID-19. The methods described in this application have been adapted from the "CDC 2019 Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" document effective March 30, 2020.

This test uses two PCR sets, the first one contains primers and probe for the simultaneous detection of N1 region of N gene from SARS-CoV-2 and human RNase P gene (RP) used as an internal control. The second PCR set contains primers and probe for the detection of N2 region of N gene from SARS-CoV-2. RNA is isolated from upper respiratory specimens and reverse transcribed to cDNA. Amplification and detection of the SARS-CoV-2 markers and control targets are performed using the AriaMx Real-time PCR System (Agilent) or StepOnePlus™ Real-Time PCR System.

2) Description of Test Steps:

a) RNA isolated and purified from upper respiratory specimens (such as nasopharyngeal, oropharyngeal and nasal swabs) is obtained using the TAAG VRE RNA Extraction kit (TAAG Genetics). Briefly, the TAAG VRE RNA Extraction kit is based on columns for RNA purification.

b) The purified nucleic acid is reverse transcribed to cDNA, followed by two real time PCRs reactions. Both RT-PCRs are based on the standard TaqMan® Technology. One RT-PCR reaction is a multiplex real time PCR which simultaneously amplifies and detects N1 and RP genes using probes labelled with FAM fluorophore and ROX fluorophore, respectively.

c) The second PCR reaction is a monoplex real time PCR which detects N2 gene using a probe labelled with FAM fluorophore.

d)Fluorescence intensity is monitored at each PCR cycle by the AriaMx Real-time PCR System (Agilent) or StepOnePlus™ Real-Time PCR System (Thermo Fisher).

3.- Warnings and precautions

A. The nPLEX SARS-CoV-2 Detection Kit has not been FDA cleared or approved. The test has been validated but FDA's independent review of this validation is pending. Laboratories using the nPLEX SARS-CoV-2 kit should include the statement above in patient reports to healthcare providers.

B. The nPLEX SARS-CoV-2 Detection Kit has been validated only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

C. All samples shall be considered potentially infectious and shall be operated and handled in strict accordance with the laboratory's bio-safety requirements. The experimental personnel should receive professional training (including sample processing, reagent preparation, instrument operation and software setting, etc.). For the laboratory management specifications, please strictly follow the relevant management specifications for gene amplification test laboratories issued by local regulatory agencies.

D. The laboratory should be separated by reagent preparation area, sample preparation area and amplification detection area. All the articles in each area are for special purposes, and they shall not be used for other purposes, as to avoid contamination. The suggested PPE (Personal Protective Equipment) for a laboratory worker are gowns or closed lab coats, hair nets, gloves, eye protection (face shield or goggles) and surgical facemasks or fit-tested N95 masks. Laboratory clothes, hats, shoes, gloves, etc. shall be fully equipped during operation to avoid direct contact of reagents or samples with skin. In case of liquid leakage, wash with plenty of water immediately. In case of contact with skin wounds, inform local health and epidemic prevention department in time.

E. The real-time fluorescent quantitative PCR analyzer should be calibrated regularly.

F. Please dispose of pipette and kit waste in a designated manner as per company safety policies, and in accordance with local, regional, and federal regulations.

G. Clean the working area immediately after the experiment. The areas and surfaces should be disinfected with 1% sodium hypochlorite, 75% alcohol or UV light regularly.

H. The quality of the test results (for samples and controls) are related to the collection, transportation, treatment and preservation of samples. If any of the sampling, storage, or testing process is improperly executed, this may lead to false negative or false positive results of the test.

4.- Kit components and packaging specification

The nPLEX SARS-CoV-2 Detection Kit (catalog number: TAGK01029) contains two PCR sets, sufficient for 96 reactions each. The components of the multiplex real time PCR set (N1 and RP) are shown in Table 1. The components of the real time PCR set for N2 detection are shown in Table 2.

Table 1. Components of multiplex real time PCR for simultaneous detection of N1 and RP gene.

Component	Vial size
nPLEX SARS-CoV-2 Multiplex primers and probes (N1 and RP)	950 µl
nPLEX SARS-CoV-2 Multiplex Master Mix with dUTP (N1 and RP)	500 µl
nPLEX DNA Polymerase (N1 and RP)	20 µl
nPLEX Mix for 1-Step RT-qPCR (N1 and RP)	20 µl
N1 and RP positive control	200 µl
Negative control (nuclease free water)	200 µl

Table 2. Components of real time PCR for detection of N2 gene.

Component	Vial size
nPLEX SARS-CoV-2 primer and probes for N2	950 µl
nPLEX SARS-CoV-2 Master Mix with dUTP for N2	500 µl
nPLEX DNA Polymerase (N2)	20 µl
nPLEX Mix for 1-Step RT-qPCR (N2)	20 µl
N2 positive control	200 µl
Negative control (nuclease free water)	200 µl

Note.
1. It is recommended to run a negative control (nuclease free water or buffer) that participate in nucleic acid extraction as a sample (not included in the kit).
2. Positive control does not participate in the extraction of nucleic acid and should be directly added into the PCR reaction solution.

5.- Reagent storage and handling

nPLEX SARS-CoV-2 Detection Kit should be stored at -20°C and the reagents are valid for 6 months. Kit materials are stable until the expiration date printed on the label under un-opened condition.

Use the reagents within eight (8) weeks of opening and limit freeze/thaw cycles for kit reagents.

6.- Instruments and materials required but not provided

Components required but not included with the test:

- AriaMx Real-time PCR System (Agilent) or StepOnePlus™ Real-Time PCR System (Thermo Fisher).
- RNA extraction kits: TAAG VRE RNA Extraction kit (TAAG Genetics; catalog #TAGK01019).
- RNAse-free pipette tips (P200. P20. P2)
- 1.5 mL microcentrifuge tubes

- Vortex
- P200 micropipette
- P20 micropipette
- P2 micropipette
- 0.2 mL PCR reaction plates (Applied Biosystems; catalog #4346906 or #4366932).
- MicroAmp Optical 8-cap Strips (Applied Biosystems; catalog #4323032).

7.- Sample type

The recommended sample type for nPLEX SARS-CoV-2 Detection Kit is an upper respiratory specimen, such as nasopharyngeal or nasal swab. Swabs should have an Universal Transport Media (UTM™) or equivalent.

8.- Procedure

8.1. RNA extraction from samples

RNA extraction is performed using the TAAG VRE RNA Extraction kit (TAAG Genetics; catalog #TAGK01019) according to the package insert using 100uL sample input volume and elution in 80uL elution buffer.

The extraction must include one negative control per batch of extracted samples consisting of 200 µl of nuclease free water or TE buffer and taken through the entire sample processing procedure.

8.2. Setting up the nPLEX SARS-CoV-2 multiplex real time PCR RT PCR

The preparation of PCR reagents is performed in the reagent preparation area.
Prepare all the devices and reagents before use.
Place the kit on ice when thawing components and preparing PCR Master Mix.
After preparing PCR Master Mix, place them on ice.

Caution: Do not freeze/thaw more than four times.

8.2.1. PCR Mix preparation

1. Prepare the PCR Master Mix for both PCR sets as described in the Table 3 and Table 4.

Table 3. PCR master mix for multiplex PCR set (N1 and RP).

Component	Volume (µl)
nPLEX SARS-CoV-2 Multiplex primers and probes (N1 and RP)	9.6
nPLEX SARS-CoV-2 Multiplex Master Mix with dUTP (N1 and RP)	5.0
nPLEX DNA Polymerase (N1 and RP)	0.2
nPLEX Mix for 1-Step RT-qPCR (N1 and RP)	0.2
Total volume	**15**

Table 4. PCR master mix for PCR set N2.

Component	Volume (µl)
nPLEX SARS-CoV-2 primer and probes for N2	9.6
nPLEX SARS-CoV-2 Master Mix with dUTP for N2	5.0
nPLEX DNA Polymerase (N2)	0.2
nPLEX Mix for 1-Step RT-qPCR (N2)	0.2
Total volume	**15**

Note: Calculate the required amount of each reagent based on the number of reactions (patient samples + controls).

2. Vortex and briefly centrifuge the PCR Master Mix using a microfuge.

3. Place 15µL aliquots of the PCR Master mix into 0.2ml PCR tubes and close the lids. This step should be performed on ice.

4. To prepare the patient samples, add 5µl of each extracted patient sample nucleic acid to its respective tubes as described in the Table 5.

Table 5. Volume for PCR Mixes and nucleic acid from patients

Component	Master mix (N1 and Rp)	Master mix (N2)
PCR master mix	15uL	15uL
Nucleic acid (either extracted patient specimen or control)	5uL	5uL
Total reaction volume	**20uL**	**20uL**

Caution:
It is recommended that the PCR mixture be prepared just before use.
Aerosol-resistant filter tips and tight gloves should be used when preparing samples. Take great care to avoid cross contamination.
Defrost the reagents completely.
Centrifuge the reagent tubes briefly to remove the drops from the inside of the lids.

To prepare the controls, add 5µl of positive control or negative control (RNase-free water) to its respective tube as shown in Table 5.

Caution:
Use a new pipette tip with each different sample.
Avoid cross-contamination of PCR Master mix and samples with Positive Control.
Do not label on the cap of the reaction tubes as fluorescence is detected through the cap.
Centrifuge the PCR tube thoroughly for 30 seconds

8.2.3. PCR program setup

1. Set up and run the AriaMx Real-time PCR System (Agilent) or StepOnePlus™ Real-Time PCR System (Thermo Fisher). Follow the instrument Reference Guides for detailed instructions.

2. Select fluorescence channels according to Table 6 and program the PCR protocol as shown in Table 7 .

Table 6. fluorescence channels for each target

	N1	RP	N2
Fluorescence channels	FAM	ROX	FAM

Table 7. Thermal cycling conditions

Temperature	Time	Cycles
45°C	30 minutes	1
95°C	5 minutes	1
95°C	15 seconds	45
60°C	1 minute	
Fluorescence acquiring in FAM and ROX		

9.- Test controls

Control Material(s) to be used with nPLEX SARS-CoV-2 Detection Kit:

Controls that will be provided with the test kit include:

a) A “no template” control (NTC) serves as a negative control and is included in every assay plate to identify specimen contamination. Molecular grade. nuclease free water is used as the NTC.

b) A positive template control (N1 and RP positive control) is included in each assay plate to ensure the reagents and instruments are performing optimally. The N1 and RP positive control is a mixture of two synthetic DNA plasmids. One contains the entire sequence of gene N of the COVID-19 virus (3x LoD) and the other the human RNase P mRNA (RP. 3x LoD).

c) A positive template control (N2 positive control) is included in each assay plate to ensure the reagents and instruments are performing optimally. The N2 positive control is a synthetic DNA plasmid that contains the entire sequence of gene N of the COVID-19 virus (3x LoD).

10.- Interpretation of results

All test controls should be examined prior to interpretation of patient results. If the controls are not valid. the patient results cannot be interpreted.

1) nPLEX SARS-CoV-2 multiplex real time PCR *Controls – Positive, Negative and Internal*

- **No Template Control (NTC):** No template controls should be negative for all targets. If any of the N1, N2 or RP NTC reactions exhibit positive fluorescence (Ct <39 N1/N2; Ct < 39 RP), the RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result is positive, re-extract and re-test all samples.

- **Positive template control (PTC):** Positive template controls should be positive for both the N1 and N2 targets (Ct <39 N1/N2). If the results are not as expected, the RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result does not yield expected results, re-extract and retest all samples

- **RNase P (Internal Control):** This control should be positive for the RP target (Ct < 39 RP). Failure to detect RNase P in any clinical specimens may indicate: improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation, absence of enough human cellular material due to poor collection or loss of specimen integrity, improper assay set up and execution, reagent or equipment malfunction.

2) *Examination and Interpretation of Patient Specimen Results:*

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Please see the Table 8 for guidance on interpretation and reporting of results.

Table 8. Patient Specimen Result Interpretation Algorithm

N1 (FAM)	N2 (FAM)	RP (ROX)	Interpretation
Ct N1 <= 35	N.A.	N.A	SARS-CoV-2 Positive
Ct N1 > 35 y <= 38	Ct N2 <= 38	N.A	SARS-CoV-2 Positive
Ct N1 > 35 y <= 38	Ct N2 >= 39	N.A	Indeterminate
Ct N1 >= 39	Ct N2 >= 39	Ct RP <= 38	Negative
Ct N1 >= 39	Ct N2 >= 39	Ct RP >= 39	Invalid/Re-test

11.- Limitations

Assay limitations:

1. The performance of the nPLEX SARS-CoV-2 Detection Kit was established using nasopharyngeal swab samples. Other specimen types have not been evaluated.
2. Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
3. Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction kits have not been evaluated.
4. This kit is intended for classification and detection of SARS-CoV-2. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses.
5. Although the detected target sequences of this kit are the conservative region of SARS-CoV-2's gene, the missed detection of coronavirus types with rare mutations in the conservative region can't be completely avoided in theory.
6. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARSCoV-2 have not been determined.

12.- Assay performance

12.1. Analytical Sensitivity: Limit of Detection (LoD)

The LoD of the nPLEX SARS-CoV-2 Detection Kit was determined using quantified whole viral SARS-related coronavirus 2 (USA-WA1/2020) RNA obtained from BEI Resources (NR-52285).

A preliminary LoD was determined by testing 10-fold serial dilutions of RNA (0.050-5.000 genomic copies/µL) spiked into pooled nasopharyngeal matrix in triplicate. Spiked samples were tested with the nPLEX SARS-CoV-2 Detection Kit following extraction with the TAAG VRE RNA Extraction Kit. The lowest concentration of SARS-CoV-2 RNA that yielded a detection rate of ≥95% was 50 genomic copies/µl.

The LoD was verified by testing 20 additional extraction replicates consisting of pooled nasopharyngeal matrix spiked at the preliminary LoD concentration of 50 copies/µl. Samples were spiked with RNA prior to extraction with the TAAG VRE RNA Extraction Kit. The results of the summary are summarized in Table 9.

Table 9. Ct values for final LoD confirmatory test results using viral RNA (50 copies/uL)

Replicant	Marker N1 (Ct)	Marker N2 (Ct)
1	35.5	35.1
2	36.0	34.8
3	35.5	34.7
4	34.6	35.4
5	37.2	37.3

6	35.9	36.4
7	37.5	37.4
8	33.9	36.3
9	36.9	36.6
10	37.3	37.9
11	36.0	37.8
12	35.5	35.7
13	34.4	35.8
14	35.2	36.2
15	35.0	36.1
16	37.4	35.1
17	35.0	35.6
18	35.3	35.5
19	36.8	34.9
20	34.9	35.9

Table 10. Summary of the LoD confirmation for nPLEX SARS-CoV-2 Detection Kit using viral RNA (50 copy/uL)

	Marker N1 (Ct)	**Marker N2 (Ct)**
Positive detection/ total	20/20	20/20
Mean Ct	35.8	36.0
Standard deviation Ct	1.1	1.0

12.2. Analytical Sensitivity: Inclusivity

An in-silico inclusivity analysis was performed by aligning each of the primer and probe sequences to all 1298 complete (>29kb), "high coverage only" hCoV-19 sequences submitted to GISAID (https://www.gisaid.org/) as of March 31, 2020 ("hCoV-19" is the name GISAID uses instead of SARS-CoV2). All primers and probes have perfect identity to >99% of the 11,689 sequences.

12.3. Analytical Specificity: Cross reactivity

To assess cross-reactivity, an in-silico analysis was performed using the primer and probe sequences in the nPLEX SARS-CoV-2 Detection Kit. In-silico cross-reactivity was defined as greater than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism (Table 11).

The in-silico analysis confirmed that there are no significant homologies with the human genome, other coronaviruses, or human microflora that would generate potential false positive test results

Table 11. Organisms and viruses evaluated for potential cross-reaction

Target organism	Target organism
Adenovirus	MERS-coronavirus
Enterovirus	Parainfluenza 1-4
Human coronavirus 229E	Parechovirus
Human coronavirus HKU1	Respiratory Syncytial Virus A and B
Human coronavirus NL63	Rhinovirus/Enterovirus
Human coronavirus OC43	SARS-coronavirus
Human Metapneumovirus (hMPV)	Bordetella pertussis
Influenza A, B and C	Chlamydophila pneumoniae
Haemophilus influenzae	Mycobacterium tuberculosis
Legionella pneumophila	Mycoplasma pneumoniae
Moraxella catarrhalis	Neisseria meningitidis
Pseudomonas aeruginosa	Staphylococcus aureus
Staphylococcus epidermidis	Streptococcus pneumoniae
Candida albicans	Pneumocystis jirovecii (PJP)
Streptococcus pyogenes	Streptococcus salivarius

12.4. Interference substances studies

Interference substances studies were conducted using negative nasopharyngeal clinical specimens spiked with SARS-CoV-2 RNA at 2x LOD concentration. Potential interfering substances, at the indicated concentrations, were added to RNA spiked specimens. These specimens were then extracted with the TAAG VRE RNA Extraction Kit and were tested using the TAAG SARS-CoV- 2 Kit. The following interference substances were tested at the stated concentrations in the interference study:

0.9 g/mL sodium chloride (100µg/mL), Mucin (60µg/mL), blood (5% v/v), oxymetazoline (0.05% v/v), beclomethasone, zanamivir (100µg/mL) oseltamivir (100µg/mL), meropenem (100µg/mL) and tobramycin, (0.25 g/L). None of the evaluated substances interfered with the nPLEX SARS-CoV-2 Detection Kit.

12.5. Clinical evaluation – Prospective Clinical Study

A prospective clinical study was conducted in one healthcare institution located in an epicenter for the COVID-19 outbreak in Santiago - Chile. Patients suspected of COVID-19 were tested using two methods: the CDC 2019-nCoV real-time RT-PCR diagnostics panel and the nPLEX SARS-CoV-2 kit. The nPLEX SARS-CoV-2 test results were compared to the results from the healthcare institution's standard using CDC kit. There was 100% agreement for positive cases and 98% agreement for negative cases (Table 13) and the CTs between both methods is equivalent.

Table 12. Results comparison between CDC 2019-nCoV real-time RT-PCR diagnostics panel and the nPLEX SARS-CoV-2 kit

		CDC 2019-nCoV real-time RT-PCR diagnostics panel			
		Positive	**Negative**	**Indeterminate**	**Total**
kit nPLEX SARS-CoV-2 kit	**Positive**	148	0	12	160
	Negative	0	558	0	558
	Indeterminate	0	12	38	50
	Total	148	570	50	768

Tabla 13. Cts comparison

	TAAG Kit: kit nPLEX SARS-CoV-2	**CDC 2019-nCoV real-time RT-PCR diagnostics panel**
Mean Ct	30,45	30,61
Standard deviation Ct	4,35	4,91

12.6. Clinical evaluation – Contrived Samples

Performance of the nPLEX SARS-CoV-2 Detection Kit was evaluated using individual clinical nasopharyngeal swab specimens spiked with whole viral SARS-CoV-2 RNA obtained from BEI Resources (NR-52285). In total, 30 negative clinical matrix samples and 30 contrived positive clinical matrix samples were tested. Of the 30 contrived positive clinical samples, 24 were prepared with concentrations of SARS-CoV-2 RNA at the assay LoD (50 copies/µl).

Half of the remaining six samples contained RNA at concentrations equivalent to 10X the assay LoD, while the other half contained RNA at concentrations equivalent to 100X the assay LoD. Prepared samples were randomized and blinded and RNA was extracted using the TAAG VRE RNA Extraction Kit. Results of the study are summarized below.

Table 14. Contrived Clinical Evaluation Summary Data.

SARS-CoV-2 concentration	Number of samples	Positive rate		Mean Ct		
		N1	N2	N1	N2	RP
1X LoD	24	24/24	24/24	35.85	36.06	25.78
10X LoD	3	3/3	3/3	34.10	33.95	25.62
100X LoD	3	3/3	3/3	31.41	31.45	25.54
Negative	30	0/30	0/30	>40	>40	25. 67

13. Manufacturer and distributor

Manufacturer information

TAAG Genetics S.A.
Río Refugio 9641 - Núcleo Empresarial ENEA,
Pudahuel - Santiago , Chile
E-mail: info@taag-genetics.com

Distributor information

TAAG Genetics Corp.
3710 Illinois Avenue, Unit A, St. Charles, IL.
60174 United States
E-mail: support-USA@taag-genetics.com

14. Symbols

The following symbols may appear on the labeling or in this document.

In vitro diagnostics medical device

Manufacturer

Catalogue number

Batch code