COVID-19 Real-Time PCR Test

REF P0180

2°C 40°C

INTENDED USE

The *Aridia* COVID-19 Real-Time PCR Test is designed for specific and qualitative detection of the novel coronavirus SARS-CoV-2, responsible for COVID-19, in respiratory specimens including oropharyngeal swabs, nasopharyngeal swabs and sputum, as an aid in the diagnosis of COVID-19 infections, alongside all available clinical and epidemiological data, patient history, and other laboratory test outcomes. The product is intended for use by healthcare professionals specifically trained in nucleic acid amplification techniques and *in vitro* diagnostic procedures.

SUMMARY AND EXPLANATION OF THE TEST

SARS-CoV-2 belongs to the broad family of viruses known as coronaviruses. It is a positive-sense single-stranded RNA (+ssRNA) virus. Other coronaviruses are capable of causing illnesses ranging from the common cold to more severe diseases, such as Middle East Respiratory Syndrome (MERS). SARS-CoV-2 is the seventh known coronavirus to infect people, after 229E, NL63, OC43, HKU1, MERS-CoV, and the original SARS-CoV virus. Protein modeling experiments on the spike (S) protein of the virus suggest that it has sufficient affinity to the angiotensin converting enzyme 2 (ACE2) receptors of human cells to use them as a mechanism of cell entry. Studies have shown that SARS-CoV-2 has a higher affinity to human ACE2 than the original SARS virus strain[1].

SARS-CoV-2 infections cause COVID-19 disease. People who have confirmed COVID-19 have a wide range of clinical symptoms, from little or no symptoms, to fever, tiredness and dry cough, and possibly leading to severe sickness and death. Some patients may develop aches and pains, nasal congestion, runny nose, sore throat or diarrhea. These symptoms are usually mild and begin gradually. Most infected patients (about 80%) recover from the disease without needing special treatment. Approximately 1 out of every 6 patients who get COVID-19 becomes seriously ill and develops difficulty breathing. Older people and those with underlying medical problems, like high blood pressure, heart problems or diabetes, are more likely to develop serious illness. Up to date, about 2-3% of people diagnosed with the disease have died.

Human-to-human transmission of the virus has been confirmed and occurs primarily via respiratory droplets from coughs and sneezes within a range of about 6 feet (1.8 m). Viral RNA has also been found in stool samples from infected patients. It is possible that the virus can be infectious even during the incubation period, but this has not been proven[2,3].

The *Aridia* COVID-19 Real-Time PCR Test is designed for specific detection of SARS-CoV-2 in respiratory specimens, to diagnose COVID-19 infection.

TEST PRINCIPLE

The *Aridia* COVID-19 Real-Time PCR Test is designed for the detection of SARS-CoV-2 in respiratory samples. The detection is done in a one-step real time RT-qPCR format where the reverse transcription and the subsequent amplification of specific target sequence occur in the same reaction well. The isolated RNA target is transcribed by reverse transcriptase generating complementary DNA, followed by the amplification of conserved regions of the SARS-CoV-2 ORF1ab and N genes using specific primers and fluorescently labeled probes. The *Aridia* COVID-19 Real-Time PCR Test also targets the housekeeping human *RNase P* gene as an internal control to monitor sample quality, reverse transcription, PCR amplification, and to ensure the correct interpretation of results.

The assay is based on 5' nuclease chemistry which utilizes SARS-CoV-2-specific primers and a hydrolysable fluorogenic probe (dual-labeled with a reporter and quencher) to detect the accumulation of target sequence amplified during the PCR reaction. Upon extension of the primers by DNA polymerase, the fluorogenic probe is hydrolyzed by the 5' to 3' exonuclease activity of the polymerase, causing spatial separation of the reporter and quencher. The resulting increase in fluorescence signal is measured by the real-time PCR thermocycler and is proportional to the amount of amplified product (and thus the target template in the sample).

The *Aridia* COVID-19 Real-Time PCR Test comes in a ready-to-use format. All real-time PCR components, including DNA polymerase, reverse transcriptase, primers, probes, and dNTPs, are stabilized in tubes with lyophilized PCR Mix sufficient to perform 24 PCR reactions. *ORF1ab* gene is amplified and detected in HEX, JOE, or VIC channel, *N* gene is amplified and detected in FAM channel and internal control *RNase P* gene is amplified and detected in ROX channel. For more information on detection channels, see the "DETECTION CHANNELS FOR REAL-TIME PCR THERMOCYCLERS" section.

REAGENTS AND MATERIALS PROVIDED

Item	Kit Components	Quantity	Catalog No.
1.	COVID-19 PCR Mix (24 tests per vial)	4 vials, 1 per pouch	PCRM180-LYO
2	COVID-19 Positive Control*	1 vial in pouch	PC0180-LYO
3.	COVID-19 Negative Control*	1 vial in pouch	PNC180-LYO
4.	PCR-Grade Water	1.8 mL	PGW002
5.	Instructions for Use	1	PI-P0180-LYO

* After their respective rehydration following the described assay procedure, the Positive Control and Negative Control vials will have material to perform 20 tests each.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Real-Time PCR thermocycler compatible with FAM, ROX and HEX (JOE or VIC) channels (check "DETECTION CHANNELS FOR REAL-TIME PCR THERMOCYCLERS" section)
2. RNA purification kit
3. Sterile RNase-free 1.5 mL centrifuge tubes
4. PCR tubes/strips/plate and optical caps/film
5. Centrifuge for 1.5 mL tubes and PCR-well strips or 96-well plate (if available)
6. Vortex
7. Micropipettes (0.5-20 µL, 20-200 µL and 1000 µL)
8. Filter micropipette tips
9. Gloves and other recommended PPE

WARNINGS AND PRECAUTIONS

1. For use by professionals specifically trained in nucleic acid amplification techniques and *in vitro* diagnostic procedures.
2. Do not use the test after expiration date.
3. Do not use the test if product packaging (foil pouches and/or tube caps) is compromised.
4. Avoid prolonged exposure of the tests to humidity, which can affect product performance. Ensure that desiccant is present in all foil pouches prior to using the kit for the first time.
5. Do not mix reagents from different products and/or lots.
6. Follow Good Laboratory Practices: wear appropriate protective clothing and use disposal gloves and protective eyewear. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and kit reagents.
7. The testing workflow must be one-directional to minimize contamination risk (allocate segregated areas for each step): it should begin in the RNA Purification Area, move to the Reaction Setup Area, followed by Amplification and Detection Area. Do not bring samples, equipment, and reagents to the area in which the previous step was performed and always change gloves between areas.
8. Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
9. Treat specimens as well as all reagents and materials that have been exposed to the samples as potentially infectious and handled in the same manner as an infectious agent. Take appropriate precautions during specimen collection, storage, handling, and disposal in accordance with local and national regulations.
10. To ensure optimal performance of the test, always follow appropriate procedures for specimen collection, transport, storage, and processing. Improper procedures may lead to false negative results.
11. Do not use the test directly with specimens. Nucleic acids must first be purified from specimens using an RNA purification kit.
12. Appropriate precautions should be exercised to monitor contamination and preserve the purity of kit components and reactions. Avoid microbial and nuclease (RNase/DNase) contamination of specimens and kit components. Avoid the spread of aerosols when loosening or uncapping specimens.

TRANSPORT AND STORAGE INSTRUCTIONS

The *Aridia* COVID-19 Real-Time PCR Test can be shipped and stored at 2°C to 40°C until the expiration date stated in the label. Keep all reagents away from direct sunlight. Once the PCR Mix and Positive and Negative Controls have been rehydrated, they can be stored at 2-8°C for 24 hours or at -20°C or below for longer storage to the expiration date, minimizing freeze-thaw cycles (up to 5 freeze-thaw cycles are allowed).

SPECIMEN TYPE, HANDLING AND NUCLEIC ACID PURIFICATION

Specimen type: Respiratory specimens including oropharyngeal swabs, nasopharyngeal swabs and sputum.

Specimen preservation: Process specimens for RNA purification and detection as soon as possible after collection. If not processed immediately, specimens should be stored frozen at -70°C or below. Avoid repeated freeze-thaw cycles during transport and storage of the specimens.

RNA purification: For sample pretreatment and nucleic acid isolation, it is recommended to use the existing, optimized manual or automatic system in your laboratory, compatible with oropharyngeal swabs, nasopharyngeal swabs or sputum specimens. Alternatively, commercially available RNA purification kits may be used. However, always perform sample preparation (specimen collection, transport, storage, etc.) and RNA purification according to manufacturer's recommendations provided in the instructions for use of the kit.

After nucleic acid purification, it is recommended to proceed directly to reverse transcription and PCR amplification. Avoid freezing purified RNA prior to amplification, as freeze-thaw cycles can degrade RNA and lead to false-negative results, particularly in samples with low viral load.

Note: No nucleic acid purification is required for the *Aridia* COVID-19 Real-Time PCR Positive Control and Negative Control.

ASSAY PROCEDURE

1. PREPARATION OF POSITIVE AND NEGATIVE CONTROLS

The COVID-19 Positive Control contains a high copy number of the SARS-CoV-2 partial gene fragments and internal control template. Contamination of the PCR environment, equipment, and/or kit components with the Positive Control can lead to false-positive results. Thus, the Positive Control should be opened and handled in a separate laboratory area, away from the PCR amplification area and other kit components.

1.1. Negative Control Preparation: Rehydrate the dry Negative Control (vial with green cap) with 220 µL of PCR-Grade Water (vial with blue cap). To ensure complete rehydration, incubate for 5 minutes, pulse-vortex the tube thoroughly and centrifuge briefly.

1.2. Positive Control Preparation: Rehydrate the dry Positive Control (vial with red cap) with 110 µL of the reconstituted Negative Control. To ensure complete rehydration, incubate for 5 minutes, pulse-vortex the tube thoroughly and centrifuge briefly.
Note: Keep the rehydrated Positive Control and Negative Control on ice during assay set up. The rehydrated controls are sufficient to run 20 tests each.

2. PCR PROTOCOL

2.1 Program your real-time PCR thermocycler
Calculate the number of required reactions, including samples and controls (Positive and Negative Control reactions must be included with each RT-qPCR run). Program your thermocycler to the following conditions below:

Cycles	Step	Time	Temperature
1	Reverse transcription	10 minutes	50°C
1	Initial denaturation	2 minutes	95°C
45	Denaturation	5 seconds	95°C
	Annealing/Extension (Data collection*)	20 seconds	60°C

Set the fluorescence data collection during the extension step (*) through the HEX, JOE or VIC (*ORF1ab* gene), FAM (*N* gene), and ROX (Housekeeping *RNase P*) channels. When using the Applied Biosystems 7500 Fast Real-Time PCR System or the Agilent Mx3005P™ Real-Time PCR System, ensure that the passive reference option ROX is set to "none".

2.2 PCR Mix Preparation
Rehydrate the COVID-19 PCR Mix (vial with white cap) with 375 µL of supplied PCR-Grade Water (vial with blue cap). To ensure complete rehydration, incubate at room temperature for 10 minutes and mix thoroughly by pipetting up and down at least 5 times. Centrifuge briefly. Keep reagents on ice until ready to dispense.

2.3 Add PCR mix to the PCR wells
Pipette 15 µL of PCR Mix into each well of the PCR tubes/strips/plate which are compatible with your Real-Time PCR thermocycler. Ensure that the Positive and Negative Controls are included with each PCR run.

2.4 Add samples or controls to the appropriate wells
Pipette 5 µL of rehydrated Negative Control (vial with green cap) into each negative control well.
Pipette 5 µL of purified RNA sample into each sample well.
Pipette 5 µL of rehydrated Positive Control (vial with red cap) into each positive control well.
Cover each well with the Optical Caps or seal plates with plate sealer. Centrifuge briefly.

2.5 Starting the real-time PCR run
Place the tubes/strips/plate into the Real-Time PCR thermocycler. Ensure that the configuration/order of the samples and control wells matches the real-time PCR experimental plate setup in the software. Start the assay run.

QUALITY CONTROL

The *Aridia* COVID-19 Real-Time PCR Test contains a Positive and a Negative Control that must be included in each run to correctly interpret the results. The test also targets the housekeeping *RNase P* gene as an internal control to monitor sample quality, reverse transcription and PCR amplification, and to ensure the correct interpretation of the test results.

INTEPRETATION OF TEST RESULTS

Fluorogenic data analysis of the samples and controls is performed by the real-time PCR thermocycler software, according to the manufacturer's instructions.

For a valid diagnostic test run, both control conditions must be met according to the Cq result of each target gene in the following table (see amplification examples in figures 1 and 2):

Controls	Target Gene Cq Value			Interpretation of Controls
	ORF1ab gene (HEX)[1]	*N* gene (FAM)[1]	*RNase P* gene (ROX)[1]	
Positive Control (PC)	< 40	< 40	< 40	Valid
Negative Control (NC)	≥ 40 or no signal	≥ 40 or no signal	< 40	Valid

Note: [1] In cases where either or both of the control assays have failed (HEX or/and FAM amplification signals observed in the negative control and/or signals absent in the positive control well for any channel), all results must be reported as 'invalid' and retesting is required.

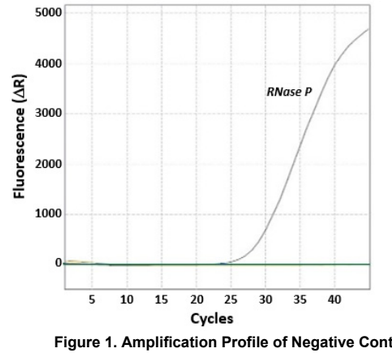

Figure 1. Amplification Profile of Negative Control

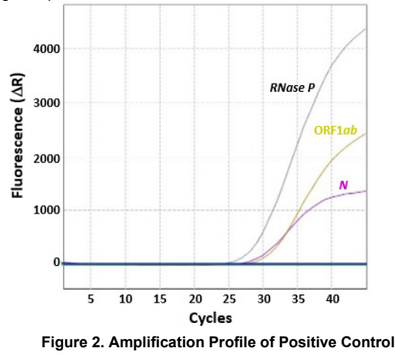

Figure 2. Amplification Profile of Positive Control

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Assessment of clinical test results should be performed after the positive and negative control results have been examined and determined to be valid and acceptable. If one or both control results are not valid, the run is considered invalid and patient's results cannot be interpreted. If both control results are valid, the run is considered valid and interpretation of patient sample results should be done following the table below:

Target Gene Cq Value			Interpretation of patient's samples	
***ORF1ab* gene (HEX)**	***N* gene (FAM)**	***RNase P* gene (ROX)**		
< 40	< 40	< 40	**Positive**	SARS-CoV-2 RNA Detected
< 40	≥ 40 or no signal	< 40	**Inconclusive**	If only one SARS-CoV-2 target gene amplifies, repeat test depending on the available material: a) Obtain a new specimen, re-extract and retest (ideally) or, b) Re-extract and retest another aliquot of the same specimen or, c) Repeat RT-qPCR with the same isolated RNA sample. After retesting once, if at least one target gene is positive, the sample should be considered SARS-CoV-2 positive.
≥ 40 or no signal	< 40	< 40	**Inconclusive**	
≥ 40 or no signal	≥ 40 or no signal	< 40	**Negative**	No SARS-CoV-2 RNA Detected
Any	Any	≥ 40 or no signal*	**Invalid**	Test Failure-Repeat Testing

Note: * If SARS-CoV-2 targets genes (HEX and FAM) show negative results, internal control *RNase P* gene must show an amplification signal with Cq < 40. In this case, if there is an absence of signal or Cq value ≥ 40 of the Internal Control, the result is considered "Invalid," and retesting is required. It is recommended to repeat the RT-qPCR diluting the RNA sample 1:10 and/or 1:100, or re-purify and retest RNA sample to check for possible failure in the purification procedure.

In case of a continuous ambiguous result, it is recommended to review the instructions for use and the nucleic acid purification process used, to verify the correct performance of each RT-qPCR steps and review the parameters, and to check the sigmoid shape of the curve and the intensity of fluorescence.

PERFORMANCE CHARACTERISTICS

1. Clinical Performance

The clinical performance of the *Aridia* COVID-19 Real-Time PCR Test was validated in respiratory specimens collected from 128 symptomatic patients with suspicion of COVID-19. In addition, the clinical performance of the *Aridia* COVID-19 Real-Time PCR Test was validated in 407 respiratory specimens collected from healthy, asymptomatic individuals, without suspicion of COVID-19. The results, compared to a reference Real-Time PCR Test are shown in the table below:

	***Aridia* COVID-19 Real-Time PCR Test**		
Reference RT-qPCR Test	**Positive**	**Negative**	**Total**
Positive	127	1	128
Negative	0	407	407
Total	127	408	535

Relative Sensitivity: 99.2% (95% CI: 95.7-100%); **Relative Specificity:** 100% (95% CI: 99.1-100%); **Overall Agreement**: 99.8% (95% CI: 99.0-100%)

2. Analytical Sensitivity

The *Aridia* COVID-19 Real-Time PCR Test has a detection limit below 10 RNA copies per reaction for *ORF1ab* and *N* genes, as shown on the table below, and shows the expected amplification profiles for both target genes over a 10000X dilution series (figures 3 and 4).

Concentration	Replicates	Positive Results	% Detected
10 copies/reaction	25	25	100%
5 copies/reaction	25	24	96%
2.5 copies/reaction	25	18	72%

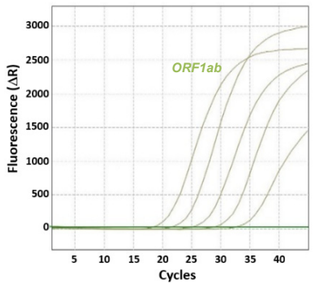

Figure 3. Dilution series of *ORF1ab* gene (10^5-10^1 copies/reaction; HEX)

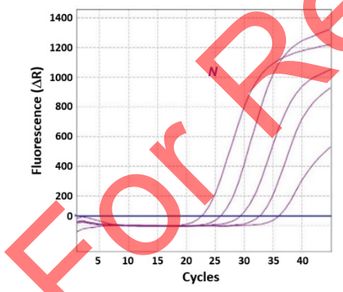

Figure 4. Dilution series of *N* gene (10^5 - 10^1 copies/reaction; FAM

3. Analytical Specificity

The analytical specificity for the *Aridia* COVID-19 Real-Time PCR Test was confirmed by testing a panel consisting of different microorganisms representing the most common respiratory pathogens. No cross-reactivity was detected between any of the following microorganisms tested:

Coronavirus 229E	Influenza B virus Yamagata	HMPV
Coronavirus OC43	Influenza B virus Victoria	*Legionella pneumophila*
Coronavirus HKU1	Enterovirus A	*Mycobacterium tuberculosis*
Coronavirus SARS	Enterovirus B	*Mycoplasma pneumoniae*
Coronavirus MERS	Respiratory syncytial virus A	*Pneumocystis jirovecii*
Coronavirus NL63	Rhinovirus A	*Pseudomonas aeruginosa*
Adenovirus type 1	Pooled human nasal wash	*Staphylococcus aureus*
Parainfluenza virus type 1	*Bordetella pertussis*	*Staphylococcus epidermidis*
Influenza A virus (H1N1 pdm09)	*Candida albicans*	*Streptococcus pneumoniae*
Influenza A virus (seasonal H1N1)	*Chlamydia pneumoniae*	*Streptococcus pyogenes*
Influenza A virus (H3N2)	*Haemophilus influenzae*	*Streptococcus salivarius*

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2°C 40°C IVD

4. Analytical Reactivity to newly identified SARS-CoV-2 variant

The reactivity of the *Aridia* COVID-19 Real-Time PCR Test was evaluated against the Alpha (U.K) variant of the SARS-CoV-2 virus using synthetic RNA controls. It demonstrated that the performance of the test remains the same as the wild-type strain (10 copies/ reaction). *In silico* analysis was performed on the SARS-CoV-2 variants circulating globally and classified as variants of concern. Results demonstrated that there will be no impact on the test's sensitivity for the Alpha (U.K), Beta (South Africa), Gamma (Brazil), Epsilon (California), Eta (Nigeria), Kappa (India) and Delta (India) variants.

DETECTION CHANNELS FOR REAL-TIME PCR THERMOCYCLERS

The fluorescence detection channels used by *Aridia* kits and their corresponding detection channels on commonly-used real-time PCR thermocyclers are listed in the following table:

Real-Time PCR Thermocycler	*Aridia* Channel	Detection Channel	Notes
Bio-Rad CFX96™	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
Applied Biosystems ABI 7500	FAM	FAM	Passive reference option ROX is set to "none"
	HEX	HEX	
	ROX	ROX	
Roche LightCycler® 480II	FAM	465/510	Color Compensation required
	HEX	533/580	
	ROX	533/610	
Cepheid Smartcycler®	FAM	Channel 1	
	HEX	Channel 2	
	ROX	Channel 3	
Abbott m2000rt	FAM	FAM	
	HEX	VIC	
	ROX	ROX	
Agilent Mx3000P™ / Mx3005P™	FAM	FAM	Passive reference option ROX is set to "none"
	HEX	VIC	
	ROX	ROX	
Agilent AriaMx	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
Qiagen Rotor-Gene®Qvalidated	FAM	Green	
	HEX	Yellow	
	ROX	Orange	
Bioneer Exicycler™ 96	FAM	FAM	
	HEX	JOE	
	ROX	ROX	

LIMITATIONS OF TEST

1. This test provides a presumptive diagnosis of COVID-19 infection. Negative test results do not preclude COVID-19 infection. All test results should be evaluated by healthcare professionals in the context of clinical symptoms, epidemiological information, patient history, and other diagnostic test results as the basis for patient management decisions.
2. Factors that may lead to false negative results must be excluded, including poor specimen quality; specimens collected too early or too late; specimens not properly stored, transported or processed; virus variation, interfering substances to PCR, etc.
3. If test results are negative but clinical symptoms persist, follow up with additional serological diagnostic testing.
4. The Assay Procedure and the Interpretation of Test Results sections must be followed closely when testing. Failure to follow the procedure may lead to inaccurate results.
5. This test should be used only with respiratory specimens. The use of other specimen types has not been validated.
6. The quality of the sample impacts the quality of the test; improper specimen collection, storage and/or transport, and improper RNA purification may yield false negative results.
7. In some samples, extremely low levels of target (below the limit of detection) may yield an amplification signal, but results may not be reproducible.
8. Cross-contamination by samples containing high copies of SARS-CoV-2, SARS-CoV-2 particles or amplification products from previous reactions can yield false positive results. Take proper precautions to monitor contamination and preserve the purity of the kit components/ reactions.

REFERENCES

1. Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. Nature, 581, 221–224.
2. Cascella, M., Rajnik, M., Cuomo, A., Dulebohn, S. C., & Di Napoli, R. (2020). Features, evaluation and treatment coronavirus (COVID-19). In Statpearls [internet]. StatPearls Publishing.
3. Healthcare Professionals: Frequently Asked Questions and Answers. (2020, March 22). Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/hcp/faq.html
4. Corman, V.M., et al. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. European communicable disease bulletin, 2000045

Index of CE Symbols

	Consult instructions for use		Use by	CONTROL +	Positive control
REF	Catalog number	N	Tests per kit	CONTROL -	Negative control
IVD	For *in vitro* diagnostic use only		Do not reuse	2°C 40°	Store between 2°C and 40°C
LOT	Lot Number	EC REP	Authorized Representative		
	Manufacturer		Date of manufacture		

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Date released: 2021-11-18
English Version

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