NIPD GENETICS

SARS-CoV-2 RT-qPCR Kit

CE IVD

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Table of Contents

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Version History

Version Number	Section and Designation
2020-11-30	Release Section

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Trademarks and Disclaimers

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Symbols


Reagents/sample


Manufacturer


1.5ml Tube


Keep dry


Telephone number


Expiration date


Consult Instructions For Use (IFU)


Email address

Contains sufficient for N tests


Temperature limits

Lot number

Item number

Material consists of polypropylene and can be recycled with plastic (PMD)

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Definitions

NTC	No (Negative) Template Control
PTC	Positive Template Control
qPCR	Quantitative PCR
RT-qPCR	Real-time quantitative PCR
cDNA	Complementary DNA
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
UTM	Universal Transport Medium
VTM	Virus Transport Medium
GLP	Good Laboratory Practice

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Intended Use

The SARS-CoV-2 RT-qPCR Kit is a CE marked, in vitro diagnostic real-time reverse transcriptase PCR (RT-PCR) assay intended to be used for identification and quantification of COVID-19 viral RNA extracted from nasopharyngeal swabs and/or oropharyngeal swabs. The assay is intended for use with the extraction systems and the designated PCR platforms. The SARS-CoV-2 RT-qPCR Kit (CE IVD) assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Purpose, Scope And Users

SARS-CoV-2 was initially named as 2019 novel coronavirus (2019-nCoV) and was identified by Chinese authorities after a pneumonia outbreak took place in December 2019 in Wuhan, Hubei Province, China. The WHO declared an international health emergency on January 31, 2020. The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend random testing for the asymptomatic population and testing for anyone who exhibits symptoms from specimens collected from the upper respiratory tract and/or lower respiratory for the identification of SARS-CoV-2. RT-qPCR is the gold-standard method for identifying active infections.

NIPD Genetics SARS-CoV-2 RT-qPCR Kit is a multiplex real-time RT-qPCR for the direct qualitative detection of RNA from the novel coronavirus (SARS-CoV-2) from human respiratory samples collected via the nasopharyngeal swab and/or oropharyngeal method. The validation was performed on real-time PCR instrument Bio-Rad CFX384™ and QIAquant 96 5plex Real-Time PCR Detection System. RNA is isolated from respiratory specimens, reverse transcribed and amplified using RT-qPCR.

Rationale

Early diagnosis is important for disease management and treatment, therefore, real-time PCR assay can be a sensitive and reliable method for detecting SARS-CoV-2 virus. NIPD Genetics takes no responsibility for the outcome of patient treatment if the results are being used to guide medical treatment from healthcare professionals.

Quality Control And Validity Of Results

The SARS-CoV-2 RT-qPCR Kit contains positive (PTC) and negative (NTC) template controls to monitor PCR amplification. Both controls must be included in each run for appropriate QC, results interpretation and for the results to be considered valid.

Introduction

Coronaviruses are single-stranded, positive-sense, non-segmented RNA viruses that belong to the ***Coronaviridae*** family. They are the largest known RNA viruses with genomes ranging between 27–31.5 kb. There are six coronavirus species known to cause human diseases 229E, OC43, NL63, HKU1, MERS-CoV and SARS-CoV. SARS-CoV-2, named as a 2019 novel coronavirus (2019-nCoV) is responsible for the current pandemic affecting millions of people worldwide. The virus is transmitted from human-to-human through respiratory droplets via direct or indirect contact. Symptoms of SARS-CoV-2 may appear from 2 to 14 days after exposure to the virus and the most common symptoms are fever, dry cough and fatigue. Less common symptoms include aches and pains, nasal congestion, sore throat, diarrhea, loss of smell (anosmia) or loss of taste (ageusia) etc. The strongest and most consistent evidence for increased risk comes from patients with BMI > 30 kg/m^2, diabetes mellitus type 2 and with underlying lung and heart conditions, who are more likely to develop severe complications and require ICU admission and oxygen support. SARS-CoV-2 virus is transmitted among humans in three ways: (1) by direct contact with infected individuals; (2) by inhalation of virus-laden aerosols and (3) by contact with contaminated objects (such as toys, doorknobs).

Product Description

The SARS-CoV-2 RT-qPCR Kit is designed for the detection of SARS-CoV-2 virus in respiratory specimens. The assay is a real-time one-step reverse transcription polymerase chain reaction (RT-qPCR) test that uses fluorescent dye probes specific for the virus. Targeted RNA isolated from the respiratory specimens is reverse transcribed into complementary DNA (cDNA) and then amplified by polymerase chain reaction (PCR). Four sets of primers and probes were selected for the detection of SARS-CoV-2: two sets of primers and probes were selected from a conserved region of the N gene (N1 and N2), one set of primers and probes from E gene and one set from RdRp gene for the detection of SARS-CoV-2. Additionally, SARS-CoV-2 RT-qPCR Kit contains primers and probe that target the human RNAse P (RP) gene to assess specimen quality. During DNA amplification, DNA polymerase cleaves the probe bound to the complementary DNA sequence causing the quencher dye to detach from the reporter producing a fluorescent signal. This process is repeated with each cycle resulting in an analogous increase in fluorescence intensity. Therefore, the fluorescence is proportional to the quantity of target RNA as it is measured by the qPCR instrument in real-time following every cycle.

General Guidelines

Transport And Storage Conditions

The reagents are shipped and stored at -20°C until the expiration date, as stated on the label. To minimize freeze and thaw cycles and preserve the integrity of the PTC, we recommend aliquoting and storing at -20°C. All components must be kept away from sunlight.

Training Requirements

Testing for presence of viral RNA should be performed in a properly equipped laboratory with staff trained to carry out the relevant technical procedures according to the Occupational Safety and Health Administration (OSHA) Laboratory standards. Refer to the World Health Organization Interim guidance on laboratory biosafety[1] and Centers for Disease Control and Prevention (CDC) guidelines for Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2[2].

Precautions And Recommendations

- The procedures in this handbook must be followed as described. Any deviations may result in assay failure or cause erroneous results and interpretation.
- GLP is required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components thought to have become contaminated should be discarded as standard laboratory waste in a sealed pouch or zip-lock plastic bag.
- Specimen samples must be collected, transported, and stored according to appropriate laboratory guidelines. For details, refer to the CDC guidelines for "Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19".
- Samples should be processed within 4 hours post collection. Samples that will be processed after the 4-hour window post-collection need to be stored between 2-8°C for up to 72 hours.
- Thaw reagents at room temperature prior to use and keep reagents on ice.
- Shelf-life of reagents is 6 months when properly stored.
- Do not use reagents past the expiration date. After the expiration date, the quality guarantee is no longer valid.
- Do not mix reagents from different kits and/or lots and/or another supplier.
- Wear personal protective equipment (PPE), such as disposable gloves, goggles, and mask during collection and sample processing.
- Handle all specimens as if infectious using GLP and the Occupational Safety and Health Administration (OSHA) Laboratory standard (29 CFR 1910.1450).
- Perform all manipulations of potential live virus samples within a class II (or higher) biological safety cabinet.
- Dispose of waste in compliance with local, state, and federal regulations.

- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces with at least 70% (v/v) ethanol. If you are working with RNA, to prevent degradation, it is recommended for the processing area benches to be wiped with RNase AWAY ® or 10% freshly prepared Bleach.
- Please consult the safety data sheet (SDS) before using this kit, which is available on request.
- Consult each Real-Time qPCR instrument's reference manual for additional warnings, precautions, procedures, and data analysis.

[1] https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance-publications (13 May 2020)

[2] https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Flab-biosafety-guidelines.html

[3] https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

Test Procedure

NIPD Genetics SARS-CoV-2 RT-qPCR Kit has been validated on nasopharyngeal and oropharyngeal specimens collected with synthetic fiber swabs and placed immediately into a sterile transport tube containing Universal Transport Medium (UTM) or Viral Transport Medium (VTM) or PBS.

Specimen Collection And Storage

- Nasopharyngeal and/or oropharyngeal specimens should be collected using only swabs with a synthetic tip, and an aluminum or plastic shaft and placed in sterile tube containing viral transport medium or PBS.
- Follow specimen collection device manufacturer instructions for proper collection methods.
- Samples shall be processed within 4 hours after collection. Samples that will not be processed within 4 hours after collection will be stored at 2-8°C for up to 72 hours after collection.

Extraction Kits

NIPD Genetics SARS-CoV-2 RT-qPCR Kit has been tested using the following extraction systems:

- Quick-DNA/RNA™ Viral MagBead (Cat. No. R2141, Zymo Research)
- Magno-Virus (K-2-16/1000, Sacace Biotechnologies)
- MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (A48310, ThermoFisher Scientific)
- TANBead OptiPure Viral Auto Plate (96 test) (Cat. No.:301009 - 665A46, TAN Bead)

Real-Time PCR Instruments

NIPD Genetics SARS-CoV-2 RT-qPCR Kit has been tested on the following equipment:

- Bio-Rad CFX384™ Real-Time PCR Detection System and
- QIAquant 96 5plex (230 V)

Required Equipment And Consumables (Not Provided)

The following list includes the materials that are required for use but not included in SARS-CoV-2 RT-qPCR Kit:

- Real-Time qPCR instrument
- RNA extraction reagents
- Specimen collection and transport system: Sterile synthetic fiber nasopharyngeal and/or oropharyngeal swabs, Universal Viral Transport Tube, Viral Transport Medium (VTM)
- Laboratory freezers: - 30°C to - 10°C and/or ≤ –70°C
- Centrifuge for 1.5 ml tubes and PCR strips or 96-well plates
- Vortex instrument
- Micropipettes (2- 20µl, 20-200µl)
- Filter tips
- PPE

Compatibility With Common Real-Time PCR Equipment

The SARS-CoV-2 RT-qPCR Kit is compatible with the following real-time PCR instruments. Please consult the instrument's manual for use and parameter settings.

Agilent Technologies	AriaMx/AriaDx Real-Time PCR System Mx3000P™ Real Time PCR System Mx3005P™ Real Time PCR System
Applied Biosystems	7500 Fast & 7500 Real-Time PCR System QuantStudio™ 5 Fast/QuantStudio™ 5 Real-Time PCR System QuantStudio™ 6 Flex 96-well Fast
Bio-Rad	CFX96TM Real-Time PCR Detection System CFX384TM Real-Time PCR Detection System iCycler iQTM Real-Time PCR Detection System iCycler iQTM5 Real-Time PCR Detection System
Qiagen	Rotor-Gene® Q
Abbott	Abbott m2000 RealTime System

Consumables

Kit Components

Kit size: 96 Reactions

Lid color	Component	Description	Quantity x Volume
Black	RT-qPCR Mix	DNA Polymerase, dNTPs, and all required buffer components	1 vial x 960µl
Blue	Reverse Transcriptase	Reverse Transcriptase & RNase Inhibitor	1 vial x 96µl
Green	Primer/Probe Mix	Primers complementary to specific regions for SARS-CoV-2 and human RNAse P gene	1 vial x 68µl
Red	Positive Template Control (PTC)	Mix of non-infectious cDNA from artificial sample including targets of SARS-CoV-2 and human RNAse P gene	1 vial x 480µl
Natural	Nuclease-free water (NTC)	RNAse/DNAse-free water	1 vial x 797µl

Table 1

Technical Procedure

1. Collect nasopharyngeal and/or oropharyngeal samples in VTM or UTM and extract viral RNA using desired extraction and purification method as per manufacturer's instructions.
2. Thaw reaction components at room temperature, then place on ice. After thawing, briefly mix each component by inversion, pipetting or gentle vortexing.
3. Determine the total volume for the appropriate number of reactions, plus 5% extra and prepare assay mix of all components except RNA template. Mix thoroughly, but gently, by pipetting or vortexing.
4. Collect assay mix to the bottom of the tube by brief centrifugation.

Reaction Mix Component	1X Reaction (µl)
RT-qPCR Mix	10
Reverse Transcriptase	1
Primer/Probe Mix	0.7
Nuclease-free water	3.3
Template RNA	5
Total reaction volume	**20**

Table 2

5. Aliquot assay mix into qPCR tubes or plate. For best results, ensure you use calibrated micropipettes and minimize bubbles during transfer.
6. Add RNA samples to qPCR tubes or plate. Seal tubes with flat, optically transparent caps or transparent film. If you are using film, properly seal plate edges and corners to prevent artifacts caused by evaporation.
7. Spin tubes or plate briefly for 1 minute at 2,500–3,000 rpm to remove bubbles and collect liquid at the bottom.
8. Program real-time instrument with indicated thermocycling protocol (see Table 3 below). Fluorogenic data are collected during the annealing/extension step: FAM for N1/N2, HEX for RdRp, Cy5 for E gene and ROX for RP.

Cycles	Step	Time	Temperature
1	Reverse Transcription	10 min	55°C
1	Initial Denaturation	1 min	95°C
40	Denaturation	10 sec	95°C
	Annealing/Extension (Data Collection)	15 sec	55°C

Table 3

Results Interpretation

The analysis of the controls and clinical samples is done by the software of the PCR instrumentation according to manufacturer´s instructions. It is recommended to set the threshold values for each channel independently. Use the PTC amplification curve (Fig.1) as a starting point during the run validation to ensure that thresholds fall within the linear phase of the fluorescence curve and above any background signal. The threshold value for different instruments may vary due to different signal intensities. Interpretation of clinical samples should only be performed after the PTC, NTC and clinical samples have been determined to be valid as shown in Table 4. In cases where one or more controls and/or clinical samples fail the acceptance criteria, they are reported as 'Invalid' and retesting is required. For detailed results interpretation refer to Table 5.

Sample or Control	N1/N2 (FAM)	RdRp (HEX)	E gene (Cy5)	RP (ROX)	Test Interpretation
NTC	NA	NA	NA	NA	Valid
PTC	Ct ≤35	Ct ≤35	Ct ≤35	Ct ≤35	Valid
Clinical samples	Ct ≤37	Ct ≤37	Ct ≤37	Ct ≤35	Valid

Table 4

N1/N2 (FAM)	RdRp (HEX)	E gene (Cy5)	RP (ROX)	Result	Action
+	+/-	+/-	+/-	Positive for SARS-CoV-2	Report results to the healthcare provider
-	+	+	+/-	Positive for SARS-CoV-2	
-	-	-	+	Negative for SARS-CoV-2	
-	+	-	+/-	Undetermined	Repeat test by re-extracting the sample and repeating the RT-qPCR
-	-	+	+/-	Undetermined	
-	-	-	-	Invalid	Repeat test by re-extracting the sample and repeating the RT-qPCR. If the repeat result remains invalid, consider collecting a new specimen.

Table 5

Note:

- A positive sign (+) indicates Ct value ≤37 for viral targets (N1/N2, RdRp and E gene) and Ct value ≤33 for human target RP.
- A negative sign (-) indicates no amplification, or Ct value >37 for viral targets (N1/N2, RdRP and E gene) and Ct value >33 for human target RP.

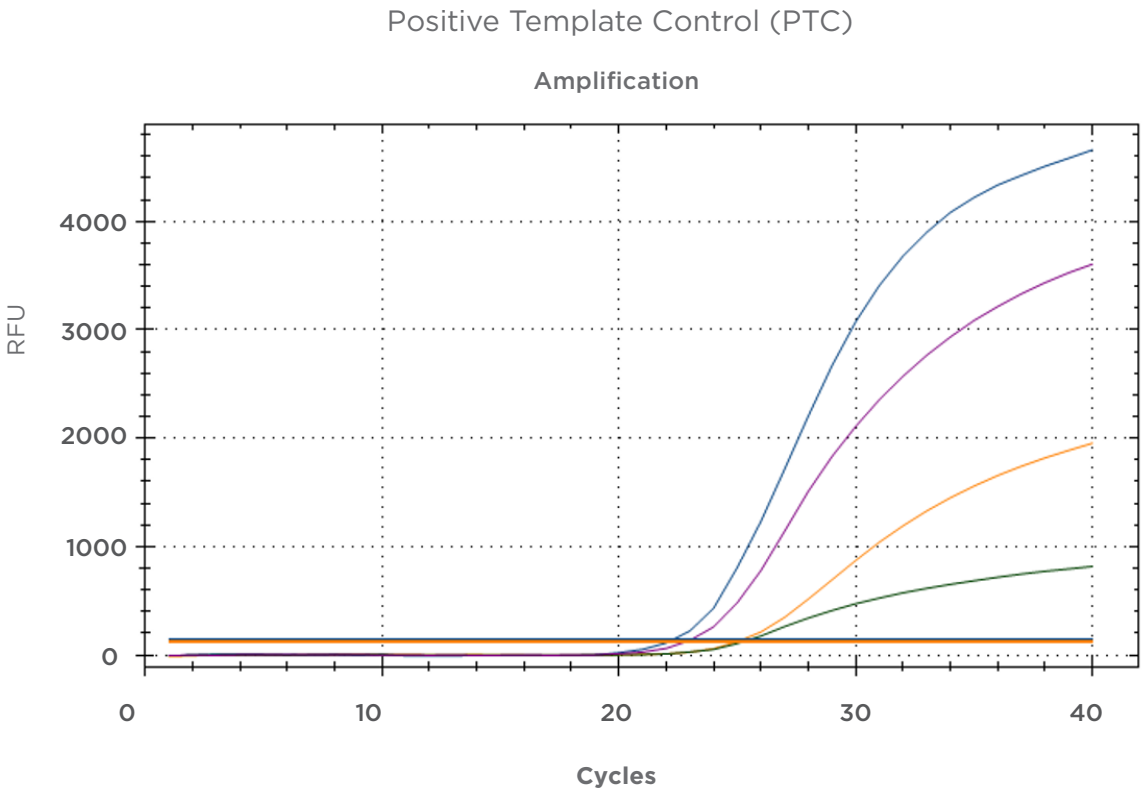

Figure 1: Positive Template Control (PTC). Amplification is detected for all targets.

Limitations Of The Procedure

- This test is intended only for human respiratory samples.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if amplification inhibitors are present or if inadequate numbers of viral genome copies are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence. Negative predictive value is lower during peak activity when prevalence of disease is high. Positive predictive value is lower during periods of low SARS-CoV-2 activity when prevalence is moderate to low.
- Mutations or polymorphisms in the primer or probe binding sites can interfere with the detection of new or unknown virus variants and can lead to false negative results.
- Extremely low concentrations of the target sequences can be detected under the limit of detection (LoD). This is termed as a false positive.
- A positive test result does not necessarily indicate the presence of viable virus. A positive result indicates that the target genes are present.
- An interference study evaluating the effect of common cold medications was not performed.
- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- This test cannot rule out diseases caused by other pathogens.

Performance Characteristics

Analytical Sensitivity

Analytical sensitivity of the SARS-CoV-2 RT-qPCR Kit was tested using RNA purified from complete genome of SARS-CoV-2 virus to determine the lowest detectable concentration and the limit of detection (LoD). The estimated LoD was defined as the lowest concentration in which each target demonstrated 100% positivity. NIPD Genetics SARS-CoV-2 RT-qPCR Kit can detect as low as 10 copies/µl of SARS-CoV-2 virus.

Analytical Specificity

The specificity of the SARS-CoV-2 RT-qPCR Kit was confirmed by using a panel of genomic material representing the most common respiratory pathogens. No cross-reactivity was detected between any of the following microorganisms.

Bocavirus
Bordetella bronchiseptica
Bordetella holmesii
Bordetella parapertussis
Bordetella pertussis
Chlamydia caviae
Chlamydia psittaci genotype A and C
Chlamydophila pneumoniae CM-1
Human coronavirus 229E, OC43, NL63 and HKU1
MERS Coronavirus
Haemophilus influenzae (MinnA strain)
Legionella bozemanii
Legionella dumoffii
Legionella longbeachae
Legionella micdadei
Legionella pneumophila
Human metapneumovirus A and B
Influenza A H1N1
Influenza A H3N2
Influenza A H5N1
Influenza B
Respiratory syncytial virus (RSV) A
Respiratory syncytial virus (RSV) B

Table 6

Clinical Evaluation

Clinical performance of the NIPD Genetics SARS-CoV-2 RT-qPCR Kit was evaluated with human upper respiratory specimens. A total of 23 SARS-CoV-2 positive samples and 52 negative samples were correctly classified showing 100% agreement with FDA-authorized CDC 2019-nCOV, SARS-CoV-2-Real-Time RT-qPCR Diagnostic Panel (Table 7).

Clinical Specimen Panel

	N1/N2	E gene	RdRp	Negative
SARS-CoV-2 Positive	23/23	21/23	21/23	0/23
Negative	0/52	0/52	0/52	52/52
Total	**23**	**21**	**21**	**52**

Table 7

Appendix Data

A. Positive Clinical samples

Ct Values for all clinical samples tested				
Sample (n)	N1/N2 (Ct)	E gene (Ct)	RdRp (Ct)	RP (Ct)
S1	14	16	18	27
S2	23	24	26	31
S3	14	16	17	25
S4	31	34	34	23
S5	26	27	29	24
S6	21	22	28	29
S7	14	17	26	23
S8	23	25	29	25
S9	24	26	31	29
S10	20	22	27	25
S11	17	19	23	28
S12	27	28	35	28
S13	21	22	24	32
S14	15	16	17	27
S15	18	19	20	30
S16	20	21	22	31
S17	23	24	25	26
S18	21	22	23	29
S19	18	20	25	26
S20	19	20	21	26
S21	36	N/A	N/A	29
S22	29	31	N/A	27
S23	33	N/A	39	27

B.Negative Clinical Samples

Ct Values for all clinical samples tested				
Sample (n)	N1/N2 (Ct)	E gene (Ct)	RdRp (Ct)	RP (Ct)
S1	N/A	N/A	N/A	25
S2	N/A	N/A	N/A	30
S3	N/A	N/A	N/A	28
S4	N/A	N/A	N/A	26
S5	N/A	N/A	N/A	26
S6	N/A	N/A	N/A	28
S7	N/A	N/A	N/A	25
S8	N/A	N/A	N/A	26
S9	N/A	N/A	N/A	26
S10	N/A	N/A	N/A	26
S11	N/A	N/A	N/A	26
S12	N/A	N/A	N/A	26
S13	N/A	N/A	N/A	26
S14	N/A	N/A	N/A	25
S15	N/A	N/A	N/A	29
S16	N/A	N/A	N/A	26
S17	N/A	N/A	N/A	26
S18	N/A	N/A	N/A	25
S19	N/A	N/A	N/A	28
S20	N/A	N/A	N/A	27
S21	N/A	N/A	N/A	26
S22	N/A	N/A	N/A	25
S23	N/A	N/A	N/A	30
S24	N/A	N/A	N/A	27
S25	N/A	N/A	N/A	26
S26	N/A	N/A	N/A	27
S27	N/A	N/A	N/A	29
S28	N/A	N/A	N/A	25
S29	N/A	N/A	N/A	29
S30	N/A	N/A	N/A	26

S31	N/A	N/A	N/A	26
S32	N/A	N/A	N/A	25
S33	N/A	N/A	N/A	26
S34	N/A	N/A	N/A	25
S35	N/A	N/A	N/A	25
S36	N/A	N/A	N/A	29
S37	N/A	N/A	N/A	28
S38	N/A	N/A	N/A	25
S39	N/A	N/A	N/A	27
S40	N/A	N/A	N/A	25
S41	N/A	N/A	N/A	28
S42	N/A	N/A	N/A	25
S43	N/A	N/A	N/A	26
S44	N/A	N/A	N/A	27
S45	N/A	N/A	N/A	25
S46	N/A	N/A	N/A	30
S47	N/A	N/A	N/A	25
S48	N/A	N/A	N/A	27
S49	N/A	N/A	N/A	26
S50	N/A	N/A	N/A	25
S51	N/A	N/A	N/A	25
S52	N/A	N/A	N/A	29

Table 8: A. Ct values for positive clinical samples (n=23) tested for the clinical evaluation study of the NIPD Genetics SARS-CoV-2 RT-qPCR Kit. B. Ct values for negative clinical samples (n=52) tested for the clinical evaluation study of the NIPD Genetics SARS-CoV-2 RT-qPCR Kit.