NovaTec Immundiagnostica GmbH

GSD NovaPrime® SARS-CoV-2 (COVID-19)

RT-PCR

(PCOV603x)

Performance Characteristics

Table of Contents

1 Introduction

End of 2019, a novel respiratory disease emerged in the city of Wuhan, Hubei Province of the People's Republic of China, and soon spread rapidly within the country and worldwide. The causative agent was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 (2019-nCoV), like the closely related SARS coronavirus (SARS-CoV), belongs to the genus Betacoronavirus within the family of coronaviruses. The zoonotic reservoir of the virus appears to be bats.

Coronaviruses are enveloped, positive single-stranded large RNA viruses that infect humans, but also a wide range of animals. The common human coronaviruses NL63, 229E, OC43 and HKU1 are widespread especially throughout the winter months. They are responsible for up to one third of all acute respiratory diseases, typically with mild symptoms (common cold). More than 80 % of the adult population have antibodies against human coronaviruses. The immunity from previous infections lasts only for a short period of time. Therefore, reinfections with the same pathogen are possible just after one year.

SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected patients. In theory, smear infection and infection through the conjunctiva of the eyes are also possible.
The incubation period is in the median 5–6 days (and up to 14 days maximum).

The clinical manifestations of SARS-CoV-2-related COVID-19 disease include fever, cough, respiratory problems and fatigue. In most patients the infection manifests with symptoms of a mild febrile illness with irregular lung infiltrates.

The initial clinical sign of COVID-19 which allowed case detection was pneumonia. But it turned out that the course of the disease is non-specific and varies widely, from asymptomatic courses to severe pneumonia with lung failure and death. However, based on current knowledge, around 80 % of the illnesses are mild to moderate.

Although severe courses of the disease also occur in younger patients and people without previous illness, the following groups of people have an increased risk of serious forms of the disease: elderly people (with a steadily increasing risk from around 50-60 years of age), smokers and people with certain diseases of the cardiovascular system or the lungs, patients with chronic liver diseases, diabetes mellitus, cancer, or patients with a weakened immune system (e.g. due to immune deficiencies or by taking drugs that suppress the immune system).

Currently, there is no specific treatment for SARS-CoV-2 infection established; an approved vaccine is not yet available.

Table 1: SARS-CoV-2 - Symptoms and Transmission Routes

Species	Disease	Symptoms (e.g.)	Transmission route
SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2)	COVID-19	the course of the disease is unspecific, diverse and varies greatly, from asymptomatic courses to severe pneumonia with lung failure and death	primary mode of transmission: droplet infection; smear infections and infections via the conjunctiva of the eyes are theoretically possible

The presence of pathogen or infection may be identified by

- Nucleic acid testing (NAT): e.g. RT-PCR
- Serology: detection of antibodies by e.g. ELISA

2 Intended Use

The GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR is intended for the qualitative determination of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) genomic RNA extracted from human respiratory (nasal wash/swab, nasopharyngeal wash/swab, oropharyngeal swab and bronchoalveolar lavage) specimen types.

3 Principle of the Assay

The qualitative determination of specific RNA is based on Real-Time Reverse-Transcription Polymerase Chain Reaction (RT-PCR) technology. The kit contains specific primers and probes labelled with fluorescent reporter and quencher dyes for amplification and simultaneous detection of specific RNA sequences which represent **two specific regions of the SARS-CoV-2 N gene**. Furthermore, the assay contains a heterologous amplification target (Extraction Control, EC) to identify possible RT-PCR inhibition by interfering substances contained in the sample or failure of the preceding RNA extraction. Therefore, the EC is added to the specimen during RNA isolation.

The gene of interest specific probes are labelled with the fluorophore FAM™. The EC specific probe is labelled with the fluorophore Cy5™ thereby allowing parallel detection of both amplicons in the corresponding detection channels.

4 Performance Characteristics

4.1 Reproducibility (Precision)

Material

GSD NovaPrime® SARS-CoV-2 (COVID-19) Lot: PCOV-002
Production date: 2020-04 Expiry date: 2020-05-31

3 samples (High positive, medium positive, low positive)

Test Description

The reproducibility of the GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR kit was determined by comparing 3 replicates of 3 different samples in one assay (within-run) and by comparing 3 different samples assayed in 3 different runs (between-run).

Acceptance Criterion: Intra-Assay Variance: CV ≤ 5 %
Inter-Assay Variance: CV ≤ 7 %

Results

Within-run and between-run precision were estimated by analysis of variance and are presented in table 2.

Table 2: Within-run and between-run precision of batch PCOV-002

SARS-CoV-2	n run per day/replicates	Mean [Ct] (day1/2/3)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability				
High positive sample	1/3	15.35 / 15.82 / 15.84	0.16 / 0.04 / 0.12	**0.39 / 0.24 / 0.73**
Medium positive sample	1/3	21.47 / 21.70 / 21.79	0.16 / 0.43 / 0.11	**0.74 / 0.43 / 0.52**
Low positive sample	1/3	27.30 / 27.27 / 27.55	0.21 / 0.01 / 0.01	**0.75 / 0.04 / 0.05**
Inter-Assay Variability				
High positive sample	3/9	15.67	0.10	**0.67**
Medium positive sample	3/9	21.65	0.12	**0.56**
Low positive sample	3/9	27.37	0.08	**0.28**

Conclusion

For Intra-Assay Variability the highest CV obtained was 0.75 % and for Inter-Assay Variability it was 0.67 %. Therefore, the acceptance criteria were met for all samples tested.

4.2 Analytical Sensitivity

Limit of Detection

The aim of the study was to evaluate the lowest amount of SARS-CoV-2 RNA which can be securely detected by the GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR kit.

Test Description

Known concentrations of SARS-CoV-2 RNA have been serially diluted and measured in five-fold determination to estimate a preliminary LoD.

The lowest concentration at which all five replicates gave a positive result, as well as the next higher and next lower concentration have been tested further.

A 20-fold determination of these three concentrations has been performed. The concentration at which 95 % of the replicates gave a positive result was determined as Limit of Detection.

Acceptance Criterion: ≥ 19 out of 20 replicates with positive result

Material

GSD NovaPrime® SARS-CoV-2 (COVID-19)		Lot:	PCOV-002
Production date:	2020-04	Expiry date:	2020-05-31

Results

For the concentration of 3.75 SARS-CoV-2 RNA copies per reaction, 19 out of 20 replicates gave a positive result.

Table 3: Limit of Detection of GSD NovaPrime® SARS-CoV-2 (COVID-19)

	LoD [copies/reaction]
Standard cycling	3.75

Conclusion

The lowest concentration which fulfilled the acceptance criterion was 3.75 copies of SARS-CoV-2 RNA per reaction. Therefore, this concentration was determined as Limit of Detection for the GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR kit.

4.3 Cross-Reactivity

Test Description

Cross-reactivity was evaluated by *in silico* analysis against normal flora or pathogens that cause similar symptoms or pathogens related to SARS-CoV-2. Primer-probe-sets not exceeding 80 % identity to a potential cross-reacting sequence are not predicted to cause a cross-reaction.
In addition to *in silico* analysis, the GSD NovaPrime® SARS-CoV-2 (COVID-19) assay was performed on nucleic acid from respiratory pathogens including the human coronaviruses 229E,

NL63, OC43, and SARS. All these pathogens tested by the GSD NovaPrime® SARS-CoV-2 (COVID-19) assay did not generate detectable amplification signals.

A number of individual primers or probes had > 80 % identity, however, potential cross-reactivity was not identified in full primer/probe sets so detection is not predicted. To confirm this conclusion, amplification of these pathogens with the SARS-CoV-2 assay was performed to assess cross-reactivity empirically with results shown in Table **4**: Measurement results with potential cross-reactive nucleic acid sequences. Additionally, the common respiratory coronaviruses 229E, NL63, and OC43 and DNA templates corresponding to the N gene sequence of SARS (position 29034 – 29233 and 28669 – 28868 of NC_004718.3) were tested empirically.

Results

All pathogens tested by the GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR kit assay did not generate detectable amplification signals.

Table 4: Measurement results with potential cross-reactive nucleic acid sequences

Pathogen	Concentration	SARS-CoV-2 [Ct]	EC [Ct]
Coronavirus 229E	$1x10^{4.10}$ $TCID_{50}$/mL	N.D.[2]	29.47
Coronavirus NL63	$1x10^{3.75}$ $TCID_{50}$/mL	N.D.	30.39
Coronavirus OC43	$1x10^{4.10}$ $TCID_{50}$/mL	N.D.	28.83
SARS NC_004718	$5x10^4$ copies/mL	N.D.	N.A.
Parainfluenza virus 1	$1x10^4$ PFU/mL	N.D.	29.56
Enterovirus	$5x10^4$ copies/mL	N.D.	30.07
Rhinovirus	$1x10^4$ PFU/mL	N.D.	29.80
Haemophilus influenzae	$5x10^4$ CFU/mL	N.D.	29.61
Legionella pneumophila	$5x10^4$ CFU/mL	N.D.	29.71
Mycobacterium tuberculosis	$5x10^4$ GEq/mL	N.D.	N.A.[3]
Streptococcus pneumoniae	$5x10^4$ CFU/mL	N.D.	32.70
Streptococcus pyogenes	$5x10^4$ CFU/mL	N.D.	29.82
Pseudomonas aeruginosa	$5x10^4$ CFU/mL	N.D.	29.67
Streptococcus salivarius	$5x10^4$ CFU/mL	N.D.	29.77
Pooled human nasal wash	N.A.[1]	N.D.	30.57
Pooled human NP swab (UTM)	N.A.	N.D.	32.17
Pooled human BAL	N.A.	N.D.	32.02

[1]Not applicable
[2]Not detected
[3]Obtained as a genomic DNA sample therefore extraction was not performed

Conclusion

Cross reactions with nucleic acid sequences of other respiratory pathogens could not be detected.

4.4 Inclusivity

The analytical sensitivity of the GSD NovaPrime® SARS-CoV-2 (COVID-19) is first and foremost ensured by the thorough selection of the oligonucleotides. Inclusivity was evaluated by *in silico* analysis using all publicly available SARS-CoV-2 sequences (available on the 26th of April 2020) to determine the percent identity matches for targeted sequences of the GSD NovaPrime® SARS-CoV-2 (COVID-19) assay. In total 73 SARS-CoV-2 genome sequences have been analyzed by alignment to primers and probes.

4.5 Negative and Positive Percent Agreement

The positive and negative percent agreement (PPA and NPA, respectively) are the proportions of positive and negative results in statistics and diagnostic tests that are true positive and true negative results, respectively. The PPA and NPA describe the performance of a diagnostic test.

The positive percent agreement (PPA) is defined as:

$$PPA = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false positives}}$$

where a "true positive" is the event that the test makes a positive prediction, and the subject has a positive result under the gold standard, and a "false positive" is the event that the test makes a positive prediction, and the subject has a negative result under the gold standard. The ideal value of the PPA, with a perfect test, is 1 (100 %), and the worst possible value would be zero.

The negative predictive value is defined as:

$$NPA = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false negatives}}$$

where a "true negative" is the event that the test makes a negative prediction, and the subject has a negative result under the gold standard, and a "false negative" is the event that the test makes a negative prediction, and the subject has a positive result under the gold standard. The ideal value of the NPV, with a perfect test, is 1 (100 %), and the worst possible value would be zero.

Test Description

30 defined negative and 30 defined positive samples have been tested with the GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR kit. The samples have been measured as duplicates.

Material

GSD NovaPrime® SARS-CoV-2 (COVID-19)		Lot:	PCOV-002
Production date:	2020-04	Expiry date:	2020-05-31

30 defined positive samples and 30 defined negative samples

Results

100 % agreement was achieved for all 60 samples tested.

Literature

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