LÆGEMIDDELSTYRELSEN
DANISH MEDICINES AGENCY

# Free Sales Certificate

The Danish Medicines Agency hereby certifies that the medical devices specified in the attached list are manufactured by:

**PentaBase ApS**
**Lumbyvej 19E-G**
**5000 Odense C**
**Denmark**

Medical devices which are CE marked in conformity with Directive 98/79/EEC meet the essential requirements for safety and performance. They may therefore be manufactured and marketed in Denmark and exported without any approval from the Danish Medicines Agency.

LÆGEMIDDELSTYRELSEN
DANISH MEDICINES AGENCY

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Valid from: 28 May 2020
Valid Until: 28 May 2022

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CoviDetect™ COVID-19 RT-qPCR Multiplex Assay CE IVD

LÆGEMIDDELSTYRELSEN
DANISH MEDICINES AGENCY

| PENTABASE APS | GENERAL DOCUMENTATION | | |
|---|---|---|---|
| | Document Name/Description<br>**EC Declaration of Conformity** | | Pages<br>1 (1) |
| | | Document number<br>05-20-2020-1 | Issue<br>02 |

**EU Declaration of Conformity (Directive 98/79/EC)**

**Manufacturer** PentaBase ApS
Lumbyvej 19E-G
5000 Odense C
Denmark
CVR: 30197852

**Competent Authority:** The Danish Health and Medicines Authority

**Product Details:** CoviDetect™ COVID-19 RT-qPCR Multiplex Assay CE IVD

**Classification:** General IVD (Non-Annex II), Self-Declaration

**GMDN code:** 64747

**GMDN term:** A collection of reagents and other associated materials intended to be used for the qualitative and/or quantitative detection of nucleic acid from severe acute respiratory syndrome-associated coronavirus 2 (SARS-CoV-2) in a clinical specimen, using a nucleic acid technique (NAT). This test is used to aid the diagnosis of coronavirus disease (COVID-19) infection.

**Conformity Assessment Route:** Annex III IVDD

We hereby declare that CoviDetect™ COVID-19 RT-qPCR Multiplex Assay CE IVD complies with the requirements of DIRECTIVE 98/79/EC, on in vitro diagnostics.

Odense – Denmark, 20 May 2020

Ulf Bech Christensen
CEO

**PentaBase ApS, Lumbyvej 19G, 5000 Odense C - Denmark**

# BasePurifier™

## Nucleic Acid Extraction Instrument


The BasePurifier™ instrument is designed for automated extraction of nucleic acids using magnetic beads. The instrument provides a high degree of automation, fast extraction speed, reliable results and ease of use. DNA or RNA extracted on the BasePurifier™ can be used directly for PCR or RT-PCR applications.

www.pentabase.com

# SPECIFICATION

| | |
|---|---|
| Capacity | 1 to 32 samples |
| Format | 6-strips or 96-well plates |
| Processing volume | 30 -1000 µl |
| Consumables | BasePurifier™ Nucleic acid extraction kits |
| Extraction method | Magnetic rods and beads |
| Magnetic rods | 32 rods in frame |
| Speed | 30-60 minutes |
| Heating capacity | Up to 120 ˚C |
| Inter-well purific. accuracy | CV < 3% for identical samples |
| Collection efficiency | >95% for optimal samples |
| Disinfection | UV-lamp |
| Program storage | Up to 15 programs on instrument firmware<br>More than 500 programs on BasePurifier™ App |
| Connection options | 3.5-inch LCD screen<br>Connection to Android OS-based tablet with BasePurifier App via Wi-Fi |
| Power failure protection | The unfinished experiment will continue when the instrument is restarted |
| Operating noise | <65 db(A) |
| Power | 240V |
| Warranty | 2 years |

# BaseTyper™

## Real-Time PCR Instrument


The BaseTyper™ Real-Time PCR Instrument is intended for performing rapid, accurate real-time PCR using DNA-binding fluorescent dyes or labeled probes to comparative quantitative readouts of DNA or reverse transcribed RNA. The BaseTyper™ Real-Time PCR Instrument is validated for use with PentBase's IVD assays including our novel CoviDetect™ COVID-19 RT-qPCR Multiplex assay.


www.pentabase.com

# SPECIFICATION

| | |
|---|---|
| Number of reactions | 48 |
| Format | 0.2 ml tubes<br>8 well strips |
| Reaction volume | 10-100 µl |
| Temperature ctrl method | Peltier |
| Speed | 8°C/s heating<br>8°C/s cooling |
| Uniformity | +0.1°C |
| Accuracy | ≤ 0.1°C |
| Resolution | ≤ 0.1°C |
| Detection | 510 to 750 nm LED<br>Photodiodes<br>Light filters |
| Multiplex level | Up to 4 channels |
| Connection options | LAN - up to 10 instruments per computer<br>Stand alone - 7.0-inch touch screen USB stick |

Dyes and Channels

| Channel | Dye |
|---|---|
| 1 | FAM, SYBR Green I, etc. |
| 2 | VIC, HEX, TET, JOE, etc. |
| 3 | ROX, Texas Red, etc. |
| 4 | Cy5, etc. |

| | | |
|---|---|---|
| Choice of custom dyes | Yes | |
| Supported assay formats | MicroSight® MSI | SimpleProbes |
| | PlentiPlex™ | Intercalators |
| | SensiScreen® | HRM |
| | CoviDetect™ COVID-19 | |
| | HydrolEasy probes™ | |
| | TaqMan® | |
| | EasyBeacons™ | |
| | Molecular Beacons | |
| | HybProbes | |
| Operating noise | <65 db(A) | |
| Power | 240V | |
| Warranty | 2 years | |

# CoviDetect™ COVID-19 Multiplex RT-qPCR Test

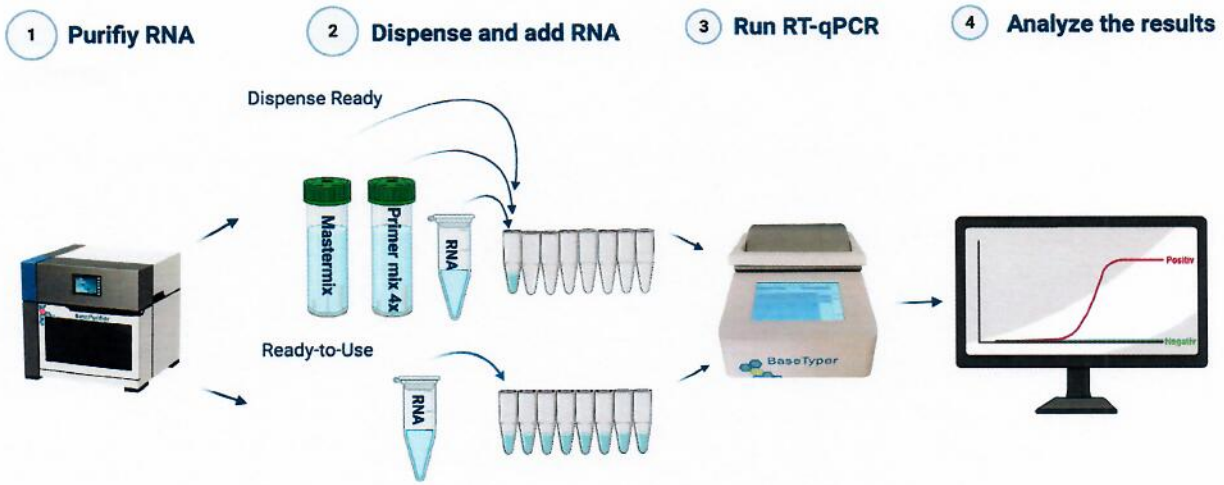
## Detection of SARS-CoV-2 RNA from individuals suspected of COVID-19

CoviDetect™ COVID-19 Multiplex RT-qPCR Test is a Danish in vitro diagnostic test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in individuals suspected of COVID-19.

The CoviDetect™ COVID-19 Multiplex RT-qPCR Test is CE IVD certified and developed in collaboration with the Danish National Hospital and Novo Nordisk.

CoviDetect™ is the most used SARS-CoV-2 RT-qPCR test by the national Danish healthcare system and it allows an increase of the test capacity, as one patient sample is analyzed in a single PCR tube, instead of three tubes.

CoviDetect ™ is the only open-platform multiplex assay, designed for use on standard real-time PCR systems with FAM, HEX/VIC and Cy5 channels. The test meets CDC's and WHO's recommendations, and it analyzes two SARS-CoV-2 sequences (N1 and N2 in the N-gene), as well as human RNase P.




CoviDetect ™ COVID-19 Multiplex RT-qPCR Test is available either as Dispense Ready (DR) or Ready-To-Use (RTU) versions. The Dispense Ready version includes Primer-Probe Mix and Master Mix in separate tubes for dispensing in own plastic products before adding RNA. The Ready-To-Use version is pre-dispensed into 8-tube PCR strips, and only RNA must be added before RT-qPCR analysis.

## CoviDetect™ Multiplex Assay provides results within 1 hour and 10 minutes

- Multiplex RT-qPCR assay for increased testing capacity
- Designed for use on standard laboratory equipment
- Based on WHO and CDC guidelines (N1, N2 and RNAse P)
- Includes SARS-CoV-2 RNA positive control panels
- Produced in Denmark
- LOD < 20 copies per RT-qPCR reaction

v.C1.0

PentaBase

More information at www.pentabase.com

04th June 2020

To whom it may concern,

We hereby confirm that our declaration of conformity (Directive 98/79/EC) for CoviDetect™ COVID-19 Multiplex RT-qPCR Assay includes the entire workflow using the BasePurifier™ Nucleic Acid Extraction Instrument and the BaseTyper™ Real-time PCR instrument.

Performance evaluation data for the entire workflow can be found in the CoviDetect™ Instructions for use version 2.4 or later.

Thanking you,
Yours truly

.....

Michael Børgesen

Chief Product Officer

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DK-5000 Odense
Denmark
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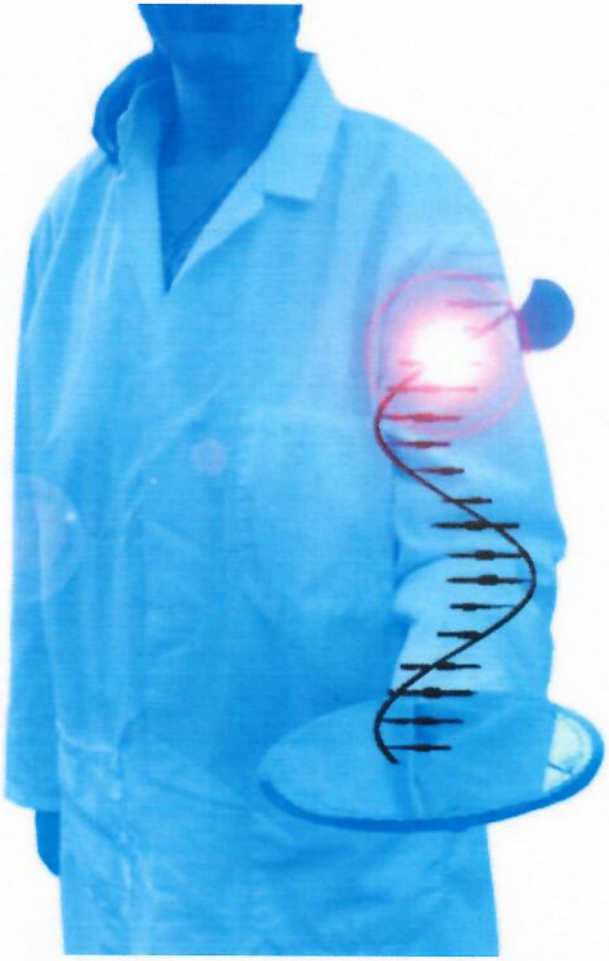
# CoviDetect™ COVID-19 Multiplex RT-qPCR Assay

## In Vitro Diagnostic Assay for Detection of SARS-CoV-2

## Instructions for use



CE IVD

**PentaBase ApS | Lumbyvej 19G | DK-5000 Odense | Denmark**

Last revised: July 2020
Version 2.7

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**Δ IMPORTANT:**

Please read these instructions carefully before using PentaBase's CoviDetect™ COVID-19 Multiplex RT-qPCR Assay. It is recommended to save the "Instructions for use" for future use. Purchasers of PentaBase's CoviDetect™ COVID-19 Multiplex RT-qPCR Assay are only granted the right of use, but no general licensing or patent rights.

# 1 INTENDED USE

PentaBase's CoviDetect™ COVID-19 Multiplex RT-qPCR diagnostic assay is a real-time RT (reverse transcriptase) PCR assay intended for detection of nucleic acids from the SARS-CoV-2 virus. SARS-CoV-2 RNA can be found in liquid from upper or lower respiratory tracts of infected individuals. Samples can be obtained by nasopharyngeal or oropharyngeal swabs or from sputum.

Positive PCR results indicate an infection with SARS-CoV-2 virus, but do not eliminate the possibilities of co-infections with other viruses or bacteria. Note that infection with SARS-CoV-2 can occur without showing any symptoms.

Negative PCR results do not exclude present or hinder future infection with SARS-CoV-2 virus and the result should always be combined with clinical observations, patient history and epidemiological information. The test should be performed by trained laboratory personnel in a professional laboratory environment.

The test is provided in one or more boxes containing all described components for use including an "Instructions for Use" and a "Quick guide". The "Instructions for Use" is also available for download on our website: www.pentabase.com.

### 1.1 INDICATIONS FOR USE

On December 31 2019, China alerted the WHO to several cases of unusual pneumonia in Wuhan, a city of 11 million people. The virus was unknown. This infection has since been identified to be caused by a novel coronavirus (2019-nCoV/SARS-CoV-2). The virus has now spread across the world and caused millions of confirmed human infections. The virus has a fast spread rate and can cause severe illness and deaths, putting extra pressure on the hospitals and healthcare suppliers. PentaBase's CoviDetect™ COVID-19 Multiplex RT-qPCR test is intended to identify the presence of SARS-CoV-2 virus RNA in humans by a fast and reliable method.

## 2 SUMMARY AND EXPLANATION OF THE ASSAY

The CoviDetect™ COVID-19 Multiplex RT-qPCR assay is a molecular *in vitro* diagnostic test based on PentaBase's highly sensitive technology to identify the presence of SARS-CoV-2 RNA in patients. The assay is in a multiplex format, which means that one sample from a patient can be analyzed in one tube.

| Targeted Regions | Description | Fluorophore |
|---|---|---|
| N1 | Nucleocapsid protein gene marker | FAM |
| N2 | Nucleocapsid protein gene marker | HEX |
| RNP | Human RNase P (Extraction Control) | Cy5 |

**Table 1.** List of detected regions in the CoviDetect™ COVID-19 Multiplex RT-qPCR assay

## 3 TECHNOLOGY AND REAGENTS

The CoviDetect™ COVID-19 Multiplex RT-qPCR assay combines real-time PCR with PentaBase's novel and selective technologies comprising both standard synthetic oligonucleotides as well as proprietary modified synthetic oligonucleotides such as HydrolEasy™ probes and SuPrimers™ for specific and sensitive amplification. The technology is applicable on standard real-time PCR equipment using standard procedures. Pentabase-modified oligos contain synthetic DNA analogues comprising a flat heteroaromatic, hydrophobic molecule and a linker. These modifications are inserted into the oligonucleotides at fixed positions during synthesis. Using the CoviDetect™ COVID-19 Multiplex RT-qPCR test, the presence of virus RNA in a sample can be detected quickly, sensitively and selectively, by real-time RT-PCR analysis.

### 3.1 HYDROLEASY™ PROBES

A **HydrolEasy™** probe is similar to a standard hydrolysis probe (also referred to as a TaqMan® probe) labeled with a fluorophore at the 5' end, a quencher at the 3'end, but is based on

pentabase-modified oligos giving the probe a significantly improved signal-to-noise ratio, higher specificity and higher sensitivity compared to conventional hydrolysis probes. HydrolEasy™ probes in the CoviDetect™ COVID-19 Multiplex RT-qPCR assay are labeled with either FAM, HEX or Cy5.

### 3.2 SUPRIMERS™

**SuPrimers™** are standard DNA primers modified with one or more Pentabases. The Pentabases provide increased specificity and sensitivity and reduce primer-dimer formation.

## 4 ASSAY FORMAT AND DESIGN

### 4.1 FORMAT

CoviDetect™ COVID-19 Multiplex RT-qPCR assay is supplied as either Dispense Ready (DR) or Ready-To-Use (RTU) versions. The Dispense Ready version includes Primer-Probe Mix and Master Mix in separate tubes to be dispensed in own plasticware before addition of RNA. The Ready-To-Use version is pre-dispensed in 8-tube PCR strips and needs only addition of RNA before running.

### 4.2 CONTROL ASSAY

Detection of amplified human RNase P is done with a Cy5-labeled HydrolEasy™ probe and used to assess whether an amplification has taken place in reactions with no signal from the FAM and HEX labeled assays in the same reaction. Since the RNase P assay can amplify both RNA and genomic DNA it acts only as a sample loading control.

### 4.3 VIRUS SPECIFIC ASSAY

The CoviDetect™ COVID-19 Multiplex RT-qPCR assay targets two markers, N1 and N2, of the SARS-CoV-2 nucleocapsid protein gene. Amplification of N1 and N2 markers is done using HydrolEasy™ probes labeled with FAM and HEX, respectively.

### 4.4 CONTROL SAMPLES

The kit include SARS-CoV-2 RNA positive and negative control samples which should be included in the RNA extraction procedure as well as in each RT-qPCR run for validation of the complete workflow. .

### 4.5 EQUIPMENT AND REAGENTS

The use of PentaBase's CoviDetect™ COVID-19 Multiplex RT-qPCR assay will require the following equipment and consumables not provide in the kit:

- Template (Extracted patient RNA)
- Nucleic acid extraction system and associated RNA extraction kit such as the BasePurifier™ Nucleic Acid Extraction Instrument and the Viral RNA/DNA extraction Kit
- Real-Time PCR instrument with at least three channels (such as the PentaBase BaseTyper™, PentaBase)

- Plasticware compatible with the PCR instrument*
- Dedicated pipettes and tips for preparing PCR mixes
- Dedicated pipettes and tips for addition of template
- Centrifuge for spinning tubes/plates
- Nuclease free $H_2O$

*Only when using Dispense Ready version

# 5 SAFETY, SHIPMENT AND STORAGE

General laboratory precautions should be taken. The CoviDetect™ COVID-19 Multiplex RT-qPCR assay should only be used by personnel who have been trained in the appropriate techniques. All chemicals and biological material should be considered as potentially hazardous. When working with the assay, suitable personal protective equipment (lab-coat, disposable gloves and safety glasses) should be used. It is recommended that all work is carried out in appropriate facilities.

## 5.1 WARNINGS AND PRECAUTIONS

- All specimens should be considered as potentially infectious and handled with precautions.
- Use personal protection wear (lab coat, gloves, eye protection etc.) when handling the specimens and reagents.
- Use extreme caution not to contaminate the reagents and samples. PCR runs and addition of samples should therefore be prepared in different areas.
- Change pipette tips between all liquid transfers.
- Limit freeze/thaw cycles of the reagents as this might impair the performance of the assay.
- Assays should be protected from light due to the presence of HydrolEasy™ probes.
- The delivered reagents should not be diluted to a lower concentration than stated in the protocol. This may affect the performance of the assay.
- Use the volumes specified in the protocol. Using different volumes may affect the result.
- Do not substitute the reagents with others, as it may affect the performance of the assay.
- Do not open the PCR tubes/unseal wells after completing the PCR program.

## 5.2 SHIPMENT

- CoviDetect™ COVID-19 Multiplex RT-qPCR assays will be shipped on dry ice or ice bricks cooled to below -40°.

## 5.3 STORAGE OF REAGENTS

- CoviDetect™ COVID-19 Multiplex RT-qPCR Assay components should be stored at -20°C immediately after arrival.
- Repeated thawing and freezing should be kept to a minimum and should not exceed 10 freeze-thaw cycles.
- Return reagents to -20°C immediately after use.

- The long-term stability of CoviDetect™ is currently being investigated. Based on stability analysis of other PentaBase IVD assays, the stability of CoviDetect™ COVID-19 Multiplex RT-qPCR assay is expected to be at least 9 months at -20°C.

# 6 SPECIMENS

Specimen should be nasopharyngeal or oropharyngeal swabs or sputum. Ineffective or inappropriate collection, storage, and transport of specimens can result in false test results. Training in specimen collection is therefore recommended to ensure the best quality.

## 6.1 RECOMMENDED PROCEDURE FOR EXTRACTION OF NUCLEIC ACIDS

It is recommended to use validated nucleic acid extraction methods such as the Viral RNA/DNA Extraction Kit on the BasePurifier™ Instrument provided by PentaBase.

## 6.2 TRANSPORTATION OF SPECIMEN

Specimens should be packaged, shipped and transported according to guidelines provided by the manufacturer.

## 6.3 STORAGE OF SPECIMEN

- Specimens can be stored in suitable buffers at 2-8°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or lower.
- Extracted RNA should always be stored at -70°C or lower.

# 7 RT-QPCR PROTOCOL

CoviDetect™ COVID-19 Multiplex RT-qPCR assay is supplied in a Dispense-Ready version suited for automatized workflows handling a large number of samples as well as in a Ready-To-Use version pre-dispensed in 8-tube PCR strips suited for manual handling of samples.

## 7.1 REAGENT PREPERATION – DISPENSE-READY ASSAY

a. Add **10 µL** 2X AmpliSmaRT™ One Step RT-qPCR Master Mix to each PCR tube or well.
b. Add **5 µL** 4X primer/probe multiplex mix to the PCR tubes or wells.
c. Add **5 µL** of template to each PCR tube. One patient is analyzed in a single PCR tube.
d. Seal all tubes.

## 7.2 REAGENT PREPERATION – READY-TO-USE ASSAY

a. Add **5 µL** of template to each PCR tube. One patient is analyzed in a single PCR tube.
b. Seal all tubes.

## 7.3 POSITIVE CONTROL

A positive control is provided (approx. 20 copies/µl) and 200 µl should be added during the RNA extraction procedure. **Note:** the positive control contains high amounts of SDS and cannot be

added directly to the CoviDetect™ Multiplex Rt-qPCR Assay but needs to be subject to a nucleotide extraction procedure first.

### 7.4 REAL-TIME RT-qPCR RUN

a. Spin down the PCR tubes/plates (2 min, full speed) to ensure that all reagents are collected in the bottom of the tubes/wells and to eliminate air bubbles in the mixes.
b. Place the PCR tubes or plates in the real-time PCR instrument and run the program shown in Table 2.

| Protocol | Temperature [°C] | Time [sec] | Cycles | Ramping [°C/sec] | Comments |
|---|---|---|---|---|---|
| **Stage 1** | | | | | |
| Hold | 52 | 300 | 1 | 2 | |
| **Stage 2** | | | | | |
| Hold | 95 | 10 | 1 | 2 | |
| **Stage 3 (Cycle 1-7)** | | | | | |
| 2-step amplification | 95<br>66 | 5<br>30 | 7 | 2<br>2 | |
| **Stage 4 (Cycle 1-38)** | | | | | |
| 2-step amplification | 95<br>60 | 5<br>30 | 38 | 2<br>2 | Acquire on:<br>FAM (green)<br>HEX/VIC (yellow)<br>Cy5 (red) |

**Table 2.** RT-qPCR protocol for running CoviDetect™ COVID-19 Multiplex Assay on BaseTyper™ Real-time PCR instrument.

## 8 DATA ANALYSIS

In CoviDetect™ COVID-19 Multiplex RT-qPCR assay, determining the cycle threshold (Cq) is a central part of the data analysis procedure. The Cq is defined as the cycle in which the fluorescence signal of a given assay exceeds the threshold value which is set as part of the analysis procedure. Cq values of the PCR program stage 4 are compared to predefined cutoff values to determine if the individual samples are positive or negative for SARS-CoV-2 (Section 8.3). An overview of the possible outcomes of the analysis is shown in Table 3.

| Target | Positive Case 1 | Positive Case 2 | Positive Case 3 | Positive Case 4 | Negative | Invalid |
|---|---|---|---|---|---|---|
| RNase P | + | -* | + | + | + | - |
| N1 | + | + | - | + | - | - |
| N2 | + | + | + | - | - | - |

**Table 3**. Analysis outcomes based on target amplification curves. Conclusions are based on target Cq values compared to the cutoffs found in Table 4. *The RNase P signal may be suppressed when N1 and N2 are positive, especially in samples with high amounts of viral RNA.

## 8.1 INSTRUMENTS

CoviDetect™ COVID-19 Multiplex RT-qPCR assay is designed to run on open platforms and has currently been validated on ABI 7500 (Applied Biosystems™), CFX96 (BioRad), LightCycler® 480 II (Roche) and BaseTyper™ (PentaBase) Real-Time PCR instruments. Optimal PCR profiles are developed for each validated instrument. Please write to info@pentabase.com for current instrument-specific instructions for use available. More instruments and settings will be added when they are validated. To run CoviDetect™ COVID-19 Multiplex RT-qPCR assay on other instruments, you must validate settings yourself. It is recommended to perform a specific validation using patient samples and synthetic controls in order to set cycle threshold and cutoffs correctly. Please contact PentaBase or your local distributor for support.

## 8.2 BASELINE AND THRESHOLD SETTINGS

Results from CoviDetect™ COVID-19 Multiplex RT-qPCR assay can be analysed using both automatic and manual baseline and threshold settings. If automatic baseline and threshold settings are used, it is recommended to also perform a visual inspection of the amplification curves since some cases might need manual adjustment of baseline and/or threshold due to baseline drift and/or incorrect baselining. When setting the baseline manually, it is recommended to use 5 cycle intervals such as from cycle 10 to cycle 15 depending on the amplification curve of the sample. When setting the threshold manually, the threshold should be set to cross in the beginning of the exponential PCR phase and above any background or baseline fluorescence. If there is significant background or baseline fluorescence, adjust the baseline interval. Please refer to the troubleshooting section (Section 11) for more guidance on correcting improper analysis settings.

## 8.3 INTERPRETATION OF RESULTS

An overview of the possible outcomes of the analysis is shown in Table 3. The results are only valid if the included positive control Cq values are below 35 for N1 and N2, and below 28 for RNase P internal control. No template (NTC) negative control should produce no Cq values. Cq cutoff values for CoviDetect™ COVID-19 Multiplex assay are shown in Table 4.

### 8.3.1 POSITIVE SAMPLES

The sample is positive for SARS-CoV-2 when Cq values for both viral N1 and N2 assays are below 35. Please notice that the RNase P signal may be repressed in some samples and particularly when containing large amounts of viral RNA. These samples are considered valid if the Cq values of both N1 and N2 are below 35 even when RNAse P is negative.

The sample is also considered positive if either N1 or N2 are positive when RNase P is positive. The lack of signal in either N1 or N2 may be due to mutations present in the target regions of the assay. In case of a confirmed positive sample where there is only signal in either N1 or N2, it is recommended to send the sample for sequencing if possible and report the mutated strain to support@pentabase.com.

### 8.3.2 NEGATIVE SAMPLES

The sample is considered SARS-CoV-2 negative if the sample is positive for RNase P but negative for N1 and N2.

### 8.3.3 INVALID SAMPLES

In case of no or late amplification of RNase P (Cq≥28), the test is invalid unless both N1 and N2 are positive (Cq < 35). If more specimen is available, repeat the extraction and run the test again. If all markers remain negative after repeating the test, no diagnosis can be concluded, and if possible, a new specimen should be collected for testing.

| Interpretation of RT-qPCR results (Stage 4) | | | |
|---|---|---|---|
| **Assay** | **Cq** | **Conclusion** | **Comments** |
| N1 | <35 | SARS-CoV-2 positive | Cq values should be below 35 for both N1 and N2 for samples to be positive for SARS-CoV-2. |
| N2 | <35 | | |
| RNase P | <28 | SARS-CoV-2 positive | The sample is positive if only N1 is positive and RNAse P is positive. |
| N1 | <35 | | |
| N2 | ≥35 | | |
| RNase P | <28 | SARS-CoV-2 positive | The sample is positive if only N2 is positive and RNAse P is positive. |
| N1 | ≥35 | | |
| N2 | <35 | | |
| RNase P | <28 | SARS-CoV-2 negative | A positive RNase P signal is required for a sample to be considered negative. |
| N1 | ≥35 | | |
| N2 | ≥35 | | |
| RNase P | ≥28 | Invalid | The sample does not contain enough material for the analysis. Take a new specimen if possible. |
| N1 | ≥35 | | |
| N2 | ≥35 | | |

**Table 4.** Cq cutoff values for CoviDetect™ COVID-19 Multiplex Assay.

# 9 PERFORMANCE EVALUATION

## 9.1 ANALYTICAL SENSITIVITY – LIMIT OF DETECTION

The limit of detection (LOD) of CoviDetect™ COVID-19 Multiplex RT-qPCR assay has been evaluated by spiking synthetic SARS-CoV-2 RNA (Twist Bioscience, Cat. no. 102015) into negative clinical oropharyngeal matrix. Based on an initial dilution series, 500 and 250 copies of SARS-CoV-2 RNA were each spiked into 20 oropharyngeal specimens. RNA was extracted using the BasePurifier™ Nucleic Acid Extraction Instrument and viral DNA/RNA extraction kit (Table 5).

| Instrument | Measurement | N1 | | N2 | | N1 or N2 | |
|---|---|---|---|---|---|---|---|
| BaseTyper™ | RNA (copies per extraction) | 500 | 250 | 500 | 250 | 500 | 250 |
| | RNA concentration (copies/µl) | 2.5 | 1.25 | 2.5 | 1.25 | 2.5 | 1.25 |
| | Positives/Total | 20/20 | 18/20 | 20/20 | 18/20 | 20/20 | 19/20 |
| | Mean Ct (Stage 4) | 30.0 | 31.1 | 28.5 | 29.2 | NA | NA |
| | Standard Deviation (Ct) | 1.2 | 1.7 | 1.1 | 1.9 | NA | NA |
| CFX96 | RNA (copies per extraction) | 500 | 250 | 500 | 250 | 500 | 250 |
| | RNA concentration (copies/µl) | 2.5 | 1.25 | 2.5 | 1.25 | 2.5 | 1.25 |
| | Positives/Total | 20/20 | 17/20 | 20/20 | 18/20 | 20/20 | 19/20 |
| | Mean Ct (Stage 4) | 28.3 | 28.7 | 27.2 | 27.8 | NA | NA |
| | Standard Deviation (Ct) | 1.0 | 1.7 | 0.9 | 1.9 | NA | NA |
| LightCycler® 480 | RNA (copies per extraction) | 500 | 250 | 500 | 250 | 500 | 250 |
| | RNA concentration (copies/µl) | 2.5 | 1.25 | 2.5 | 1.25 | 2.5 | 1.25 |
| | Positives/Total | 20/20 | 16/20 | 19/20 | 15/20 | 20/20 | 16/20 |
| | Mean Ct (Stage 4) | 29.2 | 30.5 | 30.0 | 29.7 | NA | NA |
| | Standard Deviation (Ct) | 2.6 | 1.4 | 1.2 | 0.7 | NA | NA |

**Table 5.** Limit of detection (LOD) of CoviDetect™ COVID-19 Multiplex Assay using SARS-CoV-2 RNA spiked into oropharyngeal matrix. RNA was extracted using the BasePurifier™ nucleic acid extraction instrument.

The limit of detection of CoviDetect™ COVID-19 Multiplex RT-qPCR Assay on using BaseTyper™ real-time PCR instrument independent of extraction method was determined using SARS-CoV-2 RNA (Twist Bioscience, Cat. no. 102015) diluted in a 25 ng wild type human genomic DNA background (Table 6).

| RNA (Copies per reaction) | Observations (n) | Assay | Positives | Positives (%) |
|---|---|---|---|---|
| 0 | 18 | N1 | 0 | 0 |
| | | N2 | 0 | 0 |
| | | N1 or N2 | 0 | 0 |
| 1 | 12 | N1 | 2 | 17 |
| | | N2 | 2 | 17 |
| | | N1 or N2 | 2 | 17 |
| 5 | 30 | N1 | 19 | 63 |
| | | N2 | 19 | 63 |
| | | N1 or N2 | 21 | 70 |
| 10 | 36 | N1 | 27 | 75 |
| | | N2 | 31 | 86 |

| | | | | |
|---|---|---|---|---|
| | | N1 or N2 | 32 | 89 |
| 20 | 28 | N1 | 21 | 75 |
| | | N2 | 26 | 93 |
| | | N1 or N2 | 27 | 96 |
| 50 | 10 | N1 | 10 | 100 |
| | | N2 | 10 | 100 |
| | | N1 or N2 | 10 | 100 |
| 100 | 8 | N1 | 8 | 100 |
| | | N2 | 8 | 100 |
| | | N1 or N2 | 8 | 100 |

**Table 6.** Limit of detection (LOD) of CoviDetect™ COVID-19 Multiplex Assay using SARS-CoV-2 RNA spiked into wild type human DNA. RT-qPCR of SARS-CoV-2 RNA was performed using the BaseTyper™ instrument.

## 9.2 INCLUSIVITY

CoviDetect™ COVID-19 Multiplex assay oligo sequences have been aligned with Global SARS-CoV-2 sequences from GISAID (excluding Denmark, see section 9.2.2). Mismatch frequencies were found to be less than 5%.

**N1 Forward Primer**

```
GACCCCAAAATCAGCGAAAT

|||||||||||||  ||||||  >hCoV-19/USA/WA-UW112/2020|EPI_ISL_416650|2020-03-10
||||  ||||||||||||||||  >hCoV-19/pangolin/Guangdong/1/2019|EPI_ISL_410721|2019
|||||||||||||  ||||||  >hCoV-19/Netherlands/ZuidHolland_28/2020|EPI_ISL_415532|2020-03-09
||||||||||  |||||||||  >hCoV-19/Senegal/026/2020|EPI_ISL_418209|2020-03-03
||||  ||||||||||||||||  >hCoV-19/Foshan/20SF207/2020|EPI_ISL_406534|2020-01-22
||||||||||  ||  ||||||  >hCoV-19/bat/Yunnan/RaTG13/2013|EPI_ISL_402131|2013-07-24
|||||||||||||  ||||||  >hCoV-19/USA/WA-UW112/2020|EPI_ISL_416650|2020-03-10
||||  ||||||||||||||||  >hCoV-19/pangolin/Guangdong/1/2019|EPI_ISL_410721|2019
|||||||||||||  ||||||  >hCoV-19/Netherlands/ZuidHolland_28/2020|EPI_ISL_415532|2020-03-09
||||||||||  |||||||||  >hCoV-19/Senegal/026/2020|EPI_ISL_418209|2020-03-03
||||  ||||||||||||||||  >hCoV-19/Foshan/20SF207/2020|EPI_ISL_406534|2020-01-22
||||||||||  ||  ||||||  >hCoV-19/bat/Yunnan/RaTG13/2013|EPI_ISL_402131|2013-07-24
```

**N1 Reverse Primer**

```
CGCAGTATTATTGGGTAAACC
```

No mismatches found

**N1 Probe**

```
ACCCCGCATTACGTTTGGTGGACC
||||||  ||||||||||||||||||  >hCoV-19/USA/TX_2967/2020|EPI_ISL_420798|2020-03-01
|||||||||||||||  |||||||  >hCoV-19/France/ARA11997/2020|EPI_ISL_419176|2020-03-21
||||||  ||||||||||||||||||  >hCoV-19/Tianmen/HBCDC-HB-07/2020|EPI_ISL_412983|2020-02-08
||||||  ||||||||||||||||||  >hCoV-19/Australia/VIC41/2020|EPI_ISL_419760|2020-03-11
||||||  ||||||||||||||||||  >hCoV-19/USA/TX_2967/2020|EPI_ISL_420798|2020-03-01
|||||||||||||||  |||||||  >hCoV-19/France/ARA11997/2020|EPI_ISL_419176|2020-03-21
||||||  ||||||||||||||||||  >hCoV-19/Tianmen/HBCDC-HB-07/2020|EPI_ISL_412983|2020-02-08
||||||  ||||||||||||||||||  >hCoV-19/Australia/VIC41/2020|EPI_ISL_419760|2020-03-11
```

**N2 Forward primer**

```
AGGAACTGATTACAAACATTGGC

|||||||||||||||||| ||||    >hCoV-19/Estonia/ChVir1985/2020|EPI_ISL_420067|2020-03
|||||||||||||||||| ||||    >hCoV-19/pangolin/Guangdong/1/2019|EPI_ISL_410721|2019
```

**N2 Reverse Primer**

```
TGTAGGTCAACCACGTTCCC

|||||||||| ||||||||      >hCoV-19/USA/WI-43/2020|EPI_ISL_421301|2020-03-19
||||||| ||||||||||       >hCoV-19/Hungary/2/2020|EPI_ISL_418183|2020-03-17
```

**N2 Probe**

```
TGCACAATTTGCCCCCAGCG

|||||||||||||| ||||      >hCoV-19/Wales/PHWC-255AC/2020|EPI_ISL_421008|2020-03-23
|||||||||| |||||||||      >hCoV-19/Iceland/30/2020|EPI_ISL_417773|2020-03-03
|||||||||||||| ||||      >hCoV-19/Chongqing/YC01/2020|EPI_ISL_408478|2020-01-21
||||||||| || |||||||      >hCoV-19/bat/Yunnan/RaTG13/2013|EPI_ISL_402131|2013-07-24
```

## 9.3 ANALYTICAL SPECIFICITY

CoviDetect™ COVID-19 Multiplex assay oligo sequences have been aligned with common Betacoronaviruses. There is only potential binding of the N1 assay to the original SARS coronavirus (SARS-CoV) when including less than 5 mismatches. Subsequent wet test of cross-reactivity to SARS-CoV was negative.

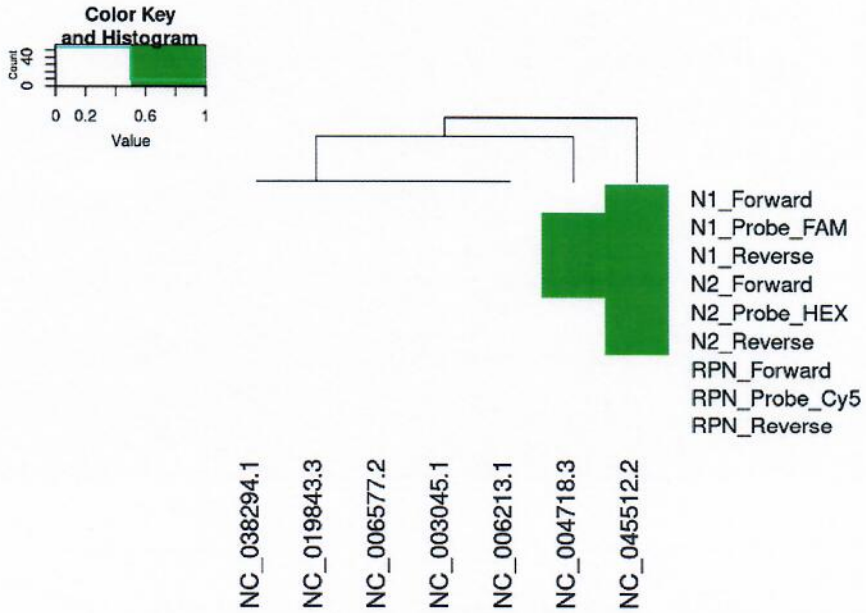



**Figure 1**. In silico analysis of cross-reactivity to betacoronaviruses including the common cold. NC_038294.1: Betacoronavirus England 1, NC_019843.3: Middle East Respiratory syndrome coronavirus, NC_006577.2: Human coronavirus HKU1, NC_003045.1:Bovine coronavirus, NC_006213.1:Human coronavirus OC43 strain ATCC VR-759, NC_004718.3: SARS coronavirus, NC_045512.2: SARS-Coronavirus 2 (SARS-CoV-2).

### 9.3.1 DANISH CASES

CoviDetect™ COVID-19 Multiplex assay oligo sequences have been aligned with +300 Danish SARS-CoV-2 sequences from GISAID. Two and one mutation(s) were identified in the target sequences of the N1 and N2 probes, respectively.

**N1 Forward Primer**

GACCCCAAAATCAGCGAAAT

No mismatches found

**N1 Reverse Primer**

CGCAGTATTATTGGGTAAACC

No mismatches found

**N1 Probe**

ACCCCGCATTACGTTTGGTGGACC

One Danish strain has been identified with an **insertion of a T at position 23**:

ACCCCGCATTACGTTTGGTGGACC | N1_Probe_FAM

ACCCCGCATTACGTTTGGTGGATCC | hCoV-19/Denmark/ALAB-SSI480/2020|EPI_ISL_429559|2020-03-25

Two Danish strains have been identified with **a G -> T mutation at position 22**:

ACCCCGCATTACGTTTGGTGTACC | hCoV-19/Denmark/ALAB-SSI209/2020|EPI_ISL_429405|2020-03-10

ACCCCGCATTACGTTTGGTGTACC | hCoV-19/Denmark/ALAB-SSI201/2020|EPI_ISL_429399|2020-03-09

The effect of the identified mutations on N1 probe affinity has been investigated using synthetic DNA (Figure 2). The T23Ins and G22T mutations reduce the melting temperature from 78.5°C (Yellow line) to 76°C (Blue) and 72.5°C (Purple), respectively. Based on these findings the effect on the performance of the assay is considered to be low.

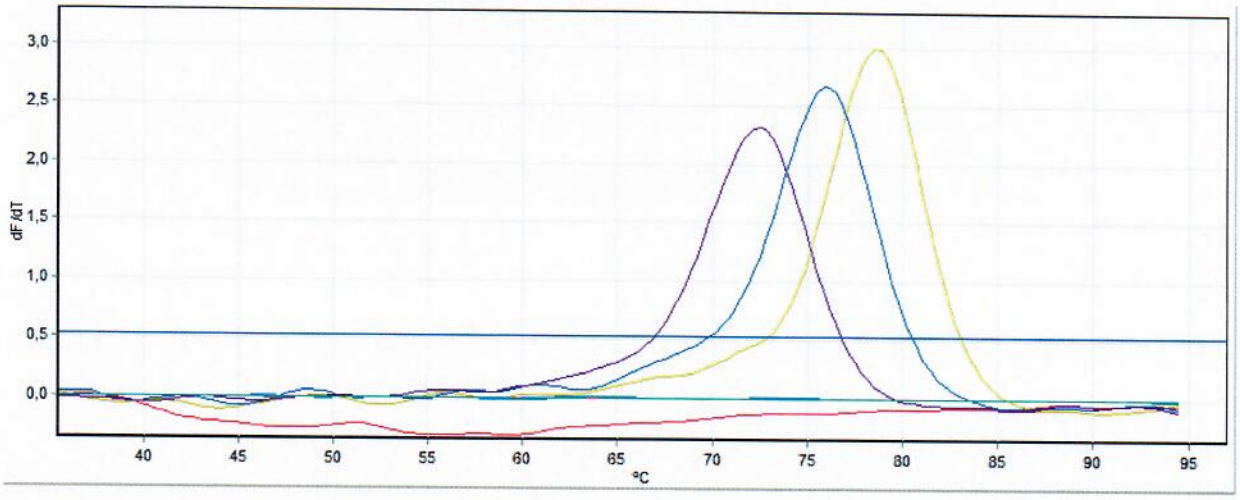



**Figure 2.** Melt studies using CoviDetect™ COVID-19 Multiplex assay N1 probe and synthetic DNA strands containing the T23Ins and G22T mutations identified in Danish SARS-CoV-2 strains.

**N2 Forward primer**

AGGAACTGATTACAAACATTGGC

No mismatches found

**N2 Reverse Primer**

TGTAGGTCAACCACGTTCCC

No mismatches found

**N2 Probe**

TGCACAATTTGCCCCCAGCG

Four Danish strains have been identified with **a C -> T mutation at position 16**:

TGCACAATTTGCCCCTAGCG hCoV-19/Denmark/alab-hh89/2020|epi_isl_429329|2020-03-15

TGCACAATTTGCCCCTAGCG hCoV-19/Denmark/alab-ssi414a/2020|epi_isl_429512|2020-03-23

TGCACAATTTGCCCCTAGCG hCoV-19/Denmark/alab-ssi413a/2020|epi_isl_429510|2020-03-23

TGCACAATTTGCCCCTAGCG hCoV-19/Denmark/alab-ssi595/2020|epi_isl_429590|2020-03-28

The impact of this mutation on probe affinity is currently under investigation.

## 9.4 CLINICAL EVALUATION

The clinical performance of CoviDetect™ COVID-19 Multiplex RT-qPCR Assay was evaluated using leftover oropharyngeal swabs and expectorate clinical specimens from patients suspected of COVID-19. Specimens were previously analyzed for the presence of SARS-CoV-2 using the comparator RT-qPCR method at a clinical laboratory in Denmark. Stored samples were collected for subsequent analysis by CoviDetect™ COVID-19 Multiplex RT-qPCR Assay. Extraction of RNA was performed using the Viral DNA and RNA Extraction Kit for the BasePurifier™ Nucleic Acid Extraction Instrument. RT-qPCR was performed using the CFX96 Real-Time PCR Detection System (BioRad) and data analysis was performed using software version 3.1. Standard analysis settings were used except that the threshold for the FAM channel was set to 100 RFU.

| | Assay | CoviDetect™ | Comparator Method 1 | Agreement |
|---|---|---|---|---|
| Oropharyngeal swabs | SARS-CoV-2 positive | 26 | 30 | 87% (PPA) |
| | SARS-CoV-2 negative | 55 | 51 | 100% (NPA) |
| Expectorates | SARS-CoV-2 positive | 4 | 5 | 80% (PPA) |
| | SARS-CoV-2 negative | 0 | 0 | NA |

**Table 7**. Summary of clinical evaluation of CoviDetect™ COVID-19 Multiplex RT-qPCR Assay using cohort 1.

The Ct values of CoviDetect™ (Ct + 7) compared to Comparator Method 1 of agreed positive and discrepant samples is illustrated in Figure 3. Discrepant samples are shown as data points with CoviDetect™ Ct values of 0. Due to lack of material, it has not been possible to confirm the results by a third method.

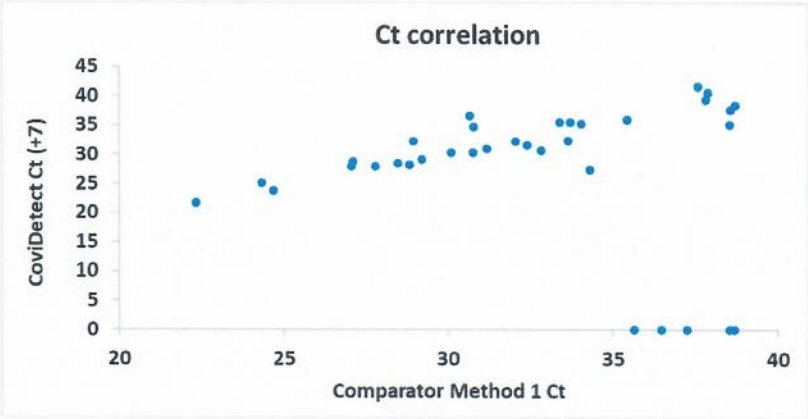



**Figure 3.** Correlation of Ct values between CoviDetect™ and Comparator Method 1 using oropharyngeal swabs and expectorate clinical samples (Cohort 1). Discrepant cases are illustrated as datapoints with CoviDetect™ Ct values of 0.

Correlation of Ct values between CoviDetect™ and Comparator Method 1 of the shared positive samples is illustrated in Figure 4.

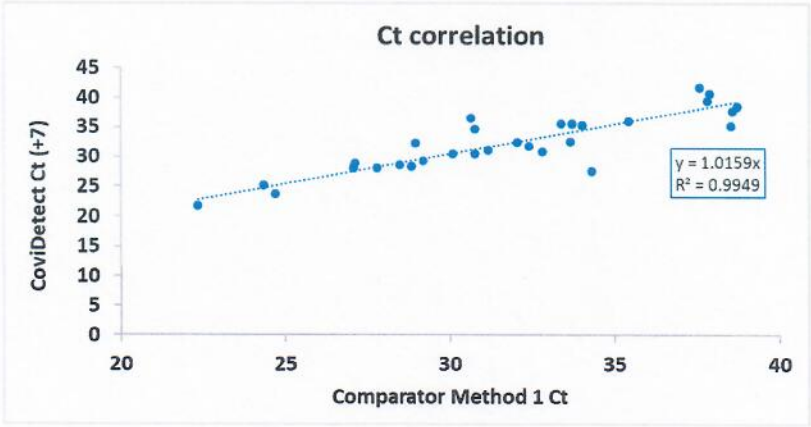



**Figure 4.** Correlation of Ct values between CoviDetect™ and Comparator Method 1 using leftover oropharyngeal swabs and expectorate clinical samples (Cohort 1).

### 9.4.1 COHORT 2

In cohort 2, leftover nasopharyngeal swab specimens from patients suspected of COVID-19 were extracted using the Viral DNA and RNA Extraction Kit for the BasePurifier™ Nucleic Acid Extraction Instrument. RT-qPCR was performed using CoviDetect™ COVID-19 Multiplex RT-qPCR Assay and Comparator Method 2 on the BaseTyper™ Real-Time PCR Instrument. Analysis was performed using automatic baseline and threshold settings. Evaluation summary is shown in Table 8.

| | Result | CoviDetect™ | IDT 2019-nCoV CDC EUA Kit | Agreement |
|---|---|---|---|---|
| Nasopharyngeal swab specimens | SARS-CoV-2 positive | 27 | 27 | 100% (PPA) |
| | SARS-CoV-2 negative | 62 | 62 | 100% (NPA) |

**Table 8.** Comparison of clinical performance of CoviDetect™ COVID-19 Multiplex RT-qPCR Assay to IDT 2019-nCoV CDC EUA Kit using Cohort 2 samples.

Correlation of Ct values between CoviDetect™ and Comparator Method 2 is illustrated in Figures 5-7.

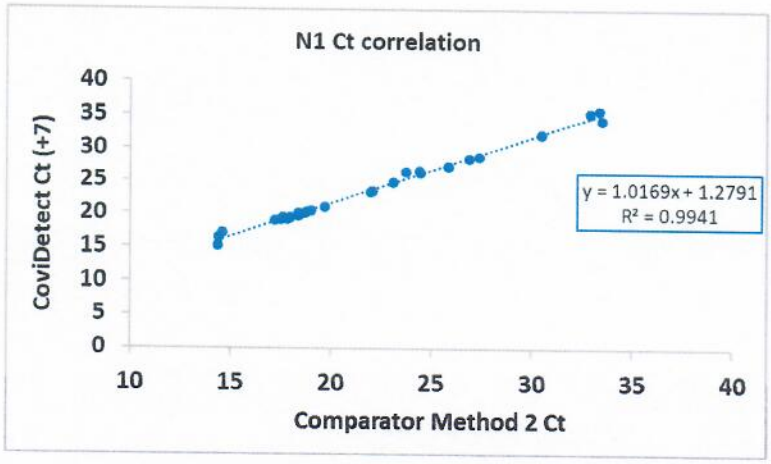
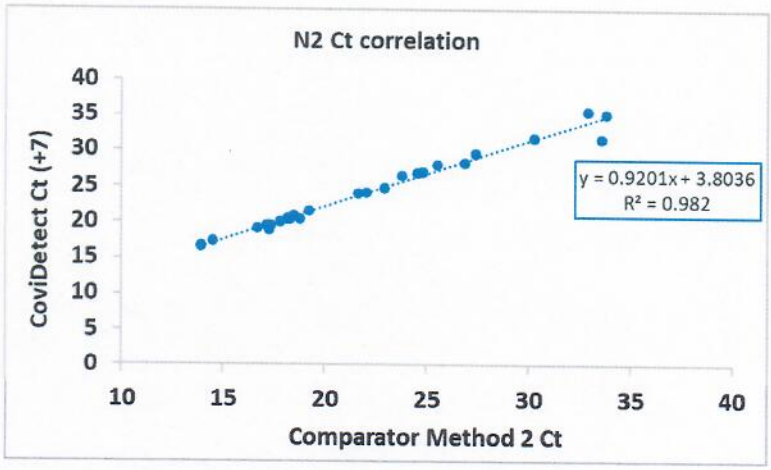



**Figure 5**. Correlation of N1 assay Ct values between CoviDetect™ and Comparator Method 2 using SARS-CoV-2 positive leftover oropharyngeal swab specimens (Cohort 2).

**Figure 6**. Correlation of N2 assay Ct values between CoviDetect™ and Comparator Method 2 using SARS-CoV-2 positive leftover oropharyngeal swab specimens (Cohort 2).



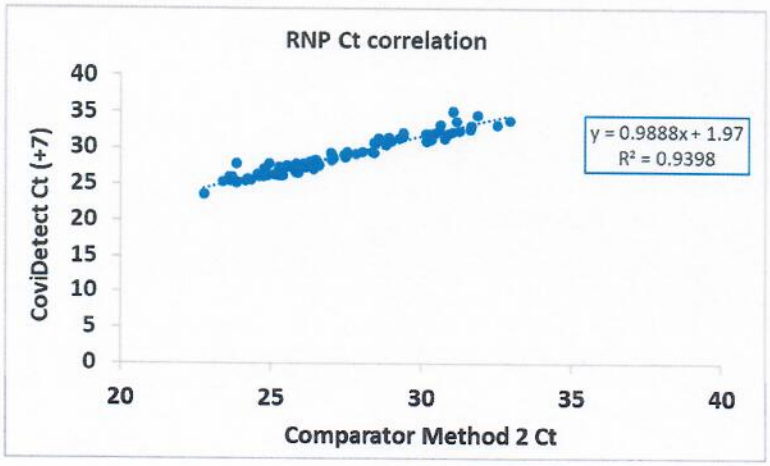




**Figure 7**. Correlation of RNP assay Ct values between CoviDetect™ and Comparator Method 2 using leftover oropharyngeal swab specimens (Cohort 2).

## 10 LIMITATIONS

- Performance of the CoviDetect™ COVID-19 Multiplex RT-qPCR assay has only been tested on specimens from nasopharyngeal or oropharyngeal swabs or sputum.

- A negative test result does not exclude infection with SARS-CoV-2 and treatment of a patient should not exclusively be based on the test result. Multiple specimens collected at different times from the same patient may be necessary to detect the virus, since it is unknown when the viral levels in the body will peak.
- Incorrect collection, transportation or handling of the sample could cause false negative test results. Also, very low amount of virus RNA in the specimen or amplification inhibitors could give false negative test results.
- Do not use reagents that have expired.
- If mutations occur in the targeted region of the virus (N1 and N2 markers) it may affect the sensitivity of the test and may result in false negative results.
- The test cannot exclude that the patient is infected with other viruses or bacteria.

# 11 TROUBLESHOOTING

The troubleshooting guide covers some of the most frequent questions and problems that can occur when using the CoviDetect™ COVID-19 Multiplex RT-qPCR assay and how these may be solved.

| Problem | Solution |
|---|---|
| No extraction control signal | Make sure that the PCR program has been defined correctly and that the instrument is acquiring on FAM, HEX/VIC and Cy5 channels in Step 2 of Stage 4. |
| No sample signal | The concentration or the quality of the RNA in the sample is too low. Add more sample if possible or collect a new specimen. |
| Signal in NTC | Make sure that the threshold has been set correctly above any background fluorescence. If this is the case, the reagents may be contaminated. Find the cause of contamination by checking or replacing all potential sources of the contamination such as pipettes and instruments. If the contamination cannot be located, contact PentaBase ApS or your local distributor. |
| Baseline drift | Baseline drift is a slowly rising signal in the amplification plot with no or late exponential phase. Baseline drift can occur when baselining has not been done properly. Baseline drift can be corrected by adjusting the baseline interval manually or applying baseline drift correction as part of the analysis settings. In both cases the amplification curve should be aligned at or close to the baseline but should not go below before any subsequent exponential phase. If baseline drift cannot be corrected and/or there is any doubt about the quality of the amplification curve, the sample should be rerun. |

**Table 9.** Troubleshooting guide

# 12 MANUFACTURER AND DISTRIBUTORS

## 12.1 MANUFACTURER

PentaBase Aps
Lumbyvej 19G
5000 Odense C, Denmark

## 12.2 TECHNICAL ASSISTANCE

For technical assistance in Denmark, please contact PentaBase ApS:
Webpage: www.pentabase.com
Email: support@pentabase.com
Phone: +45 36 96 94 96

For technical assistance in all other countries, contact your local distributor. A complete list of distributors is available at www.pentabase.com.