SARS-CoV-2 One-Step RT-PCR Kit, IVD

Viral RNA-dependent RNA polymerase (RdRp) gene

REF MD03201, 96 Determinations

For professional* in vitro *diagnostic use only

CE IVD

EN

Instructions for Use

MD0320_IM_en

VERSION 2401, January 2024

NZYtech genes & enzymes
Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal
Tel.: +351.213643514 Fax: +351.217151168
www.nzytech.com

Contents

1. Introduction

In late 2019 a novel acute respiratory disease, termed Coronavirus Disease 2019 (COVID-19), was reported in China and rapidly spread worldwide. The causative agent was identified as Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2). The virus (previously named 2019-nCoV), like the closely related SARS coronavirus (SARS-CoV), belongs to the genus *Betacoronavirus* within the family of coronaviruses. Coronaviruses are enveloped, positive, single-stranded large RNA viruses that infect humans, but also a wide range of animals. SARS-CoV-2 is thought to be of zoonotic origin and likely to have spread from large seafood and animal markets by human-animal contact in the city of Wuhan. The novel coronavirus is highly contagious and is primarly transmitted via respiratory droplets (coughing and sneezing). Early detection of SARS-CoV-2 is vital in providing rapid treatment to infected patients and, thus, to reduce the spread of infections. The most common clinical manifestations of COVID-19 include fatigue, fever and lower respiratory symptoms, such as dry cough and dyspnea. Loss of smell and taste can also occur. In the most critical situations, the infection progresses to severe pneumonia with life-threatening complications such as acute respiratory disease syndrome, organ dysfunction and death. Based on current knowledge, around 80% of infections are mild or asymptomatic. A percentage of the population is more vulnerable to the severe form of disease, including older adults (50 years and older), smokers and people with chronic diseases such as heart or lung disease, cancer, diabetes and patients with a weakened immune system.

2. Intended Use

NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD is a molecular test intended for the rapid qualitative detection of Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) nucleic acids in nasopharyngeal and oropharyngeal swabs or sputum samples collected from patients. A positive result indicates the presence of SARS-CoV-2 RNA, which results from infection with the novel human coronavirus. Testing is limited for use by laboratory trained personnel, specifically instructed in real-time PCR techniques and *in vitro* diagnostics.

3. Principles of the Assay

NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD provides the complete set of reagents and probes to qualitatively detect the SARS-CoV-2 genome, through common real-time PCR platforms (see required instrument specifications in **Section 6**). Specifically, the kit detects the presence of an RNA sequence from the SARS-CoV-2 gene encoding the viral RNA-dependent RNA polymerase (RdRp). An internal control is included to confirm efficient RNA extraction from human biological samples, as well as the absence of PCR inhibitors, among others. In addition, the test uses external controls (positive low titer control provided with the kit and negative control), as described below. SARS-CoV-2 primers and probe have 100% homology with >95% of the >2500 genome sequences available on the GISAID database as of 29 March 2020. In addition, primers and probe targeting SARS-CoV-2 genome display no significant homology with unrelated genomes rendering this highly specific test as there is no cross reactivity with any other Coronavirus sequenced thus far. The natural evolution of SARS-CoV-2 implies that new sequence information will become available after the initial design of this kit, which reflects SARS-CoV-2 adaptation strategies. Thus, NZYtech periodically revisits SARS-CoV-2 targets and, if required, will release new versions of this kit.

The qualitative determination of specific RNA is based on the one-step real-time RT-PCR technology, as this is a fast and reliable method to perform an accurate detection of SARS-CoV-2. By using NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD, RNA isolated and purified in association with a CE IVD extraction system is retrotranscribed (RT) to cDNA and subsequently amplified by PCR, in a single reaction, using two highly specific primers/probe sets exploiting the so-called TaqMan® principle. When the RNA extracted from respiratory samples is collected from an infected patient, a TaqMan® probe specifically binds to a conserved region of the SARS-CoV-2 RdRp gene circumscribed by an equally specific pair of primers. An additional primers/probe set acts as an internal control to detect a nucleic acid sequence of the human ribonuclease P [RNase P gene (RP)], which allows confirming the efficacy of the extraction process from human derived biological material. In addition, this internal control allows to demonstrate that no reaction inhibition has occurred by PCR inhibitors potencially present in the clinical samples. The two probes are differently labelled, with FAM™ and JOE™ reporter dyes, to allow identifying the individual amplification of the viral and human targets in a single reaction, respectively. Furthermore, they are provided in optimized concentrations to make sure amplification of human mRNA, even when present at very high concentrations, does not limit the efficiency of the SARS-CoV-2 primers/probe set. NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD includes a Positive control that allows controlling the performance of RT-PCR reactions. This control corresponds to a synthetic nucleic acid molecule carrying sequences that are homologous to both SARS-CoV-2 and RP targeted sequences included in this detection assay. The SARS-CoV-2/RP Positive control should be used directly with the SARS-CoV-2/RP primers/probe mix each time an array of samples is tested. A positive result indicates that the primers and probe sets for detecting the target SARS-CoV-2 and RP genes worked properly in the included master mix in that particular experimental scenario. If a negative result is obtained, the test results are invalid and must be repeated.

4. Kit Composition

NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD provides a comprehensive set of reagents sufficient to perform 96 RT-PCR reactions in a single step.

KIT COMPONENT		NUMBER OF VIALS	VOLUME (PER VIAL)
SARS-CoV-2 MMix	NZYSpeedy One-step RT-qPCR Master Mix	1	1050 µL
SARS-CoV-2 PPMix	SARS-CoV-2/RP primers/probe Mix	1	205 µL
SARS-CoV-2 Pos	SARS-CoV-2/RP Positive Control (1 x 10^4 copies/µL)	1	105 µL
SARS-CoV-2 Neg	RNase/DNase free water	1	105 µL

5. Storage, Stability and Handling Conditions

The SARS-CoV-2 One-Step RT-PCR Kit, IVD is shipped refrigerated. All components should immediately be stored at -85 °C to -15 °C upon arrival. When in use, the kit components should be returned to the freezer promptly after use to minimise the time at room temperature.

- Minimise the number of freeze-thaw cycles by storing in working aliquots. If appropriate, kit components may be aliquoted into smaller volumes after thawing.
- The SARS-CoV-2/RP primers/probe mix should be stored protected from light. Particularly, do not expose the NZYSpeedy One-step RT-qPCR Master Mix to direct sun light after combining with primers/probe mix.
- Immediately contact NZYtech if, upon arrival, the package that protects the kit is damaged.
- Beware to the expiry date indicated on the packaging. NZYtech does not recommend using the kit after the expiry date. On this date, the kit must be discarded following the disposal instructions in **Section 8.2**.

6. Materials and Instrumentation Required but Not Provided

- Real-time PCR Instrument that detects FAM™ and JOE™ fluorescent dyes (at emission wavelenghts of 520 and 555 nm, respectively). See in **Section 11** the instrument models for which the kit was validated.
- Equipment and consumables for isolating viral RNA from respiratory specimens.
- RNase/DNase free qPCR plasticware: PCR tubes, strips, caps, 96-well plates, adhesive films.
- Pipettors and filter tips (RNase/DNase free).
- Disposable gloves.
- Vortex and centrifuge.

7. Sample Collection and Preparation

Different factors, such as protocol for sample collection from human respiratory specimen (nasopharyngeal or oropharyngeal swabs, or sputum), sample transport, storage and processing time, are critical to achieve optimal results. Sputum samples must be from the lower respiratory tract. The collected samples should be tested as soon as possible. Samples shall be transported and stored at low temperatures in accordance with biosafety regulations. RNA or total nucleic acids extracted following a CE IVD protocol are the starting material for NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD. Please ensure RNA samples are suitable in terms of purity, concentration and nucleic acid integrity. An $A_{260/280}$ ratio of ~2 is generally accepted for pure RNA. Since ethanol is a strong Real-Time PCR inhibitor, it is necessary to completely eliminate it prior to the elution of the nucleic acid during extraction. NZYtech kit integrates an internal RNA extraction control reaction that targets human RNA, which is co-purified with viral RNA. Human RNA is amplified with the RNase P (RP) primers/probe set. This is useful for checking the efficiency of RNA isolation and/or the presence of inhibitors during sample processing.

8. Precautions and Warnings

As in any analytical testing procedure, good laboratory practices are essential. Carefully follow the procedures and guidelines provided in this handbook to ensure that the test is performed correctly. Any deviation from them may result in assay failure or cause erroneous results. Due to high sensitivity of the kit, special care must be taken to keep reagents and amplification mixes free from contamination.

8.1 Safety Information

Before using the kit please consult the Safety Data Sheet (SDS) that is available at NZYtech website (www.nzytech.com). Detection of SARS-CoV-2 virus should be developed only by staff trained in the relevant technical and safety procedures in appropriately equipped laboratories. International and national guidelines on laboratory biosafety should be followed in all circumstances.

8.2 Handling and Procedural Requirements

- Only for professional *in vitro* diagnostic use.
- Do not use this kit after expiration date.
- Do not use the test components if kit sealing is damaged.
- Do not interchange reagents of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- DNase/RNase free disposable plasticware and pipettes should be used in all procedures.
- Use DNase/RNase free filter tips throughout the protocol to prevent aerosol and liquid contamination.
- Sample preparation, reaction set up and amplification should be performed in different working areas.
- Positive control template contains a high copy number of templates; It should be opened and processed away from test samples and kit components to avoid cross-contamination.
- Always use the water provided in the kit (SARS-CoV-2 Neg - RNase/DNase free water).
- At the end of each testing, clean work surfaces and equipment with a DNA/RNA remover.
- Handle post-amplification plates with care and dispose them immediately after the end of the testing; plates should be always discarded into a proper biohazard container after use.
- Biological samples should be handled as if they are infectious following proper biosafety precautions.
- Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations.
- All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.
- Follow good laboratory practices, wear protective clothing, permanently wear disposable powder-free gloves, use goggles and mask. Do not eat, drink or smoke in the working area.

9. Testing Procedure

Please read the instructions for use carefully before performing the assay. Beware that all pipetting steps and experimental plate set-up should be performed on ice. After the plate is poured start immediately to the one-step RT-PCR protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artefacts that reduce the sensitivity of detection. Previous to the experiment, start to gently mix the reaction tubes provided, centrifuge for 5 seconds to collect contents at the bottom of the tube and place tubes on ice. **Always pipette the SARS-CoV-2/RP Positive Control last to avoid contamination events.**

9.1 Reaction set-up

1. Prepare a RT-PCR mix enough for the number of SARS-CoV-2/RNase P tests to be performed with a 5% additional volume for pipetting losses. Proceed according to the table below that specify the volumes for 1 and *n* tests (where *n* corresponds to the total number of reactions):

COMPONENT	1 TEST VOLUME (µL)	*n* TESTS (*) VOLUME + 5% (µL)
SARS-CoV-2 MMix	10	*n* x 10.5
SARS-CoV-2 PPMix	2	*n* x 2.1
FINAL VOLUME	**12**	***n* x 12.6**

(*) To calculate the total number of reactions needed for each assay, count the number of samples and add two more for the Negative and Positive controls, respectively.

2. Pipette 12 µL of the RT-PCR mix into individual wells according to your real-time PCR experimental plate set-up.
3. For the negative control, add 8 µL of SARS-CoV-2 Neg instead of RNA template into the negative control well. The final volume should be 20 µL.
4. For the biological samples, add 8 µL of each RNA sample into the SARS-CoV-2/RNase P wells, according to your experimental plate set-up. The final volume in each well should be 20 µL.
5. For the positive control, add 8 µL of SARS-CoV-2 Pos instead of RNA template into the positive control well. The final volume should be 20 µL.
6. Cover and seal the plate with an appropriate optical adhesive film before proceeding with the RT-PCR and detection steps.
7. Place the reaction plate in the real-time PCR instrument and run the RT-PCR protocol according to the section below.

9.2 Programming the real-time PCR instrument

The table below displays a standard protocol optimized on a number of platforms. However, these conditions may be adapted and validated to suit different machine-specific protocols.

Suggested RT-PCR Run Settings

CYCLES	TEMPERATURE	TIME	STEP
1	50 °C	20 min	Reverse Transcription
1	95 °C	2 min	Polymerase activation
40	95 °C	5 s	Denaturation
	60 °C	30 s	Annealing/Extension

Fluorescent Detectors/Dyes

TARGET	FLUORESCENT DYE	DETECTION CHANNELS
SARS-CoV-2 & RNase P targets	FAM™ & JOE™	FAM
Positive/Negative controls	FAM™ & JOE™	JOE, VIC or HEX

NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD was validated for the following Real Time PCR Systems: Applied Biosystems® 7500, Applied Biosystems® StepOnePlus and Bio-Rad® CFX96™. If other equipment is used, the kit should be validated by the user by using previous characterised samples (both positive and negative).

10. Data Analysis

10.1 Run Validation Criteria

Before analysing samples results, we recommend to verify if the real-time PCR test is valid. Thus, for each plate, please confirm if the results for Positive and Negative controls performed as expected, according to the following criteria:

Positive control: the amplification curves of FAM (SARS-CoV-2) and JOE (RP) are positive. Positive control is expected to amplify at a Ct<28, both in the FAM and JOE channels. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

Negative control (no template reaction): no amplification is detected. If the negative control has one or two amplification curves (FAM and/or JOE) with a sigmoidal shape, sample contamination may have occurred. Repeat the test following good RT-PCR practices.

If the controls are according with expected, the test is **valid**. Please proceed with interpretation of results for the tested samples.
If any of the controls do not exhibit the expected performance, the assay was compromised or executed improperly and should be considered **invalid**. **Please, repeat the test** *If the problem persists contact the manufacturer*

10.2 Test Results Interpretation

SARS-CoV-2 is detected if the FAM amplification curve displays a sigmoidal shape with a Ct<36, regardless of what result is obtained for the RP (JOE) assay.

SARS-CoV-2 is not detected if FAM curve is not positive (Ct≥36) while the RP (JOE) displays a positive sigmoidal curve (Ct<40).

The **test is invalid** if the SARS-CoV-2 and RP assays are both negative. The test should be repeated with nucleic acid re-purified from the sample.

The following table summarises the interpretation of principal results (evaluate the overall shape of the amplification curves; **only sigmoidal amplification curves are indicative of true amplification**).

SARS-CoV-2 RESULT SARS-CoV-2, CT (FAM)	**RP RESULT** RP, CT (JOE)	**RESULTS INTERPRETATION**
+ (Ct<36)	+ (Ct<40)	SARS-CoV-2 detected → POSITIVE
+ (Ct<36)	- (Ct>40)	SARS-CoV-2 detected → POSITIVE
- (Ct≥36)	+ (Ct<40)	SARS-CoV-2 not detected → NEGATIVE
- (Ct≥36)	- (Ct>40)	Invalid test, repeat extraction and repeat test

Note: *Interpretation of results must account for the possibility of false negative and false positive results.*

- False negative results may be caused by:
  - Unsuitable collection, handling and/or storage of samples.
  - Sample outside of viraemic phase.
  - Failure to follow procedures in this handbook.
  - Use of unauthorised extraction kit or real-time PCR platform.

- False positive results may be caused by:
  - Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template.
  - Unsuitable handling of the SARS-CoV-2 Pos tube.
  - Unsuitable handling of amplified product (post-amplification plate).

11. Performance Evaluation

Evaluation of the NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD performance was performed on the Applied Biosystems® 7500 and Bio-Rad® CFX96™ Real Time PCR Systems with additional testing on the Applied Biosystems® StepOnePlus Real Time PCR system. If other equipment is used, the kit should be validated by the user by using previous characterised samples (both positive and negative).

11.1 Expected Results

A typical amplification plot, observed for a clinical sample containing SARS-CoV-2 nucleic acids, is presented below.

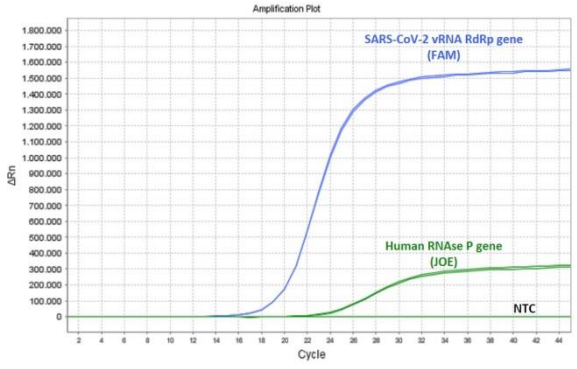
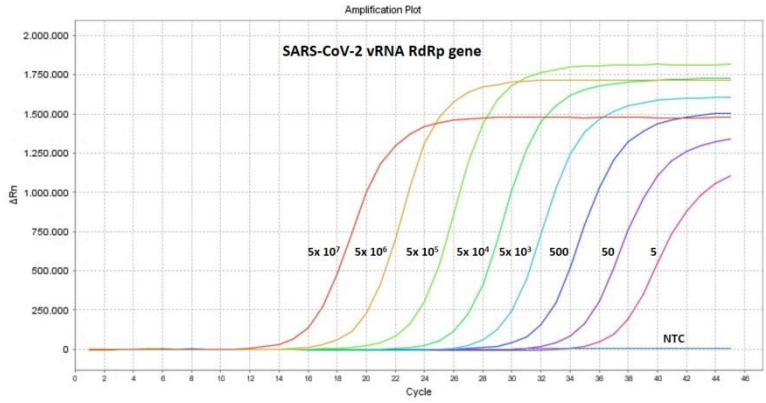


Figure 1. Simultaneous detection of SARS-CoV-2 and Human RNase-P targets from a positive clinical sample (patient infected with SARS-CoV-2). Blue curve: detection of the SARS-CoV-2 viral RNA target (RdRp gene) through the FAM channel; Green curve: detection of the human RNase P gene through the JOE channel. NTC, No Template Control (negative control).

11.2 Analytical Sensitivity

The analytical sensitivity was defined as the lowest concentration of analyte that could be reliably detected with 95% confidence. This was assessed by testing SARS-CoV-2 nucleic acids at different copy numbers (5 x 10^4 to 2.5 copies per reaction; 2.5 x 10^3 to 0.125 copies/µL), individually or spiked into RNA extracted from negative oropharyngeal samples, using 3 different kit lots following typical testing reaction conditions. Tests were repeated twice a day over 5 days, producing 60 replicates for each SARS-CoV-2 concentration tested. Together, the data revealed that NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD detects 0.25 copies/µL of SARS-CoV-2 viral RNA with a confidence ≥95%. Thus, the analytical sensitivity of the kit, expressed as the Limit of Detection (LoD), is 0.25 copies/µL or 250 copies/mL. The kit LoD was re-validated by two different operators, using three kit lots, in an experiment with a total of 48 tests, ensuring that the kit analytical sensitivity was maintained accross different testing situations. The capacity of NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD to detect the virus at different loads (from 5 x 10^7 to 5 copies per reaction) is presented in the Figure below.

Figure 2. Sensitivity of the SARS-CoV-2 One-Step RT-PCR Kit, IVD. Amplification plot (cycle number *versus* fluorescence - ΔRN) of 1:10 serial dilutions of the SARS-CoV-2 vRNA, from 5 x 10^7 copies to 5 copies per reaction through the FAM channel. NTC, No Template Control (negative control).



11.3 Analytical Reactivity (Inclusivity) and Analytical Specificity

Inclusivity and cross-reactivity were evaluated by *in silico* analysis against pathogens related to SARS-CoV-2 and normal pathogens that cause infection with similar symptoms, respectively. Upon *in silico* analysis the assay design was found to detect all SARS-CoV-2 virus strains and exhibited no reactivity with non-SARS-CoV-2 species. In addition to *in silico* analysis, SARS-CoV-2 RT-PCR was performed on nucleic acids of common oral and respiratory tract microbes, including *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, *Burkholderia vietnamiensis*, *Dickeya dadantii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Mycobacterium intracellulare*, *Mycobacterium mageritense*, *Mycobacterium smegmatis*, *Nocardia nova*, *Pseudomonas mendocina*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptomyces avermitilis*, *Streptomyces albidoflavus*. None of the pathogens tested by the SARS-CoV-2 (COVID-19) RT-PCR assay generated a detectable amplification signal.

11.4 Precision

Assay precision for the NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD was determined by the repeated testing of SARS-CoV-2 nucleic acids representing two viral load levels, 5 (LoD) and 150 copies per reaction (0.25 and 7.50 copies/µL), individually or spiked into RNA extracted from negative oropharyngeal samples, using 3 different kit lots and following typical testing reaction conditions. Precision was evaluated by measuring Cq average, Cq coefficient of variation and % of replicate detection, as described below for each case. The data is resumed in the table displayed next page.

11.4.1 Repeatability

Repeatability was assessed by one operator through by analysing 48 replicates of each sample (5 and 150 copies per reaction), accounting for a final number of 96 tests performed.

11.4.2 Daily Reproducibility

Daily reproducibility was assessed by one operator through the by analysing 120 replicates of each sample (5 and 150 copies per reaction), for 5 days with 24 replicates of each concentration per day (a total of 240 assays were performed).

11.4.3 Lot-to-lot Reproducibility

Reproducibility between lots was assessed by one operator through the analysis of 270 replicates of each sample (5 and 150 copies per reaction) using 3 different kit lots with 90 replicates per lot.

11.4.4 Operator Reproducibility

Operator reproducibility was assessed by testing 96 replicates of each sample (5 and 150 copies per reaction), by four different operators, with 48 replicates per operator.

Precision of NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD

VARIABLE TESTED		SARS-CoV-2 (COPIES/REACTION)	
		5	**150**
REPEATABILITY	n	48	48
	Mean Cq	34.61	30.82
	Coefficient of Variation (%)	2.99	1.80
	% Replicate Detection	100	100
DAILY REPRODUCIBILITY	n	120	120
	Mean Cq	33.02	30.49
	Coefficient of Variation (%)	4.21	4.95
	% Replicate Detection	100	100
LOT-TO-LOT REPRODUCIBILITY	n	270	270
	Mean Cq	33.41	30.15
	Coefficient of Variation (%)	4.35	4.33
	% Replicate Detection	100	100
OPERATOR REPRODUCIBILITY	n	96	96
	Mean Cq	33.40	30.13
	Coefficient of Variation (%)	4.76	3.73
	% Replicate Detection	100	100
INTER-INSTRUMENT REPRODUCIBILITY	n	72	72
	Mean Cq	33.44	29.98
	Coefficient of Variation (%)	5.24	3.72
	% Replicate Detection	100	100

11.4.5 Inter-instrument Reproducibility

Inter-instrument reproducibility was measured by one operator through the testing 36 replicates of each sample (5 and 150 copies per reaction), in two different qPCR instruments (Applied Biosystems® 7500, Applied Biosystems® StepOnePlus), in a total of 72 tests per sample.

11.5 Clinical evaluation

The performance of NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD with collected respiratory clinical samples was evaluated in two different laboratories. In total, 100 clinical negative and 100 clinical positive samples have been tested. The data revealed that 100% agreement was achieved for all 200 samples tested.

12. Quality Control

All components of NZYtech SARS-CoV-2 One-Step RT-PCR Kit, IVD are tested following the protocols described above. The duplex real-time PCR system allows the detection of targets described for the identification of SARS-CoV-2 viral RNA (RdRp gene) and human mRNA (RNase P gene, RP). Positive amplifications are observed for target genes, positive control and internal controls through FAM and JOE/HEX/VIC channels, according to respective primers/probe set reporter dyes.

13. Technical Support

For Technical support, please contact our dedicated technical support team by Phone: +351 (0) 21 364 35 14 or Email: info@nzytech.com.

14. Trademarks and Disclaimers

All trademarks that appear in this manual are the property of their respective owners.

15. Explanation of Symbols

IVD	*In vitro* diagnostic medical device		Consult instructions for use
REF	Catalogue number		Manufacturer
LOT	Batch code		Use by
	Temperature limitation		Sufficient for
CONTROL +	Positive control		Keep away from the sun light (primer/probe mix)
CONTROL –	Negative control		

16. Conformity Declaration

Product Name: SARS-CoV-2 One-Step RT-PCR Kit, IVD

Catalogue Number: MD03201

Intended use: SARS-CoV-2 qualitative detection.

Classification: Others (not covered by Annex II or not intended to self-testing) according to the EC Directive 98/79/EC.

Manufacturer: NZYtech - Genes & Enzymes,

Estrada do Paço do Lumiar, Campus do Lumiar

Edifício E, R/C,

1649-038, Lisboa

Portugal

We, NZYtech, Lda – Genes & Enzymes, hereby declare that this product, to which this declaration of conformity relates, is in conformity with the following standards and other normative documents ISO 9001:2015 and ISO 13485:2016, following the provisions of the 98/79/EC Directive and of the Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices as transposed into the national laws of the Member States of the European Union.

The product technical file is maintained at NZYtech, Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal.

Joana Brás, PhD

Technical Director

17. References

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