WHITE PAPER

APPLICATION NOTES

Extraction-free RT-PCR Assays for quick and accurate COVID-19 testing

CONTENT

1) ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic has caused increasing challenges for healthcare professionals on a global scale. High-throughput clinical labs processing thousands of samples per day require faster and less-expensive workflows to allow an efficient and reliable screening of SARS-CoV-2 infections. NZYTech's SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N target genes, IVD (MD0483) is a molecular test intended for the qualitative detection of SARS-CoV-2 nucleic acids in nasopharyngeal or oropharyngeal swab samples.

Here, we demonstrate the viability and robustness of a new extraction-free protocol for MD0483 in which detection of SARS-CoV-2 by direct RT-PCR in clinical samples stored in VACUETTE® Virus Stabilization Tube (VST) is not compromised, thus reducing the time-to-result and costs for clinical diagnostics labs.

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2) INTRODUCTION

In December 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). COVID-19 is typically diagnosed by reverse transcription polymerase chain reaction (RT-PCR) to detect viral RNA in patient samples, but RNA extraction step constitutes a major bottleneck to perform diagnostic. The high transmission rate of the virus led to a massive, worldwide need for rapid, cost effective and efficient diagnostic tests. System simplicity increases productivity and speeds-up pipelines and thus protocols to maximize time-to-results and reduce costs for reagents is paramount.

Here, we describe a SARS-CoV-2 RT-PCR protocol to be used with **SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N target Genes, IVD (MD0483)** on heat-inactivated nasopharyngeal swab samples transported and stored in **VACUETTE® Virus Stabilization Tube, Greiner Bio-One (456161)**. RT-PCR results obtained for the RNA extracted samples and for the correspondent direct unextracted samples were compared, demonstrating the reliability and robustness of this extraction-free protocol.

SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD)

Triple target assay SARS-CoV-2, RdRp and N genes, and human RP gene

Positive control to validate assay included

Internal control to confirm extraction / inhibition included

High Sensitivity: Early virus detection and diagnosis, detects as low as 150 copies of viral RNA /mL (LoD) sample (equivalent to 3 copies per reaction)

Precision: Coefficient of variation (CV) < 2%, allowing reproducible test results

High Throughput: Up to 94 clinical samples (96-well plate); up to 382 clinical samples (384-well plate)

High Specificity: Proven by in silico analysis as well as wet lab testing

Flexibility: Assay validated on widely used qPCR instruments including ABI® 7500, ABI® StepOnePlus™, ABI® QuantStudio™ 6, Bio-Rad® CFX96, Qiagen® Rotor-Gene Q, Roche® LC 480 II

Fast Time: Fast Time for Direct RT-PCR: <2h from sample collection to results

Minimal Sample Input: 4 µL direct sample needed

3) OBJECTIVE

A direct RT-PCR protocol was designed and validated specifically for the MD0483 kit to ensure that clinical diagnostics institutions and laboratories who need a faster extraction-free workflow with uncompromised sensitivity and specificity can test accurately and quickly.

This protocol, designed for high-frequency testing, was exclusively validated (IVD) for nasopharyngeal samples transported and stored in the VACUETTE® Virus Stabilization Tube, Greiner Bio-One (456161).

greiner BIO-ONE

VACUETTE® Virus Stabilization Tube, Greiner Bio-One Reference 456161

4) ASSAY & PROCEDURE

4.1) Sample collection and preparation

Nasopharyngeal samples used to validate the direct RT-PCR protocol were collected using Greiner Bio-One swabs (reference CM-FS913) and placed directly into VACUETTE® Virus Stabilization Tube, Greiner Bio-One (reference 456161). Aliquots containing 50 µL of each sample were pipetted and transferred to 96-well PCR plates that were immediately sealed and subjected to a heat treatment step at 95ºC, for 5 minutes, using a thermal cycler with a heated lid. Please notice that this simple heat inactivation step allows eliminating the RNA extraction phase. Experiments were performed with 10 µL SARS-CoV-2 MMix (RdRp & N), 2 µL SARS-CoV-2 PPMix (RdRp & N), 4 µL DEPC treated water (NZYTech, reference MB43701), plus 4 µL of the previously heat-inactivated nasopharyngeal swab samples collected in VACUETTE® Virus Stabilization Tube. RNA of all samples was isolated and purified in parallel using NZY Mag Viral RNA/DNA Isolation Kit, IVD (MD0488). The thermal cycling steps were: 50 ºC for 20 min, 95ºC for 2 minutes and 40 cycles of 95 ºC for 5 seconds and 60 ºC for 30 seconds. All RT-qPCR assays were performed on Applied Biosystem® 7500 FAST real-time PCR machine (Applied Biosystems).

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4.2) Analytical sensitivity

The analytical sensitivity was first evaluated by comparing the Ct values of clinical samples subjected and not subjected to the extraction process. 50 clinical samples previously classified as positive were selected. The RT-PCR assay compared SARS-CoV-2 detection results obtained for the RNA extracted samples and for the correspondent direct unextracted samples, using the protocol described above.

The LoD of the kit was evaluated and confirmed by two different operators, for a total of 48 replicates. Experiment was performed with 10 µL SARS-CoV-2 MMix (RdRp & N), 2 µL SARS-CoV-2 PPMix (RdRp & N), 4 µL DEPC treated water, plus 4 µL negative pool of nasopharyngeal swab samples collected in VACUETTE® Virus Stabilization Tube, spiked with a plasmid DNA that includes RdRp & N target genes corresponding to 3 copies of virus per reaction.

4.3) Clinical evaluation

Nasopharyngeal swab samples were collected in VACUETTE® Virus Stabilization Tube, Greiner Bio-One. Samples were tested directly with the direct RT-PCR protocol and were, in parallel, subject to a prior nucleic acid extraction step followed by RT-PCR. In total, 104 negative clinical samples and 51 positive clinical samples were evaluated in parallel.

5) RESULTS

5.1) SARS-CoV-2 Positive clinical samples – Extraction & RT-PCR versus Direct RT-PCR

Typical amplification plots, observed for clinical samples containing SARS-CoV-2 nucleic acids, are presented in Figure 1. The two scenarios represent examples of clinical samples presenting high, medium and low viral SARS-CoV-2 loads that were subjected to the two protocols - RNA extraction followed by RT-PCR and direct RT-PCR. Data show that SARS-CoV-2 detection is not compromised when using the direct RT-PCR protocol.

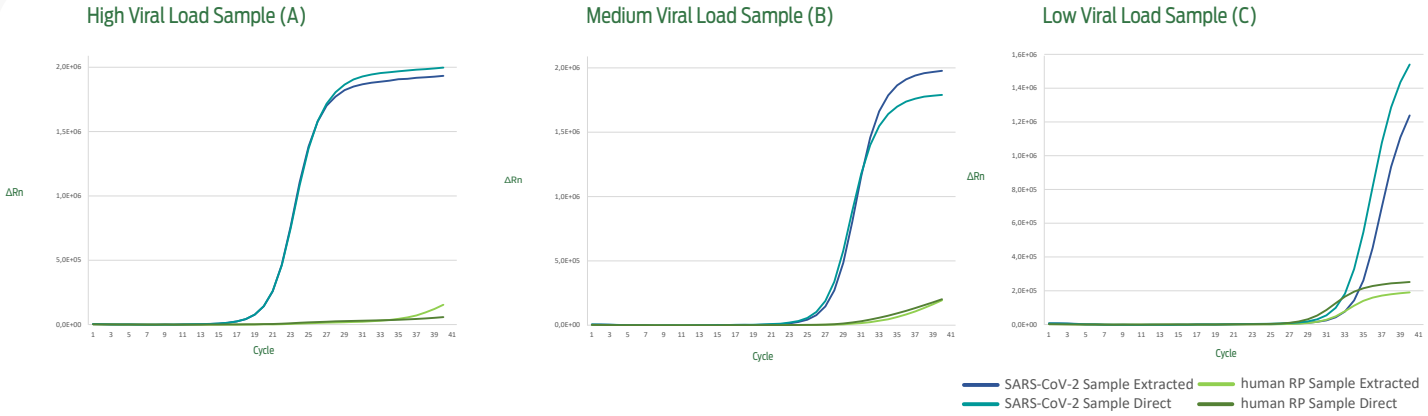

Figure 1. Amplification plot (results for three positive clinical samples containing high (A), medium (B) and low (C) viral SARS-CoV-2 loads. Light blue curves: detection of the SARS-CoV-2 viral RNA targets (RdRp and N genes) through the FAM channel for samples screened using direct RT-PCR protocol. Dark blue curves: detection of the SARS-CoV-2 viral RNA targets (RdRp and N genes) through the FAM channel for samples screened using conventional protocol that includes RNA extraction followed by RT-PCR. Dark green curves detection of the human RNase P gene through the JOE/VIC/HEX channel for samples screened using direct RT-PCR protocol. Light green curves: detection of the human RNase P gene through the JOE/VIC/HEX channel for samples screened using conventional protocol that includes RNA extraction followed by RT-PCR.

5.2) Analytical sensitivity

For the 50 positive clinical samples tested subjected to extraction plus RT-PCR or with the direct RT-PCR protocol the minimum Ct observed was 16.1 and the maximum Ct was 34.9. The results indicate that, for SARS-CoV-2 detection, the mean difference in Cts between the two procedures was 0.1. This result suggests that the LoD does not change wether the sample is subjected or not to a previous RNA extraction step. As such, the LoD of the kit was re-evaluated and confirmed and results confirmed that when using the direct test format, the kit detects 0.15 copies/µL of SARS-CoV-2 viral RNA with ≥95% confidence.

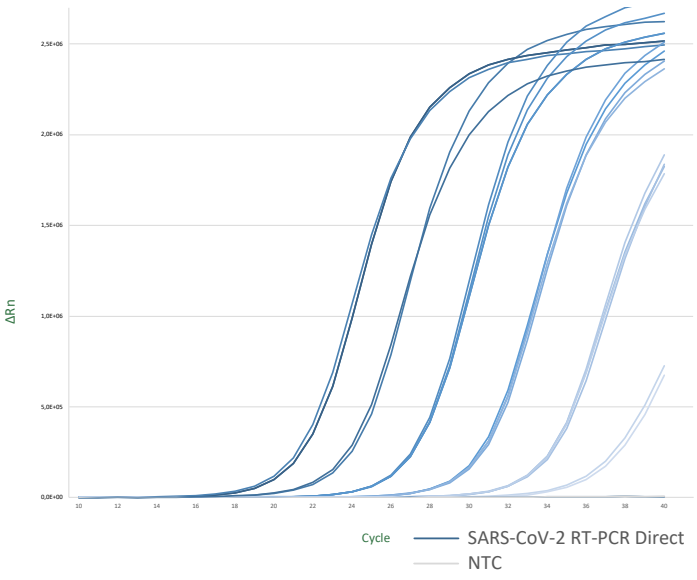

Figure 2. Sensitivity of the SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N target genes, IVD using RT-PCR direct protocol. Amplification plot (cycle number versus fluorescence - **Δ**RN) of 1:10 serial dilutions of spiked SARS-CoV-2 RNA directly in VACUETTE® Virus Stabilization Tube (VST), from 5 x 10^5 copies to 5 copies per reaction through the FAM channel. NTC, No Template Control (negative control).

5.3) Clinical Evaluation

The performance evaluation of the SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N target genes, IVD for the direct RT-PCR protocol, was performed from nasopharyngeal swab samples. In total, 104 negative clinical samples and 51 positive clinical samples were tested. The results revealed a 100% agreement for the positive and negative samples analyzed.

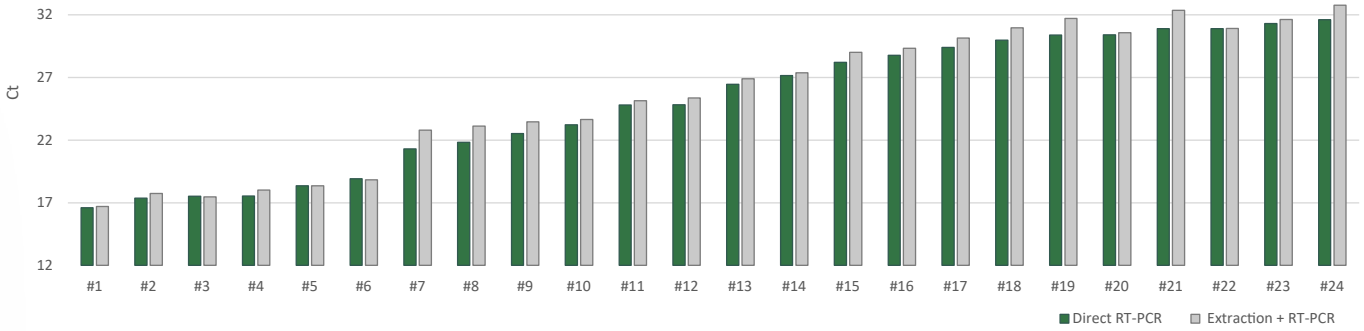

Figure 3. Ct values comparison for standard and direct RT-PCR methods for 24 samples positive for SARS-CoV-2, part of the 51 positive samples tested. Ct values obtained from the RT-PCR experiment in which 24 clinical positive samples were subjected to both extraction methods: RNA extraction plus RT-PCR protocol (grey bars) and direct RT-PCR method (blue bars) from nasopharyngeal swab samples transported and stored in VACUETTE® Virus Stabilization Tube, Greiner Bio-One. Ct values range from 16,7 to 32,8. Data show that SARS-CoV-2 detection is not compromised when using the direct RT-PCR protocol.

6) RESULTS

The direct RT-PCR pipeline for COVID-19 testing using NZYTech's kit SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N target genes, IVD can be implemented by collecting nasopharyngeal swab samples into VACUETTE® Virus Stabilization Tube. The comparison between the extraction-based and the direct RT-PCR protocols showed matching sensitivity and specificity between the two methods. Overall, the RT-PCR direct method will allow massive, fast and cost-effective screening of the population.

> "Confirmation that both specifity and sensitivity of SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N target genes, IVD are not compromised allow us to conclude that this direct RT-PCR protocol, specifically IVD validated for the VACUETTE® Stabilization Tube, can play an important role in rapid diagnosis of SARS-CoV-2."

Joana Brás, PhD
IVD Director
NZYTech

7) REFERENCES

Smyrlaki I, Ekman M, Lentini A, Rufino de Sousa N, Papanicolaou N, Vondracek M, Aarum J, Safari H, Muradrasoli S, Rothfuchs AG, Albert J, Högberg B, Reinius B. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. Nat Commun. 2020 Sep 23;11(1):4812. doi: 10.1038/s41467-020-18611-5. PMID: 32968075; PMCID: PMC7511968.

Greiner Bio-One. Stability Testing of SARS-COV-2, Influenza A and B in VACUETTE® Virus Stabilization Tubes (VST) White Paper.

Taipale, J., Romer, P. & Linnarsson, S. Population-scale testing can suppress
the spread of COVID-19. medRxiv.org, 2020.04.27.20078329 (2020).

8) PRODUCT INFORMATION

Catalogue number	Product name	Number of reactions	SARS--CoV-2	Target Genes	Target Channels	Internal Control (IC)	IC Channel
MD04831	SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N target genes, IVD	96	√	RdRp and N	FAM	Rnase P	JOE/VIC/ HEX
MD04832	SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N target genes, IVD	4 x 96	√	RdRp and N	FAM	Rnase P	JOE/VIC/ HEX

SARS-CoV-2
One-Step RT-PCR Kit,
RdRp and N target genes,
IVD

MD0483