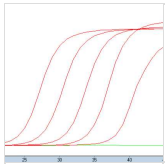

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Modified Zhen et al. SARS-CoV-2 Spike-Gene qRT-PCR assay for highly sensitive detection of the HV69/70 deletion

d.noerz [1]

[1]University Medical Center Hamburg-Eppendorf

1 *Works for me* This protocol is published without a DOI.

Coronavirus Method Development Community

d.noerz

SUBMIT TO PLOS ONE

ABSTRACT

The SARS-CoV-2 B.1.1.7 lineage (British variant) features a number of hallmark mutations, which can be used for screening by conventional Taqman RT-PCR. A recently published highly sensitive diagnostic assay by Zhen et al. (J Mol Diagn, 2020) is coincidentally well suited to detect the HV69/70 deletion as its probe is located right on top of the mutation. In a sample with B.1.1.7 lineage, the assay returns a completely flat amplification curve, representing an assay drop-out phenomenon similar to what can be observed with the Thermo Fischer Taqpath test. We created a second Taqman prove (Probe-2) using locked nucleic acid technology to specifically target the same region including the deletion. In this way, the assay can be used (i.e. as part of a typing panel or multiplex) to detect the HV69/70-deletion in order to screen for different SARS-CoV-2 variants.

PROTOCOL CITATION

d.noerz 2021. Modified Zhen et al. SARS-CoV-2 Spike-Gene qRT-PCR assay for highly sensitive detection of the HV69/70 deletion. **protocols.io** https://protocols.io/view/modified-zhen-et-al-sars-cov-2-spike-gene-qrt-pcr-bseynbfw

KEYWORDS

SARS-CoV-2, Spike, HV69/70, RT-PCR

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47288

1 Modified Primer/Probe set based on Zhen et al., (J Mol Diagn, 2020): (5' - 3')

A	B
S-gene fwd	TCAACTCAGGACTTGTTCTTAC
S-gene rev	TGGTAGGACAGGGTTATCAAAC
S-gene P-1	FAM-TGGTCCCAGAGACATGTATAGCAT-BHQ1
S-gene P-2 (new)	Yak-TGGTCCCAG(+A)(+G)AT(+A)GC(+A)T-BHQ1

Yak, YakimaYellow. +X, locked nucleic acid. Primers and Probes were custom made and ordered from TIB MOL (Berlin, Germany) and Ella Biotech (Martinsried, Germany)

2 Prepare a 4x primer/probe stock (500µL, for 100 reactions)

Oligo	stock conc. [µM]	final conc. [nM]	add volume to stock
S-gene fwd	100	400	8
S-gene rev	100	400	8
S-gene P-1	100	100	2
S-gene P-2	100	100	2
PCR-grade water	-	-	482

3 Prepare RT-qPCR reaction-mix (100 reactions, final volume 1500µL). For this example, "RNA process control kit" (Roche) is used as one-step RT-PCR master.

Reagent	add volume [µL]
5x Roche one-step RNA process control master	400
200x Roche one-step RNA process control RT-Enzyme	10
4x primer/probe stock	500
PCR-grade water	590

4 Add 5µL of sample RNA extract to 15µL of RT-qPCR reaction-mix per well in a 96-well PCR-plate. A z480 analyzer (Roche) was used to run the reaction according to the following protocol:

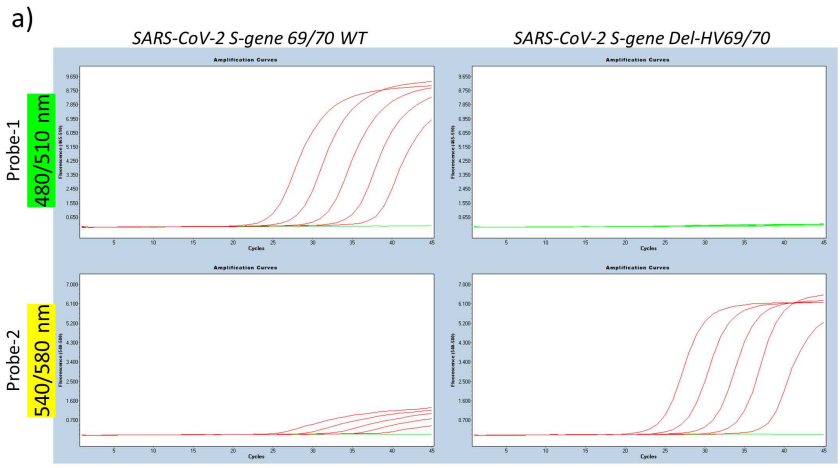
Step	temperature	duration	cycles
RT	50°C	30 minutes	
Denature	95°C	15 minutes	
Cycle - anneal	60°C	30 seconds	|
Cycle - denature	95°C	5 seconds	45

5 Possible outcomes:

Channel (Probe)	HV69/70 WT (non-B.1.1.7)	Del-HV69/70 (B.1.1.7 and others)
FAM (Probe-1)	strong signal	negative
YAK (Probe-2)	weak signal	strong signal

Exemplary amplification curves and results of a clinical sample set using the protocol described above:

a)



b)

Samples	Probe-1 (original design)	Probe-2 (Del-HV69/70)	Total
SARS-CoV-2 positive (HV69/70 WT)	42/42	0*/42	42
SARS-CoV-2 positive (Del-HV69/70)	0/6	6/6	6
Negative	0/48	0/48	48
Total			96

* Weak signals of Probe-2 in wild type strains are eliminated by fit-points analysis, using a del-HV69/70 positive control as reference for signal levels.

a) Amplification curves as displayed by the LightCycler480 software. A 10-fold dilution series of SARS-CoV-2 strain HH-1 (HV69/70 wild type) and a clinical isolate positive for del-HV69/70 (B.1.1.7) was prepared and subjected to testing with the dual-probe assay. Signals of Probe-1 (original by Zhen et al.) can be detected in the 480/510-channel (label: FAM-BHQ1). Signals of Probe-2 (modified for del-HV69/70) can be detected in the 540/580-channel (label: Yak-BHQ1). Probe-2 generates weak signals in wild type samples, which can be manually eliminated by fit-points analysis and a positive control.
b) A total of 96 clinical samples were subjected to the dual-probe assays. 48 samples were positive for SARS-CoV-2 (predetermined by cobas SARS-CoV-2 IVD assay or inhouse methods), 6 of which were of the B.1.1.7 lineage and harbouring a del-HV69/70 mutation. The dual-probe assay correctly identified all clinical samples. Notably, the 6 B.1.1.7 samples would have been missed by the original assay (including only Probe-1).

Detection of the HV69/70 is not sufficient to confirm SARS-CoV-2 B.1.1.7 lineage in clinical samples. Screening for N501Y and P681H SNPs can be used to increase confidence prior to NGS.