One-step RT-PCR Ins214EPE assay for Omicron (B.1.1.529) variant detection V.1

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On 26 November 2021 WHO designated a new variant of concern B.1.1.529 named Omicron. This variant has a large number of mutations, some of which are concerning. Preliminary evidence suggests an increased risk of reinfection with this variant and reduced neutralization by convalescent and vaccinated sera, as compared to other VOCs. Implementation of the high-throughput rRT-PCR screening for Omicron is of great importance for monitoring the spread of this VOC in the population, especially in resource-limited countries lacking sufficient sequencing capacity.

Omicron lineage B.1.1.529 (BA.1) has some indels that turned out to be a good target for its detection. In the current protocol, we use ins214EPE for this purpose. Here we describe the 1-step quantitative multiplex RT-qPCR assay consisting of the newly developed Ins214EPE detection set and widely used Hong Kong University N gene assay for SARS-CoV-2 detection (Chu et al., 2020).

Alignment of the ins214EPE primers and probe to the B.1.1.529 (BA.1) lineage sequence

The assay was validated on the Omicron variant RNA kindly provided by the Pathogenic Microorganisms Variability Laboratory (Dr. Vladimir Guschin, Gamaleya Institute, Moscow, Russia) and RNA from the collection of Smorodintsev Research Institute of Influenza.

Virus name	GISAID Isolate ID
hCoV-19/Russia/MOW-Moscow_PMVL-O11/2021	EPI_ISL_7263932
hCoV-19/Russia/MOW-Moscow_PMVL-O16/2021	EPI_ISL_7263933
hCoV-19/Russia/SPE-RII-MH44356S/2021	EPI_ISL_7717296

Virus RNA specimens used for rRT-PCR ins214EPE assay validation

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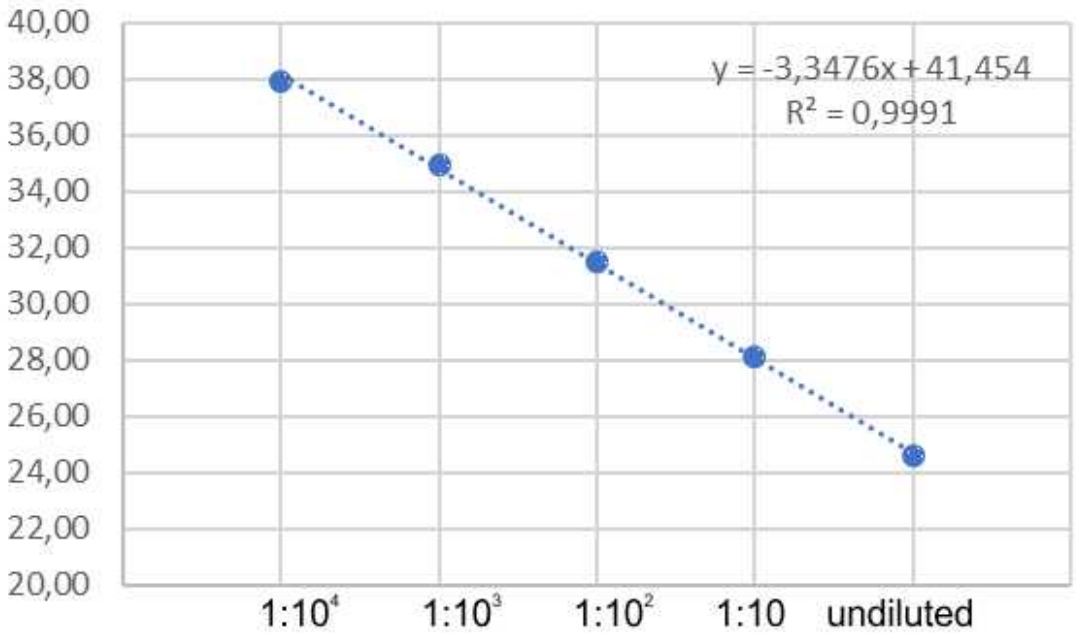
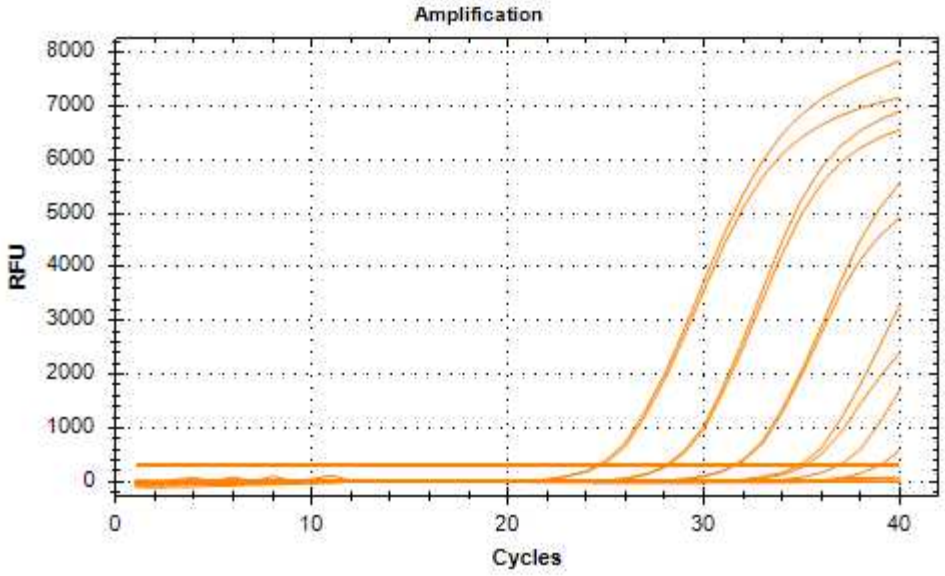
Omicron RNA specimens were positive in the assay as expected. Negative controls were found negative. 10-fold serial dilutions of Omicron RNA were used to assess ins214EPE assay amplification efficiency. The amplification efficiency was 98,9% (R^2 = 0,99).



Amplification efficiency of ins214EPE assay



ins214EPE assay amplification curves on omicron RNA serial dilutions

The developed rRT-PCR assay demonstrates high specificity. It was tested on 26 clinical samples (RNA extracted from oropharyngeal swabs) with previously

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characterized viruses belonging to 8 different SARS-CoV-2 lineages (including Delta B.1.617.2+AY.*) Specific signal was detected only in samples with SARS-CoV-2 Omicron lineage RNA (confirmed by whole-genome sequencing). Specificity was additionally tested on clinical samples positive for other respiratory viruses from the collection of Smorodintsev Research Institute of Influenza - influenza, parainfluenza, human seasonal coronaviruses (OC43, NL63, 229E, HKU1), hRSV, rhinoviruses, bocaviruses, metapneumovirus (33 in total) - with no false-positive results.

PANGO lineage	HKU SARS-CoV-2 N gene assay, Ct value	ins214EPE assay, Ct value
B.1.1.7	15,30	-
B.1.351	16,08	-
P.1	16,15	-
B.1.617.2	16,70	-
B.1	17,47	-
AT.1	18,36	-
B.1.617.2	21,87	-
B.1.1.7	22,49	-
B.1.617.2	24,79	-
B.1.617.2	26,16	-
B.1.1.7	26,24	-
B.1.1.529	26,24	26,29
B.1.617.2	28,06	-
B.1.1.7	28,54	-
B.1.1.529	28,84	28,72
AT.1	29,65	-
AY.122	31,18	-
AY.122	31,67	-
AY.122	33,51	-
B.1.1.7	33,63	-
AT.1	33,80	-
AT.1	34,01	-
AT.1	35,02	-
B.1.1.7	35,60	-
B.1.351	36,33	-
B.1.617.2	37,30	-

Analytical specificity of the assay was checked on 26 samples of 8 different SARS-CoV-2 lineages

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clinical samples positive for	RP assay, Ct value	HKU SARS-CoV-2 N gene assay, Ct value	ins214EPE assay, Ct value
hRV	32,85		
hRV	33,76		
hRV	27,75		
hRSV B	31,49		
hRSV B	30,98		
hRSV B	32,33		
hRSV A	28,76		
hRSV A	30,56		
hRSV A	27,70		
hPIV4	23,90		
hPIV3	27,01		
hPIV2	24,72		
hPIV1	28,63		
hCoV OC43	30,34		
hCoV OC43	30,69		
hCoV OC43	28,64		
hCoV NL63	32,20		
hCoV NL63	30,42		
hCoV NL63	24,95		
hMPV	30,12		
hCoV HKU1	30,06		
hCoV HKU1	28,30		
hCoV HKU1	30,73		
Human influenza A (H3N2)	28,16	39,06	
Human influenza A (H3N2)	29,13		
Human influenza A (H3N2)	28,45		
SARS-CoV-2 B.1.1.529	34,15	26,61	28,44
hBoV	32,26		
hBoV	30,75		
hBoV	27,25		
hAdV	29,47		
hCoV 229E	29,11		
hCoV 229E	32,52		
hCoV 229E	29,37		

Analytical specificity of the assay was checked on 33 clinical samples positive for other respiratory viruses. RP assay (human RNAse P assay) developed by US CDC was used to check the presence of human RNA in clinical samples

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Analytical sensitivity determination is underway.

We consider developed assay to be useful in wide populational RT-PCR screening to assess the spread of Omicron variant.

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SARS-CoV-2 RT-qPCR, Omicron lineage detection, VOC detection, 215EPEins, Ins214EPE

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https://www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf

1 Order oligonucleotides with following sequences 5'->3':

A	B
Ins214EPE F	ATATTCTAAGCACACGCCTATT
Ins214EPE R	GGCAAATCTACCAATGGTTCTA
Ins214EPE P	ROX-TGCGTGAGCCAGAAGATCTCCCT-BHQ-2
HKU-NF	TAATCAGACAAGGAACTGATTA
HKU-NR	CGAAGGTGTGACTTCCATG
HKU-NP	FAM-GCAAATTGTGCAATTTGCGG-BHQ

Ins214EPE oligonucleotide set developed in Smorodintsev Research Institute of Influenza (St.Petersburg,

Russia) tfor specific detection of B.1.1.529 (Omicron) lineage
HKU oligolucleotide set was developed by the University of Hong Kong to detect SARS-CoV-2 in human clinical specimens (Chou et al., 2020)
Please, change the dye if you use ROX as passive reference dye

2 Briefly vortex and centrifuge reagents before use.
Prepare the PCR reaction mixture following the specifications below:

A	B
2X RT-qPCR Buffer	12.5 µl
25X RT-qPCR Enzyme Mix	1 µl
Ins214EPE F	400 nM
Ins214EPE P	400 nM
Ins214EPE R	400 nM
HKU-NF	400 nM
HKU-NR	400 nM
HKU-NP	400 nM
RNA template	5 µl
Nuclease-free water	to 25 µl

We used Biomaster qRT-PCR mix (Biolabmix, Russia)

A

3 Perform the amplification in a qPCR thermocycler with appropriate temperature profile:

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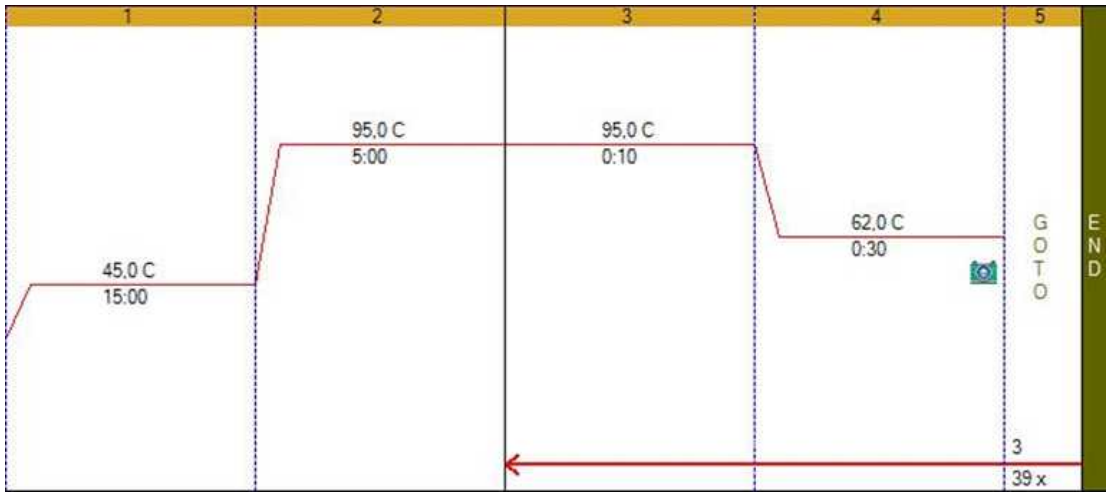
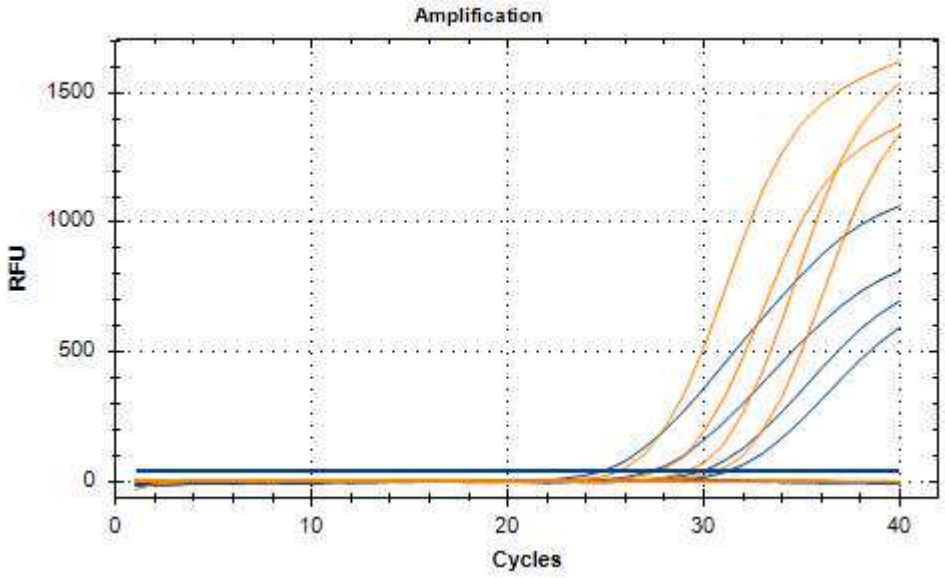


Read a plate at the annealing and elongation step in FAM and ROX channels. Developed rRT-PCR assay was validated for Bio-Rad CFX96, but is believed to work well at any device.

4 How to interpret the results: detection of fluorescence of FAM probe (HKU-NP) means thepresence of SARS-CoV-2 RNA in the sample, detection of fluorescence in ROX channel (Ins214EPE probe) means the presence of B.1.1.529 (BA.1)(omicron) RNA.



Amplification curves (HKU SARS-CoV-2 N-gene assay - blue, ins214EPE assay - orange)

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