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Multiplex SARS-CoV-2 RT-qPCR protocol

In 1 collection

Peter H L Krijger¹, Tim A Hoek¹, Sanne Boersma¹, Lieke I P M Donders², Maaïke M C Broeders², Mark Pieterse¹, Pim W Toonen¹, Ive Logister¹, Bram M P Verhagen¹, Marjon J A M Verstegen¹, Thomas W van Ravesteyn¹, Rene J T M Roymans², Francesca Mattioli¹, Jo Vandesompele^{3,4}, Monique Nijhuis⁵, Stefan Meijer⁶, Anton van Weert⁷, Edwin Dekker⁸, Fred J Dom⁹, Rob Ruijtenbeek¹⁰, Lieven B J van der Velden², Jeroen H B van de Bovenkamp², Martijn Bosch¹⁰, Wouter de Laat¹, Marvin E Tanenbaum¹

¹Onco Institute, Hubrecht Institute–KNAW and University Medical Center Utrecht, Utrecht, the Netherlands;

²Laboratory of Medical Microbiology, Stichting PAMM, Veldhoven, the Netherlands.; ³InActiv Blue, Beernem, Belgium;

⁴Biogazelle, Zwijnaarde, Belgium.; ⁵Dept of Medical Microbiology, University Medical Center, Utrecht, the Netherlands.;

⁶Tecan labwerx, Tecan Trading AG, Männedorf, Switzerland.;

⁷National Screening Laboratory Sanquin, Sanquin Diagnostics, Amsterdam, the Netherlands.;

⁸Bodegro B.V., Breda, the Netherlands.; ⁹HiFiBio Therapeutics, Saint-Jacques, France.;

¹⁰Department of Laboratory Automation, Genmab B.V., Utrecht, the Netherlands.

1 Works for me

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 Peter Krijger

DISCLAIMER

J.V. is co-founder and CSO of InActiv Blue; S.M. is an employee of Tecan Trading AG; L.M.P.D., M.M.C.B., R.J.T.M.R., L.B.J.V. and J.H.B.B. are employees of Stichting PAMM Veldhoven; A.vW. is an employee of Sanquin; E.D. is a founder and employee of Bodegro; and F.J.D. is CEO and co-founder HiFiBio France.

ABSTRACT

An in-house multiplex RT-qPCR, targeting SARS-CoV-2 and PDV as internal control [1][2], developed on QuantStudio 7 Pro Real-Time PCR Systems using Life Technologies Taqman FastVirus 1-step mastermix with E-gene primers and probe as described by Corman *et al.* and N1 primers and probes as described by the CDC[3, 4].

1. Clancy, A. *et al.*, *The development of a qualitative real-time RT-PCR assay for the detection of hepatitis C virus* European Journal Microbial Infectious Diseases, 2008. **27**(12): p. 1177.
2. Wolters, F., *et al.*, *Multi-center evaluation of cepheid xpert® xpress SARS-CoV-2 point-of-care test during the SARS-CoV-2 pandemic*. Journal of Clinical Virology, 2020. 128: p. 104426
3. Corman, V.M., *et al.*, *Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR*. Euro Surveill, 2020. **25**(3).
4. Lu, X., *et al.*, *US CDC Real-Time Reverse Transcription PCR Panel for Detection of Severe Acute Respiratory Syndrome Coronavirus 2*. Emerging Infectious Disease journal, 2020. **26**(8): p. 1654.

PROTOCOL CITATION

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COLLECTIONS ①

 **STRIP: Systematic Testing using Robotics and Innovation during Pandemics**

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PARENT PROTOCOLS

In steps of

[Step 2: RNA extraction and RT-qPCR](#)

Part of collection

[STRIP: Systematic Testing using Robotics and Innovation during Pandemics](#)

MATERIALS TEXT

4x 1-step Fast viral mastermix ThermoFisher #4444434
Nuclease-Free Water, ThermoFisher, #AM9932.

Primers/probes:

E_Sarbeco_F1: 5'-ACA GGT ACG TTA ATA GTT AAT AGC GT 3'
E_Sarbeco_R2 :5'-ATA TTG CAG CAG TAC GCA CAC A 3'
E_Sarbeco_P1: 5'-FAM-ACA CTA GCC ATC CTT ACT GCG CTT CG -BHQ1-3'
2019-nCoV_N1-F1:5'-GAC CCC AAA ATC AGC GAA AT-3'
2019-nCoV_N1-R1:5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'
2019-nCoV_N1-P: 5'-YY-ACC CCG CAT TAC GTT TGG TGG ACC -BHQ1-3'
tm PDV F1: 5'-CGGGTGCCTTTTACAAGAAC-3'
tm PDV R1: 5'-TTCTTTCCTCAACCTCGTCC-3'
tm PDV P:5'-Cy5-ATGCAAGGGCCAATTCTTCCAAGTT-BHQ2-3'

microAmp optical 384 reaction plate with barcode, ThermoFisher, #4309849.

Quantstudio 7 thermocycler

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- 1 Reconstitute the oligos and probes to 100uM with nuclease-free water, vortex briefly and prepare the primer/probe mix:

A	B	C
100uM stock	Volume (1 reaction)	Volume (1000 reactions)
tm PDV F1 (100 nM/reaction)	0.015 µl	15 µl
tm PDV R1 (100 nM/reaction)	0.015 µl	15 µl
tm PDV P (100 nM/reaction)	0.015 µl	15 µl
2019-nCoV_N1-F1 (600 nM/reaction)	0.09 µl	90 µl
2019-nCoV_N1-R1 (600 nM/reaction)	0.09 µl	90 µl
2019-nCoV_N1-P (200 nM/reaction)	0.03 µl	30 µl
E_Sarbeco_F1 (400 nM/reaction)	0.06 µl	60 µl
E_Sarbeco_R2 (400 nM/reaction)	0.06 µl	60 µl
E_Sarbeco_P1 (200 nM/reaction)	0.03 µl	30 µl
Nuclease-free water	0.345 µl	345 µl
Total	0.75 µl	750 µl

Note: The primer/probe mix can be prepared in advance, aliquoted in microcentrifuge tubes, and stored at -20°C for 1 year.

2 Prepare the RT-qPCR master mix:

A	B
Reagents	Volume (1 reaction)
4x TaqMan Fast Virus 1-Step Master Mix	3.75 µl
primer/probe mix	0.75 µl
Nuclease-free water	3.0 µl
RNA	7.5 µl
Total	15 µl

3 Run the RT-qPCR program:

A	B	C
	Time (s)	Temp (°C)
1x	300	55
1x	20	95
40x	3	95
	30	58

RT-qPCR program Quantstudio 7 thermocycler