Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Infection 83 (2021) e19–e21

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letter to the Editor

Development of a multiplex RT-qPCR using the drop out strategy to screen the SARS-CoV-2 South African 501Y.V2 variant

Emergence of SARS-CoV-2 variants may have implications for virus transmissibility and immune response, as well as for the vaccination strategy, creating the need to rapidly screen for the presence of variants in COVID-19 samples([1,2]). Among them, much attention has been paid to the 501Y.V1 (also named B.1.1.7), 501Y.V2 and 501Y.V3 (also named P1) variants detected in UK, South Africa and Brazil, respectively([3,4,5]). High throughput sequencing (HTS) technologies represent the gold standard for variantsidentification, but they are resource intensive, require expertise and have a long turnaround time(i.e. several days or weeks).

It is therefore essential to combine the routine genomic surveillance of SARS-CoV-2 variants with targeted methods such as RT-PCR that are simpler, more rapid and less expensive to implement.Recent PCR-based protocols targeted different mutant regions within the SARS-CoV-2 genome, in particular ORF1 and Spike (S) genes. Common mutations (e.g. Δ3675–3677 in ORF1a gene; N501Y in S gene) were used to detect all three variants of interest, without distinction([6,7,8]). Specific detection of some SARS-CoV-2 variants were also investigated, using unique mutations or deletions (e.g. Δ69/70 HV for UK variant) ([8]), but so far no protocol has been specifically developed for the South African variant.

To differentiate between501Y.V2and the two other main variants (501Y.V1 and 501Y.V3),literatureexamination and nucleotide alignment of the SARS-CoV-2 reference towardsthe three main variantsgenomes allowedus to design primers flanking the specific Δ242–244 LLA deletion from the 501Y.V2 spike gene, and a probe that covered the deletion.This strategy, also named "drop-out",allowes for amplification and detects all SARS-CoV-2 viruses that do not contain the deletion, while the South African 501 V.V2 variant is not amplified. A multiplex RT-qPCR was thus developed to detect four targets: i) the N1/N2 genes of SARS-CoV-2; ii) the drop-out approach for 501 V.V2 variant (Δ242–244 LLA deletion); iii) the drop out approach for 501 V.V1 variant (Δ69/70 HV deletion) developed in[8]b); and iv) RNA quality control using Rnase P gene (Table 1).

The multiplex assay was tested on fifty-eight SARS-CoV-2 samples: twenty samples belonging to a variety of strains without the Δ242–244 LLA andΔ69/70 HV deletions), twenty samples belonging to 501Y.V1 lineage(Δ69/70 HV deletion), eight samples belonging to the 501Y.V3 lineage, and ten samples belonging to 501Y.V2 lineage (Δ242–244 LLA deletion). All samples were nasal or nasopharyngeal swabs, and nucleic acid was extracted from 300 µL viral transport medium and eluted in 50µL using the Maxwell® RSC Viral TNA kit (Promega, USA). SARS-CoV-2 lineage was firstly determined by Whole Genome Sequencing using Minion (Oxford Nanopore Technologies, USA)([9]). The multiplex RT-qPCR was performed in duplicates using the TaqMan™ Fast Virus 1-Step Master Mix (Applied Biosystems – Life Technologies, USA) with 400 nM of each primer and 200 nM of each probe per reaction, and 5 µL of RNA in a total reaction volume of 20 µL. PCR cycler conditions were reverse transcription for 10 min at 55 °C, initial denaturation for 1 min at 95 °C, followed by 45 cycles of 10 s at 95 °C and 1min15 at 55 °C, and a final cooling of 30 s at 40 °C on the LightCycler480 (Roche, USA).

The results showed that our 242/244 del "drop-out" primer/probe set can serve as a screening tool to specifically

Table 1
Primer and probe sequences tested for the proposed SARS-CoV-2 multiplex detection (N1/N2, 501 V.V1 and 501 V.V2 variants, RNase P control).

Set name	Nt positions	TM	Primers/probe	Sequence
CDC	28,287–28,306	53.6	Forward primer	GAC CCC AAA ATC AGC GAA AT
N1	28,335–28,358	57.7	Reverse primer	TCT GGT TAC TGC CAG TTG AAT CTG
	28,309–28,332	63.3	Probe	**FAM**-ACC CCG CAT TAC GTT TGG ACC-**BHQ1**
CDC	29,164–29,183	52.3	Forward primer	TTACAA ACA TTG GCC GCA AA
N2	29,213–29,230	54.1	Reverse primer	GCG CGA CAT TCC GAA
	29,188–29,210	63.2	Probe	**FAM**-ACA ATT TGC CCC CAG CGC TTC AG-**BHQ1**
Liege	22,239–22,261	55.2	Forward primer	TGG TAG ATT TGC CAA TAG GTA TT
242/244del	22,365–22,387	55.1	Reverse primer	AAA AGT CCT AGG TTG AAG ATA AC
	22,274–22,298	53.5	Probe	**ROX**-TTT CAA ACT TTA CTT GCT TTA CAT A-**BHQ2**
Yale	21,710–21,733	59.3	Forward primer	TCA ACT CAG GAC TTG TTC TTA CCT
69/70del	21,795–21,817	57.4	Reverse primer	TGG TAG GAC AGG GTT ATC AAA C
	21,755–21,779	61.2	Probe	**Cy5**-TTC CATG CTA TAC ATG TCT CTG GGA-**BHQ2**
Rnase		56.5	Forward primer	AGA TTT GGA CCT GCG AGC G
P		59.5	Reverse primer	GAG CGG CTG TCT CCA CAA GT
		63.5	Probe	**HEX**-TTC TGA CCT GAA GGC TCT GCG CG-**BHQ1**

https://doi.org/10.1016/j.jinf.2021.04.035

Table 2
Validation results of the multiplexed 501 V.V2 variant screening RT-qPCR assay on different SARS-Cov-2 lineages. Cycle threshold values are indicated for the four targets: N1/N2 genes, drop-out 242/244 deletion, drop-out 69/70 deletion and the RNase P control. No detection was indicated by "ND".

Samples ID	SARS-CoV-2 Lineages	Detection (Ct values) N1/N2	Liege 242/244del	Yale 67/70del	RNase P
Liege-1	B.1.351 501 V.V2	7.78	ND	10.81	31.45
Liege-2		11.20	ND	14.87	28.82
Liege-3	South African variant	12.70	ND	14.28	29.76
Liege-4		15.31	ND	18.17	26.42
Liege-5		15.36	ND	19.77	25.33
Liege-6		16.83	ND	18.08	27.18
Liege-7		17.42	ND	18.78	28.34
Liege-8		17.78	ND	19.16	28.11
Liege-9		22.50	ND	24.87	29.02
Liege-10		22.86	ND	26.23	23.45
Liege-11	P1 501 V.V3	9.17	14.77	12.45	32.14
Liege-12	Brazilian variant	9.73	15.22	12.99	32.07
Liege-13		13.43	18.73	17.21	29.1
Liege-14		14.17	18.7	16.88	31.58
Liege-15		14.23	18.59	16.99	27.93
Liege-16		14.55	20.89	18.63	27.17
Liege-17		17.23	22.37	20.49	25.93
Liege-18		18.8	22.05	20.36	31.93
Liege-19	B.1.1.7 501.V1	11.69	15.95	ND	34.13
Liege-20	UK variant	12.16	15.99	ND	30.79
Liege-21		12.45	17.35	ND	30.61
Liege-22		13.53	18.88	ND	22.64
Liege-23		17.27	19.76	ND	32.81
Liege-24		17.46	20.80	ND	31.44
Liege-25		17.60	21.38	ND	26.61
Liege-26		17.88	20.76	ND	32.63
Liege-27		18.16	20.51	ND	33.64
Liege-28		18.96	23.15	ND	25.79
Liege-29		19.27	22.56	ND	33.81
Liege-30		19.76	21.72	ND	28.32
Liege-31		20.37	23.92	ND	31.79
Liege-32		20.58	25.41	ND	29.82
Liege-33		21.73	23.53	ND	32.87
Liege-34		22.31	26.11	ND	27.43
Liege-35		23.01	27.22	ND	27.32
Liege-36		23.60	27.62	ND	24.54
Liege-37		23.84	26.98	ND	26.27
Liege-38		29.16	33.18	ND	29.60
Liege-39	B.1.221	10.87	13.84	12.64	26.47
Liege-40	B.1.221	12.66	15.79	14.49	32.66
Liege-41	B.1.221	12.82	16.26	15.27	30.33
Liege-42	B.1.221	13.57	16.33	14.97	26.81
Liege-43	R.1	13.74	16.95	15.43	31.34
Liege-44	B.1.214.2	14.18	19.16	16.73	27.58
Liege-45	B.1.221	15.19	19.06	16.53	34.03
Liege-46	B.1.221	18.69	21.22	18.81	30.62
Liege-47	B.1.221	18.75	21.59	18.87	28.73
Liege-48	B.1.221	19.41	21.95	20.54	33.49
Liege-49	B.1.221	19.75	22.59	21.55	28.8
Liege-50	B.1.221	21.29	24.98	22.58	28.77
Liege-51	B.1.160	21.8	24.17	22.51	29.61
Liege-52	B.1.221	22.51	26.66	24.53	27.41
Liege-53	B.1.177	22.98	27.25	24.57	31.07
Liege-54	B.1.221	24.93	27.67	26.44	25.68
Liege-55	B.1	25.05	29.84	28.51	27.44
Liege-56	B.1.221	25.55	28.6	26.82	24.72
Liege-57	B.1.177	27.55	32.21	27.35	21.38
Liege-58	B.1.221	31.25	35.92	32.65	35.65

detect viruses with the Δ242–244 deletion, in multiplex with the detection of the UK 501 V.V1 variant and the standard detection of SARS-CoV-2 N1/N2 genes proposed by the centre for Disease Control and Prevention (CDC) (Table 2). No amplification was observed for any 501 V.V2 samples, while all other SARS-CoV-2 virusesincluding 501 V.V1 and 501 V.V3 samples were amplified. Concerningsensitivity of this multiplex RT-qPCR,fifty-eight infected samples with Cycle threshold (Ct) values ranging from 7.78 to 31.25 for the N1/N2 genes were tested and they were properly detected by the 242/244del primer/probe set with a mean delay of 3.78Ct values compared to the standard N1/N2 genes detection (see Table 2).Sensitivity limit of themethod was also assessed and samples were properly amplified down to 100 viral copies per ml. Testing samples below this threshold could lead to false drop-out results.Moreover, this method could potentially detect other viruses with the Δ242/244 LLA deletion.

Our multiplex RT-qPCRcan thus serve as a screening assay to prioritize samples presenting the Δ242/244 LLA deletion for sequencing and to confirm their identification as the emerging 501 V.V2 lineage.

Declaration of Competing Interest

None

References

1. Davies NG, Barnard RC, Jarvis CI, Kucharski AJ, Munday J, Pearson CAB, Russell TW, Tully DC, Abbott S, Gimma A, Waites W, Wong KLM, van Zandvoort K, Eggo RM, Funk S, Jit M, Atkins KE, Edmunds WJ, Houben R, Meakin SR, Quilty BJ, Liu Y, Flasche S, Lei J, Sun FY, Krauer F, Lowe R, Bosse NI, Nightingale ES, Sherratt K, Abbas K, O'Reilly K, Gibbs HP, Villabona-Arenas CJ, Waterlow NR, Medley G, Brady O, Williams J, Rosello A, Klepac P, Koltai M, Sandmann FG, Foss AM, Jafari Y, Prem K, Chan YWD, Hellewell J, Procter SR, Jombart T, Knight GM, Endo A, Quaife M, Showering A, Clifford S. Estimated transmissibility and severity of novel SARS-CoV-2 Variant of Concern 202012/01 in England. *medRxiv* 2020. doi:10.1101/2020.12.24.20248822.
2. Lauring AS, Hodcroft EB. Genetic Variants of SARS-CoV-2 - What Do They Mean? *JAMA - J. Am. Med. Assoc.* 2021. doi:10.1001/jama.2020.27124.
3. Tang JW, Tambyah PA, Hui DS. Emergence of a new SARS-CoV-2 variant in the UK. *J. Infect.* 2021. doi:10.1016/j.jinf.2020.12.024.
4. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, Doolabh D, Pillay S, San EJ, Msomi N, Mlisana K, von Gottberg A, Walaza S, Allam M, Ismail A, Mohale T, Glass AJ, Engelbrecht S, van Zyl G, Preiser W, Petruccione F, Sigal A, Hardie D, Marais G, Hsiao M, Korsman S, Davies MA, Tyers L, Mudau I, York D, Maslo C, Goedhals D, Abrahams S, Laguda-Akingba O, Alisoltani-Dehkordi A, Godzik A, Wibmer CK, Sewell BT, Lourenço J, Alcantara LCJ, Kosakovsky Pond SL, Weaver S, Martin D, Lessells RJ, Bhiman JN, Williamson C, de Oliveira T. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *medRxiv* 2020. doi:10.1101/2020.12.21.20248640.
5. Voloch CM, da Silva Francisco R, de Almeida LGP, Cardoso CC, Brustolini OJ, Gerber AL, Guimarães APde C, Mariani D, da Costa RM, Ferreira OC, Frauches TS, de Mello CMB, Leitão Ide C, Galliez RM, Faffe DS, Castiñeiras TMPP, Tanuri A, de Vasconcelos ATR. Genomic characterization of a novel SARS-CoV-2 lineage from Rio de Janeiro, Brazil. *J. Virol.* 2021 </bib>. doi:10.1128/JVI.00119-21.
6. Perchetti GA, Zhu H, Mills MG, Shrestha L, Mohamed Bakhash S, Lin M, Xie H, Huang ML, Bedford T, Jerome KR, Greninger AL. 2021. Specific allelic discrimination of N501Y and other SARS-CoV-2 mutations by ddPCR 1 detects B.1.1.7 lineage in Washington State 2 3 Running Title: ddPCR assay for SARS-CoV-2 N501Y mutation 32. *medRxiv* 2021;**03** 10.21253321. doi:10.1101/2021.03.10.21253321.
7. Vogels CB, Breban MI, Alpert T, Petrone ME, Watkins AE, Ott IM, Goes de Jesus J, Morales Claro I, Magalhães Ferreira G, Crispim MA, CADDE Genomic Network B-U, Singh L, Tegally H, Anyaneji UJ, Hodcroft EB, Mason CE, Khullar G, Metti J, Dudley JT, MacKay MJ, Nash M, Wang J, Liu C, Hui P, Murphy S, Neal C, Laszlo E, Landry ML, Muyombwe A, Downing R, Razeq J, de Oliveira T, Faria NR, Sabino EC, Neher RA, Fauver JR, Grubaugh ND. 2021a. PCR assay to enhance global surveillance for SARS-CoV-2 variants of concern. *medRxiv* 2021a 01.28.21250486.. doi:10.1101/2021.01.28.21250486.
8. Vogels CB, Fauver J, Grubaugh N. Multiplexed RT-qPCR to screen for SARS-COV-2 B1.1.7, B.1.351, and P.1 variants of concern V.3 Coronavirus Method Development Community. *medrxiv* 2021b. doi:10.17504/protocols.io.br9vm966.
9. Artesi M, Bontems S, Göbbels P, Franckh M, Maes P, Boreux R, Meex C, Melin P, Hayette MP, Bours V, Durkin K. A recurrent mutation at position 26340 of SARS-CoV-2 is associated with failure of the E Gene quantitative reverse transcription-PCR utilized in a commercial dual-target diagnostic assay. *J. Clin. Microbiol.* 2020;**58**. doi:10.1128/JCM.01598-20.

François Maclot, Sébastien Bontems*, Cécile Meex
Department of Clinical Microbiology, Centre for interdisciplinary research medecines (CIRM), University Hospital of Liège, Liège, Belgium

Maria Artesi, Pablo Beckers, Vincent Bours, Keith Durkin
Laboratory of Human Genetics, GIGA Research Institute, Liège, Belgium

Marie-Pierre Hayette*
Department of Clinical Microbiology, Centre for interdisciplinary research medecines (CIRM), University Hospital of Liège, Liège, Belgium

*Corresponding authors.
E-mail addresses: sbontems@chuliege.be (S. Bontems), mphayette@chuliege.be (M.-P. Hayette)