Advanced *SARS-CoV-2* Variants of Concern (VOC) whole genome materials for use as verification, external quality assessment (EQA) and prospective quality control samples

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OBJECTIVES

The *SARS-CoV-2* virus has evolved into several variants of concern (VOC), which pose a serious threat to public health. These variants, including B.1.1.7 (a.k.a. 501Y.V1), B.1.351(a.k.a. 501Y.V2), and P.1(a.k.a. 501Y.V3), are particularly dangerous due to their increased viral transmissibility, disease severity, and ability to reduce the efficacy of *SARS-CoV-2* vaccines and diagnostic tests.
Major goals of the study were to show VOC sample usability in:

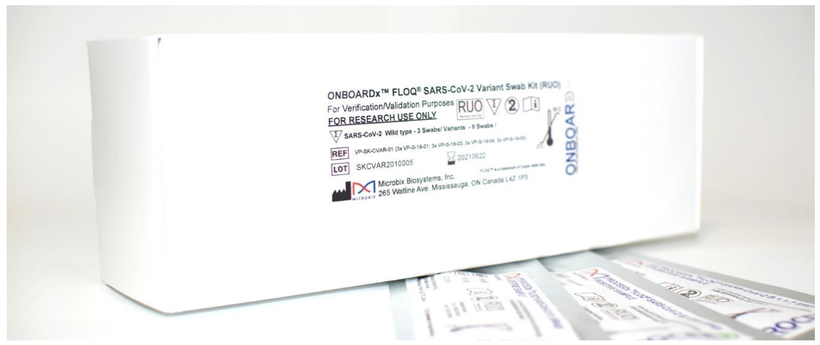
- Verifying the performance of VOC-specific *SARS-CoV-2* NAAT.
- Confirming the performance of *SARS-CoV-2* Nucleic Acid Amplification Tests (NAAT) when challenged with VOC samples.
- Evaluating sample processing workflow, from swab elution in transport medium to final test results.

MATERIALS AND METHODS

Microbix formulated novel swab-based positive VOC samples, which contain whole genome cDNA of *SARS-CoV-2* B.1.1.7, B.1.351 or P.1 variant types. The material is desiccated on Copan FLOQSwab® in a proprietary matrix. The performance of the swab-based SARS-CoV-2 VOC samples (containing all the components found in the infected patient specimen—human cells, and viral nucleic acid) was evaluated in Original Equipment Manufacturer (OEM) and clinical laboratories as RUO and PT/EQA samples.

Cat#	Variant	List of SARS-CoV-2 Mutations					
		ORF1ab	S	ORF8	ORF3a	N	E
VP-S-19-03 PT-S-19-03	B.1.1.7	4 SNPs, 1 deletion	7 SNPs, 2 deletion	3 SNPs		2 SNPs	N/A
VP-S-19-04 PT-S-19-04	B.1.351	3 SNPs, 1 deletion	7 SNPs	N/A	2 SNPs	1 SNP	1 SNP
VP-S-19-05	P.1	2 SNPs, 1 deletion	11 SNPs	1 SNP 1 Insertion	N/A	1 SNP	
VP-S-19-01 PT-S-19-01	WT	N/A	N/A	N/A		N/A	N/A

Table 1: Mutation Map for Microbix's VOC Samples
Note: Samples are comprised of more extensive mutation maps; the mutations listed above are the most relevant to these studies.



RESULTS

1. Verifying the Performance of VOC-Specific SARS-COV-2 NAAT

Seegene

A. Allplex™ SARS-CoV-2 Variants I Assay

Product Cat# PROCEEDFLOQ	Assay Targets				Output
	E484K	HV69/70 Del.	N501Y	RdRP Gene	
VP-S-19-03	-	+	+	+	B.1.1.7 VOC
VP-S-19-04	+	-	+	+	B.1.351 VOC
VP-S-19-05	+	-	+	+	P.1 VOC
VP-S-19-01	-	-	-	+	SARS-CoV-2 WT

Table 2: Detecting SARS-CoV-2 Mutations using Microbix's VOC Samples

RESULTS CONTINUED

ELITechGroup EMPOWERING IVD

B. SARS-CoV-2 Variant Typer Assay

PROCEEDFLOQ	Channel 1: E484K			Channel 4: N501Y		
	Ct Value	Tm Value	Output	Ct Value	Tm Value	Output
VP-S-19-03	29	66°C	WT	30	62°C	Mutant
VP-S-19-04	30	59°C	Mutant	30	62°C	Mutant
VP-S-19-05	31	58°C	Mutant	31	62°C	Mutant
VP-S-19-01 (+ D614G)	30	66°C	WT	ND	55°C	WT

Table 3: Ct and Melting Curve Values for Microbix's VOC Samples

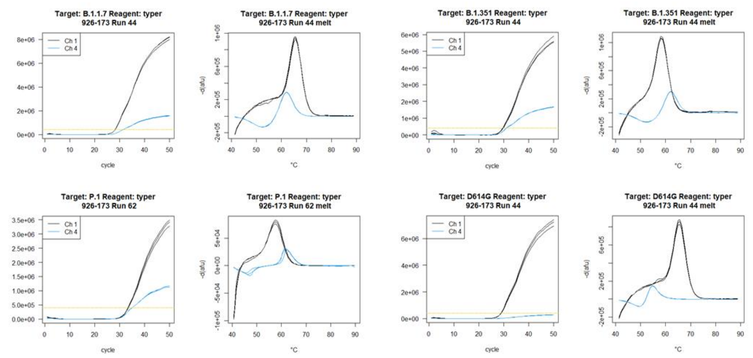


Figure 1: Realtime and Post-Amplification Melting Curves

Dynacare

C. PHO Multiplex RT-PCR SARS-CoV-2 Allelic Discrimination Assay

Product Cat# PROCEEDFLOQ	Assay Targets			Output
	E484K	N501Y	N501	
VP-S-19-03	-	+	-	B.1.1.7 Variant
VP-S-19-04	+	+	-	B.1.351 Variant
VP-S-19-01	-	-	+	WT

Table 4: Detection of E484K and N501Y Mutations with a Lab Developed Test

Product Cat# PROCEEDFLOQ	Detection Rate (%) 1:20 Dilution			Detection Rate (%) 1:100 Dilution		
	E484K	N501Y	N501	E484K	N501Y	N501
VP-S-19-03	-	94.4%	-	-	55%	
VP-S-19-04	100%	100%	-	90%	85%	-
VP-S-19-01	-	-	100%	-	-	55%

Table 5: Successful Detection of E484K and N501Y Mutations Following Sample Dilution

2. Confirming the Performance of SARS-CoV-2 NAAT When Challenged with VOC Samples

ELITechGroup EMPOWERING IVD

A. SARS-CoV-2 Plus ELITe MGB Assay

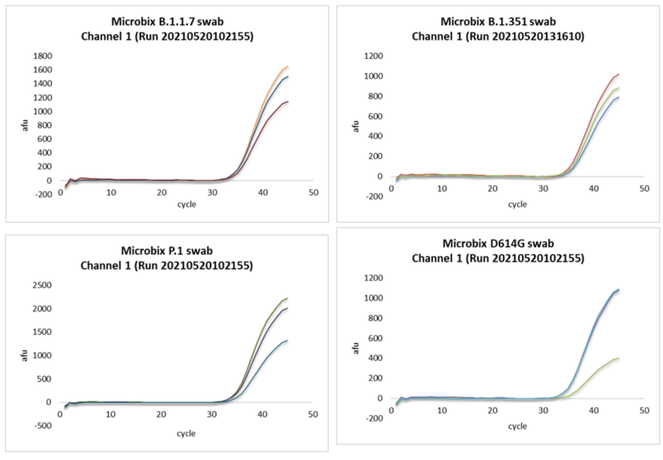


Figure 2: Microbix VOC Samples were Successfully Detected by the SARS-CoV-2 plus ELITe MGB Assay at 3x LOD Concentrations

RESULTS CONTINUED

B. Data From PT/EQA

Platforms with Confirmed Performance by EQA Samples- Data Combined from Several EQA Accreditors				
Manufacturer	Name of test	B.1.1.7 Variant; Number of Users	B.1.351 Variant; Number of Users	SARS-CoV-2 WT; Number of Users
Abacus Diagnostica	GenomEra SARS-CoV-2	13	11	25
Abbott	Alinity m-Resp-4-plex Alinity m SARS-CoV-2 ID NOW COVID-19 RealTime SARS-CoV-2	1261	19	39
Anatolia Geneworks	Bosphore 2019-nCoV	9	5	14
Applied Biosystems	TaqMan 2019-nCoV TaqPath COVID-19	12	13	26
Astra Biotech	SARS-CoV-2 Triplex	7	1	8
Atila Biosystems	iAMP COVID-19	13	-	-
Becton Dickinson	BD-SARS-CoV-2	9	9	17
BioFire Diagnostics	BioFire Respiratory Panel 2.1	17	14	31
Cepheid	Xpert Xpress SARS-CoV-2 Xpert Xpress SARS-CoV-2/Flu/RSV	97	79	177
CerTest Biotec	VIASURE SARS-CoV-2	14	12	26
DiaSorin	Molecular Simplexa COVID-19	5	4	9
EliTechGroup	GeneFinder COVID-19 Plus SARS-CoV-2 ELITe	25	9	34
Mesa Biotech	Accula SARS-CoV-2	30	-	-
Mobidiag	Amplidiag COVID-19 Novodiag COVID-19	12	16	28
NeuMoDx	SARS-CoV-2	6	7	13
PerkinElmer	SARS-CoV-2 Nucleic Acid Detection	6	5	11
Quidel	Lyra Direct SARS-CoV-2	12	-	-
Roche	cobas SARS-CoV-2 & Influenza A/B cobas SARS-CoV-2 Liat cobas SARS-CoV-2 & Influenza A/B	18	19	37
Seegene	Allplex 2019-nCoV Allplex SARS-CoV-2 Allplex SARS-CoV-2/FluA/FluB/RSV	30	30	60
Other		130	98	218
Total		**1726**	**351**	**773**

Table 6: Combined Users and Platforms for EU EQA and API

C. Statistical Data – EU EQA

299 Laboratories / 25 Countries		515 Number of Responses / >70 Participating Assays	
100% Success Rate for B.1.1.7 Positive Samples	99.1% Success Rate for B.1.351 Positive Samples	100% Success Rate for SARS-CoV-2 WT Positive Samples	98.1% Success Rate for Negative Samples

D. Statistical Data – API

1343 Laboratories / 3 Countries	1343 Number of Responses / 5 Participating Assays	98.8% Success Rate for B.1.1.7 Positive Samples	98.8% Success Rate for Negative Samples

CONCLUSIONS

SARS-CoV-2 VOC samples formulated on desiccated swabs showed compatibility with OEM and lab developed test platforms utilizing qPCR and mTOCE™ VOC methods of detection. The successful detection of various gene mutation targets demonstrates the achievement of constructing cross-platform compatible SARS-CoV-2 VOC samples for use as prospective quality controls and verification panels. Furthermore, EQA schemes with VOC samples confirmed that laboratories can successfully detect general SARS-CoV-2 infection when challenged with whole-process VOC samples.

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