NOVEL SARS-CoV-2 FULL-GENOME MATERIAL FOR USE IN A SWAB-BASED FORMULATION AS A MULTI-PLATFORM NAAT QUALITY CONTROL

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OBJECTIVES

To demonstrate the performance of a novel whole-genome (cDNA) *SARS-CoV-2* material, designed as a desiccated swab formulation (REDx™FLOQ® SARS-CoV-2 Swab Positive Control), in multiple nucleic acid amplified detection methodologies.

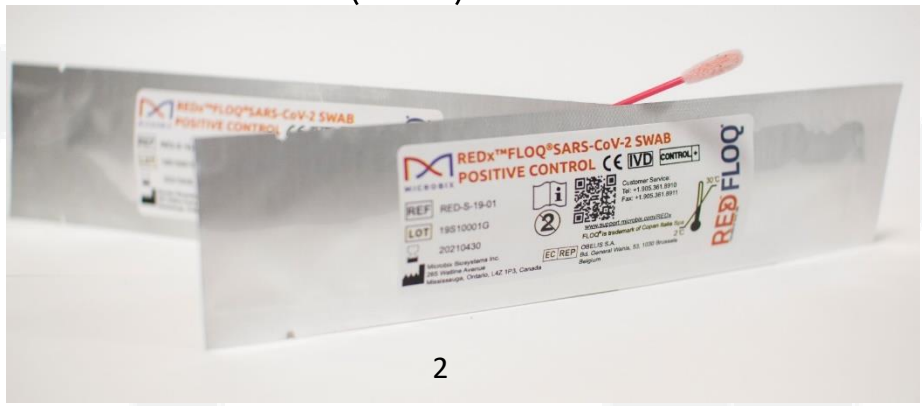
MATERIALS AND METHODS

REDx™FLOQ® SARS-CoV-2 Swab Controls IVD (Microbix Biosystems Inc.) were manufactured using a proprietary formulation allowing stable desiccation of the whole-genome *SARS-CoV-2* cDNA and human fibroblast cells on COPAN FLOQSwabs® (Cat # 502CS01) Regular Flocked Swab with 80mm Breakpoint. The resulting preparation is stable at ambient temperature and contains all the nucleic acid targets normally found in patient specimen samples.

All the samples were benchmarked internally with the CDC 2019-Novel Coronavirus (2019-nCoV) based RT-PCR test and sample performance for N1 (Ct= 31), N2 (Ct=31) and RNase P (Ct=33) genes was confirmed prior to the feasibility and validation studies.

Further, the positive and negative preparations were evaluated by using a two-step approach; first with a whole genome sequence confirmation (NC_04551 confirmed with one benign SNP ACA → ACG mutation), followed by blinded feasibility and validation studies in Clinical IVD laboratories, Original Equipment Manufacturer (OEM) laboratories. The following products were used in the studies:

Product Name	Catalogue	Viral genome/Cell culture	Format
RED FLOQ SARS-CoV-2 Swab Positive Control	RED-S-19-01	NC_04551/MRC-5	COPAN FLOQSwabs®
RED FLOQ SARS-CoV-2 Swab Negative Control	RED-S-99-M4	MRC-5	



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RESULTS

1. Feasibility study supported by Labquality Oy (n=24)

OEM	Test	Platform	Target	SARS-CoV-2 Result
Cepheid	Xpert® Xpress SARS-CoV-2	RT-PCR	N2 and E genes	Detected
ELITech Group	Genefinder™ Plus RealAmp Kit	RT-PCR	RdRp , N and E genes	Detected
NeuMoDx	NeuMoDx™ SARS-CoV-2 Assay	RT-PCR	nsp2 and N gene	Detected
Mobidiag	Amplidiag® COVID-19	RT-PCR	orf1ab and N genes	Detected
	Novodiag® COVID-19	RT-PCR	orf1ab and N genes	Detected
BioRad	SARS-CoV-2 ddPCR	RT-PCR	RNase P and N genes	Detected
Qiagen	QIAstat-Dx® Respiratory SARS-CoV	RT-PCR	orf1b poly gene (RdRp) and E genes	Detected

2. Clinical validation study - Johns Hopkins School of Medicine (n=12)

OEM	Test	Platform	Target	SARS-CoV-2 Result
Altona	RealStar® SARS-CoV-2	RT-PCR	E and S gene	Detected
BD Life Science	BD SARS-CoV-2	RT-PCR	N gene (N1 and N2 regions)	Detected
Cepheid	Xpert® Xpress SARS-CoV-2	RT-PCR	N2 and E genes	Detected
GenMark	ePlex® SARS-CoV-2 Test	RT-PCR	N gene	Detected
NeuMoDx	NeuMoDx™ SARS-CoV-2 Assay	RT-PCR	nsp2 and N gene	Detected

3. Clinical validation study – LifeLabs (n=25)

OEM	Test	Platform	Target	SARS-CoV-2 Result
Seegene	Allplex™ 2019-nCoV Assay	DPO RT-PCR	RdRp, N and E genes	Detected

4. OEM validation studies Quidel, R-Biopharm and EliTech (each n=12)

OEM	Test	Platform	Target	SARS-CoV-2 Result
Quidel	Lyra® SARS-CoV-2 Assay	RT-PCR	orf1ab	Detected
R-Biopharm	RIDA®GENE SARS-CoV-2	RT-PCR	E gene	Detected
ELITech Group	ELITe MGB® Assay	RT-PCR	orf8, orf1ab, RNase P genes	Detected

5. OEM (POCT) validation studies Abbott, Cepheid, DiaSorin and Mesa Biotech (each n=12)

OEM	Test	Platform	Target	SARS-CoV-2 Result
Abbott	ID NOW™ COVID-19	RT-NEAR	RdRp	Detected
Cepheid	Xpert® Xpress SARS-CoV-2	RT-PCR	N2 and E genes	Detected
DiaSorin	Simplexa™ COVID-19 Direct	RT-PCR	orf1ab and S gene	Detected
Mesa Biotech	Accula SARS-CoV-2 Test	RT-PCR	N gene	Detected

6. Combined data from sample performance on multiple platforms*

Multi-assay and multi-target sample variance n=115			
Target	Average (Ct)	SD	CV%
N gene [1]	34.77	0.92	2.64%
N gene [2]	29.92	0.78	2.59%
N gene [3]	27.75	0.44	1.60%
E gene [1]	30.69	0.58	1.89%
E gene [2]	28.06	0.70	2.50%
E gene [3]	31.43	0.74	2.35%
E gene [4]	28.03	0.48	1.73%
S gene [1]	28.03	0.80	2.86%
S gene [2]	26.82	0.98	3.67%
orf1ab [1]	26.84	0.97	3.63%
orf1ab [2]	29.11	0.43	1.48%
orf1ab [3]	26.16	0.67	2.57%
orf8	27.49	0.67	2.44%
nsp2	27.68	0.45	1.64%
RNase P	30.03	0.45	1.50%

*Platforms are not specified in order to avoid Ct comparison.
Ct data was generated following the instructions for processing patient specimens for each platform by the end-users, the preparations were provided as unassayed blinded samples.
RNase P is the human housekeeping gene, and it is not a target from the viral genome.

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CONCLUSIONS

Novel *SARS-CoV-2* QC material formulated using a widely-accepted swab-based format showed excellent compatibility with multiple OEM platforms utilizing qPCR, isothermal and TOCE-DPO detection methods. Calculated Sensitivity (Detectability) of the samples was 100%, with zero false positives and negatives reported (n=139).

The successful detection of various N, E, S, RdRP, orf1ab and nsp2 gene targets and RNaseP human housekeeping genes demonstrates the achievement of constructing cross-platform compatible SARS-CoV-2 samples for use as prospective unassayed workflow quality controls (QC). Furthermore, low variance of the eluted material reported by the end-users, gives additional opportunity for use of the QC as a reproducible validation material for comparing the performance of different Viral/Sample Transport Medium with various NAAT.

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