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Advanced SARS-CoV-2 nucleocapsid protein swab-based formulation for use as cross-platform EQA sample and as a prospective Quality Control

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INTRODUCTION

The need for fast, reliable, and widely implemented methodology for surveillance testing during the COVID-19 pandemic was fulfilled by immunodiagnostic Point of Care Testing (POCT) that detect SARS-CoV-2 nucleocapsid protein (antigen).

Unfortunately, SARS-CoV-2 Antigen POCT methods have limited options for External Quality Assessment (EQA) and third-party quality controls. Therefore, there is an urgent need for cross-platform EQA and quality control (QC) materials within the current IVD ecosystem.

AIM

To develop a non-infectious SARS-CoV-2 nucleocapsid protein formulation desiccated on a swab and evaluate its performance and reproducibility in monitoring the entire SARS-CoV-2 antigen detection workflow (sample elution, matrix separation and detection).

MATERIALS & METHODS

Microbix's advanced SARS-CoV-2 nucleocapsid protein desiccated on Copan FLOQSwabs® was initially developed with five levels of positivity. Two levels representing high (H) and medium-low (ML) positive samples (Table 1 and 2) were chosen for further feasibility and EQA studies with semiquantitative and qualitative test methods (Table 3, 4 and 5).

The performance of the novel SARS-CoV-2 swab-based formulations was evaluated by Original Equipment Manufacturer (OEM) and clinical laboratories (n=3740 samples) in 17 countries, as well as internally by various POCT detection and semiquantitative methodologies (Euroimmun and Roche). The samples were used as patient swab specimens with no additional preparation steps required.

Product Name	Cat#	Level
REDx™FLOQ® SARS-CoV-2 Ag Swab Positive Control	RED-S-19-02	H
PROCEEDx™FLOQ® SARS-CoV-2 Ag Swab Positive Sample	VP-S-19-02	H
PROCEEDx™FLOQ® SARS-CoV-2 Ag Swab Positive L1 Sample	VP-S-19-02L1	ML

Table 1. Samples used for OEM and internal feasibility studies

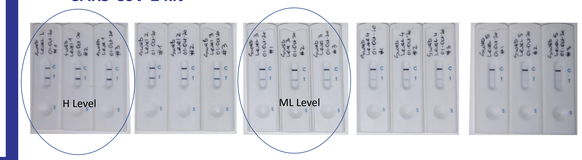
EQA Product Name	Cat#	Level
SARS-CoV-2 Ag Swab Positive Sample	PT-S-19-02	H
SARS-CoV-2 Ag Swab Negative Sample	PT-S-99-01	NA

Table 2. Samples used for EQA and pilot programs

REDFLOQ® PROCEEDFLOQ® PTD®

RESULTS

1. Internal data with the levels (1-5) done by BTNX Rapid Response SARS-CoV-2 kit



2. Data from OEMs – Qualitative Tests

REDx™FLOQ® SARS-CoV-2 Ag Swab Positive Control (RED-S-19-02)/ PROCEEDx™FLOQ® SARS-CoV-2 Ag Swab Positive Sample (VP-S-19-02)			
OEM	**Test**	**Platform**	**Result**
Becton Dickinson	Veritor COVID-19	LFT	+++
R-Biopharm	RIDA®QUICK SARS-CoV-2	LFT	+++
Abbott	BINAX NOW COVID-19	LFT	+++
BTNX	COVID-19	LFT	+++
iXensor	SARS-CoV-2	LFT	+++
DIALAB	DIAQUICK COVID-19 Ag	LFT	+++
Thermabright	ACUVID COVID-19 Saliva Antigen kits	LFT	+++
Nanōmix's	eLab COVID-19 Rapid Antigen test	LFT	+++
LumiraDx	SARS-CoV-2 Ag Test	FIA	+++
LightDeck	COVID-19 Test	Planar waveguide	+++
Quidel	Sofia SARS	FIA	+++
	Sofia SARS/FLU AB	FIA	+++
Actim	SARS-CoV-2 Ag Test	LFT	+++

Table 3. POCT qualitative tests with confirmed performance with H Level samples from REDx™ (IVD) and PROCEEDx™ (RUO) product lines (Interpretation +++ = High Positive)

3. Data from OEMs – Semiquantitative Tests

A. Euroimmun - SARS-CoV-2 Antigen ELISA

Microbix swabs in 0.5 ml UTM: Comparison	Ratio	
Product Cat#	VP-S-19-02L1 (ML)	VP-S-19-02 (H)
Undiluted	**9.37**	17.36
1:10	**1.43**	15.22
1:100	0.36	**2.93**
1:1000	0.28	0.55
1:10,000	0.35	0.21
Interpretation		
Ratio < 0.50: negative		
Ratio ≥0.5 to < 0.60: borderline		

Table 4. Results with interpretation - Euroimmun

B. Roche - Elecsys® SARS-CoV-2 Antigen

Instrument: Cobas e 801 analytical unit **Assay:** Elecsys® SARS-CoV-2 Antigen				
Interpretation:				
Non-reactive	COI < 1.0			
Reactive	COI ≥ 1.0			
Elution Medium	**Sample**		**COI**	**Interpretation**
UTM	RED-S-19-02	Eluted in 3.0 mL	544	Reactive
			543	Reactive
	RED-S-19-02	Eluted in 0.5 mL	888	Reactive
			877	Reactive
	VP-S-19-02L1	Eluted in 3.0 mL	7.2	Reactive
			7.1	Reactive
	VP-S-19-02L1	Eluted in 0.5 mL	30	Reactive
			30	Reactive
Roche SARS-CoV-2 Extraction Solution	RED-S-19-02	Eluted in 3.0 mL	280	Reactive
			270	Reactive
	RED-S-19-02	Eluted in 0.5 mL	786	Reactive
			802	Reactive
	VP-S-19-02L1	Eluted in 3.0 mL	7.7	Reactive
			7.5	Reactive
	VP-S-19-02L1	Eluted in 0.5 mL	16	Reactive
			16	Reactive

Table 5. Results with interpretation - Roche

4. Data from EQA

A. Platforms with confirmed performance (combined) by EQA samples

Test used by participants in SARS-CoV-2 Antigen pilot study		
Manufacturer	**Name of test**	**Number of users**
Quidel	Sofia, 2 SARS Ag	1072
BD Veritor, Plus	SARS Ag	255
BinaxNOW COVID-19 Ag Card	Abbott	128
LumiraDx SARS-CoV-2 Ag Test	LumiraDx	52
Quidel	Sofia,2 Flu + SARS	51
AccessBio	CareStart COVID19 Ag	22
Panbio COVID-19 Ag Rapid Test Device	Abbott	13
VITROS SARS-CoV-2 Antigen Test	Ortho Clinical Diagnostics	12
mariPOC SARS-CoV-2	ArcDia	9
Quidel	QuickVue SARS Antigen	7
STANDARD F COVID-19 Ag FIA	SD BIOSENSOR	5
STANDARD Q COVID-19 Ag Test	SD BIOSENSOR	5
mariPOC Respi+	ArcDia	2
NADAL COVID-19 Ag Rapid Test	Nal von Minden	2
AMP Rapid Test SARS-CoV-2 Ag	AMP Diagnostics	1
NowCheck COVID-19 Ag Test	BioNote	1
BIOSYNEX COVID-19 Ag BSS	BIOSYNEX	1
LIAISON SARS-CoV-2 Ag	DiaSorin	1
SARS-CoV-2 Antigen ELISA	EUROIMMUN	1
Lumipulse G SARS-CoV-2 Ag	Fujirebio	1
Coronavirus Ag Rapid Test Cassette	Menarini Diagnostics	1
SARS-CoV-2 Rapid Antigen Test	Roche	1
QuickStripe SARS-CoV-2 Antigen Test	Savyon Diagnostics	1

Table 6. Combined users and platforms for EU EQA and API

B. Statistical data EU EQA Pilot Study(2020 EU)

47 Participants	50 Number of Responses	98% Success Rate for Positive Sample	100% Success Rate for Negative Sample
16 Countries	18 Participating Assays		

C. Statistical data API (combined pilot study 2020 and first run 2021 US)

1595 Participants	1617 Number of Responses	98.83% Success Rate for Positive Sample	99.38% Success Rate for Negative Sample
1 Country	9 Participating Assays		

CONCLUSIONS

Advanced SARS-CoV-2 nucleocapsid protein formulations desiccated on a widely accepted swab-based format showed excellent compatibility with several OEM platforms that utilize colloidal gold lateral flow tests, fluorescent, and planar waveguide methodologies. The successful detection of desiccated antigen epitopes after reconstitution in various elution buffers demonstrates that newly developed SARS-CoV-2 nucleocapsid protein formulations desiccated on Copan FLOQSwabs® are cross-platform compatible with SARS-CoV-2 antigen assays. These swabs can be used as prospective cross-platform EQA samples and quality controls.

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Actim - Espoo, Finland
LightDeck - Colorado, United States
Nanōmix – California, US
Thermabright – Ontario, Canada
DIALAB (represented by Zafena, Sweden)- Neudorf, Austri
iXensor - Taipei City, Taiwan
BTNX – Ontario, Canada
Labquality – Helsinki, Finland
API – Michigan, US

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