EMERGENCY RESPONSE VALIDATION

AOAC® *Performance Tested Method*℠
CERTIFICATION

The AOAC Research Institute hereby certifies that the performance has been evaluated and found to perform as stated in the applicability of the method. Approval has been granted with the following certificate number:

022102

SureFast® SARS-CoV-2 PLUS Test

manufactured by:

Congen Biotechnologie GmbH
Robert-Roessle-Straße 10
13125 Berlin,
Germany

distributed by

R-Biopharm AG
An der neuen
Bergstraβe 17
64297 Darmstadt
Germany

R-Biopharm Inc.
870 Vossbrink Drive
Washington, MO
63090 USA

This certificate signifies that an AOAC® Certification Mark License Agreement has been executed, which authorizes the manufacturer to display the AOAC *Performance Tested Methods* ℠ certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND FOUND TO PERFORM AS STATED IN THE APPLICABILITY STATEMENT" - on the above-mentioned method. Renewal may be granted at the end of one calendar year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date: February 10, 2021
Expiration Date: June 30, 2021

METHOD TYPE: Emergency Response Validation, In Silico, Single Site

METHOD AUTHORS
R-Biopharm AG and R-Biopharm Inc.: Markus Lacorn, Martin Mehl, Caroline Knoll, and Patricia Meinhardt

SUBMITTING COMPANIES
R-Biopharm AG
An der neuen Bergstrasse 17
64297 Darmstadt Germany
and
R-Biopharm Inc.
870 Vossbrink Drive
Washington, MO 63090 USA

MANUFACTURING COMPANY
Congen Biotechnologie GmbH
Robert-Roessle-Straße 10
13125 Berlin,
Germany

KIT NAME
SureFast® SARS-CoV-2 PLUS Test

CATALOG NUMBERS
SureFast® SARS-CoV-2 PLUS F7110; SureFast® Prep F1051; SureFast® PCR 7710

INDEPENDENT LABORATORY FOR R-BIOPHARM
MICOBAC® Labs
640 Spence Lane Suite 121
Nashville, TN 37217 USA

INDEPENDENT LABORATORY
MRIGlobal
425 Volker Blvd
Kansas City, MO 64100

AOAC EXPERTS AND PEER REVIEWERS
William Burkhardt[1], Jacquelina Woods[2], John SantaLucia[3]
[1] United States Food and Drug Administration, College Park, MD, USA
[2] United States Food and Drug Administration, Dauphin Island, AL, USA
[3] Wayne State University, Ann Arbor, MI, USA

APPLICABILITY OF METHOD
Analytes – SARS-CoV-2 virus

Matrixes – Stainless steel surface (2" by 2" swab)

Performance claims - Performance comparable to the U.S. Centers for Disease Control and Prevention 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel, Revision 04 (2).

REFERENCE METHOD
Centers for Disease Control and Prevention (2020). *CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.* Revision 5. (2)

ORIGINAL CERTIFICATION APPROVAL DATE	**CERTIFICATION RENEWAL RECORD**
February 10, 2021	**New Approval 2021**

METHOD MODIFICATION RECORD	**SUMMARY OF MODIFICATION**
NONE	**NONE**

Under this AOAC® *Performance Tested℠* License Number, 022102 this method is distributed by:	**Under this AOAC® *Performance Tested℠* License Number, 022102 this method is distributed as:**
1. R-Biopharm AG	**1. SureFast® SARS-CoV-2 PLUS Test**

PRINCIPLE OF THE METHOD (1)
The SureFast® SARS-CoV-2 PLUS is a real-time RT-PCR for the direct, qualitative detection of intact novel coronavirus (SARS-CoV-2) RNA from stainless steel swab samples. Each reaction contains an Internal Control RNA (ICR, consisting of MS2-bacteriophage) as an internal control of sample preparation procedure and to monitor possible PCR-inhibition. The RT-qPCR assay can be performed with commonly used real-time PCR instruments, equipped for detection of two fluorescence emissions at the channels FAM and VIC/HEX simultaneously.

DISCUSSION OF THE VALIDATION STUDY (1)
Results from the POD analysis demonstrated that the the SureFast® SARS-CoV-2 RT-PCR better at detecting low concentrations (2×10^3 GU/ 2" x 2" test surface) of deposited SARS-CoV-2 on a stainless steel surface compared to the CDC reference method when using the same swabbing sample preparation and swabbing procedure for the both the RT-qPCR primers and probes of the candidate method and the reference method.
The *in silico* analysis of the primers and probes utilized in the SureFast® SARS-CoV-2 RT-PCR test method are specific and sensitive enough (99.99% binding of the oligomer and the target binding region) to detect low levels of SARS-CoV-2 without exhibiting false negatives when compared to the CDC reference method. The high level of specificity could be due to the novel single target assay (E gene) requirement of the SureFast® SARS-CoV-2 RT-PCR test method in comparison to the double-target assay (N1 and N2 SARS-CoV-2 gene targets) of the CDC reference method. Competition in amplification efficiency between two targets and/or RNA degradation on surfaces may contribute to a single target assay readily detecting one target over a double-target assay. Another possibility for the SureFast® SARS-CoV-2 PLUS RT-PCR test method providing better results that the CDC reference method may be due to the difference in swabs used. The swab in the SureFast® method may have better recovery of the virus from the stainless steel surface; since there is no prescribed swabbing method in the CDC reference method it is unknown what role the swab material plays in virus recovery.

Inclusivity, Exclusivity and Background Organism Summary (1)

In Silico Analysis	
Inclusivity	
15,764 unique SARS-CoV-2 strain accessions[a]	
Exclusivity	
Human coronavirus (229E, OC43, NL63, HKU1), SARS-coronavirus, MERS-coronavirus, Porcine delta coronavirus	
Background Organisms	
Viruses:	Bovine coronavirus, Human respirovirus 3, Enterovirus, Infectious bronchitis virus, Enterovirus D68, Human adenovirus 1, Human alphaherpesvirus 3, Human bocavirus, Human metapneumovirus, Human orthorubulavirus 2, Human orthorubulavirus 4, Human respirovirus 1, Influenza A H7N9 subtype, Influenza A virus, Influenza A H1N1, Influenza B virus, Norovirus, Respiratory syncytial virus, Simplexvirus, Transmissible gastroenteritis virus
Bacteria and Fungi:	*[Candida] glabrata, Acinetobacter baumannii, Acinetobacter baylyi, Acinetobacter bereziniae, Acinetobacter calcoaceticus, Acinetobacter chinensis, Acinetobacter cumulans, Acinetobacter defluvii, Acinetobacter disperses, Acinetobacter equi, Acinetobacter guillouiae, Acinetobacter haemolyticus, Acinetobacter junii, Acinetobacter lactucae, Acinetobacter lanii, Acinetobacter larvae, Acinetobacter nosocomialis, Acinetobacter phage ZZ1, Acinetobacter pittii, Acinetobacter schindleri, Acinetobacter seifertii, Acinetobacter shaoyimingii, Acinetobacter wanghuae, Bacillus cereus, Bacillus thuringiensis, Bordetella pertussis, Candida albicans, Chlamydia pneumoniae, Clostridioides difficile, Enterococcus casseliflavus, Enterococcus cecorum, Enterococcus faecium, Enterococcus hirae, Enterococcus lactis, Enterococcus mundtii, Enterococcus rotai, Enterococcus saigonensis, Enterococcus thailandicus, Enterococcus wangshanyuanii, Escherichia coli O157:H7 str. Sakai, Escherichia coli str. K-12 substr. MG1655, Haemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Mycobacterium tuberculosis, Mycoplasma pneumoniae, Pneumocystis jirovecii MT seq, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus salivarius*
Fungi and Eukaryotes:	*Homo sapiens aedes aegypti, Aedes albopictus, Dermatophagoides pteronyssinus, Musa domestica, Drosophila, Chlorocebus sabaeus*

[a] Accessions acquired from the Global Initiative on Sharing Avian Influenza Data (GISAID) database from December 2019 to 26 June 2020.

Table 14. Stainless Steel Candidate vs. Reference Method – POD Results (1)

Matrix	Strain	GU/Test Area[a]	N[b]	Candidate SureFast® SARS-CoV-2			Reference			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Stainless Steel (2" x 2")	SARS-CoV-2 BEI NR-52281	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		2.0 x 10^3	20	20	1.00	0.84, 1.00	11	0.55	0.34, 0.74	0.55	0.20, 0.66
		2.0 x 10^4	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.43, 0.43

[a] GU/Test Area = Results of the GU/Test area were determined by plating the inoculum for each matrix in triplicate
[b] N = Number of test portions
[c] x = Number of positive test portions
[d] POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e] POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f] dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

REFERENCES CITED

1. Lacorn, M., Mehl, M., Knoll, C., and Meinhardt, P., Validation of the SureFast® SARS-CoV-2 PLUS Test Method for the Detection of SARS-CoV-2 Virus on Stainless Steel Surfaces, AOAC® *Performance Tested*SM Emergency Response Validation certification number 022102.
2. Centers for Disease Control and Prevention (2020). *CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.* Revision 5. 07/13/2020. https://www.fda.gov/media/134922/download (Accessed October 2020).