FloodLAMP EasyPCR™ COVID-19 Test

Instructions for Use v1.1

IVD

COVID-19 Emergency Use Authorization Only
For *in vitro* diagnostic (IVD) Use

www.floodlamp.bio
FloodLAMP Biotechnologies, PBC | 930 Brittan Ave. San Carlos, CA 94070 USA

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FloodLAMP EasyPCR™ COVID-19 Test

For COVID-19 Emergency Use Authorization Only

Instructions for Use

Intended Use

FloodLAMP EasyPCR™ COVID-19 Test is a real-time reverse transcriptase polymerase chain reaction (RT-qPCR) assay intended for the qualitative detection of RNA from SARS-CoV-2 in upper respiratory specimens including nasopharyngeal swabs, anterior nasal and mid-turbinate nasal swabs from individuals suspected of COVID-19 by their healthcare provider and from individuals without symptoms or other epidemiological reasons to suspect COVID-19 infection, when such individuals are tested as part of a testing program that includes testing at regular intervals, at least once per week, such as those implemented by schools, workplaces and community groups. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens including nasopharyngeal swabs, anterior nasal and mid-turbinate nasal swabs during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The FloodLAMP EasyPCR™ COVID-19 Test is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of *in vitro* diagnostic procedures. The FloodLAMP EasyPCR™ COVID-19 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Principles of Procedure

The FloodLAMP EasyPCR™ COVID-19 Test is a RNA extraction-free, duplexed RT-qPCR assay which indicates whether SARS-CoV-2 RNA is present. It can widely and rapidly be scaled

because 1) it does not require nucleic acid extraction equipment, 2) it utilizes reagents and supplies readily available in large quantities, and 3) it is a straightforward protocol with minimal steps that can be executed quickly and reliably. It also utilizes the same streamlined sample preparation as the FloodLAMP QuickColor™ COVID-19 Test. Both are supply chain robust, "open source" protocol tests, meaning designated laboratories may obtain the test components directly from vendors. Further, the FloodLAMP QuickColor™ COVID-19 Test is isothermal, does not require any instrumentation and has a visual readout. Together, the two tests can be used in an integrated program for screening and rapid confirmation in large populations by a broad range of laboratories.

In the FloodLAMP EasyPCR™ COVID-19 Test, samples are first treated with a TCEP-based Inactivation Solution followed by a heat inactivation step, and the resulting inactivated sample is directly used as input in the duplexed RT-qPCR test. The test does not use new primers and probes for RT-qPCR testing, but rather uses previously validated primer and probe sets (2019-nCoV_N1 and RP) developed by the US CDC, which are readily available from multiple commercial suppliers. The human Ribonuclease P (RP) probe was modified with a different fluorophore so that the primer/probe set could be combined in a duplex assay, reducing the number of tests to 1 assay with 2 sets.

Materials Provided and Storage

The FloodLAMP EasyPCR™ COVID-19 Test utilizes standard chemicals available from multiple vendors, with the exception of the PCR Kit. Designated CLIA labs may order components directly from vendors.

Materials Required but Not Provided

The FloodLAMP EasyPCR™ COVID-19 Test is to be used with the reagents or equivalents listed in Table 1. The FloodLAMP EasyPCR™ COVID-19 Test is to be used with RT-PCR Instruments such as Applied Biosystems Quantstudio™ Systems and Bio-Rad CFX™ Systems.

Table 1: Validated reagents used with Test

Item	Concentration	Chemical Composition	Vendor	Catalog Number
TCEP	.5 M	tris(2-carboxyethyl)phosphine hydrochloride	Sigma-Aldrich Millipore Sigma	646547-10X1ML
EDTA	.5 M	Ethylenediaminetetraacetic acid	Thermo Fisher	15575020
NaOH	10 N	Sodium Hydroxide	Sigma-Aldrich	SX0607N-6
Nuclease-free Water		Ultrapure Water, nuclease free	Thermo Fisher	10977015
NaCl	5 M	Sodium Chloride	Thermo Fisher	24740011
PCR Kit*		Luna® Universal Probe One-Step RT-qPCR Kit	New England Biolabs	E3006

* Item may not be substituted for equivalent.

The FloodLAMP EasyPCR™ COVID-19 Test uses the validated primer and probe sets (2019-nCoV_N1 and RP) developed by the US CDC. The human Ribonuclease P (RP) probe was modified with a different fluorophore so that the primer/probe set could be combined in a duplex assay, detecting the 2 targets in a single well. This configuration is described in the SalivaDirect™ EUA Authorized test (www.fda.gov/media/141192/download).

The complete set of 6 primers and probes may be purchased from the vendor Eurofins Genomics using the catalog number 12YS-010YST. This product contains primers and probes suspended at 100µM and is enough for 12,500 reactions. The contents can be mixed along with nuclease-free water to create the primer stocks used in the FloodLAMP EasyPCR™ COVID-19 Test. See Table 6 below for details. A larger volume of primer-probe stock can be prepared in advance and stored at -20°C for up to 1 year.

Table 2: Primers and probes

Target	Primer/Probe	Sequence
CDC-N1	2019-nCoV_N1-F	GACCCCAAAATCAGCGAAAT
CDC-N1	2019-nCoV_N1-R	TCTGGTTACTGCCAGTTGAATCTG
CDC-N1	2019-nCoV_N1-P	**FAM**-ACCCCGCATTACGTTTGGTGGACC-**IBFQ**
Human RNAseP	RP-F	AGATTTGGACCTGCGAGCG
Human RNAseP	RP-R	GAGCGGCTGTCTCCACAAGT
Human RNAseP	RP-P	**Cy5**-TTCTGACCTGAAGGCTCTGCGCG-**IBRQ**

Standard Lab Equipment and Consumables

- 70% ethanol
- 10% bleach, prepared daily
- 96-well PCR reaction plates (Applied Biosystems # 4346906, 4366932, 4346907, Eppendorf # 951020303 or equivalent)
- Optical strip caps (Applied Biosystems # 4323032 or equivalent)
- Optical plate seal (Applied Biosystems # 4311971 or equivalent)
- PCR strip tubes and caps (USA Scientific catalog # 1402-2500 or equivalent)
- 5 mL transport tubes or equivalent (sterile)
- 1.5 mL microcentrifuge tubes or equivalent (nuclease-free)
- Aerosol resistant micropipette tips (nuclease-free)
- Micropipettes (calibrated)
- Bottle top dispenser for 1 mL volume (optional, calibrated)
- 96-well cold block
- Cold blocks for 5 mL and 1.5 mL - 2.0 mL tubes, or ice
- Vortex mixer

- 96-well plate centrifuge or equivalent
- Mini centrifuge for 1.5 mL tubes or equivalent
- Thermal cycler, water bath, dry heat bath or equivalent (calibrated)
- Class II Biological Safety Cabinet (BSC)
- PCR Instrument (choose one)
    - QuantStudio™ 6 Flex
    - QuantStudio™ 7 Pro
    - Bio-Rad CFX96 Touch™

Warnings and Precautions

Materials or chemicals required for the use of the FloodLAMP EasyPCR™ COVID-19 Test should be closely examined by the user. The user should carefully read all warnings, instructions or Safety Data Sheets provided by the supplier and follow the general safety precautions when handling biohazards, chemicals and other materials.

General Precautions

- The FloodLAMP EasyPCR™ COVID-19 Test is for *in vitro* diagnostic use (IVD) only. Rx Only.
- For use under COVID-19 Emergency Use Authorization Only.
- Standard precautions and procedures should be taken when handling and disposing of human samples.
- This test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Standard precautions and procedures should be taken when handling and extracting human samples.
- Standard precautions and procedures should be taken when using laboratory equipment.
- Standard precautions and procedures should be taken when disposing of waste.
- Dispose of reagents according to local regulations.
- Do not use reagents after their recommended stability time frame.
- Ensure reagents are stored at the recommended temperatures as described below and in the vendor product information and manuals.

Contamination Precautions

- Avoid contamination by following good laboratory practices, wearing proper personal protective equipment, segregating workflow, and decontaminating workspace appropriately.
- Ensure that surfaces and equipment used for all test steps have been properly cleaned with 10% bleach and 70% ethanol.
- Ensure all consumables are DNase and RNase free except for sample collection tubes which may be sterile.
- Use only calibrated pipettes and filter tips that are sterile and PCR clean.
- After completion of the test, dispose of the amplification reaction plates or tubes. Do not open tubes or remove the seals on plates after heating amplification reactions.

Limitations

- The use of this assay as an *in vitro* diagnostic under the FDA COVID-19 Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests by Rx only.
- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may lead to erroneous results.
- The performance of the FloodLAMP EasyPCR™ COVID-19 Test was established using Nasopharyngeal Swab specimen type collected in saline. Nasal swabs, oropharyngeal swabs, mid-turbinate nasal swabs specimens are also considered acceptable specimen types for use with the test but performance has not been established.
- Samples must be collected according to recommended protocols and transported and stored as described herein.
- Samples should not be collected in UTM or VTM or Liquid Amies transport media.
- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- False-positive results may arise from various reasons, including, but not limited to the following:
    - Contamination during specimen collection, handling, or preparation
    - Contamination during assay preparation
    - Incorrect sample labeling
- False-negative results may arise from various reasons, including, but not limited to the following:
    - Improper sample collection or storage
    - Degradation of SARS-CoV-2 RNA

  - Presence of inhibitory substances
  - Use of extraction reagents or instrumentation not approved with this assay
  - Incorrect sampling window
  - Failure to follow instructions for use
  - Mutations in SARS-CoV-2 target sequences
- Nucleic acid may persist even after the virus is no longer viable.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Performance has not yet been established in asymptomatic individuals and will be established during a post-authorization study.
- Use of the test in a general, asymptomatic population for serial screening is intended to be used as part of an infection control plan, that may include additional preventative measures, such as a predefined serial testing plan or directed testing of high-risk individuals. Negative results should not be treated as definitive and do not preclude current or future infection obtained through community transmission or other exposures. Negative results must be considered in the context of an individual's recent exposures, history, and presence of clinical signs and symptoms consistent with COVID-19.
- This test should not be used within 30 minutes of administering nasal or throat sprays.
- Positive results must be reported to appropriate public health authorities, following state and national guidelines.
- The clinical performance of the test has not been established in all circulating variants, and test performance may vary depending on the prevalence of variants circulating at the time of patient testing.
- Negative test results do not exclude possibility of exposure to or infection with SARS-CoV-2 virus. Patient handling will be directed by healthcare professionals.

Conditions of Authorization for the Laboratory

The FloodLAMP EasyPCR™ COVID-19 Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

However, to assist clinical laboratories running the FloodLAMP EasyPCR™ COVID-19 Test, the relevant Conditions of Authorization are listed below:

- Authorized laboratories[1] using the FloodLAMP EasyPCR™ COVID-19 Test will include all authorized Fact Sheets with test result reports. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories[1] using the FloodLAMP EasyPCR™ COVID-19 Test will use the FloodLAMP EasyPCR™ COVID-19 Test as outlined in the FloodLAMP EasyPCR™ COVID-19 Test Instructions for Use. Deviations from the authorized procedures, including the

authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the test are not permitted.

- Authorized laboratories must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the FloodLAMP EasyPCR™ COVID-19 Test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and FloodLAMP Biotechnologies, PBC support center (via email: eua.support@floodlamp.bio) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in molecular assay techniques and use appropriate laboratory and personal protective equipment when handling these test components, and use the test in accordance with the authorized labeling.
- FloodLAMP Biotechnologies, PBC authorized distributors, and authorized laboratories using the FloodLAMP EasyPCR™ COVID-19 Test will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] For ease of reference, this will refer to, "Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified laboratories with FDA Emergency Use Authorization FDA for performing SARS-CoV-2 testing

Specimen Collection and Storage

Upper respiratory specimens including nasopharyngeal swabs, anterior nasal and mid-turbinate nasal swabs should be collected using standard procedures and recommendations. Swab specimens should be collected in 0.9% saline, PBS, or dry tubes. Specimens should not be collected in UTM, VTM, or Liquid Amies.

Please refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19:
https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

The stability study of the nasal swab sample transported in saline has been conducted by Quantigen Biosciences, with support from The Gates Foundation and UnitedHealth Group. Quantigen Biosciences has granted a right of reference to any sponsor wishing to pursue an EUA to leverage their COVID-19 swab stability data as part of that sponsor's EUA request.

- Samples can be stored at room temperature for 56 hours after collection prior to inactivation.
- For longer term storage, samples can be stored at ≤-70° C.

Note: Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens.

Running Tests

Reagent Preparation

The FloodLAMP EasyPCR™ COVID-19 Test is to be used with the reagents or equivalents listed in Table 1.

Table 1: Validated reagents used with Test

Item	Concentration	Chemical Composition	Vendor	Catalog Number
TCEP	.5 M	tris(2-carboxyethyl)phosphine hydrochloride	Sigma-Aldrich Millipore Sigma	646547-10X1ML
EDTA	.5 M	Ethylenediaminetetraacetic acid	Thermo Fisher	15575020
NaOH	10 N	Sodium Hydroxide	Sigma-Aldrich	SX0607N-6
Nuclease-free Water		Ultrapure Water, nuclease-free	Thermo Fisher	10977015
NaCl	5M	Sodium Chloride	Thermo Fisher	24740011
PCR Kit*		Luna® Universal Probe One-Step RT-qPCR Kit	New England Biolabs	E3006

* Item may not be substituted for equivalent.

Stocks of TCEP, EDTA, NaOH, and NaCl may be prepared from powder form at the specified concentration using nuclease-free, MilliQ or equivalent molecular biology grade water.

0.9% Saline (154 mM) may be prepared by diluting 15.4 mL of 5 M NaCl in MilliQ or equivalent molecular biology grade water to a final volume of 500 mL. Equivalent preparations or commercial saline products may be utilized, with appropriate validation.

The 100X Inactivation Solution is prepared by mixing the components in Table 3 and vortexing for 30 seconds. Equivalent preparations utilizing components with different source concentrations may be used such that the final 100X Concentration is achieved. Aliquots of 100X Inactivation Solution should be stored in the dark at -20° C for up to 3 months. Upon thaw, working aliquots of 100X Inactivation Solution should be stored in the dark at room temperature for up to 1 month.

Table 3: 100X Inactivation Solution

Component	Source Concentration	Volume	100X Concentration
TCEP	0.5 M	10 mL	250 mM
EDTA	0.5 M	4 mL	100 mM
NaOH	10 N	2.3 mL	1.15 N
Nuclease-free Water		3.7 mL	
TOTAL VOLUME		**20 mL**	

For swabs that are collected or eluted in 0.9% saline solution or equivalent, the 100X Inactivation Solution should be added at 1/100th the sample solution volume.

For dry swabs, a preparation of 1X Inactivation Saline Solution should be prepared per Table 4. 1X Inactivation Saline Solution should be kept at room temperature and used within 24 hours of preparation from components or 100X Inactivation Solution.

Table 4: 1X Inactivation Saline Solution

Component	Volume
0.9% Saline (154 mM NaCl) in MilliQ Water	1000 mL
100X Inactivation Solution	10 mL
TOTAL VOLUME	**1010 mL**

Controls Preparation

One **Positive Template Control and one Negative (No Template) Control** will be included on every 96-well plate with up to 94 samples, or with every batch of strip tubes. An **Internal Process Control** is included in every PCR reaction.

a) A **"No Template" (Negative) Control (NTC)** is needed to assure the absence of cross-contamination from positive samples, positive controls, or amplicons and is used to determine if sample results are valid. It consists of nuclease-free water.

b) A **Positive Template Control** is needed to assure proper functioning of reagents and the absence of significant RNAse contamination. It consists of synthetic viral RNA at a concentration of approximately 100,000 cp/mL diluted in total human RNA and nuclease-free water. Stock and working aliquots of the positive control are produced from the sources listed in Table 5 or equivalents. Working aliquots should be diluted prior to use to 100,000 cp/mL. Positive control aliquots should be stored for at most 3 months at -80°C, or at most 1 month at -20°C.

c) An **Internal Process Control** is needed to assure sufficient specimen quantity and quality. It consists of a primer/probe set targeting the human RNaseP gene that is included in a single PCR amplification reaction. The RNAseP Internal Process Control uses a fluorescent reporter in a separate channel from the SARS-CoV-2 channel (i.e. in duplex).

Table 5. Components for Positive Template Control

Material	Vendor	Catalog #	Volume
SARS-CoV2 Positive Control RNA	Twist	102019	5 µL
Total Human RNA	Thermo Fisher	4307281	100 µL
Nuclease-free Water	Thermo Fisher	10977015	4,895µL

PCR Primer Stock Preparation

The FloodLAMP EasyPCR™ COVID-19 Test uses the validated primer and probe sets (2019-nCoV_N1 and RP) developed by the US CDC. The human Ribonuclease P (RP) probe was modified with a different fluorophore so that the primer/probe set could be combined in a duplex assay, detecting the 2 targets in a single well. This configuration is described in the SalivaDirect™ EUA Authorized test (www.fda.gov/media/141192/download).

The complete set of 6 primers and probes may be purchased from the vendor Eurofins Genomics using the catalog number 12YS-010YST. This product contains primers and probes suspended at 100µM and is enough for 12,500 reactions. The contents can be mixed along with nuclease-free water to create the primer stocks used in the FloodLAMP EasyPCR™ COVID-19 Test. See Table 6 below for details. A large volume of primer-probe stock can be prepared in advance and stored at 4°C for one month or -20°C for up to 1 year. Vendors for the Primer and Probe sets are below in Table 7.

Table 6: 5X PCR Primer Stock Preparation from Eurofins Genomics Product

Component	5X PCR Primer Stock Concentration	Volume (1 reaction)	Volume (3,125 reactions)
2019-nCov_N1-F	2 µM	0.4 µl	1,250 µl
2019-nCov_N1-R	2 µM	0.4 µl	1,250 µl
2019-nCov_N1-P	1 µM	0.2 µl	625 µl
RP-F	0.75 µM	0.15 µl	469 µl
RP-R	0.75 µM	0.15 µl	469 µl
RP-P	1 µM	0.2 µl	625 µl
Nuclease-free water		2.5 µl	7,813 µl
Total Volume		**4 µl**	**12,500 µl**

Table 7: PCR Primer Stock Components

Vendor	Item	Catalog number	Quantity	# Reactions
Order one of the following primer and probe sets				
Eurofins Genomics	SalivaDirect™ complete set of 6 primers and probes	12YS-010YST	50-100 nmol	12,500
Integrated DNA Technologies	nCov_N1 Forward Primer Aliquot	10006821	50 nmol	6,2500
		10006830	100 nmol	12,500
	nCov_N1 Reverse Primer Aliquot	10006822	50 nmol	6,250
		10006831	100 nmol	12,500
	NCov_N1 Probe Aliquot	10006831	25 nmol	6,250
		10006832	50 nmol	12,500
	RNaseP Forward Primer Aliquot	10006827	50 nmol	16,600
		10006836	100 nmol	33,300
	RNaseP Reverse Primer Aliquot	10006828	50 nmol	16,600
		10006837	100 nmol	33,300
	RNase P Probe	Custom order (Cy5)	25 nmol	6,250
		Custom order (Cy5)	50 nmol	12,500
		10007061 (ATTO647)	25 nmol	6,250
		10007062 (ATTO647)	50 nmol	12,500
LGC Biosearch Technologies	nCov_N1 Forward Primer	nCoV-N1-F-100	100 nmol	12,500
		nCoV-N1-F-1000	1000 nmol	125,000
	nCov_N1 Reverse Primer	nCoV-N1-R-100	100 nmol	12,500
		nCoV-N1-R-1000	1000 nmol	125,000
	NCov_N1 Probe	nCoV-N1-P-25	25 nmol	6,250
		nCoV-N1-P-250	250 nmol	62,500
	RNaseP Forward Primer	RNP-F-20	20 nmol	6,660
		RNP-F-100	100 nmol	33,300
		RNP-F-1000	1000 nmol	333,300
	RNaseP Reverse Primer	RNP-R-20	20 nmol	6,660
		RNP-R-100	100 nmol	33,300
		RNP-R-1000	1000 nmol	333,300
	RNase P Probe	RNP-PQ670-25	25 nmol	6,250
		RNP-PQ670-250	50 nmol	12,500

Sample Preparation

* For wet swab specimens (swabs in saline or unprocessed swab elution):
1) If samples are frozen, thaw unless no ice crystals are present and then keep on ice, cold block or at 4°C.
2) Pulse vortex each sample and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
3) Wipe the outside of the sample tube with 70% ethanol.

For dry swab specimens:
1) Wipe the outside of the sample tube with 70% ethanol.

Sample Inactivation

1) Place the inactivation heater (a thermal cycler, water bath, dry heat bath or equivalent) in the BSC, turn on, and set the temperature to hold at 100°C.
2) * For wet swab specimens: transfer 1 mL or available volume of each sample to an appropriately labeled 1.5 mL or 5mL tube and securely cap.
3) * For wet swab specimens: add 10µL per 1 mL sample volume of 100X Inactivation Solution to each sample tube.
4) For dry swab specimens (DO NOT DO FOR WET SWAB SPECIMENS): add 1 mL of 1X Inactivation Solution to each sample tube.
5) Vortex for 30 seconds.
6) Place sample tubes into the inactivation heater for 8 minutes.
7) Remove sample tubes from the inactivation heater and let cool at room temperature for 10 minutes.
8) Place sample tubes on ice, in the refrigerator, or on a cold block at 4°C until ready to perform amplification reaction.
   Note: Testing of inactivated specimens must be conducted the same day inactivation is performed. For long term storage, keep the original specimen at ≤-70°C.

Preparing to Run Assay for the First Time

Note: Any instrument running the FloodLAMP EasyPCR™ COVID-19 Test must be calibrated for the following dyes: FAM and Cy5.

Download the Template Run File

The Template Run File contains all the parameters preconfigured to run the FloodLAMP EasyPCR™ COVID-19 Test. These parameters can be seen in more detail under "Create the Run File ..." headings below.

To download the Template Run File:
1) Go to www.floodlamp.bio/euas

2) Download the Template Run file(s) for the instrument type and assay to be run.

Table 7: RT-PCR Instrument Template Run Files

Instrument	Setup Template Filename
ABI QuantStudio™ 7 Pro	FloodLAMP_QS7Pro_PCR_template_run.edt
ABI QuantStudio™ 6 Flex	FloodLAMP_QS6Flx_PCR_template_run.edt
Bio-Rad CFX96 Touch™	FloodLAMP_BRCFX_PCR_protocol.prcl
	FloodLAMP_BRCFX_PCR_template_run.pltd

Note: Template Run Files only need to be downloaded once upon first use.

Alternatively Create the Template Run File on QuantStudio™ 6 Flex or 7 Pro

1) Open the Design and Analysis Software.
2) Select the "SET UP PLATE" option.
3) From the sidebar on the screen, select the following properties to filter:
    a) Instrument – choose the appropriate instrument
    b) Block – choose the appropriate block
    c) RunMode – Standard
    d) Analysis options are left blank
4) From the plate sections present on the screen, select the correct System Template and the system will automatically navigate to the "Run Method" tab.
    a) "Presence/Absence" for QuantStudio™ 7 Pro
    b) "Presence-Absence Standard Pre PCR Post" for QuantStudio™ 6 Flex
5) Change Run Parameters as shown below:

- Run Method:

- 20µL Rxn Vol.
- 105° C Heated Cover Temp
- Ramp Rate: 1.6° C/s

Table 8 : Thermal cycling and plate read steps for QuantStudio™ Systems for PCR

Stage	Temperature	Time	Reps
1	52° C	10 min	1
2	95° C	2 min	1
3	95° C	10 sec	44
	55° C *	30 sec	
4	55° C **	30 sec	1

** This step should be the optical read step*
*** This step is only required for QuantStudio™ 6 Flex*

- Plate Setup

- Targets: FAM (N1) & Cy5 (RP)
- Quencher: None

- Passive Reference: ROX

6) Once done setting up the template, go to "Actions" in the top right corner and hit "Save As":
   a. On Connect: Save to template folder.
   b. Offline: Save to preferred location.

Create the Template Run File on Bio-Rad CFX96 Touch™

1) Launch the CFX96 Touch™ software package.
2) In the Startup Wizard pop-up window select the instrument "CFX96" from the drop down menu.
3) Under "Select Run Type" press the "User-defined" button.
4) Create a new thermocycler protocol by selecting "Create New" from the Run Setup window.
5) Under Tools in the top left toolbar select "Run Time Calculator" and check "96 Wells-All Channels".
6) Make the following changes to the cycling conditions in the Protocol Editor:
   - Sample Volume to 20 µL
   - Lid Setting to 105°C
   - Change cycles to be as shown below:

Table 9 : Thermal cycling and plate read steps for the Bio-Rad CFX96 Touch™

Stage	Temperature	Time	Reps
1	52° C	10 min	1
2	95° C	2 min	1
3	95° C	10 sec	44
	55° C *	30 sec	

** This step should be the optical read step*

7) Click "OK" to save the protocol as type Protocol File (*.prcl) and return to the Protocol tab in Run Setup.

Create the Plate Layout Map

QuantStudio™ 6 Flex or 7 Pro Option 1: Sample Name Input

For this option, sample names (i.e. specimen IDs) are directly input into the instrument software prior to starting the run.

1) Open template in Design and Analysis app and go to the "Plate Setup" tab.
2) On the right side of the screen ensure the "Samples" tab is highlighted and press the addition icon to add the number of samples being tested.
3) Click on the "Sample 1" box to rename the sample.
   a) Repeat this step for all subsequent samples being entered.

4) Click the well located in the plate map then check the box next to the sample name from the right side bar to associate the name to the well.
    a) There is also the option to highlight the well location in the plate map and click on the "Enter sample" box. Enter the sample ID and press tab to continue to the next well in the plate map. This will automatically load the sample name into the sidebar.
5) Once the sample names have been entered, the wells may be highlighted by left clicking the mouse over the starting well and dragging the mouse across all wells associated in run. The targets are then chosen by clicking the check boxes next to each target in the sidebar.
    a) FAM & Cy5 targets should be chosen and named "N1" and "RP" respectively.
6) Once done setting up the template, go to "Actions" in the top right corner and hit "Save As," a pop-up window will appear directing the user to title the file according to information pertaining to the sample run.
    a) Connect: Save to the desired folder (only applicable for 7 Pro).
    b) Offline: Save to a USB that is inserted into the computer.
7) Use your plate layout to load your samples and controls after preparing the amplification reaction mix.

QuantStudio™ 6 Flex or 7 Pro Option 2: Lookup Based on Well Position

For this option, a single generic sample name is applied to all wells, and subsequently, outside of the instrument software, the results are linked to the actual sample name via a lookup table to the well position.

1) Open the template in the Design and Analysis app and go to the "Plate Setup" tab.
2) Highlight the entire plate and add 1 sample to all wells, with the same sample name in every well.
3) Once the sample name has been entered, the targets are chosen by clicking the check boxes next to each target in the sidebar.
    a) FAM & Cy5 targets should be chosen and named "N1" and "RP" respectively.
4) Go to "Actions" in the top right corner and hit "Save As" and name the Template Run File as desired and the software will automatically save as a .edt file.
    a) Connect: Save to desired location (only applicable for QuantStudio 7 Pro).
    b) Offline: Save to a USB that is inserted into the computer.

This process only needs to be done once – all subsequent runs can use the same Template Run File.

Bio-Rad CFX96 Touch™:

1) Launch the CFX96 Touch™ software package and open the correct protocol template.
2) Review the details of the protocol. If correct, click "Next" to proceed to the Plate tab.
3) On the Plate tab, click "Create New" and the Plate Editor appears.

4) Use the Plate Editor to create a new plate.
5) To ensure the correct plate size is selected, go to "Settings > Plate Size" and check that 96-well or 384-well is selected from the drop-down menu.
    a) The plate size selected must correspond to the block size of the instrument being used.
6) To set the plate type, select" Settings > Plate Type" and select "BR Clear" or "BR White" from the drop-down menu.
    a) The plate type selected should match the plate type used in the run.
7) To set the scan mode, select the "All Channels" scan mode from the Scan Mode drop-down list in the Plate Editor toolbar.
8) Select the "Select Fluorophores" button on the upper right of the Plate Editor window
    a) De-select all default fluorophores and select "FAM" and "Cy5" and click OK.
9) In the Plate Editor window highlight the whole plate and click the checkbox in front of FAM and Cy5.
10) Select the "Experiment Settings" button in order to define the Targets.
    a) In the lower left of the Experiment Settings window in the New box type in "N1" and select "Add".
    b) Repeat this step and type in "RP".
    c) Select "OK".
11) In the Plate Editor window next to FAM in the drop-down menu under Target Name select "N1" and for Cy5 select "RP".
12) Click OK to save changes and close the "Select Fluorophores" dialog box.

Bio-Rad CFX96 Touch™ Option 1: Sample Name Input

For this option, sample names (i.e. specimen IDs) are directly input into the instrument software prior to starting the run.

1) Load the appropriate sample type to each well by selecting the well and selecting the appropriate Sample Type (Unknown, NTC, or Positive Control) from the drop-down menu.
2) Multiple wells can be selected at once to load the sample type.
    a) Note: The EC can be listed as an Unknown sample.
3) In the "Target Names" section confirm that the necessary fluorophores are assigned to each well.
4) Name each well by typing in the sample name and pressing "Enter" in the Sample Names dropdown list in the right pane.
5) Click OK to save the Plate File (*.pltd)and return to the Plate tab in Run Setup. When prompted, specify a name for the plate and a save location.

Bio-Rad CFX96 Touch™ Option 2: Lookup Based on Well Position

For this option, a single generic sample name is applied to all wells, and subsequently, outside of the instrument software, the results are linked to the actual sample name via a lookup table to the well position.

1. Name the file as desired and save as type "Plate File (*.pltd)"
2. Select "Save", click "OK" in the Plate Editor window and exit the software.

This process only needs to be done once – all subsequent runs can use the same Template Plate File.

PCR Amplification Reaction Preparation

1) Place a 96-well PCR plate or PCR strip tubes onto a cold block or ice.
2) Thaw frozen reagents until ice crystals are not present.
3) Pulse vortex thawed reagents for 3 seconds and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
4) Store on ice or in the refrigerator or on a cold block at 4°C until ready to use.
5) Prepare the PCR Amplification Reaction Mix by combining the components listed below in Table 10.
   NOTE: Component volumes should be scaled proportionally for the number of reactions.
6) Vortex the PCR Amplification Reaction Solution for 10 seconds and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
7) Add 18 µL of the PCR Amplification Reaction Solution into the wells of the PCR plate or PCR strip tubes.

Table 10: PCR Amplification Reaction Mix

Component	Volume (1 reaction)	Volume (1 reaction x 100) 1 x 96-plate w/ 4% overage
5X PCR Primer Stock	4 µL	400 µL
Nuclease-free Water	3 µL	300 µL
PCR Master Mix*	10 µL	1000 µL
PCR RT*	1 µL	100 µL
SUBTOTAL VOLUME	**18 µL**	**1800 µL**
Sample	2 µL	
REACTION VOLUME	**20 µL**	

* in Luna® Universal Probe One-Step RT-qPCR Kit

Sample Addition

NOTE: Ensure that positive and negative controls are included in each batch run (i.e. in each PCR plate or group of strip tubes that are heated together).

1) Add 2 µL of each sample into a separate tube in the amplification reaction PCR plate or strip tubes.
2) Mix by pipetting.
3) If using PCR plate, seal with optical film (optionally using heat sealer). If using PCR strip tubes, cap strips.
4) Pulse vortex and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
5) Continue to section "Run the Assay".

Run the Assay

Refer to Specific Instrument User Manuals for full system usage and maintenance details.

On QuantStudio™ 6 Flex

1) To transfer templates from a USB drive plug a USB drive into the USB port below the touchscreen.
2) If the instrument is in standby, touch the touchscreen to activate it and then press the green power icon.
3) In the Main Menu, press "View Templates".
4) In the Browse Experiments screen, select the template:
    a) Press the Folder icon, then choose "USB".
    b) Press the desired template, then press "Save".
5) In the Save Experiment As screen, set the name for the file.
    a) Press the "New Template Name" field, then enter a name for the copied file.
    b) Press the "Save to Folder" field, then select the folder to receive the file
    c) Hit "Save".
6) Press the Home icon to return to the Main Menu.
7) Navigate to the Main Menu screen, then press the red eject icon.
8) When the side door opens, load the appropriate plate or PCR strips. Ensure that the consumable is properly aligned in the holder.
9) In the Main Menu, press "Browse Experiments".
10) In the Experiments screen, choose the desired experiment and then click the Folder icon to choose where to save the experiment.
11) Then press "Start Run" to start the run immediately.

On QuantStudio™ 7 Pro

1) Log into user on instrument.
2) USB: Plug in USB with saved template on it.
3) From the options on the instrument's screen press "Load plate file".
    a) The QuantStudio™ 7 is a touchscreen device.
4) From the "Run Queue" screen,

   a) USB: press “USB drive” on the left side.
   b) Connect: press Cloud icon on the left side.
5) This will bring up any plate files saved.
6) Press the plate file associated with the run to be performed.
7) A new window will appear requesting location of results once the run is complete.
   a) Connect: Press the “Cloud Connect” icon, press again to verify location the files will be uploaded to and then press “Done”.
   b) USB: Press the “USB drive Connected” if the icon is not already highlighted and press “Done”.
8) Press the double-arrow icon located at the top right sided corner of the screen on the instrument.
   a) The instrument drawer will open from the front.
9) Place the plate/strips into the plate holder ensuring proper orientation of the plate.
   a) A1 well should be in the position of the top left corner.
   b) The plate/strips will appear slightly suspended above the block due to two silicone strips above and below this plate. This is to be expected and the instrument lid will press the plate down once the drawer has closed.
10) Press “Start Run” on the screen of the instrument.
   a) A pop-up window will appear asking the user to confirm the plate has been loaded.
   b) If the plate has been loaded, press “Start Run” again or press “Open Drawer” to place the plate into the block and then press “Start Run”.

On Bio-Rad CFX96 Touch™

1) Open the correct .pcrl file and review the protocol details. If correct click “Next” to proceed to the Plate tab.
2) When prompted, open the correct .pltd file and review the plate details in the Run Information section.
3) Select the checkbox for the appropriate block (CFX96 or CFX384) on which to perform the run.
4) To insert the plate or 8-tube strips into the block, click Open Lid.
5) Insert the plate or 8-tube strips into the block. Ensure the plate or 8-tube strips are properly oriented.
6) Click Close Lid.
7) Click Start Run at the bottom right of the screen.
8) When prompted, save the data file (.pcrd) to the desired location.

Analyzing Data

Exporting Data from QuantStudio™ 6 Flex or 7 Pro

Using USB

1) Confirm Quant says “File Transferred - USB”.
2) Take USB from Quant and plug it into computer.
3) Export data off of USB onto computer.

Using Cloud Connect with QuantStudio™ 7 Pro

1) Go to Cloud Connect and log in.
2) Go to files and find the data that was just uploaded by the Quant, it will be in the folder chosen previously chosen while running the Quant.
3) Download .xlsx file.

Exporting Data from Bio-Rad CFX96 Touch™

1) After the run has completed, open the data file (.pcrd) by going to Select File > Open > Data File in the Home window and locating the desired data file. Adjust the following settings as described below.
2) Select Settings > Cq Determination mode and select Single Threshold.
3) Select Settings > Baseline Setting and select Baseline Subtracted.
4) Select Settings > Analysis Mode and select analysis by fluorophore.
5) Select Settings > Cycles to Analyze and the Cycles to Analyze dialog box appears. Confirm that all cycles are being analyzed and click “OK”.
6) Cq values of each well are displayed in the Quantification Data tab.
7) Export .xlsx files and select Export > Export all Data Sheets to Excel (Cq values are available in "Quantification Plate View Results").

Compiling Results Option 1: Lookup Based on Well Position

For this option, outside of the instrument software the results are linked to the actual sample name via a lookup table to the well position. An example spreadsheet to perform this lookup and results compilation is available with instructions at www.floodlamp.bio/euas.

Compiling Results Option 2: Sample Name Input

For this option, sample names (i.e. specimen IDs) are directly input into the instrument software prior to starting the run. Open the results file and continue to “Analyzing Data” section to score results.

Results Interpretation

Test Controls

All test controls should be examined prior to interpretation of patient specimen results. If the controls are not valid and the expected result, the specimen results cannot be interpreted. Target results for the controls will be interpreted according to Table 5 below.

1) The "No Template" (Negative) Control (NTC) should yield a negative "not detected" result for both the N1 and RNaseP targets.

2) The Positive Template Control should yield a positive "detected" result for the N1 target and a negative "not detected" for the RNaseP control.

3) The Internal Process Control should yield a positive "detected" result for RNaseP. Detection of RNaseP is required to report a negative SARS-CoV-2 result.

If the negative and positive controls do not appear as expected, the specimen results of the corresponding plate or batch should be considered invalid. In the event of a failure of either the positive or negative control, the lab should discard some or all of the consumables utilized for associated run, including the filter tips, tubes, plates, seals, and aliquots of reagents. Additionally, all pipettes, BSC, and appropriate lab surfaces should be thoroughly cleaned with freshly made 10% bleach solution, 70% ethanol, and (optionally) RNAseZAP™ product. In the event of the failure of the positive control, the working aliquot of positive control material should be discarded. Additionally, the lab should review the expiration of the batch of positive control aliquots and verify their integrity by performing qualification reactions of one or more positive control aliquots. If controls continue to fail, labs should not perform additional tests on clinical specimens or report results. Invalid test results should be repeated by performing another amplification reaction.

Patient Specimen Results Interpretation

NOTE: Patient specimen results can only be interpreted if the positive and negative controls in the plate or group of strip tubes have the expected results.

Use Table 11 below to assign a result to each sample.

Table 11: Interpretation of Assay Results

ABI QuantStudio™ 7 Pro		
Result	**Ct Value: N1**	**Ct Value RP**
Positive	<38.0	Any Value
Negative	≥38.0	<35.0
*Invalid	≥38.0	≥35.0

Bio-Rad CFX96 Touch™ ABI QuantStudio™ 6 Flex		
Result	**Ct Value: N1**	**Ct Value RP**
Positive	<40.0	Any Value
Negative	≥40.0	<35.0
*Invalid	≥40.0	≥35.0

*Invalid test results should be repeated by rerunning the primary sample if available, otherwise the inactivated sample. Results from retested samples will follow the same interpretation as listed in Table 11.

If the final interpretation of the test result is invalid, then "Invalid/Inconclusive" should be reported and retesting of the individual is recommended.

Performance Evaluation

Analytical Sensitivity: Limit of Detection (LoD)

The Limit of Detection (LoD) for the FloodLAMP EasyPCR™ COVID-19 Test was established using gamma-irradiated SARS-CoV-2 virus cell lysate (BEI NR-52287) spiked into negative real specimens. The gamma-irradiated virus was spiked into the specimen prior to the heat inactivation step, and carried through the entire assay. The concentration of spike was such that the contrived positive sample was at 100,000 copies/mL after the inactivation step. The stock contrived positive was diluted into inactivated negative sample matrix to produce the concentrations for the LoD study. A preliminary LoD run was performed using the concentrations ranging from 100,000 copies/mL to 3,100 copies/mL. Concentrations of 6,300, 3,100 and 1,600 copies/mL were selected for confirmatory LoD runs. LoD run details are provided in Supporting Data, with the results summarized below in Table 12. The LoD, defined as the concentration at which at least 95% of the samples are positive, was determined at 3,100 copies/mL.

Table 12: Confirmatory LoD Data Results

Instrument	LoD	Positive Replicates
ABI QuantStudio™ 7 Pro	3,100 copies/mL	95% (20/21)
ABI QuantStudio™ 6 Flex	3,100 copies/mL	100% (21/21)
Bio-Rad CFX96 Touch™	3,100 copies/mL	95% (20/21)

Analytical Sensitivity: Inclusivity

FloodLAMP EasyPCR™ COVID-19 Test includes a modified RT-qPCR assay by duplexing the previously authorized CDC N1 and human RNase P primer-probe sets. Inclusivity was tested in the original US CDC EUA with all publicly available SARS-CoV-2 genomes as of 1 February 2020. The initial analysis showed 100% homology between the N1 primer-probe set and

available genomes, except for one low frequency mismatch with the N1 forward primer. However, this was not expected to affect performance of the primer-probe set due to annealing temperatures of 55°C which tolerate 1-2 mismatches. Indeed, performance of the N1 primer-probe set was shown to be high in the previous comparison of primer-probes sets (https://www.nature.com/articles/s41564-020-0761-6). GISAID continuously evaluates mismatches between newly available SARS-CoV-2 genomes and primer-probe sets and confirms a low frequency of nucleotide mismatches (<5%) with the N1 primer-probe set.

Analytical Specificity: Cross-Reactivity

The primer and probe sets used in FloodLAMP's duplex assay were developed by the US CDC and have been EUA certified. The CDC reported no cross-reactivity with other human coronaviruses (229E, OC43, NL63, and HKU1), MERS-coronavirus, SARS-coronavirus, and 14 additional human respiratory viruses (see https://www.fda.gov/media/134922/download).

Analytical Specificity: Interfering Substances

Exogenous and endogenous substances were tested for potential interference with the FloodLAMP EasyPCR™ COVID-19 Test. Gamma-irradiated SARS-CoV-2 virus cell lysate (BEI NR-52287) was spiked onto dried AN swab specimens to produce contrived Positve Controls. Negative Control dried swabs obtained simultaneously were confirmed to be SARS-CoV-2 negative by PCR using the CDC primers. The gamma-irradiated SARS-CoV-2 virus and interfering substances were spiked into the dried swabs prior to the heat inactivation step, and carried through the full test protocol.

All interfering substance testing showed no disagreement with expected positive and negative results, as shown in Table 13.

Table 13: Interfering Substances Results

Interfering Substance	Active Ingredient	Concentration	% Agreement with Expected Results	
			Positive Control Spiked	Negative Control Unspiked
Blood	N/A	1% v/v	100% (3/3)	100% (3/3)
Nasal Congestion Spray	Acetaminophen, Guaifenesin, Phenylephrine HCl	20% v/v	100% (3/3)	100% (3/3)
Nasal Allergy Spray	Oxymetazoline HCl	15% v/v	100% (3/3)	100% (3/3)
Lozenges	Menthol	10% w/v	100% (3/3)	100% (3/3)
Mucin	N/A	0.5% w/v	100% (3/3)	100% (3/3)

Clinical Evaluation

The clinical evaluation of the FloodLAMP EasyPCR™ COVID-19 Test utilized confirmed clinical anterior nares swab specimens. 40 positive and 40 negative clinical specimens were evaluated and compared to a high sensitivity EUA authorized test run on the original fresh samples. The FloodLAMP EasyPCR™ COVID-19 Test showed a positive agreement of 97.5% and a negative agreement of 100%. The single false negative result was a specimen with a high Ct value as previously measured by the comparator test, indicating low viral load. A summary of the clinical performance is shown below in Table 14.

Table 14: Clinical Evaluation Results

FloodLAMP EasyPCR™ COVID-19 Test Results	Comparator - High Sensitivity EUA Authorized Test		
	Positive	Negative	Total
Positive	39	0	39
Negative	1	40	41
Total	40	40	80
Positive Agreement	97.5% (39/40) 95% CI: 86.8% to 99.9%		
Negative Agreement	100% (40/40) 95% CI: 91.2% to 100%		

Support

FloodLAMP Biotechnologies, PBC support center
eua.support@floodlamp.bio
650-394-5233

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