EUA Submission - FloodLAMP EasyPCR™ COVID-19 Test

A. PURPOSE FOR SUBMISSION

Emergency Use Authorization (EUA) request for distribution and/or use of the **FloodLAMP EasyPCR™ COVID-19 Test** for the *in vitro* qualitative detection of RNA from the SARS-CoV-2 in in upper respiratory specimens including oropharyngeal and 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**. Additional testing and confirmation procedures should be performed in consultation with public health and/or other authorities to whom reporting is required. Test results should be reported in accordance with local, state, and federal regulations.

B. MEASURAND

Specific nucleic acid sequences from the genome of the SARS-CoV-2, targeted by the previously certified **2019-nCoV_N1 primers and probe set as part of the CDC qPCR assay**. Primer names and sequences are listed in Table 1.

Table 1: 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**

C. APPLICANT

FloodLAMP Biotechnologies, a DE Public Benefit Corporation

Mailing Address:
4860 Alpine Rd.
Portola Valley, CA 94028

Laboratory Address:
930 Brittan Ave
San Carlos, CA 94070

Randall J. True, CSO
Phone: (415) 269-2974
Email: randy@floodlamp.bio

D. PROPRIETARY AND ESTABLISHED NAMES

Proprietary Name - **FloodLAMP EasyPCR™ COVID-19 Test**
Established Name - **FloodLAMP EasyPCR™ COVID-19 Test**

E. REGULATORY INFORMATION

Approval/Clearance Status:
The **FloodLAMP EasyPCR™ COVID-19 Test** is not cleared, CLIA waived, approved, or subject to an approved investigational device exemption.

Product Code:

QJR

F. PROPOSED INTENDED USE

1) 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**. 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.

2) Special Conditions for Use Statements:

For Emergency Use Authorization (EUA) only
For prescription use only
For in vitro diagnostic use only

3) Special Instrument Requirements:

The **FloodLAMP EasyPCR™ COVID-19 Test** test is to be used with the RT-PCR instruments listed in Table 2.

Table 2. RT-PCR Instruments validated for test

Manufacturer	Instrument
Thermo Fisher Scientific	Applied Biosystems QuantStudio™ 6 Flex
Thermo Fisher Scientific	Applied Biosystems QuantStudio™ 7 Pro
Bio-Rad	CFX96 Touch™ Real-Time PCR Detection System

Designated laboratories will receive an FDA accepted instrument qualification protocol included as part of the **FloodLAMP EasyPCR™ Covid-19 Test** IFU and will be directed to execute the protocol prior to testing clinical samples. Designated laboratories must follow the authorized IFU, which includes the instrument qualification protocol, as per the letter of authorization.

G. DEVICE DESCRIPTION AND TEST PRINCIPLE

1) Product Overview/Test Principle:

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 scale because 1) it does not require nucleic acid extraction equipment, 2) it utilizes reagents and supplies readily available in large quantities, and 3) is a very 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.

2) Description of Test Steps:

Specimens including **nasopharyngeal swabs, anterior nasal and mid-turbinate nasal swabs** are collected in **sterile collection tubes.** Swabs are transported and stored dry prior to processing. At the laboratory, an inactivation solution at 1X containing TCEP (2.5 mM), EDTA (1 mM), and NaOH (11 mM) in 0.9% saline (154 mM) is added to the container with the swab, at the volume of 1 ml. Alternatively, for swabs that are collected or eluted in a saline solution or equivalent, the inactivation solution at 100X concentration should be added at 1/100th the sample solution volume.

The container with the specimen and inactivation solution is mixed by vortexing for 30 seconds. Subsequently, the container is heated in a 95°C water bath or dry heat block for 8 minutes. The now inactivated specimen container is allowed to cool at room temperature for 10 minutes and then stored on ice or at 4°C until amplification.

An amplification reaction mix (18 µl) is prepared per manufacturer's specifications, containing the New England Biolabs Luna® PCR Master Mix (New England Biolabs E3006, 10 µl), New England Biolabs Luna® RT (New England Biolabs E3006, 1 µl), a primer solution (4 µl of 5X PCR primer stock w/ N1-F & N1-R at 2 µM, N1-P & RP-P at 1 µM and RP-F & RP-R at 0.75 µM), and nuclease-free water (3 µl).

2µl of the inactivated sample is added to 18µl of the amplification reaction mix. The reaction is then run with the following thermocycler conditions in Table 3:

Table 3: Thermal cycling conditions and plate read steps

Step	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*

3) Control Materials to be Used with FloodLAMP EasyPCR™ COVID-19 Test:

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 4 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 4. Components for Positive Template Control

Material	Supplier	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	4,895 µL

H. INTERPRETATION OF RESULTS

1) FloodLAMP EasyPCR™ COVID-19 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.

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

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

c) 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.

2) Examination and Interpretation of Patient Specimen Results:

Assessment of clinical patient specimen test results should be performed after the positive, negative, and internal controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Patient specimen results will be interpreted according to Table 5 below.

Table 5: Interpretation of Patient Specimen 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 will be repeated by rerunning the primary sample if available, otherwise by rerunning the inactivated sample. Results from retested samples will follow the same interpretation as listed in Table 5.

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

I. PRODUCT MANUFACTURING

1) Overview of Manufacturing and Distribution:

The **FloodLAMP EasyPCR™ COVID-19 Test** utilizes standard chemicals available from multiple vendors, with the exception of the New England Biolabs Luna® Universal Probe One-Step RT-qPCR Kit.

The **FloodLAMP EasyPCR™ COVID-19 Test** utilizes the same primer and probes as the EUA authorized SalivaDirect™ test, which derive from the CDC primer set. These are available in very large quantities with immediate distribution from multiple vendors including Eurofins Genomics, Integrated DNA Technologies, and LGC Biosearch.

New England Biolabs has expressed strong support for the **FloodLAMP EasyPCR™ COVID-19 Test** and FloodLAMP's other open source protocol EUA submissions that incorporate their products. New England Biolabs has very large quantities of the Luna® Universal Probe One-Step RT-qPCR Kit prepared and ready for immediate distribution, typically with 24 hour shipping. Their manufacturing capacity is among the largest in the United States and can surge to meet increased demand.

***Under the Emergency Use Authorization (EUA) any of the 21 CFR Part 820 Quality System Regulation (QSR) requirements can be waived for the duration of the EUA but FDA recommends that developers follow comparable practices as much as possible if such requirements are waived. Among other things, FDA may consider previous compliance history when determining whether or not to waive certain QSR requirements for a specific product. Please note adverse events, as per 21 CFR Part 803, have to be reported for authorized devices (see Section P).**

2) Components Included with the Test

None. Designated CLIA labs may order components directly from vendors.

3) Components Required But Not Included with Test:

The **FloodLAMP EasyPCR™ COVID-19 Test** is to be used with the following reagents or equivalents listed in Table 6.

Table 6: Validated reagents used with Test

Item	Concentration	Chemical Composition	Vendor	Catalog Number
TCEP	.5 M	tris(2-carboxyethyl)phosphine hydrochloride	Sigma-Aldrich Millipore	646547-10X 1ML
EDTA	.5 M	Ethylenediaminetetraacetic acid	Thermo Fisher	15575020
NaOH	10 N	Sodium Hydroxide	Sigma-Aldrich	SX0607N-6
Nuclease-free Water		Ultrapure Water, DNAse RNAse free	Thermo Fisher	10977015
NaCl	5M	Sodium Chloride	Thermo Fisher	24740011
PCR MasterMix *		2X PCR Master Mix (in Luna® Universal Probe One-Step RT-qPCR Kit)	New England Biolabs	E3006
PCR RT *		Reverse Transcriptase (in Luna® Universal Probe One-Step RT-qPCR Kit)	New England Biolabs	E3006

* Item may not be substituted for equivalents. Only the specified vendor and catalog number may be utilized.

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 7. Equivalent preparations utilizing components with different source concentrations may be used such that the final 100X Concentration is achieved.

Table 7: 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 8. 1X Inactivation Saline Solution should be kept at room temperature and used within 24 hours of preparation from components or 100X Inactivation Solution.

Table 8: 1X Inactivation Saline Solution

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

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 9 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 10.

Table 9: 5X PCR Primer Stock Preparation from Eurofins Genomics Product

Component (final concentration)	Volume (1 reaction)	Volume (3,125 reactions)
2019-nCov_N1-F (10 µM)	0.4 µl	1,250 µl
2019-nCov_N1-R (10 µM)	0.4 µl	1,250 µl
2019-nCov_N1-P (5 µM)	0.2 µl	625 µl
RP-F (3.75 µM)	0.15 µl	469 µl
RP-R (3.75 µM)	0.15 µl	469 µl
RP-P (5 µM)	0.2 µl	625 µl
Nuclease-free water	2.5 µl	7,813 µl
TOTAL VOLUME	**4 µl**	**12,500 µl**

Table 10: Primer and Probe Set Products

Vendor	Item	Catalog number	Quantity	# Reactions
Order one of the following primer and probe sets				
Eurofins Genomics	SalivaDirect™ complete set of the 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

The final amplification reaction components are listed in Table 11. PCR plates or strip tubes used for the amplification reactions should be maintained on ice or a cold block.

Table 11: PCR Amplification Reaction

Component	Volume (1 reaction)	Volume (100 reactions)
5X PCR Primer Stock	4 µL	400 µL
New England Biolabs PCR MM	10 µL	1000 µL
New England Biolabs PCR RT	1 µL	100 µL
Nuclease-Free Water	3 µL	300 µL
SUBTOTAL VOLUME	**18 µL**	**2250 µL**
Sample	2 µL	
REACTION VOLUME	**20 µL**	

4) Software Validation

The **FloodLAMP EasyPCR™ COVID-19 Test** has been validated on the RT-PCR instruments listed in Table 2 using the baseline threshold settings unless otherwise noted in the Instructions for Use. The test does not require any additional software.

5) Testing Capabilities

The **FloodLAMP EasyPCR™ COVID-19 Test** has been optimized for a robust, streamlined workflow and for rapid turnaround time on results. The total time to perform the test is dependent upon the following factors: number of lab technicians, batch size of samples, and in advance preparation of reaction mixes. The minimum turnaround time is approximately 1 hour and 45 minutes, of which approximately 1 hour and 20 minutes is the RT-PCR instrument run time. Automation can greatly increase overall throughput.

6) Reagent Stability:

A stability test plan for the components of the **FloodLAMP EasyPCR™ COVID-19 Test** will be developed during an interactive review. Briefly, the proposed study includes assessing all prepared solutions including: 100X Inactivation Solution, 1X Inactivation Saline Solution, 5X PCR Primer Stock, and the full PCR Amplification Mix. Prepared solutions will be assessed both for long term storage stability (typically 1-3 months at -20ºC) and short term storage stability prior to usage (typically hours to several days at room temperature, 4ºC or -20ºC).

The proposed study uses a contrived positive sample consisting of gamma-irradiated SARS-CoV-2 virus cell lysate (BEI NR-52287) spiked into negative specimens at approximately 50,000 copies/mL (4X LoD). The contrived positive stability study samples will be prepared, aliquoted and stored at -80ºC to permit repeated testing of the various solutions at the appropriate step of the test protocol.

For test components supplied by vendors, such as New England Biolab's Luna® Universal Probe One-Step RT-qPCR Kit, the manufacturer's recommended storage conditions and duration will be followed.

7) Sample Stability:

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 ≤-70ºC.

J. PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical Sensitivity:

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/mLwere 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	Mean Ct Value (SD)
ABI QuantStudio™ 7 Pro	3,100 copies/mL	95% (20/21)	36.5 (.8)
ABI QuantStudio™ 6 Flex	3,100 copies/mL	100% (21/21)	36.9 (1.1)
Bio-Rad CFX96 Touch™	3,100 copies/mL	95% (20/21)	37.2 (.9)

2) Inclusivity (analytical sensitivity):

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.

3) Cross-reactivity (Analytical Specificity):

The primer and probe sets used in the 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).

Endogenous Interference Substances Studies:

Exogenous and endogenous substances were tested for potential interference with the **FloodLAMP EasyPCR™ COVID-19 Test.** 10 µL of each stock of interfering substance was spiked on dried AN swab specimens. A contrived positive control was produced by spiking gamma irradiated SARS-CoV-2 virus cell lysate (BEI NR-52287) onto dried AN swab specimens. 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. The contrived Positive Control Spiked comprised 20 µL of 8e6 copies/mL irradiated virus stock spiked in, producing after elution of the swab in 1 mL of Inactivation Saline Solution at most a concentration of 160,000 copies/mL in the sample input into the amplification reaction.

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

Table 13: Interfering Substances Results

Interfering	Active	Concentration	% Agreement with Expected Results

Substance	Ingredient		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)

4) 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 Tes**t 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 very low viral load. A summary of the clinical performance is below in Table 14.

Anterior nares swab specimens were collected from patients in phosphate buffered saline by the Stanford COVID-19 clinical testing program. Specimens were initially tested by the Stanford clinical laboratory using the Hologic Panther Fusion and Aptima SARS-CoV-2 Assays, which serves as the high sensitivity comparator test.

For the **FloodLAMP EasyPCR™ COVID-19 Test**, materials and the Instructions For Use were provided to the Stanford clinical laboratory. The materials provided consisted of the validated reagents listed in Table 6, the Eurofins Genomics primers and probes, and an aliquot of the positive control. The Bio-Rad CFX96 instrument was used to perform the RT-PCR. After thawing the frozen specimens, 1 mL of each specimen was transferred to 5mL tubes for the inactivation step. The positive and negative clinical specimens were assigned a new ID in a random order, then transferred to new tubes that were barcoded and labeled with the new ID. Line Item data are provided in the Supporting Data.

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%		

K. UNMET NEED ADDRESSED BY THE PRODUCT

This section will be completed by FDA.

L. APPROVED/CLEARED ALTERNATIVE PRODUCTS

Currently no methods for the detection of the SARS-CoV-2 have been approved/cleared by FDA.

M. BENEFITS AND RISKS:

This section will be completed by FDA.

N. FACT SHEET FOR HEALTHCARE PROVIDERS AND PATIENTS:

Fact Sheets for Patients and Healthcare Providers attached.

O. INSTRUCTIONS FOR USE/ PROPOSED LABELING/PACKAGE INSERT:

Instructions for Use attached.

P. RECORD KEEPING AND REPORTING INFORMATION TO FDA:

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.

QuantStudio is a trademark of Thermo Fisher Scientific (NYSE: TMO)

Bio-Rad and Bio-Rad CFX96 Touch is a trademark of Bio-Rad Laboratories, Inc. (NYSE: BIO)