

FloodLAMP Test Validation Guide

EasyPCR™ COVID-19 Test

QuickColor™ COVID-19 Test

Excerpts from DRAFT Instructions for Use*

Protocol & Materials for Test Validation

* Tests have been submitted to the FDA but have not been authorized or approved.

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Overview

FloodLAMP's QuickColor™ and EasyPCR™ COVID-19 Tests are streamlined, direct extraction-free molecular assays for the qualitative detection of RNA from the SARS-CoV-2 virus in upper respiratory specimens. They are validated for use with nasal swabs and full EUAs have been submitted to the FDA, including all *in silico*, wet testing, and clinical evaluation data. A summary of the EUA validation data is below. In addition, a pre-EUA for the FloodLAMP Home Collection Kit DTC was submitted on [5-10-2021], describing the clinical study design for unsupervised collection of pooled nasal swabs (up to 5). The clinical study also includes usability testing for the FloodLAMP Mobile App. FloodLAMP will conduct the clinical study under an approved IRB Protocol (from WIRB #20210401) in coordination with a testing program to enrich for asymptomatic positives.

FloodLAMP EUA Submission Validation Summary

	EasyPCR™ COVID-19 Test	QuickColor™ COVID-19 Test
Limit of Detection	3,100 copies/mL	12,500 copies/mL
Clinical Sensitivity	97.5% (39/40)	90.0% (36/40)
Clinical Specificity	100% (40/40)	100% (40/40)

The FloodLAMP QuickColor™ and EasyPCR™ COVID-19 Tests use the same inactivated sample, making validation and troubleshooting more straightforward. The inactivation process consists of adding freshly prepared 1X Inactivation Saline Solution, produced by adding 100X Inactivation Solution to 0.9% Saline. 1 mL of 1X Inactivation Saline Solution is used in each tube of up to 5 dry nasal swabs. The tube is vortexed for 30 seconds to elute the specimen from the swab. Next it's heated at a nominal 95°C for minutes and allowed to cool at room temperature for 10 minutes. The inactivated sample is then ready to be added to a prepared amplification reaction mix.

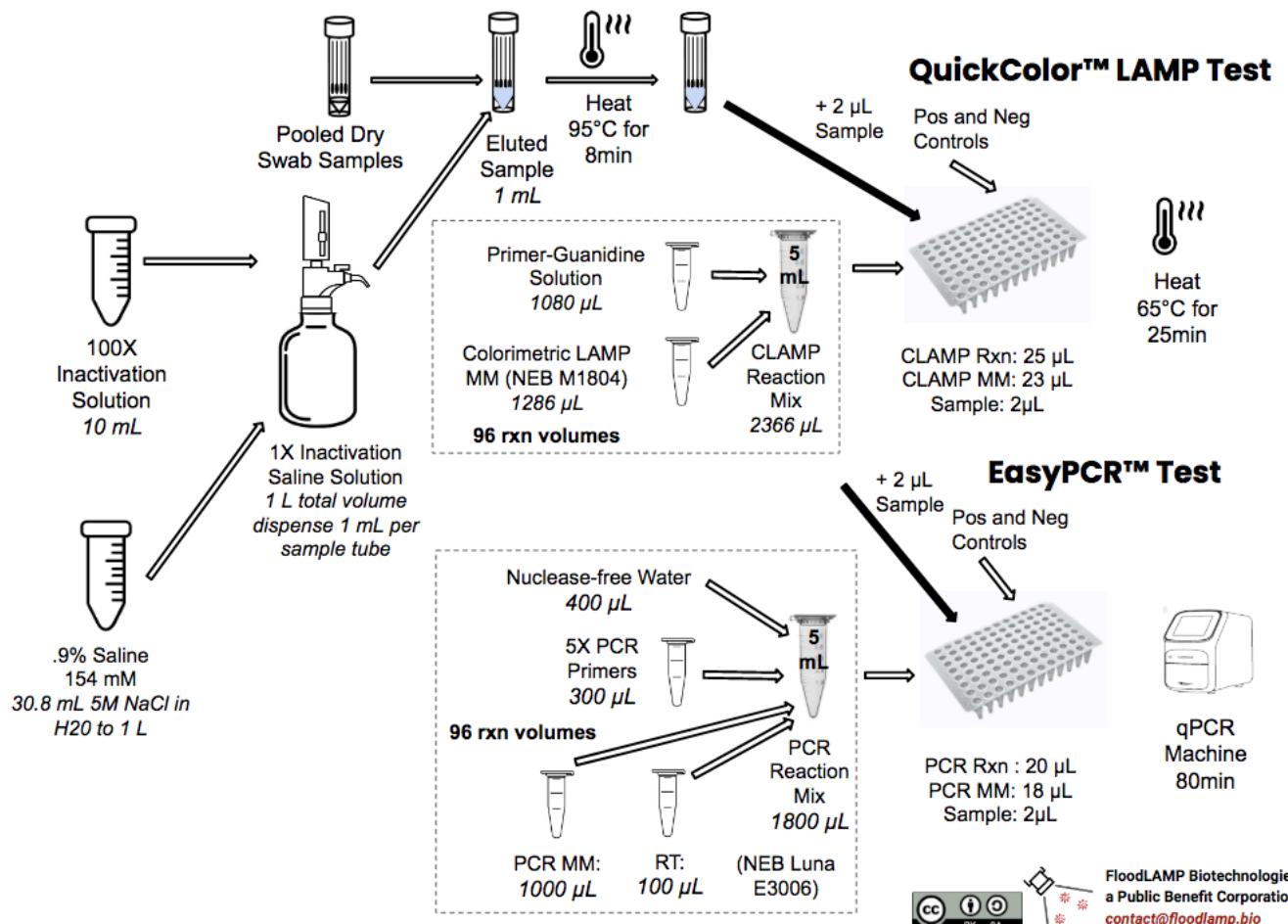
The tests only require a single pipette step and one filter tip per pool and the 1X Inactivation Saline Solution is efficiently dispensed by bottle top dispenser. Small batch sizes can be run in as little as 45 minutes from sample to result for the QuickColor™ test, 1 hour and 40 min for EasyPCR™.

A team of 4 newly trained technicians can process approximately 2,500 tubes per 8 hr shift, totaling 10K people screened in pools of 4. Given the low infrastructure needed to run the QuickColor™ LAMP test, a distributed network of basic labs or processing sites can be supplied from a single lab that prepares ready to use assay plates and controls in bulk.

FloodLAMP's QuickColor™ and EasyPCR™ COVID-19 tests have very low consumable cost, on a per reaction and per person basis. Additionally, both tests are supply chain robust, using readily available chemicals and supplies.



FloodLAMP Direct RNA Assays



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a Public Benefit Corporation
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Validation Runs

The FloodLAMP validation process for the EasyPCR™ and QuickColor™ tests comprises 2 runs:

- **1st Run** - alternating positive and negative amplification controls and only materials provided in the FloodLAMP Validation Kit. 8 reactions in a single strip tube.
- **2nd Run** - inactivation of a contrived positive sample and preparation of dilution series between 100 and 1 cp/ul. Run consists of a preliminary LoD (full dilution series in triplicate) and a confirmatory LoD of 22 reps at 12.5 cp/µL for LAMP QuickColor™ and and 3 cp/µL for EasyPCR™. 48 reactions in each PCR plate.

The contrived positive in the 2nd Run is made by spiking a self-collected nasal swab sample with inactivated virus (provided, gamma-irradiated SARS-CoV-2 virus cell lysate BEI NR-52287). The spiked sample is inactivated along with 3 unspiked samples, which are used together to make a dilution series to be used for the complete preliminary and confirmatory LoD runs (see Dilution Series spreadsheet,



LoD plate map, and GCP100 Batch Record below). A positive and negative amplification control is also included in the 2nd Run.

Validation Kit Prep: Dilution Series

STEP 1) DILUTION SERIES			sample vol/well	2 uL			
Sample Label	cp/uL	reps	Spike Used	Spiked uL	Unspiked uL	Make uL	Final uL
100cp	100	50	100cp	100	0	386	100
50cp	50	50	100cp	286	286	572	100
25cp	25	250	50cp	472	472	944	500
12.5cp	12.5	250	25cp	444	444	888	500
6.3cp	6.3	250	12.5cp	388	388	775	500
3.1cp	3.1	250	6.3cp	275	275	550	500
1.6cp	1.6	50	3.1cp	50	50	100	100
0cp	0	50	None	0	100	100	100
Amount of Sample Used: (uL)						Formulas work from bottom up	
100	3.9	GCP	386			Requires Last to be zero	
500	4.03	NSS		2014			

Process

Step 1) Prep dilution series per above in 1.5ml EDLB tubes in cold block

Vortex all samples 3s and spindown

Add the spiked first then unspiked

Vortex 5s after dilution is made and spindown

Step 2) Run on PCR using PCRP_210427

Step 3) Run on CLAMP using PGS_210427

QuickColor LoD Configuration

Row	A	B	C	D	E	F
1	100cp	100cp	100cp	12.5cp	12.5cp	12.5cp
2	50cp	50cp	50cp	12.5cp	12.5cp	12.5cp
3	25cp	25cp	25cp	12.5cp	12.5cp	12.5cp
4	12.5cp	12.5cp	12.5cp	12.5cp	12.5cp	12.5cp
5	6.25cp	6.25cp	6.25cp	12.5cp	12.5cp	12.5cp
6	3.1cp	3.1cp	3.1cp	12.5cp	12.5cp	12.5cp
7	1.6cp	1.6cp	1.6cp	12.5cp	12.5cp	1X Saline Inactv
8	0cp	0cp	0cp	12.5cp	12.5cp	TPC

CLAMP Amplification Reaction Mix

Num Samples	Overage:
24	9.1%
PGS	275 uL
CLAMP MM	327 uL
Total	602 uL
Fill Strip / Tube	73 uL

EasyPCR LoD Configuration

	A	B	C	D	E	F
1	100cp	100cp	100cp	3.1cp	3.1cp	3.1cp
2	50cp	50cp	50cp	3.1cp	3.1cp	3.1cp
3	25cp	25cp	25cp	3.1cp	3.1cp	3.1cp
4	12.5cp	12.5cp	12.5cp	3.1cp	3.1cp	3.1cp
5	6.25cp	6.25cp	6.25cp	3.1cp	3.1cp	3.1cp
6	3.1cp	3.1cp	3.1cp	3.1cp	3.1cp	3.1cp
7	1.6cp	1.6cp	1.6cp	3.1cp	3.1cp	NF H2O
8	0cp	0cp	0cp	3.1cp	3.1cp	TPC

PCR Amplification Reaction Mix

Num Samples	Overage:
24	9.4%
PCRP Primers	105 uL
NF Water	79 uL
Luna MM	263 uL
Luna RT	26.3 uL
Total	473 uL
Fill Strip Per Tu	57 uL



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GCP100 Batch Record v1.3

Last Updated: 05-05-2021 by RT

Changes: Highlighted

Prepares 1 x GCP100 at 800uL and 3 x NSS 800uL = 2400uL total

- Safety Procedures: long pants, closed toed shoes, lab coat, gloves, mask
- Pre-Set Up: Set heat block to 105°C and let warm up for ~1 hour prior to inactivation

Components

GBD	Batch Record ID: _____
100X Inactivation Solution	Batch Record ID: _____
.9% Saline	Batch Record ID: _____

Make 1X Inactivation Saline Solution (1X ISS)

- Add 4.5 mL .9% Saline to 5mL Screw Cap
- Add 45 uL 100X Inactivation Solution to .9% Saline
- Vortex 30s

Swab Preparations

- Swab four swabs, each one in one nostril
- Dry on aluminum foil, minimum 20 minutes

GCP Sample Elution

- Add 900 uL of 1X ISS to 5mL Transport tube
- Add 100 uL Gamma Blue Dil (GBD 1kcp/uL) to 1X ISS {vortex 3s} Change gloves
- Add dried swab (snip shaft if necessary) and change gloves

NSS Sample Elution

- Add 1 mL of 1X ISS to 5mL Transport tube
- Add dried swab (snip shaft if necessary)

Inactivation

- Vortex GCP together with NSS samples for 30s in flipper rack
- Add to heat for 8 minutes
- Remove from heat and take QC temperature
- Let sit at room temperature 10 minutes

Aliquot

- Combine NSS samples in 5mL Screw Cap and vortex
- Aliquot out into 1.5 mL Screw Cap tubes at 500 uL
- Aliquot GCP sample into 1.5mL Screw Cap tubes at 150 uL

Batch ID format: "GCP"+YYMMDD & "NSS"+YYMMDD

Batch ID	Prep By	GBD Batch	100XIS Batch	SAL Batch	Swab	QC Temp

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Equipment & Materials for Test Validation

Equipment Required but not Provided

- Pipettors and filter pipette tips
- Thermal Cycler or Heat Block - set at 65°C for LAMP Amplification Reaction (do not use heated lid)
- BioRad CFX96 RT-PCR Instrument (or similar, see EasyPCR™IFU for instructions on BioRad CFX96, QuantStudio 7 Pro and QuantStudio6 Flex)
- Water Bath or Heater - set 99.9°C for Inactivation, prepare rack to hold 5ml transport tubes securely (recommend 4-way flipper racks). For heat block, it must hold 16mm tubes properly.
- Bottletop Dispenser (optional) - typically used for larger runs (> 100 samples)
- Vortexer
- Centrifuge

Reagents Provided in FloodLAMP Validation Kit (room temp)

Reagent Name (Label)	Photo	Amount	Conc.	Num Rxns	Purpose
0.9% Saline Solution		50 mL	0.9% = 154 mM	50 at 1 mL/rxn	Making 1X Inactivation Saline Solution
5M NaCl Thermo 24740011		15 mL	5M	500	Source for making 0.9% Saline Solution (1 part 5M NaCl to 31.5 water) and then 1X Inactivation Saline Solution



100X Inactivation Solution (100XIS)		4 x 1 mL	100X	400	Making 1X Inactivation Saline Solution
Nuclease-free Water Thermo 10977015		1 mL			

Reagents Provided in FloodLAMP Validation Kit (dry ice)

Reagent Name (Label)	Storage	Photo	Amount	Conc.	Num Rxns	Purpose
5X PCR Primers (PCRP) Eurofins 12YS-010YST	-20°C		10 x 105µL	5X	10 x 24 = 240	EasyPCR™ Reaction Mix
PCR MM (NLMM) NEB Luna E3006	-20°C		737µL		~72	EasyPCR™ Reaction Mix



PCR RT (NLRT) NEB Luna E3006	-20°C		74µL		~<72	EasyPCR™ Reaction Mix
Primer-Guanidine Solution for LAMP (PGS) FloodLAMP PGS_210427	-20°C		10 x 275µL		10 x 24 = 240	Colorimetric LAMP QuickColor™ Reaction Mix
Colorimetric LAMP MM NEB M1804	-20°C		1mL	2X	72	Colorimetric LAMP QuickColor™ Reaction Mix
Amp Positive Control (TPC) Twist 102019 & Thermo 4307281	-80°C		10 x 10µL	~100 cp/µL	10 x 4 rxn @ 2ul/rxn	Amplification Positive Control (should give ~32 Ct on EasyPCR™)
Inactivated Virus (GBD) BEI NR-52287	-80°C		4 x 100µL	1K cp/µL	4	Contrived Positive Sample creation. In 1ml, final conc. is 100 cp/µL



Assay Supplies Provided by FloodLAMP

Item Description	Number	Vendor	Catalog Number
8-Well PCR Strip & Cap	10	USA Scientific	1402-2500
96-Well PCR Plate	4	Eppendorf	951020303
Optical Plate Seal	3	Thermo	4311971
Foil Plate Seal	3	Sigma	Z721549
1.5 mL Snap Cap	20	Eppendorf	022431021
1.5 mL Screw Cap Tube	5	MTC Bio	C3150-SL
5 mL Transport Tubes	15	MTC Bio	C1811
30 mL Tube	3	MTC Bio	C2630

Nasal Swabs Provided by FloodLAMP

Item Description	Number
Micro swab	20

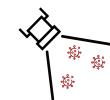
Inactivation Solution

100X Inactivation Solution is provided. Active ingredients are TCEP and EDTA.

Sample Preparation (wet swabs)

* 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.



Sample Inactivation (wet swabs, previously eluted)

- 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) **Transfer 1 mL** or available volume of each sample to an appropriately labeled 1.5 mL or 5mL tube and securely cap.
- 3) **Add 10µL per 1 mL sample volume of 100X Inactivation Solution, or 2µL per 0.1 mL sample volume of 50X Inactivation Solution** to each sample tube.
- 4) **Vortex for 30 seconds.**
- 5) Place sample tubes into the inactivation **heater for 8 minutes**.
- 6) Remove sample tubes from the inactivation heater and let **cool at room temperature for 10 minutes**.
- 7) Place sample tubes on ice, in the refrigerator, or on a cold block at **4°C until ready** to perform an 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 $\leq 70^{\circ}\text{C}$.

Preparing to Run Assay for the First Time

For PCR and Fluorimetric LAMP tests that use RT-PCR Instruments, complete this section of the corresponding IFU prior to continuing.

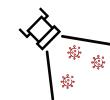
Amplification Reaction Preparation

Common to all assays:

- 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 reagents on ice, in the refrigerator, or on a cold block at 4°C until ready to use.
- 5) Prepare 96-well PCR plate or PCR strip tubes with amplification reaction mix

NOTE: For Colorimetric LAMP, optionally seal 96-well PCR plate with foil seal and add sample by piercing seal.

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).



NOTE: Colorimetric LAMP Negative Control must be prepared from 0.9% Saline and 100X Inactivation Solution.

Controls and Primers

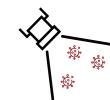
	PCR	Colorimetric LAMP
Contrived Positive	Gamma BEI - GCP	Gamma BEI - GCP
Amp Positive Control	Twist - TFPC	Twist - TFPC
Negative Control	nuclease-free water	0.9% saline with 1X Inactivation Solution
Primers (Tube Label)	PCRP (5X PCR Primer Stock)	PGS (Primer-Guanidine Solution)

EasyPCR™ Amplification Reaction Preparation

- 1) Prepare the PCR Amplification Reaction by combining the components listed in the table below.
NOTE: Component volumes should be scaled proportionally for the number of reactions.
NOTE: For 24 reaction scale, the PCR Master Mix and RT should be added to the tube containing the 5X PCR Primer Stock.
- 2) 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.
- 3) Add 18 µL of the PCR Amplification Reaction Solution into the wells of the PCR plate or strip tubes.

EasyPCR™ Amplification Reaction Mix

Component	Volume (1 reaction)	Volume (1 x 24 rxn w/ 9% overage)	Volume (1 x 96 rxn w/ 4% overage)
5X PCR Primer Stock	4 µL	105 µL	400 µL
Nuclease-free Water	3 µL	79 µL	300 µL
PCR Master Mix	10 µL	263 µL	1000 µL
PCR RT	1 µL	26.3 µL	100 µL
SUBTOTAL VOLUME	18 µL	473 µL	1800 µL
Sample	2 µL		
REACTION VOLUME	20 µL		



Sample Addition (EasyPCR™)

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

- 1) Add 2 uL 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, optical seal (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.

Continue to section “Run the Assay” on pg. 20 of FloodLAMP EasyPCR™ PCR COVID-19 Test IFU.

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	

Test Controls (EasyPCR™)

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 the table below.

- The “No Template” (Negative) Control (NTC) should yield a negative “not detected” result for both the N1 and RNaseP targets.
- The Positive Template Control should yield a positive “detected” result for the N1 target and a negative “not detected” for the RNaseP control.
- 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.

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 (EasyPCR™)

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 the below to assign a result to each sample.

EasyPCR™: 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

QuickColor™ Colorimetric LAMP Amplification Reaction

- 1) Prepare the Colorimetric LAMP Amplification Reaction Mix by adding the Colorimetric LAMP MM to the Primer-Guanidine Solution per the volumes listed in the Table below.

NOTE: Component volumes should be scaled proportionally for the number of reactions.

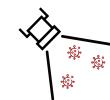
NOTE: For 24 reaction scale, the LAMP MM should be added to the tube containing the Primer-Guanidine Solution.

- 2) Vortex the Colorimetric LAMP Amplification Reaction Solution by for 10 seconds and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
- 3) Add 23 µL of the Colorimetric LAMP Amplification Reaction Solution into the wells of the PCR plate or PCR strip tubes.

NOTE: Reaction plates/strip tubes comprising the Colorimetric LAMP Amplification Reaction Solution may be prepared in advance, capped/sealed, and stored at -20°C for up to 3 days prior to addition of the sample.

QuickColor™ Colorimetric LAMP Amplification Reaction

Component	Volume (1 reaction)	Volume (1 x 24 rxn w/ 9%)	Volume (1 x 96 rxn w/ 9%)



		overage)	overage)
Primer-Guanidine Solution	10.5 µL	275 µL	1100 µL
Colorimetric LAMP MM	12.5 µL	327 µL	1310 µL
SUBTOTAL VOLUME	23 µL	602 µL	2420 µL
Sample	2 µL		
REACTION VOLUME	25 µL		

Sample Addition and Heating (QuickColor™)

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) Turn on the amplification heater (a thermal cycler, water bath, dry heat bath or equivalent) and set the temperature to hold at 65°C.
NOTE: Amplification heater should be located in a separate, dedicated BSC or area of the lab. Proper cross contamination prevention practices are required, such as glove changes, to prevent amplicon contamination.
- 2) Add 2 µL of each sample into a separate tube in the amplification reaction PCR plate or strip tubes.
- 3) Mix by pipetting.
- 4) If using PCR plate, seal with foil seal, optical seal (optionally using heat sealer). If using PCR strip tubes, cap strips.
- 5) Pulse vortex and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
- 6) Place the plate or strip tubes in the heater and set timer for 25 minutes.
- 7) Remove the plate or strip tubes from the heater after 25 minutes.
- 8) Let cool for 1 minute and then interpret the test results.

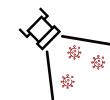
Continue to section "Test Controls" on pg. 17 of FloodLAMP QuickColor™ COVID-19 Test IFU.

Patient Specimen Results Interpretation (QuickColor™)

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.

Test results should be read at least 1 minute and no more than 8 hours after plates or tubes have been removed from heat. Test results may be determined directly from visual inspection of the color of the reaction tubes:

- yellow - result is positive
- bright pink or red - result is negative
- any other color - result is invalid.



Examples are shown below. Edge cases for positive and negative results are shown below. Any color variance stronger than the edge cases should be interpreted as inconclusive. In order to reduce the chance of both false negative and false positive results, this window for color variance is intentionally set to be small.

If the initial test is inconclusive, then one of the following should be performed:

- 1) repeat the Colorimetric LAMP Amplification Reaction on the inactivated sample. If the repeat test has a positive result then the final interpretation of the test is positive. If the repeat test has a negative or another inconclusive result, then the final interpretation is inconclusive.
- 2) follow-up test the inactivated sample with the FloodLAMP EasyPCR™ COVID-19 Test or another high sensitivity EUA authorized test that comprises the same inactivation protocol. The final interpretation is the result of the follow-up test.

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



**Figure 2. Example of Test Results
(Left 2 Negative, Right 2 Positive)**



**Figure 3. Edge Case Test Results
(Left Negative, Right Positive)**



Validation Kit Photos



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Notes and Suggestions