Proposed Pooling Study

From FDA Guidance Document "Pooling and Serial Testing Amendment Letter" (4-20-2021) - Appendix B: Swab pooling up to n=5

“Validation of Expected Limit of Detection (LoD)” and “Validation of High Viral Concentrations”. As part of the notification to FDA, summary data must be submitted, including the percent of positive pools detected, the Ct score difference, and the line data including the Ct score for each pool and individual sample tested.

1) Validation of Expected Limit of Detection (LoD)

- Use inactivated virus of a known quantity represented as copies/mL
  - Zeptometrix NATSARS(COV2)-ST at 1e6 copies/mL OR
  - BEI gamma-irradiated SARS-CoV-2 virus cell lysate (BEI NR-52287).
- Prepare negative swabs by collecting from healthy individuals.
- Sample volumes for 4 swab pools of 2 mL of 1X Inactivation Saline Solution.
- Prepare positive swabs by spiking a known amount of inactivated virus onto the swab prior to immersion in the 1X Inactivation Saline Solution.
- Add remaining 4 negative swabs.
- The final concentration of inactivated virus in the 1X Inactivation Saline Solution is approximately 3x the LoD of the assay.
  - **FloodLAMP EasyPCR™ COVID-19 Test**: 10,000 copies/mL (~3 x 3,100)
  - **FloodLAMP QuickColor™ COVID-19 Test**: 40,000 copies/mL (~3 x 12,500)
- Test 20 independent extraction replicates of individual positive swabs in 1 mL of 1X Inactivation Saline Solution (same volume used in the LoD study as described in single swab protocol in EUA submission and IFU).
- Test 20 replicates of 4 swab pools each containing a single positive swab and 3 negative swabs in 2 mL of 1X Inactivation Saline Solution.
- Results criteria:
  - ≥95% of pooled replicates are detected as positive using the swab pooling protocol;
  - The Ct score difference for **FloodLAMP EasyPCR™ COVID-19 Test** between the pooled and single swab protocols does not exceed 1.7 Ct;
  - The invalid rate in the swab pooling protocol does not exceed 5%.

2) Validation of High Viral Concentrations

- Prepare 3 positive swabs simulating high viral concentrations by spiking to a final concentration of 1e6 copies/mL of inactivated virus in 1X Inactivation Saline Solution.
- Prepare 2 negative swabs by collecting from healthy individuals.
- Combine 4 swabs into high viral concentration 4 swab pool and add 2 mL of 1X Inactivation Saline Solution.
- Test 10 replicates of inactivated high viral concentration sample.
- Results criteria:
  - All 10 replicates are detected as positive;
  - The invalid rate does not exceed 5%.

Proposed Asymptomatic Screening Study

The following clinical study is proposed as a condition of authorization for the serial screening indication of **FloodLAMP EasyPCR™ and QuickColor™ COVID-19 Tests** which specifies anterior nasal respiratory specimens from individuals without known or suspected COVID-19 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. A point-in-time asymptomatic claim will be requested provided the performance evaluation data from the study supports such a claim.

FloodLAMP has obtained IRB approval to conduct the following clinical study from WCG IRB ("FloodLAMP COVID-19 Test Validation Protocol" 20210401, Study Number 1306140). The IRB Protocol includes several variations in sample collection sites and methods.

Inclusion and Exclusion Criteria for Asymptomatic Screening Study

Inclusion Criteria	Exclusion Criteria
The specimen is an anterior nasal swab using the swab and QR code labeled tubes, nasal swabs included with the FloodLAMP Pooled Swab Collection Kit DTC.	The specimen was not properly collected, identified, transported, processed, or stored according to the instructions provided by the sponsor.
The swab specimen can be tested within 56 hours or less after collection. If frozen, specimens are to be stored at <-70℃ until tested.	The specimen was not collected under informed consent.
The subject is suspected of COVID, whether or not symptomatic.	
The subject is not experiencing symptoms and/or has not notified a physician that they suspect they have COVID .	
The subject has had a positive COVID test within the last 10 days.	
The specimen is from a consenting male or female (including pregnant women) subject.	
The specimen is from a subject ages 2 to 13 and has been collected by a parent or guardian OR age 14 and above and has been self-collected.	

The study will utilize an enrichment strategy to obtain specimens from 20 positive and 20 negative subjects. Subject recruitment will be conducted via internet advertisements seeking participation by individuals who are asymptomatic and have recently received a COVID-19 test result. An eligibility questionnaire will select for subjects to meet the 20 positives and 20 negatives. The questionnaire will include symptom screening questions.

Eligible subjects will register using the **FloodLAMP Mobile App**, sign the Research Subject Information and Consent Form, then be mailed a version of the FloodLAMP Home Collection Kit for self-collection and sample return.

Duplicate anterior nares swabs will be self-collected with the order being randomized and at least 10 but no more than 30 minutes between collection of the two swabs. One swab will be used to run the comparator test (EUA purified PCR test) and the other will be used to run the **FloodLAMP EasyPCR™ and QuickColor™ COVID-19 Tests**.