



April 20, 2021

To: Developers of Molecular-Based Diagnostic Tests Authorized for  
Emergency Use for Coronavirus Disease 2019 (COVID-19) as of  
Today's Date

Re: Amending Certain EUAs for RT-PCR Molecular-Based Diagnostic  
Tests to Authorize the Detection of Nucleic Acid from SARS-  
CoV-2 from Pooled Anterior Nasal Respiratory Specimens for  
Screening When Used as Part of a Serial Testing Program

On February 4, 2020, pursuant to Section 564(b)(1)(C) of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. § 360bbb-3(c)), the Secretary of the Department of Health and Human Services (HHS) determined that there is a public health emergency that has a significant potential to affect national security or the health and security of United States citizens living abroad, and that involves the virus that causes COVID-19. Pursuant to Section 564 of the Act, and on the basis of such determination, the Secretary of HHS then declared that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of the virus that causes COVID-19 subject to the terms of any authorization issued under Section 564(a) of the Act.<sup>1</sup>

On April 20, 2021, pursuant to Section 564 of the Act, in response to public health needs to expand the nation's testing capacity,<sup>2</sup> FDA is issuing this letter to authorize additional indications for EUAs that are within the Scope of this Amendment (Section II). For such indications, use is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests, except that tests authorized for use in specific named or designated high complexity laboratories can only be used in such laboratories.

As set forth throughout, this authorization amends certain EUAs to authorize the tests for additional indications for use. There are multiple indications for use in the appendices but they all contain these essential elements: tests with EUAs that are amended by this letter are authorized for use with pooled anterior nasal specimens for screening (i.e., testing individuals without symptoms or other epidemiological reasons to suspect COVID-19) when used as part of

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<sup>1</sup> U.S. Department of Health and Human Services, *Determination of a Public Health Emergency and Declaration that Circumstances Exist Justifying Authorizations Pursuant to Section 564(b) of the Federal Food, Drug, and Cosmetic Act*, 21 U.S.C. § 360bbb-3. 85 FR 7316 (February 7, 2020)

<sup>2</sup> Based on the production and testing capacity estimates provided by test developers and the anticipated demand by schools, workplaces and other groups setting up testing programs, the need for testing remains greater than available resources. If such testing programs are scaled up using individual tests, it would likely overwhelm the supply chain for many of the consumables used for diagnostic testing. Pooled testing strategies are intended to address public health needs by mitigating potential shortages.

a serial testing program. This means that tests with EUAs that are amended by this authorization may be used with pooled 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. The indications in each appendix (A-H) differ in the number of specimens that can be pooled (i.e., up to 3, up to 5, or up to 10) and the type of pooling that can be done (i.e., media or swab pooling). Tests with EUAs that are amended by this authorization will be added to Exhibit 1, annotated with the authorized indication(s) for each test, which will be maintained on FDA’s webpage.<sup>3</sup> FDA’s determination that the indications added by this amendment meet the criteria for issuance under section 564(c) of Act is based on the available scientific evidence, including recent studies involving antigen tests used in a serial manner, knowledge of recently authorized antigen and molecular tests, as well as our experience with SARS-CoV-2 tests over the past year.

Having concluded, based on the available scientific evidence, that the criteria for issuance of this authorization under Section 564(c) of the Act are met, I am authorizing the emergency use of the tests identified in the Scope of this Amendment (Section II) and subject to the Conditions of Authorization (Section IV), for use in laboratories certified under the CLIA, 42 U.S.C. § 263a, that meet the requirements to perform high complexity tests to detect SARS-CoV-2 in anterior nasal respiratory specimens from individuals, except that tests authorized for use in specific named or designated high complexity laboratories can only be used in such laboratories.<sup>4</sup>

## **I. Criteria for Issuance of Authorization**

I have concluded that the emergency use of the authorized tests meets the criteria for issuance of an authorization under Section 564(c) of the Act, because I have concluded that:

1. The SARS-CoV-2 can cause a serious or life-threatening disease or condition, including severe respiratory illness, to humans infected by this virus;
2. Based on the totality of scientific evidence available to FDA, it is reasonable to believe that the authorized tests may be effective in diagnosing COVID-19 for the indications set forth in the appendices, and that the known and potential benefits of the authorized tests when used for such use, outweigh the known and potential risks of the authorized tests; and
3. There are no adequate, approved, and available alternatives to the emergency use of the authorized tests.<sup>5</sup>

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<sup>3</sup> CDRH IVD EUA webpage <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>.

<sup>4</sup> For ease of reference, this letter uses the phrase “authorized tests” to refer to molecular-based tests authorized by this amendment to detect nucleic acid from SARS-CoV-2 with pooled anterior nasal respiratory specimens for screening when used as part of a serial testing program.

<sup>5</sup> No other criteria of issuance have been prescribed by regulation under Section 564(c)(4) of the Act.

## II. Scope of this Amendment

I have concluded, pursuant to Section 564(d)(1) of the Act, that the scope of this amendment is limited to authorized tests identified below for the additional indications for use<sup>6</sup> given in the applicable appendix(ces) for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests, except that tests authorized for use in specific named or designated high complexity laboratories can only be used in such laboratories.

### Authorized Tests

The emergency use authorization (EUA) of a molecular diagnostic SARS-CoV-2 test is amended consistent with this letter where:

1. The test is an RT-PCR test authorized under an EUA specific to that test as of today's date for the qualitative detection of SARS-CoV-2 in respiratory specimens collected by anterior nasal swabs, with a PPA  $\geq$  95% based on validation with positive patient specimens as stated in the test's authorized labeling;
2. The test as authorized is designed to detect two or more viral targets on the SARS-CoV-2 genome;
3. The test as authorized includes a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction);
4. The test is authorized for detecting only SARS-CoV-2;
5. Prior to notification pursuant to Condition of Authorization C of this letter, either validation data has not been submitted to FDA for testing pooled specimens, or such validation data has been submitted to FDA and FDA authorized the test for testing pooled specimens<sup>7</sup>; and
6. Prior to notification pursuant to Condition of Authorization C of this letter, either validation data has not been submitted to FDA for testing specimens from asymptomatic individuals, or such validation data has been submitted to FDA and FDA authorized the test for screening (i.e., testing individuals without symptoms or other reasons to suspect COVID-19).<sup>8</sup>

As set forth in Condition of Authorization A of this letter, and notwithstanding other statements and conditions concerning authorized labeling in the EUAs being amended, developers must make the following changes to the test's authorized labeling before distributing or using the test for any indication added by this authorization:

- Update your Instructions for Use and/or laboratory procedure to add the following language:
  - The following indication is authorized under the Pooling and Serial Testing

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<sup>6</sup> The additional indications for use authorized by this amendment are limited to anterior nasal swab specimens and do not include any additional indications for other specimen types, such as saliva.

<sup>7</sup> In other words, this amendment does not amend EUAs for tests for which validation data for pooling was submitted, reviewed, and not authorized for pooling.

<sup>8</sup> In other words, this amendment does not amend EUAs for tests for which validation data from asymptomatic individuals was submitted, reviewed, and not authorized for screening.

Amendment [include link to this letter] for use in [laboratories certified under CLIA to perform high complexity tests] OR [for tests authorized for use in specific named or designated high complexity laboratories, insert the language included under “Authorized Laboratories” in your EUA] : [insert indication from applicable appendix]

- This indication is authorized with the following validated protocol: [insert the relevant validated protocol for testing with pooled specimens that meets Condition of Authorization D]; and
- Update your Fact Sheet for Health Care Providers and Fact Sheet for Patients with the relevant additional information set forth in Appendix I.

This labeling will be added to FDA’s webpage and posted with the EUA being amended after it is submitted to FDA per the process outlined below.

A test may not be distributed or used for any additional indications authorized by this letter until the pooling protocol for that indication is validated in accordance with the applicable appendix and the required notification is submitted and confirmed to be complete by FDA in accordance with Condition of Authorization C of this letter.

Tests limited for use at specific named or designated laboratories that are not authorized to be distributed per the EUA being amended continue to be authorized for use only in those laboratories and not for distribution even if the EUAs are amended as set forth in this authorization.

### **Addition to Exhibit 1**

An EUA that meets the above will be added to Exhibit 1 after FDA confirms that the required documentation set forth below has been submitted to FDA. At that time, FDA will notify the developer of the inclusion of its test(s) in Exhibit 1 by replying to the email. Please note that being added to Exhibit 1 does not necessarily mean that FDA has reviewed the underlying validation data submitted or confirmed that the test is appropriately validated. Instead, being added to Exhibit 1 only means that the developer has submitted complete documentation. FDA may revise or revoke an EUA under certain circumstances, such as when there are concerns with the test’s performance.

As set forth in Condition of Authorization C of this letter, developers must notify FDA by sending a message to FDA with the subject line “Addition to Exhibit 1 of the Pooling and Serial Testing Amendment” to [CDRH-EUA-Templates@fda.hhs.gov](mailto:CDRH-EUA-Templates@fda.hhs.gov) with the information below before the test can be distributed (if authorized to be distributed per the EUA being amended) and used for the additional indication(s):

- Developer (company) name
- Contact individual’s name, address, phone number, email address
- Test name
- EUA# and link to the EUA being amended
- The specific new indication(s), referenced by noting the applicable appendix

- The required validation data as set forth in the applicable appendices
- Revised (redline and final) labeling as set forth above and required by Condition of Authorization A
- The following information on testing capacity:
  - i. The number of individual tests that can be run with normal operation in a 24-hour period;
  - ii. The number of patient samples that can be tested in a 24-hour period if all samples are pooled at the maximum ratio permitted by the additional indication(s) added by this amendment; and
  - iii. For distributed test kits, the number of laboratories in the United States with the required platforms installed.
- Statement certifying that the six criteria above are met, no changes have been made to the authorized test other than to add the indication(s) for use set forth in the applicable appendix, no changes have been made to the authorized labeling other than those required by this amendment, and that all information submitted is truthful and accurate, for example:
  - I certify that, in my capacity as [the position held in company] of [company name], I believe to the best of my knowledge that all six criteria described in this amendment have been met, no changes have been made to the authorized test other than to add the indication(s) for use set forth in the applicable appendix(ces), no changes have been made to the authorized labeling other than those required by this amendment, and that all data and information I am submitting are truthful and accurate and no material fact has been omitted.

Authorized tests are qualitative tests for the detection of nucleic acid from SARS-CoV-2 in anterior nasal respiratory specimens from individuals without symptoms or other reasons to suspect COVID-19, when tested with pooled testing at least once per week as part of a serial testing program, as set forth in the applicable appendix.

Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be reported as presumptive positive or tested individually prior to reporting a result. Individuals included in a pool that returns a positive or invalid result should be treated as a presumptive positive unless or until they receive a negative result when re-tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, they should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result. Specimens with low viral loads may not be detected with pooled testing due to decreased sensitivity or increased interference from pooled testing.

For serial testing programs, additional confirmatory testing for negative results may be necessary, if there is a high likelihood of COVID-19, such as an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high

prevalence of infection. Additional confirmatory testing for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposure to COVID-19 or residing in communities with low prevalence of infection.

The above described tests, with the authorized labeling provided as set forth in the Scope of this Amendment (Section II) and subject to the Conditions of Authorization (Section IV) of this letter, are authorized to be distributed (if previously authorized to be distributed per the underlying EUA) and used in accordance with the Scope of this Amendment (Section II) and the Conditions of Authorization (Section IV), despite the fact that such tests do not meet certain requirements otherwise required by applicable federal law.

I have concluded, pursuant to Section 564(d)(2) of the Act, that it is reasonable to believe that the known and potential benefits of the authorized tests, when used consistent with the Scope of this Amendment (Section II), outweigh the known and potential risks of such authorized tests.

I have concluded, pursuant to Section 564(d)(3) of the Act, based on the totality of scientific evidence available to FDA, that it is reasonable to believe that the authorized tests may be effective in diagnosing COVID-19 as set forth in Section I of this letter, when used consistent with the Scope of this Amendment (Section II), pursuant to Section 564(c)(2)(A) of the Act.

FDA has reviewed the scientific information available to FDA, including the information supporting the conclusions described in Section I above, and concludes that the authorized tests (as described in the Scope of this Amendment (Section II)) meet the criteria set forth in Section 564(c) of the Act concerning safety and potential effectiveness.

The emergency use of the authorized tests under this amendment must be consistent with, and may not exceed the terms of this letter, including the Scope of this Amendment (Section II) and the Conditions of Authorization (Section IV). Subject to the terms of this amendment and under the circumstances set forth in the Secretary of HHS's determination under Section 564(b)(1)(C) described above and the Secretary of HHS's corresponding declaration under Section 564(b)(1), the authorized tests are authorized for the additional indications set forth in the applicable appendix.

### **III. Waiver of Certain Requirements**

This amendment does not change the waiver of any requirements included in the EUAs being amended.

### **IV. Conditions of Authorization**

Pursuant to Section 564(e) of the Act, I am establishing the conditions below, which are specific to the indications added by this amendment for which developers intend to distribute or use their test. The conditions in the EUAs being amended continue to apply to all authorized indications for use, including any indications added by this amendment for which developers intend to distribute or use their test.

**Developer (You)**

- A. You must update your labeling in accordance with Section II of this letter.
- B. You must provide any authorized distributor(s) with a copy of this amendment and its authorized accompanying materials (e.g., Fact Sheets).
- C. In order for your test to be distributed (if authorized to be distributed per the underlying EUA) and used for any of the indications authorized by this letter, you must first validate your test in accordance with the requirements in the applicable appendix and notify FDA by submitting the information set forth in Section II to FDA at [CDRH-EUA-Templates@fda.hhs.gov](mailto:CDRH-EUA-Templates@fda.hhs.gov) using the subject line “Addition to Exhibit 1 of the Pooling and Serial Testing Amendment”.
- D. You must have a pooling protocol that includes instructions for follow-up for positive and invalid pools, including follow-up instructions to be provided to the organizer of the testing program. For media pooling, the instructions for follow up for positive and invalid pools must include deconvoluting to retest individual samples. For swab pooling, the instructions for follow up for positive and invalid pools must include reporting as “presumed positive” unless or until the individual is re-tested individually and must include instructions to collect a new specimen to be tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, the instructions must indicate that such individuals should isolate until receiving a negative result when re-tested individually and should **not** be cohorted with other individuals who have received a positive or presumptive positive result.
- E. You must immediately cease distribution and use of your test for an indication added by this amendment upon written notification by FDA that the test has not been validated for that indication in accordance with the applicable requirements, and must notify authorized laboratories to which your test has been distributed that they must immediately cease use of your test for that indication. You must also relabel any tests that have been distributed for that indication to no longer include such indication.
- F. If, prior to the date of this letter, your test was not authorized for screening (i.e., testing individuals without symptoms or other reasons to suspect COVID-19), you must further evaluate the clinical performance of your test to support the serial testing claim in an FDA agreed upon post authorization clinical evaluation study within 6 months of submitting the notification pursuant to Condition C of this letter (unless otherwise agreed to with DMD/OHT7-OIR/OPEQ/CDRH). Results from this study must be submitted to FDA at [CDRH-EUA-Templates@fda.hhs.gov](mailto:CDRH-EUA-Templates@fda.hhs.gov). After FDA concurs with the data, you must update your authorized labeling to reflect the additional testing. Such labeling updates must be made only after concurrence by DMD/OHT7-OIR/OPEQ/CDRH. If FDA does not concur with the results from the study, then you must cease distributing or using the test for screening and relabel such tests that have already been distributed to reflect this change.

### Authorized Laboratories<sup>9</sup>

- G. Authorized laboratories that use a test that has been amended by this letter must notify the relevant public health authorities of their intent to run the authorized test for the new indication(s) set forth in this amendment prior to initiating testing.
- H. Authorized laboratories testing pooled specimens with your test must include with test result reports for specific individuals whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that “Individual specimens with low viral loads may not be detected due to the decreased sensitivity or increased interference when tested with pooled testing.”
- I. Authorized laboratories must follow your pooling protocol that includes instructions for follow up for positive and invalid pools, including follow-up instructions to be provided to the organizer of the testing program. For media pooling, the instructions for follow up for positive and invalid pools must include deconvoluting to retest individual samples. For swab pooling, the instructions for follow up for positive and invalid pools must include reporting as “presumed positive” unless or until the individual is re-tested individually and must include instructions to collect a new specimen to be tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, the instructions must indicate that such individuals should isolate until receiving a negative result when re-tested individually and should **not** be cohorted with other individuals who have received a positive or presumptive positive result.
- J. Authorized laboratories testing pooled specimens with your test must include with test result reports for specific individuals whose specimen(s) were the subject of pooling, a notice that their test result is “presumed positive” unless or until they are re-tested individually if the pool in which they were included returns a positive or invalid result. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, the report must include instructions to collect a new specimen to be tested individually and must indicate that such individuals should isolate until receiving a negative result when re-tested individually and should **not** be cohorted with other individuals who have received a positive or presumptive positive result.
- K. Authorized laboratories testing specimens using pooled testing with your test must monitor the positivity rate of the specimens tested using pooled testing by calculating the percent positive results using a moving average (such as a rolling average updated daily using data from the previous 7-10 days).
- L. Authorized laboratories must keep records of specimen pooling test result data, daily testing totals including number of pooled test results, number of individuals tested and daily running average of percent positive results. For the first 12 months from the date of their creation, such records must be made available to FDA upon request within 48

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<sup>9</sup> These conditions also apply to the test developer where the test developer is also an authorized laboratory.

business hours for inspection. After 12 months from the date of their creation, upon FDA request, such records must be made available for inspection within a reasonable time.

Sincerely,

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RADM Denise M. Hinton  
Chief Scientist  
Food and Drug Administration

Enclosures

**Appendix A**  
**Swab pooling up to n=3**

Subject to the terms of this EUA, a test that is within the Scope of this Amendment (Section II) is authorized for the following additional indication without needing additional validation and may be distributed (if previously authorized for distribution per the EUA being amended) and used for this indication after a notification has been submitted to FDA and confirmed by FDA to be complete in accordance with Condition of Authorization C of this letter:

Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing up to 3 individual human anterior nasal swabs placed in a single vial containing transport media after being collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19, when tested at least once per week as part of a serial testing program.

This indication is authorized for use in laboratories certified under CLIA to perform high complexity tests, except that tests authorized for use in specific named or designated laboratories can only be used in such high complexity laboratories.

Tests will be added to Exhibit 1 after FDA determines it has received a complete notification.

## **Appendix B**

### **Swab pooling up to n=5**

Subject to the terms of this EUA, a test that is within the Scope of this Amendment (Section II) is authorized for the following additional indication and may be distributed (if previously authorized for distribution per the EUA being amended) and used for this indication after validation is completed as set forth in this Appendix and a notification has been submitted to FDA and confirmed by FDA to be complete in accordance with Condition of Authorization C of this letter:

Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing up to 5 individual human anterior nasal swabs placed in a single vial containing transport media after being collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19, when tested as part of a serial testing program including testing at least once per week.

This indication is authorized for use in laboratories certified under CLIA to perform high complexity tests, except that tests authorized for use in specific named or designated laboratories can only be used in such high complexity laboratories.

Tests will be added to Exhibit 1 after FDA determines it has received a complete notification.

Validation for this indication includes both validation protocols outlined below, “*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 either diluted positive clinical samples quantified in copies/mL using a standard curve, or inactivated virus of a known quantity represented as copies/mL. Prepare samples using negative anterior nasal clinical matrix (collection from healthy individuals is acceptable) using sample volumes you intend to include in your IFU or procedure for testing 5-swab pools.

To generate a 5-swab pool positive for SARS-CoV-2, prepare a single positive swab by spiking a known amount of inactivated virus or quantified positive patient sample onto the swab prior to immersion in the volume of transport media you intend to include in your IFU or procedure for 5-swab pooling. The remaining four swabs added to transport media should only contain negative patient clinical matrix. The final concentration of the transport media must be approximately 3x the LoD of your previously authorized assay. To ensure this concentration is achieved, factor in how much volume the swab absorbs, and the SARS-CoV-2 concentration needed on the single positive swab to achieve a final concentration of approximately 3x the LoD in the transport media. This is the concentration you should use to prepare the positive swabs for your validation study.

Test at least 20 independent extraction replicates of individual swabs in the same volume of buffer used in the LoD study as described in your authorized test's IFU or procedure, using the testing protocol in your authorized test's IFU or procedure. Test 20 paired 5-swab pools in parallel, each containing a single positive swab and 4 individual negative swabs in the volume of transport media you intend to include in your IFU or procedure for testing 5-swab pools, using the testing protocol you intend to include in your IFU or procedure for testing 5-swab pools.

Your validation must demonstrate that:

- $\geq 95\%$  of pooled replicates are detected as positive using the swab pooling protocol;
- The Ct score difference between the pooled and single swab protocols does not exceed 1.7 Ct; and
- The invalid rate in the swab pooling protocol does not exceed 5%.

## 2) *Validation of High Viral Concentrations*

Prepare three swabs simulating high viral concentrations by spiking  $10^6$  copies/mL of inactivated virus or quantified positive patient sample. Test 10 replicates of viral transport material containing the three spiked positive swabs and 2 individual negative swabs in the volume of transport media you intend to include in your IFU or procedure for testing 5-swab pools, using the testing protocol you intend to include in your IFU or procedure for testing 5-swab pools.

Your validation must demonstrate that:

- All 10 replicates are detected as positive; and
- The invalid rate in the swab pooling protocol does not exceed 5%.

### **Appendix C** **Swab pooling up to n=10**

Subject to the terms of this EUA, a test that is within the Scope of this Amendment (Section II) is authorized for the following additional indication and may be distributed (if previously authorized for distribution per the underlying EUA) and used for this indication after validation is completed as set forth in this Appendix and a notification has been submitted to FDA and confirmed by FDA to be complete in accordance with Condition of Authorization C of this letter:

Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing up to 10 individual human anterior nasal swabs placed in a single vial containing transport media after being collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19, when tested as part of a serial testing program including testing at least once per week.

This indication is authorized for use in laboratories certified under CLIA to perform high complexity tests, except that tests authorized for use in specific named or designated laboratories can only be used in such high complexity laboratories.

Tests will be added to Exhibit 1 after FDA determines it has received a complete notification.

Validation for this indication includes both validation protocols outlined below, “*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 either diluted positive clinical samples quantified in copies/mL using a standard curve, or inactivated virus of a known quantity represented as copies/mL. Prepare samples using negative anterior nasal clinical matrix (collection from healthy individuals is acceptable) using sample volumes you intend to include in your IFU or procedure for testing 10-swab pools.

To generate a 10-swab pool positive for SARS-CoV-2, prepare a single positive swab by spiking a known amount of inactivated virus or quantified positive patient sample onto the swab prior to immersion in the volume of transport media you intend to include in your IFU or procedure for 10-swab pooling. The remaining four swabs added to transport media should only contain negative patient clinical matrix. The final concentration of the transport media must be approximately 3x the LoD of your previously authorized assay. To ensure this concentration is achieved, factor in how much volume the swab absorbs, and the SARS-CoV-2 concentration needed on the single positive swab to achieve a final concentration of approximately 3x the LoD in the transport media. This is the concentration you should use to prepare the positive swabs for your validation study.

Test at least 20 independent extraction replicates of individual swabs in the same volume of buffer used in the LoD study as described in your authorized test's IFU or procedure, using the testing protocol in your authorized test's IFU or procedure. Test 20 paired 10-swab pools in parallel, each containing a single positive swab and 9 individual negative swabs in the volume of transport media you intend to include in your IFU or procedure for testing 10-swab pools, using the testing procedure you intend to include in your IFU or procedure for testing 10-swab pools.

Your validation must demonstrate that:

- $\geq 95\%$  of pooled replicates are detected as positive using the swab pooling protocol;
- The Ct score difference between the pooled and single swab protocols does not exceed 1.7 Ct; and
- The invalid rate in the swab pooling protocol does not exceed 5%.

## 2) *Validation of High Viral Concentrations*

Prepare three swabs simulating high viral concentrations by spiking  $10^6$  copies/mL of inactivated virus or quantified positive patient sample. Test 10 replicates of viral transport material containing the three spiked positive swabs and 7 individual negative swabs in the volume of transport media you intend to include in your IFU or procedure for testing 10-swab pools, using the testing protocol you intend to include in your IFU or procedure for testing 10-swab pools.

Your validation must demonstrate that:

- All 10 replicates are detected as positive; and
- The invalid rate in the swab pooling protocol does not exceed 5%.

**Appendix D**  
**Media pooling up to n=3**

Subject to the terms of this EUA, a test that is within the Scope of this Amendment (Section II) is authorized for the following additional indication without needing additional validation and may be distributed (if previously authorized for distribution per the underlying EUA) and used for this indication after a notification has been submitted to FDA and confirmed by FDA to be complete in accordance with Condition of Authorization C of this letter:

Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing aliquots of transport media from up to 3 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19 and placed in individual vials containing transport media when tested as part of a serial testing program including testing at least once per week.

This indication is authorized for use in laboratories certified under CLIA to perform high complexity tests, except that tests authorized for use in specific named or designated laboratories can only be used in such high complexity laboratories.

Tests will be added to Exhibit 1 after FDA determines it has received a complete notification.

**Appendix E**  
**Media pooling up to n=5 – validation option 1**

Subject to the terms of this EUA, a test that is within the Scope of this Amendment (Section II) is authorized for the following additional indication and may be distributed (if previously authorized for distribution per the underlying EUA) and used for this indication after validation is completed as set forth in this Appendix and a notification has been submitted to FDA and confirmed by FDA to be complete in accordance with Condition of Authorization C of this letter:

Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing aliquots of transport media from up to 5 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19 and placed in individual vials containing transport media, when tested as part of a serial testing program including testing at least once per week.

This indication is authorized for use in laboratories certified under CLIA to perform high complexity tests, except that tests authorized for use in specific named or designated laboratories can only be used in such high complexity laboratories.

Tests will be added to Exhibit 1 after FDA determines it has received a complete notification.

Validation for this indication includes the validation protocol outlined below, “*Validation of Expected Limit of Detection (LoD)*”. 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 either diluted positive clinical samples quantified in copies/mL using a standard curve, or inactivated virus of a known quantity represented as copies/mL. Prepare samples using negative anterior nasal clinical matrix (collection from healthy individuals is acceptable) using sample volumes you intend to include in your IFU or procedure for testing 5-sample pools.

Prepare positive samples for your validation study at 5x the LoD of your previously authorized assay.

Test at least 20 independent extraction replicates of individual samples using the testing protocol in your authorized test’s IFU or procedure. Test 20 paired 5-sample pools in parallel, each containing aliquots from a single positive sample and 4 individual negative samples, using the volumes and testing protocol you intend to include in your IFU or procedure for testing 5-sample pools.

Your validation must demonstrate that:

- $\geq 95\%$  of pooled replicates are detected as positive using the sample pooling protocol;
- The Ct score difference between the pooled and single swab protocols does not exceed 1.7 Ct; and
- The invalid rate in the swab pooling protocol does not exceed 5%.

**Appendix F**  
**Media pooling up to n=10 – validation option 1**

Subject to the terms of this EUA, a test that is within the Scope of this Amendment (Section II) is authorized for the following additional indication and may be distributed (if previously authorized for distribution per the underlying EUA) and used for this indication after validation is completed as set forth in this Appendix and a notification has been submitted to FDA and confirmed by FDA to be complete in accordance with Condition of Authorization C of this letter:

Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing aliquots of transport media from up to 10 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19 and placed in individual vials containing transport media, when tested as part of a serial testing program including testing at least once per week.

This indication is authorized for use in laboratories certified under CLIA to perform high complexity tests, except that tests authorized for use in specific named or designated laboratories can only be used in such high complexity laboratories.

Tests will be added to Exhibit 1 after FDA determines it has received a complete notification.

Validation for this indication includes the validation protocol outlined below, “*Validation of Expected Limit of Detection (LoD)*”. 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 either diluted positive clinical samples quantified in copies/mL using a standard curve, or inactivated virus of a known quantity represented as copies/mL. Prepare samples using negative anterior nasal clinical matrix (collection from healthy individuals is acceptable) using sample volumes you intend to include in your IFU or procedure for testing 10-sample pools.

Prepare positive samples for your validation study at 10x the LoD of your previously authorized assay.

Test at least 20 independent extraction replicates of individual samples using the testing protocol in your authorized test’s IFU or procedure. Test 20 paired 10-sample pools in parallel, each containing aliquots from a single positive sample and 9 individual negative samples, using the volumes and testing protocol you intend to include in your IFU or procedure for testing 10-sample pools.

Your validation must demonstrate that:

- $\geq 95\%$  of pooled replicates are detected as positive using the sample pooling protocol;
- The Ct score difference between the pooled and single swab protocols does not exceed 1.7 Ct; and
- The invalid rate in the swab pooling protocol does not exceed 5%.

## **Appendix G**

### **Media pooling up to n=5 – validation option 2**

Subject to the terms of this EUA, a test that is within the Scope of this Amendment (Section II) is authorized for the following additional indication and may be distributed (if previously authorized for distribution per the underlying EUA) and used for this indication after validation is completed as set forth in this Appendix and a notification has been submitted to FDA and confirmed by FDA to be complete in accordance with Condition of Authorization C of this letter:

Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing aliquots of transport media from up to 5 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19 and placed in individual vials containing transport media, when tested as part of a serial testing program including testing at least once per week.

This indication is authorized for use in laboratories certified under CLIA to perform high complexity tests, except that tests authorized for use in specific named or designated laboratories can only be used in such high complexity laboratories.

Tests will be added to Exhibit 1 after FDA determines it has received a complete notification.

Validation for this indication includes the validation protocol outlined below, “*Validation of the Effect on the Percent Agreement*”. 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 the Effect on the Percent Agreement*

Test at least 20 individual positive clinical specimens using the testing protocol in your authorized test’s IFU or procedure. Test 20 paired 5-sample pools in parallel, each containing aliquots from a single positive specimen and 4 randomly selected individual negative clinical specimens (collection of negative anterior nasal specimens from healthy individuals is acceptable), using the testing protocol you intend to include in your IFU or procedure for testing 5-sample pools.

20 unique positive specimens and 80 unique negative specimens are needed to comprise twenty 5-sample pools. At least 20% of positive clinical specimens used for this validation study should be low positives, where, for 5-sample pooling, a low positive is within 2.32 Ct of the mean Ct at LoD for your previously authorized test.

Archived individual clinical anterior nasal specimens are acceptable for this validation study, if available, given they contain enough volume for both individual and 5-sample pool testing. If archived specimens are used, the original diagnostic results are acceptable in lieu of repeating the individual specimen testing, if the original diagnostic results were acquired according to your previously authorized test’s IFU or procedure for

individual testing.

If you cannot acquire at least 20% low positive samples as natural clinical specimens, you may dilute positive clinical specimens into pooled negative anterior nasal clinical matrix prepared as described here. Use either diluted positive clinical samples quantified in copies/mL using a standard curve, or inactivated virus of a known quantity represented as copies/mL. Prepare samples using negative anterior nasal clinical matrix (collection from healthy individuals is acceptable) using sample volumes you intend to include in your IFU or procedure for testing 5-sample pools.

Your validation must demonstrate that:

- $\geq 85\%$  agreement between pooled testing and individual testing; and
- The invalid rate in the pooling protocol does not exceed 5%.

## **Appendix H**

### **Media pooling up to n=10 – validation option 2**

Subject to the terms of this EUA, a test that is within the Scope of this Amendment (Section II) is authorized for the following additional indication and may be distributed (if previously authorized for distribution per the underlying EUA) and used for this indication after validation is completed as set forth in this Appendix and a notification has been submitted to FDA and confirmed by FDA to be complete in accordance with Condition of Authorization C of this letter:

Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing aliquots of transport media from up to 10 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19 and placed in individual vials containing transport media, when tested as part of a serial testing program including testing at least once per week.

This indication is authorized for use in laboratories certified under CLIA to perform high complexity tests, except that tests authorized for use in specific named or designated laboratories can only be used in such high complexity laboratories

Tests will be added to Exhibit 1 after FDA determines it has received a complete notification.

Validation for this indication includes the validation protocol outlined below, “*Validation of the Effect on the Percent Agreement*”. 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 the Effect on the Percent Agreement*

Test at least 20 individual positive clinical specimens using the testing protocol in your authorized test’s IFU or procedure. Test 20 paired 10-sample pools in parallel, each containing aliquots from a single positive specimen and 9 randomly selected individual negative clinical specimens (collection of negative anterior nasal specimens from healthy individuals is acceptable), using the testing protocol you intend to include in your IFU or procedure for testing 10-sample pools.

20 unique positive specimens and 180 unique negative specimens are needed to comprise twenty 10-sample pools. At least 20% of positive clinical specimens used for this validation study should be low positives, where, for 10-sample pooling, a low positive is within 3.32 Ct of the mean Ct at LoD for your previously authorized test.

Archived individual clinical anterior nasal specimens are acceptable for this validation study, if available, given they contain enough volume for both individual and 10-sample pool testing. If archived specimens are used, the original diagnostic results are acceptable in lieu of repeating the individual specimen testing, if the original diagnostic results were acquired according to your previously authorized test’s IFU or procedure for

individual testing.

If you cannot acquire at least 20% low positive samples as natural clinical specimens, you may dilute positive clinical specimens into pooled negative anterior nasal clinical matrix prepared as described here. Use either diluted positive clinical samples quantified in copies/mL using a standard curve, or inactivated virus of a known quantity represented as copies/mL. Prepare samples using negative anterior nasal clinical matrix (collection from healthy individuals is acceptable) using sample volumes you intend to include in your IFU or procedure for testing 10-sample pools.

Your validation must demonstrate that:

- $\geq 85\%$  agreement between pooled testing and individual testing; and
- The invalid rate in the pooling protocol does not exceed 5%.

## Appendix I Required Changes to Authorized Labeling – Fact Sheets

Per Condition of Authorization A and as set forth in the Scope of this Amendment (Section II), your labeling must be updated in the following ways:

- A) Fact Sheet for Healthcare Providers (a sample updated Fact Sheet for Healthcare Providers is included in Appendix J):
- 1) Include the additional authorized indication(s) from the applicable appendix in all places where the Fact Sheet includes the indications.
  - 2) In the section titled “What does it mean if the specimen tests positive for the virus that causes COVID-19?” add the following paragraph immediately following the first paragraph:

*Individuals included in a pool that returns a positive or invalid result should be treated as a presumptive positive unless or until they receive a negative result when re-tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, they should isolate until receiving a negative result when re-tested individually and should **not** be cohorted with other individuals who have received a positive or presumptive positive result.*

- 3) In the section titled “What does it mean if the specimen tests negative for the virus that causes COVID-19?” add the following three paragraphs immediately following the first paragraph:

*In addition, asymptomatic people infected with COVID-19 may not shed enough virus to reach the limit of detection of the test, giving a false negative result. In the absence of symptoms, it is difficult to determine if asymptomatic people have been tested too late or too early. Therefore, negative results in asymptomatic individuals may include individuals who were tested too early and may become positive later, individuals who were tested too late and may have serological evidence of infection, or individuals who were never infected.*

*Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity or increased interference of pooled testing. Your interpretation of negative results should take into account clinical and epidemiological risk factors.*

*If COVID-19 is suspected based on exposure history together with other clinical findings, re-testing using a new sample with a sensitive method or without pooling should be considered by healthcare providers in consultation with public health authorities. Additional testing may be helpful to ensure testing was not conducted too early.*

B) Fact Sheet for Patients (a sample updated Fact Sheet for Healthcare Providers is included in Appendix K):

- 1) In the section titled “Why was my sample tested?” add the following to the beginning of the section:

*You are being tested at regular intervals (serial testing) even though you do not have symptoms or risk factors for COVID-19; or*

And add the following to the end of the section:

*Laboratories may use pooling when testing your specimen, which means they combine your sample with other individuals samples prior to testing and test them as a “pool”. The laboratory may return a result for the entire pool together or may return individual results.*

- 2) In the section titled “What does it mean if I have a positive test result?” add the following to the beginning of the section:

*If you were tested as part of a pool that returned a positive or invalid test result, you may have COVID-19 and should consider yourself to have a positive test result unless or until you receive a negative test result when re-tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, you should isolate until receiving a negative result when re-tested individually and should **not** be grouped with other individuals who have received a positive or presumptive positive result.*

- 3) In the section titled “What does it mean if I have a negative test result?” add the following after the second paragraph of the section:

*In particular, people infected with COVID-19 but who have no symptoms may not shed enough virus to trigger a positive test.*

*If your test result indicates your specimen was pooled and you have a negative test result there a small chance that your result is incorrect. You should talk with your healthcare provider if you are concerned.*

And add the following paragraph and text box to the end of the section:

*If you have no symptoms but have been tested because your doctor thought you may have been exposed to COVID-19, you should continue to monitor your health and let your healthcare provider know if you develop any symptoms of COVID-19. If you develop symptoms you may need another test to determine if you have contracted the virus causing COVID-19.*

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**If you develop symptoms or your symptoms get worse you should seek medical care. If you have the following symptoms you should seek immediate medical care at the closest emergency room:**

- Trouble breathing
  - Persistent pain or pressure in the chest
  - New confusion
  - Inability to wake up or stay awake
  - Bluish lips or face
-

**Appendix J**  
**Sample Updated Fact Sheet for Health Care Providers**

**Appendix K**  
**Sample Updated Fact Sheet for Patients**