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Puerto Rico Diagnostic Test Evaluation Commission

Molecular Diagnostic Template for Laboratories[1]

This template (the "template") includes FDA's and PRoDTEC's current recommendations for laboratories concerning what data and information they should submit to support an EUA request for a molecular diagnostic for SARS-CoV-2 developed for use in a single CLIA certified high-complexity laboratory. As outlined in Section V.A. of the FDA guidance document: *Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)*, [2] FDA recommends that the following validation studies be conducted for a SARS-CoV-2 molecular diagnostic assay: Limit of Detection, Clinical Evaluation, Inclusivity, and Cross-reactivity. This template is intended to help laboratories provide these validation data and other information to FDA, but alternative approaches can be used. It reflects FDA's and PRoDTEC's current thinking on the topic, and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* means that something is suggested or recommended, but not required. For more information about EUAs in general, please see the FDA Guidance document: *Emergency Use Authorization of Medical Products and Related Authorities*.[3]

GENERAL INFORMATION ABOUT THIS TEMPLATE

- This EUA review template (EUA template) is only intended for use by CLIA certified high-complexity laboratories who intend to submit a pre-EUA or EUA to FDA for a SARS-CoV-2 molecular diagnostic test. Use of the template is applicable only for testing of respiratory specimens, e.g., nasopharyngeal, sputum, and BAL specimens.
- Text highlighted in yellow ***[Text]*** should be completed by the laboratory (sponsor) as applicable to their specific test. Text in **bold** outlines the Food and Drug Administration's (FDA) recommendations for the sponsors' consideration when providing the suggested information in a specific section.
- A test authorized under an EUA is only authorized for emergency use while the EUA is in effect.
- This is an EUA interactive review template for Pre-EUA/EUA submissions. We plan to update the template as appropriate as we learn more about the COVID-19 disease and gain experience with the EUA process for this test.

[1] This template is part of the Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised) - Immediately in Effect Guidance for Clinical Laboratories, Commercial Manufacturers, and Food and Drug Administration Staff
[2] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-coronavirus-disease-2019-tests-during-public-health-emergency-revised
[3] https://www.fda.gov/media/97321/download

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EXAMPLE TEMPLATE

A. PURPOSE FOR SUBMISSION

Emergency Use Authorization (EUA) request for use of a SARS-CoV-2 molecular diagnostic test to be performed for the in vitro qualitative detection of RNA from the SARS-CoV-2 in respiratory samples from patients as recommended for testing by public health authority guidelines or for screening of individuals without symptoms or other reasons to suspect COVID-19. The test will be performed in CLIA certified high-complexity laboratories. Additional testing and confirmation procedures should be performed in consultation with public health and/or other authorities to whom reporting is required.

Positive results should also be reported in accordance with local, state, and federal regulations.

If you plan to include a sample pooling protocol in your procedures please include a brief description of the pooling strategy in your EUA request.

If you plan to request authorization to test specimens collected with a home specimen collection kit, please refer to the Home Specimen Collection Molecular Diagnostic Template ***and include any relevant information in this request.***

B. MEASURAND

Specific nucleic acid sequences from the genome of the SARS-CoV-2 ***[please specify the targeted gene(s) of the pathogen; assays with more than one target are recommended].***

C. LABORATORY/SPONSOR

[Official name, address and contact information of applicant and all locations where specimen testing will be performed]

D. REGULATORY INFORMATION

Approval/Clearance Status:
The SARS-CoV-2 assay test is not cleared, CLIA waived, approved, or subject to an approved investigational device exemption.

Product Code:
QJR

E. PROPOSED INTENDED USE

The proposed IU will be finalized based on the performance data and recommendations from Public Health authorities at the time of authorization – example text is provided below for a qualitative molecular test that detects organism RNA but may be adapted according to the specific emergency situation addressed by the device.

1) Intended Use:

The SARS-CoV-2 assay is a [***specify test technology such as, real-time RT-PCR test***] intended for the [***presumptive***] qualitative detection of nucleic acid from the SARS-CoV-2 in [***list respiratory specimens e.g., nasopharyngeal, nasal, and oropharyngeal swab specimens and lower respiratory tract, BAL, sputum***] ***[If your test is intended for testing multiple respiratory pathogens, please list the specific analytes detected by your test.] [describe intended use population, e.g., from individuals suspected of COVID-19 by their healthcare provider or for screening of individuals without symptoms or other reasons to suspect COVID-19]***. Testing is limited to [***Name of Clinical Laboratory***] that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, ***to perform high complexity tests***. ***[Describe the sample pooling approach and maximum number of specimens which can be pooled, as applicable.]***

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in [***name specimen type, e.g. upper respiratory***] 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.

The assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

[Depending on the performance data submitted and patient population included in the clinical evaluation, additional limitations may be recommended and/or your intended use may be modified to include the following, as applicable:

- ***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.***

- ***Negative results from pooled samples should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, pooled samples should be tested individually. Negative results do not preclude SARS-CoV-2 infection and must not be used as the sole basis for patient management decisions. Negative results must be considered in the context of a patient's recent exposures, history, presence of clinical signs and symptoms consistent with COVID-19.***

- ***Use of the [test name] in a general, asymptomatic screening population is intended to be used as part of an infection control plan, that may include additional preventative measures, such as a predefined serial testing plan or directed testing of high-risk individuals. Negative results should be considered presumptive and do***

not preclude current or future infection obtained through community transmission or other exposures. Negative results must be considered in the context of an individual's recent exposures, history, presence of clinical signs and symptoms consistent with COVID-19.]

2) Instruments Used with Test:
The **[*test name*]** test is to be used with the **[*list all RT-PCR Instruments, software, automated extraction instruments*]**.

F. DEVICE DESCRIPTION AND TEST PRINCIPLE

Example text has been added under each of the sub-headings below for a fluorescence based rRT-PCR test for detection of organism RNA. If a different test principle is used by the test for the detection of a specific analyte please modify the description accordingly to capture the salient points in each of the sub-headings below. Please note this template is intended for use only with existing, well-established technologies.

1) Product Overview/Test Principle:
Please note if your test is a previously FDA-authorized modified test. The assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

2) Description of Test Steps:
[Please describe in abbreviated form the steps for performing your assay in sequential order as a numbered list, including extraction methods. This should include the names of the instruments used in your assay, e.g., ABI 7500. A copy of your laboratory procedure would be acceptable and can be appended to this form.]

3) Control Material(s) to be Used:
[Please describe the assay controls to be performed in the laboratory, including the following:

- ***The positive and negative control; ideally the positive control will be used to confirm performance near the test LoD. If a template control is used, please describe in general terms the sequence used.***
- ***The extraction control.***
- ***The internal control, if present.***

Your description should also include the frequency that controls will be performed.]

4) Assay results and interpretation
All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. ***[Please describe the results of your assay procedure, e.g., reactive (positive/detected), non-reactive (negative/non-detected), or Invalid (no result reported).] Please describe if a Ct cutoff is used as part of your testing algorithm. When applicable, provide a table clearly describing***

the possible combinations of test result values for each primer/probe set, and how they should be combined into a final interpretation of the result for your test.

G. PERFORMANCE EVALUATION

The following validation studies should be performed during your assay development:

1) Limit of Detection (LoD) -Analytical Sensitivity:
Laboratories should document the limit of detection (LoD) of their SARS-CoV-2 assay. It is recommended to spike quantified inactivated virus (e.g., heat treated or irradiated virus) into real clinical matrix (e.g., BAL fluid, sputum, nasopharyngeal swab, etc.) for LoD determination, since the inactivated virus most closely reflects live virus in a clinical sample. If you are unable to acquire inactivated virus, FDA believes that viral genomic RNA is the next best material to use to generate contrived samples for LoD determination. As positive natural clinical specimens are increasingly becoming available, a known positive clinical specimen as determined by an EUA-authorized test can also be used in generating dilutions in clinical matrix for LoD determination. Respiratory swab matrix should derive from swab specimens collected from SARS-CoV-2 negative individuals.

FDA recommends that preliminary LoD be determined by testing a 2-3 fold dilution series of three extraction replicates per concentration. The lowest concentration that gives positive results 100% of the time is defined as the preliminary LoD. The final LoD concentration should be confirmed by testing 20 individual extraction replicates at the preliminary LoD. FDA defines LoD as the lowest concentration at which 19/20 replicates are positive. If multiple clinical matrices are intended for clinical testing, you should submit to FDA the results from one representative matrix of each claimed clinical matrix type. For example:
- If testing common upper respiratory tract specimens (e.g., nasopharyngeal (NP) swabs, oropharyngeal (OP), swabs, nasal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, and nasal washes etc.), please submit results from the most challenging upper respiratory matrix. FDA considers nasopharyngeal (NP) swabs to be the most challenging upper respiratory matrix.
- If claiming common lower respiratory tract specimens (e.g., tracheal aspirates, sputum, etc.), please submit results from the most challenging lower respiratory matrix. FDA considers sputum to be the most challenging lower respiratory matrix.
- If claiming both upper and lower respiratory matrixes, submitting results from sputum samples may suffice to support both upper and lower respiratory matrices.
- If claiming alternative respiratory specimens, such as saliva, oral fluid, buccal swab, etc., please submit results from testing each of the claimed uncommon respiratory specimen type.
- If needed, we recommend that you follow the most current version of the CLSI standard, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures (CLSI EP17)

[Please describe in abbreviated form your LoD study, the specific material used (e.g., live or inactivated viral stocks, viral RNA, or in vitro transcripts), the specific clinical matrix used, and the LoD (with appropriate units) for your assay]

2) Inclusivity (analytical sensitivity):

Laboratories should document the results of an inclusivity study that demonstrates the strains of SAR-CoV-2 that can be detected by the proposed molecular assay. It is acceptable to conduct an in silico analysis of published SARS-CoV-2 sequences using the assay's primers and probes. FDA anticipates that 100% of published SAR-CoV-2 sequences will be detectable with the selected primers and probes.

[Please describe in abbreviated form your Inclusivity study and confirm that there was 100% detection of all SARS-CoV-2 strains.] If sequences with less than 100% homology with any of the primers and probes in your test are identified, please provide a thorough risk assessment on how such mismatches may impact the performance of your test.

3) Cross-reactivity (Analytical Specificity)
At a minimum, an in silico analysis of the assay primer and probes compared to common respiratory flora and other viral pathogens, listed in the table below for respiratory specimen claims, should be performed. FDA defines in silico cross-reactivity as greater than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism. Laboratories should follow recognized laboratory procedures in the context of the sample types intended for testing for any additional cross-reactivity testing.

Recommended List of Organisms to be Analyzed in silico and by Wet Testing*

Other high priority pathogens from the same genetic family	High priority organisms likely present in a respiratory specimen.
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Pooled human nasal wash – *to represent diverse microbial flora in the human respiratory tract*

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Other high priority pathogens from the same genetic family	High priority organisms likely present in a respiratory specimen.
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Streptococcus salivarius

* For wet testing, concentrations of 106 CFU/ml or higher for bacteria and 105 pfu/ml or higher for viruses is recommended.

[Please describe in abbreviated form your cross-reactivity study and list the microorganisms tested, indicating whether this was performed either in in silico or wet testing. Organisms recommended for testing are listed in the table above]

4) Clinical Evaluation

a) Testing patients suspected of COVID-19 by their healthcare provider:
FDA recommends using natural clinical specimens in the clinical evaluation. Please refer to the following table for additional information regarding clinical study design:

Note: Clinical study recommendations listed in the table below do not apply to claims for screening individuals without symptoms or other reasons to suspect COVID-19 and to saliva or other alternative respiratory specimen type claims.

Minimum Number of Positive Specimens	***A minimum of 30 natural (prospective or retrospective or leftover samples) positive clinical specimens should be collected from patients suspected of SARS-CoV-2 infection by a healthcare provider in COVID-19 disease endemic region(s).*** ***Samples can be a mixture of specimen types, if you are seeking an upper respiratory claim (e.g., nasopharyngeal (NP) swab, oropharyngeal (OP) swab, nasal swab (NS)).*** ***If you are seeking a sputum claim, and any other respiratory specimen claim except alternative respiratory specimen types (e.g., saliva), we recommend a combination of 15 NP and 15 sputum samples.*** ***Specimens collected from different anatomical sites from the same patient may be used to support claims for multiple specimen types.*** ***The use of frozen samples is acceptable.*** ***Specimens representing a wide range of viral load including low positive samples should be tested.*** ***The use of samples previously tested positive by another EUA RT-PCR assay may be acceptable without additional comparator testing. You should indicate the source of the samples, provide results for each tested sample, indicate specimen type, and initial test date.***
Minimum Number of Negative Specimens	***A minimum 30 individual negative samples acquired from the following sources are acceptable; (1) archived/retrospective respiratory samples collected from patients with signs and symptoms of respiratory infection, and (2) other subjects that are expected to be negative for SARS-CoV-2.***

Recommended Comparator Method for percent agreement performance calculations	***Positive percent agreement should be calculated in comparison to an EUA RT-PCR test. We recommend using only a high sensitivity EUA RT-PCR assay which uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction). If available, FDA recommends selecting a comparator assay that has established high sensitivity with an internationally recognized standard or FDA SARS-CoV-2 Reference Panel. Please contact approvals@prodtec.org to discuss options to establish the sensitivity of your comparator method. Please see the following website for the most recent list of FDA authorized 2019-nCoV tests: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations.*** ***Negative result agreement may be calculated in comparison to an EUA RT-PCR test (prospectively collected samples) or as agreement with expected results if samples were collected from individuals known to be negative for SARS-CoV2 (e.g. collected before December 2019).*** ***The comparator assay may have the same, or different, targets as your assay. False results can be investigated using an additional EUA RT-PCR assay, and/or Sanger sequencing. The results of the discordant analysis can be footnoted in your final performance table but cannot be used to change the final performance calculations.***
Acceptance Criteria	***FDA believes a minimum of 95% positive and negative agreement is acceptable clinical performance.***
Natural Clinical Specimens IRB/Informed Consent Note	***Prospective collection of clinical specimens to support the EUA request should be done in accordance with regulations for human subject protection, including IRB approval and informed consent.*** ***Use of leftover de-identified samples may follow the policy outlined in the FDA Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable (https://www.fda.gov/media/122648/download).***
Testing Approach Note 1	***All clinical specimens tested in your study should be evaluated in accordance with your proposed diagnostic algorithm (i.e., tested using your laboratory procedure), including retesting when appropriate. The limited volume of natural specimens may preclude retesting. In instances where retesting is indicated but not performed, for the purposes of performance evaluation, initial results will be analyzed for performance and equivocal/indeterminate/inconclusive results should count against your final performance.***
Testing Approach Note 2	***Specimens should be tested in a blinded fashion, e.g., positive and negative samples should be presented to the end user in***

	a blinded fashion. The end user should also be blinded to the results of any comparator method testing.

b) **Testing alternative specimens (i.e., other than respiratory specimens) from patients suspected of COVID-19 by their healthcare provider:**

If you seek a claim for alternative specimens, such as saliva, oral fluid, buccal swabs, etc., you should test two paired specimens from at least 30 positive and 30 negative patients. Consecutively collected specimens are preferred. Specimens representing a wide range of viral load including low positive samples should be tested. One specimen from each patient should be collected by a healthcare worker using a nasopharyngeal (NP) swab and tested with an assay authorized for use with NP specimens. FDA recommends selecting a comparator assay that has established high sensitivity with an internationally recognized standard or FDA SARS-CoV-2 Reference Panel. Please contact approvals@prodtec.org to discuss options to establish the sensitivity of your comparator method. The other specimen from each patient should be the alternative specimen and should be tested with your candidate EUA assay, provided it is authorized for testing of NP specimens, or using a previously authorized test with an NP swab claim. To minimize the occurrence of discordant results due to biological variability, both samples should be collected within a short time period. FDA believes ≥95% positive percent agreement with similar Ct values for the paired specimen types is acceptable performance.

Please provide detailed information regarding the type of collection device and transport media you propose to validate for use with your assay. Please note that some transport media may not be compatible with assays that do not use a nucleic acid extraction step. In addition, some transport medium may not be acceptable for use for at-home collection due to the presence of hazardous chemicals. For additional information that may be needed to support at-home sample collection and transport, please review the Home Specimen Collection Molecular Diagnostic Template or contact FDA at approvals@prodtec.org.

c) Screening individuals without symptoms or other reasons to suspect COVID-19 with a previously unauthorized test

The recommendations below reflect FDA's current thinking. The study design and recommendations may change as additional information becomes available regarding asymptomatic infections, including but not limited to viral titer dynamics and transmission rates in this population.

If you seek to have your test authorized for screening individuals without symptoms or other reasons to suspect COVID19, FDA recommends that you conduct a clinical study in the intended population. In the clinical study, you should compare results for your assay and a comparator assay for each patient enrolled. Please consider the following when designing your clinical validation study:

- ***The number of enrolled patients should be sufficient to ensure at least 20 positive samples are prospectively collected in the intended use population and be sufficient to demonstrate the following minimum performance:***

PPA ≥95% (Lower Bound of the two-sided 95% confidence interval >76%)
NPA ≥98% (Lower Bound of the two-sided 95% confidence interval >95%)

The total number of samples needed will depend on the prevalence of SARS-CoV-2 in the intended use population.

- ***Samples for the candidate test should be collected according to the instruction for use.***
- ***Samples for comparator method testing should be healthcare provider collected NP swabs. If an NP swab cannot be collected, a nasal swab may be used, however, both anterior nares should be sampled with the same swab. Sampling for the candidate test and comparator method should occur within a short timeframe to avoid biological variability in viral load.***
- ***If available, FDA recommends selecting a comparator assay that has established high sensitivity with an internationally recognized standard or the FDA SARS-CoV-2 Reference Panel. Please contact approvals@prodtec.org to discuss options to establish the sensitivity of your test.***
- ***In general, we recommend that you collect samples at a minimum of three geographically diverse sites, especially if you are planning to use the same data to support a subsequent De novo/510k submission. If this is not possible, FDA will consider samples collected at one or two sites in the context of an EUA.***
- ***It may be possible to use archived samples that were collected from asymptomatic patients. We recommend you contact FDA to discuss such an approach prior to initiating your study.***

d) Adding population screening of individuals without symptoms or other reasons to suspect COVID-19 to an authorized test

Alternative approaches may be acceptable for tests that have been previously authorized with clinical data for symptomatic patients. For example:

- ***If your assay is highly sensitive as determined by testing with the FDA SARS-CoV-2 Reference Panel or a recognized international standard, a post-authorization study may be appropriate. We recommend testing a minimum of 20 consecutively collected asymptomatic positive specimens and at least 100 consecutively collected negative specimens based on the results of the candidate test. All specimens should then be tested with another EUA authorized molecular assay. Using estimates of the predictive values and the percentage of positive results, this study can be used to establish the sensitivity (PPA) and specificity (NPA) of your test in a general, asymptomatic population, as this is an important performance metric for tests intended for screening of large populations without symptoms or other reasons to suspect COVID-19. The FDA expectation is that PPA should be ≥95% (lower bound of the two-sided 95% confidence interval >76%) and NPA should be ≥98% (with a lower bound of the two-sided 95% confidence interval >95%). If you do not have access to either the FDA SARS-CoV-2 Reference Panel or a recognized international standard then please contact approvals@prodtec.org to discuss options.***

- ***If you can demonstrate that performance of your assay in both populations is likely similar (i.e., the percent of positive individuals with Ct values representing low viral loads are similar in individuals suspected and not suspected of COVID19 by their healthcare provider) you may include both populations in your evaluation. We encourage the use of historic data (i.e., existing or published data) for this evaluation.***

FDA is open to considering additional alternative study designs to demonstrate that the performance of your assay is appropriate for screening individuals without symptoms or other reasons to suspect COVID-19. We recommend contacting FDA to discuss alternative study designs prior to beginning such a study.

c) Specimen Pooling

- ***The recommendations below reflect FDA's current thinking. The study design and other recommendations may change as additional information becomes available. At this time, the need for testing remains greater than available resources. Combining multiple patient samples to create one pooled sample for testing could enable broader access to testing.***

- ***To establish performance of your test with pooling, FDA recommends conducting a clinical validation study in the intended use population that includes testing each sample individually and using your proposed pooling strategy.***

- ***Currently FDA recommends two approaches to patient specimen pooling: 1) pooling aliquots of transport media which each contain a single patient sample (sample/media pooling) or 2) adding swabs from multiple patients into a single volume of transport media (swab pooling). As more data become available and new approaches are identified, our recommendations may evolve.***

Monitoring:

- ***Laboratories should incorporate ongoing monitoring of the pooling strategy by addressing the following in their procedures:***
  - ***Before implementation of pooling, evaluate existing test data in the testing population from the previous 7-10 days to estimate the initial positivity rate.***
  - ***When implementing a pooling strategy, continue to test a random sampling of patient samples without pooling to:***
    - ***evaluate the positivity rate and percent of weak positive samples in the testing population and***
    - ***identify differences in positivity rate between those tested individually and those tested through pooling.***

- ***Calculate the percent of positive results after implementation of pooling using a moving average (such as a rolling average updated daily using data from the previous 7-10 days) to determine whether there is a change in the positivity rates between individual testing and pooled testing. Reevaluate testing strategy if the moving average of the positivity rate for pooled samples starts trending in a positive or negative direction.***
- ***Finally, when resource availability is sufficient to meet testing demand, FDA recommends considering whether the risks of reduced test sensitivity with pooling continue to outweigh the benefits of resource conservation.***

e.1) Sample/Media Pooling
A simple, or Dorfman, approach involves testing an "n-sample pool," where n is the number of transport media samples included in the pool. A negative result implies that all samples in the pool are negative. A positive result indicates that at least one sample in the pool is positive. When an n-sample pool is positive, each sample within the pool must be individually tested to determine which is/are positive. When used effectively, n-sample pooling can generally enable testing of more individuals despite limited testing resources.

- ***When pooling transport media, rather than swabs, one individual sample is defined as a single specimen swab collected from a subject and placed in a specific volume of transport media. In this type of pooling, an aliquot of each individual sample is combined into non-overlapping pools of n samples and each n-sample pool is tested. Therefore, the volume of samples initially collected from an individual must be sufficient for both the pooled testing and individual follow-up testing, if needed.***

- ***N-sample pooling should be considered in the context of the positivity rate of a test in the test population, analytical sensitivity of the test, and the percent of weak positive subjects in the tested population. Pooling of n samples reduces the analytical sensitivity of the test (increase in the LoD) because samples are diluted. The impact of decreased analytical sensitivity depends on the percent of subject specimens with viral genetic material concentrations close to the LoD (weak positives) in the tested population. Therefore, analytical sensitivity of the test with n-sample pools should be evaluated.***

- ***FDA believes an n=5 is a reasonable starting point for validation of pooling for a high-sensitivity test in populations with a positivity rate of approximately 5% to 6%. In populations with lower prevalence, larger sample pools may be feasible. In populations with higher prevalence, smaller sample pools may be needed. FDA recommends that developers begin by validating their tests for pooling using an n=5. Tests validated and***

authorized for n=5 can then be used with any n≤5 depending on testing needs and taking into consideration local prevalence. In cases where a developer wants to validate an n>5, or is considering alternate pooling schemes, FDA recommends that developers reach out to FDA at approvals@prodtec.org or submit a pre-EUA to discuss their approach and validation plan.

- ***The table below presents calculated n-sample pool sizes with the maximal efficiency (a maximum increase in the number of tested patients because of n-sample pooling strategy) for different positivity rates P. This n with maximal efficiency ($n_{maxefficiency}$) should be a starting pool size for validation of pooling with positivity rate P. If the accuracy of the test with regard to missed positive patients because of $n_{maxefficiency}$ samples pooling is not acceptable, $n < n_{maxefficiency}$ should be considered and accuracy of pooling with this n should be evaluated.***

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P, percent of positive subjects in the tested population	$n_{maxefficiency}$ ***(n corresponding to the maximal efficiency)***	***Efficiency of n-sample pooling (a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)***
1%	11	5.11
2%	8	3.65
3%	6	3.00
4%	6	2.60
5%	5	2.35
6%	5	2.15
7%	4	1.99
8%	4	1.87
9%	4	1.77
10%	4	1.68
11%	4	1.61
12%	4	1.54
13%	3	1.48
14%	3	1.43
15%	3	1.39
16%	3	1.35
17%	3	1.31
18%	3	1.28
19%	3	1.25
20%	3	1.22
21%	3	1.19
22%	3	1.16
23%	3	1.14
24%	3	1.12
25%	3	1.10

Because a single positive sample in a pool requires individual retesting of each sample in the pool, the efficiency of any pooling strategy depends on the positivity rate. The efficiency (F) of n-sample pooling for positivity rate (P) can be calculated with the following formula $F=1/(1+1/n-(1-P)^n)$. The efficiency (F) indicates how many more patients can be tested with n-sample pools compared to individual testing. For example, a 3-sample pooling strategy increases the number of tested patients by 1.48 times for positivity rate P of 13% (F=1.48) and by 1.22 times for positivity rate P of 20% (F=1.22).

At F=1.48, 1,000 tests can cover testing of 1,480 patients. Likewise, at F=1.22, 1,000 tests can cover testing of 1,220 patients.

- ***A test validated for a specific n-sample pooling strategy is also considered to be validated for any number of pooled samples below n. For example, a test validated for a 5-sample pooling strategy can be performed for any n≤5.***

- ***Different specimen types should not be pooled together.***

- ***FDA recommends that your procedures specify a sample volume great enough to allow for individual and pooled testing so that, during clinical use, any samples in a positive pool can be re-tested without the need for a second sample collection.***

- ***Due to the reduction in analytical sensitivity, a pooling strategy should include risk mitigations such as additional language in the report noting that pooling was used during testing.***

Validation:

- ***Test developers should characterize the reduction in assay analytical sensitivity (i.e., shift in Ct value for RT-PCR assays) with respect to the number (n) of samples to be pooled to ensure the selected n-sample pooling strategy will maintain appropriate sensitivity. This maximum number of samples acceptable to pool should be determined and validated using the recommendations below for each specimen type you intend to pool.***

- ***We strongly recommend that you develop and validate a system for deconvoluting pooled test data which is intended to accurately identify individual patient samples composing each pooled sample. If you plan to use a software solution intended to deconvolute pooled SARS-CoV-2 diagnostic test data then we recommend providing validation data establishing that the software can achieve its intended use. For example, we recommend providing evidence that the software has been validated to ensure that:***
    - ***The inputs and outputs of the software are appropriate for the intended use of the assay;***
    - ***All expected inputs produce the expected outputs for all functions critical for system operation; and***
    - ***The system will be provided to the customer free of defects or defects will be known and mitigated.***

A) Sample pooling: adding a pooling strategy to a previously authorized (EUA) test

When requesting to add an n-sample pooling strategy to the authorized uses and the authorized procedures for your own previously authorized assay, you should submit an EUA amendment request with the appropriate validation data as described below. To leverage the previous individual sample testing validation data for a different developer's assay, please provide a Right of Reference from that EUA holder. To add a pooling strategy to a previously authorized test, you generally do not need to establish performance with a separate comparator assay.

You should conduct a clinical study with at least 20 individual positive samples, comparing the performance of the EUA-authorized assay when testing single specimens according to the authorized procedures to the performance of the assay when testing n-sample pools. We strongly encourage you to work with your customers to gather existing data (e.g., 100 Ct values from individually tested positive patient samples) and evaluate the percentage of samples with Ct values close to your assay LoD (i.e., weak positives). A theoretical Ct shift of $Log_2(n)$ can be estimated for most RT-PCR tests (e.g., for n=5, a Ct shift of 2.3 would be expected). Therefore, if a large percentage of positive patient samples are close to your assay LoD, you may want to consider a smaller n, which will reduce the observed Ct shift and maintain higher sensitivity.

Please consider the following when designing your clinical validation study with 20 individually tested positive samples:

- ***If archived individual samples are available and have enough volume for testing with n-sample pools, we recommend that you use at least 20 archived positive samples. If these samples are not available with sufficient volume, we recommend that you enroll enough patients to collect at least 20 positive samples and an appropriate number of individual negative samples from the intended use population. For example, for a 5-sample pooling strategy, a total of 80 unique comparator method negative samples are recommended in order to make up 20 5-sample pools with the 20 positive samples (20 positives + 4x20 negatives). Additionally, 100 comparator method negative samples are recommended to make up 20 5-sample negative pools (5x20 negatives) as described below. If there is sufficient volume, the same negative patient samples can be used to create positive and negative pooled samples.***
- ***We recommend that at least 25% of the validation samples be within 2-3 Ct of the cut off, and no more than within 2-4 Ct.***
- ***Samples should be collected according to the procedures, keeping in mind that additional sample volume will be needed to test using an n-sample pooling strategy (n-sample pooling will need 1+1/n times the volume needed for individual testing).***
- ***All samples should be individually tested by your assay, either previously for archived specimens or prospectively, and have recorded Ct values if using an RT-PCR test.***

- ***To characterize the performance of your assay when testing pooled samples, those samples with positive results when tested individually should each be pooled with n-1 (e.g., where n=5, n-1=4) randomly selected negative samples. The resulting 20 pools, each consisting of 1 positive sample and n-1 negative samples, should be tested by your assay.***
- ***To confirm that negative samples remain negative in n-sample pools, we recommend testing 20 pools each consisting of n (e.g., n=5) negative samples. If there is sufficient volume, the same negative patient samples can be used to create positive and negative pooled samples.***

Analysis of data

- ***You should report estimates of positive and negative percent agreement comparing the performance of your test for pooled samples to the expected result. With regard to positive percent agreement (PPA), using a study design with 20 positives, you should calculate the percent of pools (1 positive and n-1 negative) with positive results. It is anticipated that all samples that were identified individually as positive by your test should still be positive when tested in pools with n-1 negative samples (PPA=100%); lower levels of PPA in the range of 85-90% may be acceptable depending on pooling efficiency and other factors. The n that allows a test to meet 85% or higher PPA should be validated for each test.***
- ***Additionally, for RT-PCR tests, you should provide an analysis of Ct values for each target detected by your test. We recommend presenting the Ct values for the n-sample pools on the Y-axis and Ct values for the individually tested samples on the X-axis. The clinical validation study should demonstrate that individual positive samples with viral loads close to the assay's LoD (i.e., weak positives) are accurately detected by your test in a pool with (n-1) negative samples.***
- ***We recommend that you provide an appropriate type of regression analysis with slope and intercept along with 95% confidence interval. Using regression analysis, we recommend that you evaluate the shift in Ct values for the positive patient samples diluted with negative patient samples.***

B) Sample pooling: new test (not previously authorized)

When requesting to include an n-sample pooling strategy for a new test, you should submit an EUA request with the appropriate validation data for individual testing in your proposed intended use population and for pooled testing, as described below. This should involve using a high-sensitivity comparator assay to characterize performance of your candidate test.

You should conduct a clinical study with at least 30 individual positive samples, as identified by the comparator assay, comparing the performance of the candidate assay both when testing single specimens and when testing n-sample pools to the performance of the comparator assay.

Please consider the following when designing your clinical validation study:

- ***The number of enrolled patient specimens should be sufficient to ensure at least 30 comparator method positive samples and an appropriate number of comparator method negative samples are collected from the intended use population. The number of comparator method negative samples depends on the pooling strategy. For instance, for a 5-sample pooling strategy, a total of 120 unique comparator method negative samples are recommended in order to make up 30 5-sample pools with the 30 positive samples (30 positives + 4x30 negatives). Additionally, 150 comparator method negative samples should make up 30 5-sample negative pools (5x30 negatives) as described below. If there is sufficient volume, the same negative patient samples can be used to create positive and negative pooled samples.***
- ***Samples for comparator method testing should be healthcare provider collected NP swabs. If an NP swab cannot be collected, a nasal swab can be used however both anterior nares should be sampled with the same swab. Sampling for the candidate test and comparator method should occur within a short timeframe, such as during the same visit, to avoid biological variability in viral load.***
- ***If available, FDA recommends selecting a comparator assay that has established high sensitivity with an internationally recognized standard or the FDA SARS-CoV-2 Reference Panel. Please contact approvals@prodtec.org to discuss options to establish sensitivity.***
- ***Samples for the candidate test should be collected according to the procedures. Depending on the sample volume required for your test, a single specimen collected from each study participant may be sufficient for individual and pooled sample testing.***
- ***In general, we recommend that you collect samples at a minimum of three geographically diverse sites, especially if you are planning to use the same data to support a subsequent De novo/510k submission. If this is not possible, FDA recommends samples collected at one or two sites in the context of an EUA.***
- ***It may be possible to use archived positive samples that were collected from the intended use population. We recommend you contact FDA to discuss such an approach prior to initiating your study. If archived samples are available, we recommend that at least 25% of the validation samples should be within 2-3 Ct of the cut off, and no more than within 2-4 Ct.***
- ***All samples should be individually tested by the comparator assay and individually tested by the candidate assay to characterize the performance of your assay when testing individual samples.***
- ***To characterize the performance of your assay when testing n-sample pools, those samples with positive results by the comparator method should each be pooled with n-1 (e.g., where n=5, n-1=4) randomly selected comparator method negative samples. The resulting 30 pools, each consisting of 1 comparator method positive sample and n-1 comparator method negative samples, should be tested by your candidate assay.***

- ***To confirm that samples with comparator method negative results remain negative in n-sample pools, we recommend testing 30 pools each consisting of n (e.g., n=5) comparator method negative samples.***

Analysis of data

- ***You should report estimates of positive and negative percent agreement comparing individual results from your test and the comparator test, as well as performance of pooled samples to the expected results (i.e., a pool which includes a comparator method positive sample is expected to remain positive when pooled). With regard to positive percent agreement (PPA), using a study design with 30 positives, you should calculate the percent of pools (1 positive and n-1 negative) with positive results. It is anticipated that all samples that were identified individually as positive should still be positive when tested in pools with n-1 negative samples (PPA=100%); lower levels of PPA in the range of 85-90% may be acceptable depending on pooling efficiency and other factors. The n that allows a test to meet 85% or higher PPA should be validated for each test.***
- ***Additionally, for RT-PCR tests, you should provide an analysis of Ct values of each target detected by your test. We recommend presenting the Ct values for the n-sample pools on the Y-axis and Ct values for the individually tested samples on the X-axis. The clinical validation study should demonstrate that individual positive samples with viral load close to the assay's LoD (i.e., weak positives) are accurately detected by your test in a pool with (n-1) negative samples.***
- ***We recommend that you provide an appropriate type of regression analysis with slope and intercept along with 95% confidence interval. Using regression analysis, we recommend that you evaluate the shift in Ct values for the positive patient samples diluted with negative patient samples.***

C) Example of validation and data presentation.

The information below is included as an example of how data can be presented to FDA in a pre-EUA or EUA request. It is for illustrative purposes only and is not reflective of data from any specific test nor the only way to present such information. This example is based on a 5-sample pooling strategy using an extraction method requiring a 500 uL sample.

1) Used the candidate assay to individually test 500 uL aliquots of 30 comparator positive samples and 150 comparator negative samples.

Example of table for presenting calculation of PPA and NPA of the candidate test results for samples tested individually vs the comparator test results:

Samples Tested Individually	***Comparator Method Result***	
Candidate Test Result	***Positive***	***Negative***

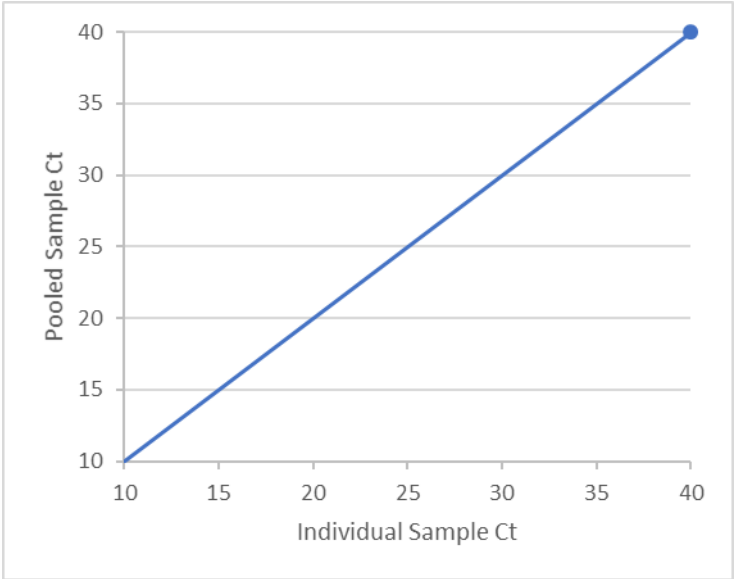
Positive		
Negative		

2) ***Created expected positive 5-sample pools by combining 100 uL of one (1) individual positive patient sample with 100 uL aliquots from each of four (4) unique comparator method negative patient samples. This was done for all positive patient samples thereby creating 30 5-sample pools (i.e., a total of 30 positives combined with a total of 120 negatives).***
3) ***Created expected negative 5-sample pools by combining 100 uL of five (5) individual negative patient samples using a total of 150 unique negative samples. When there was sufficient volume, the same negative patient samples were used to create positive and negative pooled samples.***
4) ***Tested all 5-sample pools by following the procedures of the candidate test. All previous results were unknown to the user (i.e., an individual other than the user performing the testing prepared the samples such that testing was performed "blinded").***
5) ***Calculated the percent agreement of the pooled samples with respect to the expected results (i.e., if a positive patient sample was included in the 5-sample pools, the expected result was positive).***

Example of table for presenting calculation of PPA and NPA of the candidate test results for samples tested in 5-sample pools vs expected results (where expected results are based on the individual testing):

Samples Tested in 5-sample pool	***Expected Result***	
Pooled Test Result	***Positive***	***Negative***
Positive		
Negative		

6) ***If the candidate assay is an RT-PCR test and cycle threshold values (Ct value) are available, we recommend that you provide a data plot (example below) of the positive sample Ct values of an individual tested positive (i.e., the Ct value of the individual positive sample used to create the positive pooled sample) and the positive pooled sample. We recommend that you include a diagonal line with a slope of 1 and a y-intercept of 0. We recommend that you provide an appropriate type of regression analysis with slope and intercept along with 95% confidence interval. Using the regression analysis, we recommend that you evaluate the shift in Ct values for the positive patient samples diluted with negative patient samples.***



7) ***Agreement should also be presented in a stratified manner so that performance over the range of Ct values can be evaluated. For example, if the cut-off for the candidate test is Ct = 40 then the following table should be provided:***

Samples Tested in a 5-sample Pool	***Expected Result Individual Samples with 37 < Ct ≤ 40***	
Pooled Test Result	***Positive***	***Negative***
Positive		
Negative		
	Expected Result Individual Samples with 34 < Ct ≤ 37	
Positive		
Negative		
	Expected Result Individual Samples with Ct ≤ 34	
Positive		
Negative		
	Expected Result All Individual Samples	
Positive		
Negative		

e.2) Swab Pooling

Swab pooling is an approach which conserves transport media and has the potential to maintain sensitivity of the test; however, deconvolving which swab was positive cannot be done without collecting another specimen. This approach also results in a high concentration of swab specimen in transport media, therefore inhibition may be observed. The effects of inhibition due to high concentrations of swab specimens (e.g., mucin) and high concentrations of virus when there are multiple positive swabs in the swab pool should be

investigated. We recommend performing swab pooling validation using the two studies described below using the highest number of swabs that is both desired and deemed feasible. If the data does not meet the acceptance criteria noted below we recommend evaluating a lower number of swabs until the recommended acceptance criteria are met. Laboratories can proceed testing with any number of pooled swabs up to the highest number of pooled swabs that was successfully validated.

In your procedures, you should provide a detailed procedure describing a method to combine swabs into a single volume of transport media. The procedure should include recommendations to maximize the amount of specimen resuspended into the transport media from the swab and help ensure that the user performs sample and swab handling in a manner consistent with current infection control procedures, which should also reduce the chance of carryover between sample pools.

The maximum number of swabs that can be pooled for maximum efficiency can be calculated the same way as the maximum number of samples as discussed above for Dorfman pooling.

To establish performance of your test with swab pooling, FDA recommends conducting a clinical validation study in the intended use population that includes testing each sample individually and using your proposed pooling strategy. Examples of clinical validation studies for adding pooling to a previously authorized (EUA) tests or to include pooling in an EUA request for a new test are included in the sample/media pooling section above. These studies can be adjusted to validate a swab pooling strategy.

For n-swab pooling strategies, the two studies below should also be conducted:

1) ***We recommend establishing performance related to test interference from multiple swab specimens in a single volume of transport media. N-swab samples containing the maximum number of swabs you intend to validate in the minimum volume of transport media you intend to validate should be tested with an analyte concentration of 2-3X LoD. The swabs should contain clinical matrix negative for SARS-CoV-2. The acceptable range of transport media volume should be noted in your procedures and interference performance should be validated by testing in the minimum recommended volume. We recommend testing replicates of three n-swab pooling samples at the same analyte concentration both with and without clinical matrix. Each n-swab pooling sample should contain maximum number of swabs you recommend pooling in your procedures.***

   For example, if you recommend pooling three swabs (n = 3) then we recommend acquiring a total of nine confirmed negative swabs from individual subjects and adding three unique swabs to three unique tubes of transport media thereby making three n-swab pooling samples. Each n-swab pooling sample should be spiked with either positive patient sample (in transport media), live virus, or inactivated virus at a concentration of 2-3X the LoD of your assay. We recommend testing a

total of at least 20 replicates which can be composed of equal numbers of aliquots taken from each n-swab pooling sample (i.e., 7 replicates from each sample in this example). Ideally, negative n- swab sample matrix should be tested prior to spiking to ensure that the matrix is negative. Acceptance criteria should be at least 95% agreement with the expected results and an invalid rate of < 5%. We recommend providing the Ct value line data (if applicable) for analysis.

2) ***We recommend evaluating the effect of high viral concentrations on assay performance. It appears that patients with SARS-CoV-2 infection can exhibit unusually high viral loads. This, combined with the possibility of pooling multiple positive swabs into a single volume of transport media, could result in unexpectedly high viral titer in the pooled sample. We recommend evaluating existing data on viral loads in infected subjects and, in combination with your existing LoD data, propose a maximum expected viral titer per swab. Using this number, estimate the expected viral titer in transport media with at least three positive swabs. For instance, if you expect a maximum of 100,000X LoD per swab we recommend spiking a single negative n-swab sample with 300,000X LoD target analyte and testing with 10 replicates. It is anticipated that all replicates are either positive or have an invalid rate of ≤5%.***

Multi-analyte Respiratory Panels Under EUA:

An emergency declaration by the HHS Secretary allowing for the issuance of EUAs is typically specific for a pathogen/ disease (i.e., there is a publicly declared health emergency involving a particular etiologic agent). Therefore, for tests, the EUA pathway is generally only an option for testing patients for that single agent in a given emergency. Given the overlap in signs and symptoms between SARS-CoV-2 and other respiratory viral infections, including influenza, FDA has authorized multi-analyte respiratory panels for the qualitative detection and differentiation of nucleic acid from multiple pathogens, including the SARS-CoV-2 virus. These panels are useful to efficiently detect and differentiate between multiple pathogens that are relevant to the event/disease outbreak that is the subject of the specific emergency declaration. They may also be useful in preserving critical testing resources during the public health emergency by reducing the number of tests, and therefore supplies, needed per patient.

When determining whether to issue an EUA for a multi-analyte respiratory panel FDA takes into consideration the use of the test (multi-analyte pathogen detection as an aid in differential diagnosis), clearance/approval status of IVDs for the other panel members, whether the proposed Intended Use fits within the HHS emergency declaration and how the panel test would fit into current public health authority patient testing algorithm recommendations. If you are requesting an EUA for a multi-analyte respiratory panel, analytical and clinical evaluations for each target analyte should be provided. We recommend you contact FDA at approvals@prodtec.org for specific feedback on this type of EUA request.

1) ***Addition of SARS-CoV-2 to previously FDA-cleared Multi-Analyte Respiratory Panels***

To add the SARS-CoV-2 target to respiratory panels previously cleared by the FDA where the SARS-CoV2 reagents are run in a separate well (or tube) and no modifications are required to the cleared portion of the assay, only studies for validation of the SARS-CoV-2 reagents described in this template are recommended.

To add the SARS-CoV-2 target to respiratory panels previously cleared by the FDA where the SARS-CoV-2 reagents are combined in the same well as the reagents for previously cleared analytes (in a multiplex reaction), the following studies should be conducted to validate the SARS-CoV-2 reagents and the modifications made to the cleared respiratory panel:

- ***Studies described in this template to validate the SARS-CoV-2 reagents***
- ***LoD confirmation of the previously cleared analytes by conducting side by side testing of 3-5 replicates of serially diluted viruses with modified and original versions of the test to show that the LoD is unchanged due to modifications***
- ***Testing 10 retrospective positive samples for each previously cleared analyte***
- ***Competitive inhibition study with clinically relevant titers of each analyte in the panel (viruses 10^5 PFU/mL, bacteria 10^6 CFU/mL)***

2) Multi-analyte Panels not Previously Cleared by the FDA

To support an EUA for a multi-analyte respiratory panel that was not previously cleared by FDA, analytical and clinical evaluations for each target analyte should be provided. The following analytical studies should be conducted and data provided to the FDA for review:

- ***Limit of Detection (Analytical Sensitivity)***
- ***Cross-Reactivity / Microbial Interference***
- ***Inclusivity / Analytical Reactivity***
- ***Collection Media Equivalency - each claimed additional sample collection media not used in your clinical study should be validated (if appropriate for study designs)***
- ***Co-infection (Competitive Interference)***
- ***Interfering Substances Study (Endogenous and Exogenous)***
- ***Clinical Specimen Stability***
- ***Reagent Stability testing protocol***
- ***Carry over/Cross-Contamination (if a new instrument previously not reviewed by the FDA is used)***
- ***Reproducibility and Repeatability (if a new instrument previously not reviewed by the FDA is used)***
- ***Fresh vs. Frozen If you intend submit data testing archived frozen specimens in support of your EUA, please conduct an analytical study to demonstrate that preservation of samples (e.g., by freezing at ≤-70°C) does not affect the accuracy of test results compared to freshly collected samples.***

Clinical Performance

To evaluate the clinical performance of your multi-analyte test, a prospective clinical study should be conducted. Considering the public health needs in the current emergency, a clinical performance study in support of the EUA application may be conducted at one site testing archived positive and negative clinical samples with known

specimen types. The pre-selection of archived positive samples should represent a range of viral load or Ct values including low positive samples near the assay cut-off.

Since your device has not been FDA-cleared for the respiratory pathogens included in your test, and it is likely that your test would be used in patients with respiratory symptoms in lieu of an FDA-cleared respiratory panel, FDA generally intends to include a condition of authorization that you conduct a post EUA prospective clinical study. The prospective clinical study should include a minimum of three sample collection sites and three testing sites, prospectively enrolling patients with general respiratory symptoms. You may consider conducting a prospective clinical study in Southern Hemisphere countries during their typical influenza/respiratory season to increase the likelihood of obtaining a sufficient number of positive samples (e.g., for influenza at least 50 positive Flu A and 30 positive Flu B samples) in a timely fashion.

The FDA performance expectation for SARS-CoV-2 is that PPA and NPA should be ≥95% (with a lower bound of the two-sided 95% confidence interval >85%); for Flu A/B, and other respiratory viruses, PPA should be ≥90% (with a lower bound of the two-sided 95% confidence interval ≥80%), and the NPA should be ≥95% (with a lower bound of the two-sided 95% CI ≥90%) in comparison to an EUA RT-PCR test. We recommend using only a high sensitivity EUA RT-PCR assay which uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction).

We recommend that you submit a Pre-EUA with an outline of the studies that you plan to conduct to support the FDA-authorization or contact FDA at approvals@prodtec.org for specific feedback.

Claiming Multiple Instruments and/or Extraction Methods:
FDA recommends the following analytical and clinical validation for use of multiple instruments and/or extraction methods where the elution volumes from the extraction methods and PCR volumes on the different RT-PCR instruments are identical.

- ***Limit of Detection (LoD): These studies should be repeated for each clinical matrix claimed in the Intended Use. Pick one RT-PCR instrument and determine the tentative LoD (using 5 replicates in 10-fold dilution) followed by the confirmatory LoD (20 replicates spiked at tentative LoD) for each extraction method on the chosen instrument. Note: If you detect 20/20 replicates in your confirmatory LOD study you should test the next lower concentration, using a 3-fold dilution, until you achieve a hit rate of <20/20.***
    - ***If the different extraction methods yield the same LoD (≤3xLOD) on the RT-PCR instrument chosen for initial testing, pick one extraction method for further LoD determination on the remaining RT-PCR instruments and follow the recommendations below.***
    - ***If the extraction methods do not yield the same LoD on the chosen RT-PCR instrument, please choose the extraction method with the worst LoD for further comparison of the LoD on all RT-PCR instruments.***

  For all other RT-PCR instruments you should use the following adaptive LoD study design:
    - ***Please perform a refined tentative LoD study with 5 replicates at 0.5x, 1x,***

and 1.5 to 2x LoD. If you detect 4/5 replicates as positive at all the tested levels, you need to include the next higher concentration (i.e., 3x LoD). If you obtain 5/5 replicates at 0.5x LoD, you need to test the next lower concentration (i.e., 0.25x LoD). You will test in this manner until you find the lowest concentration that gives you 5/5 positive results for the tested RT-PCR instrument. This concentration should be used for a confirmatory LoD study for the given RT-PCR instrument using 20 replicates.

Final reported LoD: Please list all RT-PCR instruments with their respective LoDs if different LoDs are obtained. LoDs are considered comparable if they are between 1-3xLoD. These studies should be repeated for each clinical matrix claimed in the Intended Use.

- ***Interference Substances Studies (if applicable): FDA recommends evaluating interfering substances with the extraction method and RT-PCR instrument combination that has the worst overall LoD.***

- ***Inclusivity Testing (if applicable): FDA recommends evaluating inclusivity with the extraction method and RT-PCR instrument combination that has the worst overall LoD.***

- ***Exclusivity Testing (if applicable): FDA recommends evaluating exclusivity with any extraction/instrument combination.***

- ***Clinical study: If an LoD study confirms equivalency for all RT-PCR instruments (between 2-3xLoD), then the clinical study may be conducted with any RT-PCR instrument. If one or more RT-PCR instruments have different LoDs, we recommend conducting the clinical study with the extraction method / RT-PCR instrument combination with the worst LoD.***

Note, if there are differences in the extraction input volume, extraction elution volume and PCR input volume (extracted nucleic acid) then the LoD should be confirmed for each.

H. UNMET NEED ADDRESSED BY THE PRODUCT

This section will be completed by FDA.

I. APPROVED/CLEARED ALTERNATIVE PRODUCTS

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

J. BENEFITS AND RISKS:

This section will be completed by FDA.

K. FACT SHEET FOR HEALTHCARE PROVIDERS AND PATIENTS:

Include proposed Fact Sheets for Patients and Healthcare Providers - ***see examples for authorized EUA tests on our website and templates will be made available.***

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

In lieu of a package insert or labeling please include your Laboratory SOP/protocol.

M. RECORD KEEPING AND REPORTING INFORMATION TO FDA:

The laboratory will track adverse events and report to FDA under 21 CFR Part 803. A website is available to report on adverse events, and this website is referenced in the Fact Sheet for Health Care providers. The laboratory will maintain will information on the performance of the test, and report to FDA any suspected change in performance of which they become aware. The laboratory will maintain records associated with this EUA and ensure these records are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.