SARS-CoV-2 Reference Panel Comparative Data

The FDA SARS-CoV-2 Reference Panel allows for a more precise comparison of the analytical performance of different molecular in vitro diagnostic (IVD) assays intended to detect SARS-CoV-2. The Reference Panel contains common, independent, and well-characterized reference material that is available to developers of SARS-CoV-2 nucleic acid-based amplification tests (NAATs) for which Emergency Use Authorization (EUA) was requested.

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Background

During the early months of the Coronavirus Disease 2019 (COVID-19) pandemic, clinical specimens were not readily available to developers of IVDs to detect SARS-CoV-2. Therefore, the FDA authorized IVDs based on available data from contrived samples generated from a range of SARS-CoV-2 material sources (for example, gene specific RNA, synthetic RNA, or whole genome viral RNA) for analytical and clinical performance evaluation. While validation using these contrived specimens provided a measure of confidence in test performance at the beginning of the pandemic, it is not feasible to precisely compare the performance of various tests that used contrived specimens because each test validated performance using samples derived from different gene specific, synthetic, or genomic nucleic acid sources.

From February through the middle of May, the FDA issued a total of 59 EUAs for IVDs for the qualitative detection of nucleic acid from SARS-CoV-2 based on validation data using contrived specimens derived from SARS-CoV-2 viral RNA. As the pandemic progressed and more patient specimens became available, on May 11, 2020, the FDA recommended in the Policy for Coronavirus Disease-2019 Tests (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-coronavirus-disease-2019-tests-during-public-health-emergency-revised)that developers obtain and use patient specimens to validate their tests.

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Recognizing the value to healthcare professionals, laboratories, and patients in understanding the relative performance of NAATs for SARS-CoV-2, the FDA obtained live virus in February to develop a reference panel. Reference panels are a fundamental tool for performance assessment of molecular tests (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas), and the use of the same reference material across different tests allows a direct comparison of analytical sensitivity performance across these tests. As was done for the evaluation of NAATs for Zika (https://www.fda.gov/vaccines-blood-biologics/science-research-biologics/fda-zika-virus-reference-panel-molecular-based-diagnostic-devices-supports-product-testing-emergency), the FDA is again providing a tool for a comparative analysis of the performance of different tests. Such comparison has shown to be useful to health care providers and laboratories implementing these tests.

The FDA SARS-CoV-2 Reference Panel is shared with developers who have interacted with FDA through the review process.

Development of the FDA SARS-CoV-2 Reference Panel

To more precisely compare the performance of NAAT SARS-CoV-2 assays, through a collaboration between the Center for Devices and Radiological Health (CDRH) and the Center for Biologics Evaluation and Research (CBER), the FDA established a Reference Panel composed of standardized material, suitable for the determination and direct comparison of analytical sensitivity and cross-reactivity of nucleic acid-based SARS-CoV-2 assays.

The panel contains one heat-inactivated SARS-CoV-2 strain and one heat-inactivated MERS-CoV strain in cell culture media. The panel is composed of five tubes (T1 to T5): T1 contains the SARS-CoV-2 strain (2019-nCoV/USA-WA1/2020) at a concentration of ~1.8x108 RNA NAAT detectable units/mL (NDU/mL); T2, T3, T4, and T5 contain blinded samples, meaning that, although the FDA knows the concentration, the developer testing the samples does not. Based on a standard protocol provided by the FDA for T1, the developers are asked to perform a range finding Limit of Detection (LoD) study followed by a confirmatory study to further define and corroborate the LoD of their assay. The blinded samples (T2 to T5) are also tested per a protocol provided by the FDA, to confirm the LoD determined for T1 and evaluate cross-reactivity with MERS-CoV virus. Depending on the test, the number of tests performed on different amounts of viral replicates can range from over 40 to over 150.

The FDA SARS-CoV-2 Reference Panel was first provided to all developers of authorized IVD EUAs that used contrived samples to validate their assay and is provided to all developers who request an EUA for SARS-CoV-2 NAATs. In general, FDA's EUAs require developers to evaluate and submit the analytical limit of detection and assess traceability of their product with any FDA-recommended reference material as a condition of the authorization. As explained above,

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assessment of assay performance using the FDA SARS-CoV-2 Reference Panel allows for a consistent determination of the relative sensitivity of these tests and cross-reactivity with MERS-CoV virus.

While the FDA SARS-CoV-2 Reference Panel helps determine the comparative performance among authorized tests, the panel is not a replacement for the analytical and clinical validation recommendations the FDA has provided in the EUA templates (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#covid19ivdTemplates). For example, the panel only includes one strain of SARS-CoV-2 and one cross-reactant, MERS-CoV. Recent mutations reported for SARS-CoV-2 (e.g., D614G), which may impact molecular testing, are not included.

Distribution and Testing of the FDA SARS-CoV-2 Reference Panel

FDA began distribution of the FDA SARS-CoV-2 Reference Panel in May 2020. As of September 10, 2020, the FDA has contacted developers of 176 authorized assays for shipping information and has sent the reference panel to developers of 152 authorized assays. The FDA is reviewing results as they are returned, and continues to send the reference panel out to additional developers.

As of August 15, 2020, the FDA has contacted developers of 154 authorized assays for shipping information and by August 17, 2020 sent the reference panel to developers of 137 authorized assays which are included in the tables below. Developers who received the reference panel were asked to conduct testing and return results within two weeks of receiving the panel. Many developers returned data to the FDA by August 31, 2020, but in some cases, FDA did not receive the data, or the data was uninterpretable, or is still under interactive review. All contacted developers are listed in Table 1 along with the current status of their Reference Panel testing. Confirmed results of the relative sensitivity of EUA authorized assays provided by developers as of August 31, 2020, are displayed in Tables 2A, 2B, and 2C. The results are presented in three tables according to the clinical matrix used in the study: swab in transport media, direct swabs (dry swabs), or saliva.

Following a protocol provided by the FDA with the reference panel, developers conducted testing with 3 replicates of serial dilutions of the SARS-CoV-2 virus provided in T1 in clinical negative specimens. The developers identified a provisional LoD and then performed confirmatory testing. To corroborate the LoD identified from testing T1, developers then diluted in clinical negative specimens and tested the blinded samples (T2-T5) according to the protocol provided by the FDA.

Table 1. Status of Developers of Authorized EUAs contacted for distribution of the FDA SARS-CoV-2 Reference Panel*

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Developer	Authorized Test	Status**
SEASUN BIOMATERIALS	U-TOP COVID-19 Detection Kit	Results in Table 2A
Seasun Biomaterials, Inc	AQ-TOP COVID-19 Rapid Detection Kit	Results in Table 2A

Showing 1 to 2 of 2 entries (filtered from 154 total entries)

* Developers who were contacted for distribution of the FDA SARS-CoV-2 Reference Panel as of 8/15/2020 for panel shipping no later than 8/17/20.

**Status of “Did not provide shipping information” indicates that no shipping information was received by 8/15/2020. Status of “Data not returned” indicates that no data was received as of 8/31/2020.

*** No patient matrix was used

**** Nasal swab

FDA SARS-CoV-2 Reference Panel Results

Confirmed results from reference data sets received as of August 31, 2020, are shown in Table 2. No cross-reactivity with MERS-CoV has been observed.

Note: In the data in Table 2, a lower LoD represents a test’s ability to detect a smaller amount of viral material in a given sample, signaling a more sensitive test. The FDA will continue to update the table as it receives results to provide laboratories, health care providers, and patients with a resource they can use to better inform which tests they select to use.

Table 2. Sensitivity* Mean Estimates of the EUA authorized SARS-CoV-2 molecular diagnostic tests using the FDA SARS CoV-2 Reference Panel

A - Swabs in Transport Media**

Swabs are collected and transported in media as per the Instructions for Use of each EUA. If several specimen types are authorized, nasopharyngeal (NP) swabs were recommended to use. The clinical matrix is spiked following the FDA-proposed dilution protocol and tested.

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Product LoD (NDU/mL***)	Developer	Test
600	SEASUN BIOMATERIALS	U-TOP COVID-19 Detection Kit

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Product LoD (NDU/mL***)	Developer	Test
6000	Seasun Biomaterials, Inc	AQ-TOP COVID-19 Rapid Detection Kit

Showing 1 to 2 of 2 entries (filtered from 55 total entries)

*The current performance reflects the extraction/ instrument combination with the least sensitive LoD.
**Nasopharyngeal swabs, unless otherwise noted. Transport media refers to Viral Transport Media (VTM), Universal Transport Media (UTM), phosphate-buffered saline (PBS), saline, etc.
***NAAT Detectable Units/ mL
**** Nasal swab

B-Direct Swabs (Dry Swabs) **
For devices authorized for use with dry swab specimens only, mock swabs are prepared by pipetting 50 µL of each diluted virus stock onto a swab. Dry swabs are let to dry for a minimum of 20 minutes, and the swab is tested following the Instructions for Use for the device.

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Product LoD (NDU/mL***)	Developer	Test
300000	Abbott Diagnostics Scarborough, Inc.	ID NOW COVID-19
540000	Quidel Corporation	Lyra Direct SARS-CoV-2 Assay

Showing 1 to 2 of 2 entries

**Nasopharyngeal swabs, unless otherwise noted
***NAAT Detectable Units/ mL

C-Saliva
Corroborated negative saliva is pooled. The volume of saliva that goes into the collection device is then spiked with the material at the same dilution as indicated for NP swabs (considering the replicates needed and an excess volume for the serial dilution). If the collection device is a dry container with nothing in it, the saliva is spiked and tested. However, if the collection device contains liquid, the saliva is mixed with virus and then the normal volume of collected saliva is added to the container to mimic the workflow. The volumes in the FDA-proposed dilution protocol may need to be adjusted to follow the device Instructions for Use.

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