Understanding, verifying and implementing Emergency Use Authorization molecular diagnostics for the detection of SARS-CoV-2 RNA

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Abstract

The SARS-CoV-2 pandemic has brought a new wave of challenges to health care, particularly in the area of rapid diagnostic test development and implementation. Acute diagnosis of COVID-19 infection is critically dependent on detection of SARS-CoV-2 RNA from clinical specimens (e.g. nasopharyngeal swabs). While laboratory-developed testing for SARS-CoV-2 is an essential component of diagnostic testing for this virus, the majority of clinical microbiology laboratories are dependent on commercially available SARS-CoV-2 molecular assays. In contrast to assays approved or cleared by the Food and Drug Administration for in vitro diagnostic use, assays for the detection of SARS-CoV-2 nucleic acids have Emergency Use Authorization (EUA) from the FDA. Outside of highly specialized academic and commercial laboratory settings, clinical microbiology laboratories are likely unfamiliar with EUA classification and thus assay verification can be daunting. Further compounding anxiety for laboratories are major issues with supply chain that are dramatically affecting the availability of test reagents and requiring laboratories to implement multiple commercial EUA tests. Here, we describe guidance for the verification of assays with EUA for the detection of SARS-CoV-2 nucleic acid from clinical specimens.
The coronavirus disease (COVID-19) pandemic due to SARS-CoV-2-associated respiratory tract illness has created unprecedented demand for diagnostic testing. Infection with SARS-CoV-2 leads to a range of outcomes from asymptomatic infection to mild and moderate symptoms including fever and cough to the requirement of intensive respiratory support and death. According to the Johns Hopkins University COVID-19 Dashboard, as of May 6, 2020, there are over 3.7 million confirmed cases of COVID-19 and more than 258,000 deaths worldwide, with the U.S. representing the country with the largest number of reported cases and deaths to date. Unfortunately, many cases are likely to have gone undocumented because testing resources have been limited such that testing has been predominantly restricted to the most at-risk individuals. As more resources are produced and more testing is implemented, less acute cases of COVID-19 should be able to be tested and identified.

The slow implementation of testing and the lack of testing capacity has repeatedly made headlines. Initial problems with the CDC test led to delayed deployment to public health labs and restricted testing to the CDC for several weeks. Further delaying implementation of testing was the requirement for laboratory-developed tests (LDTs) to be submitted for U.S Federal Drug Administration (FDA) Emergency Use Authorization (EUA) review (discussed below) and shortages of collection devices, extraction kits, mastermix and other commercial reagents. Additionally, commercial manufacturers of diagnostic devices took several additional weeks to develop and submit assays to the FDA for EUA review and were unable to meet supply demands for reagents or instruments once tests were approved. Not only was the demand for test components greatly increased worldwide, the manufacturing facilities for many products were located in parts of the world that were hardest hit by the pandemic. The combination of widespread lockdowns impacting transportation and production and the excess expenditure...
associated with COVID-19 medical responses has led to a downturn of the global economy and a broken supply chain (1). Additional laboratory challenges to implementing molecular testing for SARS-CoV-2 include lack of: positive control materials, personnel/time, primers/probes, specificity panel, funds, quality control system, commercial tests, procurement procedures, training and equipment (2).

The continued need to increase testing capacity is based on the desire to test both asymptomatic and symptomatic individuals, particularly as countries begin to restart their economies. Detection of SARS-CoV-2 RNA from respiratory tract samples is an important component of outbreak management. Rapid and accurate identification of infected individuals, whether symptomatic or asymptomatic, is critical to enforce self-isolation recommendations, contact tracing in the community and the use of appropriate enhanced personal protective equipment (PPE) in healthcare settings. Positive SARS-CoV-2 RNA detection is also important for therapeutic clinical trials. Negative results obtained from highly sensitive tests on inpatients with symptoms of COVID-19 can help prevent overuse of enhanced PPE by medical providers. Similarly, negative results obtained on healthcare personnel can assist in maintaining adequate levels of staffing. It is important to note that the reliability of negative test results can be confounded by stage of disease, adequacy of sampling, performance characteristics of the test and disease prevalence.

Laboratory-developed nucleic acid amplification tests have been a mainstay for academic medical centers and commercial laboratories for decades. They have been particularly helpful in rapidly deploying diagnostics for new or emerging infectious diseases, like SARS-CoV-1, MERS CoV, influenza A(H1N1)pdm09 and enterovirus D68 (3). Usually, laboratories can develop and validate LDTs under Clinical Laboratory Improvement Amendments (CLIA)
regulation without direct oversight by the FDA. However, when a Public Health Emergency (PHE) is declared for a new infectious disease of public health importance, laboratories cannot offer LDTs for the PHE agent without applying for, and being granted, EUA by FDA. The concept behind the FDA’s EUA process is to strengthen public health protections and facilitate the availability and use of medical countermeasures (MCMs) such as drugs, vaccines, medical equipment and supplies and diagnostics (4). The goal is to make MCMs readily accessible during a public health emergency while still protecting the public, by providing a less stringent and more streamlined authorization process than that usually required for full FDA approval.

On February 4, 2020, the Secretary of Health and Human Services declared potential for a PHE from COVID-19 and EUA authority for associated MCMs including diagnostics. Any laboratory or commercial company developing molecular tests for detection of SARS-CoV-2 had to submit an application to FDA and obtain EUA status in order to offer the test for diagnostic purposes. Most laboratories lacked experience with the EUA process and found the prospect daunting because most only had experience with clinical trials leading to in vitro diagnostic (IVD) submissions to FDA, which are quite complex. The prospect of overseeing such a process while directing a laboratory in the midst of a pandemic was intimidating, causing many LDTs to remain unused and unimplemented. On February 29, 2020, the FDA issued new guidance and streamlined the EUA process for CLIA-certified laboratories that allowed clinical testing to begin, as long as the abbreviated EUA application was submitted within 15 days. Several laboratories began offering their LDT through the streamlined EUA process, though FDA review was still required. On March 16, 2020, the guidance was further updated, giving States the option of overseeing the validation process for CLIA-certified laboratories. States that have notified FDA that they will take responsibility for COVID-19 testing in their State as of May 6, 2020 are:
Connecticut, Maryland, Mississippi, Nevada, New Jersey, New York and Washington. An important addition to the March 16th guidance was the ability of commercial manufacturers to distribute their test kits after validation as “research use only” while the EUA submission was being prepared and under review. This led to a rapid increase in the number of commercially available EUA tests for the detection of SARS-CoV-2 RNA for clinical testing, which was a critical step towards increasing testing capacity in the U.S. However, despite the addition of numerous commercial tests with EUA, a testing gap remains due to chronic shortages of testing reagents, materials and instruments, as well as specimen collection swabs and transport media.

The EUA process does not include classifying tests into traditional categories such as CLIA waived or FDA cleared and is a temporary designation that is usually discontinued once the PHE is declared over. It should be noted that the studies performed by laboratories and manufacturers to define performance characteristics for EUA tests are less stringent than those required of an IVD test. Studies for EUA submission primarily focus on analytic sensitivity (i.e., limit of detection) and analytic specificity (i.e., cross-reacting organisms). Under EUA, clinical trials to establish clinical sensitivity and specificity are not required due to the novelty of the pathogen and the absence of both reference methods and a clearly defined disease state. As of May 2020, the clinical sensitivity of assays that have received FDA EUA have not been determined by extensive study. However, as for most diseases, there are reports of individuals with COVID-19 who have negative SARS-CoV-2 RNA tests, and thus a negative nucleic acid test does not definitively rule out COVID-19 (5).

Commercial EUA tests are not FDA-cleared/approved tests, nor are they considered laboratory-developed tests, so there can be confusion regarding how to verify these tests. Verification of commercial EUA tests should include assessment of accuracy and precision in a
similar fashion to IVD assays, as long as there is no modification to the test as described under the EUA. Furthermore, attention should be paid when considering an EUA assay on a device that is typically considered to be “point of care” (POC). It is recommended to review the FDA letter of authorization to determine if the EUA allows for testing in a true POC setting (e.g. physician office, usually run by non-laboratory staff) or requires the testing to be conducted within a lab certified to perform moderate or high complexity testing. These letters of authorization can currently be found at the FDA website (6). It is important to keep in mind that while the instrument may be POC, the assay itself is not and the test, even if on a POC device, requires a verification prior to being used. Many current CLIA-waived assays do not require a verification prior to clinical testing, so the concept of verification to establish test performance may be a new concept to those running the test. Clinical microbiology laboratories may consider assisting POC departments with the verification process to ensure the EUA test performs as expected.

Of the commercial molecular SARS-COV-2 assays receiving EUA status, there are direct sample to answer, POC devices, as well as those designed for high-complexity batched-based testing. Some commercial molecular assays offer rapid diagnosis of a viral pathogen directly from patient specimens (7). However, it is important to consider the limitations of these assays that are included in the Instructions for Use document (i.e. package insert) provided by the manufacturer. These limitations may include the acceptable specimen types, specific extraction and amplification procedures, availability of trained staff, and laboratories where tests can be performed (6). Because of variations among tests between different vendors with respect to approved sample types, collection/transport media, swabs and test performance, review of the FDA authorization letter is recommended before deciding to implement a test. A bridging study or independent EUA submission to the FDA may be required if a laboratory modifies a
commercial EUA test from its Instructions for Use in any way. Bridging studies and the
processes for developing a laboratory developed test for EUA submission will not be discussed
here. Guidance on how to perform these studies can be reviewed on the FDA website (8).

Readers are directed to contact the FDA at COVID19DX@FDA.HHS.GOV with additional
questions regarding these. Here, we provide guidance for the verification of non-modified
commercial EUA tests for the detection of SARS-CoV-2 RNA.

**Biosafety**

Commercial EUA test verification requires the use of appropriate biosafety and
protective equipment while handling, manipulating, and testing specimens. An internal risk
assessment of the device and its potential use in the user’s location, particularly POC devices,
should be done to ensure proper biosafety and PPE are employed. Risk identification and
mitigation are critical in maintaining a safe laboratory environment. Therefore, risk assessment
plans should be in place to identify and mitigate risks associated with SARS-CoV-2 diagnostic
testing procedures. A plan to mitigate risks should be documented after prioritizing the risks and
should be communicated to laboratory personnel performing the tests. Risk assessment and
mitigation strategies are dependent on the procedures performed, hazard identification associated
with the procedures, the competency level of the laboratory staff, the laboratory equipment and
facility and the available resources (9).

Good laboratory practices should be followed at all stages of SARS-CoV-2 diagnostic
molecular testing. Appropriate disinfectants should be used for decontamination of work surfaces
and equipment (9, 10). For laboratory-based testing, a certified Class II biological safety cabinet
(BSC) should be used for procedures with the potential to generate aerosols and droplets. In
locations without a BSC, additional precautions should be implemented to reduce the risk of
exposure, which may include additional PPE, such as surgical mask and face shield or splash shield. Specimens may be heat inactivated (56°C for 30 minutes), but a bridging study is required if this is not specified in the Instructions for Use. A biohazard risk assessment plan should be in place to identify any additional risks and precautions. All laboratory waste generated from testing confirmed or suspected SARS-CoV-2 patient specimens should be considered as biohazardous waste and handled in a similar manner as biohazard waste.

General Considerations for Verification

Acceptable specimens should be collected in the transport media recommended by the FDA or manufacturer (11). Samples used for the verification should be the same or similar to samples that will be used for clinical testing. The primary materials required to conduct verification procedure include, at a minimum, ten positive and ten negative specimens, commercially available reference material (e.g. Accuplex, Exact, NAtrol) or residual patient samples, and SARS-CoV-2 reagents authorized by FDA EUA. The instruments used for testing should be based on those specified in the FDA EUA authorization documents for the commercial tests (6). Verification is a two-step process: The first is performance of the manufacturer’s described quality control (QC) instructions to verify that controls have produced the expected results. The second includes accuracy and precision studies and should proceed after successful completion of the QC verification.

Quality Control

QC is a critical step to any verification procedure to ensure that cartridges, reagents and instrument are working properly. The manufacturer should be contacted if QC is not successful. QC material is also required for developing an Individual Quality Control Plan (IQCP). The material can be synthetic commercial products, residual patient samples, genomic or in vitro
transcribed RNA. However, the latter is generally not recommended on account of stability issues. The use of quantified, inactivated virus QC material is preferred over non-quantified material or RNA and should contain every SARS-CoV-2 target detected by the assay. If QC material is included in the commercial EUA test kit, it should be used per manufacturer’s instructions. Otherwise, when it is not included with test kit, or if the QC included does not include the SARS-CoV-2 target(s) (e.g. a processing control only) it must be purchased independently. Recommendations on the use of external quality control material can be obtained from the EUA test manufacturer. In the absence of commercial QC material, residual patient samples can be used for external quality control. A large batch of positive controls (1:10 diluted, heat-inactivated, moderate positive patient sample) should be made, aliquoted for single use and stored at -80°C. Residual negative patient samples should be used for negative control. The use of purified genomic viral RNA may also be an option for QC purposes. However, its use is more challenging compared to working with intact virus as RNA is fragile and degrades more readily than intact virus. This requires special handling and storage conditions that may present challenges for laboratories unaccustomed to working with RNA. Recombinant or in vitro transcribed RNA, when used, should match the target gene(s) of the assay. A large batch of positive control should be made by spiking the quantified RNA into pre-lysed negative sample matrix (moderate positive, 5000 genome copies/ml), aliquoted for single use and stored at -80°C. Spiking of RNA into lysed samples is suggested to prevent RNA degradation. Of note, viral RNA is not recommended as QC material in verification samples for cartridge-based assays because RNA degradation begins as soon as patient samples are spiked directly with RNA. External QC must be run every day of patient testing or according to the manufacturer’s instructions. An IQCP can be developed for EUA tests with an internal control that could serve...
as the daily control. CLIA requirements should be followed for implementing and monitoring IQCP (12). While the number of days of continuous QC that is required for IQCP is up to the laboratory medical director to determine, a minimum of 20-days is commonly practiced and generally considered acceptable.

**Accuracy**

The verification procedure for batched and non-batched (i.e., random access, sample to answer) testing should be similar in approach (13). During accuracy and precision studies, it is recommended to assess for carryover contamination by alternating positive and negative specimens during verification for both batched and non-batched assays. Limit of detection (LOD) verification is not required for on-label use of EUA assays. Verification of accuracy can be performed using two options: (1) contrived patient samples or (2) remnant patient samples tested for SARS-CoV-2 RNA by another EUA test.

In option one, contrived patient samples are created by spiking pooled negative matrix (i.e., pooled residual negative patient samples) with commercial materials, ideally quantified by the manufacturer. Blank transport media can be used as a pooled negative matrix if residual negative patient samples are not available. It is important to note that in transport media, the internal control will be negative if it targets a human gene [e.g. RNase P]. Positive reference material can be run neat or diluted 1:2 or 1:10 to represent a strong positive or a moderate positive, respectively. It is recommended to contact the manufacturer or refer to manufacturer’s instructions to determine if the product can be diluted with transfer media or must be diluted with the commercial synthetic negative matrix. Testing should include a minimum of 10 positive samples, including five strong positive and five moderate positive samples, which can also be used for precision studies (see below). In addition, at least 10 negative remnant patient
specimens should be tested. If patient specimens are not available or are limited, contrived
negative samples can be created by pooling fewer specimens and testing ten times. If discordant
results are obtained, the specimen should first be repeated by the test under verification. If the
discordance is resolved, additional training and/or additional specimens may need to be tested to
complete the verification. If the discordance is not resolved, consider testing the specimen by an
alternative method, or contact the manufacturer for additional guidance.

In option two, residual patient samples tested by another EUA method can be used for
verification. While most PCR-based EUA methods provide a PCR cycle threshold (C\text{r}) value,
some instruments may not allow such data to be accessed and therefore the strength or weakness
of the sample cannot be assessed. Additionally, laboratories that initially used reference labs for
testing may not have C\text{r} values available for reference. In this instance, when saving samples for
verification of a commercial EUA test, it is recommended to reach out to the respective reference
lab to gather the C\text{r} values for the few positive samples that will be used for verification
purposes. Testing should be performed on 10 positive (independent patient samples that are not
pooled, or dilutions of a strongly positive sample) and 10 negative residual samples (independent
patient samples that are not pooled). If ten independent positive specimens are not available, a
strong positive sample can be diluted to obtain five strongly positive samples and five
moderately positive samples. The identification of a strongly positive sample is based on the
PCR threshold value: C\text{r} range from 15-24 for strong positive samples and 25-30 for moderate
positive samples. Ten-fold serial dilutions of positive specimens can be made in remnant pooled
negative residual patient samples or transport media to achieve the desired C\text{r} value. LOD is an
important parameter to consider when making dilutions to ensure that dilutions do not reach or
go past the LOD of the assay. Every 10-fold dilution will approximately increase the C\text{r} by three
cycles (Table 1). For example, a 10-fold dilution of a sample with an initial $C_T$ of 20 will result in a $C_T$ of approximately 23. It is critical to note that an initial $C_T$ value of $>$31 may approach the LOD, after delayed transport or multiple freeze-thaw cycles. It is recommended that such samples not be included in accuracy studies.

**Precision**

The general recommendation for precision studies is to test one strong, one moderate positive and one negative sample, each in triplicate. The negative sample can be either an individual negative or a pooled negative, based on available resources. It is recommended that precision be performed by different operators over either different shifts or different days. While inter- (reproducibility) and intra-precision ( repeatability) is recommended for high complexity testing, intra-precision does not apply for cartridge-based assays where each cartridge is independent from each other. It is recommended that inter-precision be determined for all commercial EUA assays, regardless of test format. However, for commercial EUA assays that are in a batched format (eg. 96-well plate), intra-precision should also be assessed in a similar manner to inter-precision. The precision studies can be performed by comparing the $C_T$ values of the same positive samples run in triplicate.

**Additional Considerations**

For laboratories that are a part of a large healthcare system or reference laboratories that aim to implement the same cartridge-based or “POC” EUA assay throughout the system, in efforts to conserve testing supplies while still ensuring the assay performs as expected, a full verification (as described above) may be performed at the main or core laboratory before any clinical testing begins. If the full verification meets expectations, then a verification using a smaller panel with 6 positive and 4 negative specimens can be performed at each affiliated site.
within the system using the same instrumentation. Of the 6 positive samples, 3 should be
strongly positive and 3 should be moderate to low positive as described previously. One positive
patient sample tested in triplicate, or replicates of QC material, can be used for precision.
Negative samples should be negative residual patient samples used for accuracy. The main
laboratory can develop and distribute mini-verification panels to aid the process. The steps for
the mini-verification at each site are similar to the main verification, where QC is performed and
deemed acceptable before proceeding with additional testing. Each individual site should use the
mini-verification panel to verify their device, which can also be used as part of the technical staff
training. Before taking this approach, each lab or system should consider the degree of medical
oversight, staffing, workflow, availability of biosafety equipment and appropriate quality
assurance procedures at each remote laboratory. Note, this approach is not recommended for
either high-complexity EUA tests or laboratory-developed tests. Staff training and competency
should be documented appropriately as for all other assays in the clinical laboratory. If required
by the health system, results should be submitted to the main/central laboratory for review and
approval. The full verification summary from the main laboratory should be made available to
satellite labs in case requested or required by regulatory agencies. QC should be run at each site
per the manufacturer’s instructions. If an IQCP is an option, each site must perform their own
risk assessment before developing and implementing an IQCP.

If a laboratory intends to implement a multiplexed pathogen panel to which SARS-COV-2 has been added, the verification approach may vary depending on how the new target is
incorporated in the panel. If it has been added as a separate reaction, the verification can be
performed on the SARS-CoV-2 target alone, as described above. If it is incorporated as a
multiplexed assay with any of the other target assays in the panel, the new target (SARS-CoV-2)
must be verified as above, and additionally, the performance of the assay for the other targets
that it is multiplexed with, must be verified by running low-level positive controls for those
targets and demonstrating no loss of detection by the addition of the SARS-CoV-2 assay.

**Reporting**

After the assay has been verified and clinical testing beings, there are considerations
regarding how the results should be reported. Table 2 lists comment examples that could be
customized and added to all test reports. Other resources are available to clinical labs verifying
and performing EUA SARS-COV-2 diagnostics. FDA requires fact sheets to be shared with
providers and patients. These should be included in all test reports or physically distributed to the
individuals. Fact sheets can currently be found here: [https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd](https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd).

COVID-19 is currently a reportable disease; a system should therefore be in place to properly
notify the appropriate public health agencies of cases in which SARS-CoV-2 RNA is detected.

The rapid emergence and spread of SARS-CoV-2 has posed unprecedented challenges
for assay development and deployment; regulatory oversight systems; reagent, materials and
instrument resource allocation; the validation, verification and implementation of testing services
and the response to major surge testing loads. The cooperation of partners and stakeholders
across clinical, public health and commercial laboratories, as well as industry and regulatory
authorities, has been critical in order to navigate the myriad of issues with the necessary speed
and scope of changes that have occurred. Clarification, further discussions and resolution will
continue in the coming months and future years, as we continue to learn from the events of
SARS-CoV-2 pandemic.
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Table 1. Dilutions based on CT values

| Sample | Reference Method | Expected CT Value |
|--------|------------------|-------------------|
|        | Neat 1:10 1:100 1:1,000 1:10,000 1:100,000 1:1,000,000 1:10,000,000 |
| 1      | 15 18 21 24 27 30 33 |
| 2      | 25 28 31 34 37 40 43 46 |
| 3      | 30 33 36 39 42 45 48 51 |

Red strikethrough values may approach the LOD of the assay and should not be used in verification studies.
| Result                      | Comment                                                                                                                                                                                                 |
|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Negative for SARS-CoV-2 RNA** | This assay is designed to detect the XXXX and/or XXXX genes of SARS-CoV-2 using nucleic acid amplification. A [not detected or negative] result does not preclude the possibility of SARS-CoV-2 infection since the adequacy of sample collection and/or low viral burden may result in the presence of viral nucleic acids below the analytical sensitivity of this test method. Test results should be used with other clinical and laboratory data in making the diagnosis. |
| **Universal Comment**       | This test has received FDA Emergency Use Authorization and has been verified by XXXXXXXX laboratory. This test is only authorized for the duration of the public health emergency declaration and the circumstances that exist to justify the authorization of the emergency use of in vitro diagnostic tests for the detection of SARS-CoV-2 virus and/or diagnosis of COVID-19 infection under section 564(b)(1) of the Act, 21 U.S.C. 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. |
| **Universal Comment**       | This testing was performed in the XXXXXXXX laboratory located at [Anywhere, USA 12345] (CLIA Certificate #XXXXXXXX, Accreditation #XXXXXX, other as appropriate).                                                                                                           |