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Verification procedure for commercial tests with Emergency Use Authorization for the detection of SARS-CoV-2 RNA

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I. Introduction

Numerous molecular SARS-COV-2 assays are receiving Emergency Use Authorization from the FDA. These include direct sample to answer and "point of care" devices, as well as high-complexity batched-based testing. While there are advantages to these diagnostics, there can be limitations. The Instructions for Use (i.e. package insert) and the FDA authorization letter should be reviewed before deciding to implement a test, as approved sample types, collection/transport media, swabs and performance varies between vendors. Any deviation from what is specifically described under the EUA requires either a bridging study or submission to the FDA for independent EUA approval.

It is prudent to keep in mind that authorization of EUA does not classify tests into traditional assay categories (e.g. CLIA waived, FDA cleared). EUA can be a temporary designation, and it is likely that the EUA will eventually be discontinued by the FDA once FDA-cleared in vitro diagnostic (IVD) assays become available. If an assay is granted an EUA on a device that is typically considered to be used at the "point of care", it is critical that the FDA letter of authorization be reviewed. This will describe if the assay EUA allows for testing in a point of care setting (i.e. physician office, 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 here: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

All commercial EUA tests should be verified for accuracy and precision in a similar fashion to FDA-cleared diagnostic assays. It should be noted that the studies performed by manufacturers for EUA are not the same extent as an FDA-cleared/approved product.

II. Materials Required

- Appropriate biosafety and protective equipment should be used when handling, manipulating, and testing specimens. The CDC offers interim laboratory biosafety guidelines: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html
- Transport media
- Ten positive and ten negative specimens

- Test tubes/pipette tips
- Reference material kit (Examples: Accuplex, Exact)
- SARS-CoV-2 reagents with FDA EUA

Quality Control and Individual Quality Control Plan (IQCP)

1. Sourcing Quality Control Material

i. Commercial Synthetic

Many SARS-COV-2 synthetic commercial products have become available to use for assay verification and quality control. Quantified material is preferred over non-quantified material. Some EUA test kits will include QC material, which should be used per manufacturer's instructions. However, some EUA test kits do not contain QC and material must be purchased independently. When choosing commercial controls, be sure the material includes the genes targeted by the desired EUA assay. Contact the test manufacturer for recommendations on external quality control material.

ii. Residual patient samples

If commercial QC is not available, residual patient samples can be used for external quality control. A moderate positive patient sample should be diluted 1:10 for QC. A large batch of positive controls should be made, dispensed into single use aliquots and stored at -80°C.

Residual negative patient sample should be used for negative control.

iii. Genomic or in vitro transcribed RNA

Viral RNA may also be used to make controls. If using recombinant RNA, be sure that the RNA used matches the target gene(s) of the assay. Please note that RNA degrades more readily than intact virus, so RNA should be diluted and stored in RNA storage solutions. In general, using viral RNA is more challenging that using intact virus, so this approach may be challenging for laboratories unaccustomed to working with RNA. Quantified RNA can be spiked into pre-lysed negative sample matrix to make a moderate positive (5,000 genome copies/ml) control. A large batch of positive controls should be made, dispensed into single use aliquots and stored at -80°C. **Note**: Patient specimens cannot be spiked directly with RNA as degradation begins immediately. RNA should only be spiked into lysed samples. Because of this, viral RNA is not recommended as control material in verification samples for cartridge-based assays.

2. Frequency of QC

External QC must be run every day of patient testing or no less then manufacturer's instructions. Refer to the test's Instructions for Use for QC requirements. An individual quality control plan (IQCP) may be established for EUA assays that do not specifically require daily external quality control. If an assay qualifies for IQCP, follow CLIA

requirements for implementing and monitoring of IQCP (https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Individualized_Quality_Control_Plan_IQCP).

III. Instruments Required

- Based on FDA EUA authorized available tests.

IV. Verification Procedure

Verification procedure for batched and non-batched (i.e., random access, sample to answer) assays should be similar in approach. Testing to assess carryover by alternating positive and negative specimens during verification is recommended for batched testing. Similarly, alternating the testing of positive and negative specimens throughout the verification is recommended for non-batched testing. Determination of limit of detection is not required for on-label use.

Step 1. Perform one positive and one negative QC

This should be done before proceeding with additional testing to ensure the cartridges and reagent are working appropriately. Run QC according to the manufacturer's instructions. Verify that controls have produced the expected results. If QC is successful, proceed to Step 2. If QC is not successful, contact the manufacturer.

Step 2. Option 1: Verification panel with commercial synthetic material

Contrived patient samples can be created by spiking pooled negative matrix with commercial materials. Working with RNA can be challenging (see QC section above). Commercial materials may or may not be quantified for verification, but it is strongly recommended to use quantified material. Ideally, negative matrix is comprised of pooled residual negative patient samples. However, if residual negative patient samples are not available, blank transport media is acceptable (note: internal control will be negative if the internal control targets a human gene [e.g. RNase P]).

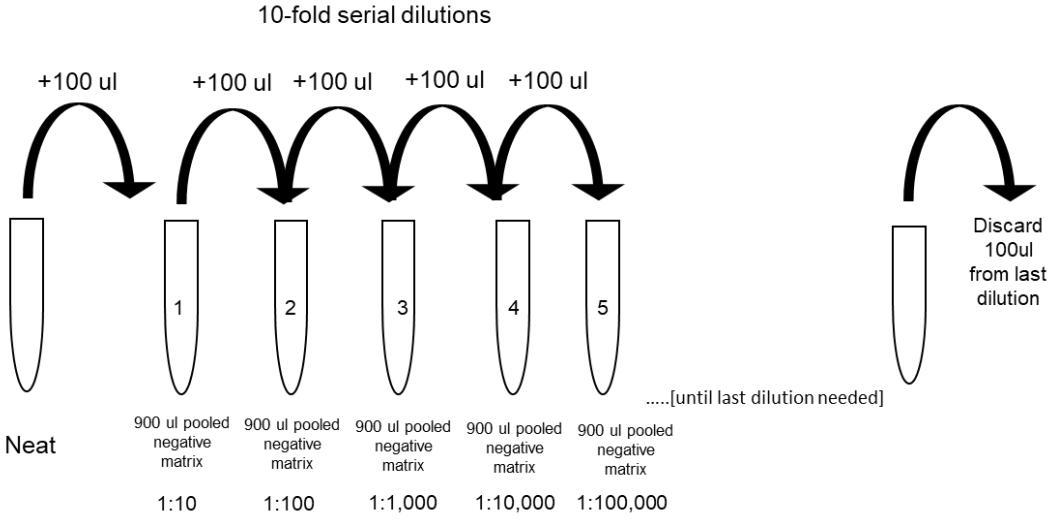
If the commercial product is quantified, dilutions of positive material should be made to test 5 high and 5 moderate viral loads. Positive reference material can be run neat or diluted 1:2 to represent a strong positive and diluted 1:10 for a moderate positive. Refer to the manufacturer's instructions or contact the manufacturer technical support to determine if the product can either be diluted into transfer media or must be diluted into the commercial synthetic negative matrix. A minimum of 10 positive samples should be tested; five strong positive and five moderate positive. These replicates can also be used for precision studies. For contrived negative samples, pool at least two vials of the negative material together and mix well. From this pooled sampled, test a minimum of 10 negative samples.

Step 2. Option 2: Verification panel with residual patient samples

Residual patient samples tested by another EUA method can be used for verification. 10 positive and 10 negative residual samples should be tested. Negative samples should be 10 independent patient samples (i.e. not pooled). Positive samples can be either 10 independent patient samples or dilutions of a strongly positive patient sample. This may be helpful when limited positive samples are available. A strongly positive sample can be identified based on the PCR threshold value (C_T). Strong positive samples typically have a C_T range from 15-24, where moderate positives range from 25-30. If diluting a strong positive sample for verification, 5 samples should still be strongly positive after dilution and 5 should be moderately positive. Ten-fold serial dilutions can be made in pooled negative residual patient samples to achieve the desired C_T value. Caution should be taken when making dilutions to not reach or go past the limit of detection (LOD) of the assay. Generally speaking, every ten-fold dilution will increase the C_T by 3 cycles. For example, a sample with an initial C_T of 20 will have a C_T of approximately 23 after making one 10-fold dilution. Be aware that an initial C_T value >31 may approach the LOD, after delayed transport or multiple freeze-thaw cycles, and it is recommended that those should not be included in accuracy studies. The positive replicates can also be used for precision studies by comparing replicate C_T values. Note, that not all methods yield a C_T value.

EXAMPLE

Serial Dilutions



Dilutions based on CT values

Sample	Reference Method CT Value	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~~	~~36~~
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.

Other considerations

1. SARS-COV-2 detection in a multiplexed pathogen panel

If SARS-COV-2 is included as a new target on a larger multiplex panel, the extent of verification depends on how the assay is incorporated in the panel. If it is incorporated 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.

2. Healthcare Systems

For large healthcare institutions containing multiple hospital labs that plan to implement the same diagnostic assay throughout the system, a full verification (as described above) should be performed at the main or core laboratory before testing is started at any of the laboratories. If the full verification meets expectations, then a verification using a smaller number of specimens can be performed at each site within the system. For this smaller verification, a total of 10 samples can be tested; 6 positive and 4 negatives. Of the 6 positives, 3 should be strongly positive and 3 should be moderate to low positive. Negatives should be negative residual patient samples. The main laboratory can develop and distribute mini-verification panels to aid the process. This verification testing can also be documented as part of the training of technical staff using the new method.

Below are recommendations for the mini-verification.

Step 1. Perform one positive and one negative QC

This should be done before proceeding with additional testing to ensure the instrument, cartridges and reagent are working appropriately. Run QC according to the manufacturer's instructions. Verify that controls have produced the expected results. If QC is successful, proceed to Step 2. If QC is not successful, contact the manufacturer.

Step 2. Test using a Mini-Verification Panel

Each individual site should use the mini-verification panel to verify their device. Results should be submitted to the main laboratory for review and approval. 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 implementing an IQCP.

V. Safety Requirements/Precautions

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. According to the CDC guidelines, 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.

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. For laboratory-based testing, a certified Class II biological safety cabinet (BSC) should be used for procedures involving generation of 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 inactivated by incubating at 56°C for 30 minutes prior to testing. 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.

VI. Limitations

- Assay/Technical limitations
  Refer to the package inserts for the test that is being implemented.
- Staff training
  Staff training and competency should be documented appropriately as for all other assays in the clinical laboratory.

- As of April 2020, the clinical sensitivity of assays that have received FDA EUA have not been determined by extensive study. Many reports of individuals with COVID-19 have had specimens that test negative for SARS-CoV-2, so a negative nucleic acid test does not definitively rule out COVID-19.

VII. Supplementary Information

- FDA requires fact sheets to be shared with providers and patients. These can 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
  - Examples to include in the patient report:

    Fact sheet for providers: www.fda.gov/media/XXXXXXX/download

    Fact sheet for patients: www.fda.gov/media/YYYYYYY/download

- As of April 2020, supplies and reagents needed for specimen collection and testing are limited. The FDA provides insight regarding what labs and providers can use when they encounter shortages: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2
- COVID-19 is currently a reportable disease. Be sure that a system is in place to properly notify the appropriate public health agencies of cases in which SARS-CoV-2 is detected.
- The following comments are examples that could be customized and added to all test reports:

  This assay is designed to detect the XXXX and/or XXXX genes of SARS-CoV-2 using nucleic acid amplification. A Not Detected result does not preclude the possibility of 2019-nCoV 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 along with other clinical and laboratory data in making the diagnosis.

  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 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.

  XXXXXXXX laboratory is certified under CLIA-88 as qualified to perform high complexity testing. This testing was performed in the XXXXXXX laboratory located at [Anywhere, USA 12345] (CLIA License #XXXXXXXX, CAP #XXXXXX, other as appropriate).

- Guidance for COVID-19 testing for CAP accredited laboratories https://www.cap.org/laboratory-improvement/news-and-updates/guidance-for-covid-19-testing-for-cap-accredited-laboratories

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