Contains Nonbinding Recommendations

Validation of Certain In Vitro Diagnostic Devices for Emerging Pathogens During a Section 564 Declared Emergency

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Guidance for Industry and Food and Drug Administration Staff

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For questions about this document, contact IVDguidance@fda.hhs.gov.



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health

Preface

Public Comment

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Guidance for Industry and Food and Drug Administration Staff

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff or Office responsible for this guidance as listed on the title page.

I. Introduction

The Food and Drug Administration (FDA or Agency) plays a critical role in protecting the United States from threats such as emerging infectious diseases, potential public health emergencies, and public health emergencies. FDA is issuing this guidance to describe recommendations for validation of certain in vitro diagnostic devices (IVDs) for emerging pathogens when the Secretary of Health and Human Services has declared that the circumstances exist justifying emergency use authorizations (EUAs) for such IVDs under section 564 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (hereafter referred to as an "applicable 564 declaration"), based on an underlying determination under section 564(b)(1) of the FD&C Act.

For the current edition of the FDA-recognized consensus standards referenced in this document, see the FDA Recognized Consensus Standards Database. For more information regarding use of consensus standards in regulatory submissions, please refer to the FDA guidance entitled "Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices."

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. Background

The Emergency Use Authorization (EUA) authority under section 564 of the FD&C Act allows FDA to help strengthen the nation's public health protections against chemical, biological, radiological, and nuclear (CBRN) threats by facilitating the availability and use of medical countermeasures (MCMs) needed during an actual or potential emergency or material threat. Under section 564 of the FD&C Act, when the Secretary of Health and Human Services (HHS) declares that the circumstances exist justifying the issuance of EUAs, FDA may authorize certain unapproved medical products or unapproved uses of approved medical products to diagnose, treat, or prevent serious or life-threatening diseases or conditions caused by CBRN agents when certain criteria are met, including when there are no adequate, approved, and available alternatives. For information on FDA's implementation of this and other emergency use authorities, see FDA's guidance "Emergency Use Authorization of Medical Products and Related Authorities."

Accurate and reliable IVDs are critical to the diagnosis, tracking, treatment, and interruption of transmission of infectious diseases during outbreaks, as well as for diagnosing and treating diseases or conditions caused by CBRN threats. In the public health emergencies of COVID-19[1] and monkeypox (mpox),[2] FDA issued guidances that included enforcement discretion policies for certain unauthorized tests to help rapidly increase national testing capacity early in the outbreaks. Certain tests were made available prior to or without an EUA as described in those policies. Regardless of whether a test is issued an EUA or offered as described in an enforcement discretion policy, it is critical that the test be appropriately validated. FDA may take action, as appropriate, against violative tests, including those that lack appropriate validation. This guidance and associated template(s) are intended to help test manufacturers better prepare for future public health emergencies by including FDA's recommendations for test validation during an applicable 564 declaration.

Also, this guidance and associated templates address the recommendations received from two independent assessments of FDA's response to COVID-19. Specifically, FDA selected Booz Allen Hamilton to do such an independent assessment, which culminated in an October 2021 report, "Emergency Use Authorization Assessment – Final Report," recommending that FDA "develop a framework for how to conduct validation of diagnostic tests for emerging pathogens in the setting of a declared PHE." Similarly, the HHS Office of the Inspector General's September 2022 report, "FDA Repeatedly Adapted Emergency Use Authorization Policies To Address the Need for COVID-19 Testing," recommended that FDA "develop a suite of EUA templates for future emergencies involving novel pathogens" and "expand and improve resources" on the EUA process, among other actions FDA has taken or is taking.

III. Scope

This guidance describes general recommendations for validation of certain IVDs for emerging pathogens during an applicable 564 declaration. The IVDs in the scope of this guidance are

[1] See FDA guidance document "Policy for Coronavirus Disease-2019 Tests (Revised)."
[2] See FDA guidance document "Policy for Monkeypox Tests to Address the Public Health Emergency."

diagnostic tests[3] intended to detect a pathogen(s) to aid in the diagnosis of a serious or life-threatening infectious disease or condition for which an applicable 564 declaration has been issued.[4] The recommendations in this guidance apply to test data and information submitted in a pre-EUA[5] or an EUA request, or for a test offered as described in an applicable enforcement discretion policy. This guidance does not address the EUA regulatory process; refer to "Emergency Use Authorization of Medical Products and Related Authorities" for additional information.

The information and recommendations provided in this guidance are intended to be broadly applicable to potential future emerging pathogens. This guidance applies to all stages of an outbreak and includes discussion about when appropriate validation may depend on the stage of the outbreak. Additionally, FDA recognizes that outbreaks inherently come with uncertainty and that recommended validation approaches should be adjusted for certain situations. For example, different considerations may be needed in certain situations, such as matters of national security. In any outbreak, FDA continually monitors and assesses the testing landscape in the U.S. and will update its policies and recommendations as appropriate.

Due to differences across tests, including technology and indications for use, as well as different circumstances across outbreaks, some sections of this guidance may not be applicable to all tests. Test manufacturers should consider which sections are applicable based on the stage of the outbreak, availability of validation materials, and the design and proposed indication for use of their test, among other considerations.

FDA generally will work interactively with the manufacturer during the development and review of an EUA request to help ensure appropriate validation of a test, particularly given potential changes in recommendations due to the changing circumstances of any outbreak. Test manufacturers may also refer to the mpox and COVID-19 EUA templates on FDA's website for additional examples of approaches taken during prior emergencies.[6]

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[3] These IVDs are in vitro diagnostic products as defined in 21 CFR 809.3 that are intended to aid in the diagnosis of disease (referred to herein as "diagnostic tests"), such as molecular or antigen tests, which are also devices as defined in section 201(h) of the FD&C Act. Screening tests, which are used for testing individuals without symptoms or other reasons to suspect illness, are a subset of diagnostic tests. In contrast, serology/antibody and other adaptive immune response tests generally are not used to diagnose a current acute infection and are outside the scope of this guidance. Diagnostic tests may be designed for use in various settings, such as in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory, at the point of care site covered by a laboratory's CLIA certificate, or at home.

[4] The guidance also includes some recommendations regarding home collection kits and certain software that are used with diagnostic tests.

[5] A pre-EUA, a submission useful for facilitating early interaction between a sponsor and FDA about potential EUA products, can be submitted *prior to* or *during* an applicable 564 declaration before submitting an EUA request. A pre-EUA can only transition to an EUA request if there is a current applicable 564 declaration. The recommendations in this guidance may be helpful to manufacturers preparing for early engagement such as a pre-EUA, even prior to an applicable 564 declaration, as it could help facilitate the completeness of a potential future EUA request. Refer to Section III.C of the FDA guidance document "Emergency Use Authorization of Medical Products and Related Authorities" for more information about pre-EUA activities.

[6] See mpox templates at: https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-mpox-emergency-use-authorizations-medical-devices#templates and COVID-19 templates at: https://www.fda.gov/medical-devices/covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas#covid19ivdtemplates.

FDA recommends that test manufacturers consult with FDA regarding alternative approaches via CDRH-IVD-EUA@fda.hhs.gov.

IV. Availability of Templates

FDA has found the use of templates to be beneficial during prior emergencies, for both manufacturers and FDA reviewers, to help facilitate the preparation and submission of pre-EUAs and EUA requests to FDA, and any resulting authorization. Templates should be viewed only as recommendations, and alternative approaches can be used unless specific regulatory or statutory requirements are cited.

A generic template entitled "General IVD Emergency Use Authorization (EUA) Request/Pre-EUA Template" is made available through download from our website,[7] and it reflects FDA's current thinking on data and information that should be submitted in pre-EUAs and EUA requests. FDA may provide more tailored recommendations for tests for a specific outbreak through separate guidance or pathogen-specific templates, as appropriate.

Additional templates may be added to our website. For example, FDA may provide updated templates as appropriate in the event of a specific outbreak.

V. Validation Study Recommendations

Validation should objectively demonstrate that a finished device can consistently fulfill defined user needs and its intended use. The validation recommendations discussed here reflect that the "may be effective" standard for EUAs[8] provides for a lower level of evidence than the "effectiveness" standard that FDA uses for product approvals,[9] as discussed in the FDA guidance, "Emergency Use Authorization of Medical Products and Related Authorities." As such, the validation should demonstrate that the device may be effective for its intended use and that the known and potential benefits of the device when used as intended outweigh the known and potential risks of the device. This guidance also reflects the understanding that, in rapidly developing or unexpected emergency circumstances, it may not be possible for a manufacturer to collect and/or provide all of the recommended data, including in the format suggested in a timely manner. In such cases, additional information may become available and/or FDA might request such information at a later date.

Ideally, validation testing is performed using the final design of the device and the proposed instructions for use. This ensures that validation is most reflective of performance of the device following authorization, although an equivalency study can sometimes be used to address device changes following validation. Validation protocols and reports should be provided, including study objectives, test procedures, and information on the instrument(s), reagents, software, and

[7] Available at: https://www.fda.gov/media/184828/download.
[8] See section 564(c)(2)(A) of the FD&C Act.
[9] See, e.g., sections 513 and 515 of the FD&C Act and 21 CFR 860.7.

any other test materials or components needed to perform the test, whether provided with the test or that are required but not provided.

For innovative technologies, FDA might request technology-specific studies to assess the known and potential benefits and risks associated with the test. However, for rapid response to an emergency, tests are often designed using existing instruments and other components, such as reagents, that are lawfully marketed[10] for clinical use. In such cases, additional validation data to support issuance of an EUA may not be needed for the components that are already lawfully marketed for clinical use. For example, if a test is designed for use on a lawfully marketed instrument, instrument specific validation may not be needed to support issuance of an EUA for the test.

Additionally, certain validation studies described in Section V and 0 may be applicable only for certain device types or technologies. FDA recommends that manufacturers consider the validation studies applicable to their device type, as discussed in subsections A and B.

A. Clinical Performance Evaluation

A clinical performance evaluation is used to assess the accuracy of a test in detecting the analyte of interest, often through method comparison or comparison of test results to known positive and negative specimens. This type of study typically should characterize the performance of the device as it would be expected to perform in actual clinical use. The number of samples and the design of the study, such as an appropriate comparator method, will depend on many factors including the target pathogen, type of test, availability of clinical specimens containing the pathogen, availability of appropriate comparator methods, the stage of the outbreak, and overall testing needs.

For example, for a viral nucleic acid test using established technologies known to be capable of high sensitivity detection, at a stage of the outbreak where clinical samples and comparators are generally available, at least 30 positive and 30 negative specimens (as defined by the comparator) from the intended use population and using the intended specimen type are typically adequate to demonstrate the performance of the test compared to a highly sensitive comparator method. Evaluation of fewer specimens may not accurately characterize the true performance of the test.[11]

[10] A "lawfully marketed" device means a device that is in compliance with FDA requirements, which may include premarket authorization.

[11] For additional context, FDA issued EUAs for tests in public health emergencies prior to COVID-19 based on 50 contrived positive and 50 contrived negative clinical specimens. For similar products outside a declared emergency, FDA generally has expected an "all-comers" study of natural clinical specimens until at least 50 positives were obtained. The 30 positive and 30 negative described represents what FDA generally considers to be the minimum number of specimens needed to provide appropriate assurance of performance in an outbreak. Evaluation of fewer specimens may not accurately characterize the true performance of the test. For example, FDA received an EUA request for a molecular test for COVID-19 that included validation with only 12 positive samples, showing perfect performance among this limited sample set. FDA requested evaluation of additional specimens to confirm. When an additional 12 samples were evaluated, the cumulative performance dropped to an unacceptable positive percent agreement (PPA) of 71%, and the EUA request was withdrawn.

Early in an outbreak, however, clinical samples and an appropriate comparator may not be available, so initial test validation could be limited to contrived sample evaluation as discussed in subsection A(1).

For infectious disease indications, a highly sensitive comparator method is often a molecular method (e.g., RT-PCR) that utilizes a nucleic acid isolation method and multiple target regions for the detection of the analyte with a high sensitivity based on clinical performance (e.g., positive percent agreement, or PPA ≥ 95%) from testing natural clinical specimens of the appropriate specimen type.

(1) Initial Stages of the Outbreak - Alternative Specimen Types

Natural clinical specimens are the preferred sample type for validation of a diagnostic test. However, in the early stages of an emerging disease outbreak, natural clinical specimens may not be readily available. In such cases, the use of archived positive and negative clinical specimens[12] could be a reasonable alternative. Ideally, archived specimens should be accompanied by information to determine sample adequacy, such as the specimen collection date, specimen storage conditions, and date of onset of symptoms.

When prospective and archived samples are not available, use of contrived (e.g., spiked) samples may be acceptable. Contrived samples are constructed in the laboratory by placing known concentrations of a microorganism or analyte into individual (not pooled) human specimens known to be negative for that microorganism or analyte (i.e., negative clinical matrix[13]). In situations where pathogen stocks are not available, such as at the early stages of an outbreak, FDA would likely consider use of synthetic material to be acceptable.[14] When synthetic material is used, it should closely mimic natural materials. For example, if the pathogen is an RNA virus, then synthetic RNA, rather than synthetic DNA, should be used in most cases.

Due to limitations of validation with contrived samples, including those prepared using synthetic or natural materials, emergency use authorization of such tests would likely include a Condition of Authorization (CoA) requiring a post-authorization clinical performance evaluation with natural patient specimens when it becomes feasible. When a clinical performance evaluation is not a prospective study, FDA recommends that manufacturers ensure that their evaluation includes samples that appropriately represent the range of pathogen levels expected in clinical specimens in the intended use population.

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[12] For purposes of this guidance, archived specimens, sometimes referred to as retrospective or banked specimens, are defined as specimens collected from a human subject that are known to harbor the analyte of interest (i.e., positive) or not harbor the analyte (i.e., negative). Archived samples should be selected to minimize bias; for instance, samples should not be selected for archiving based on the candidate test result. The appropriateness for use of archived samples, such as length of time in storage or other factors, will vary based on the individual emerging pathogen during an outbreak.

[13] For purposes of this guidance, clinical matrix is defined as a specimen taken from a human subject. Negative clinical matrix is a clinical specimen taken from a human subject which does not harbor the analyte of interest. Liquid transport media without clinical matrix and specimen transport medium included in a collection kit that was not used to collect a clinical specimen are not considered real clinical matrix. Generally, FDA recommends individual negative matrix for studies such as confirmatory LoD and for constructing contrived specimens to represent a range of sample components (e.g., mucus, particulate matter) which may be present in samples.

[14] For example, FDA authorized certain COVID-19 tests that were validated with synthetic material through April 2020.

(2) Study Design

Ideally, clinical performance should be established through a prospective, all-comers clinical study in the intended use environment, by the intended user(s), and with natural clinical specimens from the intended use patient population(s).

In some cases, it might be appropriate for the clinical performance evaluation to include only the most challenging clinical matrix type proposed in the intended use of the device[15] (e.g., nasopharyngeal (NP) swabs for common upper respiratory types, sputum for common lower respiratory types). For atypical specimen types, the clinical performance evaluation should evaluate each specimen type included in the intended use of the device.[16] In addition, specimens from the same anatomical site but different in collection or transport methods, such as with and without liquid transportation medium, are considered as two distinct types of specimens and should each be validated.

Subjects should be enrolled in the clinical performance evaluation to reflect the intended use population(s). For example, if the test is intended for use with asymptomatic individuals, the study protocol and report should document how individuals were screened and confirm that all enrolled individuals were free of any symptoms of the target infection prior to enrollment and sample collection. In cases where obtaining positive samples from asymptomatic individuals is challenging, enrichment strategies that minimize potential biases could be considered (e.g., conducting the study in an asymptomatic screening population that is under quarantine due to possible exposure may increase the likelihood of obtaining more positive specimens). Manufacturers should consult FDA prior to implementing enrichment approaches in your clinical performance evaluation.

(3) Clinical Data Analysis

Generally, all samples meeting pre-defined inclusion criteria for the clinical performance evaluation should be included in the analysis. Justification should be provided for exclusion of any such samples from data analysis. FDA generally recommends that clinical data analysis include the calculation of positive percent agreement (PPA) and negative percent agreement (NPA) with a highly sensitive comparator method, and corresponding 95% confidence intervals using the Wilson Score method. Target PPA and NPA to support adequate performance of a diagnostic test depend on the test type and indications for use as well as a benefit/risk assessment in the context of the emerging outbreak. FDA may consider lower PPAs acceptable for different test types and intended use populations with certain mitigations, such as reporting of negative test results as "presumptive" based on benefit-risk considerations. In certain cases, such as for screening tests for asymptomatic individuals, a higher NPA has generally been expected. If the test is intended for symptomatic individuals, the data should include time from symptom onset to test for each enrolled subject. The data analysis should account for performance shifts in relation to time from symptom onset to support adequate clinical performance.

[15] For example, for respiratory viruses, examples of the most challenging clinical matrix type include nasopharyngeal (NP) swabs for common upper respiratory types or sputum for common lower respiratory types.
[16] For example, for respiratory viruses, examples of atypical specimen types include saliva, oral fluid, and buccal swabs.

(4) Human Subject Protection

Studies involving clinical specimens (human specimens) are subject to applicable requirements for Institutional Review Board (IRB) review and approval and informed consent (*see* 21 CFR parts 50, 56, and 812). In December 2023, FDA published a final rule[17] that permits an IRB to waive or alter informed consent requirements for certain minimal risk clinical investigations that meet the conditions in 21 CFR 50.22. FDA anticipates that this new provision may be applicable to certain IVD studies involving clinical specimens. In addition, the FDA guidance "Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable," describes a policy about informed consent requirements for certain IVD studies that use leftover, de-identified specimens. Use of such specimens does not obviate consideration of the other recommendations in this guidance document. Please note that additional requirements in the Investigational Device Exemption (IDE) regulations (21 CFR part 812) may be applicable to certain IVD clinical studies.

B. Analytical Validation Testing

In addition to the clinical performance evaluation, analytical validation studies assessing analytical performance should also be provided, as recommended in this section.

(1) Limit of Detection (LoD) (Analytical Sensitivity)

The LoD provides a measure of the analytical sensitivity of a test for a particular target analyte and is defined as the lowest concentration of target analyte that is consistently detected by the test in 95% of the specimen replicates.[18] The LoD results guide additional validation studies, including the clinical performance evaluation. LoD should be determined using the entire test from specimen preparation and extraction through detection and the result interpretation algorithm. In some cases, it may be appropriate to determine LoD only for the most challenging negative clinical matrix type included in the intended use of the device. For atypical specimen types, the LoD should be determined with each specimen type included in the intended use of the device.

In situations where neither live stocks, inactivated stocks, nor known positive clinical specimens are available, use of synthetic material[19] may be considered for use in the LoD evaluation in real clinical matrix. When synthetic material is used, it should closely mimic the natural target analyte.[20] Manufacturers should discuss potential use of alternative matrices for unique circumstances with FDA. As more specimens become available, FDA generally recommends that LoD be evaluated by spiking individual or pooled natural negative clinical matrix with well

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[17] See FDA's final rule on *Institutional Review Board Waiver or Alteration of Informed Consent for Minimal Risk Clinical Investigations*, 88 FR 88228, 88241, Dec. 21, 2023.

[18] See definition and additional considerations in CLSI EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.*

[19] Please refer to HHS guidance on the use of synthetic nucleic acids, found here: Synthetic Nucleic Acid Screening | ASPR. At the time of issuance, the current version of the HHS guidance is: 2023 HHS Screening Framework Guidance for Providers and Users of Synthetic Nucleic Acids | ASPR.

[20] Simulated or artificial specimen matrix (e.g., clean liquid transport media spiked with mucin, human DNA, and HeLa cells) or recombinant antigen should not be used in an LoD study as this material does not accurately mimic actual patient samples, and testing with this material may not accurately reflect performance of the device.

characterized, quantified natural stocks of the target analyte, for each clinical specimen type included in the intended use of the device.

Generally, the preliminary LoD is determined by testing a 2-3-fold dilution series of three replicates per concentration. The lowest concentration at which all tested replicates are positive is considered the preliminary LoD. The preliminary LoD study should include at least one concentration that does not yield 100% positive results. The LoD should then be confirmed by testing a minimum of 20 individual replicates of the concentration determined to be the preliminary LoD. The final LoD is the lowest concentration resulting in positive detection of at least 95% of the replicates (e.g., at least 19 out of 20 replicates). In the case where the final LoD study achieves a positivity of 100%, a lower concentration (using a 3-fold dilution) should be tested (with 20 replicates) until < 95% positivity is obtained. While the LoD for an entire analyte is most critical for test validation, FDA might also request the LoD for individual targets for multi-target tests to help the Agency evaluate the performance of the device.[21]

(2) Inclusivity (Analytical Reactivity)

An inclusivity study shows reactivity of the test with additional related (e.g., taxonomic, immunological, and genetic composition) target species or isolates.

For molecular-based tests, FDA generally recommends 100% nucleotide sequence identity, meaning that the test reagents have no mismatches with known published sequences and therefore can likely detect all known species or isolates. If a test has less than 100% nucleotide sequence identity to a significant number of published sequences, FDA recommends performing a risk assessment on how such mismatches may impact the performance of the test. Inclusivity of molecular-based tests should be evaluated through *in silico* analysis of the test primer and probes with all known sequence variants. *In silico* data should be supplemented with wet testing of currently circulating variants, when available, by testing clinical isolates and/or inactivated materials spiked into clinical matrix at or near the test LoD.

Inclusivity of antigen-based tests should be evaluated through wet testing of currently circulating variants. The methods and results of this analysis should show the extent to which variation in the target genome may affect clinical and analytical sensitivity. FDA recommends monthly evaluation of inclusivity to account for new and emerging clinically significant mutations. This could include, for example, assessing the prevalence of mutations in well-established publicly available sequence databases and monitoring for credible reports that a given variant or mutation has the potential to increase pathogenicity, increase transmission, or otherwise increase the risk to public health. FDA might request additional testing based on its own monitoring. The aggregate impact of the mutations should be evaluated and should not result in the clinical performance point estimates for the test dropping below the clinical performance recommendations described in Section V.A. If a greater than 3-fold reduction in analytical sensitivity is observed when comparing pathogens with and without the mutation, manufacturers should conduct a risk analysis for the observed difference in performance, consider further risk mitigations, and assess whether the known and potential benefits of the test continue to outweigh the known and potential risks.

[21] Multi-target tests detect multiple sites for the same analyte. For example, a multi-target SARS-CoV-2 test may detect N-gene, S-gene, and E-gene targets of the SARS-CoV-2 virus.

(3) Specimen Stability

Degradation of a specimen prior to testing can lead to false results. The Centers for Disease Control and Prevention (CDC) often communicates acceptable specimen stability conditions for certain specimen types to be tested for specific analytes.[22] In such cases, when a manufacturer uses CDC's specifications for specimen stability, additional data are likely not needed to support validation. For additional or extended specimen stability conditions not supported by CDC's specifications, or in cases where CDC has not communicated specifications, the manufacturer should validate their proposed specimen stability conditions in a specimen stability study, as described in this section.

When such a specimen stability study is performed, the stability of specimens collected and stored should be evaluated in real-world conditions.[23] The study should include several time points throughout the duration of the proposed storage time and at least one time point beyond the proposed stability, as well as temperatures at the upper and lower limits of the proposed temperature ranges for storage and transportation (e.g., 15°C and 30°C for extremes of temperature ranges when stored at room temperature). When a test is intended to be performed on the specimen immediately or shortly after obtaining the specimen, the specimen stability testing timeframe should reflect a shorter storage time (e.g., 2 hours at room temperature).

The study should include contrived positive specimens prepared by spiking live or inactivated pathogen into an individual or pooled negative clinical matrix around the LoD and a minimum of 10 negative specimens. If live or inactivated pathogen are not available, we recommend manufacturers contact FDA to discuss potential options.

Specimen stability should be demonstrated for each proposed transport method (e.g., VTM/UTM, saline, and dry swabs). Specimen stability should be demonstrated for each atypical specimen type included. Specimens from the same anatomic site but transported in different ways are considered different specimen types. If a test is intended for use with multiple commonly used specimen types, specimen stability could be demonstrated using the most challenging intended specimen type (e.g., NP swabs for common upper respiratory types, sputum for common lower respiratory types).

(4) Basic Safety, Software Validation, Cybersecurity, and Electromagnetic Compatibility (EMC) Testing

Basic safety hazards such as electrical hazards, fire hazards, and mechanical hazards should be addressed for a test that includes instrumentation and/or batteries that has not been previously

[22] See CDC Infectious Diseases Laboratories Test Directory, available at: https://www.cdc.gov/laboratory/specimen-submission/list.html.

[23] For example, the expected environmental conditions at the storage and/or shipping specifications (e.g., temperature and time specifications) recommended in labeling. Specimen stability evaluation for home collection should be designed to simulate home specimen collection and shipping/transport (e.g., storage of specimens before the home user ships the specimen, specimen stored in a mailbox or drop box waiting for pick-up, shipping conditions after pick-up when the specimen is shipped to the testing lab).

cleared, approved, or authorized by the FDA for use with the test.[24] Instruments with device software functions[25] should be validated to ensure that software inputs and outputs are appropriate to fulfill test requirements, all expected inputs produce the expected outputs for all functions important for proper test operation and for defined user needs and intended uses (e.g., verification and validation), and the test will be free of defects or defects will be known and mitigated to an acceptable level (e.g., via risk assessment). The cybersecurity[26] of instruments or software device functions used with the test with any external wired and/or wireless communication interfaces[27] should be evaluated to ensure user and patient safety in the intended use environment. Instruments or other components that are electrically-powered should be designed and validated to demonstrate adequate Electromagnetic Compatibility (EMC).[28]

(5) Usability, User Comprehension, and Flex Studies

For Point-of-Care (POC) home collection, and home use tests, a usability study should be conducted to ensure lay users can complete all steps of the workflow in an actual or simulated use environment.[29] For tests that do not involve a healthcare provider, a user comprehension study should also be conducted to assess risks associated with misinterpretation and misuse of test results. This study should evaluate the lay user's understanding and comprehension of critical elements and concepts in the labeling. [30] It may be possible to combine the usability study, user comprehension study, and/or the clinical performance evaluation study since these all include representative operators under intended use settings.

Flex studies are intended to demonstrate the robustness of a test, including the test's ability to maintain performance through environmental and usage variations under conditions of stress. These studies should be designed to identify the maximum deviation in conditions reasonably expected for POC or home use environments that will still generate accurate results (e.g., operating temperature). A hazard risk analysis should first be conducted to identify the most common or likely sources of error based on the use locations and test procedures.[31]

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[24] Consider International Electrotechnical Commission (IEC) 60601-1 *Medical electrical equipment – Part 1: General requirements for basic safety and essential performance*, which defines basic safety as freedom from unacceptable risk directly caused by physical hazards when medical electrical equipment is used under normal condition and single fault condition.

[25] Device software functions are software functions that meet the definition of a device under section 201(h) of the FD&C Act. Device software functions may include software as a medical device (SaMD) and software in a medical device (SiMD). See also FDA guidance documents "General Principles of Software Validation," "Content of Premarket Submissions for Device Software Functions," "Policy for Device Software Functions and Mobile Medical Applications," "Off-The-Shelf Software Use in Medical Devices," and 21 CFR 820.30.

[26] See FDA Guidance document "Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions."

[27] For example, Wired: USB, ethernet, SD, CD, and RGA; Wireless: Wi-Fi, Bluetooth, Radio Frequency, inductive communication, Near Field Communication (NFC), and Cloud.

[28] For more information on EMC testing, consult the FDA guidance document "Electromagnetic Compatibility (EMC) of Medical Devices."

[29] See also FDA guidance document "Applying Human Factors and Usability Engineering to Medical Devices."

[30] For example, understanding the intended use of the test, instructions for use, the warnings and precautions, and comprehension of the test results (e.g., positive, invalid, and negative results and the impact of each).

[31] See also FDA guidance document "Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices" and FDA website resource regarding CLIA Waiver by Application Decision Summaries.

As discussed earlier in this guidance, FDA recognizes that validation recommendations may be adjusted based on circumstances at different stages of an outbreak, such as emergent public health needs, availability of validation materials, and the important role of POC tests and home testing options for infectious diseases. FDA generally will work interactively with a manufacturer during the development and review of an EUA request to help ensure appropriate validation of a test, particularly given potential changing circumstances of an outbreak.

(6) Potential Additional Testing

In addition to the recommended analytical validation discussed throughout this guidance, additional testing may be recommended in certain circumstances. Some examples are outlined briefly here: Antigen tests should generally be evaluated for high-dose hook effect, which occurs when the presence of very high levels of the target analyte in the patient specimen interferes with the binding ability of the capture antibody and causes false negative results. If the clinical evaluation does not use real clinical specimens, then cross-reactivity, microbial interference, and endogenous/exogenous interference studies should generally be performed, typically with wet testing using the most sensitive extraction/instrument combination and the Limit of Detection (LoD) with the lowest analyte concentration. FDA may also find it appropriate to request studies such as these in other circumstances where there may be a question or concern about accuracy or reliability, such as when novel approaches are used or where there are signals of potential impacts on test performance. Cross-reactivity should be evaluated to establish that the test does not react with related non-target microorganisms, high prevalence disease causing agents, and commensal or pathogenic flora that are likely to be in the clinical specimen. In addition, microbial interference should be evaluated to establish test performance when the target analyte is present in a clinical specimen with other relevant non-target microorganisms. Endogenous/exogenous interference studies assess the effects of endogenous and exogenous substances on test performance. Tests at risk for cross-contamination from high-positive specimens adjacent to negative specimens (e.g., automated liquid handling systems) should undergo carry-over testing to check for false positive results. Comparable performance between fresh and frozen specimens should be demonstrated for tests intended for use, or otherwise validated using, frozen specimens. For some tests with optional/additional components/workflows (e.g., multiple thermocyclers, multiple extraction methods, different collection media, extraction and/or PCR instruments), FDA may recommend an analytical equivalency study to reduce the likelihood of additional clinical performance evaluation being requested.

C. Change Protocols

Manufacturers seeking an EUA might consider developing a change protocol[32] for potential future modifications. When a change protocol is reviewed by FDA and included in the initial authorization, changes implemented pursuant to the change protocol are considered to be covered by the initial authorization. Change protocols should include specific anticipated

[32] In the draft of this guidance, FDA referred to predetermined change control plans (PCCPs). FDA has revised this discussion to refer to "change protocols" to distinguish these from PCCPs and, among other things, avoid suggesting that such change protocols are being implemented in the same way as PCCPs, which are applicable only to marketing submissions (see, e.g., "Marketing Submission Recommendations for Predetermined Change Control Plan for Artificial Intelligence-Enabled Device Software Functions").

modifications the manufacturer would make, the steps that will be taken to validate the modifications, and the performance metrics that would be considered an indication of successful validation (e.g., acceptance criteria). The change protocol should also specify any changes to labeling that would be made if the validation is successful. All modifications included in a change protocol should maintain the device within the device's intended use. Examples of modifications that might be in a change protocol include adding new instruments or collection devices. In general, requirements for implementation of the change protocol would be specified in a CoA for the EUA.

VI. Additional Considerations for Certain Test Types

A. Multi-Analyte Panels

During an outbreak it may be beneficial to have a multi-analyte panel using one specimen to detect and differentiate among pathogens that can cause similar symptoms. Multi-analyte tests may alleviate the need for multiple samplings and may decrease use of medical supplies, reducing pressure on supply chains during an emergency. Each analyte of a multi-analyte test should be validatedVValidation Study Recommendations. Manufacturers should address the potential for cross-reactivity and microbial interference (including competitive inhibition) between analytes. Generally, the validation needed to support EUA issuance for multi-analyte panels depends on several factors, including, but not limited to:

- State of scientific knowledge for each pathogen,
- Whether target analytes have been the subject of previously FDA cleared/approved/authorized tests,
- Whether the test is a modification of a multi-analyte test previously FDA cleared/approved/authorized for other pathogens,
- Test format [e.g., individual wells used to test for each target analyte or one single well used to test for all target analytes together (i.e., multiplex reaction)],
- Specimen types the test will be used with, and
- Disease prevalence/availability of clinical specimens.

B. Home Specimen Collection and Home Use Tests

Home specimen collection and home use tests may be beneficial during an outbreak by increasing patient access to testing and by reducing potential exposures. Home specimen collection involves the use of a home collection kit to collect a specimen and send it to a laboratory for testing. A home use test involves collecting a specimen and testing it at home, without needing to send it anywhere else. FDA recommends that manufacturers of home collection kits and home use tests consider incorporating design features to address user accessibility (e.g., for vision or hearing deficits). Home collection kits and home use tests should use only non-invasive specimen collection methods that require no specialized training to be safely and correctly performed. The home collection kit or home use test should be safe for home use and for lay-user collection of specimens from the intended anatomical site. Home collection kits and home use tests should be assessed for toxicology. Home collection kits or home use tests that contain hazardous materials may not be appropriate without adequate risk mitigations such as safety features to limit the risk of patient exposure. Labeling should inform users of the risks associated with using the device. Instructions for lay users should be written as Quick Reference Instructions (QRI) limited to one to two pages, at no higher than a 7th grade reading level, and

should include pictures and diagrams to facilitate lay person use. Inadequate specimen collection or use of home tests by a lay user can introduce risks of false results. These risks can be mitigated in multiple ways, such as video observation of the user by a trained healthcare professional and/or inclusion of an internal control in the test design to indicate that adequate human specimen was collected and placed into the test for analysis.

Home collection kits can be paired with a single test or multiple tests. Validation of home collection kits should support the proposed intended use and should evaluate the entire testing workflow following the validation recommendations discussed earlier in this guidance. This should include distribution of the home collection kit to an individual, collection and storage of a clinical specimen at home using the materials provided, and sending the specimen to a CLIA-certified clinical laboratory for testing. Where home collection kit and test manufacturers separately seek EUAs, a right of reference[33] shared between the manufacturers may help streamline the review process by allowing data from each EUA request to be incorporated by reference into the other.

In general, a home use test should be simple to perform, and its results should be simple to interpret. Home use tests can be prescription use or over the counter (OTC) and can be used in non-laboratory settings, such as offices, sporting events, airports, schools, where a lay person collects a sample, performs the test, and interprets test results themselves. The clinical performance evaluation of home use tests should be conducted at U.S. sites representative of the intended use setting (i.e., that mimic a home use environment) and with users who represent the intended use population (e.g., including different socioeconomic and educational backgrounds and range of ages). Generally, for OTC tests, the intended use patient population includes adults (and older pediatric individuals) who can perform self-collection and testing, pediatric individuals who may be able to self-collect and perform the test under supervision of an adult, and younger pediatric individuals (and some adults) who need their specimen collected and tested by an adult caregiver. Each patient population proposed should be evaluated following the validation recommendations discussed elsewhere in this guidance. The entire workflow should be performed by each individual participant including, as applicable, test registration, specimen collection, testing, and results interpretation. Testing sites should be set up in a way that precludes a user from seeing or hearing other users performing the test (e.g., in separate rooms). Specimens collected for use with the comparator methods should be collected according to comparator device labeling (e.g., by a health care provider).

When using smartphone software applications to facilitate performing the test and/or providing test results, the display should promote easy interpretation (e.g., positive, negative, and invalid) and understanding of results and what lay users should do next, including how to care for themselves and when to seek follow up care. Error messages should be readily understandable, and troubleshooting should be included in the instructions for use. The software application

[33] A manufacturer seeking to leverage data regarding another manufacturer's EUA-authorized assay should obtain a right of reference from that manufacturer. A manufacturer that has provided data to the FDA may grant a right of reference to other manufacturers, either broadly or to individual manufacturers. A right of reference provides a manufacturer the ability to rely upon, and otherwise use, existing information and data in one regulatory submission for the purpose of supporting a different regulatory submission. If the data is applicable to a new manufacturer's test, a new manufacturer may not have to repeat validation to support its submission to FDA, or FDA may recommend only a bridging study.

should be capable of capturing and transmitting test results and associated diagnostic data when appropriate in accordance with applicable local, state, and federal requirements. Automation, data harmonization, and integration of software in the diagnostic workflow should be optimized to lessen burden on the test user, minimize the potential for data entry errors, and improve the overall quality and utility of data captured. Software applications intended to interpret test results or otherwise impact test results should be evaluated in analytical validation and clinical performance studies.

C. Point-of-Care (POC) Tests

Point-of-Care (POC) tests are tests intended for use in professional near patient settings, such as hospitals, urgent care centers, and emergency rooms. POC tests can be beneficial during an outbreak because they provide more immediate results compared to laboratory-based tests.

Clinical performance evaluation of POC emergency use tests that are intended for use in a CLIA Certificate of Waiver setting should be conducted at one or two U.S. sites representative of anticipated real-world settings, with four to six operators without laboratory training, and who are representative of intended operators. For example, this may include: using the device in a healthcare setting, such as at a hospital bedside, by non-laboratorian healthcare professionals; in a non-traditional healthcare setting, such as at a school, by untrained users who are not healthcare professionals; or in a temporary testing site setting, such as a tent set up at a non-healthcare workplace, by users who have limited or no training or hands-on experience in conducting laboratory testing. The clinical performance evaluation for POC tests should include all steps in specimen collection and handling described in labeling, including addition of the specimen to the specimen port/well of the test. Testing should be performed in real time after specimen collection. Study operators should only rely on QRI and should receive no additional training or intervention on how to use the device. The QRI should be written for untrained users at no higher than a 7th grade reading level, limited to one to two pages, and include pictures and diagrams to facilitate use.

In addition to the clinical performance evaluation described above in Section V.A, for POC tests, performance around the LoD should be evaluated by the intended POC users with contrived specimens in real clinical matrix. Testing should include 10 samples near the LoD (e.g., ≤ 3-fold of the LoD), and 10 negative specimens per site. All contrived specimens should be blinded, randomized, and tested as part of the normal workflow of the site by the intended POC users, each of whom tests at least three positive samples near the LoD and three negative samples. Results that do not match the expected result should be investigated. Testing should demonstrate positive and negative agreement of at least 95%. If this is not achieved, the LoD should be re-evaluated.

Guidance History*	Date	Description
Level 1 Final Guidance	November 2026	See Notice of Availability for more information.**
Level 1 Draft Guidance	January 2025	See Notice of Availability for more information.**

**The Notice of Availability is accessible via the Search for FDA Guidance Documents webpage.