

VERIFICATION AND VALIDATION TOOLKIT

Verification and Validation 101

To ensure correct diagnosis and treatment, clinical laboratory testing must be accurate and reliable. A key component of the quality assurance process is the verification or validation of new instruments and tests to confirm their ability to perform prior to implementation.

The Verification and Validation Toolkit walks users through this process and provides additional resources, templates and examples for use in the laboratory.

Find the complete toolkit at aphl.org/VV-Toolkit

The toolkit has eight sections:

1. **Verification and Validation 101**
2. Verification and Validation Process Checklist
3. Obtaining Appropriate Test Samples
4. Qualitative Assays
5. Quantitative Assays
6. Related Processes
7. Safety Considerations and Risk Assessments
8. Cost Analysis and Budget

Laboratory testing compliance requirements are defined by the Clinical Laboratory Improvement Amendments (CLIA) regulations in [Title 42 of Code of Federal Regulations Section 493, 42 CFR 493](#).¹ The (CLIA) regulatory requirements related to establishment and verification of performance specifications of clinical test systems prior to reporting patient test results are found in [Section 493.1253](#).²

CLIA defines a ‘test system’ as instructions and all the instrumentation, equipment, reagents, and supplies needed to perform an assay or examination and generate test results. As explained by CLIA in their [Survey Procedures and Interpretive Guidelines](#),³ a clinical test system verification or validation may be required for:

- Any test system first used in a laboratory to measure a new analyte
- A test currently performed by a laboratory but on a new test system
- An analyte added to a system currently used by the laboratory to perform other testing,
- A modification to a test system already being used in the laboratory (e.g., different specimen type or specimen volume) or
- Multiple instruments used to perform the same tests.

The implementation of a new or modified test method to the laboratory test menu involves a step-by-step process to demonstrate the performance of the test and is required for a CLIA Certificate of Compliance (CoC) or Certificate of Accreditation (CoA). There are two different processes:

- **Verification:** The one-time process by which a laboratory determines that an unmodified US Food and Drug Administration (FDA) cleared or approved test performs according to the manufacturer’s specifications when used as directed.
- **Validation:** The process used to confirm with objective evidence that a laboratory-developed test (LDT) or modified FDA-cleared or approved test method or instrument system delivers reliable results for the intended application.

Laboratories that operate under a CoA are subject to the accrediting body regulatory requirements such as College of American Pathologists (CAP) which, in some cases, may be more stringent than CLIA. In these instances, the laboratory should ensure compliance by confirming these requirements with the accrediting organization.

Verification or Validation Process Determination

The choice of which process is followed depends on the new or modified method that will be implemented, which will dictate the type of performance characteristics to evaluate. **Table 1** shows whether a verification or validation is recommended based on the category of test. The FDA maintains [searchable databases](#) to determine if a medical device is approved, cleared or authorized.⁴

Table 1. Recommended Process Based on Categories of Laboratory Tests*

Method Type	Definition	Recommended Process
FDA Approved	The device has been approved through the Premarket Approval (PMA) Process , which evaluates the safety and effectiveness of Class III medical devices.	Verification
FDA Cleared	The device has been cleared as a substantially equivalent to a legally marketed device through Section 510(k) of the Food, Drug and Cosmetic Act.	Verification
FDA Authorized (EUA)	During a declared public health emergency, a device that is neither authorized nor cleared, has been evaluated by FDA through the Emergency Use Authorization (EUA) process and found acceptable for use to prevent serious or life-threatening diseases when no alternatives exist.	Verification**
FDA Modified	Any modification to an FDA Approved or Cleared test. Modifications can include the intended use, sample types, patient age, collection device, etc. These modifications require that the method is evaluated as a high complexity laboratory developed test.	Validation
Laboratory Developed Test	A test used for analyzing samples that is: <ol style="list-style-type: none"> 1. Performed by the clinical laboratory that developed the test and 2. Is neither FDA approved nor FDA cleared, or 3. Is an FDA approved or FDA cleared test that has been modified. This may include analyte specific reagents (ASR) or adoption of another laboratory’s LDT or non-cleared or approved test. These tests are considered high complexity.	Validation

* Bolded words are defined in the Glossary

** FDA authorized methods require minimal evaluation of performance, typically accuracy and precision. If an EUA expires, a complete validation is required if the method is not approved or cleared.

Required Performance Characteristics Determination

A range of performance characteristics may be required to be verified or validated (see the **Glossary (page 4)** for definitions):

- Accuracy
- Precision (reproducibility)
- Analytical sensitivity
- Analytical specificity
- Reportable range/intervals (normal values)
- Reference range
- Any other performance characteristic required for test performance

Requirements may depend on intended use, type of assay, applicable manufacturer’s studies and EUA instructions.

Table 2 outlines the performance characteristics generally required for test systems; more information about requirements is provided in the [Qualitative](#) and [Quantitative Assays](#) sections.

There are other variables in a verification or validation process such as the sample type(s), sample number(s) (see [Obtaining Appropriate Test Samples](#)) and whether the test method is a [qualitative](#) or [quantitative assay](#), which are discussed in other sections of this toolkit.

Table 2. General Summary of Performance Characteristics Required for Test Systems

Test Type	Accuracy	Precision	Analytical Sensitivity	Analytical Specificity	Reportable Range	Reference Range
FDA Approved	Required	Required			Required	Required
FDA Cleared	Required	Required			Required	Required
FDA Modified or LDT	Required	Required	Required	Required	Required	Required
FDA Authorized (EUA)***	Required	Required			Required	Required

General Steps of a Verification or Validation Plan

After determining whether a verification or validation is needed and which performance characteristics must be verified, the following are the general steps of a verification or validation plan:

1. Develop a plan or proposal
 - Reason for study
 - Safety Considerations
 - Methodology
 - Acceptance criteria
 - Data analysis and acceptance plan
2. Plan approval by laboratory director
3. Initiate Plan
4. Analyze
5. Re-evaluate and modify, if necessary
6. Complete testing and create a summary report
7. Complete additional supporting documents (SOPs, training, LIMS updates)
8. Summary report review and approval by laboratory director
9. Test Implementation

Details on these steps are available in other sections of this toolkit.

***Requirements may vary depending on EUA. Performance characteristics will be defined by the EUA Instructions for Use and the laboratory director. Learn more about implementing a test under emergency use conditions at [csi.org/standards/products/method-evaluation/documents/ep43/](https://www.cdc.gov/epi/standards/products/method-evaluation/documents/ep43/)

Glossary

Accuracy

An analytical performance measurement that assesses the ability of a method to produce correct results, as compared to a reference standard. Diagnostic sensitivity (known absence for target analyte) and diagnostic specificity (known presence of target analyte) are used.

Analytical Sensitivity (Limit of Detection)

The lowest concentration, or amount of an analyte, that can be measured and distinguished from a blank (i.e., minimum detection limits).

Analytical Specificity (Interfering Substances)

The ability of an instrument or test system to measure only the intended organism or substance. Tests the ability to discriminate between the target analyte and other related, but non-target analytes (i.e., cross-reactivity, interfering substances).

Emergency Use Authorization (EUA)

An EUA is a mechanism that enables the FDA to facilitate the availability and use of medical countermeasures during declared public health emergencies.

FDA Approved

The device has been approved through the Premarket Approval process.

FDA Authorized

The device has been reviewed by FDA through the EUA mechanism.

FDA Cleared

The device has been cleared as a substantially equivalent device through Section 510(k) of the Food, Drug and Cosmetic Act.

FDA Modified

Any modification to an FDA approved or cleared test. The modification should be handled as a laboratory developed test.

High Complexity

The most complex testing category assigned to a test by the FDA, based on seven scored criteria. CLIA requirements for laboratories will vary based on the assigned complexity of a test with more stringent requirements for high complexity testing.

Laboratory Developed Test

Test developed wholly, or in part, by the performing laboratory. This may include analyte specific reagents (ASR) or adoption of another laboratory's LDT or non-cleared or approved test.

Precision

An analytical performance measurement that assesses the closeness of agreement between independent results of measurements obtained under stipulated conditions. Assesses the inherent random error of a test system to determine how close two or more repeated measurements are to each other, regardless of accuracy.

Premarket Approval (PMA) Process

This is the FDA process of scientific and regulatory review to evaluate the safety and effectiveness of Class III Medical Devices.

Reference Range

The typical result (qualitative) or range of values (quantitative) expected in a non-diseased population that do not have the condition for which the test is performed, including variation due to type of specimen and demographic variables such as age and sex, as applicable.

Reportable Range

The span of test result values over which the laboratory can establish or verify the accuracy of the instrument or test system measurement response. For a qualitative test, the reportable range could be the limit of detection, the cutoff value, or the 95% confidence interval.

Validation

The process used to confirm with objective evidence that a laboratory-developed test (LDT) or modified FDA-cleared or approved test method or instrument system delivers reliable results for the intended application.

Verification

The one-time process by which a laboratory determines that an unmodified FDA-cleared or approved test performs according to the manufacturer's specifications when used as directed.

References

- 1 Code of Federal Regulations. 42 CFR 493 Laboratory Requirements. National Archives and Records Administration. Accessed Oct 1, 2023: www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493
- 2 Code of Federal Regulations. 42 CFR 493.1253 Standard: Establishment and verification of performance specifications. National Archives and Records Administration; 2003. Accessed Oct 1, 2023: www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493/subpart-K/subject-group-ECFRc96dae380f6ed/section-493.1253
- 3 CMS CLIA. State Operations Manual Appendix C - Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services, Rev. 166, 02-03-17. Accessed Jan 15, 2024: www.cms.gov/regulations-and-guidance/guidance/manuals/downloads/som107ap_c_labpdf.pdf
- 4 FDA. Are There “FDA Registered” or “FDA Certified” Medical Devices? How Do I Know What Is FDA Approved? Accessed Oct 8, 2023: www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm



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