



**Method Validation of Molecular
Diagnostic Tests at the North
Carolina State Laboratory of
Public Health**

CLIA

Clinical Laboratory Improvement Act

- Congress passed the Clinical Laboratory Improvement Amendments (CLIA) in 1988 establishing quality standards for all laboratory testing to ensure the accuracy, reliability and timeliness of patient test results regardless of where the test was performed.

- CLIA rules and regulations apply to all facilities (laboratory or clinical laboratory) that perform:

“biological, microbiological, serological, chemical, immuno-hematological, hematological biophysical, cytological, pathological, or other examination of materials derived from the human body for the purpose of providing information for the diagnosis, prevention or treatment of any disease or impairment of, or the assessment of the health of, human beings”

- CLIA rules are applicable to all clinical laboratory testing

Cumitech 31

“Verification and Validation of Procedures in the Clinical Microbiology Laboratory” 1997

“The key desirable attribute of a laboratory test is its ability to produce accurate and precise results consistently over extended periods of time with an appropriately rapid turnaround time so that the test results are of clinical utility.”

Laboratories must have in place:

- detailed methods for evaluating and analyzing the accuracy and precision of new tests prior to these tests being offered or replacement of older tests (verification)
- methods for demonstrating that the laboratory can obtain performance specifications comparable to those reported by the manufacturer (verification)
- process for continued review of established tests to assure consistency results and personnel remain competent to perform tests (validation)

CLIA regulations (42 CFR Section 493.1253) indicate for an unmodified FDA cleared or approved systems, the following performance characteristics of a new molecular test method must be performed:

- **accuracy**
- **precision**
- **reportable range**
- **reference range (normal values)**

CLIA regulations (42 CFR Section 493.1253) indicate for modified FDA or non-FDA cleared or approved systems, the following performance characteristics of a new molecular test method must be performed:

- **accuracy**
- **precision**
- **reportable range**
- **reference range (normal values)**
- **analytic sensitivity**
- **analytic specificity**
- **limit of detection**

Definitions

Accuracy – The degree to which a test result corresponds to the “true” concentration of the analyte in a sample. Validation of accuracy will establish that the molecular method is producing equivalent results with the current method or reference method (usually culture) being used. Accuracy can be assessed by comparative method analysis. This study is performed to estimate systemic bias in the methods.

- Comparing PCR results to culture results

Precision – The ability of a test to produce the same result upon repeat testing of the sample. The day to day or run to run variability is measured when the same sample is analyzed repeatedly over a short period of time. Precision is checked by using two different levels of controls (normal and abnormal).

- Reproducibility

More Definitions

Sensitivity – The ability of a test to correctly identify individuals who have a given disease or disorder. The more sensitive the test, the fewer false negative results are produced. A false negative result fails to expose disease states that may be present. Also serves as **limit of detection**.

- Dilutions of bacteria, viruses, or nucleic acid

Specificity – The ability of a test to detect the target of interest. The more specific a test, the fewer false positive results it produces.

- Competitor organisms
- Challenge inoculums
- Effect of interfering substances

More Definitions

Reportable range – The span of test result values which the laboratory can use to establish or verify the accuracy of the test system measurement. The manufacturer establishes a reportable range for the test; the CLIA regulations require each laboratory to demonstrate that it can duplicate the manufacturer's reportable range prior to testing.

Reference range – Also called the “normal range”. The laboratory can use the manufacturer's reference range.

More Definitions

Modified test – any change to the assay or the intended use of the assay that could effect performance specifications.

- change in specimen
- change in manufacturer's cut-off
- change in instrumentation
- change in calibrators and reference material
- change in intended- "Off-label" use
 - change of sample matrix
 - using the test for another purpose (screening vs. diagnostic)

Non-FDA cleared or approved systems – any laboratory developed assay.
Also referred to as home brewed assay.

- Norovirus
- Influenza

Designing a Validation Study for Molecular Testing

Prepare and submit a “New Test/Method Checklist”

Establish number and type of specimens to be tested (agent dependent)

- stock isolates/cultures, clinical samples, nucleic acid
- minimum of 20 patient samples, preferably 40 depending on testing volume, are analyzed by the new molecular method and an established method available to the laboratory.

Establish acceptance criteria

- for molecular assays CLIA does not specify
- rule of thumb: 95% + agreement (90-95% acceptable)
depends on the analyte and type of test (screening vs confirmatory)
- calculate accuracy (sensitivity/specificity), precision, reportable range

Designing a Validation Study for Molecular Testing

Establish how to resolve discrepancies

- refer to a reference laboratory
- characterize product

Molecular methods verification/validation proposal –reviewed and approved

QA manager

Molecular QA Public Health Scientist

Laboratory Director

COMPLETED VALIDATION STUDY

Review of validation of new molecular method prior to implementation

QA manager

Molecular QA Public Health Scientist

Laboratory Director

Public Health Laboratory Exceptions

Bioterrorism threat or novel pathogens

LRN assays

SARS

avian influenza

Public health surveillance testing (serotyping or genogrouping)

Result reports should have disclaimers

“This test was developed and its performance characteristics determined by the North Carolina State Laboratory of Public Health. It has not been cleared or approved by the U.S. Food and Drug Administration and should not be used for diagnostic purposes”

Considerations for Assays of Public Health Significance

If rapid deployment of assays are necessary for the benefit of the public's health and/or reference or control materials are not available, public health labs:

May receive temporary exemption from CLIA requirements for establishing performance characteristics

Should use competent and trained personnel to perform testing

Must perform testing exactly as written in method

Examples: Influenza A/H5, mumps outbreak, agents of terrorism, SARS, WNV

References and additional resources

NCSLPH Quality Assessment S:\Quality Assessment

Code of Federal Regulations, Title 42, Part 493 revised 2004

Clinical and Laboratory Standards Institute TM. *Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline-Second Edition*. CLSI document MM3-A2. Clinical and Laboratory Standards Institute TM, 6940 West Valley Road, Suite 1400, Wayne, Pennsylvania, 2006.

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