DCLS

Division of

Consolidated Laboratory Services

DGS

DEPARTMENT OF GENERAL SERVICES

Validation/Verification of Analytical Methods

Definitions

- **Validation:** The process of establishing the performance characteristics and limitations of a test system and the identification of the influences which may change these characteristics and to what extent (In house developed, Non-FDA methods)
- **Verification:** The evaluation of a test system to assure it complies with regulations, requirements or specifications (FDA approved methods)
- **Re-Verification:** Re-evaluation of a test system
- **Method/Instrument Emergency Use Approval:** Process to allow for the testing of emergency samples prior to method, matrix, instrument being fully validated or verified. Must be approved by the Laboratory Director

Validation/Verification Levels

- **Level I (Re-verification):**
  - Intermittent or sporadically used method
  - instrument is moved to another location
- **Level II (Verification CLIA or Validation TNI/ISO):**
  - New instrument
  - Component change on an instrument
  - Regulatory approved method (no modifications allowed for CLIA), TNI/ISO Reference Method with or without modifications
- **Level III (Validation –CLIA & TNI/ISO):**
  - Laboratory Developed Methods
  - Non-Standard or Non-Reference Methods
  - Non-FDA approved or modified FDA methods (Clinical)

Proposal

- **Proposal:** Used to outline what will be done in the validation and is approved prior to start of the validation and should include:
  - ❖ Validation/Verification Level
  - ❖ Summary describing purpose of the validation
  - ❖ Plan for validation to include the following:
    - ✓ samples and reference materials that will be used
    - ✓ number of replicates
    - ✓ analyses will be done on different days
    - ✓ number of analysts
    - ✓ Method requirements (how you know it will meet the need)
  - ❖ Safety
  - ❖ Resources required to include personnel, time, cost
  - ❖ Waste management
  - ❖ Timeline explaining estimated time to acquire supplies/instrumentation, and completing analysis

Level I Requirements

- Intermittent SOP or Replacement Verification
  - ❖ Review the current method to evaluate updates and applicability
  - ❖ Retrieve previous performed validation studies for review
  - ❖ Perform an analytical run to include quality controls, past proficiency tests, previously tested samples, and MDLs (if applicable)
  - ❖ SOP updated
  - ❖ Competency assessment of staff
  - ❖ Proficiency testing or alternative assessment must be performed within 30 days prior to restarting testing

Level I Requirements

- Relocation of Instrumentation
  - ❖ CLIA
    - ✓ accuracy
    - ✓ precision
    - ✓ reportable range (if applicable)
  - ❖ TNI/ISO
    - ✓ run QC
    - ✓ calibration standards

Level II – TNI Chemistry

- Validation Data - Minimum of 3 days with 20 data points required
  - ❖ Analyze individual samples or individual samples their duplicates
    - ✓ Samples may be reference material, previously tested de-identified client samples
  - ❖ Samples should have been previously analyzed by the current validated method or instrument before the change or modification
  - ❖ Must run blank samples and quality controls (ICV,CCV, MS/MSD, duplicates)
  - ❖ Analyze Initial MDL samples if applicable
  - ❖ Analyze Initial LOQ
  - ❖ Perform Initial DOC
  - ❖ Evaluation of Precision and Bias
  - ❖ Evaluate Selectivity

Level II – TNI Chemistry

- Final Data
  - ❖ Calculate relative percent difference:
    - ✓ Between the results of the currently validated method or instrument and the new proposed method or instrument
    - ✓ duplicates, matrix spike/matrix spike duplicates
  - ❖ Calculate percent recovery for matrix spike/matrix spike duplicate, known control sample, ICV, CCV
  - ❖ Calculate MDL/LOD and LOQ
  - ❖ Calculate the estimation of uncertainty
  - ❖ Calculate the XY plot, linear regression, correlation coefficient, and paired t-test to determine statistical difference (if applicable)
  - ❖ For some environmental methods, LDR (linear dynamic range) and LCR (linear calibration range) must also be used for method validation.

Level II – TNI Chemistry

- Additional information
  - ❖ State if there are any spectral, physical, chemical or memory interferences by analyzing a sample containing various suspected interferences in the presence of the target analyte
  - ❖ State the frequency for calibration and control performance
    - ✓ Include the type, number, and concentration of calibration and quality control samples
    - ✓ The frequency for calibration and control performance must not be less than the frequency specified in the manufacturer's instructions or less than the requirements stated in the method

Level II – TNI Microbiology

- Validation Data - Minimum of 3 days with 20 data points required
- Must contain positive and negative control samples
- Accuracy
  - ❖ Use at least one known positive reference culture as part of 10 samples uses
- Precision
  - ❖ Perform 10 replicate analyses
  - ❖ Target microorganism must be used
- Selectivity
  - ❖ 10 samples with mixed cultures to include target organism
- Initial DOC

Level II – TNI Microbiology

- To meet ruggedness or robustness, the analyses may be performed with different analysts, reagent lots, at different temperature or other variable conditions. Known interferences may be introduced during validation
  - ❖ Analyze individual samples or individual samples their duplicates
    - ✓ Samples may be reference material, previously tested de-identified client samples
  - ❖ Samples should have been previously analyzed by the current validated method or instrument before the change or modification
  - ❖ Analyze negative and positive control samples
  - ❖ Analyze quality controls that will be used (ex: duplicates)
    - ✓ Matrix spikes/matrix duplicates

Level II – TNI Microbiology

- Final Data
  - ❖ State if there are any spectral, physical, chemical or memory interferences by analyzing a sample containing various suspected interferences in the presence of the measure
  - ❖ For qualitative methods, the laboratory must verify that a method will identify the presence/absence of the analyte
  - ❖ Calculate relative percent difference
    - ✓ Between the results of the currently validated method or instrument and the new proposed method or instrument
    - ✓ If applicable, calculate relative percent difference for duplicates, matrix spike/matrix spike duplicates
  - ❖ Calculate percent recovery for positive controls, duplicates and other controls, if applicable

Level II-ISO/IEC 17025 Food Chemistry

- Chemical analytical methods fulfill many purposes in testing. Most methods will fall into the following six (6) categories:

Category	Name
1	Confirmation of identification- confirms detection of target analyte
2	Quantification of analyte at a low concentration
3	Determination if analyte is present below or above a low concentration (Limit Test)
4	Quantification of analyte at a high concentration
5	Determination if analyte is present below or above a high concentration (Limit Test)
6	Qualitative Test

Level II-ISO/IEC 17025 Food Chemistry

Table 2. Category 1: Confirmation of Identity—A method that ensures a material is what it purports to be or confirms the detection of the target analyte

Performance Characteristic	Verification	Verification Activities	Reason for Verification
Specificity	No—if the lab's samples are identical to those in the standard method and if any differences in instrumentation do not impact specificity.	NA	If the samples have the same matrix, the specificity which is based on basic principles, will not be impacted. Basic principles are chemical reactions, e.g. reaction of Ag with Cl to create a precipitate.
	Yes—if the lab's samples differ from those in the standard method.	Same as those required for validation.	
	Yes–if differences between instruments could affect specificity.	The activity need only deal with the unique aspect's of the lab's samples or instrumentation.	Specificity can be impacted by differences in instrumentation.

Level II-ISO/IEC 17025 Food Chemistry

Table 3. Category 2: Analyte at Low Concentration, Quantitative

Performance Characteristic	Verification	Verification Activity	Reason for Verification
Accuracy	Yes	If the concentration range for which the method is validated is narrow (<1 order of magnitude), analyze one reference material/standard/spike at one concentration. Otherwise, demonstrate accuracy at each concentration level (low, middle and high) by analyzing one reference material/standard/spike at each level.	Over a narrow concentration range, the accuracy and precision should not vary, therefore, the demonstration at one concentration is sufficient. Over a wide concentration range, the accuracy and precision can vary, thus they need to be verified at the different concentration levels.
Precision	Yes	Perform the repeatability test once. If the method covers a concentration range >1 order of magnitude, then the repeatability test must include low, middle and high concentrations.	Over a narrow concentration range, the accuracy and precision should not vary, therefore, the demonstration at one concentration is sufficient. Over a wide concentration range, the accuracy and precision can vary, thus they need to be verified at the different concentration levels. Intermediate precision, between analysts, is handled by making sure the analysts are trained and can adequately perform the method.
Specificity	No/Yes	*See* Specificity in General Requirements	*See* Specificity in General Requirements
LOD	Yes	Run a sample close to LOD	LOD is very likely to be matrix and instrument specific
LOQ	Yes	Run a sample close to LOQ	LOQ is very likely to be matrix and instrument specific

Level II-ISO/IEC 17025 Food Chemistry

Table 4. Category 3: Analyte is present above or below a specified, low concentration (Limit Test)

Performance Characteristic	Verification	Verification Activity	Reason for Verification
LOD	Yes	Run a sample close to LOD	LOD is very likely to be matrix and instrument specific
LOQ	Yes	Run a sample close to LOQ	LOQ is very likely to be matrix and instrument specific
Specificity	No/Yes	*See* Specificity in General Requirements	*See* Specificity in General Requirements

Level II-ISO/IEC 17025 Food Chemistry

Table 5. Category 4: Quantifying an analyte at high concentration and Category 5: Analyte above or below a specified, high concentration (often called a Limit Test)

Performance Characteristic	Verification	Verification Activity	Reason for Verification
Accuracy	Yes	If the method is a limit test or if the concentration range for which the method is validated is narrow (<1 order of magnitude), analyze one reference material/standard/spike at one concentration. Otherwise, demonstrate accuracy at each concentration level, low middle and high by analyzing one reference material/standard/spike at each level.	Over a narrow concentration range, the accuracy and precision should not vary, therefore, the demonstration at one concentration is sufficient. Over a wide concentration range, the accuracy and precision can vary, thus they need to be verified at the different concentration levels.
Precision	Yes	Perform the repeatability test once. If the method covers a concentration range >1 order of magnitude, then the repeatability test must include low, middle and high concentrations.	Over a narrow concentration range, the accuracy and precision should not vary, therefore, the demonstration at one concentration is sufficient. Over a wide concentration range, the accuracy and precision can , thus they need to be verified at the different concentration levels. Intermediate precision, between analyst, is handled by making sure the analysts are trained and can adequately perform the method.
Specificity	No/Yes	*See* Specificity in General Requirements	*See* Specificity in General Requirements

Level II-ISO/IEC 17025 Food Chemistry

Table 6. Category 6: Qualitative Tests

Qualitative tests are used to identify a specific element or compound (analyte) based on the response of a material to the test. The most important characteristic of a qualitative test is its ability to reliably identify the analyte in the presence of other substances. This is referred to as the "specificity."

Method validation includes determining any cross reactivity with other known entities. The lack of cross reactivity demonstrates the specificity of the method. If samples are identical to those for which the method is intended, no verification of specificity is required. If any matrix components are unique to the lab's samples, the lab will need to demonstrate there is no impact on specificity.

The method precision of qualitative tests is generally expressed as false-positive/false-negative rates and is determined at several concentration levels. Verification of a lab's ability to properly operate a qualitative method can be demonstrated by analyzing populations of negative and positive fortified samples. For example, for each different sample matrix, duplicate samples are analyzed at three levels. Suggested levels are blanks (no analyte), low level (near the lower range of the method) and high level (near the high end of the range). Standard additions can be used to obtain the correct concentration levels. Rates comparable to those stated in the validated method demonstrate the labs ability to operate the method.

Level II-ISO/IEC 17025 Food Chemistry

- Final Data
  - ❖ Current operating conditions
  - ❖ Calculate the relative percent difference between the results of the currently validated method or instrument and the new proposed method or instrument
  - ❖ If applicable, Precision and Bias results
  - ❖ If applicable, calculate the XY plot, linear regression, correlation coefficient, and paired t-test to determine statistical difference
  - ❖ If applicable, calculate percent recovery for matrix spike/matrix spike duplicate, known control sample, ICV, CCV
  - ❖ Calculate the LOQ

Level II-ISO/IEC 17025 Food Microbiology

- Verification data for each method, matrix, and instrument
  - ❖ Accuracy
    - ✓ When possible include a high spike, low spike, and no spike
    - ✓ Low spike close to Detection Limit (DL)
    - ✓ High spike 1 log higher than low spike
    - ✓ No spike is an uninoculated sample
  - ❖Precision
    - ✓ Repeatability
      - ➢ Qualitative - analyze at a minimum one (1) target organism on different days (minimum 2) by same scientist
      - ➢ Quantitative – Approximately 20 samples are tested in duplicate by a first analyst, with each plate being counted twice
    - ✓ Reproducibility
      - ➢ Qualitative - analyze at a minimum one (1) target organism on different days (minimum 2) by at least two (2) scientists
      - ➢ Quantitative - A second analyst then also counts each plate with approximately 20 samples in duplicate on a different day

Level II-ISO/IEC 17025 Food Microbiology

- LOD for purpose if applicable (Qualitative and Quantitative)
- LOQ if applicable (Quantitative only)
- Measurement of Uncertainty if applicable
- Initial Demonstration of Capability

Level II-ISO/IEC 17025 Food Microbiology

- Matrix Extension (Qualitative)
  - The verification of method performance with a new matrix is intended to assure that the new matrix will produce neither high false positive or high false negative results as defined for the level of validation and the intended use of the method
  - Analyze the matrix in question concurrently with a matrix spike
  - Negative spike results invalidate the analysis and the sample must be analyzed using the conventional culture procedure
  - The laboratory may continue to perform the individual sample matrix spikes for products that have not been fully validated for the method. When a specific food has yielded at least seven (7) positive and no negative matrix spikes, or a >95% confidence level, the food product will be considered validated

Level II - Clinical

- Validation Data - Minimum of 3 days with 20 data points required
  - ❖ Analyze individual samples
    - ✓ Samples may be calibrators/calibration material, previously tested de-identified client samples, proficiency tests, and quality control with known values
  - ❖ Samples should have been previously analyzed by the current validated method or instrument before the change or modification
  - ❖ Analyze negative and positive control samples
  - ❖ Initial comparison between multiple instruments
- Analysis must be performed on 3 separate days by at least 2 different analysts

Level II - Clinical

- Final Data
  - ❖ Accuracy
    - ✓ The laboratory needs to compare the accuracy of the test results it obtains when using a test system with the manufacturer's accuracy claims to the sample results from previous validated method or instrument
  - ❖ Precision
    - ✓ Repeatability- Same sample run numerous times on same day by same analyst to see if comparable results are obtained
    - ✓ Reproducibility - Samples run on different days by different analysts to see if comparable results are obtained
  - ❖ Reportable Range
    - ✓ Choose samples at the highest and lowest levels stated by manufacturer
    - ✓ May only report test results that fall within the verified levels
    - ✓ Laboratory Director will decide how to report results outside these levels
  - ❖ Reference Ranges (Normal Values)
    - ✓ Do the reference ranges provided by the test manufacturer fit your patient population

Level III- General

- Validation Data – 5 days minimum of 50 data points
  - ❖ Analyze samples to obtain a minimum of 3 replicates of samples which consists of an individual sample and its duplicate
    - ✓ Samples may be reference materials, proficiency samples or previously tested de-identified client samples
    - ✓ When applicable, these samples must have been analyzed by the previously validated method or instrument before the change or modification
    - ✓ If applicable, the analytical run must include a blank sample
    - ✓ Analyze quality controls that will be used
  - ❖ The range and accuracy (e.g. specificity, sensitivity, the uncertainty of the results, detection limit, selectivity of the method, linearity, limit of repeatability and/or reproducibility, robustness against external influences and/or cross-sensitivity against interference from the matrix of the sample/test object), are assessed for the intended use and whether the method is relevant to the customers' needs

Level III- TNI Chemistry, Micro

- All performance measures in Level II verification will need to be performed as well as the additional measures listed below
- Validation Data
  - ❖ Range and accuracy
  - ❖ Evaluation of Precision and Bias
  - ❖ Estimation of Analytical Uncertainty
- Calculations:
  - ❖ For each analyte, calculate the mean recovery for each day, each level over days, and all samples
  - ❖ Calculate the relative standard deviation for each of the separate means obtained
  - ❖ Compare the standard deviations for the different days and the standard deviations for the different concentrations using analysis of variance (e.g. F-test)
  - ❖ If the different standard deviations are all statistically insignificant, then compare the overall mean and standard deviation with the established criteria from above

Level III- TNI Chemistry, Micro

- Final Data should also include:
  - ❖ Calibration using reference standards or reference materials
  - ❖ State if there are any spectral, physical, chemical or memory interferences
  - ❖ Calculate the relative percent difference between the results of the currently validated method or instrument and the new proposed method or instrument
  - ❖ Reference range
  - ❖ Upper and lower limits of the test system
  - ❖ Specificity
  - ❖ If applicable, calculate percent recovery for duplicate and control sample

Level III- ISO/IEC 17025 Food Chemistry and Microbiology

- All performance measures in Level II verification will need to be performed as well as the additional measures listed below.
- Validation Data
  - ❖ Categories of Chemical Test Methods: Performance Characteristics Included in a Validation Study

	Performance Characteristics Included in a Validation					
Performance Characteristic	Identification 1	Analyte at Low Concentration Quantitative 2	Analyte at Low Concentration Limit Test 3	Analyte at High Concentration Quantitative 4	Analyte at High Concentration Limit Test 5	Qualitative 6
Accuracy	No	Yes	No	Yes	Yes	No
Precision	No	Yes	No	Yes	Yes	No
Specificity	Yes	Yes	Yes	Yes	Yes	Yes
LOD	No	Yes	Yes	Yes/No	No	No
LOQ	No	Yes	No	Yes/No	No	No
Ruggedness	No	Yes	No	Yes	No	No
Linearity/Range	No	Yes	No	Yes	No	No

Level III- ISO/IEC 17025 Food Chemistry and Microbiology

- Categories of Microbiological Test Methods: Performance Characteristics Included in a Validation Study

Performance Characteristic	Identification	Quantitative	Qualitative (P/A)
Relative Accuracy	Yes	Yes	No
Matrix Effects	No	Yes	Yes
Precision	No	Yes	No
Selectivity	No	Yes	Yes
Specificity	Yes	Yes	Yes
Inclusivity	Yes	Yes	Yes
Exclusivity	Yes	Yes	Yes
False-Positive Rate	No	Yes	Yes
False-Negative Rate	No	Yes	Yes
LOD	No	Yes	Yes
LOQ	No	Yes	No
Ruggedness	Yes	Yes	Yes
Linearity/Range	No	Yes	No

Level III- ISO/IEC 17025 Food Chemistry and Microbiology

- Final data should also include:
  - State if there are any spectral, physical, chemical or memory interferences
  - Successful PT results
  - Calculate the relative percent difference between the results of the currently validated method or instrument and the new proposed method or instrument
  - Reference range
  - Upper and lower limits of the test system
  - Specificity
  - If applicable, calculate percent recovery for duplicate and known control sample

Level III - Clinical

- All performance measures in Level II verification will need to be performed as well as the additional measures listed below.
- Validation Data
  - Analytical Sensitivity
  - Analytical Specificity
  - Interfering Substance
  - Determination of calibration and control procedures
  - Diagnostic Sensitivity (Qualitative)
  - Diagnostic Specificity (Qualitative)
- Final Data
  - State if there are any spectral, physical, chemical or memory interferences
  - Successful PT results
  - Calculate the relative percent difference between the results of the currently validated method or instrument and the new proposed method or instrument
  - Discuss specificity of test
  - If applicable, calculate percent recovery for duplicate and known control sample

Requirements listed by Accrediting Bodies

Data Requirements	CLIA required (FDA approved methods) VERIFICATION (yes or no)	CLIA required (non-FDA approved methods or modified FDA methods) VALIDATION (yes or no)	TNI required (yes or no)	ISO required (yes or no)
Accuracy	Yes	Yes	Yes	Yes
Calibration and Controls	Yes	Yes	Yes	Yes
Estimation of Uncertainty	No	No	No	Yes
Interferences	No	Yes	No	No
Limit of Detection (LOD)	Yes	Yes	Yes	Yes/No*
Limit of Quantitation (LOQ)	No	No	Yes	Yes
Precision/Bias/Trueness	Yes	Yes (3 replicates of samples each day for 5 days)	Yes	Yes
Repeatability	Yes (included in intra-run verification)	Yes (3 replicates of samples each day for 5 days)	Yes	Yes
Reproducibility	Yes (included in intra-run verification)	Yes	Yes	Yes
Reportable Range	Yes	Yes	Yes	Yes
Reference Range	Yes	Yes	Yes	Yes
Ruggedness or robustness	No	No	No	Yes
Selectivity	No	Yes	Yes	Yes
Sensitivity	Yes	Yes	Yes	Yes
Specificity	Yes	Yes	No	No
Method Comparison	Yes	Yes	No	No

*No annual MDL is required if there is no reporting below the lowest standard of the calibration curve.

Validation Data Evaluation

- Validation packet includes:
  - ❖ summary indicating if validation meets the need
  - ❖ Signature Page
  - ❖ Signed proposal
  - ❖ Validation Summary of findings
  - ❖ Installation, Operational, Performance Qualifications
  - ❖ Raw Data
  - ❖ SOP
  - ❖ Training /Competency of staff who participated in validation

Other Types of Verifications

- Instrument Comparisons – For CLIA must be performed every 6 months

- Spreadsheet calculation cell verification

References

- US Food and Drug Administration, Guidelines for the Verification/Validation of Chemical Methods for the FDA Foods Program, 2nd ed., April 2015
- Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, 2nd ed., FDA, April 2015
- How to Meet ISO 17025 Requirements for Method Verification, AOAC
- Centers for Medicare and Medicaid Services, CLIA, Appendix C, February 3, 2017
- ISO/IEC 17025:2017
- The NELAC Institute, 2016 TNI Standards, September 2016
- The Fitness for Purpose of Analytical Methods A Laboratory Guide to Method Validation and Related Topics, 2nd ed. 2014, Eurochem
- Food and Drug Administration, ORA-LAB.5.4.5 Methods, Method Verification and Validation

Questions

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