Wake Forest Baptist Medical Center	CCL- 030 New Method Validation/Verification Non Waived	Dept: 324318	Critical Care Labs
		Effective Date:	12/12/2018
		Revised Date:	
		Contact:	Ann Shoffner
Name & Title: Gregory Pomper, MD CLIA Laboratory Director		Date:	2/25/19
Signature: GP			

1) **General Procedure Statement:**

a. Purpose: This document provides instructions for a process of validating and verifying new methods in the laboratory. It is meant to aid the laboratory to meet applicable CAP/CLIA regulatory requirements. According to the Standard CAP/CLIA: CFR 42 § 493.1253: Establishment and verification of performance specifications: states that each laboratory that introduces an unmodified, FDA-cleared or approved test system must demonstrate that it can obtain performance specifications comparable to those established by the manufacturer for the following performance characteristics before reporting patient test results: Accuracy, Precision, Reportable Range of the test results and verification that the manufacturer's reference intervals (normal values) are appropriate for the laboratory's patient population.

b. Principle: Following selection of a method, the assessment of its suitability begins with the understanding of the sources of potential analytical error. With the properly planned experiments/studies the laboratory can measure the error produced in a method and determine if it is acceptable for use in the laboratory. The Validation/Verification study will document this process.

Total error is the sum of random and systemic error and is used to make the final judgment on the acceptability of a new or modified method in the laboratory. The laboratory will assess random and systemic error and document its findings.

c. Scope: All laboratory tests must be validated or verified before being placed into routine use for testing and reporting of patient results. Method validations are required for all new tests as well as any modification of existing procedures. Equipment validation/verifications are required for all new instruments and instruments that have been moved. All validation/verifications must be approved, signed and dated by the Laboratory Section Director and CLIA laboratory medical director (LMD) prior to use.

a. **d. Responsible Department Party/Parties:**

i. Procedure owner: Greg Pomper, MD and Ann Shoffner
ii. Procedure: Critical Care Lab (CCL) Staff
iii. Supervision: Ann Shoffner
iv. Implementation: Ann Shoffner and Critical Care Lab (CCL) Staff

2) **Definitions:**

A. Accuracy - How close is the measured value to the "true" value. The difference can be described as the Systemic error (inaccuracy, bias) in the method.

B. Analytic Measurement Range (AMR) - The range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process.

C. CAP – College of American Pathologists. Deemed to be an accreditation body by CLIA and currently directs the Laboratory Accreditation Program (LAP), established in 1961.

D. CLIA- Clinical Laboratory Improvement Amendments of 1988. Responsible under the Centers for Medicare & Medicaid Services (CMS), an agency within the US Department of Health and Human Services for the regulation of clinical laboratories in the United States.

E. Correlation Coefficient - A number between -1 and 1 which measures the degree to which two variables are linearly related. A perfect linear relationship will have a correlation coefficient of 1

F. FDA – US Food and Drug Administration

Definitions continued:

G. Precision – reproducibility. The ability of the laboratory to duplicate results time after time on different days and with different operators. Measures Random error the precision or imprecision can be expressed in CV% from the calculated standard deviation SD and mean. Repeat measurements of samples at varying concentrations, within-run and between run over a period of time should be performed.

H. Quantitative results – Test results that are reported as numbers.

I. Reportable Range – Same as Analytic Measurement Range (AMR). How high and low can test result values be and still be accurate? This can be determined by a linearity study for quantitative methods.

J. Reference Range – Normal values for your patient population.

K. Analytical Sensitivity – The smallest quantity of an analyte that can be reproducibly distinguished from background levels. Positive agreement as compared to reference method. For quantitative methods this includes determining the Limit of Detection (Can be described by the slope of the calibration curve).

L. Diagnostic Sensitivity – The percentage of subjects with the target condition whose test values are positive.

M. Analytical Specificity – the ability of a method to detect only the analyte it is designed to detect. Negative agreement as compared to reference method. Can be measured with interference and recovery experiments.

N. Diagnostic Specificity – the percentage of subjects without the target condition whose test values are negative.

O. Validation – "...the process of assessing the assay and its performance characteristics to determine the optimal conditions that will generate a reliable, reproducible, and accurate...result for the intended application." The term is often used instead of Verification. This can be source of confusion. For non-FDA approved/cleared tests: the laboratory must establish the performance specifications.

P. Verification – The one-time process performed to determine or to confirm a test's expected performance compared to actual results produced by the laboratory (CAP definition). For tests cleared or approved by FDA, verification is required.

3) **Equipment:**

A. Reagents/Media/Standards:

1. The laboratory must have sufficient in-house supplies such as reagents and media to perform the validation/verification.
2. It is ideal if the same lots of reagents/media are used throughout the entire validation/verification study.
3. Expiration dates of reagents/media should be long enough to complete the validation/verification study.
4. Ensure that the media/reagents used are appropriate for the method.
5. Ensure that a sufficient quantity of purchased materials such as standards, calibrators and controls are available prior to starting the validation/verification study.

B. Equipment: Instrument to be used for method verification/validation

1. Ensure that there is sufficient space and that the environmental requirements can be met. (Example: located out of direct sunlight, humidity, temperature, etc.)
2. Ensure that proper electrical requirements, data ports, water, waste, and other manufacturer requirements are met for the proper functioning of the instrument.

C. Method Validation/Verification Software - will be available to the laboratory for most validations/verifications. The LMD will provide assistance in its use.

4) **Procedure:**

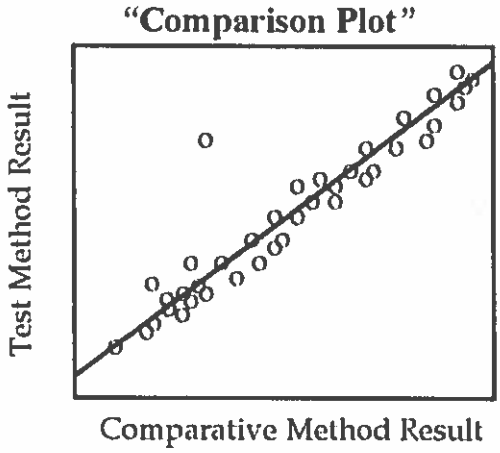
Each method validation/verification study is a collection of experiments to assess performance and error in order to judge a method's suitability for use in the laboratory. A validation/verification plan should be created and approved prior to starting the validation/verification experiments to prevent unnecessary testing and ensure that the study is complete. Acceptability Criteria – the laboratory must establish acceptance criteria as part of the validation/verification plan. The acceptance criteria for accuracy, precision, sensitivity and specificity are a

confidence level of at least 90%, or meet the claims of the manufacturer. Quantitative Methods include laboratory methods that report numbers.

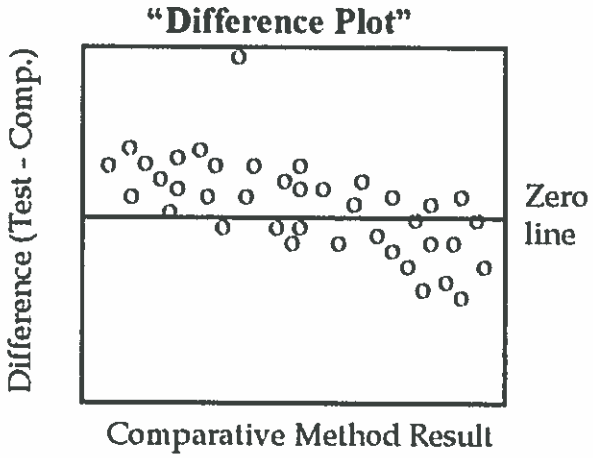
A. Accuracy (systematic error or bias): comparison of method experiment. Accuracy demonstrates how close to the "true" value the new method can achieve. A method comparison experiment is used to estimate inaccuracy or systematic error. Test material can include: calibrators/controls, reference material, outdated proficiency testing material with known values, samples tested against a reference standard, high-quality method or another lab using the same method or by comparing results to an established in-house method.

Most sources recommend comparing at least 20-40 patient specimens for a FDA-cleared or approved method. Using less than 20 samples will need to be approved by the section manager and laboratory medical director (LMD). A larger number has a better chance to detect interferences. Depending on the test system and test volume the number used can vary. The actual number is less important than the quality of the samples. The estimate of systematic error is more dependent on wide range of test results than on a large number of samples.

The method comparison experiment for accuracy is recommended to be done over a minimum of 5 days. Continue for another 5 days if discrepancies are observed. If the laboratory cannot perform the experiment for the 5 days due to lack of samples, resources or other reasons, consult with the manager or LMD.

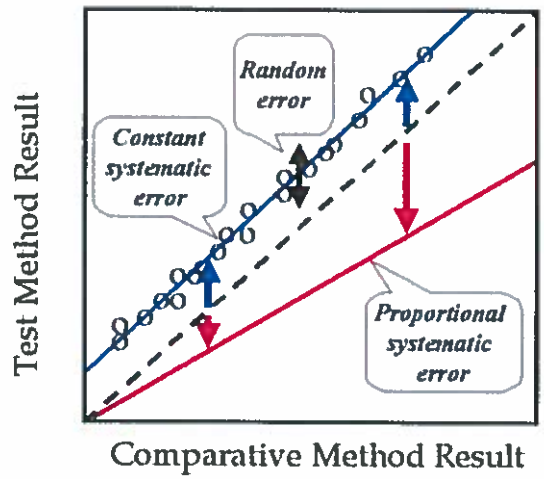


Prepare a comparison plot of all the data to assess the range, outliers, and linearity.
For methods that are not expected to show one-to-one agreement, for example enzyme analyses having different reaction conditions, the graph should be a "comparison plot" that displays the test result on the y-axis versus the gold standard comparison result on the x-axis. As points are accumulated, a visual line of best fit should be drawn to show the general relationship between the methods and help identify discrepant results.



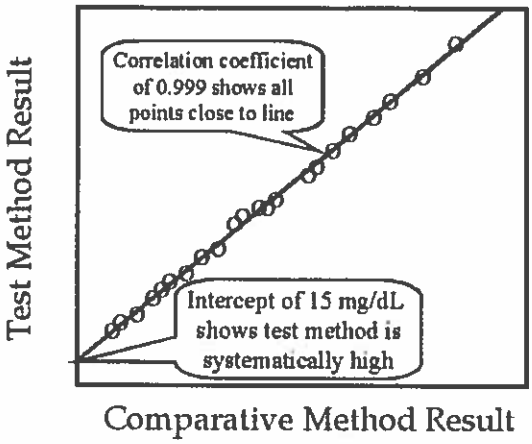
Accuracy continued:
If the two methods are expected to show one-to-one agreement, the initial graph may be a "Bland-Altman plot" or "bias plot" that displays the difference between the test method results minus the comparative results on the y-axis versus the comparative result or average of the comparative and test results on the x-axis. The differences should scatter around the line of zero differences, half being above and half being below the line. Any large differences will stand out and draw attention to those specimens whose results need to be confirmed by repeat measurements. Review the data and graphs for any outlying points that do not fall within the general pattern of the other data points. For example, in the figure above there is one suspicious point in the difference plot. In addition, there are points that tend to scatter above the line at low concentrations and below the line at high concentrations, suggesting possibility of some constant and/or proportional systematic errors.

Precision or imprecision = Random error, Accuracy/Bias = Systematic Error, can be of two types: constant systematic error or proportional systematic error. Constant and proportional systematic error can be seen on a comparison plot.



Statistics: Accuracy / Bias (= systematic error):
Run comparison of methods study (test method vs. reference method, laboratory's previous method, or manufacturer's results, etc.). The line of best fit (calculated using a statistics program) provides the linear regression equation $Y = mx + b$
Calculate correlation coefficient "r".

Analytes with a wide range (cholesterol, glucose, enzymes, etc.) tend to have a high "r" in comparison studies; analytes with a narrow range (electrolytes) tend to have low "r".



B. Precision - (random error): replication experiment, calculation of standard deviation, also known as reproducibility. Can the new method duplicate the same results? It is important to test samples that have a matrix as close as possible to the real specimens. For clinical tests, patient samples are the first choice followed by control material and reference solutions. When using quality control samples, these should be different to those used to ensure the instrument is in control at the time of the assessment. Most sources agree that a minimum of 2-3 samples near each medical decision levels run for 3-5 replicates over 5 days will provide sufficient data for within-run and between-run components to estimate precision. Having different operators perform the precision experiment is important for methods that are operator dependent.

Statistics - Precision (= random error) – Imprecision is measured, when determined within a run = repeatability; imprecision across multiple runs across multiple days = reproducibility. The latter is most reflective of actual lab practice. Random error is described quantitatively by calculating (use a statistics program) the mean (x), standard deviation (s), and coefficient of variation (CV). Compare the CV to the manufacturer's data. The laboratory should verify the manufacturer's claim for precision.

C. Reportable Range: CAP Reportable range (analytic measurement range= AMR), is the range of values that the method can directly measure without dilution or concentration. Reportable Range (AMR) can be verified by running 3 points near low end, midpoint, and high end using calibration/control/reference matrix appropriate materials. The AMR must be re-verified at least every 6 months, and following changes in major system components or lots of analytically critical reagents (unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected). Data must be within the laboratory's acceptance criteria or within the manufacturer's stated range to be acceptable.

D. Reference Range (Normal Values): Provided by the manufacturer and verified by running known normal patients. If the laboratory has a similar patient population then the manufacturer's ranges or even published reference ranges from textbooks or scientific articles may be used. The Reference Range can be verified by testing 20 known normal samples; if no more than 2 results fall outside the manufacturer/published range then that reference range can be considered to be verified. (CLSI guideline C28-A3c). Criteria for evaluation of reference intervals include: Introduction of a new analyte to the test repertoire, change of analytic methodology, or a change in patient population. If it is determined that the range is no longer appropriate for the patient population, corrective action must be taken. If the laboratory changes its analytical methodology so that test results or their interpretations may be SIGNIFICANTLY different, the change should be explained to clients. NOTE: This can be accomplished in any of several different ways, depending on local circumstances. Some methods include directed mailings, laboratory newsletters or part of the test report itself.

5) **Decision on Method Performance:**
Acceptance Criteria for most CAP/CLIA validation/verifications will meet the following:
Accuracy, precision, sensitivity and specificity parameters will match or exceed 90% as compared to the current/reference method or with reference materials with known values and/or meet or exceed the claims of the manufacturer.

Performance Characteristic	Performance Specification	Notes
Accuracy (Method Comparison)	Correlation Coefficient r +/- 0.1/bias.	Known bias can be acceptable but must be commented on. Within 10% at medical decision points
Precision	CV comparable to manufacturer	
Reportable Range: Linearity	Slope +/- 0.1 (0.9 – 1.1)	
Reference Range	Within manufacturer's or published limits	
Acceptance Criteria complete		

6) **Attachments:** none

7) **Related Procedures:**
Department of Pathology – Method Validation Process for Non Waived Tests

8) **Related CAP standards**: COM.40310, COM.40000, COM.40300, COM.40600, COM.40800, COM.40615

9) **References:**
CAP/CLIA: CFR 42 § 493.1253: Establishment and verification of performance specifications
CLSI document EP15-A2
CLSI guideline C28-A3c

10) **Review/Revision/Implementation:**
- Review Cycle: All procedures must be reviewed at least every 2 years.
- Office of Record: Department of Pathology, Critical Care Laboratory

11) **Previous Revision Date(s):**

12) **Revised/Reviewed Dates and Signatures:**

Reviewed/Revision Date:_____ Signature:_____

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