Wake Forest Baptist Medical Center	Method Validation/Re-Validation Process for Non-Waived	Department:	Pathology
		Effective Date:	Feb 2019
		Revised Date:	N/A
		Contact:	Lab Compliance, QA and Safety
CLIA Laboratory Director		Date:	2/15/19
Signature:			

TABLE OF CONTENTS

A. GENERAL PROCEDURE STATEMENT:

1) **Scope:**
42 CFR Section 493.1253 (Standard: Establishment and verification of performance specifications) dictates that prior to implementing a new or revised method, the Wake Forest Baptist Health (WFBH) Department of Pathology Section Laboratories must demonstrate the capability of the method in terms of precision, accuracy, detection limits, linearity, interferences, adequacy, sensitivity, specificity, and predictive value. Assays that are marketed by manufacturers already have established parameters prior to marketing. In these cases, the WFBH labs only have to verify precision and accuracy for marketed assays prior to implementation of these assays. Whenever the WFBH labs must verify a method, the analysts will follow this process as a template for generating the final report and data summary. The purpose and principle section is to contain the general background and overview of why the method was selected and verified for use in the lab section.

When performing validation studies and summaries for assays being performed in Molecular, Immunohistochemical (IHC), Genetic or HLA based labs, the types of specimens and numbers of specimens required may vary based on CAP/CLIA guidelines. These lab sections should refer to those specific standards for guidance as they set up their validation processes and submit summary documentation.

2) **Responsible Department/Party/Parties:**
   a) Procedure owner: Department of Pathology
   b) Procedure: Department of Pathology
   c) Supervision: Department of Pathology Section Managers, Section Medical Directors and CLIA Lab Director.
   d) Implementation: Department of Pathology CLIA Lab Director, Section Medical Director, Section Managers and Laboratory Compliance, QA, POC and Safety.

B. DEFINITION:

1) **Accuracy:** Accuracy is conformance to a value, accepted standard, or expected value. For a test result, bias is a measure of accuracy; it is the difference between the test result and the accepted reference value for an analyte. For a measurement procedure, bias is expressed as the difference between the average result obtained by a procedure under specified conditions and an accepted reference value, perhaps from an accepted comparative procedure or a certified reference material.
2) **Acceptability Criteria:** The laboratory must establish acceptance criteria as part of the validation/verification plan. Parameters for accuracy, precision, sensitivity and specificity should include a confidence level of at least 90%, or meet the claims of the manufacturer.
3) **Analytical Measurement Range (AMR):** AMR is 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. The AMR must be re-verified every six months, unless the calibration curve spans the entire AMR. Or 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 (lot comparisons).

> *"If the materials used for calibration or for calibration verification include low, midpoint and high values that are near the stated AMR [limits], and if calibration verification data are within the laboratory's acceptance criteria, the AMR has been validated; no additional procedures are required."*

4) **Analytical Sensitivity:** This is related to the detection limit of the assay for quantitative methods. The smallest quantity of an analyte that can be reproducibly distinguished from background levels.
5) **Analytical Specificity/Cross-Reactivity and Interfering Substances:** The ability of the assay to measure the analyte of interest in the presence of any other component present in the sample that may cause interference. This could be caused by cross-reactivity, inhibition or acceleration or reactivity, hemolysis, lipemia, anticoagulant, turbidity, icterus, gel barriers; patients' clinical conditions, disease states, and medications, etc.
6) **Clinical Laboratory Improvement Amendments (CLIA):** United States federal regulatory standards that apply to all laboratory testing performed on humans.
7) **College of American Pathology (CAP):** Member-based regulatory agency that advocated for the best laboratory practices in pathology and laboratory medicine.
8) **Correlation Coefficient, "r":** 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.
9) **FDA:** Us Food and Drug Administration
10) **Laboratory Developed Test (LDT):** A test used in patient management that has been developed solely or in part in the laboratory and is neither FDA-cleared nor FDA-approved. Any modification of manufacturer's instructions is considered new laboratory developed tests.

    Changes may include (but are not limited to) any change in:
    - Intended use by using a different sample matrix (e.g., plasma vs. urine)
    - Intended use by promoting the test for another purpose (e.g., screening vs. diagnostic)
    - Intended use by changing the type of analysis (e.g., qualitative results reported as quantitative)
    - Specimen handling instructions including change from duplicate to single testing
    - Incubation times or temperatures
    - Specimen or reagent dilution
    - Calibration material (e.g., using a different calibration material or changing the manufacturer's set points)
    - Antibody (e.g., introducing a different antibody, source, monoclonal vs. polyclonal)
    - Or elimination of a procedural step
    - Or addition of detector (conjugate) or substrate
    - The solid phase
    - The cutoff or method of calculating the cutoff for semi-quantitative assays
    - The endpoint or calculation of the endpoint
    - Or addition of adsorbent
    - The strain of antigen in serologic assays
    - The calibrator/reference material
11) **Limit of Detection (LoD):** Minimum amount of analyte whose presence can be qualitatively detected reliably under defined conditions.
12) **Limit of Quantitation (LoQ):** The LoQ is the lowest concentration at which analytical performance meets the laboratory's stated quality goals or quality requirements for that analyte.
13) **Linearity:** The ability of a method to produce quantitative results which are directly proportional to the concentration of the measured analyte in a sample.
14) **Precision:** The agreement among replicate measurements. Precision can refer to either repeatability (within-run precision) or within-laboratory precision. Repeatability is a value indicating the disagreement among a set of replicate measurements when all measurements

are made under identical conditions (or within a single run of a procedure). Within-laboratory precision is a value indicating the disagreement among replicate measurements over a longer time period when all known, major sources of measurement error within the laboratory are considered. Within-laboratory precision reflects the accumulation of various error sources, including repeatability. NOTE: Precision is not typically represented as a numerical value but is expressed quantitatively in terms of imprecision; the SD or CV of the results in a set of replicate measurements.

15) **Qualitative Test System:** A test system that reports observations in the form of interpretive comments. Results can also be an alpha result such as "Positive" or "Negative" or "Reactive" or "Non-reactive".
16) **Quantitative Test System:** An assay that produces measurements in continuous numerical values based on a standard curve and on a signal measured by an instrument. These measurements are reported to, and interpreted by, the authorized provider, based on numerical cutoffs (e.g., reference ranges).
17) **Quality Control (QC):** A process to ensure the test system is performing as expected.
18) **Quality Assurance (QA):** A system for ensuring a desired level of quality.
19) **Reference Interval:** The Reference Interval is defined as the central interval of values bounded by an upper and lower limit at certain designated percentiles, like the 2.5% and 97.5% to achieve a central 95% reference interval.
20) **Reference Range:** The entire range (minimum to maximum) of laboratory values of 'healthy donors without disease'. This is an all-inclusive range containing 100% of all the results, in contrast to the Reference Interval which is usually defined as a 95% central interval.
21) **Reportable Range:** Reportable range means the entire span of test result values over which the laboratory can establish or verify the accuracy of the instrument or test system measurement response. There are two components used to determine reportable range:
    i. The primary range of measurement; the Analytical Measurement Range (AMR)
    ii. Anything done to the system to expand the AMR: sample dilution, or sample concentration, each of which must be separately validated;
        - The entire span of values that can be reliably measured using these modifications is the reportable range.
22) **Re-verification:** The process by which a laboratory re-validates/re-verifies an existing LDT, modified FDA-cleared/approved test, or a FDA-cleared/approved test.
23) **Standard Deviation:** A statistic used to describe the distribution or spread in data in a population (that is shown to have the shape of a normal or Gaussian curve).
24) **Total Allowable Error (TAE):** The amount of error that meets the laboratory's stated quality goals or quality requirement for that analyte that can be tolerated without compromising the clinical usefulness of the analytical results, or the maximum amount of error allowed for successful performance in proficiency testing. The TAE for a variety of laboratory tests is defined by CLIA or other society or industry standards; reference the standard used for the validation. This error rate can be adjusted by the CLIA laboratory director.
25) **Validation:** A defined process by which a laboratory confirms that a test performs as intended or claimed.
26) **Verification:** The process by which a laboratory determines or confirms a test's expected performance compared to actual results produced by the laboratory. For FDA-cleared/approved test(s), verification is required.

C. VALIDATION PLAN

1) A formal validation plan must be submitted to and approved by the Section Medical Director and the CLIA Laboratory Director prior to starting any validation and/or re-verification.
2) The validation plan should include but is not limited to the following:
   A. Method Application, Purpose, and Scope
   B. Method Categorization (FDA Approved/Cleared, LDT)
   C. List of Personnel Involved and their Responsibilities
   D. Instrumentation Used and Laboratory(ies) Involved
   E. Define Performance Parameters and Acceptance Criteria
      i. Precision
      ii. Accuracy
      iii. Specificify
      iv. LoD
      v. LoQ
      vi. Linearity and Reference Interval
   F. Proposed Method Parameters
   G. List of Reagents, Standards, Controls, etc being used (including lot numbers, if applicable)
   H. Develop SOP(s) Draft for executing the method in routine workflow
   I. Define type and frequency of system suitability tests and/ or analytical QC checks

D. EQUIPEMNT

1) **Instrument to be Used for Method Validation/Verification:**
   A. Ensure that there is sufficient space and that the environmental requirements can be met (temperature and humidity specifications).
   B. Ensure that proper electrical requirements, data ports, water, waste, and other manufacturer requirements are met for the proper functioning of the instrument.
2) **Method Validation/Verification Software:**
   A. Software is available to the laboratory
   B. Use of EP Evaluator™ software or other linear regression (eg. MS Excel) applications.

E. VENDOR SUPPORTED VALIDATIONS

1) A vendor may participate in the laboratory's validation of a non-laboratory developed test under supervision of the technical supervisor or designee; including running samples under the direction of testing personnel.
2) Tasks or steps that involve judgment or manual methods must be performed by testing personnel. Judgment involves evaluating calibration and quality control, and judgment of adequacy of validation studies.
3) A vendor may perform validation as a part of training for testing personnel.
4) The vendor's training should use this time to guide the testing personnel through all steps necessary to perform the assay.
5) All data (originals) generated during the validation must remain on-site. The vendor may take copies of validation data if data reduction is performed off site.
6) If vendor staff must perform data entry off site, they may take copies of data provided all Patient Health Information (PHI) has first been removed by the testing personnel, supervisor, or section designee.
7) All original data must reside in the laboratory.

F. FULL VALIDAITON PROTOCOL:

1) A full validation must be performed for, but is not limited to, the following instances:
   a) Before the methodology is use for patient testing (regardless of when it was first introduced by the laboratory)
   b) With each new piece of instrumentation

c) Any methodology changes that could drastically affect patient results

2) The following validation procedures must be included in all full validations:
   a) Precision
   b) Accuracy
   c) Reportable Range
   d) Reference Interval/Range
      **Note: If a formal reference interval study is not possible or practical, then the laboratory should carefully evaluate the use of published data for its own reference intervals, and retain documentation of this evaluation.
   e) Proficiency Testing/External QA
3) The following additional validation procedures must be included for a LDT:
   a) Analytical Sensitivity
   b) Analytical Specificity
   c) Reportable Range
   d) Reference Interval/Range
   e) Other parameters considered important to assure analytical performance of the test (stability, carryover, cross-contamination, etc.)

G. RE-VERIFICATION PROTOCOL:

1) A re-verification must be performed for, but is not limited to, the following instances:
   a) When an instrument is moved
   b) Duplication of method on another instrument of same make/model as original full validation
   c) After major maintenance and/or repair
   d) Per manufacture's guidelines
2) The following validation procedures must be included in all re-verifications, including LDT:
   a) Reportable Range/Linearity
   b) Carryover
   c) Proficiency Testing/External QA

H. 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 the 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.

1) **QUALITATIVE-** includes semi-quantitative testing that use cutoff; however, no values/concentrations are included in the patient report. Test results are reported as positive/negative, normal/abnormal, reactive/nonreactive, etc.

A. <u>FDA Approved/Cleared Tests</u>

1. **Precision (Reproducibility):** Can the new method duplicate the same results? Use samples that have a matrix as close as possible to the real specimen. For clinical test, patient samples are the first choice followed by control material and reference solutions. Other acceptable sources are:
   a. Standards
   b. Previously Run Proficiency Samples

Precision should be reported as a standard deviation (SD) and a coefficient of variation (CV %), which expresses the standard deviation as a percentage of the mean value of the replicate measurements. In both cases, the mean value should be reported as well.

Analyze at least 1 run per day with at least 2 negative samples and 2 positive samples in triplicate for 5 days to provide data for within-run and between-run components.

Having different operators perform the precision experiment must be done for methods that are operator dependent. This also demonstrates reproducibility or the method.

Follow all appropriate quality control steps for each assay. Any unacceptable performance based on failed quality control should be discarded and the run(s) should be repeated. Include the daily quality control samples normally used in each run.

Calculate the percent within-run (intra), between-run (inter) and total precision by diving observed results over known results multiplied by 100. See Example 1 below:

Precision Data Table:

ID	Day 1			Day 2			Day 3			Day 4			Day 5			Between Run %
Pos Concentration 1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	100%
Pos Concentration 2	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	93%
Neg Concentration 1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	93%
Neg Concentration 2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	100%
Within Run %	100%			92%			92%			100%			100%			

2. **Accuracy:** For a measurement procedure, bias is expressed as the difference between the average result obtained by a procedure under specified conditions and an accepted reference value, perhaps from an accepted comparative procedure or a certified reference material.

   Obtain at least 20 patient specimens to be used for testing. If fewer than 20 samples are used, this must be approved by the CLIA laboratory director and the section medical director. However, depending on the test system and test volume the number of patient samples may vary.

   The method comparison experiment is should be run daily for a minimum of 5 days. If there are discrepancies observed, additional daily runs may be necessary. If the laboratory cannot run the experiment for 5 days, the CLIA laboratory director and section medical director should be notified.

Calculate the percent agreement of positive and negative results by diving observed results over known results multiplied by 100. See Example 2 below:

Method Comparison/Accuracy Table

Specimen ID	Comparison Method Result	New Method Result
Spec # 1		
Spec # 2		
Spec # 3		
Spec # 4		
Spec # 5		
Spec # 6		
Spec # 7		
Spec # 8		
Spec # 9		
Spec # 10		
Spec # 11		
Spec # 12		
Spec # 13		
Spec # 14		
Spec # 15		
Spec # 16		
Spec # 17		
Spec # 18		
Spec # 19		
Spec # 20		
Spec # 21		
Spec # 22		
Spec # 23		
Spec # 24		
Spec # 25		
Spec # 26		
Spec # 27		
Spec # 28		
Spec # 29		
Spec # 30		
Spec # 31		
Spec # 32		
Spec # 33		
Spec # 34		
Spec # 35		
Spec # 36		
Spec # 37		
Spec # 38		
Spec # 39		
Spec # 40		

New Method = 18 positives, 20 negatives
Comparison Method = 19 positives; 20 negatives

Percent Positive Accuracy = (18/19) x 100 = 94.7%
Percent Negative Accuracy = (20/20) x 100 = 100%

3. **Reportable Range (AMR):**
The CLIA laboratory director in association with the laboratory specific section medical director may establish a narrower reportable range based on clinical necessity.

   A record of established ranges should be kept for the duration of the method and for at least two years after the discontinuation of the method.

   To verify reportable range (AMR), test at least 3-5 low and high positive samples once.

   For methods depending on a cut-off value to determine positive results, testing positive specimens near the cut-off can serve as the cut-off validation.

   Data must be within the laboratory's acceptance criteria or within the manufacturer's stated range to be acceptable.

4. **Reference Range (Normal Values):** These are typically provided by the manufacturer and verified by the laboratory by running known normal patients.

   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 ban be considered to be verified.

   If the laboratory cannot reference the normal values set by the manufacturer, then the reference range will need to be established.

5. **Sensitivity and Specificity:** Documentation from manufacturers or published literature is acceptable to document sensitivity and specificity.

B. Non-FDA Approved/Cleared Test, LDTs

1. Follow the instruction above for **Precision, Accuracy, Reportable Range, and Reference Range.**

2. **Sensitivity:** Qualitative sensitivity is addressed by **Diagnostic Sensitivity.** This is the percent of subjects with the target condition whose teste values are positive. It is calculated by dividing the number of true positives by the sum of the number of true positives plus the number of false negative and multiplying by 100. See Example 3 below.

3. **Specificity:** Qualitative sensitivity is addressed by **Diagnostic Specificy**. This is the percent of subjects without the target condition whose test values are negative. Calculate by dividing the number of true negatives by the sum of the number of true negatives plus the number of false positives and multiplying by 100. See Example 3 below.

Result of Test	Positive:	Negative:
Positive:	True Positive (TP)	False Positive (FP)
Negative:	False Negative (FN)	True Negative (TN)

3) The calculations for determining sensitivity and specificity are shown below:

Calculation 2: Sensitivity: (TP / (TP+FN)) x 100
Calculation 3: Specificity: (TN / (TN+FP)) x 100

4. **Interference and Interference Studies:** The laboratory must be aware of common interfering substances by referencing literature or performing studies.

Substances to be included in the interference study can be selected from:

4) Scientific Articles
5) Literature Reference

2) **QUANTITATIVE**- includes laboratory methods that report numbers. EP Evaluator ™ is available to assist in statistical analysis.

A. FDA Approved/Cleared Tests

The same requirements apply to the Quantitative methods that were stated above with the qualitative methods. The approach to method validation is to perform a series of experiments designed to estimate certain types of errors:

- **Precision** (random error): replication experiment, calculation of standard deviation
- **Accuracy** (systematic error or bias): method comparison experiment
- **Reportable Range:** linearity experiment
- **Reference Range:** reference range experiment

1. **Precision (Reproducibility):** Can the new method duplicate the same results? Use samples that have a matrix as close as possible to the real specimen. For clinical test, patient samples are the first choice followed by control material and reference solutions. Other acceptable sources are:
    a. Standards
    b. Previously Run Proficiency Samples

Analyze at least 1 run per day with a minimum of 3 replicate samples at least 2 different concentrations daily for 5 days to provide data for within-run and between-run components.

Having different operators perform the precision experiment must be done for methods that are operator dependent. This also demonstrates reproducibility or the method.

Follow all appropriate quality control steps for each assay. Any unacceptable performance based on failed quality control should be discarded and the run(s) should be repeated. Include the daily quality control samples normally used in each run.

Use the table below and/or EP Evaluator™ to record and calculate the data. The same table can be used for each Low/Medium/High Concentration/Value Replicates. The Run Mean, SD, and CV will show the inter-day variation.

Inter-Day Precision Data Table

	Run 1	Run 2	Run 3	Run 4	Run 5
Date					
Concentration					
Operator ID					
Replicate 1					
Replicate 2					
Replicate 3					
Run Mean					
Run SD					
Run % CV					

The table below is used to evaluate the intra-day variation. Each concentration will need to be evaluated separately.

Intra-Day Precision Data Table

	Run Mean	Run SD	Run % CV
Day 1			
Day 2			
Day 3			
Day 4			
Day 5			
Total Mean			
Total SD			
Total % CV			

Statistical Analysis: Random error is described quantitatively by calculating the mean (x), standard deviation(s) (SD), and coefficient of variation (CV). Compare these calculations to the manufacturer's data.

If the calculated SD(s) and CV(s) are less than or equal to the published values, the laboratory has verified the manufacturer's claim.

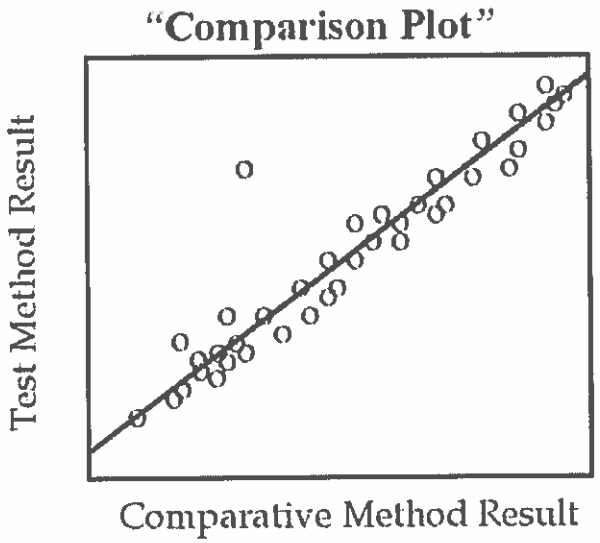
2. **Accuracy:** This will demonstrate how close to the "true" value the new method is able to achieve. A method comparison experiment is used to estimate inaccuracy or systematic error.

   Obtain at least 20 patient specimens to be used for testing. If fewer than 20 samples are used, this must be approved by the CLIA laboratory director and the section medical director. However, depending on the test system and test volume the number of patient samples may vary.

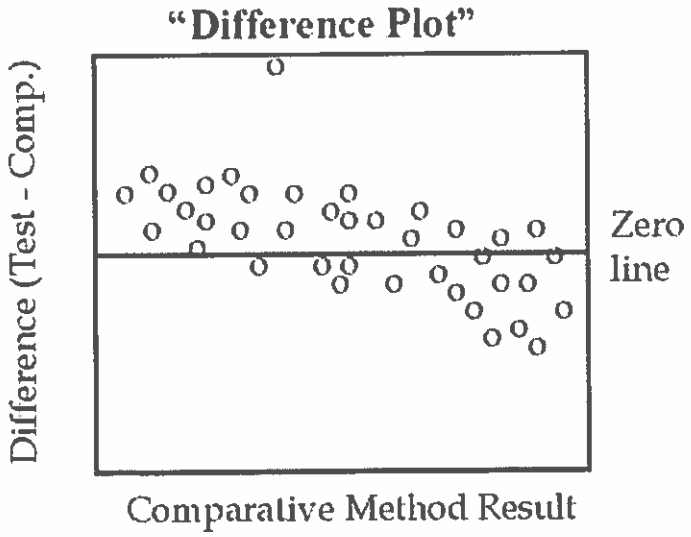
   The method comparison experiment is should be run daily for a minimum of 5 days. If there are discrepancies observed, additional daily runs may be necessary. If the laboratory

cannot run the experiment for 5 days, the CLIA laboratory director and section medical director should be notified.

Prepare a comparison plot, in EP Evaluator™ of all the data to assess the range, outliers, and linearity.



"Comparison Plot" – are used for method that are not expected to show one-to-one agreement, for example enzyme analyses having different reaction conditions. As points are accumulated, a visual line of best fit should be drawn to show the general relationship between the two methods.



"Difference Plot" – are used if the two methods are expected to show one-to-one agreement. The differences should scatter around the line of zero differences, half being above and half being below the line.

Review the two graphs for any outlying points that do not fall within the general pattern of the other data points. Document and explain any outliers.

The line of best fit will provide the linear regression, slope, equation; Y= a + bX.

Calculate the correlation coefficient "r" using EP Evaluator™ or excel. The "r" value should be greater than or equal to 0.90. The CLIA Laboratory Director and the Section Medical Director must be notified if value is less than 0.90.

Compare the calculated slope and correlation coefficient to the manufacturer's and/or published claims.

3. **Reportable Range (AMR):** The CLIA laboratory director in association with the laboratory specific section medical director may establish a narrower reportable range based on clinical necessity.

   A record of established ranges should be kept for the duration of the method and for at least two years after the discontinuation of the method.

   The AMR should be validated by running at least 3 points near the low end, midpoint, and high end using calibration/control/reference in matrix appropriate materials.

   Data must be within the laboratory's acceptance criteria or within the manufacturer's stated range to be acceptable.

4. **Reference Range (Normal Values):** This is typically provided by the manufacturer and verified by running known normal patients.

   The reference range is validated by testing at least 20 known normal samples.
   - If no more than 2 results from this patient population fall outside the manufacture/published range, then the reference range can be considered validated.
   - If the manufacturer's range cannot be validated, then the reference range will need to be established by the CLIA Laboratory Director and/or the Section Medical Director.

B. Non-FDA approved/Cleared Test, LDTs

   1. Follow the instruction above for **Precision and Accuracy.**

   - **Precision** (random error): replication experiment, calculation of standard deviation
   - **Accuracy** (systematic error or bias): method comparison experiment
   - **Reportable Range:** linearity experiment
   - **Specificity:** interference experiment
   - **Sensitivity:** LoD experiment
   - **Reference Range:** Reference range experiment

2. **Reportable Range (AMR):**

   Linearity is the measure of the degree to which a curve approximates a straight line. The linearity is also monitored prior to and during a run. The degree to which the plotted

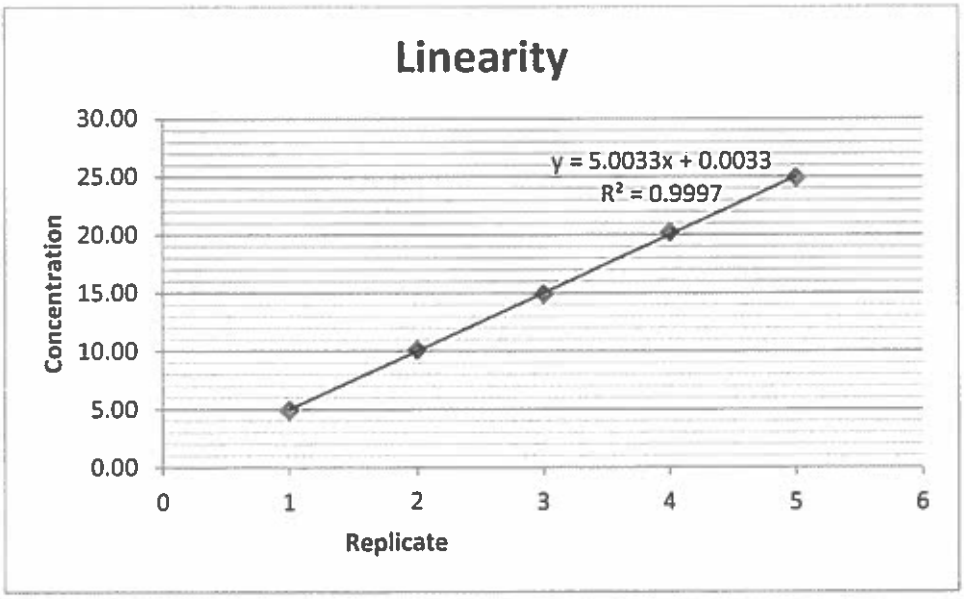
curve conforms to a straight line is a measure of the method's linearity. Conversely, the deviation of the curve from the straight line is a measure of non-linearity.

Linearity refers to the overall system response rather than simply the instrument output.

Linearity Experiment: Test a series of known dilutions of highly elevated specimen or patient pool. At least 5 different concentration levels are tested at least 3 times for at least 2 days. The measured or reported test values are compared to the assigned values or the dilution values. See table below as an example.

Replicate	Concentration 1	Concentration 2	Concentration 3	Concentration 4	Concentration 5
1	5.0	10.2	14.9	20.5	24.8
2	4.8	10.1	15.0	20.0	24.9
3	5.0	10.0	14.9	20.1	25.0
Mean	4.93	10.10	14.93	20.20	24.90
SD	0.12	0.10	0.06	0.26	0.10
%CV	2.34%	0.99%	0.39%	1.31%	0.40%
% Error	1.33%	-1.00%	0.44%	-1.00%	0.40%

Dilute the elevated sample into a series of dilutions, at least 5 levels. Run each level at least 3 times. Plot the mean of the measured values on the y-axis versus the assigned values, relative values or dilution values on the x-axis. The line of best fit, slope, can be plotted manually, through EP Evaluator™, or Excel. See the chart below as an example.



3. **Analytical Sensitivity:** Known as the limit of detection, LoD, is "the lowest concentration of the analyte which the test can reliably detect as positive in the given matrix."

   The LoD does not have to have acceptable quantitation but does have to meet all other acceptable critieria for a positive sample (i.e. Ion Ratios, Retention Time, Peak Shape, etc).

   Two different kinds of samples are generally analyzed. The first sample is a "blank" that has NO analyte of interest present. The second is a "spiked" sample that has a low concentration of the analyte of interest.The amount of analyte added to the "spiked" sample should represent the detection concentration claimed by the manufacturer and/or literature.

   It may be necessary to spike multiple samples whose concentrations are in the analytical range to the expected detection limit. Additionally, when determining the LoQ, several spiked samples at varying concentrations can be used

   The "blank" and the "spiked" samples are run at least 20 times each and the means and SDs are calculated from the values observed.

4. **Analytic Specificity:** This is the ability of the method to detect only the analyte that it is designed to detect. This includes the interference experiment.

   The interference experiment is performed to estimate the systematic error caused by other materials that may be present in the specimen being analyzed. The interference experiment must be performed for each interfering substances tested.

   Interference Experiment: Collect a minimum of 1 -2 samples that will achieve a distinctly elevated level. In to each sample aliquot the same volume of either the interfering substance (sample 1) or blank (sample 2). The amount of interfering substance should be small relative to the original test volume to minimize any matrix effect observed from the dilution.

   Both test samples are analyzed to see if there is any difference in the values due to the addition of the suspected interference.

   The mean and SD of the two samples are calculated and documented.

5. **Reference Range (Normal Values):** When the reference interval information from the manufacturer is not adequate, the laboratory must establish their own reference range.

   At least 40 patient samples who would represent the sample population are ran to establish a "normal" patient population range.

3) ADDITIONAL METHOD VALIDATION EXPERIMENTS:

A. Specimen Stability: If published data is not available on stored specimen stability for a given analyte in a given matrix, stability studies should be conducted. Prior to placing the method into production, a one-week and a one-month stability study should be conducted. After placing the method into production, a three-month, six-month, and one-year stability study should be conducted.

1. Prepare three sets of at least 20 samples (patients or controls).
2. Store one set of samples at room temperature, one at refrigerator temperature, and the third at freezer temperature.
3. Run all samples daily for a week, then once a week for the following month, three-month, six-month, and yearly increments.
4. Compare the SD and the %CV of the samples against the original validation for acceptability. Note any trends seen in the data. EP Evaluator and/or Excel may be used.

Note: If fewer than 20 samples are used, this must be approved by the CLIA Laboratory Director and the Section Medical Director.

B. Reagent Stability
See specific manufactures guidelines for purchased reagents. Refer to Reagent Labeling Policy for labeling of reagents prepared in-house. Refer to section specific procedures for preforming stability testing on calibrators, controls, reagents, and/or solutions that do not have manufacture specified stability.

C. Specimen Container Validation
If a new or different specimen collection is used, then a specimen container validation must be performed to rule out any possible interferences and/or cross-contamination.

1. Collect at least 20 samples (patient or controls across the AMR) in the old specimen container.
2. Collect at least 20 samples (patient or controls across the AMR) in the new specimen container.
3. Run all samples on the new method.
4. Compare the SD and the %CV of the two sets for acceptability. EP Evaluator and/or Excel may be used.

Note: If fewer than 20 samples are used, this must be approved by the CLIA Laboratory Director and the Section Medical Director.

D. Carryover
A carryover study is demonstrated during the method validation procedure and is monitored within each run.

A high standard and a matrix blank are alternately injected at least five times. The concentration of the high standard must be equal or greater than the highest linearity standard.

The matrix blank injected after the high standard is evaluated and must be determined to be negative. See example table below.

Replicate	High Standard	Matrix Blank
1		
2		
3		
4		
5		
Average		
SD		
%CV		

E. Cross-Contamination
Appropriate procedures should be in place to prevent any cross-contamination. These may include but are not limited to: instrument cleaning procedures, quality control procedures, following appropriate PPE procedures, etc.

F. Instrument Correlations
Appropriate procedures should be in place to ensure that all instrumentation that run the same methods are producing statistically similar results.

See section specific procedures for running parallel samples.

G. Proficiency/External QA
Provide a detailed description of what proficiency testing survey(s) will cover this method/analyte. Even if current surveys can cover, be sure to address this specifically in the summary. If current surveys do not cover, determine which survey will be used to replace the old survey method.
If there are no commercially available proficiency surveys, an alternative assessment method must be used. Describe in detail what this assessment method will be and how it will be evaluated by the lab.

Provide documentation showing that any necessary changes to the CAP instrumentation list, testing summary and proficiency summary have been completed.

4) **INSTRUMENT VALIDAITONS and RE-VERIFICATIONS**
New instruments as well as instruments that have been moved in the laboratory must be validated/verified prior to use.
A. New Instrument of a Different Make or Model of the Current Instrument: Must be validated for all method performance specification depending on complexity of the method.
B. Additional Instruments of Same Make and Model as the Current Instrument: Each instrument must be validated/verified separately. The "main" instrument must have a full validation. Additional or "back-up" instrument must have at least a re-verification. All instruments that run the same method must have an Instrument Correlation experiment ran annually.
C. Instrument that have been Moved from One Location to Another Location in the Laboratory: These must at least have a re-verification validation preformed. Running accuracy and precision is also recommended.

5) **AMR RE-VERIFICATION:**
   A. AMR Verification by Calibration Verification:
      - Run calibrator or other materials traceable to a recognized standard near the minimum, maximum, and midpoint of the AMR.
      - An average of the observed values should be calculated and fall within the acceptable TAE.
      - If any concentration exceeds the specified TAE, the assay must be recalibrated, QC checked for acceptable results and the Calibration Verification study should be repeated.
      - For FDA Approved/Cleared Methods: AMR verification at low and high points should be as close to the FDA approved AMR as possible, preferable within 10-15% depending on the analyte.

I. ACCEPTANCE CRITERIA FOR METHOD AND INSTRUMENT VALIDATION/RE-VERIFICATION

1) Acceptance Criteria for most CAP/CLIA FDA Approved/Cleared validations and re-verifications should meet at least one of the following:
   A. 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.
   B. Meet or exceed the claims of the manufacturer
2) Acceptance Criteria for most LDTs validations and re-verifications should meet the following:
   A. Method performance is acceptable when the observed errors are less than or equal to the total allowable error. Method performance is NOT acceptable when the observed errors are larger than the total allowable error.
   B. Guidelines for TAE can be found through CLIA for certain analytes, see Attachment A. For analytes not include in the table, the TAE is 10%.

J. VALIDATION AND VERIFICATION EVALUATION/SUMMARY WRITE-UP:

The final validation and/or re-verification evaluation/summary must include the following (as applicable):

1) A written assessment (general description) of each component of the validation study, including:
   1. Description of test performed
   2. Instrument identifier
   3. Reagents used
   4. Sample type(s)
   5. The acceptability criteria for the data:
      a. Accuracy (eg. method comparison using patient samples or using known concentrations)
      b. Precision
      c. Linearity (LoD and LoQ)
   6. Analytical Specificity
   7. Established Reportable Range and Reference Interval
   8. Stability (if different from package insert and for LDTs)
   9. A brief explanation of any discrepancies/issues and the resolution (if applicable)
   10. An explanation of whether the method is to be implemented in the WFBH lab.
2) A signed approval statement by the Section Medical Director, such as, *"I have reviewed the verification/validation data for accuracy, precision, reportable range, etc. for the (insert*

instrument/test name) and the performance of the method is considered acceptable for patient testing."

3) A signature of the Section Medical Director and date of implementation prior to use in patient testing.

K. Review/Revision/Implementation:

a. Review Cycle: 2 years
b. Office of Record: Department of Pathology

L. Related Policies:

Reagent Labeling Policy

M. References, National Professional Organizations, etc.:

CAP. *All Common Checklist*. College of American Pathologists; Northfield, IL. Revised 8/22/2018.
CAP. *Laboratory General Checklist*. College of American Pathologists; Northfield, IL. Revised 8/22/2018.
CLIA, 42 CFR 42 493.1253. CLIA Proficiency Testing Criteria. 02-28-1992;57(40):7002-186.
http://www.iacld.ir/DL/talar/cliproficiencytestingcriteria.pdf. Accessed 2/14/2019.
"Standardized Protocol for Method Validation/Verification."
https://www.aphl.org/mrc/documents/lss_2014_texas-clia-standardized-method-validation.pdf
Accessed 2/8/2019.
Westgard J. O.: Basic Method Validation, Westgard Quality Corporation
Ludwig, Huber PhD. "Validation of Analytical Methods and Procedures."
www.labcompliance.com Accessed 2/14/2019.

N. Attachments:

a. **Attachment A:** CLIA Proficiency Testing Criteria for Acceptable Analytical Performance

O. Revised/Review Dates and Signatures:

Review/Revision Date	Review/Revision Discription	Signature

Attachment A: CLIA Proficiency Testing Criteria for Acceptable Analytical Performance

	Routine Chemistry
Test or Analyte	Acceptable Performance
Alanine aminotransferase	Target value ± 20%
Albumin	Target value ± 10%
Alkaline phosphatase	Target value ± 30%
Amylase	Target value ± 30%
Aspartate aminotransferase (AST)	Target value ± 20%
Bilirubin, total	Target value ± 0.4 mg/dL or ± 20% (greater)
Blood gas p02	Target value ± 3 SD
Blood gas pCO2	Target value ± 5 mm Hg or ± 8% (greater)
Blood gas pH	Target value ± 0.04
Calcium, total	Target value ± 1.0 mg/dL
Chloride	Target value ± 5%
Cholesterol, total	Target value ± 10%
Cholesterol, high dens lipoprotein	Target value ± 30%
Creatine kinase	Target value ± 30%
Creatine kinase isoenzymes	MB elevated (present or absent) or Target value ± 3 SD Creatinine
Creatinine	Target value ± 0.3 mg/dL or ± 15% (greater)
Glucose	Target value ± 6 mg/dL or ± 10% (greater)
Iron, total	Target value ± 20%
Lactate dehydrogenase (LDH)	Target value ± 20%
LDH isoenzymes	LDH1/LDH2 (+ or -) or Target value ± 30%
Magnesium	Target value ± 25%
Potassium	Target value ± 0.5 mmol/L
Sodium	Target value ± 4 mmol/L
Total protein	Target value ± 10%
Triglycerides	Target value ± 25%
Urea Nitrogen	Target value ± 2 mg/dL or ± 9% (greater)
Uric acid	Target value ± 17%

Toxicology	
Alcohol, blood	Target value ± 25%
Blood lead	Target value ± 10% or ± 4 mcg/dL (greater)
Carbamazepine	Target value ± 25%
Digoxin	Target value ± 20% or ± 0.2 ng/mL (greater)
Ethosuximide	Target value ± 20%
Gentamicin	Target value ± 25%
Lithium	Target value ± 0.3 mmol/L or ± 20% (greater)
Phenobarbital	Target value ± 20%
Phenytoin	Target value ± 25%
Primidone	Target value ± 25%
Procainamide (and metabolite)	Target value ± 25%
Quinidine	Target value ± 25%
Theophylline	Target value ± 25%
Tobramycin	Target value ± 25%
Valproic acid	Target value ± 25%

Hematology	
Test or Analyte	Acceptable Performance
Cell identification	90% or greater consensus on identification
White cell differentiation	Target ± 3 SD based on percentage of different types of white cells
Erythrocyte count	Target ± 6%
Hematocrit	Target ± 6%
Hemoglobin	Target ± 7%
Leukocyte count	Target ± 15%
Platelet count	Target ± 25%
Fibrinogen	Target ± 20%
Partial thromboplastin time	Target ± 15%
Prothrombin time	Target ± 15%

Endocrinology	
Test or Analyte	Acceptable Performance
Cortisol	Target value ± 25%
Free thyroxine	Target value ± 3 SD
Human chorionic gonadotropin	Target value ± 3 SD or (positive or negative)
T3 uptake	Target value ± 3 SD by method
Triiodothyronine	Target value ± 3 SD
Thyroid stimulating hormone	Target value ± 3 SD
Thyroxine	Target value ± 20% or 1.0 mcg/dL (greater)

General immunology	
Test or Analyte	Acceptable Performance
Alpha-1 antitrypsin	Target value ± 3 SD
Alpha-fetoprotein	Target value ± 3 SD
Antinuclear antibody	Target value ± 2 dilution or (pos. or neg.)
Antistreptolysin O	Target value ± 2 dilution or (pos. or neg.)
Anti-Human Immunodeficiency virus	Reaction or nonreactive
Complement C3	Target value ± 3 SD
Complement C4	Target value ± 3 SD
Hepatitis (HBsAg, anti-HBc, HBeAg)	Reactive (positive) or nonreactive (negative)
IgA	Target value ± 3 SD
IgE	Target value ± 3 SD
IgG	Target value ± 25%
IgM	Target value ± 3 SD
Infectious mononucleosis	Target value ± 2 dilution or (pos. or neg.)
Rheumatoid factor	Target value ± 2 dilution or (pos. or neg.)
Rubella	Target value ± 2 dilution or (pos. or neg.)