TRAINING UPDATE

Lab Location:	GEC, SGMC & WAH	**Date Distributed:**	6/19/2017
Department:	Technical Mgmt, QA	**Due Date:**	6/26/2017
		Implementation:	**6/26/2017**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Policy for Laboratory Method Validation of Quantitative and Semi-Quantitative Methods SGAH.QDNQA743 v1.1

New corporate SOP to replace QDQC710

Description of change(s):

Content is very similar to the current SOP.

CQA also issued a separate document titled “Guidebook for Method Validation Data Analysis” to accompany this SOP. We have added it as an attachment (on SmearSolve, refer to attachment pane to view)

This SOP will be implemented on June 26, 2017

Document your compliance with this training update by taking the quiz in the MTS system.

Non-technical SOP

Title	**Policy for Laboratory Method Validation of Quantitative and Semi-Quantitative Methods**
Prepared by	Rob Willis, Jerry Wagner, Ph.D.

Laboratory Approval	**Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name and Title	**Signature**	**Date**

Corporate Approval	**Corporate Issue Date:**	4/3/2017
Print Name and Title	**Signature**	**Date**
Dianne Zorka **Director, Corporate Quality Assessment**	*On File*	3/31/17
Andrew Edelman, M.D. **BPT Advisor**	*On File*	3/31/17
Ronald Kennedy, M.D. **Sr. Medical Director Medical Quality**	*On File*	3/20/17

Retirement Date:	*Refer to the SmartSolve EDCS.*
Reason for retirement/replacement:	

Form ID: QDNQA305 v2 issued 2017

TABLE OF CONTENTS

1. PURPOSE

This document sets forth the policy and process for establishing and verifying performance specifications for quantitative and semi-quantitative tests or test systems introduced into Quest Diagnostics laboratories.

2. SCOPE

- This policy and process applies to:
  - All Quest Diagnostics owned and operated laboratories, including Rapid Response Laboratories (RRL).
  - All waived and non-waived quantitative test systems
  - Semi-Quantitative test systems which report a qualitative result based on numeric values results.
- This policy and process does not apply to Qualitative tests, or Semi-Quantitative systems based on non-numeric results such as titers, "+" grades, results reflected in dilutions, etc.

3. RESPONSIBILITY

Responsible Party	Task
Laboratory Director	• Approval of the initial document and any subsequent revisions. • Approval of all method verifications/validations prior to patient testing. • This task may be delegated to an individual who meets CAP director qualifications, <u>except</u> in states where the laboratory director cannot delegate this responsibility. • Consultation with Corporate Medical Operations and Quality (MOQ), if any instruction in an initial document is unclear or inaccurate.
Laboratory Director or Designee	• The recurring review of this document.
Quality Assurance Department	• Reviewing method verification/validation packages • Maintenance of method verification/validation packages (except for Rapid Response Laboratory where the original is kept at the RRL) • Updating the Proficiency Testing providers' Activity Menu and adding/removing PT survey subscriptions as required.
Technical Supervisor	• Ensuring compliance with this procedure in his/her department. • Documenting all steps of the method verification / validation process. • Implementing approved tests within the department. • Ensuring that all new or changed test systems have been approved by the Laboratory Director prior to implementation. • Ensuring all staff members are appropriately and adequately trained and competent. • Communicating with BPT if questions or concerns are noted with a rollout package or problems with the validation are experienced.
Testing Personnel	• Following the verification/validation protocol as designed. • Following the test protocol for all technical procedures. • Documenting all steps of the method verification / validation process.

4. DEFINITIONS

Refer to the *Guidebook for Method Validation Data Analysis* (QDNQA413) for a more detailed explanation of terms and additional guidance for data analysis.

Term	Definition
Accuracy	The closeness of a measurement to the target concentration.
Allowable Total Error (TEa)	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 result, or incurring unsuccessful performance in proficiency testing surveys.
Analyte Measurement Range (AMR) Verification	A study performed with matrix-appropriate materials that evaluate the low, mid, and high range of the AMR. Target values are established by comparison with peer group values for reference materials, by assignment, by reference or comparison method values, and by dilution ratios of one or more specimens of known values.
Analytical Measurement Range (AMR)	The range of analyte values that a method can directly measure without dilution or concentration.
Analytical Sensitivity and Detection Limits	The sensitivity limits for a specific method, which are determined by evaluating the Limit of Blank, Limit of Detection, and Limit of Quantitation for a method. **NOTE:** Additional information for details on the concepts of LoB, LoD and LoQ can be found in the *Guidebook for Method Validation Data Analysis.*
Analytical Specificity	The ability of an assay to accurately measure the analyte of interest in the presence of other components present in the sample. This may be caused by cross-reactivity or interfering substances.
Best Practice Team (BPT)	Cross-functional group of people managed by a National Laboratory Operations (NLO) and a designated Chairperson. The team is responsible for standardization of testing platforms, associated written procedures and general guidance to the testing sites. Members include representation from Operations, Medical Quality, Compliance and Materials Management.
Calibration Verification	The process of confirming that the current calibration settings for each analyte remain valid for a test system throughout the AMR.
Clinical Significance	Analytical performance that fails to achieve medical requirements is said to be "clinically significant."
Limit of Blank (LoB)	The minimum concentration above the ambient background "noise" of the system (i.e. the s/n of the blank) at which the test system can reliably detect the difference between background "noise" and presence of analyte.
Limit of Detection (LoD)	The calculated mean value achieved from repetitive measurements of a blank + 4 * SD of those measurements.

Term	Definition
Limit of Quantitation LoQ)	The lowest concentration at which analytical performance meets the laboratory's stated quality goals or requirements for that analyte
Linear Regression	A regression analysis between multiple sets of data pairs
Maximum Concentration/Dilution	The highest dilution allowed for achieving a result within the AMR of the method, to which the dilution factor may be applied to achieve a final result. Once it is determined that the maximum allowable dilution fails to achieve a result within the AMR, the result is typically reported as "greater than" the maximum concentration for the method.
Medical Decision Level (Xc)	The concentration of analyte at which some medical action is indicated for proper patient care. There may be multiple medical decision levels for a given analyte (e.g., the upper and lower levels of the reference interval, priority values, etc.)
Method Bias	The difference in results obtained by two different methods.
Method Validation	The defined process by which a laboratory confirms that a laboratory developed test (LDT) or Modified FDA-cleared or approved test performs as claimed.
Method Verification	The process by which a laboratory determines that an unmodified FDA Cleared or Approved test performs according to the specifications set forth by the manufacturer and as specified in this document.
Non-Procedural Changes	BPT-approved deviations from a manufacturer product insert that have no consequence for the method to achieve reliable / reproducible values near the detection limit. • Examples include, but are not limited to, changes in calibrator set points, changes to the reference interval(s), proportionate reductions in sample and reagent volumes such that all volumes remain within the exact proportions described by the manufacturer.
Non-waived Test System	Test categorization for either moderately complex (including provider-performed microscopy) or highly complex testing, as assigned by the US Food and Drug Administration (FDA).
Precision	The agreement among replicate sample measurements.
Primary Standard Material	Substance of known chemical composition and sufficient purity used in preparing a Primary Standards Solution and recognized by national or international standardization organizations (e.g., Standard Reference Materials from National Institutes of Standards and Technology (NIST), Certified Reference Materials (CRM) and International Standards from the World Health Organization (WHO).
Primary Standards Solution	Solution used as a calibration standard in which the concentration is determined solely by dissolving a weighed amount of Primary Standard Material in an appropriate solvent, and making a stated volume or weight.

Term	Definition
Procedural Changes	Any modification to instrument settings or the procedural instructions for running a test. • Examples include but are not limited to, changing from serum to plasma, changing to a different sample storage, changing detector wavelength or incubation temperature, and changing sample or reagent volume (except as noted above under **Non-Procedural Changes**),
Qualitative Test System	A test or 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."
Quantitative Test System	A test or test system that produces measurements in continuous numerical values based on a standard curve and on a signal (e.g., light) measured by an instrument (e.g., relative light units).
Random Error (RE)	An error in measurement that is unavoidable, and thus cannot be eliminated. Often this is used synonymously with imprecision.
Recovery	Linear Regression study where one set of data is anticipated to be accurate and the second set potentially lost or gained during the analysis. **NOTE:** Additional information for details on the concepts of Linear Regression, Recovery and AMR Validation can be found in the *Guidebook for Method Validation Data Analysis.*
Reference Interval	The interval of values bounded by an upper and lower limit at certain designated percentiles.
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.
Reportable Range	The entire span of result values over which the laboratory can establish or verify the accuracy of the instrument or test system.
Semi-Quantitative Test System	A test system that produces a signal that is measured and interpreted by laboratory staff based on laboratory NUMERIC cutoffs and reported as qualitative statements (e.g., negative, positive, equivocal, positive at specific dilutions etc.).
Standard Deviation	A statistic used to describe the distribution or spread of data in a population that is shown to have the shape of a normal or Gaussian curve.
Systematic Error	An error that is introduced by an inaccuracy (as of observation or measurement) inherent in the system. An error that is not determined by chance.
Test System	The process that includes pre-analytic, analytic, and post-analytic steps used to produce a test result or set of results. A test system may be manual, automated, multi-channel or single-use and can include reagents, components, equipment or instruments required to produce results. A test system may encompass multiple identical analyzers or devices. Different test systems may be used for the same analyte.

Term	Definition
Total Error (TE)	The combination of random and systematic analytical errors used to estimate the magnitude of error that might occur in a single measurement.
Total Error (TEc)	The calculated estimate of the total error of an assay.
Waived Test System	Test categorization for simple laboratory examinations and procedures which have an insignificant risk of an erroneous result.

5. POLICY

All tests and test systems must be validated or verified before they are introduced into the laboratory for patient testing. This includes the following situations:

- A test that is introduced for the first time to measure an analyte the laboratory has not previously measured.
- A duplicate test system added to the laboratory either in addition to an existing system or as a replacement for an existing system (e.g., an additional chemistry analyzer is added to support increased volume).
- Locally implemented national Quest Diagnostics standard testing procedures.
- A test system that had completed Method Validation, but due to seasonal or other causes, was not performed in routine or production testing for an extended period of time (which will be determined on a case-by-case basis).
  NOTE 1: A test is considered out of production when: (1) patient testing was not offered and (2) proficiency testing (PT) or Alternative Performance Assessments (APA), as applicable, was suspended.
  NOTE 2: This does not apply to situations where a PT or APA challenge was not performed due to a temporary, short-term situation, such as a reagent back order or an instrument breakdown. In those situations, the laboratory must perform APA for that testing event prior to reinstating the test system.
- Replacement of the entire working module of an instrument by a service engineer, such that a significant portion of the instrument is now new to the test system(s). Such changes must be reviewed by the Laboratory Director to determine if Method Verification and approval by the Laboratory Director will be required prior to the instrument being reinstated for patient testing.

6. PROCESS FOR STANDARD METHODS

6.1 Standard method verification / validation packages are authorized and distributed by NLO when a test or test system is introduced as a Quest Diagnostics national initiative.

- Such initiatives may include modifications to a manufacturer's instructions which could affect the test performance with regard to sensitivity, specificity, accuracy, or precision.
- Changes may also potentially alter the stated purpose of the test, the approved test population, or claims related to interpretation of the results.
- All authorized deviations from the manufacturer's instructions will be declared in each technical SOP and stated in the applicable NLO implementation package.

- Any further deviations from product insert must be approved by the applicable BPT, CQA and NLO. The deviations must be validated and approved by the local Laboratory Director.

6.2 A Laboratory Director and Technical Supervisor must review a national initiative for the following:

- Regulatory status of the method (i.e. FDA Cleared or Approved, modified FDA Cleared or Approved, LDT, RUO, etc.). If the method includes modifications to an FDA Cleared or Approved method, review the nature of the modifications in the documentation provided with the roll out.
- Determine if the intent is to locally deviate from the vendor product insert.
- An unmodified FDA Cleared or Approved assay requires a minimum of studies.
- Deviations from the product insert instructions may alter the regulatory status, which may in turn require additional validation studies.

6.3 Modification to Manufacturer's Instructions

A. **ANY** change to the manufacturer's supplied ingredients or modifications to the assay as set forth in the manufacturer's labeling and instructions -- including specimen type, instrumentation or procedural, that could affect sensitivity, specificity, accuracy, or precision may alter the regulatory status of the test system.

B. Changes to the stated purpose of the test, its approved test population, or claims related to interpretation of the results may also alter the regulatory status.

C. A test which has been modified may no longer be considered FDA Cleared or Approved; it must be considered Modified FDA Cleared or Approved.

D. Changes to a Test System which Require Method Validation include but are not limited to:

- Vendor changes to reagent formulation
- Different sample matrix (e.g., plasma vs. urine)
- Patient sample or reagent stability specifications
- Promoting the test for another purpose (e.g., screening vs. diagnostic)
- Changing the type of analysis (e.g., qualitative results reported as quantitative or semi-quantitative)
- Specimen handling instructions including change from duplicate to single testing
- Incubation times or temperatures
- Specimen or reagent dilution
- Changing calibrator material.
  NOTE: For those assays where the Best Practice Team (BPT) evaluates and occasionally adjust the manufacturer's specified calibrator set point(s) for new lots of calibrator, each lab must complete and maintain on file a full initial Method Validation as a <u>modified</u> FDA Cleared or Approved assay. However, as new lots of calibrator with BPT-approved set point changes are received, labs are not required to repeat full Method Validation, rather must verify method Accuracy with the new set points for <u>each instrument</u> via a correlation, or split sample comparison study against the previous lot of calibrator.

- Antibody (e.g., introducing a different source, monoclonal to polyclonal, etc.)
- Change or elimination of a procedural step
- Change or addition of detector (conjugate) or substrate
- Change in solid phase
- The cutoff or method of calculating the cutoff for semi-quantitative assays
- The endpoint or calculation of the endpoint
- Addition of adsorbent
- The strain of antigen in serologic assays

E. A modified moderate complexity test (including modifications in its intended use) is considered uncategorized for CLIA and therefore becomes a high complexity test.

6.4 Changes Requiring Focused Verification

A. Other changes that do not impact the ability of the test system to generate accurate/reliable results do not require full Method Verification of the analytical test system (pre-processing, extraction, instrumentation, etc.). Examples include:
- Significant vendor software updates
- Assay database changes
- BPT-approved changes to manufacturer's calibrator set point values
- New or revised instrument interfaces
- LIS conversions

B. Such changes may, however, be a source of pre/post-analysis errors and must be validated with a focus on the changes made.

C. A mix of before/after results must be evaluated, including as many results that span the AMR as practical.

D. Testing may also include samples below and above detection limits to ensure the proper translation keys are sent and interpreted by the LIS, and other result values that require special calculations, etc.

E. If the change is implementation of a post-instrument software upgrade, the instrument may be able to reprocess and repost/reprint data which had previously been run, in order to reanalyze it using the updated software.
- In this case, the before/after comparisons should be identical.
- If the instrument cannot reprocess and repost/reprint results, reanalysis of a mix of previously analyzed samples is required and must be compared to original results.

6.5 Validation Plan and Performance Requirements

A. It is the responsibility of the BPT and/or designee(s) to:
- Define performance requirements (consistent with Quest Diagnostics process for defining Quality Goals) and
- Design a protocol that enables the laboratory to establish or verify performance specifications.

B. Performance requirements and acceptability requirements may be derived from TEa and/or other medical quality standards in accordance with the following error budgeting approach.

Performance Characteristic	Performance requirement
Precision: Within Run SD (or CV), Intra-Assay	< TEa/4
Precision: Total SD (or CV), Includes Intra-Assay and Inter-Assay	< TEa/3
Accuracy: Bias (or average) difference at Medical Decision Level.	< TEa/4

C. When test(s) or test systems are introduced at the direction of Quest Diagnostics national programs, the laboratory must follow performance requirements defined nationally as closely as practical.

D. Precision and accuracy may be defined explicitly in terms of maximum CV and maximum bias by some agencies (e.g. NYS, etc.).

E. If a Laboratory Director wishes to define a different specification for TEa or a different error budgeting model, or define a TEa specification for which there is no previously defined specification, he/she must submit the recommendation to Corporate Quality Assessment (CQA) for consideration

F. Refer to Quest Diagnostics *Quality Goals for Method Performance Requirements for Method Evaluations and for Quality Control* (QDQC703) for details.

6.6 Components for the Method Validation

A. A method validation protocol must include a general description of the following, as applicable:

- Description of test
- Instrument identifier
- Reagents used
- Sample type (s)
- Acceptability criteria for:
  - Accuracy
  - Precision
  - Analytical Sensitivity
- Analytical Specificity - Interfering Substances Studies (if needed)
- Reportable Range
- Reference Interval
- Role and expectations of vendor supported validations.
- Sample Stability (if different from package insert or published data)
- Sample type (to include all acceptable types).
  - The number of each sample type should be proportionate to the typical numbers of each alternate sample type the laboratory is anticipated to receive. For example, if the primary sample type is serum and 10% of samples are expected to be EDTA, then the study must include EDTA sample at the same proportion.

- When samples types not included in the FDA Cleared or Approved package insert will be accepted by the laboratory, additional sample types must be included in the validation (e.g., plasma, etc.).
- Procurement for validation of unconventional samples may be difficult; hence, the Laboratory Director and/or designee should contact the BPT for assistance in sample procurement.

B. Define in writing how the laboratory will perform all the components defined in the steps above. Specific guidance for performing the validation is given in the attached Appendices. Refer to the local QA procedure "Process and Equipment Validation Protocol" (QA46).

C. If validation is being performed under the direction of the BPT, the laboratory must define any additional components and requirements that may be necessary because of local regulations

6.7 FDA Cleared or Approved Test Systems (Unmodified)

Prior to reporting test results, for unmodified FDA Cleared or Approved tests, the laboratory may use data from manufacturers' information or published reports, but the laboratory must verify:

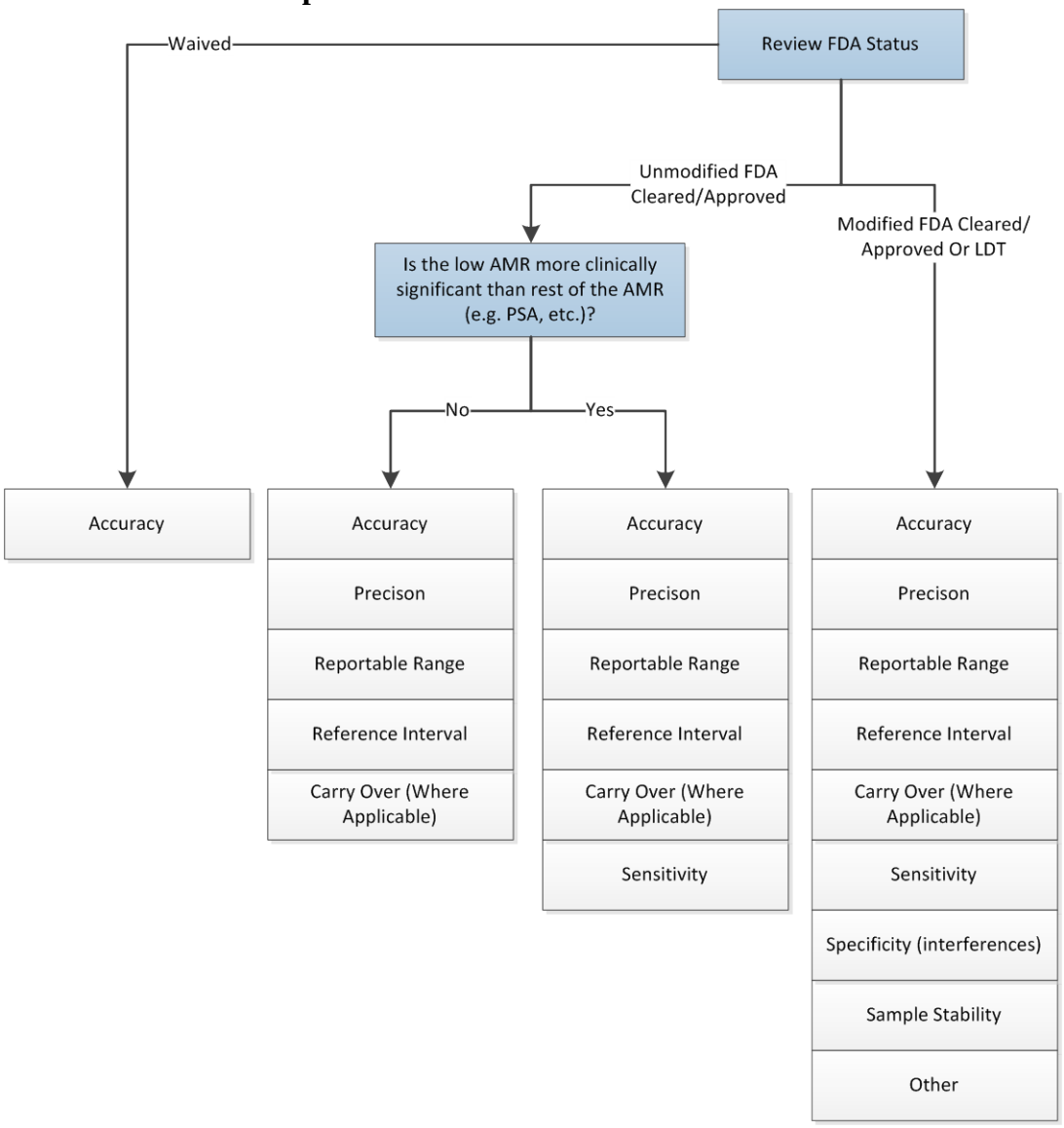
- Accuracy
- Precision
- Reportable Range
- Reference Interval
- Carryover (where applicable)
- Other

6.8 Modified FDA Cleared or Approved Test Systems or Laboratory Developed Tests (LDT)

Prior to reporting results for assays that are not FDA Cleared or Approved (including tests developed in-house), or for FDA Cleared or Approved tests modified by the laboratory, the laboratory must establish the following performance characteristics as applicable and demonstrate that they meet the laboratory's stated acceptance criteria:

- Accuracy
- Precision
- Reportable Range
- Analytical Sensitivity (Detection Limit)
- Reference Interval
- Carryover (where applicable)
- Analytical Specificity (Interferences)
- Sample Stability
- Other

6.9 General process flow



7. LABORATORY METHOD VALIDATION/ VERIFICATION STUDIES

The following sections describe components of a Method Validation/Verification study identified in the flow chart (above) and include reference to Appendices that provide additional detail.

7.1 ACCURACY

- Correlation studies are statistically evaluated to document Accuracy using the Comparison sheet for quantitative tests, or the SemiQ sheet for semiquantitative tests of the *Method Verification Template - Quantitative* workbook (QDNQA356). Data for quantitative assays should be analyzed by standard regression analysis or Deming regression. The slope and intercept with their accompanying statistical errors should be reviewed. If interpretation of the regression analysis is difficult to interpret, the analysis should be referred to Corporate Medical Operations and Quality.

- Method accuracy is verified by performing a correlation study, or split sample comparison against a validated method/instrument which has been performing the test in routine production, or another Quest Diagnostics laboratory that has completed Method Validation for the same method/instrument and has been performing the test in routine production. The selection of samples should include a mix of randomly selected samples, as well as a mix of low and high samples to evaluate the full range of AMR (as much as practical). Alternately, a correlation study may be based on patient samples provided by the reagent/instrument vendor.
- Method accuracy may be performed by spiking a sample with materials of certified purity. When this is the basis for the lab's claim to accuracy, additional external commercial verification materials must be also included to verify that the prepared materials were prepared properly (QC materials with target concentrations, etc.).
- In the case where a pre-analytical chemical or enzymatic hydrolysis step is performed to liberate the target analyte to be measured, a second recovery study must be performed to determine the efficiency and adequacy of the hydrolysis.
- Patient comparison studies for unmodified FDA Cleared or Approved tests require a minimum of 20 samples to be tested for non-waived tests, 10 for waived tests. Patient comparison studies for LDT or modified FDA Cleared or Approved tests require a minimum of 45 samples. All patient comparison studies should ideally be tested over 3 – 5 days.
- Method Verifications for testing at a Rapid Response Laboratory must include correlations with both the current platform in use and the Main Laboratory.
- **NOTE:** See additional information in the *Guidebook for Method Validation Data Analysis.*
- **Refer to Appendix A** (Accuracy: Recovery Studies) and **Appendix B** (Accuracy: Split-Sample Comparison Studies)

7.2 PRECISION

- For modified or unmodified FDA Cleared or Approved tests, total precision is determined by measuring each QC level 5 times per day for 5 separate days. If it is necessary to complete the Precision study over a shorter time frame than 5 separate days, each set of 5 QC values must be in separate runs and an acknowledgement of the shortened time-frame documented in the final precision study
- Precision studies are statistically evaluated using the Precision sheet of the *Method Verification Template - Quantitative*. Analyze data by calculating the mean and standard deviation, and hence and coefficient of variation (CV).
- A verification of precision is not required for Waived testing, unless specifically required by the applicable BPT or Laboratory Director.
- **NOTE:** See additional information in the *Guidebook for Method Validation Data Analysis.*
- **Refer to Appendix C** (Precision: FDA Cleared or Approved Tests) and **Appendix D** (Precision: Laboratory-Developed Tests)

7.3 REPORTABLE RANGE

- Reportable range is verified by (1) completing an AMR Verification study and (2) confirming that all manual and instrument on-board dilution/concentration protocols produce accurate and reproducible results.
- AMR Verification may be completed by running commercial linearity material, traceable materials, or by diluting high patients to span the AMR.
- AMR Verification must include a minimum of at least one sample within +1.5 TEa of the low AMR, one within -1.5 TEa of the high AMR, and one near the middle of the AMR.

  NOTE 1: Regulations require that "low, middle and high" values be evaluated with each AMR Verification study. The QC BPT has provided a guideline of 1.5 TEa for these tolerances.

  NOTE 2: It is understood that such "one size fits all" guidelines will not work for every test system. For example, commercial materials may not be available within these limits, or diluting samples may not be possible. Consult your Laboratory Director if you are unable to acquire a sample that falls within 1.5 TEa limit of AMR. If your Laboratory Directory determines that your data adequately achieves the regulatory / accreditation requirement of including "low, middle, high" values, you may document the issue in the *Method Verification Template - Quantitative* as an acceptable exception.
- Enter the AMR Verification data into the Recovery sheet of the *Method Verification Template - Quantitative.*
- Use the Dilutions and/or Concentrations sheet of the *Method Verification Template - Quantitative* to evaluate the statistical validity for all manual and/or instrument on-board concentrations and/or dilutions.
- AMR Verification is always required for Method Verification,
  - Once verified, subsequent AMR Verification studies may not be necessary if:
    1. Calibrations are performed successfully more often than every six months and
    2. The test includes three or more calibrators that adequately span the AMR. (Refer to the *Analytical Measurement Range (AMR) Validation and Calibration Verification* SOP (QDQC704).
- **NOTE:** See additional information in the *Guidebook for Method Validation Data Analysis.*
- **Refer to Appendix E** (Reportable Range: Analyte Measurement Range (AMR) Verification)

7.4 REFERENCE INTERVAL

- In the evaluation of the Reference Interval, as per CLSI guidelines, the following approaches may be taken: Establishing (or determining) a reference interval, Transferring a reference interval (transference applied) or Verifying (or validating) a reference interval (see Guidebook for more details).
- If the Split Sample Comparison study shows that the bias is acceptably small (Bias < TEa/4), a specific verification of the Reference Interval is not required. In this case, the Reference Interval from the current method may be transferred to the new.
- If the reference interval for the new assay differs from that of the current assay/platform, each laboratory must perform a reference interval validation study.

- If a formal reference interval study is not possible or practical, the laboratory should carefully evaluate the use of published data for its own reference intervals, and retain documentation of this evaluation. Contact Corporate CQA/MOQ for assistance.
- Reference Interval studies are statistically evaluated using the Reference Interval sheet of the Method Verification workbook.
- Refer to the table below for guidelines regarding when a Reference Interval study is required. If required, refer to **Appendix G** (Reference Interval Verification).
- **NOTE:** See additional information in the *Guidebook for Method Validation Data Analysis.*

If	Then
If the Split Sample Comparison study shows that the bias is acceptably small (Bias < TEa/4)	Validation of the Reference Interval is achieved by transferability of the current reference interval limits.
If the reference interval for the new assay differs from that of the current assay/platform	Each laboratory must perform a reference interval validation study
If a formal reference interval study is not possible or practical	The laboratory should carefully evaluate the use of published data for its own reference intervals, and retain documentation of this evaluation.

7.5 CARRYOVER

- Method Verifications must include one-time carry-over studies whenever the test system has an integrated or stand-alone automatic pipetting system that does NOT use single use tips, AND where the dynamic range of minimum to maximum values in patient samples is more than 100 fold.
- Carryover studies are statistically evaluated using the Carryover sheet of the *Method Verification Template - Quantitative*.
- **Refer to Appendix H** (Carryover Studies)

7.6 ANALYTICAL SENSITIVITY

7.6.1 Analytical Sensitivity Procedure

- Verification of Analytical Sensitivity is always required for LDTs or <u>modified</u> FDA Cleared or Approved modified methods.
- When a test is developed as an LDT or modifications are made to FDA Cleared or Approved assay, the regulatory status for that test changes and Analytical Sensitivity must be validated.
- Verification of Analytical Sensitivity is required for <u>unmodified</u> FDA Cleared or Approved assays **if** the low end clinical significance is greater than the rest of the AMR.
    - Verify Analytical Sensitivity by repetitive analysis of a single sample with a target concentration between the low AMR and +1.5 TEa.
    - Statistically evaluate data using the *Verification Near the Limit of Quantitation* sheet included in the *Method Verification Template - Quantitative.*

- Achieved SD/CV must not exceed TEa/3.

7.6.1.1 Laboratory Developed Test (LDT)

- The laboratory must specifically calculate LoQ (beyond the scope of this SOP).
- Consult the applicable BPT for further guidance as necessary.

- **NOTE:** Additional information for details on the concepts of LoB, LoD and LoQ can be found in the *Guidebook for Method Validation Data Analysis.*
- **Refer to Appendix F** *Analytical Sensitivity: Verifying Detection Limits*

NOTE: The Analytical Sensitivity studies above do not calculate the numeric value for the LoQ. The studies verify that the proximity of the LoQ is greater than or equal to LoD and less than or equal to AMR

7.7 ANALYTICAL SPECIFICITY (INTERFERENCES)

7.7.1 Unmodified FDA Cleared or Approved Methods:

- Unmodified FDA Cleared or Approved tests do not require a specific verification for Analytical Specificity. Each laboratory must be aware of all interferences listed in the manufacture's product insert. Refer to the Manufacturer's insert sheet for information on common interferences (hemolysis, icterus, lipemia).
- Verify that all potential sample condition interferences which may adversely impact the quality of the results are listed in the product insert and technical SOP (e.g. hemolysis, lipemia, etc.).
- Verify that there are processes in place to identify samples that may contain interfering substances so that samples may be appropriately processed (e.g. cancelled, qualifier messages attached to the results, etc.).
- Additional interference studies may be statistically evaluated using the Interference sheet of the *Method Verification Template - Quantitative*.
- If the laboratory determines that the interferences listed in the manufacturer's product insert are acceptable for the patient population to be tested, the notation "Accept Vendor Claims" may be documented as the conclusion.

7.7.2 Modified FDA Cleared or Approved:

- Non-procedural changes to FDA Cleared or Approved test systems (e.g. stability, reference intervals, etc.) may accept vendor claims regarding interferences. This must be declared in the Method Validation documentation.
- Procedural changes to FDA Cleared or Approved test systems (e.g. sample or reagent volumes, detector wave length, etc.) must be aware of all interferences listed by the manufacturer and other potential interferences as well. Interferences may be obtained from manufacturers or published literature. Refer to the Manufacturer's insert sheet for information on common interferences (hemolysis, icterus, lipemia).

7.7.3 LDTs:

- An interference study must be completed for any LDT test systems.
- **Refer to Appendix I** (Analyte Specificity: Interference Testing for Laboratory-Developed Tests)

7.8 STABILITY

- CAP and CLIA do not require validation of manufacturer's claims for specimen stability for an unmodified FDA Cleared or Approved assay. Therefore, additional studies are not required if the lab is following the manufacturer's instructions.
- Review the current product insert or manufacturer's instructions to determine if modifications will be required for specimen stability.
- If specimen stability is modified, stability validation studies must be performed at the testing laboratory.
- The BPT may elect to have one laboratory perform a larger stability study as part of a BPT Validation; however each testing laboratory must perform its own stability validation study to meet regulatory requirements and confirm the data obtained from the BPT stability validation. Documentation of the BPT validation should be readily available in addition to the local validation.
- Documentation of the BPT validation should be readily available in addition to the local validation.
- **Refer to Appendix J** (Specimen Stability Validation Requirements)

7.9 OTHER

- Any other performance characteristic required for test performance if different than package insert, or specifically required by regulatory/accreditation agencies for a particular technology (e.g. matrix effects in toxicology).
- See section 6.3 *Modification to Manufacturer's Instructions* above).

8. BODY FLUID VALIDATION

- Fresh specimens are required for stability studies.
- Pooled, spiked or individual samples may be acceptable.
- If possible, paired sample data from the primary sample type such as serum or plasma must be collected.
- Freeze/thaw stable fluids used for validation studies will be collected and stored frozen.
- It may be difficult to obtain specimen types other than serum, plasma, and urine for validation. This includes body fluids such as:
  - Ascitic
  - Amniotic
  - CSF
  - Cyst
  - Bronchial lavage
  - Dialysate
  - Pericardial
  - Peritoneal
  - Pleural
  - Sputum/Saliva
  - Synovial
  - Vitreous
- If the specified number of samples cannot be obtained, a variance or exception to this protocol may be approved by the applicable BPT and local Laboratory Director. Any

exception must be described in the "Comments" spreadsheet of the *Method Verification Template - Quantitative*.

- The sample-handling for each particular sample type used in the validation must be described in the protocol.
- Additional validations may be added to support compliance with state or local laboratory requirements.

9. PROCESS FOR PERFORMING A METHOD VALIDATION

Step	Action
1	Perform the method verification / validation using parameters and requirements defined in the studies above and/or provided by BPT.
2	Conduct validation studies on the laboratory's equipment and within the laboratory environment.
3	Perform validation on EACH analyte performed on EACH instrument.
4	Perform the validation process under the typical production conditions, whenever possible.
5	Define, perform and document maintenance and function checks on all equipment used for method validation.
6	Record the following for all steps of the validation process • Unique instrument identifier(s) • Reagent lot numbers and expiration dates • Quality Control material lot numbers and expiration dates • Dates of testing • Names of operators
7	Perform the test on all approved specimen types using the specified parameters for the respective assay type (i.e., quantitative, semi-quantitative) and by regulatory status. **NOTE:** Testing **must** be performed by Quest Diagnostics testing personnel who are trained, competent and licensed (if required by State regulations). Refer to the exceptions under Vendor Supported Validations below.
8	Enter test result data into the approved *Method Verification Template – Quantitative*.
9	Analyze and assemble raw data for review and approval by the Laboratory Director (or qualified designee).
10	Prepare a summary of the findings and a statement of acceptability. Refer to the *Method Verification Template - Quantitative* for an example of a summary page with all appropriate conclusions.
NOTE: Testing is permitted on patient samples ONLY after approval is complete.	

10. VENDOR SUPPORTED VALIDATION

- 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.
- All 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.
- The vendor's training is used to guide the Quest Diagnostics testing personnel through all steps necessary to perform the assay.
- All data (originals) generated during the validation must remain on-site. The official Method Verification / Validation Excel template must be used to document all Method Validations / Verifications. These templates must be considered proprietary. The vendor must be notified that the template may be used for a particular study, but cannot be shared or distributed.
- The vendor staff may take copies of data provided all Patient Health Information (PHI) has first been removed.

11. PROCEDURE NOTES

N/A

12. RECORDS MANAGEMENT

Records generated as a result of this policy/process/procedure may have different retention requirements. Refer to the Quest Diagnostics *Records Management Program Reference Guide.* http://questnet1.qdx.com/Business_Groups/legal/records/schedule.htm

13. RELATED DOCUMENTS

- Quest Diagnostics Allowable Total Error Table
- Quest Diagnostics Analytical Measurement Range (AMR) Validation and Calibration Verification SOP (QDQC704)
- Quest Diagnostics Body Fluid Validation SOP (QDQC711)
- Quest Diagnostics Guidebook for Method Validation Data Analysis (QDNQA413)
- Quest Diagnostics Method Validation Template - Quantitative (QDNQA356), available on the NQA intranet site/Method Validation
- Quest Diagnostics Nichols Institute - San Juan Capistrano Specimen Stability Study (130SM090). (Current copy available from the QA Department)
- Quest Diagnostics Quality Goals for Method Performance Requirements for Method Evaluations and for Quality Control SOP (QDQC703)
- Quest Diagnostics Verification of Performance Specification for Relocated Test Systems (QDNQA731)
- Process and Equipment Validation Protocol, local Quality Assurance procedure

Form ID: QDNQA305 v2 issued 2017

14. REFERENCES

1. CLSI. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition.* CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
2. CLSI. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.* CLSI document EP06-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2003.
3. CLSI. *Interference Testing in Clinical Chemistry; Approved Guideline-second Edition.* CLSI document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
4. CLSI. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples*; *Approved Guideline-Third Edition. CLSO document* EP09 A3. Wayne, PA: Clinical and Laboratory Standards Institute: 2013.
5. CLSI. *Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline-Third Edition.* CLSI document EP10 A3.Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
6. CLSI. *User Verification of Precision and Estimation of Bias; Approved Guideline-Third Edition.* CLSI document EP15 A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
7. CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition.* CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
8. CLSI. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition.* CLSI document) E28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
9. Code of Federal Regulations CLIA Public Health 42 CFR Part 493
10. College of American Pathologists Laboratory Accreditation Checklists

15. DOCUMENT HISTORY

Version	Date	Revision (Immediate retired and prior two versions)	Revised By
1	3/6/2017	Assigned a new document ID. Changed from QDQC710 to QDNQA743	R. Willis
1	6/8/2017	Adopting corporate version 1 Page 1: Added non-technical SOP designation and referral to EDCS per local document control. 6.6, 13: Added local validation protocol 13: Added template location 16: Added Guidebook for Method Validation	L Barrett

16. APPENDICES

Appendix	Title
A	Accuracy: Recovery Studies
B	Accuracy: Split-Sample Comparison Studies
C	Precision: FDA Cleared or Approved Tests
D	Precision: Laboratory-Developed Tests
E	Reportable Range: Analyte Measurement Range (AMR) and Dilutions
F	Analytical Sensitivity: Verifying Detection Limits
G	Reference Interval Verification
H	Carryover Studies
I	Analytical Specificity: Interference Testing for Laboratory-Developed Tests
J	Specimen Stability
K	Guidebook for Method Validation Data Analysis (see Attachment Pane of SmartSolve EDCS)

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APPENDIX A
Accuracy: Recovery Studies

Recovery of Peer Group Values for QC Samples

Step	Action
1.	The bias (or difference) of the mean value for each level of QC material from the Peer Group Mean should be < TEa/4 **NOTE:** If this is a new test system for Quest Diagnostics, there may be no prior QC data to compare. **In this case, the laboratory will evaluate InterLab QC data as soon as it becomes available**.

Recovery of Known Standards: (There is no CLSI protocol to assess this attribute)

Step	Action
1	Select at least 6 patient pools of low enough concentration so that when know amounts of a standard material are added, the higher concentration will still be near a medical decision concentration.
2	Prepare spiked aliquots of each patient sample, by adding a known volumetric amount of the standard solution to a known volume of a patient sample or pool. • Do this for each patient sample or pool. • Have the standard solution high enough in concentration so that the volume of standard added to the patient sample is not more than 10%, so as to not disrupt the patient sample matrix too much.
3	Prepare a baseline aliquot of each patient sample (or Pool) by adding an equal volume of the diluent added to separate aliquots of each patient sample.
4	Perform 4 measurements of each aliquot to minimize the effects of imprecision.
5	The amount recovered is the difference between the spiked and baseline samples.
6	Calculate the amount of analyte added from the standard material based on the volumetric details.
7	The % recovery is the ratio of the amount recovered divided by the amount added.
8	The error due to lack of perfect recovery: (amount recovered MINUS amount added) should be $\leq$ ¼ * TEa.
9	Write a conclusion as to acceptability of recovery performance.

- **NOTE:** If standard materials are not available, this study cannot be performed.
- **DO NOT** use the kit calibrators to perform this recovery, because that will not be an "independent" recovery study.

APPENDIX B
Accuracy: Split-Sample Comparison Studies

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>The comparison of methods study should be conducted with the current method/instrument, or if this is the first time the assay is being performed in the lab, then the study will be performed by comparison of results to new-method results from another Quest Laboratory. Alternately, a comparison of methods may be conducted with split samples provided by the assay vendor. Samples provided by the assay vendor may include result values not easily obtained by the Quest Laboratory (e.g. positive patients, very high or very low values, etc.). The vendor may provide all or only some of the samples to be used for a split sample comparison.</td></tr>
<tr><td></td><td>
<table>
<tr><th>IF your lab currently</th><th>THEN</th></tr>
<tr><td>sends out the test to be performed elsewhere (i.e., our lab may not have a “collection” of left over samples in storage to use)</td><td>Then either
The lab that had originally validated the method for the BPT
For RRLs, must be the Main lab to that RRL
Another Quest lab that has validated the method and currently performs that method/instrument in routine production
Select a minimum of 20 samples to send to your laboratory for the correlation study in your lab</td></tr>
<tr><td>performs testing for the analyte in question, but by a different platform</td><td>The validating laboratory should select a minimum of 20 samples for the correlation study, and compare to the current instrument/method.</td></tr>
</table>
</td></tr>
</table>

3	**Specimen Selection** • Select at least **10** samples for a Waived test system, **20** samples for an unmodified FDA Cleared or Approved test system or modified FDA Cleared or Approved test systems in which the modifications do not include changes to the procedure (e.g. stability study extensions, etc.). Select at least **45** samples **distributed over the Analyte Measurement Range (AMR)** for LDT test systems, or modified FDA Cleared or approved test systems in which the modifications DO include changes to the procedure. • As a general guide, pick 5 samples in each quartile of the AMR. DO NOT restrict to the reference interval. • Aliquot and store the specimens appropriately for shipment to the other lab. • Testing should be performed as described in the technical procedure. • Testing of these 20 samples ideally should be performed over 3 - 5 days (i.e., a minimum of 4 - 8 samples per day).

4	**Calculations: Regression Analysis.**
	• Perform linear regression using the applicable Method Verification Excel Validation template, with X being the current assay and Y being the new assay.
	• Select up to three medical decision levels to be evaluated against the regression line. Consult the Roll Out documentation to determine if the BPT assigned the medical decision values to be evaluated and use those if provided. If the medical decision fields in the Excel template are left blank, it will recalculate against three values which represent a span of low, middle, high AMR. However, the Method Verification evaluation will be more meaningful if applicable and meaningful medical decision points are provided.
	• The following are examples for the selection of medical decision points.
	  • Low and high limits of the reference interval, plus a third abnormal value.
	  • Thresholds or cutoffs where different medical decisions are made relative to patient care. For example, for Glucose, many medical decision level concentrations can be defined: 60 mg/dL (hypoglycemia), 100 (pre-diabetes), 109 (abnormal fasting glucose), 126 (diabetes), and 200 (limit for glucose tolerance).For each medical decision level, defined as Xc, the Excel template will calculate the corresponding Yc value using the equation for the regression line and the difference (Yc - Xc). The absolute difference should be < TEa/4 at each value of Xc.
	• Consult your Laboratory Director for additional guidance.
	• Examine the plot for outliers and non-linearity. If outliers are observed, investigate and document the cause.

5	**If the Accuracy Criteria is not met**
	• If the regression or difference plot shows anomalies such as non-linearity, clustering, outliers, etc. STOP and investigate for possible causes of inaccuracy, perform corrective action and repeat all affected studies in their entirety.
	  • If isolated to a single "outlier" or discrepant sample, repeat the testing of that sample by both methods to check for clerical errors.
	  • If the single sample discrepancy repeats, investigate the possible causes for the difference. Also select another sample of similar concentration for retesting in both labs, to check for possible specimen handling errors (mix-up).
	  • Recalculate the bias or difference between methods with the new value and check to see that Bias < TEa/4
	If the discrepancy appears as a general trend indicative of possible sample degradation and not the assay, perform maintenance, recalibrate the assay, run QC samples, and repeat the patient sample correlation study in its entirety on both methods (new and old).

6	Write a conclusion as to acceptability of split sample comparison studies.

APPENDIX C
Precision: FDA Cleared or Approved Tests

Step	Action
1	Select the QC materials that have been recommended for this assay for routine QC. There should be three levels. •It is acceptable to supplement the 3 QC materials with an in-house serum pool (following the procedure for maintenance and preparation of in-house QC pools. (QDQC708)
2	Measure each QC material ideally 5 times per day for 5 separate days.
3	For each material, calculate mean, SD, and %CV.
4	For each material, calculate the Total Precision based on the SD and % CV and the defined allowable total error (TEa). The Method Verification spreadsheet does this automatically. •Sigma (for precision only) = TEa (units) /SD (units) or •Sigma (for precision only) = TEa%/ CV%
5	The Method Verification template creates a spreadsheet that summarized Total Precision for each material
6	Check that each Process Sigma (precision only) is greater than 3. (This is another way of saying the maximum SD $\leq$ TEa/3) •Acceptable performance for a quantitative assay run requires at least 3 Sigma quality •If Process Sigma is < 3.0, then testing should be performed in duplicate until such time as the assay precision is improved to achieve 3 Sigma over two months of routine operation. •If Process Sigma is $\geq$ 3 Sigma at higher concentrations, but not at very low concentrations, the manager and the Local Medical Director may decide to perform replicate testing for low concentration samples, but singlicate testing for concentrations at or above the point where the Precision Process Sigma is 3.0 or greater.
7	Document your findings in the "Precision" tab of the *Method Verification Template - Quantitative.*
8	Write a conclusion as to acceptability of precision performance.

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APPENDIX D: Precision: Laboratory-Developed Tests

Studies should be performed to establish within run precision, between run, and total imprecision for lab developed tests, and those tests that are modified in a way that may affect precision. Knowledge of the magnitude of these components provides important information for effective management of the assay.

- If the majority of the variation is in the within run component, then pay close attention to those factors that cause variability within the individual test run.
- If the within-run component of variation is much larger than the between run variation, that at least indicates the assay is quite reproducible from day to day, and that the calibration procedure is quite effective.
- If there is a large between- run or between-day component, then pay close attention to factors that cause a large variation. In this case, special attention should be given to minimizing calibration variability.

1. Materials

Step	Action
1.	Prepare control materials in the same matrix similar to clinical samples. External control materials should be used when possible and may be supplemented with in-house control materials (See QC SOP for Preparation and In-House QC Pools in Related Documents Section.
2.	Use at least three levels of control, if possible. **NOTE:** When selecting the target values for QC materials, take into account the following, in this order of priority: **1st** Medical decision levels: Targets should be selected at or near concentrations where medical decisions are made. The quality should be known most specifically at these points. **2nd** Extreme limits of the AMR to document precision near the AMR limits. **3rd** Reference Interval Limits a. Low-level sample – A sample with a concentration of analyte near the low end of the measuring range. b. A sample with a concentration in the reference interval for patient results. c. A sample above the reference interval, but within the AMR.
.	For therapeutic drugs, these levels should extend from sub-therapeutic to toxic levels.

2. Within Run Precision

Step	Action
1	Test a minimum of three levels. Run all standard proposed QC materials since within-run statistics will also be used to estimate preliminary QC ranges.
2	Sequencing of samples: The QC samples should be spread out over the entire run to maximize detection of time or position dependent variables. Use a sequence like. 123123123123123 instead of 1111112222222333333. This is especially important for microtiter plates.
3	Calculate mean, SD for each control material. The SD calculated should be less than allowable SD (within run) or < TEa/4.

	NOTE: This mean and SD can also be used as a preliminary estimate of QC ranges for the remainder of the study. Use Mean +/- TEa/4 to predict the QC limits for acceptable day-day variation. One may also use information from the insert sheet for the preliminary estimation of the QC limits. (See document QDQC706 Determining QC Targets and Limits for New Materials in Related Documents Section.)

3. Total Precision

Step	Action
1	Perform a **20-Day** study with 2 runs per day for at least 3 QC levels, each level in duplicate in each run. • If the assay will be set up only once per day when it is in routine operation, set up only one run for this validation. However, if the assay will be set up several times (or on different shifts each day), this study should include 2 runs per day, as required in the **CLSI EP5** protocol. • If it is necessary to complete the study before the completion of the 20 days, data must be collected for **a minimum of 12 days to facilitate an initial decision** about the acceptability of the total SD. But then the Validation Report **must** have a supplement added that includes a summary of the precision data collected for 20 days (first month) during production, to document long term precision (defined as 20 days or 1 month).
2	**Acceptability of Precision:** Compare the total SD to the defined allowable precision or allowable total error. ***Either of the following criteria for TOTAL SD MUST BE MET prior to accepting the method and going on to other studies!*** **Acceptable:** • Six Sigma capable if Total SD ≤ 1/6 * TEa. • Good, if Total SD ≤ TEa/4. • Acceptable (fair) if Total SD ≤ TEa/3 **Marginal:** • Marginal if Total SD > 1/3 * TEa but < ½ * TEa • A marginal condition may require testing be performed in duplicate or triplicate. **Unacceptable**: • Unacceptable if Total SD ≥ ½ * TEa • The assay may be required to be re-classified as qualitative, with no numeric result provided. **OR** **Total SD must be less than a defined maximum SD or CV**. • Example: Maximum SD or CV for HDL defined by NCEP/CDC is SD < 1.7 mg/dL or CV < 4%, whichever is greater.
3	Document your findings in the "Precision" tab of the *Method Verification Template - Quantitative*.
4	Write a conclusion as to acceptability of precision performance.

APPENDIX E
Reportable Range: Analyte Measurement Range (AMR) and Dilutions

1. AMR Verification using Materials of Known Value

AMR Verification may be accomplished with either traceable materials, linearity materials, or other materials of known concentration. It may also be accomplished through a series of dilutions of a high and low patient pool across the range of the assay

Step	Action
1	Run calibrators from a different lot than used in production, or other materials (known pools) traceable to a recognized standard at the minimum, maximum, and midpoint of the claimed AMR.
2	Appropriate materials should have a target value concentration within 1.5 TEa of the respective minimum or maximum of the AMR.
3	• If each material is tested multiple times then the average of the observed values should deviate from the expected target by no more than TEa/4 • The "Recovery" sheet of the Method Verification Excel template automatically performs these calculations.
4	• If any of the differences exceed the specification, the assay must be recalibrated. Check QC for acceptable results and repeat the AMR Verification study, again with 1-3 replicates of each level. • If criteria still are not met, consult with supervisor, manager, technical director, and manufacture (as appropriate).
5	Document your findings in the "Recovery" tab of the Method Verification Excel template.
6	Write a conclusion as to acceptability of the AMR Verification.

2. AMR Verification using Dilutions of Patient Samples

Step	Action
1	Mix together a very high patient pool and a very low patient pool to achieve 5 different levels, and measure each in duplicate **within a single run**, in randomized order.
2	See the "Recovery" sheet of the Method Verification Excel Template for preparing mixtures.
3	Plot the average value of each, versus expected value or versus the dilution factor (per the CLSI EP6 protocol). Visual inspection is sufficient to identify the region for a straight line. BUT continue to Step 4.
4	Check that the difference of the averages. Each singlecate measurement must fall within full TEa, duplicate measurements within TEa/3, triplicate measurements within TEa/4.
6	Document your findings in the "Recovery" tab of the *Method Verification Template - Quantitative*.
7	Write a conclusion regarding the validation of the AMR Verification.

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Refer also to the *Analytical Measurement Range (AMR) Validation and Calibration Verification* SOP (QDQC704)

APPENDIX F
Analytical Sensitivity: Verifying Detection Limits

1. Limit of Blank (LoB):

Step	Action
1	Use zero calibrator or a blank mixture (i.e. reagent grade water or saline).
2	Measure 20 times **within a run.** Exclude any gross outlier, > 5.0 SD.
3	Calculate the mean and SD.
4	Calculate the **LoB = mean + 2.0 SD**. (note mean + 2 SD is the 97.5th percentile for a Gaussian distribution of data.)
Special Case: If the instrument does not provide a result < 0.000 or there is some other minimum threshold of limiting the display of results, then one must work in OD's or Light Units or other raw signal.	
5	Obtain data in OD's or Light Units.
6	Calculate the mean and SD in these units,
7	Calculate the [**Mean + 2SD**] in OD's or Light Units
8	Convert this value back to the corresponding concentration value, using the calibration curve to define the LoB.
If it is not possible to obtain the raw OD or Light Units for all readings because some readings are below the reporting threshold, determine LoB non-parametrically based on the **97.7%ile**.	
9	Rank all results including those for which there is a signal flag below the readout minimum. Count those with a Low Signal flag as low results.
10	Identify the upper **97.7%ile** for these blank readings.
11	This is the LoB for the blank for zero standard.

2. Limit of Detection (LoD): a Calculation only:

Step	Action
1	Assume SD for sample of very low concentration is equal to the SD of the Blank. Therefore no new measurements are required. Use the SD from Section 1
2	Calculate the **LoD = LoB + 2.0 SD**. = **mean of Blank + 4 SD of Blank**
If LoB was determined non-parametrically as the 97.7%ile of the zero standard readings, then LoD will have to be determined based on measurements of a very low sample whose concentration is just above the LoB.	
3	Prepare a sample whose concentration is just above the LoB (suggest 50% higher concentration.
4	Measure 20 times **within a run.** Exclude any gross outlier, > 5.0 SD.
5	Calculate the mean and SD.
6	Calculate the **LoD = LoB + 2.0 SD**.

NOTES:

- **LoD should be calculated for every analyte,** from the same studies for LoB (see above).
- Values at or above the LOD will be detected 97.7% of the time.

- Values between LoB and LoD are "detected", but they may not always be detected.

3. Limit of Quantitation Study (LoQ)

Step	Action
1	Prepare a low pool with a target value within 1.5 TEa of the low AMR.
2	Measure each pool 5 times on each run, over the 4 runs of the validation study (20 values).
3	After excluding no more than one gross outlier (>5 SD if there is one) calculate the mean, SD, and %CV.
4	Compare the SD to the requirement that SD $\leq$ TEa/3.
5	The SD must be $\leq$ TEa/3.
6	Write a conclusion as to acceptability of LoQ (and LoB and LoD).

NOTES:

- LoQ is the lowest value that can be reported.
- LoQ can be equal to LoD, but not less than LoD.

SPECIAL NOTE: *for esoteric tests where TEa has not been defined, and where Tonks' criteria cannot be applied to estimate TEa, apply the historical definition of LOQ as the lowest concentration where the CV equals 20%. As above, LOQ cannot be less than LoD*

APPENDIX G
Reference Interval Verification or Establishment

Step	Action

1	**Reference Interval Verification (small sample):**
	• Reference interval limits used for this study must be based on what will be used for patient testing as specified in the technical SOP and LIS.
	• Using a relatively small number of **reference individuals** (n = 20), perform the assay (optionally run the samples with both the old and new assay) and **enter results into the *RefInt* template.**
	• Count the number of results that fall outside the reference interval limits (regardless of whether low or high). If there are no more than 2 of 20 results outside the stated reference interval limits, then the reference interval limits may be considered acceptable for use by the laboratory. STOP HERE.
	• If 3 or 4 results fall outside the stated reference interval limits, another 20 samples should be collected and tested.
	  • If no more than 2 of these new 20 fall outside the stated reference interval limits, then these limits may be considered acceptable for use by the laboratory. STOP HERE.
	  • If 3 or more results are again outside the stated reference interval limits, contact CQA/MOQ or the QC BPT.
	• If 5 or more of the original 20 results fall outside the stated reference interval, notify the applicable CQA/MOQ or the QC BPT.

2	Write a conclusion regarding validation or determination of reference intervals.

APPENDIX H
Carryover Studies

Carryover studies should be performed for

- Any method employing a specimen sampling system that uses a non-disposable sample probe or set of probes to sample patient specimens.

1. **AND** where the dynamic range of minimum to maximum values in patient samples is very wide (more than 100 fold).
   The purpose of this experiment is to determine any effect on a result that could be caused by an extremely high concentration of the analyte in the previous patient specimen.

Perform the following experiment three times, to obtain an overall average effect.

Step	Action
1	**Specimen Selection.** • Obtain a patient specimen of very high concentration (near the highest value that can occur in the human body) • Obtain a specimen at a very low concentration, but above LoQ (it must be measureable). Pooled serum is OK, but it MUST be WELL-Mixed!
2	**Specimen volume** • Sufficient volume to perform at least 6 assays of the high sample • Sufficient volume to perform at least 15 assays of the low sample
3	**Perform the carryover test at least 3 separate times with the following sequence.** L1, L2, H1, H2, L3, L4, L5 Running all samples the same day is OK.
4	**Enter the data in the appropriate template in the Excel file for Laboratory Validations**
5	**The Template for Laboratory Validation performs the following calculations** **For each run, calculate the following.** • Average expected baseline value for the low sample will be the average of L1 and L5 • Average of the expected value for the High sample will be the average of H1 and H2 • Calculate the difference in the first sample after the high samples, relative to expected baseline • Calculate the difference in the second sample after the high samples, relative to expected baseline • Calculate the difference in the sample just before the high samples, relative to expected baseline, to check for pre-carryover. **Average above across runs** • Calculate the average carryover of the first low sample (L3) after the High samples

	• Calculate the average carryover of the second low sample (L4) after the High samples • Calculate the average pre-carryover of the low sample (L2) just before the high samples
6	**Data Review** • **Compare average differences to allowable difference (TEa/4)** • **Interpretations:** If Carryover is not observed, then no action needs to be taken, other than to note this in the "intended Use" section of the SOP. If Carryover is observed, and was **not** expected per the claims of the manufacturer, contact the manufacturer to ensure that all system alignments are in order. Record any limitations in the SOP, so that samples that follow high samples greater than that which will not cause unacceptable carryover, will be retested following samples of more moderate concentration.
7	Write a conclusion as to acceptability of carryover performance.

CAUTION:
For some equipment with multiple sample probes, it is possible that adjacent samples may not be pipetted by the same probe. The Evaluator should know what probe pipets what sample and arrange test samples accordingly.

APPENDIX I
Analyte Specificity: Interference Testing for Laboratory-Developed Tests

The following should be performed for laboratory-developed tests and for other special interests.

1. Screening Interference Experiment

Step	Action
1	Identify the Interfering Substances to test. Test at 2 x the highest physiologically expected concentration of the interfering substance.
2	Define the concentrations of analyte to test (at medical decision levels).
3	Define the decision criteria: ▪ Error due to interference ≤ TEa/4: Results are acceptable. ▪ Error due to interference > TEa/4: Results may be unacceptable and may require a qualifying message. Refer to the applicable BPT for guidance.
4	Prepare the solution of interfering substance, prepare Test Samples, and prepare Control Samples. • EP7 contains a large index of interfering substances and recommended preparations. Solutions of interfering substances should be of such a high concentration so as to avoid diluting the patient sample aliquot by more than 10% (by volume). Prepare Control (or baseline) samples by adding an equal volume of diluent to another aliquot of the same patient sample.
5	Perform 4 replicates for each aliquot (baseline and spiked aliquots) prepared to minimize the effects of imprecision.
6	Measure the Test and Control samples, in a randomized order in one run.
7	Calculate the average value for each aliquot (from the 4 replicates) and then
8	Determine the difference in the average value of the spiked aliquots from the baseline aliquot.
9	Compare the average difference obtained for each sample and condition to medically allowable error. The difference due to a potential interfering substance should be ≤ TEa/4 to be considered negligible or acceptable.
10	If the interference effect is > TEa/4, perform the "dose-response" test described below in Part 2 to determine thresholds for criteria defined here in this Part (Step 3).
11	Write a conclusion as to acceptability of interference performance.

2. Dose Response Interference Study

If there is a significant effect at the very high concentration of interfering substance tested in the screening protocol, then this dose response study will enable one to determine the threshold at which the effect becomes clinically significant.

Step	Action
1	Determine the highest and lowest concentration of interfering material to be tested.
2	Define the decision criteria as in Step 3 in Section 1 above.
3	**Prepare materials: (See special instructions below)** • **Base Pool**: Patient serum pool • **Stock Solution** of a high concentration of the interfering substance (20 x the intended test concentration). • **High Pool**: Base Pool plus stock solution to give a final concentration of interferent that is at least 2 x the maximum concentration expected in patient samples. • **Low Pool**: Base Pool with minimum expected concentration of interferent, prepared by adding an equal volume of diluent (used to prepare stock solution) to the base pool that was used to prepare the High pool.
5	**Prepare Intermixtures:** Intermix Low Pool and High Pool in the following format: Sample No. — Action steps 1 = 1 part Low Pool 2 = 3 parts Low and 1 part High 3 = 1 part Low and 1 part High 4 = 1 part Low and 3 parts High 5 = 1 part High
6	Perform 4 measurements of each mixture and arrange in a randomized order in one run (or alternating order: low to high, then high to low, etc., for all 4 replicates).
7	**Determine the average concentration** of the Low Pool (baseline) and subtract this value from all the other results, to calculate the interference effect.
8	**Plot the data**: • Y-axis - the difference from baseline. • X axis - the calculated concentration of the interfering substance.
9	**If the data appear linear, perform a linear regression** to estimate the slope, y-intercept, and the standard error of estimate (Sy/x).
10	Determine the x-value for which the ▪ y-value (difference from baseline) is = TEa/4 **and** ▪ y-value (difference from baseline) is = TEa/2.
11	Apply the criteria defined above in Step 3 of Section 1 above ▪ Error due to interference ≤ TEa/4 Results are acceptable. Report results. ▪ Error due to interference > TEa4, but less than TEa/2: Results may be considered "marginal". Report results with a qualifying message. ▪ Error due to interference > TEa/2: Results are not acceptable. Do not report patient results. Report with a message "Unable to determine result due to interfering substance."
12	Write a conclusion as to acceptability of interference performance.

3. Special instructions for preparation of "Stock" solutions of interfering substances

a. Lipemia

Step	Action
1	Select a specimen at the low clinical decision point and a specimen at the high clinical decision point
2	Spike each with a lipid concentrate (examples include 10% Intralipid® or 10% Lypsin®) in proportions of: 0.5mL of lipid material + 9.5 mL of specimen pool to create a stock of about 1000 mg/dL (estimated triglyceride). Label as Low Stock and High Stock**.**
3	Dilute these stock solutions with the same specimen pool respectively to create 0, 31, 62, 125, 250, 500, and 1000 mg/dL of apparent triglyceride. Describe the nature of the turbidity of each of these samples (as clear, slightly-cloudy, cloudy, opaque, or milky).
NOTE: The measurement of triglyceride is not a good surrogate for turbidity for samples prepared by the addition of lipid materials such as Intralipid, so it is important to make visual observation of the degree of turbidity	
4	Perform 4 replicate measurements of each sample prepared to minimize the effects of imprecision.
5	Continue with Step 7 in Section 1 above to analyze the data.

b. Hemolysis

Step	Action
1	Select a fresh lavender top blood sample. • Record the hemoglobin value. • The sample should be less than 24 hours old. • The hemoglobin value should be generally in the normal range but the exact value isn't critical.
2	Mix the tube well. Transfer 2.0 ml to a 13x100 tube. Mark the blood meniscus with a marker.
3	Wash the cells 3 times with normal saline. After the third wash, carefully pipet the saline off the cell pellet.
4	Reconstitute the cell pellet to the 2 ml mark on the tube with deionized water.
5	Freeze the tube for at least one hour. The combination of deionized water and freezing will completely disrupt the red cells**.**
6	Thaw and mix. Use within 3 days. **NOTE:** even when frozen the hemolysate will eventually oxidize (turn brown) making it unusable.
7	Select at least one specimen at a low clinical decision point and one specimen at a high clinical decision point.
8	Prepare mixtures of each with the above hemolysate per the following table, to achieve final approximate values for hemoglobin of 0, 150, 375, 750, and 1500 mg/dL. One may make other mixtures for additional concentrations of hemoglobin between 0 and 150 mg/dL.

SAMPLE NO.	1	2	3	4	5
µL SERUM	900	900	900	900	900
µL Hemolysate	0	10	25	50	100
µL water	100	90	75	50	0
Hgb mg/dL**	0	150	375	750	1500
Appearance	straw	light red	red	cherry	dark. Cherry

In this example, the whole blood hemoglobin value was 15 g/dL of the original sample that was lysed. When the hemolysate is added to a serum sample in the volumes indicated, the concentrations of hemoglobin are calculated. For example, for sample No. 5, Hgb = 15,000 mg/dL * 100uL/1000uL = 1,500 mg/dL. All other samples are calculated accordingly

9.	Perform 4 replicate measurements of each sample prepared to minimize the effects of imprecision
10	Continue with Step 7 in Section 1 above to analyze the data.
NOTE: The laboratory may wish to prepare a hemolysate from use of Hemoglobin crystals. This will create a sample of reduced hemoglobin, which may or may not provide all the spectral interference that oxygenated hemoglobin may have.	

c. Icterus

Step	Action
1	Select a specimen at the low clinical decision point and a specimen at the high clinical decision point. Label as Low Pool and High Pool respectively
2	Prepare a concentrated solution of bilirubin by dissolving 4 mg of bilirubin with 0.1 mL of DMSO, 0.2 mL 0.15 M Na_2CO_3 and 0.2 mL 1 N HCl. Add this to 9.5 mL of specimen pool to create a concentrated stock solution of 4 mg in 10 mL or 40 mg/dL.
3	Volumetrically, combine the Low Pool with the concentrate to create final concentrations of bilirubin of 0, 2.5, 5, 10, 20, and 40 mg/dL of bilirubin. Do the same for the High Pool.
4	Perform 4 replicate measurements of each sample prepared to minimize the effects of imprecision
5	Continue with Step 7 in Section 1 above to analyze the data.

APPENDIX J
Specimen Stability

When the BPT elects to have one laboratory perform a larger specimen stability validation as part of a BPT validation, each testing laboratory must also perform its own stability validation to meet regulatory requirements and confirm the data obtained from the BPT stability validation. Documentation of the BPT validation should be readily available in addition to the local validation data.

Stability Validation Procedure:

1. Determine the Medical Decision levels for the assay. A Stability Study evaluates stability as a property of analyte, so all samples used in a Stability Study should have measurable analyte. Do not include negative samples unless specifically directed to do so by the applicable BPT. Concentrations should include values near Medical Decision levels and those typically achieved during routine production testing.

2. Determine the conditions for the specimen stability
   a. room temperature [defined temperature must be stated in procedure],
   b. refrigerated [defined temperature must be stated in procedure],
   c. frozen [defined temperature must be stated in procedure]

3. Determine the parameters of the validation for the laboratory
   a. **For BPT Specimen Stability Validation:**
      Laboratories performing the BPT stability validation will complete a more rigorous stability study.
      i. Minimum of five samples is required
      ii. At least one of each sample type must be analyzed at every applicable storage condition / temperature

   b. **For Testing Laboratory Specimen Stability Validation:**
      Each testing laboratory must perform its own specimen stability validation to meet regulatory requirements and confirm the data obtained from the BPT stability validation.
      i. Minimum of two samples is required
      ii. At least one of each sample type must be analyzed at every applicable storage condition / temperature
      iii. If there is no **BPT Specimen Stability Validation** then:
         a. A minimum of five samples must be analyzed and
         b. At least one of each sample type must be analyzed at every applicable storage condition / temperature.

4. For each specimen type defined in the assay SOP, select specimens that have been previously analyzed within 24 hours of collection.
   a) Each sample must have adequate volume to complete the analysis (different storage condition over multiple days). If volume is an issue, like sample types may be pooled.
   b) If previously analyzed specimens are not available it is acceptable to use fresh donor samples.

Collect sufficient quantities of fresh donor samples. If more than one tube is collected from an individual donor, combine all tubes from that one donor into one larger pool. Aliquots will be made from this larger pool. Label samples without using personal identifiers to maintain confidentiality.

5. Analyze each sample as soon as possible to obtain the baseline (target) result.
NOTE: If the sample is previously analyzed and un-pooled, the original result may be used as the baseline.

6. Aliquot the number of sample sets required to test each storage condition over the number of days necessary.
   a. Split each sample (or pool) into aliquots, one aliquot for each time and temperature condition.
   b. Seal each sample tightly with a screw cap (or equivalent) to minimize evaporation.
   c. Label each sample appropriately (e.g., Donor A, Donor B) Storage Temperature and Storage Time).

7. Store all samples in the intended storage location (i.e., freezer, refrigerator, or room temperature). Ensure that the samples are stored upright and are clearly marked as a STABILITY study.

8. All preparation and testing must be performed following the SOP for patient samples. Test all samples on the same instrument to reduce variability.

9. Statistically compare the means of each day's results with Day Zero.
   a. **For BPT Specimen Stability Validation:**
   Laboratories performing the BPT stability validation will complete a more rigorous stability study.
      - A sample is considered stable as long as the average difference between the baseline value and the time/temperature sample value is $\leq$ TEa/4.

   b. **For Testing Laboratory Specimen Stability Validation:**
   Each testing laboratory must perform its own specimen stability validation to meet regulatory requirements and confirm the data obtained from the BPT stability validation.
      - A sample is considered stable as long as the average difference between the baseline value and the time/temperature sample value is $\leq$ TEa/3.

10. Write a conclusion regarding acceptability of specimen stability for each condition.

Example: 7-day stability validation where the manufacturer's instructions state 24 hours:
BPT Validation:
a) Five samples tested on days 0, 2, 4, 6, 7 at each storage condition/temperature.
b) Testing Lab Validation:
c) Three samples tested on day 0 and again on day 7 at each storage condition/temperature.

Example: 14-day stability validation where the manufacturer's instructions state 72 hours:
BPT Validation:
a) Five samples tested on days 0, 4, 8, 12, 14 at each storage condition/temperature

b) Testing Lab Validation:
c) Three samples tested on day 0, 7 and 14 at each storage condition/temperature.

Notes on Stability Studies:

Specimen stability is considered a characteristic of the analyte itself and not necessarily dependent on how it is measured. However, there are conditions where differences in measurement may impact on the observed stability of the analyte. In some cases, specimen stability information may be applied across different instrument platforms for tests that do not involve immunoassays. Testing that involves an immunoassay of any type must be considered assay-specific, because the antibody reagent may react with a different epitope, but even if the same epitope, the reaction conditions may be completely different, resulting in different Antibody-Antigen interactions. Below is a partial list of specimen stability information that can or cannot be transferred between test systems.

a. CBC's should apply the same stability, regardless of what instrument is used for cell counting.
b. Analyte stability determined by GC or LC methods should be the same across all platforms of these technologies.
c. A chemical method for Homocysteine may show a different stability than that determined by an immunoassay; therefore, stability studies would need to be performed by each type.
d. Serum total calcium and ionized calcium are not the same analyte; therefore, each must have its own independent stability study performed.
e. Estradiol -6 and Estradiol 6-III are different analytes and involved different antibody reagents; therefore, should have their own separate stability studies performed (even though they are both performed on the same platform.)
f. A Centaur-CP test that uses the same reagent(s) as the corresponding test on the Centaur may use the same stability data.

Appendix K

Title	**Guidebook for Method Validation Data Analysis**
Prepared by	Rob Willis, Kathy Grimes

TABLE OF CONTENTS

1. PURPOSE

This document is intended to provide enhanced detail for definitions and regulatory requirements for method validation. This document also provides specific details about statistical analyses utilized in Quest Diagnostics method validation studies

2. SCOPE

This document is a companion to *Policy for Laboratory Method Validation of Quantitative and Semi-Quantitative Methods* (QDNQA743).

3. EXPANDED DEFINITIONS

- **Accuracy:** The closeness of a measurement to the target concentration.

- **Allowable Total Error (TEa):** 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 result, or incurring unsuccessful performance in proficiency testing surveys. The numeric values of TEa for a particular analyte are defined a Table on the Medical Quality website and are defined according to the document *Guidelines for Defining Quality Goals for Method Performance Requirements for Method Evaluations and for Quality Control* (QDQC 703) (see Related Documents). This document describes the process to integrate information for the purpose of establishing the Allowable Total Error specifications from a variety of source**s.**

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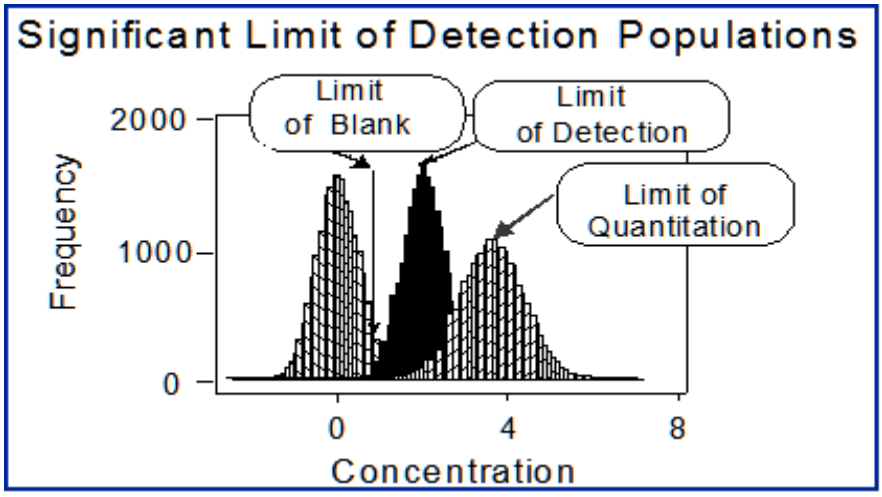
- **AMR Verification:** While Split Sample Comparison and Recovery studies evaluate for bias equally across the entire data set and often extend to a zero value, an AMR Verification study includes the additional element (to satisfy the regulatory and accreditation agency requirement) of an evaluation for accuracy specifically at concentrations near the low, middle, and high ranges of the AMR. AMR studies normally do not extend to zero. They extend only down to at or near the low AMR.

- **Analytical Measurement Range (AMR):** The range of analyte values that a method can directly measure without dilution or concentration.

- **Analyte Measurement Range Verification**: A verification study performed with matrix-appropriate materials (linearity, calibrator or QC materials) which evaluate the low, mid, and high range of the AMR. Target values are established by comparison with peer group values for reference materials, by assignment, by reference or comparison method values, and by dilution ratios of one or more specimens of known values.

- **Analytical Sensitivity and Detection Limits (CLSI, EP17):** The term Analytical Sensitivity describes the detection limits for the method. Analytical Sensitivity is evaluated by three distinct terms listed in the illustration below:



  - **Limit of Blank (LoB):**
    - LoB is the minimum concentration above the ambient background "noise" of the system (i.e. the s/n of the blank) at which the test system can reliably detect the difference between background "noise" and presence of analyte.
    - LoB is statistically considered the minimum analyte concentration that the test system can reliably detect analyte with 95% confidence. It is calculated as the mean value achieved from repetitive measurements of a blank + 2 * SD of those measurements.
    - Statistically speaking, results above the LoB have less than 2.5% probability of being absent of analyte. CLSI EP17 defines LoB as the 95th percentile of the ranked data.

- **Limit of Detection (LoD):**
  - The LoD raises the confidence that a positive result is truly positive by increasing the threshold from Negative to Positive an additional 2sd higher than the LoB.
  - LoD is calculated as the mean value achieved from repetitive measurements of a blank + 4 * SD of those measurements.
  - Results between LoB and LoD statistically suggest that analyte is "present." However, a sample that is truly negative may still be inappropriately characterized as positive since part of the 95% confidence interval around the LoD overlaps the Blank or <LoB region (see graphic above).
  - CLSI EP17 defines LoD as the mean value of a sample whose lower limit of a 95% Confidence Interval is equal to the LoB. Hence LoD is 2 SD's above LoB. LoD = Mean (of blank) + 4 * SD (of blank).
- **Limit of Quantitation (LoQ):**
  - LoQ is the lowest concentration at which analytical performance meets the laboratory's stated quality goals or requirements for that analyte (CLSI EP17). Unlike LoB and LoD, LoQ is not calculated as multiples of SD of the blank.
  - LoQ must be specifically determined by any lab developing a new method or modifying an existing method either developed by a Quest Diagnostics laboratory or a commercial vendor.
  - Labs are not required to determine the exact value for the LoQ, rather must verify that the test system is capable of achieving a reliable and reproducible result at or below AMR for EACH instrument using the same staff, equipment and instrumentation intended to be used for Production testing.
  - The LoQ study consists a precision study in which the precision at a single value just above the low AMR is calculated.
  - The LoQ is verified if the imprecision is less than TEa/3.
  - The LoQ must be at or below than AMR.
- The relationship between LoB, LoD, and LoQ may be summarized as $LoB < LoD \leq LoQ$.

- **Analytical Specificity/Cross-reactivity and Interfering Substances:** The ability of the assay to accurately measure the analyte of interest in the presence of other components present in the sample. This may be caused by cross-reactivity, inhibition or acceleration or reactivity, hemolysis, lipemia, anticoagulant, turbidity, icterus, gel barriers; patients' clinical conditions, disease states, medications, etc.

- **Best Practice Teams (BPT):** Teams who are responsible for general guidance and support of Quest Diagnostics laboratories. Each BPT is managed by a National Laboratory Operations (NLO) Manager and designated BPT Chair. These teams are cross functional in nature, and include representation from Regional Operations, Quality, Medical, Compliance and Materials Management.

- **Bias Assessment: AMR Verification, Split Sample Comparison and Recovery:** AMR Verification, **Split Sample Comparison** and Recovery are tools to evaluate for analytical bias. Each tool involves a regression analysis of target vs. achieved data pairs.

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- **Calibration Verification:** The process of confirming that the current calibration settings for each analyte remain valid for a test system throughout the AMR by assaying materials of known concentration in the same manner as patient specimens.

- **Clinical Significance:** Analytical performance that fails to achieve medical requirements is said to be "clinically significant."

- **Concentrations/Dilutions:** Any procedure applied to the system to expand the AMR by sample dilution or concentration, each of which must be separately validated.

- **Deming Regression:** Deming regression is a technique for fitting a straight line to two-dimensional data where both variables, X and Y, are assumed to possess random error. This is different from simple linear regression where only the response variable, Y, is measured with error. Deming regression is often used for method comparison studies in clinical chemistry to look for systematic differences between two measurement methods.

- **Investigational Use Only (IUO):** Investigational Use Only tests are intended for undergoing initial development and evaluation concurrent with clinical studies. These products may or may not be manufactured under FDA QSRs.

- **Linear Regression:** This describes a regression analysis between multiple sets of data pairs. Evaluating the slope and intercept of the regression is a statistically superior way to characterize "bias." For example, a slope of 1.05 would indicate a 5% increase in concentration between two sets of data, whereas a slope of 0.95 would indicate a 5% decrease in concentration.

- **Maximum Concentration/Dilution:** The maximum concentration and dilution allowable for a procedure. This is established as the highest dilution/concentration required to achieve a clinically meaningful result, or to limit the magnitude of allowable concentrations/dilutions to ensure a reliable/reproducible result.

- **Medical Decision Level (Xc):** A concentration of analyte at which some medical action is indicated for proper patient care. There may be multiple medical decision levels for a given analyte; for example, the upper and lower levels of the reference interval(s), priority values, etc.

- **Method Bias:** The difference in results obtained by two different methods. It is calculated as either the difference in mean values by each method, the average of the paired differences, or the slope and intercept achieved by a linear regression of the paired data.

- **Method Validation:** A defined process by which a laboratory confirms that a laboratory developed test (LDT) or Modified FDA-cleared or approved test perform as claimed.

- **Method Verification:** The process by which a laboratory determines that an unmodified FDA Cleared or Approved test performs according to the specifications set forth by the BPT and as specified in this document.

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- **Nonwaived Test System:** Tests categorized as either moderately complex (including provider-performed microscopy) or highly complex by the US Food and Drug Administration (FDA), according to a scoring system used by the FDA

- **Precision:** The agreement among replicate measurements. 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. Also referred to as imprecision, where the higher the imprecision, the higher the SD).

- **Primary Standard Material:** Substance of known chemical composition and sufficient purity used in preparing a Primary Standards Solution. These are recognized by national or international standardization organizations. Examples include Standard Reference Materials (SRM from NIST, National Institutes of Standards and Technology), Certified Reference Materials (CRM) and International Standards (from WHO- World Health Organization).

- **Primary Standards Solution:** Solution used as a calibration standard in which the concentration is determined solely by dissolving a weighed amount of Primary Standard Material in an appropriate solvent, and making a stated volume or weight.

- **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."

- **Quantitative Test System:** An assay that produces measurements in continuous numerical values based on a standard curve and on a signal (e.g., light) measured by an instrument (e.g., relative light units).

- **Recovery:** Recovery is where one set of data is anticipated to be accurate and the second set potentially lost or gained during the analysis. Often Recovery is the term used to measure degradation caused by analyte instability, interferences, etc. It can also be used to determine the efficiency of a chemical or enzymatic hydrolysis to liberate a target molecule which will be measured. For the purpose of this procedure, Recovery refers to the comparison of values against samples with known accurate concentrations.

- **Reference Interval:** 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.

- **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.

- **Random Error (RE):** An error in measurement that is unavoidable, and thus cannot be eliminated. Often, it is synonymous with imprecision. Typically, it assumes a symmetrical and often a Gaussian distribution about a mean value (positive and negative values).

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- **Reportable Range:** The entire span of result values over which the laboratory can establish or verify the accuracy of the instrument or test system measurement response.

- **Research Use Only (RUO):** Research Use Only labeled products are those that are intended and marketed by the manufacturer for performing basic scientific research in support of a diagnostic hypothesis or intended use for a new diagnostic device. These products are usually not manufactured under FDA QSRs.

- **Semi-Quantitative Test System:** A test system that produces a signal that is measured and interpreted by laboratory staff based on laboratory cutoffs and reported as qualitative statements (e.g. “negative,” “positive,” “equivocal,” “positive” at dilutions, titers, etc.).

- **Standard Deviation:** A statistic used to describe the distribution or spread of data in a population (that is shown to have the shape of a normal or Gaussian curve).

- **Systematic Error:** An error that is not determined by chance but is introduced by an inaccuracy (as of observation or measurement) inherent in the system. See definition for Bias above.

- **Test System:** The process that includes pre-analytic, analytic, and post-analytic steps used to produce a test result or set of results. A test system may be manual, automated, multi-channel or single-use and can include reagents, components, equipment or instruments required to produce results. A test system may encompass multiple identical analyzers or devices. Different test systems may be used for the same analyte.

- **Total Error (TE, TEc, TEa):** The combination of random and systematic analytical errors: an estimate of the magnitude of error that might occur in a single measurement. TEc refers to the calculated estimate of the total error of an assay. TEa refers to the maximum allowable total error of an assay.

- **Waived Test System:** A category of tests defined as "simple laboratory examinations and procedures which have an insignificant risk of an erroneous result." Laboratories performing waived tests are subject to minimal regulatory requirements.

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4. REQUIREMENTS

Below is an excerpt from the CAP All Common checklist. CAP recognizes only two classifications: (1) unmodified FDA Cleared or Approved, and (2) all others which include any Modified FDA Cleared or Approved and LDT.

> *For unmodified FDA-cleared or approved tests, the laboratory may use information from manufacturers, or published literature, but the laboratory must verify such outside information on accuracy, precision and reportable range.*
>
> *For tests that are not FDA-cleared or approved (including tests developed in-house), or for FDA-cleared/approved tests modified by the laboratory, the laboratory must establish accuracy, precision, analytical sensitivity, interferences, analytical specificity, and reportable range, as applicable; data on interferences may be obtained from manufacturers or published literature, as applicable.*

- **FDA Cleared or Approved Test Systems:** Each laboratory must complete verifications for accuracy, precision, reportable range, reference interval and carry over (if applicable) before introduction into the laboratory.
- **LDTs or Modified FDA-cleared or approved Test Systems:** In addition to verifications listed above for unmodified FDA Cleared or Approved test systems, each laboratory must complete additional verifications for analytical sensitivity, interferences, and analytical specificity.
- **Waived Test Systems:** Prior to implementing a Waived test system (see definitions), laboratories must verify accuracy by performing a split sample comparison with another instrument currently in routine use for patient testing, with another laboratory using the same equipment, by comparing to purchased traceable material, or with samples provided by the device vendor. Split sample comparison studies may be based on fewer samples than required below for FDA Cleared or Approved test systems. A verification of the applicability for the reference intervals specified by the manufacturer may be completed as part of the split sample comparison. Precision studies should also be performed for quantitative tests, where practical.
  Note 1: It is critical that laboratories follow the manufacturer's instructions for Waived test systems as written. Failing to follow the instructions could cause the test to be reclassified as a high complexity, non-waived test system, thus subjecting it to more stringent regulatory requirements.
  Note 2: If the laboratory modifies the manufacturer's instructions, the test may no longer be considered an unmodified FDA Cleared or Approved test. The test system must be validated by the laboratory, and all requirements for a high complexity, non-waived test systems apply. Changes in the sample stability, specimen type or collection device are examples of common modifications.
  Note 3: Refer to manufacturer for tools and materials for performing Method Validation studies.
- Unmodified FDA Cleared or Approved Test systems where result values near the low AMR are more clinically significant than the rest of the AMR (e.g. PSA) require that analyte sensitivity be verified.
- Additional verifications may be required by Quest Diagnostics (e.g. reference intervals, carryover, etc.).

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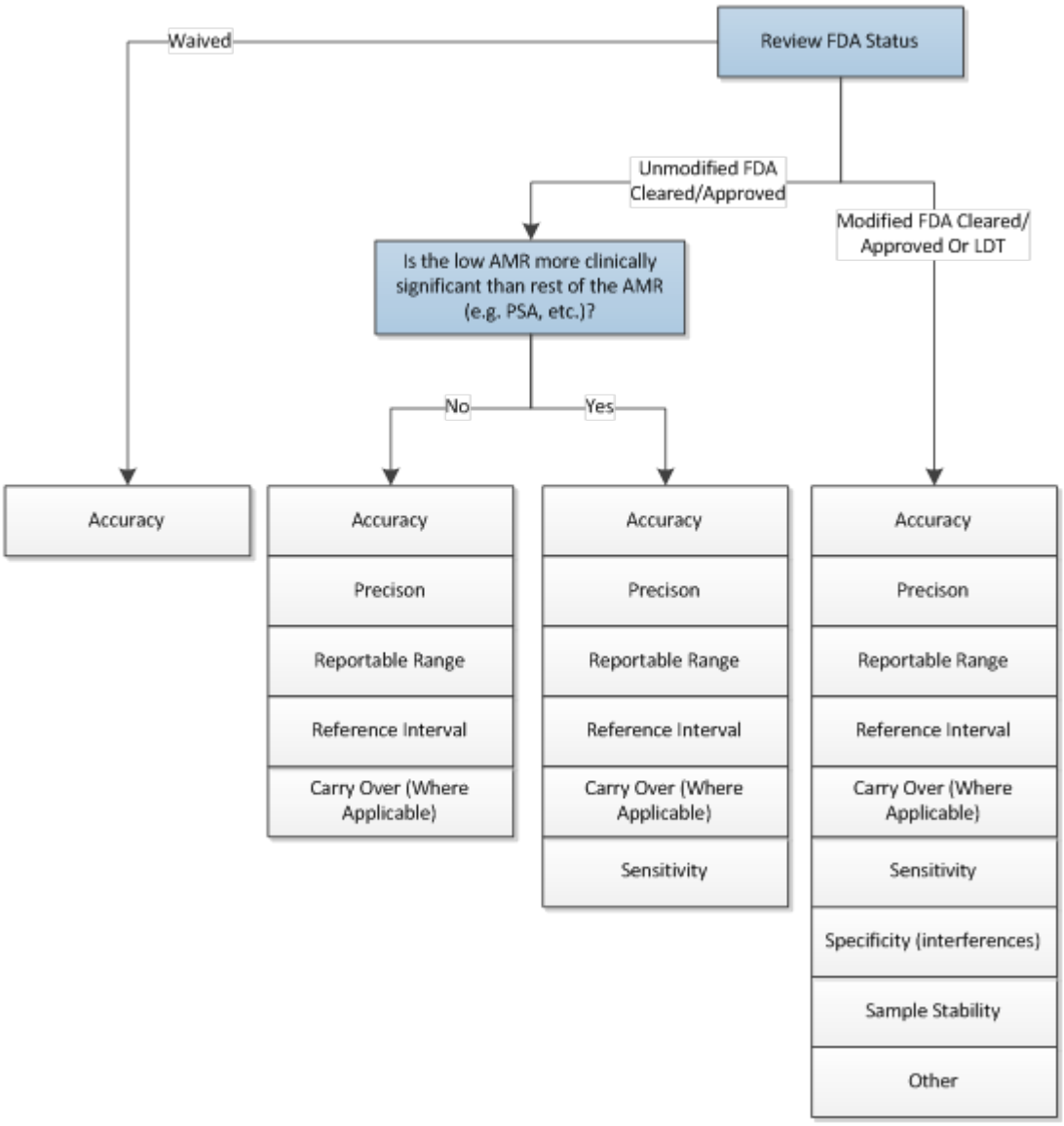
- All method verifications/validations must be approved by the Laboratory Director prior to implementing the test or test system for patient care.

5. METHOD VERIFICATION / VALIDATION STUDIES

The following provide a description of each Method Verification study and a reference to the addendum which provides additional detail.



6. ACCURACY

Correlation studies are statistically evaluated to document Accuracy using the Comparison sheet of the *Method Verification Template - Quantitative (QDNQA356).* Data should be analyzed by Least Squares or Deming regression. The slope and intercept with their accompanying statistical errors should be reviewed. If interpretation of the regression analysis is difficult to interpret, the analysis should be referred to Corporate Medical Operations and Quality.

7. PRECISION

- Precision studies are statistically evaluated using the Precision sheet of the Method Verification /Validation workbook. Analyze data by calculating the mean and standard deviation, and hence and coefficient of variation (CV) for each control level.
- For modified or unmodified FDA Cleared or Approved tests, total precision is determined by measuring each QC level 5 times per day for 5 separate days. If it is necessary to complete the Precision study over a shorter time frame than 5 separate days, each set of 5 QC values must be in separate runs and an acknowledgement of the shortened time-frame documented in the final precision study.
- A verification of precision is not required for Waived testing, unless specifically required by the applicable BPT or Laboratory Director.

8. REPORTABLE RANGE

- Reportable range is verified by (1) completing an AMR Verification study and (2) confirming that all manual and instrument on-board dilution/concentration protocols produce accurate and reproducible results.
- AMR Verification may be completed by running commercial linearity material, traceable materials, or by diluting high patients to span the AMR.
- AMR Verification must include a minimum of at least one sample within +1.5 TEa of the low AMR, one within -1.5 TEa of the high AMR, and one near the middle of the AMR.

  Note 1: Regulations require that "low, middle and high" values be evaluated with each AMR Verification study. The QC BPT has provided a guideline of 1.5 TEa for these tolerances.

  Note 2: It is understood that such "one size fits all" guidelines will not work for every test system. For example, commercial materials may not be available within these limits, or diluting samples may not be possible. Consult your Laboratory Director if you are unable to acquire a sample that falls within 1.5 TEa limit of AMR. If your Laboratory Directory determines that your data adequately achieves the regulatory / accreditation requirement of including "low, middle, high" values, you may document the issue in the Method Verification workbook as an acceptable exception.

- AMR Verification is always required for Method Verification, but once verified, subsequent AMR Verification studies may not be necessary if (1) successful calibrations are performed more often than every six months and (2) the test includes three or more calibrators that adequately span the AMR. (See Corporate SOP QDQC704 – "Analytical Measurement Range (AMR) Validation and Calibration Verification").

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9. REFERENCE INTERVAL

- In the evaluation of the Reference Interval, as per CLSI guidelines, the following approaches may be taken:
  - **Establishing (or determining) a reference interval** –the process used in creating a reference interval de novo, encompassing all of the steps from selection of reference individuals, through exact details of the analytical methods, and concluding with data collection and analysis.
  - **Transferring a reference interval (transference applied)** – the process by which one may be able to adapt a previously established reference interval to a new analytical method or to a new location.
  - **Verifying (or validating) a reference interval** – the process by which one ensures, with reasonable confidence, using relatively few reference individuals (e.g., n = 20), that a reference interval established elsewhere, or transferred from another study, can be used locally.
    - BPT validations may undergo full multicenter reference interval studies (as per CLSI). Once the common set of reference intervals is ***established***, each individual laboratory then has only to ***validate*** these reference intervals in its own environment
    - The transference of reference values requires that certain conditions be fulfilled in order to be acceptable. Assuming the original reference value study was done properly, the transference of the respective reference interval involves two distinct issues:
      - Comparability of the analyte system
      - Comparability of the test subject population
    - If the reference interval for the new assay differs from that of the current assay/platform, each laboratory must perform a reference interval verification/validation study.
    - If a formal reference interval study is not possible or practical, the laboratory should carefully evaluate the use of published data for its own reference intervals. Contact CQA/MOQ for assistance.
    - Reference Interval verification studies are statistically evaluated using the Reference Interval sheet of the *Method Verification Template - Quantitative*. Contact CQA/MOQ for assistance with establishing reference interval statistical evaluation.

If	Then
If the Split Sample Comparison study shows that the bias is acceptably small (Bias < TEa/4)	Validation of the Reference Interval is achieved by transferability of the current reference interval limits.
If the reference interval for the new assay differs from that of the current assay/platform	Each laboratory must perform a reference interval validation study (cannot apply the principle of transference towards a new assay with a different reference interval).
If a formal reference interval study is not possible or practical	The laboratory should carefully evaluate the use of published data for its own reference intervals, and retain documentation of this evaluation.

10. RELATED DOCUMENTS

- Quest Diagnostics Policy for Laboratory Method Validation of Quantitative and Semi-Quantitative Methods (QDNQA743)
- Quest Diagnostics Method Validation Template - Quantitative (QDNQA356)
- Quest Diagnostics Guidebook for Method Validation Data Analysis (QDNQA413)

11. REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Wayne, PA,
2. CLIA 88 Laboratory Regulations 42 CFR (Code of Federal Regulation), 493.1253
3. CAP Checklists: All Common, Laboratory General and other Specific checklists as appropriate.

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