TRAINING UPDATE

Lab Location:	GEC, SGAH & WAH	**Date Distributed:**	7/1/2014
Department:	Mgmt, QA and Technical Specialist	**Due Date:**	7/31/2014
		Implementation:	**8/1/2014**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Laboratory Method Validation Protocol

GEC / SGAH / WAH. QDQC710v4.1

Description of change(s):

Section	Revision
4 *	Added definitions for reportable range, analytical sensitivity, verification, validation and removed CRR
8 *	Revised Vendor Supported Validations
9, 13 17	Adopting corporate issued version 4 with revisions dated 3/14/13 above. Additional local revisions: ** Added local validation protocol Added local file locations

* Revisions to section 4 and 8 were made by the QC BPT

** Local revisions are **NOT** new; these were previously added to version 3 of the BPT SOP.
The validation spreadsheet has **NOT** been revised

This revised SOP will be implemented on August 1, 2014

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

Non-Technical SOP

Title	**Laboratory Method Validation for Quantitative and Semi-Quantitative Methods**
Prepared by	Carl Garber, Ph.D., Rob Willis, Dianne Zorka, QCBPT **Date: 3/17/2014**

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: 5/5/2014**
Print Name and Title	**Signature**	**Date**
Jerry Wagner, Ph.D. **Owner**	*On file*	**4/29/2014**
Martin Kroll, M.D. **BPT Medical Advisor**	*On file*	**4/29/2014**
Stephen Suffin, M.D. **Chief Laboratory Officer/Designee**	*On file*	**5/2/2014**

Retirement Date:	
Reason for retirement/replacement:	

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1. PURPOSE

This document describes the requirements for the establishment and verification of performance specifications for any quantitative or semi-quantitative test or test system introduced into the laboratory. Within this laboratory, the establishment and/or verification of test performance specifications is equated with the phrase "Method Validation".

2. SCOPE

- This document applies to all tests and / or test systems as described below that are introduced into the laboratory. This includes:
  - A test that is introduced for the first time to measure an analyte the laboratory has not previously measured.
  - A test that is introduced for the first time into the laboratory for an analyte that the laboratory currently performs on an alternative test system (e.g., instrument A has been used for cholesterol and now instrument B will be used).
  - 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).
  - An analyte is added to a test system that can measure multiple analytes which the laboratory has been using for patient testing but has not previously reported patient results for the particular analyte.
  - A modification to a test or test system that the laboratory has been using for patient testing (See definitions for Modification of Manufacturer's Instructions).
  - Local implementation of national Quest Diagnostics standard testing processes.
  - Quantitative and Semi-Quantitative systems.
- This document applies to Quest Diagnostics owned and operated laboratories including Rapid Response Laboratories (RRL).
- This document **does not** address the method validations of qualitative or other unique test systems (i.e., Microbiology, Cytogenetics).

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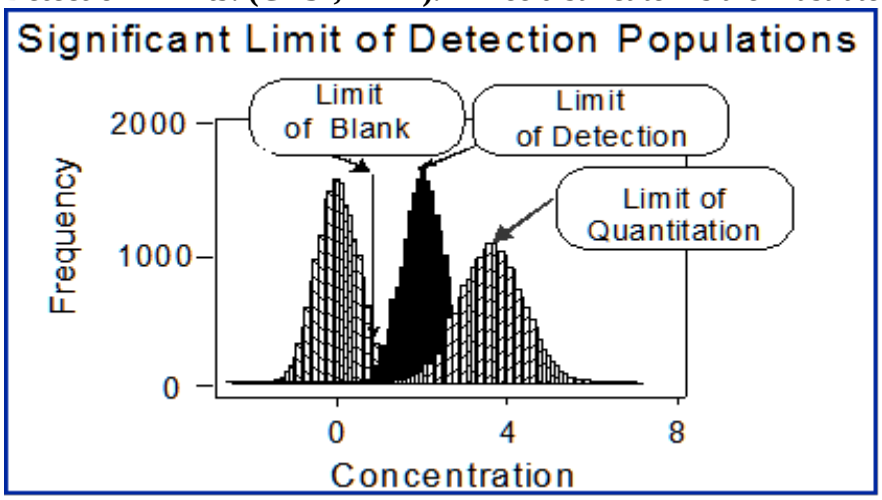
3. RESPONSIBILITY

- **Testing Personnel** are responsible for:
  - Following the verification/validation protocol as designed.
  - Following the test protocol.
  - Documenting all steps of the method verification/validation process.
- The **Department Technical Supervisor** is responsible for:
  - 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 all staff is appropriately trained.
- The **Quality Assurance Department** is responsible for:
  - Reviewing method verification/validation packages
  - Maintaining method verification/validation packages (except for Rapid Response Laboratory (RRL) packages where the original is kept at the RRL)
- The **Laboratory Director** is responsible for:
  - Approval of the initial document and any subsequent revisions.
  - Approval of all method verifications/validations prior to patient testing.
    - **Note:** This may be designated to an individual who meets CAP director qualifications, except in states where the laboratory director cannot delegate this responsibility.
- The **Laboratory Director or Designee** is responsible for recurring review of this document.

4. DEFINITIONS

- **Accuracy:** The closeness of a measurement to the true concentration, trueness.
- **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 the maximum amount of error allowed for successful performance in proficiency testing. The numeric values of TEa for a particular analyte are defined in the Table of this name on the Medical Quality website and are defined according to the document "Guideline for Defining Allowable Total Error for Method Validation and Quality control (QDQC 703). This document describes the process to integrate information from a variety of source**s.**
- **Analytical Measurement Range (AMR):** AMR is the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process.
- **Analyte Measurement Range Validation**: A validation study performed with matrix-appropriate materials (calibration materials or control materials) which include the low, mid, and high range of the AMR. Target values can be 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:** The term Analytical Sensitivity is related to the detection limit.
- **Analytical Specificity/Cross-reactivity and Interfering Substances:** The ability of the assay to measure the analyte of interest in the presence of any other component present in the sample that may cause interference. This could be caused by cross-reactivity, inhibition or acceleration or reactivity, hemolysis, lipemia, anticoagulant, turbidity, icterus, gel barriers; patients' clinical conditions, disease states, and medications, etc.

- **Best Practice Teams (BPT):** Teams who are also responsible for general guidance and support of Quest Diagnostics laboratories. Each BPT is managed by a National Testing Operations (NTO) Manager and designated BPT Chair. These teams are cross functional in nature, and include representation from BU Operations, Quality, Medical, Compliance and Materials Management.
- **Calculated Total Error (TEc):** The calculated total error is a calculated estimate of the total error of an assay, the combination of imprecision and inaccuracy (or bias).
- **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 of materials of known concentration in the same manner as patient specimens.
- **Clinical Significance:** Analytical performance that exceeds medical requirements is said to be "clinically significant". Clinical Significance as opposed to statistical significance (see below), is the main issue that needs to be addressed in the assessment of method performance.
- **Detection Limits: (CLSI, EP17).** Three distinct terms are illustrated in this graphic.



- **Error Budgeting:** A process of allocating a certain portion of the total error to different causes, so that the sum total of all causes does not add up to a quantity that is greater than the allowed total error for a particular measurement.
- **Establish:** To perform studies to determine performance specifications which provides evidence that the accuracy, precision, analytical sensitivity, and analytical specificity of the procedure is adequate to meet the authorized provider needs as determined by the laboratory director or clinical consultant. This applies to laboratory developed tests and modified FDA Cleared or Approved tests.
- **Limit of Blank (LoB):** "highest measurement result that is likely to be observed (with a stated probability) for a blank sample" (CLSI EP17). We interpret this to mean the upper limit of a 95% confidence interval of a series of measurements of a zero standard or blank sample. Results less than or equal to the LoB are indicative of analyte being absent or "not detected". Results above the LoB have less than 2.5% chance of being zero, so should be reported as "detected". Typically this is calculated as the mean value of a blank sample + 2 * SD of the measurements of that blank sample. For non-Gaussian data, the LoB can be defined as the 95th percentile of the ranked data.
- **Limit of Detection (LoD):** Minimum amount of analyte whose presence can be qualitatively detected reliably under defined conditions (CLSI EP17). This is 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. Assuming the SD of the "LOD sample" is about the same as the SD of the blank, then LoD = Mean (of blank) + 4 * SD (of blank).
    - Results between LoB and LoD indicate analyte is "present". But a sample in this region may not always be detected since part of its 95% confidence interval overlaps the Blank region.
    - Results at the LoD or greater, will be "detected" at least 97.5% of the time.
    - Note, at LoD, we cannot be sure of the value; only that analyte is present or "detected".
- **Limit of Quantitation (LoQ):** LoQ is the lowest concentration at which analytical performance meets the laboratory's stated quality goals or quality requirements for that analyte (CLSI EP17).
    - If bias is small, then the LoQ is the lowest concentration at which Total SD < TEa/3.
    - The LoQ may be equal to the LoD (if the data warrants), but cannot be less than LoD.
    - The lower limit of the Analyte Measurement Range (AMR) cannot be less than LoQ.
    - When specifying the LoQ, restate the quality goal (TEa) that is being met by the LoQ.
    - **Relationship between LoB, LoD, and LoQ: LoB < LoD </= LoQ**
- **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; at a minimum the upper and lower levels of the reference interval(s) should be included.
- **Method Bias:** Difference in results obtained by two different methods. It is calculated as the difference in mean values by each method, or the average of the paired differences.
- **Method Validation:** Within this laboratory, a method validation is understood to mean either the verification or establishment of performance specifications as outlined in this policy document.
- **Modification of Manufacturer's Instructions**: 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 procedure that could affect its performance specifications for sensitivity, specificity, accuracy, or precision or any change to the stated purpose of the test, its approved test population, or any claims related to interpretation of the results

  Changes may include (but are not limited to) any change in:
    - Intended use by using a different sample matrix (e.g., plasma vs. urine)
    - Intended use by promoting the test for another purpose (e.g., screening vs. diagnostic)
    - Intended use by changing the type of analysis (e.g., qualitative results reported as quantitative)
    - Specimen handling instructions including change from duplicate to single testing
    - Incubation times or temperatures
    - Specimen or reagent dilution
    - Calibration material (e.g., using a different calibration material or changing the manufacturer's set points)
    - Antibody (e.g., introducing a different antibody, source, monoclonal vs. polyclonal)
    - Or elimination of a procedural step
    - Or addition of detector (conjugate) or substrate
    - The solid phase
    - The cutoff or method of calculating the cutoff for semi-quantitative assays
    - The endpoint or calculation of the endpoint
    - Or addition of adsorbent
    - The strain of antigen in serologic assays
    - The calibrator/reference material

- **NTO:** National Testing Operations is the organization within Quest Diagnostics that supports and directs the selection of tests and test methods.
- **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 to be used in preparing a Primary Standards Solution. These should be 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 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). These measurements are reported to, and interpreted by, the authorized provider, based on numerical cutoffs (e.g., reference ranges).
- **Random Error (RE):** An error, either positive or negative, the direction and exact magnitude of which cannot be predicted. It is usually expressed as some multiple of SD or Coefficient of Variation (%CV) and measured by within-run and between-run precision studies; random error is commonly referred to as "imprecision."
- **Reference Interval:** The term "Normal Range" is obsolete and should not be used. The Reference Interval is defined as the central interval of values bounded by an upper and lower limit at certain designated percentiles, like the 2.5% and 97.5% to achieve a central 95% reference interval.
- **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.
- **Reportable Range:** Reportable range means the entire span of test result values over which the laboratory can establish or verify the accuracy of the instrument or test system measurement response. There are two components used to determine reportable range:
    1. The primary range of measurement; the Analytical Measurement Range (AMR)
    2. Anything done to the system to expand the AMR: sample dilution, or sample concentration, each of which must be separately validated;
        - The entire span of values that can be reliably measured using these modifications is the reportable range.
- **Semi-Quantitative Test System:** A test system that produces a signal that is measured and interpreted by laboratory staff based on laboratory cutoffs without a standard curve and reported to the authorized provider as qualitative statements selected by laboratory staff (e.g. "negative," "positive," "equivocal," "positive" at dilutions, titers, etc.).
- **Standard Deviation:** A statistic used to describe the distribution or spread in data in a population (that is shown to have the shape of a normal or Gaussian curve).
- **Statistical Significance:** A level of performance that causes the rejection of a null hypothesis for a given statistical test of significance, such as an F-test, or a t-test, or a chi square test. Such

a test is "interesting", but is not the fundamental issue in assessment of method performance relative to medical requirements. (See Clinical Significance above.)

- **Systematic Error (SE):** An error that is always in one direction: inaccuracy.
- **Test System:** The complete testing system used to analyze a patient sample or batch of samples in order to produce a test result. This system may include reagents, culture media, manufactured kits, major and minor instrumentation and sampling devices such as pipettes.
- **Total Error (TE):** 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.
- **Validation:** A defined process by which a laboratory confirms that a laboratory developed test (LDT) or modified FDA-cleared/approved test performs as intended or claimed.
- **Verification:** The process by which a laboratory determines that an FDA-cleared/approved test performs according to the specifications set forth by the manufacturer.

5. 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 laboratorymust verify:

- Accuracy
- Precision
- Reportable Range (AMR and dilution/concentration protocols and maximum allowable dilution/concentration)
- Reference Interval:
    - If a formal reference interval study is not possible or practical, then the laboratory should carefully evaluate the use of published data for its own reference intervals, and retain documentation of this evaluation.

In addition the following must also be verified, as applicable:

- Carry Over: If test the system uses an integrated or stand-alone automatic pipetting system that does NOT use single use (disposable) tips, and if there is more than a 100 fold difference between the upper and lower limits of the Reportable Range.

6. Modified FDA-Cleared or Approved Test Systems OR Laboratory Developed Tests (LDT)

Prior to reporting test results 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 the following performance characteristics as applicable and show that they meet the laboratory's stated acceptance criteria:

- Accuracy
- Precision
- Reportable Range (AMR and dilution/concentration protocols and maximum allowable dilution/concentration)
- Analytical Sensitivity (applicable when low end values are clinically significant).
- Analytical Specificity (including interfering substances as applicable; data on interferences may be obtained from manufacturers or published literature)
- Reference Interval (If a formal reference interval study is not possible or practical, then the laboratory should carefully evaluate the use of published data for its own reference intervals, and retain documentation of this evaluation).

In addition the following must also be validated, as applicable:

- Any other performance characteristic required for test performance (including Stability if different than package insert; see also definition of **Modification of Manufacturer's Instructions** above). Stability studies performed in another Quest Diagnostics owned laboratory can be used provided the testing and its conditions are identical (documentation of the studies must be available in the laboratory).
- Carry Over (if test system uses an integrated or stand-alone automatic pipetting system that does NOT use single use (disposable) tips)

7. Quest Diagnostics Standard Testing Processes

- When tests or test systems are introduced at the direction of the Quest Diagnostics national initiatives, standard method validation packages will be used as directed by NTO.
- Stability studies may be performed in one lab and used in other Quest labs provided the conditions in which the test is performed or the specimen is processed do not change at any of those other sites; i.e., test kit used, equipment, shipping, handling, immediate environment, etc. If conditions are different, then each lab would have to perform their own establishment for stability (stability studies). Stability studies must be included in the laboratory validation package.
- Additional validations may be added to support compliance with state or local laboratory requirements.

8. VENDOR SUPPORTED VALIDATIONS

(This section is not applicable for laboratories located in California)

- 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.
- 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.
- A vendor may perform validation as a part of training for Quest Diagnostics testing personnel.
- The vendor's training should use this time 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 vendor may take copies of validation data if data reduction is performed off site. These copies must be returned to the lab when the data reduction is completed.
- If vendor staff must perform data entry off site, they may take copies of data provided all Patient Health Information (PHI) has first been removed by the supervisor.
- A vendor may perform special studies, such as the "evaluation" of a new lot of reagent prior to its being "accepted" for shipment to Quest Diagnostics labs under the following guidelines:
- The vendor technical representative may perform these studies under the direction of local laboratory supervision.
- Testing is performed per specific protocols agreed to by Quest Diagnostics and the vendor
- All original data must reside in the Quest Diagnostics laboratory.
- Every laboratory (including those where this acceptance testing was performed) will perform the standard new lot checkout procedure per Quest Diagnostics Standard Procedures upon receipt of these previously accepted lots.

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9. DEFINE THE VALIDATION PROTOCOL

A. Define Performance Requirements

- It is the responsibility of the Best Practice Team and/or designee(s) to define performance requirements (consistent with Quest Diagnostics process for defining Quality Goals) and to design a protocol that enables the laboratory to establish or verify performance specifications.
- 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 Total CV), Includes Intra-Assay and Inter-Assay	< TEa/3
Accuracy: Bias (or average) difference at Medical Decision Level.	< TEa/4

- When test(s) or test systems are introduced at the direction of Quest Diagnostics national programs, the laboratory will follow performance requirements defined nationally as closely as practical.
- Precision and accuracy may be defined explicitly in terms of maximum CV and maximum bias by some agencies.
- If any Laboratory Director wishes to:
  - Define a different specification for TEa or a different error budgeting model, that Laboratory Director should submit the recommendation to Corporate Medical and to the QC BPT, to consider updating the standard table;
  - Define a TEa specification for which there is no previously defined specification at Quest Diagnostics, that Laboratory Director should submit their recommended specification to Corporate Medical and the QC BPT for consideration to expand that standard table.

See the separate Quest Diagnostics policy on *Quality Goals for Method Performance Requirements for Method Evaluations and for Quality Control* for more details.

B. Define in writing the components to be included in the Method Validation. Refer to the local QA procedure "Process and Equipment Validation Protocol" (QA46).

- The protocol must include (as applicable) a general description of the Method Validation:
  - Description of test
  - Instrument identifier
  - Reagents used
  - Sample type (s)
  - Acceptability criteria for:
    - Accuracy
    - Precision
    - Analytical Sensitivity (LoQ)
  - 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)
- Any other performance characteristic required for test performance

NOTE: When samples types not included in the FDA approved/cleared package insert will be accepted by the laboratory, additional sample types will be included in the validation (e.g., body fluids, fecal samples, etc.). (See also QDQC711_Body Fluid Validation.doc.). Procurement of unconventional samples may be difficult for validation; hence, the Laboratory Director and/or designee should contact the Best Practice Team for assistance in sample procurement.

- Specific details of a Method Validation may vary. The following table gives guidance for consideration when defining required elements of a validation.

If…	**Then…**
Low values are not important, such as serum Ca or serum Total Protein.	Not necessary to perform LoQ, but Still perform LoB and LoD.
Analyte can't be diluted, such as for Prothrombin Time (PT), aPTT or T3Uptake.	Can't perform AMR verification (previously known as linearity).
There are no calibrators or standard materials for the assay	Cannot perform "calibration verification".
Lab is performing the test on a platform different from the standard platform recommended by the BPT.	• Laboratory should apply interference data and stability information from their platform's package insert (PI). • Differences from the standard platform PI must be reviewed with the BPT.
There is no commercial Proficiency Testing program for this analyte.	An acceptable Alternate Performance Acceptance (APA) process must be documented.
A main lab laboratory is performing this validation study.	The patient sample correlation study: • New assay versus the current assay if currently being tested by a different platform. • If the assay is new to the laboratory correlation is performed with a laboratory that has been performing the test.
A Rapid Response Laboratory (RRL) is performing this validation.	The patient sample correlation study: Current platform in use at the RRL AND their main lab.
For all patient comparison studies	A minimum of 20 samples must be tested over a minimum of 3 – 5 days. Testing all samples on one day is **not acceptable**.
The Split Sample Correlation versus the current assay 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. Per CLSI C28-A2 "How to define and Determine Reference Intervals in the Clinical Laboratory" Section 8: "Transference and Validation". A separate reference interval study is not necessary.
If the reference interval for the new assay is different from that for the current assay/platform	Each laboratory performs a reference interval validation study, since it is not possible to apply the principle of transference for a new assay that is different.
There may be other special cases that the BPT with their Medical Advisor may define that may impact on the specific details of the lab validation study for a given analyte.	

C. Define in writing how the laboratory will perform all the components defined in the steps above. Guidance for performing the validation is given in the attached addenda.
D. NOTE: 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.

10. PERFORM METHOD VALIDATION

A. Perform the method validation using parameters and requirements defined in the steps above and/or provided by BPT.
B. Validation experiments are to be conducted on the laboratory's equipment and within the laboratory environment.
C. Validation must be performed on EACH analyte performed on a platform.
D. Whenever possible, the validation process should mimic the expected operations specimen number per run. This will help to determine the presence and extent of any front to back assay variations (caused by dilutors, etc.) in high volume assays.
E. The laboratory must define, perform and document maintenance and function checks on all equipment used for the method validation.
F. For all steps of the validation process, the laboratory must document:
   - Unique instrument identifier(s)
   - Reagent lot numbers and expiration dates
   - Quality Control material lot numbers and expiration dates
   - Dates of testing
   - Names of operators
G. All approved specimen types are to be validated using the stated parameters for the respective assay type; i.e., quantitative, semi-quantitative and qualitative and by regulatory status.
H. Competent and licensed (if required by State regulations) Quest Diagnostics staff are responsible for performing validation studies; see exceptions under Vendor Supported Validations above.
I. Method Validations 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 very wide (more than 100 fold).

11. METHOD VALIDATION REPORT

A. Enter data into the Validation of a Quantitative Assay Template (See Appendix A), the template provided by BPT.

B. Collect, analyze and present the method validation data for the approval process. Final method validation package must include a summary of findings and a statement of acceptability. For an example of a summary page with all appropriate conclusions, see summary or cover page in Appendix A.

C. All method validations studies must have Laboratory Director (or designee) approval prior to patient results being tested.

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12. RECORDS MAINTENANCE

Records are maintained according to the requirements for Method Validation records published in the Quest Diagnostics Records Management Program Reference Guide.

13. RELATED DOCUMENTS

- QDMD731_Body Fluid Validation.doc and template QDMD731 Body Fluid Template.xls (These will be renumbered to QDQC711)
- QDQC703a Quality Goals for Method Performance Requirements for Method Evaluations and for Quality Control. http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/Quality_Goals.doc
- Allowable Total Error Table http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
- 130SM090 Specimen Stability, San Juan Capistrano. Please contact the QA Department at San Juan Capistrano for a current copy.
- Additional, educational material may be available on the Corporate Medical Intranet Website.
- Process and Equipment Validation Protocol, local Quality Assurance procedure

14. REFERENCES

- Clinical and Laboratory Standards Institute (CLSI) guideline EP5, Evaluation of Precision Performance of Quantitative Measurement Methods, Wayne, Pa,
- Clinical and Laboratory Standards Institute (CLSI) guideline EP6, Evaluation of the linearity of quantitative analytical methods, Wayne, Pa,
- Clinical and Laboratory Standards Institute (CLSI) guideline EP7, Interference testing in clinical chemistry, Wayne, Pa,
- Clinical and Laboratory Standards Institute (CLSI) guideline EP9, Method comparison and bias estimation using patient samples, Wayne, Pa.
- Clinical and Laboratory Standards Institute (CLSI) Guideline EP10, Preliminary Evaluation of Quantitative Clinical Methods, Wayne, PA,
- Clinical and Laboratory Standards Institute (CLSI) guideline EP15: User Demonstration of Performance for Precision and Accuracy, Wayne, PA,
- Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation, Wayne, PA,
- Clinical and Laboratory Standards Institute (CLSI) guideline C28-A2: How to Define and Determine Reference Intervals in the Clinical Laboratory, Wayne, PA,
- Garber CC and Carey RN, "Evaluation of Methods" In: Kaplan LA, Pesce AJ, and Kazmierczak SC, Eds. Clinical Chemistry: theory, analysis and correlation. 4th Edition. Mosby Co., St. Louis, 2003 (Ch 22): 402-426.
- Carey RN, Garber CC, and Koch DD (Method Evaluation Workshop, presented at AACC annual meetings, 1976 to 2007)
- ISO/IEC Guide 99: International vocabulary of metrology – Basic and general concepts and associated terms (VIM), International Organization of Standardization (ISO), Geneva, 2007 draft. (Final version printed/issued in 2008)
- Sasse EA "Reference Intervals and Clinical Decision Limits" In: Kaplan LA, Pesce AJ, and Kazmierczak SC. Eds. Clinical Chemistry: theory, analysis and correlation. 4th Edition. Mosby Co., St. Louis, 2003 (Ch 20): 362 – 378.
- CLIA 88 Laboratory Regulations 42 CFR (Code of Federal Regulation), 493.1253
- CAP Checklists: All Common, Laboratory General and other Specific checklists as appropriate.

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15. DOCUMENT HISTORY

Version	Date	Section	Revision	Revised By	Approved By
3.0	06/02/11		Revised Addendum L for TDM per recommendation by Dr. Suffin and added Addendum M for Multiplexed HPLC Carryover	S Daly, C Garber, and S Wernlund	S. Suffin, M.D.
4.0	3/14/2014		General formatting changes	C. Garber, R. Willis, D. Zorka	S. Suffin, M.D.
4.0	3/14/2014	4	Added definitions for reportable range, analytical sensitivity, verification, validation and removed CRR	C. Garber, R. Willis, D. Zorka	S. Suffin, M.D.
4.0	3/14/2014	8	Revised Vendor Supported Validations	C. Garber, R. Willis, D. Zorka	S. Suffin, M.D
3.3	6/3/2014	9, 13 17	Adopting corporate issued version 4 with revisions dated 3/14/13 above. Additional local revisions: Added local validation protocol Added local file locations	L Barrett	C. Bowman-Gholston

16. ADDENDA

Addendum	Title
A	Precision Studies for FDA Cleared or Approved tests
B	Precision Studies for Laboratory-Developed Tests
C	Detection Limits
D	Analyte Measurement Range (AMR) Validation
E	Interference Studies
F	Interference Testing for Laboratory-Developed tests
G	Recovery Studies
H	Split-Sample Comparison Studies
I	Carryover Studies
J	Reference Interval Validation
K	Specimen Stability
L	Alternate Specimen Validation
M	Carryover Studies on a Four Channel Multiplexed HPLC system using an automated liquid pipetting system for patient sample preparation

ADDENDUM A: Precision Studies for 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 material 5 times per day for 5 separate days
3	For each material, calculate mean, SD, and %CV.
4	For each material, calculate the Process Sigma based on the SD and % CV and the defined allowable total error (TEa). The spreadsheet does this automatically. • Sigma (for precision only) = TEa (units) /SD (units) or • Sigma (for precision only) = TEa%/ CV%
5	The template creates a spreadsheet that summarized Process Sigma 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 at two months of routine operation. • If Process Sigma is $\geq$ 3 Sigma at higher concentrations, but not at very low concentrations, the manager may the Local Medical Director) decide to perform replicate testing for low concentration samples, but singlicate testing for concentrations at or above where the Precision Process Sigma is 3.0 or greater.
7	Write a conclusion as to acceptability of precision performance.

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ADDENDUM B: Precision Studies for 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 we know we need to 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 we need to pay close attention to factors that cause a large variation. In this case, special attention should be given to minimizing calibration variability.

1. Materials

1. Control materials should be prepared 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. At least three levels of controls should be used if possible. Selection of the target values for QC materials should take into account the following in 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 Analyte Measurement Range (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.

3. Within Run Precision

There is no standard template for this addendum. Create a separate Excel spreadsheet.

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 11111122222223333333. This is especially important for microtiter plates.
3	Calculate mean, SD for each control material • The SD calculated should be less than allowable SD(wr) or < TEa/4.
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 +/- 4 * SD(wr) 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.)

4. Total Precision (per CLSI EP5)

1	Perform The **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, then set up only one run for this validation. However, if the assay will be set up several times (or on different shifts each day) then 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	• Plot the data **daily** on Levy Jennings plots, so that there is an immediate sense of the acceptability of the testing for that day. • Identify instances of shift and drift in the data and investigate causes. This will be extremely important information for routine operation.
3	**Calculate The Data Using ANOVA (analysis of variance) Software** that is consistent with the CLSI EP5 Precision Protocol. • **It is insufficient to just calculate the overall SD.**
4	**Acceptability of Precision:** Compare the total SD to the defined allowable precision or allowable total error. **Acceptable conditions are these** • Six Sigma capable if Total SD $\leq$ 1/6 * TEa. • Good, if Total SD $\leq$ TEa/4. • Acceptable (fair) if Total SD $\leq$ TEa/3 **Marginal condition and will require testing be performed in duplicate or triplicate**: • Marginal if Total SD > 1/3 * TEa but < ½ * TEa **Unacceptable condition**: • Unacceptable if Total SD $\geq$ ½ * TEa (assay should 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. ***Either of these criteria for TOTAL SD MUST BE MET, before accepting the method and going on to other studies!***
5	Write a conclusion as to acceptability of precision performance.

ADDENDUM C: Detection Limits

1. Limit of Blank (LoB): check EP17

Step	Action
1	Use zero calibrator or a blank mixture.
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 **97.5%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 **97.5%ile** for these blank readings.
11	This is the LoB for the blank for zero standard.

LoB should be determined for every analyte.

- Values at or below the LOB are considered "not detected".
- Values above the LOB are considered "detected".
- For analytes for which low values are not expected (serum Calcium), LOB gives an indication of a "no sample" situation. The lab may want to check results below this limit for proper sampling.

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 97.5%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.

Step	Action
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**.

LOD should be calculated for every analyte, from the same studies for LoB (see above).

- Values at or above the LOD will be detected 97.5% 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 2 or 3 very low pools • One at the stated LoD (LoQ could be as low as LoD) • One at LoD x 2 • One at LoD x 4 (optional)
2	Measure each pool 5 times on each run, over the 5 runs of the validation study.
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 of each pool to the requirement that SD ≤ TEa/3
5	Determine the LoQ. This is the lowest concentration at which SD ≤ TEa/3. **Note, LoQ cannot be less than LoD.**
6	Write a conclusion as to acceptability of LoQ (and LoB and LoD).

- 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

ADDENDUM D: Analyte Measurement Range (AMR) Validation

The CAP checklist (CHM 13700 states that verifying the AMR can be accomplished by **either** of two approaches:

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

Therefore, either an acceptable Calibration Verification OR an acceptable AMR validation.

- Perform a **calibration verification** with standards (or known pools) at the minimum, maximum, and midpoint of the "claimed" reportable range, OR
- **AMR validation** (previously known as a linearity study) with a series of dilutions of a high and low patient pool across the range of the assay

1. AMR validation may be met by Calibration Verification

Step	Action
1	Run calibrators or other materials (known pools) traceable to a recognized standard at the minimum, maximum, and midpoint of the claimed reportable range.
2	Appropriate materials should have a target value concentration within the TEa of the respective minimum or maximum of the reportable range.
3	• If each material is tested many times then the average of the observed values should deviate from the expected target by no more than TEa/4 • The 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 this Calibration Verification study, again with 4 replicates of each level. • If criteria still are not met, consult with supervisor, manager, technical director, and manufacture (as appropriate).
5	Write a conclusion regarding Calibration Verification.

OR perform an AMR validation by dilution of a patient sample.

2. AMR Validation

Step	Action
1	Intermix 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 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	Mathematically, non-linearity may be called at the point where the observed values deviate from the straight line by more than TEa/4 for that test. • Therefore, if some curvature appears, determine the line of best fit using only the samples that appear to fall on a straight line. • Use this regression line to calculate predicted values for all the samples. • Calculate the difference between the values based on the regression line and the observed values. • Check that the difference is $\leq$ TEa/4
5	• The AMR will include those samples where the difference is $\leq$ TEa/4.
6	Write a conclusion regarding the validation of AMR

ADDENDUM E: Interference Studies

1. refer to manufacturer's literature

Step	Action
	Common Interferences
	Refer to the Manufacturer's insert sheet for information on common interferences (hemolysis, icterus, lipemia) and **record on the Interference spreadsheet.**

ADDENDUM F: Interference Testing for Laboratory-Developed tests

The following should be performed for laboratory-developed tests and for other special interests. *There is no standard template for this addendum. Create a separate Excel spreadsheet.*

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. Report results. ▪ Error due to interference > TEa/4, but less than TEa/2: Results are "marginal". Report results with a qualifying message. ▪ Error due to interference > TEa/2: Results are not acceptable. Do not report result. Report with a message "Unable to determine result due to interfering substance"
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 3 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 interferent to be tested.
2	Define the decision criteria as in Step 3 in Section 2 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 2 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 result. 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).

4	Please note, that 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
5	Perform 4 replicate measurements of each sample prepared to minimize the effects of imprecision.
6	Continue with Step 7 in Section 3 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 <u>low</u> clinical decision point and one specimen at a <u>high</u> 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 3 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 3 above to analyze the data.

ADDENDUM G: Recovery Studies

1	**Recovery of Peer Group Values for QC Samples**
	• 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**.

2	**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.

3	**Recovery of Known (Previously tested) Proficiency Test Samples**
	WARNING: This must only be done using proficiency testing samples from **previous formally evaluated surveys**, where the agency already has issued a formal evaluation report of the results for samples used in this recovery study.
1	Assay at least 5 External Proficiency Test samples (e.g., CAP) if available. Two measurements should be obtained for each sample if possible to *generate a larger database*. Note, only for the purposes of method validation studies, is it permissible to run proficiency samples multiple times! At all subsequent times, proficiency test samples must be run in the exact same way that patient samples are run.

2	A minimum of 9 out of 10 of the individual results must be within the acceptable range for your peer group. PT criteria are defined for or individual results for each sample; not for averaged results of individual samples.
3	However, it is important to consider performance across all the samples so show that the average bias is small. To do this, we look at the average difference. • The average difference from target across all 5 samples or 10 results should be < TEa/4. • One can calculate the simple average difference in results if the sample concentrations are close to each other. • If the sample concentrations cover a wide range of concentration then perform regression analysis of observed result (y) versus target value (x) and then use the regression line to predict the difference in (Yc – Xc), where Xc is a medical decision concentration
4	Write a conclusion as to acceptability of recovery performance.

ADDENDUM H: Split-Sample Comparison Studies

1 COMPARISON OF RESULTS FOR PATIENT SAMPLES

Step	Action
1	The comparison of methods study should be conducted with the current method, 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.
2	**IF your lab currently** / **THEN** 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) / Then the Primary lab (or main lab to an RRL) should select a minimum of 20 samples to send to your laboratory for the correlation study in your lab performs testing for the analyte in question, but by a different platform / Your laboratory should select a minimum of 20 samples for the correlation study, and ship aliquots to the lab that performed the primary validation (or to your main lab if you are an RRL).
3	**Specimen Management** • Select at least **20** samples **distributed over the Analyte Measurement Range (AMR)**, • 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 in singlicate by each method should be performed. • Testing of these 20 samples should be spread over the 3 - 5 days (i.e., a minimum of 4 - 8 samples per day).
4	**Calculations: average bias**: • $bias = \left\lvert \bar{y} - \bar{x} \right\rvert$ • The absolute value of the difference in averages should be less than TEa/4. (See **Section 4.1**)

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5	**Calculations: regression analysis.**
	• Perform linear regression (the template does this) with X being the current assay and Y being the new assay. *Simple regression is subject to mathematical errors if r < 0.97*5. Therefore when this occurs, and if available, do Deming's regression (for example, David Rhoads' "EP Evaluator" program, or "Analyze-it" program) instead of the traditional linear least squares to obtain a mathematically correct calculation of the slope and y-intercept.
	• Examine the plot for outliers (>3.5 Sy.x) and non-linearity. If outliers are observed, investigate and be able to explain.
	• For each medical decision level, Xc, calculate the corresponding Yc value using the equation for the regression line. Three different medical decision levels concentrations can be chosen based on the following or any combination of these:
	  • Low and high limits of the reference interval, plus a third high abnormal value.
	  • Mean values of the tri-level QC material.
	  • 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).
	• Calculate the difference (Yc - Xc)
	• The absolute difference should be < TEa/4 at each value of Xc (Section 5.1)

6	**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.

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

ADDENDUM I: 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.
- **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 3 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 The same day and run 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 the be 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 re-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.

ADDENDUM J: Reference Interval Validation

If	and	Then
The laboratory performed a Patient Sample Comparison study versus the current method	Bias is acceptable, Bias $\leq$ TEa/4	It is not necessary to perform a Reference Interval validation (by application of the Principle of Transference of Reference Intervals (CLSI C28-A2, Section 7).
For all other cases,		It is necessary to perform a Reference Interval validation study.

Step	Action
1	• Obtain 20 specimens from apparently healthy donors. • These can be from **known healthy donors** based on typical screening criteria to check for such factors as use of aspirin, multi-vitamins, and birth control pills, smoking, etc. • Or patient samples might be used that have a completely unremarkable Chemistry Panel (i.e., Comprehensive Metabolic Panel), CBC, CRP, and/or other marker(s) that are appropriate to exclude disease processes that might impact the analyte being evaluated. For example, for a thyroid function test, other thyroid function markers might be checked to assess probability for thyroid disease. For a liver function test, other liver function markers, including hepatitis virus might be checked to assess the probability for liver disease. • Or contact the Clinical Correlations Department at San Juan Capistrano for assistance in collecting "healthy donor" specimens.
2	Perform testing on each and rank the results in increasing order.

3	Count the number of results that fall outside the reference interval limits (regardless of whether low or high), per recommendations from CLSI C28-A2.
	• If there are no more than 2 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 more 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, then the laboratory should consider establishing a new reference interval as described in Step 4.

4	**Reference Interval establishment**
	• Collect at least 60 and preferably 120 samples from healthy donors
	• Analyze data using appropriate statistical methods
	  • Non-parametrically by ranking and selecting the 2.5 percentile and 97.5 percentile. For this approach, CLSI recommends 120 samples.
	  • Parametrically: This is based on the mean +/- 2SD. This approach should ONLY be used after demonstrating that the shape of distribution is Gaussian. This may require transformation by a suitable function, such as log transformation, square root transformation, or inverse transformation, or inverse-square-root transformation, etc. Consult corporate medical or the Clinical Correlations department at San Juan Capistrano for guidance for these.

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

ADDENDUM K: Specimen Stability

1. CAP and CLIA 88 do not require validation of manufacturer's claims for specimen stability for an FDA Cleared or Approved test system if this information is used exactly as claimed.

2. For an FDA Cleared or Approved test, where different stability information is being applied by the laboratory, the laboratory may refer to other stability data that has been generated by any one of the laboratories in the Quest Diagnostics network. According to CAP (December 9, 2005 special meeting)
   a. Each laboratory does not need to perform its own specimen stability studies
   b. The Stability study data and report must be readily available to every laboratory. These are usually provided in rollout packages, for each laboratory to refer to.

3. For lab developed tests, specimen stability studies must also be performed in accordance with section 7 below.

4. Use the specimen stability procedure described on this page starting with section 5 below if the assay being validated is
   a. A fully automated assay (but not micro-titer plate technology) or
   b. Where it is known that frozen samples are not acceptable (regardless of assay technology). Examples of analytes that cannot be frozen include CBC's, Total CO2, HDL, certain enzymes that are denatured when frozen, etc.

   If the test procedure is a manual procedure, or is based on micro-titer plate technology, and where it is known that freezing samples or aliquot is acceptable, use the Specimen Stability SOP developed and implemented by Quest Diagnostics Nichols Institute at San Juan Capistrano entitled "Specimen Stability" SOP NO. 130SM090. The design of that procedure is based on temporarily freezing all aliquots, so that they can all be thawed and tested on the same run, to eliminate the effects of inter-assay variation. To obtain a copy of this SOP, please contact the QA department at San Juan Capistrano for a current copy of that SOP.

5. **Specimen Stability General Procedure**:
   a. Determine the Medical Decision levels for the assay in question. If there is only one Medical Decision Level, also perform testing at 2x that medical decision concentration or 2x the upper limit of the reference interval.
   b. If two decision level concentrations are to be tested, test samples from at least three donors at or near each decision level, for a total of 6 donors. If three decision level concentrations are to be tested for stability, test samples from at least two donors near each decision level, also for a total of 6 donors.
   c. If concentrations of interest fall outside the typical donor parameters, it is acceptable to use freshly pooled or freshly spiked specimen(s) to achieve required concentrations. (See instructions at the end of this addendum for preparation of spiked samples.)
   d. Determine the conditions at which the sample stability will be checked
      room temperature [defined temperature must be stated in procedure],
      refrigerated [defined temperature must be stated in procedure],
      frozen [defined temperature must be stated in procedure]
   e. Collect sufficient quantities of fresh samples from each donor. If more than one tube is collected from an individual donor, combine both (or all tubes) from that one donor into one larger pool, from which all aliquots will then be made. Do the same for the samples collected from each other individual donor. Label each **without using personal identifiers**, such as Donor A, Donor B, etc**. Donor names must not be used to maintain confidentiality.**
   f. Analyze each sample as soon as possible after centrifugation to obtain the baseline result. Each sample should be analyzed in triplicate.

g. Split each donor sample (or donor pool) into aliquots, one aliquot for each time and temperature condition. And label appropriately, with Donor Designation, Storage Temperature and Storage Time, to ensure the right samples are tested at the designated times.
h. Each successive analysis (each day of study) should be performed on the same instrument to eliminate instrument to instrument variation, and with at least triplicate measurements of each sample aliquot.
i. Enter the data into the appropriate template entitled specimen stability, using one template for each "donor".
j. 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 for that analyte.
k. Write a conclusion regarding acceptability of specimen stability for each condition.

6. **Specimen Preservatives**:
   a. For stability of "preserved" samples or specimens with anti-coagulants or other additives, each specimen type shall have its own specimen stability data (either from the manufacturer or from a study performed in Quest Diagnostics).
   b. For potential interference of additives or anticoagulants to other analytes, see Interference Section

7. **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 general, specimen stability information may be applied across different tests and 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. This partial list illustrates specimen stability information that can or cannot be transferred between test systems.
   a. Serum creatinine stability that was determined for Olympus **CAN** be transferred to Hitachi, to Beckman, and other chemistry analyzers,
   b. CBC's should show the same stability, regardless of what instrument is used for cell counting.
   c. Analyte stability determined by GC or LC methods should be the same across all platforms of these technologies.
   d. 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.
   e. Serum total calcium and ionized calcium are not the same analyte; therefore, each must have its own independent stability study performed.
   f. 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.)
   g. An ACS-180 test that uses the same reagent(s) as the corresponding test on the Centaur/Centaur-CP **CAN** use the same stability data.
   h. A Centaur-CP test that uses the same reagent(s) as the corresponding test on the Centaur **CAN** use the same stability data.

Special Instructions for preparing a spiked sample.

1. **Base Pool**: Obtain a freshly drawn patient sample (or pool)

2. **Prepare or obtain a Stock Solution** of a high concentration of analyte that is about 10 or 20 or 30 times higher than the base pool, to achieve a decision level concentration that is 2x or 3x or 4x times higher than the base pool, respectively. This might be a high standard or calibrator material used for the calibration of the assay. Or it could be material purchased commercially. Or it could be a very high QC material.
   - ❖ Use a stock solution of about 10 times higher to spike the base pool to about 2X its concentration.
   - ❖ Use a stock solution of about 20 times higher to spike the base pool to about 3X its concentration.
   - ❖ And so on.

   Do not use stock solutions that have had stabilizers added to them by the manufacturer. Their effects will defeat the purpose of the stability study.

3. **Final Spiked Pool**: Mix 9 parts base pool with 1 part stock solution to give a final spiked pool that will be near the intended test concentration.
   - ❖ This 9:1 volume ratio is important to try to maintain the original patient sample matrix of the base pool.
   - ❖ Once this final spiked pool is prepared, go to **step 5.f** and continue through the remainder of the procedure.

ADDENDUM L: Alternate Specimen Validation

For Serum and Plasma (for all analytes except TDMs), see the following Table.

TDMs are a special case. Due to lack of alternate sample types available in the laboratory and lack of available donors taking TDM's, the laboratory can accept the manufacturer's recommendations in the product insert sheet for use of serum and various types of plasma for a given TDM. This special case is ONLY applicable to TDM's. For all other analytes that may be tested in serum and/or plasma, refer to the following Table.

For body fluids (other than blood, serum, plasma, or urine) refer to the Body Fluid Validation SOP and associated Excel Template listed in Related Documents (QDMD731 will be renumbered to QDQC711).

Write a conclusion as to acceptability of each individual specimen type.

Performance Characteristic	**Laboratory Validation Main specimen type (i.e., serum)**	**Laboratory Validation Alternate specimen type (i.e., plasma)**
Precision	5 reps x 5 days of QC material At three levels.	**For Serum and Plasma, no extra studies**, because the same QC materials would be used. *Write a sentence to the file as to why this was not repeated.*
Accuracy – Recovery	Recovery of Reference materials or CAP previously tested materials	**No extra studies**, because CAP materials are the same for serum and plasma. *Write a sentence to the file as to why this was not repeated.*
Accuracy – Split sample studies	Test at least 20 serum samples by new and old method	Test at least 20 pairs of serum and plasma samples on the new method, each pair collected from a different donor.
Analytical Measurement Range (Reportable Range)	Perform testing at least in duplicate on at least 5 concentrations that cover the AMR, using dilutions of a high standard, or a "linearity set"	**No extra studies**, if using the same set of standards. *Write a sentence to the file as to why this was not repeated.*
Reference Interval	"Transfer" Reference Interval from **previous method** if bias is small (< TEa/4). CLSI C28-A2, **or** test at least 20 healthy donors.	"Transfer" Reference Interval **from serum** if bias is small (< TEa/4). CLSI C28-A2, **or** test at least 20 healthy donors.
Analytic Sensitivity	• LoB, LoD in water blank or zero standard, by definition. • LoQ using appropriate dilutions of a standard, or a QC material or diluted patient serum pools.	• **No extra studies for LoB and LoD**. • LoQ: No extra studies if serum LoQ was based on dilution of a standard or QC material. • LoQ: If "serum" LoQ was based on diluted patient serum pools, repeat LoQ on diluted patient plasma pools.
Analytic Interferences	**Not applicable**. Refer to manufacturer's insert sheet.	**Not applicable**. Refer to manufacturer's insert sheet.
Carryover	If applicable, test with high and low serum samples	If significant for the main specimen type, perform with alternate specimen type.

This same process is performed for each different type of plasma prior to that type being accepted for testing patient samples.

ADDENDUM M: Carryover Studies on a Four Channel Multiplexed HPLC system using an automated liquid pipetting system for patient sample preparation.

This addendum covers carryover analysis of HPLC systems which employs four independent HPLC systems working in series with a single detector to reduce overall sample analysis time. The design of this protocol/template assumes the samples are pipetted by an automated liquid handling system employing 8 independent non-disposable sampling tips. Examples of such a system could be the Tecan or Perkin-Elmer Janus system.

Carryover is calculated by determining the LOD of blank material and then running high patient samples on the system followed by blank material which is measured for the possibility of carryover from the preceding high sample. The protocol and template check for carryover within a column, and carryover within a tip. Carryover is defined as an observation for a blank sample that exceeds the calculated LOD.

Prepare a sample plate and run it two times to obtain an overall average result. *Note, the purpose of performing the study two times is to obtain a better estimate of LOD. Do NOT calculate an average carryover for comparison to the LOD. The LOD is a limit for a single measurement, not applicable to averages.*

Step	Action
1	Specimen selection. • Obtain a patient pool of very high samples or spiked samples (at least twice the upper limit of the AMR). • Obtain a pool of blank material. • The amount of each pool should be enough to perform carryover analysis of all available instruments.
2	Prepare a sample plate using the blanks and patient pool in place of real patient samples. • The carryover samples must be bracketed by QC material. • The standard curve, QC material, and system blanks must follow the assay's SOP requirements. • No mispipetting by the automated liquid handling system is allowed, all samples must be pipetted by the system correctly. • Additional blanks are allowed to ensure that the first carryover sample goes onto HPLC system 1.
3	Run the sample plate. • The same plate must be run twice on the same day. • All QC and system suitability requirements according to the assay SOP must be met for the data to be considered valid.
4	Enter the data into the Excel template for Laboratory Validations.
5	The Excel template performs the following calculations: • Determines the average response for the blank material based on Tip and Column for the blanks in each run. • Determines the Grand Average based on Tip and Column for the blanks in both runs combined.

	• Determines the Overall Standard Deviation based on Tip and Column for the blanks in both runs combined. • Determines the LOD for the Tip and Column. • Determines Carryover for the Tip and Column.
6.	Review the data. • If NO carryover is detected, no action is needed. • If carryover is detected, then a supervisor must be informed and troubleshooting be performed to isolate the carryover.
7.	Troubleshooting. • Prepare another plate but pipette the carryover samples by hand • Run the plate according to this addendum. • If NO carryover is detected, then the automated liquid handling system must be inspected and serviced before patient samples are run on it. • If carryover is detected, then the HPLC system must be inspected and serviced before patient samples are run on it.
8.	Write a conclusion as to the acceptability or failure of carryover performance.

17. APPENDIX

See link to Excel Template.

This template is used for data entry of all studies described in this SOP.

http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/Method_Validation_Template_16_8.xls

The Microsoft Excel Workbook provided with this SOP will generate a summary page for the method being validated.

Local appendices locations:

Title	
Method Validation Template 16_8	See Attachments in SmartSolve
Completed Example of Method Validation Template 16_8 (for instructional use)	See Attachments in SmartSolve

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