pSMILE SAFETY·QUALITY·RESULTS

Quantitative Validation Guidelines

Validation of a quantitative system consists of an established set of required experiments. Each laboratory should first design a validation plan describing how they will satisfy each of these requirements. The validation plan must also detail the acceptability criteria for each element. After completing all of the validation experiments, results should be compiled and filed in an organized manner. Package Inserts should be included as part the validation packet and submitted with your validation summary. All validation records should be retained for the life of the instrument. A validation summary should be prepared that contains a place for the Laboratory Director to sign, indicating the validation has been reviewed and approved.

The following are the required components of validation for most quantitative instrument methodologies in chemistry, hematology and coagulation. For INR see specific Guidelines as well as Plan and Summary. For Hematology all calculated and measured analytes (i.e. percent and absolute counts) require precision, accuracy and reference range and should be included in Validation Summary Reports.

1. **Precision** is reproducibility - the agreement of the measurements of replicate runs of the same sample. Replication experiments are performed to estimate the imprecision or random error of the analytical method. There are two components for quantitative precision: short-term (also called within run) and long-term (also called between day).

    I. Short-Term (Within Run)

        a. Sample Criteria

            - Two levels (Low/High or Normal/Abnormal)
            - Chemistry- use patient samples or quality control samples.
            - Hematology- use stabilized material such as quality control material. Patient samples are also acceptable for short term.
            - Coagulation- refer to *VAL 2008_Coagulation Specimen Requirement*
            - For other quantitative systems, consult pSMILE and/or the manufacturer for suggested sample types.

        b. Testing and Results

            - Run each sample 20 times on the same run, if possible, or at a minimum within the same day.
            - Calculate the mean, standard deviation (SD) and coefficient of variation (CV) for each normal and abnormal sample.
            - Compare laboratory CV to manufacturer's stated precision claims found in the package insert. If the manufacturer describes multiple

precision specifications (e.g. different CVs at different levels), the laboratory may use the pSMILE Total Allowable Error Limits (TEa) tables.

c. Acceptability Criteria

- The CV for each assay level is expected to be equal to or less than the manufacturer's performance specifications for precision.
- In the event that an assay does not perform as expected, the CV maybe compared to the allowable random error. It is acceptable to attain short-term precision by using <25% of pSMILE Total Allowable Error Limits.
- Refer to *EQA 1380a_TEa Tables* for Total Allowable Error Limits.
- If Short -Term precision is unacceptable, consult instrument manufacturer for assistance.

II. Long-Term (Between Day)

a. Sample Criteria

- Two levels (Low/High or Normal/Abnormal)
- Chemistry- use patient samples or quality control samples.
- Hematology- use stabilized material such as quality control material.
- Coagulation- refer to *VAL 2008_Coagulation Specimen Requirements.*
- For other quantitative systems, consult pSMILE and/or the manufacturer for suggested sample types

b. Testing and Results

- Run each sample at least once but not more than 5 times per day for a total of 20 data points for each level of material used. (For example, these scenarios are all acceptable: a) run each sample two times per day for 10 days b) once per day for 20 days c) 5 times per day for 4 days.)
- Calculate the mean, SD and CV for each normal and abnormal sample.
- Compare laboratory CV to manufacturer's stated precision claims found in the package insert. If the manufacturer describes multiple

precision specifications (e.g. different CVs at different levels), the laboratory may use the pSMILE TEa tables.

c. Acceptability criteria:

- The CV for each assay is expected to be equal to or less than the manufacturer's performance specifications for precision.
- In the event that an assay does not perform as expected, the CV may be compared to the allowable random error. It is acceptable to attain long-term precision by using <33% of SMILE Total Allowable Error Limits.
- Refer to *EQA 1380a_TEa Tables* for Total Allowable Error Limits.
- If Long -Term precision is unacceptable, consult instrument manufacturer for assistance.

2. **Accuracy** is the true value of a substance being measured. Verification of accuracy is the process of determining that the test system is producing true, valid results.

   Note for Chemistry: Accuracy testing is only required for measured analytes and not required for calculated analytes. Consult the instrument user's manual to determine which analytes are measured and which are calculated.

   a. Determine the Reference Method

   - The ideal reference method is a similar instrument/method.
   - The reference method must be previously validated.
   - The reference method must currently be performing successfully on EQA.
   - Comparison to an in-house method is preferred if the in-house instrument meets the above criteria.

   b. Sample Criteria

   - The ideal number of samples is 40, however a minimum of 20 samples that cover the reportable range of the method and include points near the medical decision points, if possible, is acceptable.
   - A combination of patient samples, quality control material, and external quality assurance (EQA) samples may be used.
   - Coagulation- refer to *VAL 2008_Coagulation Specimen Requirements*
   - For other quantitative systems, consult pSMILE and/or the manufacturer for suggested sample types.

c. Testing and Results

- Run each sample in duplicate on each instrument. Average the duplicate results. Refer to *VAL 2002_Quantitative Accuracy Pack.*
- Samples should be run within 2 hours of each other ideally.
- Data should be plotted immediately to identify and correct any outliers.
- Calculate the Correlation Coefficient (R-value) and Error Index.

d. Acceptability Criteria

i. Correlation Coefficient (R-value)

- R <0.975: Data does not extend over acceptable range. More data must be evaluated over larger range.
- R >0.975: Data covers acceptable range, move onto Error Index (see below) to see if 95% of the data points from the comparative method are within Total Allowable Error limit of the reference method.

ii. Error Index

- Each Error Index pair must fall within -1 and 1. If more than 5% of the specimens have an Error Index of less than -1 or greater than 1, the accuracy experiment fails. Consider possible cause of inaccuracy. Troubleshoot before repeating accuracy studies.
- If 95% of Error Indices are acceptable: Accuracy is acceptable. Proceed with Linearity experiments.

3. **Linearity** for a quantitative analytical method is when measured results from a series of sample solutions are directly proportional to the concentration or activity in the test specimens. This means that a straight line can be used to characterize the relationship between measured results and the concentrations or activity levels of an analyte for a determined range of analyte values. Linearity testing is only required for measured analytes and not required for calculated analytes. Consult the instrument user's manual to determine which analytes are measured and which are calculated.

   Note for Coagulation: Linearity study is not applicable, skip to next section.

   Note for Hematology testing: Linearity testing performed on measured analytes as provided by the linearity material.

   Sample Criteria

   - A minimum of 5 samples that cover the reportable range of the method.

- When plotted, the values should ideally be equidistant from each other.
- Samples with known values, such as quality control, calibrators or commercial linearity standards should be used.

a. Testing and Results

- At a minimum, run each sample in duplicate and average the results.
- Data should be plotted immediately to identify and correct any outliers.
- Plot the data in a regression analysis program such as *VAL 2003_Quantitative Linearity Pack*. The spreadsheet will calculate the slope and intercept.

b. Acceptability Criteria

- The method is linear if the difference between the predicted Y-value and the measured Y-value is less than the allowable error for each specimen point. The systematic error must be less than 50% of the total error

4. **Analytical Measurement Range (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. AMR validation is the process of confirming that the assay system will correctly recover the concentration or activity of the analyte over the AMR. The manufacturer defines the AMR but it is the laboratory's responsibility to verify it. AMR testing is only required for measured analytes, and not required for calculated analytes. Consult the instrument user's manual to determine which analytes are measured and which are calculated

   Note for Coagulation testing: Measurement range study is not applicable, skip to next section.

   Note for Hematology testing: Measurement range study is not applicable for analytes that do not require linearity testing.

   a. Sample Criteria

   - Samples with known values, such as quality control, calibrators or commercial linearity standards should be used.
   - It may be necessary to dilute the lowest sample to verify the low end of the AMR.
   - The high end of the AMR will only be as high as the highest sample.

   b. Testing and Results

   - At a minimum, run each sample in duplicate, and average the results.

- Data should be evaluated immediately to identify and correct any problems.
- Refer to *EQA 1380a_TEa Tables* for Total Allowable Error Limits.

c. Acceptability Criteria

i. Upper Limit Verification

- The manufacturer's upper limit can be accepted if the known sample is within the percent TEa of your AMR upper limit.
- Your measured value must also be within TEa of the known sample.
- Upper Limit Example: (the same principles can be applied to Hematology analytes)

Bilirubin (mg/dL)		
TEa %	**TEa- Minimum Detectable Difference**	**Mfg. AMR**
20%	0.4	0-25

- Measured values needed:

Bilirubin Standard (mg/dL)			Laboratory Measured Results		
	Acceptable Range AMR ± TEa	**Known Value**	**Allowable Measured Error (Known Std ± TEa)**	**Measured Value**	**AMR Verified?**
Upper AMR	25 ± 20% Range: 20 - 30 mg/dL	24	24.0 ± 20% Range: 19.2 - 28.8 mg/dL	29.5 mg/dL	No, above Mfg. AMR

- If TEa is 20%, an upper AMR of 25 can be verified with a known sample of 20 - 30 if the measured sample is within TEa of the known sample. In the example above, the measured value must be between 19.2 - 28.8.
- If a sample within TEa cannot be obtained, the highest known sample measured and within TEa should be used as the highest reportable undiluted value.
- For example:

Bilirubin Standard (mg/dL)			Laboratory Measured Results		
	Acceptable Range AMR ± TEa	**Known Value**	**Allowable Measured Error (Known Std ± TEa)**	**Measured Value**	**Upper AMR (25 mg/dL) Verified?**
Upper AMR	25 ± 20% Range: 20-30 mg/dL	10	10.0 ± 20% Range: 8-12 mg/dL	10.5 mg/dL	No, but verification of New Upper AMR is 10.5 mg/dL

ii. Lower Limit Verification

- The manufacturer's lower limit can be accepted if the known sample is within the minimum detectable difference or percent TEa of the lower limit (whichever is greater).
- Your measured value must also be within TEa of the known sample.
- Lower Limit Example: (the same principals can be applied to Hematology analytes)

Bilirubin (mg/dL)		
TEa %	**TEa- Minimum Detectable Difference**	**Mfg. AMR**
20%	0.4	0-25

- Measured values needed:

Bilirubin Standard (mg/dL)			Laboratory Measured Results		
	Acceptable Range AMR ± TEa	**Known Value**	**Allowable Measured Error (Known Std ± TEa)**	**Measured Value**	**Upper AMR (25 mg/dL) Verified?**
Lower AMR	0-0.4 mg/dL	0.3	0.3 ± 0.4 Range: 0.0-0.7 mg/dL	0.5 mg/dL	Yes

- If TEa is 0.4, a lower AMR of 0.0 can be verified with a known sample of 0.0 to 0.4 if the measured sample is within the minimum detectable difference or TEa, whichever is greater. In the example above the measured value must be between 0.0-0.7.

- For analytes that round to the nearest whole number, a proximity limit of 1 is acceptable to verify the lower range of 0.
- Serial dilution should be used to obtain a sample within the desired range. However, if a sample within TEa cannot be obtained, the lowest known sample measured and within the acceptable TEa will be used as the lowest reportable range. For Example:

Bilirubin Standard (mg/dL)			Laboratory Measured Results		
	Acceptable Range AMR ± TEa	**Known Value**	**Allowable Measured Error (Known Std ± TEA or Min. Detectable Diff.)**	**Measured Value**	**AMR (0 mg/dL) Verified?**
Lower AMR	0 ± 0.4 Range 0 - 0.4 mg/dL	0.7	TEa- 0.7 ± 20% Range: 0.6-0.8 OR Min. Detectable Diff: 0.7 ± 0.4 Range: 0.3-1.1 mg/dL	0.7 mg/dL	No, but verification of New Lower AMR is 0.7 mg/dL

Clinical Reportable Range (CRR) is the range of analyte values that a method can report as a quantitative result, allowing for specimen dilution, concentration or another pretreatment used to extend the AMR. The laboratory should establish a CRR that covers the range of Grade 4 Adverse Events on the DAIDS Toxicity Table without exceeding manufacturer's dilution guidelines.

- The lab should establish what dilutions are necessary to cover this range, bearing in mind that a minimum amount of dilution is ideal since accuracy decreases with increasing dilution.
- The laboratory should decide the maximum value of dilution that will be allowed without exceeding the manufacturer's recommendations for dilution.
- Any samples that do not give a numerical value beyond this allowed dilution should be reported as greater than the upper end of the CRR.

5. **Analytical Sensitivity** is the lowest concentration of an analyte that can be measured (also called the Lower Limit of Detection).
    - For an FDA approved, unmodified method, the manufacturer's stated sensitivity will be used.

- For a non-FDA approved or modified method the laboratory must establish the lowest concentration that the method can accurately measure that is distinguishable from zero.

Analytical Specificity is the determination of the effect of interfering substances.

- For an FDA approved, unmodified method, the manufacturer's stated specificity will be used.
- For a non-FDA approved or modified method the laboratory must determine the effect of interfering substances.

6. **Reference Range** is the range of test values expected for a designated population where 95% of the individuals are presumed to be healthy (or normal).

   Note for Hematology testing: for WBC differential parameters, reference ranges must be determined for both absolute and percent cell counts. pSMILE recommends that separate reference ranges be used for at least the following adult male/female ranges: WBC's, RBC's, Hemoglobin, and Hematocrit. pSMILE also recommends locally established reference ranges.

   I. Transference of Reference Ranges with Verification

      a. Sample Criteria

         i. Select reference range to be verified:

            - Current laboratory ranges
            - Published reference ranges
            - Locally established reference ranges
            - Manufacturer's ranges

         ii. 20 healthy participants samples must be used

         iii. Qualify healthy volunteers. This can be done through a questionnaire or health assessment. See VAL 2005_*Reference Range Sample Health Questionnaire.*

         iv. If the analyte reference range differs for gender or age group, then 20 samples must be run for each category.

      b. Testing and Results

         - Test each sample immediately and evaluate.
         - Refer to *VAL 2004_Reference Range Pack to verify samples.*

c. Acceptability criteria

- 90% of the samples must be within the proposed reference range.
- If ≥ 90% of samples are within the reference, then the reference range is verified.
- If < 90% of samples are within the reference range: Re-evaluate the range being verified, and/or re-evaluate the healthy volunteer qualifications, and/or collect and evaluate 20 additional samples.
    - If ≥ 90% of the additional samples are within the reference range then the reference range is verified.
    - If < 90% of the additional samples are within the reference range, see below to Establish Reference Range

II. Establishment of Reference Ranges

a. Sample Criteria

- Determine population to be used to establish reference range
- Qualify healthy volunteers. This can be done through a questionnaire or health assessment. See VAL 2005_*Reference Range Sample Health Questionnaire.*
- Obtain samples from 120 healthy participants for each range to be verified.

b. Testing and Results

- Test each sample immediately after collection and evaluate. It is not advisable to collect and test all samples on the same day

c. Acceptability Criteria

- 90% of the samples must be within the reference range.
- If ≥ 90% of samples are within the reference, then the reference range is verified.
- If < 90% of samples are within the reference range: Re-evaluate the range being verified, and/or re-evaluate the healthy volunteer qualifications, and/or collect and evaluate 20 additional samples.
    - If ≥ 90% of the additional samples are within the reference range then the reference range is verified.

- If < 90% of the additional samples are within the reference range, repeat exercise.

III. Transference of Reference Ranges without Verification-

- The CLSI EP28-A3C Guideline describes different ways for a laboratory to validate the “transference” of established reference intervals. Pediatric reference intervals often require this approach because of the difficulty in obtaining sufficient specimens to establish or verify reference intervals. If a laboratory wishes to transfer a reference interval established by another laboratory or publication, the acceptability should be assessed based on several factors:
  - Similarity of geographic and demographics.
  - Similarity of test methodology.
  - Sound clinical judgment and consultation with local medical professionals.
  - Approval by the laboratory medical director is required and must be documented.

7. Method Approval

- The final decision on methodology validation and acceptance is made after a careful review of all the studies performed as part of the complete method validation process. The Laboratory Director shall make the ultimate decision on method validation.
- There must be an approval with a signature from the Medical and/or Laboratory Director and preparer of validation documents with dates.

Validation Guidelines for Instruments Post-Move, Major Instrument Part Replaced, and Test Taken Out Service

Post-Move: After moving the analyzer to a different room, floor, or building, lab should perform function checks of the system as described by the manufacturer (i.e. cleaning, mechanical, electronic, operational checks). Lab should also perform calibration, quality control, within-run precision by running controls 5 times, and accuracy testing using at least 10 patient samples.

Major Instrument Part Replaced: After the major instrument part is replaced, the laboratory should perform function checks of the system as described by the manufacturer (i.e. cleaning, mechanical, electronic, operational checks).
Lab should also perform calibration, quality control, within-run precision by running controls at least 5 times, and accuracy testing using at least 10 patient samples.

Test Taken Out of Service: If the test is taken out of the service for more than 30 days, prior to resuming testing, lab should perform function checks of the system as described by the manufacturer (i.e. cleaning, mechanical, electronic, and operational checks).
Lab should also perform calibration, quality control, precision and accuracy testing within 30 days prior to restarting patient testing. Precision and accuracy testing should be performed according to the requirements described in this guideline.
Proficiency testing (PT) should be performed within 30 days prior to restarting patient testing. If PT challenge is not offered during the 30-day period prior to restarting patient testing, the laboratory may perform an alternative assessment of the test. However, the lab must participate in the next scheduled PT event. Competency assessment of laboratory professionals should be performed within 12 months prior to restarting patient testing.

References

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4. CLSI. *Preliminary Evaluation of Quantitative Medical Laboratory Measurement Procedures Implementation Guide- 1st Edition*. CLSI document EP10-ED3-IG. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.

5. CLSI. *Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition.* CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.

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