

Assay Validation Methods - Definitions and Terms

Validation methods are completed to ensure that an analytical methodology is accurate, specific, reproducible and rugged over the specified range that a target analyte will be analyzed. Assay validation provides an assurance of reliability during normal use, and is sometime referred to as "the process of providing documented evidence that the method does what it is intended to do."

Assay Validation Levels and Steps

Assay Optimization (pre-validation): Assay optimization and pre-validation are experiments that determine how a range of matrix and sample elements, as well as assay conditions, effect assay parameters and assay performance. These data, along with scientific judgment, set the acceptance criteria for the assay validation. It is important to establish acceptance criteria before executing the validation protocol.

Assay Qualification: Assay qualification is an experimental protocol that demonstrates that an accepted method will provide meaningful data for the specific conditions, matrix and samples that the procedure is intended for. Assay Qualification may not require validation of accuracy and reliability of the method (sensitivity), but merely verify the suitability of the protocol under actual conditions (generally, specificity).

Assay Validation: Comprehensive experiments that evaluate and document the quantitative performance of an assay, including sensitivity, specificity, accuracy, precision, detection limit, range and limits of quantitation. Full Assay Validation will include inter-assay and inter-laboratory assessment of assay repeatability and robustness.

Assay Parameters DEFINITIONS

Specificity is the ability to assess unequivocally the target pathogen or analyte in the presence of components which might be expected to be present (3). The specificity of an assay is the capability of the assay to differentiate similar organisms or analytes or other interference from matrix elements that could have a positive or negative effect on the assay value.

Accuracy is the agreement between value found and an excepted reference value (3). This requires a "gold" standard or method but in the absence of a gold standard or method, comparison to established reference labs may substitute.

Precision is the variability in the data from replicate determinations of the same homogeneous sample under the normal assay conditions (3). For enzyme assays, precision is usually <10% ; 20 to 50% for in vivo and cell based assays; and >300% for virus titer assays. Precision includes within assay variability, repeatability (within-day variability), and reproducibility (day-to-day variability). Precision may be established without the availability of a "gold" standard as it represents the scatter of the data rather than the exactness (accuracy) of the reported result.

Detection Limit is the lowest amount of analyte which can be detected, but not necessarily quantitated as an exact value (3). The detection limit is a low concentration that is statistically distinguishable from background or negative control, but is not sufficiently precise or accurate to be quantitated.

Limits of Quantitation are the lowest and highest concentrations of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy (3). The lower limit of quantitation is often defined by an arbitrary cut-off such as a ratio of signal-to-noise, equal to 1:10, or a value equal to the mean of the negative control plus 5 times the standard deviation of the negative control values.

Linearity is the ability of the assay to return values that are directly proportional to the concentration of the target pathogen or analyte in the sample. Mathematical data transformations, to promote linearity, may be allowed if there is scientific evidence that the transformation is appropriate for the method.

Range is the concentrations of analyte or assay values between the low and high limits of

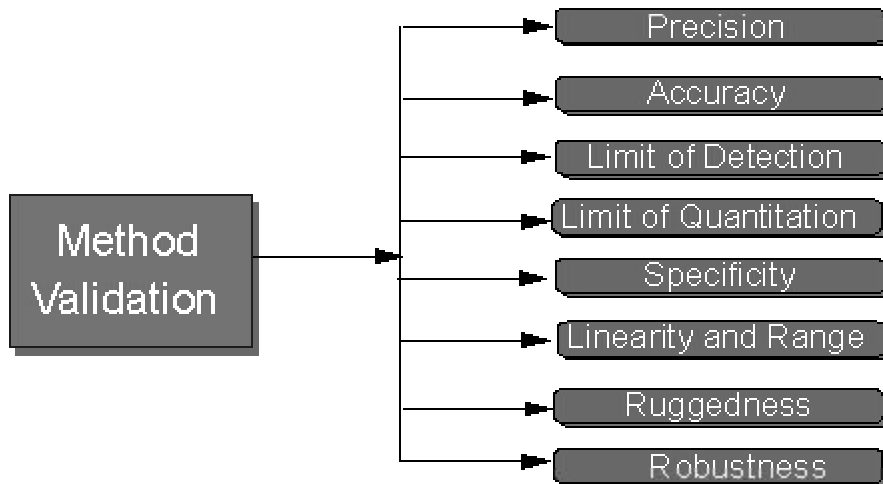
quantitation. Within the assay range, linearity, accuracy and precision are acceptable.

Ruggedness is the reproducibility of the assay under a variety of normal, but variable, test conditions. Variable conditions might include different machines, operators, and reagent lots. Ruggedness provides an estimate of experimental reproducibility with unavoidable error.

Robustness is a measure of the assay capacity to remain unaffected by small but deliberate changes in test conditions. Robustness provides an indication of the ability of the assay to perform under normal usage (3). Robustness measures the effect of deliberate changes (incubation time, **temperature, sample preparation, buffer pH**) that can be controlled through specifications in the assay protocol.

Considerations for METHOD VALIDATION STUDIES

Figure 1 - The "Eight Steps of Assay/Method Validation"



Specificity

Specificity is the ability to assess unequivocally the target pathogen or analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. This definition has the following implications:

IDENTIFICATION: To ensure the identity of the target pathogen or analyte.

ASSAY (Content or Potency): To provide an exact result which allows an accurate statement on the content or potency of the target pathogen or analyte in a sample.

Accuracy

The closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value, and the value found.

Note: When measuring accuracy, it is important to spike placebo preparations with varying amounts of active ingredient(s). If a placebo cannot be obtained, then a sample should be spiked at varying levels. In both cases, acceptable recovery must be demonstrated.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate Precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Reproducibility expresses the precision between laboratories (collaborative studies usually applied to standardization of methodology).

Precision should be investigated using homogeneous, authentic (full scale) samples. However, if it is not possible to obtain a full-scale sample it may be investigated using a pilot-scale or bench-top scale sample or sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Note: Measurements using clean standard preparations should be performed to demonstrate detector linearity, while method linearity should be determined concurrently during the accuracy study.

Classical linearity acceptance criteria require:

- the correlation coefficient of the linear regression line is not more than some number close to 1
- the y-intercept should not differ significantly from zero.

When linear regression analyses are performed, it is important not to force the origin as (0,0) in the calculation. This practice may significantly skew the actual best-fit slope through the physical range of use.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage.

Note: Ideally, robustness should be explored during the development of the assay method. By far the most efficient way to do this is through the use of a designed experiment.

Such experimental designs might include:

- a Plackett-Burman matrix approach to investigate first order effects, or
- a 2k factorial design that will provide information regarding the first (main) and higher order (interaction) effects.

In carrying out such a design, one must first identify variables in the method that may be expected to influence the result. For instance, consider an HPLC assay which uses an ion-pairing reagent. One might investigate: sample sonication or mixing time; mobile phase organic solvent constituency; mobile phase pH; column temperature; injection volume; flow rate; modifier concentration; concentration of ion-pairing reagent; etc. It is through this sort of a development study that variables with the greatest effects on results may be determined in a minimal number of experiments. The actual method validation will ensure that the final, chosen ranges are robust.

System Suitability

In addition, prior to the start of laboratory studies to demonstrate method validity, some type of system suitability must be done to demonstrate that the analytical system is performing properly. Examples include:

- replicate injections of a standard preparation for HPLC and GC methods;
- standardization of a volumetric solution followed by assays using the same buret for titrimetric methods; replicate scanning of the same standard preparation during UV-VIS assays, etc.

When the method in question utilizes an automated system such as a chromatograph or an atomic absorption spectrophotometer, a suitable standard preparation should be intermittently measured during the sample analysis run. The responses generated by the standard should exhibit a reasonable relative standard deviation. This is done primarily to demonstrate the stability of the system during sample measurements.

Protocols

Prior to initiating a validation study, a well-planned validation protocol should be written and reviewed for scientific soundness and completeness by qualified individuals. The protocol should describe the procedure in detail, and should include pre-defined acceptance criteria and pre-defined statistical methods. Following approval by the appropriate Quality Control authorities, the protocol should be executed in a timely manner. A typical assay validation will require the preparation of product placebo(s), standards, and many samples. The assay should be repeated to ensure "validity"; 3 sequential replicates are often considered the "magic number," however a far more definitive number is one produced by a sound scientific rationale, usually with the assistance of statistical analyses.

Subsequent to the execution of the protocol, the data must be analyzed with results, conclusions and deviations presented in an official validation summary report. Provided the pre-defined acceptance criteria are met, and the deviations (if any) do not affect the scientific interpretation of the data, the method can be considered valid. A statement of the method's validity should be placed at the beginning of the final summary report, along with the signatures and titles of all significant participants and reviewers.

Source/Acknowledgment

Adapted from Dr. Robert Mandle, BioSciences Research Associates, Inc.
www.cbirlabs.com/assay-validation.html