VERIFICATION AND VALIDATION TOOLKIT

Obtaining Appropriate Test Samples

To ensure correct diagnosis and treatment, clinical laboratory testing must be accurate and reliable. A key component of the quality assurance process is the verification or validation of new instruments and tests to confirm their ability to perform prior to implementation.

The Verification and Validation Toolkit walks users through this process and provides additional resources, templates and examples for use in the laboratory.

This section of the toolkit provides information on sample types, sample numbers and sample volume.

Find the complete toolkit at aphl.org/VV-Toolkit

The toolkit has eight sections:

1. Verification and Validation 101
2. Verification and Validation Process Checklist
3. Obtaining Appropriate Test Samples
4. Qualitative Assays
5. Quantitative Assays
6. Related Processes
7. Safety Considerations and Risk Assessments
8. Cost Analysis and Budget

Sample Types

Selection, sourcing or creation of the experimental samples plays a critical role in the verification or validation study of new, modified or laboratory-developed test systems and instrumentation. These samples will serve as the gold-standard comparator for validations or verifications. Sources of samples can be commercially available calibrators/calibration or quality control materials with known values, proficiency testing materials that have established values, or previously tested patient specimens with established values.

Potential clinical samples must be sufficiently characterized, or contrived samples thoughtfully designed, to allow for predictions or expectations of their performance. Poorly chosen samples could increase the rate of false positives or negatives leading to a failure in assay validation, verification, or result in misleading performance specifications. Poorly characterized samples could also introduce unknown variables or unexpected complications that compromise any derived claims based on the experimental data.

General considerations for choosing sample types:

- Examine manufacturer's statements of intended use, claims, approved specimen types and collection devices, recommended specimen preparation/handling, instructions on operating the assay, storing essential materials and any restrictions or limitations. Manufacturers' claims often exclude certain patient populations; attempts to include indicated manufacturer exclusions or clear omissions require validation and samples must be sourced from the population.
- Qualitative assay sample selection is dependent on the presence or absence of the target analyte. Knowledge of the target analyte concentration is not essential, but efforts should be made to acquire samples that span the detection range (e.g., low, medium, or high positives). Alternatively, limiting the range to clinically relevant concentrations is a viable option.
- Quantitative assay sample selection requires knowledge of the target analyte concentration. Reportable data is quantified, and an acceptance criterium can be imposed at the distinct stages of assessment (i.e., accuracy, precision, etc.).
- Clinical samples are the preferred sample type to simulate the conditions during routine testing. These samples should be well characterized including patient demographics and sample or analyte integrity. Refer to the Related Processes section of this toolkit to assess sample or analyte stability and integrity.
- Contrived samples may be utilized when clinical samples are unavailable or not suitable for the type of testing being performed. Creation of contrived samples includes spiking, or introducing, the target analyte into a matrix that is initially known not to contain the target analyte. This matrix may be an individual or homogenized (pooled) clinical sample, buffer, medium or other inert substances.

Sample Numbers

The total number of samples tested for a given study is dependent upon the evaluator's intended use of the method, the recommended statistical analysis, and the sample population. The Clinical and Laboratory Standards Institute (CLSI) has published method evaluation standards that provide explanations and instructions for evaluation of test method performance characteristics such as accuracy and precision. CLSI EP09c[1] recommends analyzing a minimum of 40 samples by both test and reference method. Ultimately, sample number is at the discretion of the CLIA Laboratory Director, and 40 samples may not be appropriate depending on the evaluator's intended use. For studies utilizing smaller sample sizes, greater statistical implications will be seen when outliers or non-correlating results are present, requiring a smaller margin of error to achieve an anticipated confidence interval. The larger the sample size, the more confident one can be that the results are reflective of the population.

Sample numbers may vary based on a number of scenarios, including:

- Category of laboratory test performed
  - FDA-approved or cleared or authorized verifications may state suggested sample numbers, but often require fewer samples than a validation.
  - FDA modified and laboratory-developed tests require validations and often larger sample sizes than a verification.
  - EUA tests often provide guidance for verification testing regarding sample size in the instructions for use (IFU) documentation.
- Qualitative assays vs quantitative assays
- Difficult to acquire specimens (e.g., rare positives, rare organisms, etc.)
- Costly test reagents (e.g., whole genome sequencing)
- Instrument verification or validation (see Related Processes)
- Reagent comparisons (e.g., discontinued products) (see Related Processes)

Sample Volume

Sufficient sample volume is a major consideration when selecting samples for a verification or validation. The laboratorian should consider the volume needed to evaluate the assay including replicate testing, use of multiple instruments and the potential need for repeat testing. Insufficient sample volume for repeat testing and/or the repeat of discrepant results could lead to insufficient investigation into any method error and, in turn, result in insufficient data to validate or verify a testing method. If comparison studies are being included as part of the validation process, the laboratory should take into consideration sample volume requirements for any additional test systems involved.

If the test method design calls for multiple replicates and instrumentation, it may be difficult to acquire sufficient volumes from clinical samples. Therefore, contrived or alternative samples may be ideal in these situations as volume issues can typically be avoided if sufficient large batches are prepared or purchased.

Reference

1 CLSI. Measurement Procedure Comparison and Bias Estimation Using Patient Samples, 3rd ed., CLSI guideline EP09c, Wayne, PA: Clinical and Laboratory Standards Institute; 2018. Available from: https://clsi.org/standards/products/method-evaluation/documents/ep09/

Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

7700 Wisconsin Avenue, Suite 1000 Bethesda, MD 20814 | 240.485.2745 | www.aphl.org

This publication was supported by Cooperative Agreements #NU60OE000104, 100% funded by the US Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC or the US Department of Health and Human Services.