



Validation of a qualitative laboratory test consists of an established set of required experiments. This overview is intended to be applicable to both Clinical Laboratory Improvement Act (CLIA) waived and non-waived methods. As defined by CLIA, waived tests are categorized as “simple laboratory examinations and procedures that have an insignificant risk of an erroneous result.” The Food and Drug Administration (FDA) determines which tests meet these criteria when it reviews manufacturer’s applications for test system waiver. As indicated in the document, additional validation testing may be required for non-FDA Approved test methods.

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. 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 qualitative methodologies

1. **Precision** is reproducibility - the agreement of the measurements of replicate runs of the same sample. There are two components for precision: short-term (also called within-run) and long-term (also called between-day). Precision testing is only required for qualitative tests that meet the following criteria:
 - Are derived from a quantitative value, such as an optical density (OD)
 - The manufacturer’s package insert describes precision specifications for the assay
 - a. Sample Criteria
 - Two levels of controls (Low/High or Normal/Abnormal)
 - b. Testing and Results
 - Short-term: Run each level of control 20 times on the same run, if possible, or at a minimum within the same day.
 - Long-term: Run each level of control at least once but not more than 5 times a day for a total of 20 runs. (For example, run each sample once per day for 20 days, OR twice a day for 10 day, but no more than 5 times per day for 4 days).
 - Calculate the mean, standard deviation (SD) and coefficient of variation (CV) of the quantitative results for each control for short-term and long-term.
 - Compare laboratory CV to manufacturer’s stated precision claims found in the package insert.



c. Acceptability Criteria

- The CV for each control 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, further troubleshooting may be needed. Consult the manufacturer and pSMILE for additional 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.

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

- A minimum of 10 samples for each expected result. For example, if a test method gives results of "Positive/Negative", the accuracy study must include 10 known positives and 10 known negatives.
- A combination of patient samples, quality control material, and external quality assurance (EQA) samples may be used.
- For HIV rapid tests: if using patient samples for accuracy testing, results must be confirmed positive or negative by an FDA approved, validated method.
- For urine hCG rapid tests: Ideally, in order to verify the manufacturer's stated cut-off, the 10 positive samples should include low as well as high positives, and at least 1 sample should be close to the manufacturer's stated cut-off.

c. Testing and Results

- Two levels of quality control must be run each day that validation testing is performed, not including controls internal to the kit cartridge/testing device
- Run each sample on each method and record results.
- Samples should be run within 2 hours of each other ideally.



- Calculate the diagnostic sensitivity and specificity rate (true positive and true negative percentage)

d. Acceptability Criteria

- The table below is a contingency table that compares the results of a qualitative test with the outcome of the diagnostic accuracy criteria. The entry in each cell of the table represents the number of specimens corresponding to the labels in the margins.

Method being Validated	Diagnostic Sensitivity and Specificity (Results from Comparison Study)		Total
	Positive	Negative	
Positive	<i># true positive (TP)</i>	<i># false positive (FP)</i>	<i>TP+FP</i>
Negative	<i># false negative (FN)</i>	<i># true negative (TN)</i>	<i>FN+TN</i>
Total	<i>TP+FN</i>	<i>FP+TN</i>	<i>N</i>

- Calculate the estimated Diagnostic Sensitivity
(True positive rate) = $100 \times [TP/(TP+FN)]$
- Calculate the estimated Diagnostic Specificity
(True negative rate) = $100 \times [TN/(FP+TN)]$
- Calculate the percent Positive Agreement
(Positive Predictive Value) = $100 \times TP/(TP+FP)$
- Calculate the percent Negative Agreement
(Negative Predictive Value) = $100 \times TN/(TN+FN)$
- Compare the results calculated above with the manufacturer's stated claims for Sensitivity, Specificity and Agreement found in the test kit package insert.
- Results must be equal to, or greater than, the manufacturer's claims for the method to be considered accurate.

3. **Linearity, Analytical Measurement Range (AMR) and Clinical Reportable Range** are not applicable for qualitative methods.

4. **Analytical Sensitivity** is the lowest concentration of an analyte that can be measured. **Analytical Specificity** is the determination of the effect of interfering substances.

- For an FDA approved, unmodified method, the manufacturer's stated analytical sensitivity and specificity will be used.



5. Reference Ranges can be determined by the laboratory with laboratory director approval. Verification of manufacturer's stated reference range is not required.

6. 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.

References

1. CLSI. *Evaluation of Qualitative, Binary Output Examination Performance*. 3rd ed. CLSI guideline EP12. Clinical and Laboratory Standards Institute; 2023.
2. EP Evaluator Release 12.0, David G. Rhoads Associates Inc., datainnovations.com/ep-evaluator-resources.
3. Westgard, James O., *Basic Method Validation: Training in Analytical Quality Management for Healthcare Laboratories*, 4th edition, 2020 Madison, WI 53717.