Urinalysis Dipstick Validation Guidelines

Validation of a method 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. All validation records should be retained for the life of the method/assay. A validation summary should be prepared that contains a place for the Laboratory Director to sign, indicating the validation has been reviewed and approved.

Analytes that may be included in urinalysis are: Nitrite, Protein, Glucose, Ketone, Bilirubin, Hemoglobin/Blood, Urobilinogen, Leukocyte Esterase, pH and Specific Gravity.

The following are the required components of validation/verification:

1. **Precision** is reproducibility - the agreement of the measurements of replicate runs of the same sample. Precision testing is not applicable for qualitative or semi-quantitative tests unless manufacturer specifications include precision data.

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

        - Accuracy studies must include a minimum of 20 replicates. These may be quality control, EQA or known patient samples.
        - Semi-quantitative analytes must include 10 negative and 10 positive replicates. Positive semi-quantitative results may include any of the following: Trace, 1+, 2+, 3+, 4+ or any numerical grouping like 250, 500 et.
        - For pH, 10 replicates are required in the normal range (5.5-7.0) and 10 replicates in the abnormal range (preferably a mix of acidic and basic).
        - For specific gravity, it is preferable to acquire 20 replicates that span the range of 1.000 to 1.035.

    c. Testing and Results

- Two levels of quality control must be run each day that testing is performed.
- It is recommended that testing should be performed by at least 2 different testing personnel.

d. Acceptability criteria:

- pH should be within +/- 1 of the expected value.
- Specific gravity should be within +/- 0.005 of the expected value.
- Semi-quantitative tests should be evaluated based on the comparison method used.
    - if using quality control material, refer to the manufacturer's acceptable range of each level of quality control.
    - If using EQA, refer to the provider's participant summary report for acceptability limits.
- For each qualitative or semi-qualitative analyte, use the contingency table below 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	***# true positive (TP)***	***# false positive (FP)***	***TP+FP***
Negative	***# false negative (FN)***	***# true negative (TN)***	***FN+TN***
Total	***TP+FN***	***FP+TN***	***N***

- Calculate the estimated Diagnostic Sensitivity
  (True positive rate) = 100 x [TP/(TP+FN)]
- Calculate the estimated Diagnostic Specificity
  (True negative rate) = 100 x [TN/(FP+TN)]
- Calculate the percent Positive Agreement
  (Positive Predictive Value)=100 x TP/(TP+FP)
- Calculate the percent Negative Agreement
  (Negative Predictive Value) =100 x 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. If this information is not stated in the package insert, results must be equal to, or greater than, 95%.

3. **Linearity, Analytical Measurement Range (AMR)** and **Clinical Reportable Range** are not applicable for qualitative or semi-quantitative tests.

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. GCLP Standards version 4.1, Test Method Validation and Verification, pages 24-30.CLSI. *Evaluation of Qualitative, Binary Output Examination Performance.* 3rd ed. CLSI guideline EP12. Clinical and Laboratory Standards Institute; 2023.
2. CLSI. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples;* 3rd ed. CLSI guideline EP09c. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
3. CLSI. *Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline-Third Edition*. CLSI document EP10-A3-AMD. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
4. EP Evaluator Release 12.0, David G. Rhoads Associates Inc., datainnovations.com/ep-evaluator-resources.
5. Westgard, James O., Basic Method Validation: Training in Analytical Quality Management for Healthcare Laboratories, 4th edition, 2020 Madison, WI 53717.