COLA Clinical Laboratory Accreditation

COLA Primers Accreditation

COLA PRIMER 13

# *Verifying Performance Specifications*

## •Overview•

Verification of performance confirms that the test method performs as expected when the test is run in your laboratory. The CLIA regulations require that you verify the manufacturer's stated performance characteristics (found in the package insert) for accuracy, precision, reportable range and reference range. This must be done and documented before you initiate patient testing, and is required when:

- You add a new test method for an analyte– if multiple instruments will be used for the same analyte, each instrument must have performance verified;
- You add a new analyte to an existing instrument;
- You switch to a new reagent manufacturer for an analyte;
- You are using a loaner instrument; and
- After relocating an instrument to a new environment (see note below).

**NOTE:** When relocating an instrument to a new location, consider that the new location may have environmental conditions that are different from the original location. After relocation, it is a good practice to test several previously tested specimens and run QC. If you obtain comparable results on the previously tested samples, if QC is acceptable, and if the Laboratory Director has determined that performance has not been affected by the move, then a full new performance verification study would not be required. It is very important that the Laboratory Director determine if this abbreviated verification is acceptable.

## • Definitions •

For FDA approved methods that have not been modified, you must verify accuracy, precision, reportable range, and reference range for quantitative tests. For qualitative tests, verification of accuracy is sufficient, since there is no reportable range and precision statistics cannot be calculated.

***Accuracy:*** This verifies that your laboratory test yields a result that reflects the true value for the analyte. Accuracy is verified by running samples with known values and comparing your results to the known values. You should verify accuracy and precision using samples with both normal and abnormal values, preferably across the reportable range for the test.

To verify accuracy for qualitative tests, your accuracy verification confirms that the test correctly identifies the presence or absence of the analyte.

***Precision:*** This verifies the reproducibility of the test. Samples should be repeated and results compared within the same run, from different runs on the same day, and from runs on different days. It is helpful to have various testing personnel who would normally run the test, perform the reproducibility runs.

For qualitative tests, you can verify precision by running the same samples multiple times, on different days. This is essentially the same as what you will do to verify accuracy for qualitative tests.

***Reportable Range:*** This is the lowest and highest result that can be accurately and reliably measured by the method. The manufacturer should include a reportable range in the package insert. Your laboratory can then only report results that fall within the range that you have been able to verify in your laboratory. You then must establish a policy for how you will report results that are outside of this range. Typically this is done using "greater than" or "less than," for example ">30 mg/dl" if 30 mg/dl is the highest value you were able to verify.

***Reference Range:*** This is the range of results that you would expect to see in healthy individuals in your patient population. Reference ranges are often referred to as "normal" ranges, and should reflect the medical decision points for clinicians. Initially, you may use the manufacturer's stated reference range. During the first month of testing, you will need to monitor your patient results to verify that the manufacturer's range is appropriate for your patient population. Make adjustments if necessary.

COLA also encourages laboratories to do method comparisons when initiating a new method for a test, to determine if medical decision points for the new method are different than a previous method. Method comparisons are required in the state of Washington.

## • Selecting Samples for Use in Verifying Performance •

Samples with known values, such as calibrators, QC materials, or previously tested PT samples are good samples to use when verifying performance. Do not use PT samples for this purpose until after your scores have been received from the PT provider.

Previously tested patient specimens can also be used, but you will need to carefully establish your own acceptable limits when using for performance verification.

It may be difficult to find suitable samples with sufficient volume to allow you to perform the studies. So planning ahead of time is key. Your Laboratory Director is responsible for determining the appropriate selection of samples to verify the performance specifications and have confidence in the test results across the reportable range.

The vendor cannot perform verification studies for you, but may assist by providing samples to use for the verification, and/or providing statistical analysis for you. The point is to verify that the test performs as intended in your laboratory when tested by your personnel.

## • Verification of Qualitative Tests •

For qualitative tests, you will need to verify accuracy by documenting that the test correctly identifies the presence or absence of the analyte. Precision, reportable range, and reference ranges do not apply to qualitative tests, as long as you test a variety of known positive and known negative samples.

The more samples and repetitions, the better the data, and the more confidence you can have in the test results. ***At the very minimum:***

- Test at least two known negative samples in duplicate on two different days, using a different testing personnel each day, if possible.
- Test at least two known positive samples in duplicate on two different days, using a different testing personnel each day, if possible.

All known negatives and known positives must achieve the expected result. If not, the test may not be suitable for your laboratory, OR you may need to evaluate if sufficient training occurred and do additional training before repeating the study.

## • Verification of Quantitative Tests •

Accuracy and precision can be performed concurrently using the same samples. For statistical significance, 20 data points is the minimum number of samples to be tested. This is certainly recommended.

You will need to verify the reportable range by including samples that are "normal," as well as samples that are at or near the lower and upper limit of the manufacturer's stated reportable range. It is a good idea to plan for this ahead of time, as you are preparing to bring on a new test method.

## • Verification Procedure •

A form template for documenting performance verification is included at the end of this document.

1. For precision, run at least five repetitions of at least two samples with different values. Do NOT use a zero value sample for this purpose. Test the repetitions on different days and if possible, have different testing personnel perform the repetitions.

   To evaluate the precision, calculate the mean, standard deviation (SD), and coefficient of variation (CV) for each set of results. The lower the CV, the better your precision. Compare your CV with the manufacturer's stated CV.

2. The results of your precision study can also be used to evaluate accuracy. Calculate the mean of the values obtained for each sample and compare to the known value of the sample. Determine the difference between your mean and the known value, and compare this to the acceptability range established by your Laboratory Director.

   Another way to evaluate the accuracy is to divide the mean you obtained on the sample by the known value and then multiply by 100. The closer the result is to 100, the more accurate the test. This is referred to as the ratio of means.

3. Remember that the reportable range is the range of values which the laboratory can accurately report results. If you used samples with values close to the lower and upper range of the manufacturer's state reportable range in steps 1 and 2 above, and those results were acceptable, you have verified the reportable range. If, however, in verifying accuracy and precision you did not cover the full range of values, including at or near the lower and upper limits, then you will need to test at least two additional samples each at or near the lower and upper limits of the reportable range. Use the same process you used to evaluate accuracy for the additional samples.

4. You may use the manufacturer's stated reference range for the test when you initially begin patient testing. Monitor patient results for the first month or so, and verify that the reference range is appropriate for your patient population. Make any necessary adjustments. You should use a reference range within which approximately 95% of healthy individuals fall.

    - Evaluate results from 20-30 "normal" patients over several days. Again, the more data the better.
    - Calculate the mean and standard deviation.
    - Calculate the range that is the mean minus and plus 2 standard deviations. Then compare this to the manufacturer's stated reference range. Discuss with the Laboratory Director to determine if an adjustment is necessary, or if more data should be evaluated.

    You should check this periodically as part of your Quality Assessment plan, to make sure that your reference ranges continue to be appropriate for your patient population.

## • Documentation •

Document your performance verification studies, and keep this documentation, including raw data, for as long as the test is performed plus two years. Following is a sample form that can be used to record and evaluate your performance verification studies.

***Verification of Performance for FDA Approved Methods  Name of Laboratory_____***

TEST_____ INSTRUMENT_____

DATE_____ PERFORMED BY_____

***Must be performed by laboratory staff.***

1. Accuracy verification

Test 2 levels of assayed control material.

| DAY | Results – level 1 | Acceptable Y/N* | Results – level 2 | Acceptable Y/N* |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| ∑ (sum) | | | | |
| $\bar{x}$ (mean) | | | | |

*Acceptable range – Each test value must be within +/- 10% of the mean of the published assay value of the control material.

The mean of the daily values must be within +/- 5% of the mean of the published assay value of the control material.

Level 1 mean published assay value_____

Level 2 mean published assay value_____

2. Precision verification

Verify precision by calculating the Standard Deviation (SD) and Coefficient of Variation (CV) for the data collected above.

| | Level 1 | Level 2 |
|---|---|---|
| SD | | |
| CV | | |

Example acceptable limits for CV:
5% for non-enzymes; 8 % for enzymes

If the CVs exceed the acceptable limits but are close, analyze the assayed control material for several more days and recalculate the CV. If the CVs are still unacceptable, you may need to troubleshoot after consulting with the manufacturer.

3. Reportable range verification

Verify reportable range by testing a High, Mid, and Low level calibrator in duplicate. It is also acceptable to use other samples of known values, such as previously tested patient samples or linearity materials. The samples used should span the manufacturer's stated reportable range.

% Difference = $\bar{x}$ - C / C x 100
% Difference must be within </= 10%

| | Result 1 | Result 2 | $\bar{x}$ (mean) | Calibrator value (C) | % Difference |
|---|---|---|---|---|---|
| Low | | | | | |
| Mid | | | | | |
| High | | | | | |

Reportable range is from the low mean value to the high mean value.

4. Reference interval verification

Test 20 patients with a condition that generally produces test results that fall within the reference interval (RI). The RI is sometimes called the reference range or normal range. If more than two values fall outside the RI, more studies are required.

| | Patient value | Within RI Y/N | | Patient value | Within RI Y/N |
|---|---|---|---|---|---|
| 1 | | | 11 | | |
| 2 | | | 12 | | |
| 3 | | | 13 | | |
| 4 | | | 14 | | |
| 5 | | | 15 | | |
| 6 | | | 16 | | |
| 7 | | | 17 | | |
| 8 | | | 18 | | |
| 9 | | | 19 | | |
| 10 | | | 20 | | |

Reference Interval = _____

Then test 10 patients with a condition that should have test results falling outside the RI. If more than two values are within RI, more studies are required.

| | Patient value | Within RI Y/N | | Patient value | Within RI Y/N |
|---|---|---|---|---|---|
| 1 | | | 6 | | |
| 2 | | | 7 | | |
| 3 | | | 8 | | |
| 4 | | | 9 | | |
| 5 | | | 10 | | |

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If results of these studies are acceptable, indicate approval by signing this form and taking the following steps:

- Make sure that a complete procedure for the test is available for lab staff.
- Make sure that testing personnel are trained on the new procedure. Document the training. Perform competency assessment as required.
- Inform the physicians who use the laboratory that the test is available and make sure they have access to the verified reference interval.
- Make sure to develop a policy for how results that fall outside of the reportable range will be handled and reported.

COMMENTS:

_____

_____

_____

Approved

**Date**_____

**Laboratory Director or Designee**_____