LabGuide 13

How to Verify Performance Specifications

This LabGuide pertains to QSE Equipment and the Analytic Phase of the Path of Workflow.

VERIFICATION OF PERFORMANCE SPECIFICATIONS

As a result of updated CLIA regulations, laboratories need to verify the performance specifications of all nonwaived, FDA approved, unmodified tests added to the laboratory menu on or after April 24, 2003. (Before April 24, 2003, this requirement only applied to new high complexity tests.)

When any of the following situations occur, CLIA requires you to verify the manufacturer's stated performance specifications for accuracy, precision, reportable range, and patient reference range for the test before you report patient results. These can be found in the package insert.

- Whenever you add a new instrument or method, including multiple versions of the same instrument or method;
- When you add a new analyte to your menu that will be performed on an existing instrument;
- When switching methods or reagent manufacturer for an analyte already on your menu;
- Before putting a "loaner" instrument in use;*
- After relocating an existing instrument to a new environment.**

The verification of performance specifications process confirms that the instrument and/or test method performs as the manufacturer intended when utilized in your laboratory environment, by your personnel, for your patient population.

The verification process confirms that the instrument and/or test method performs as the manufacturer intended when utilized in your laboratory environment, with your personnel, for your patient population.

CMS did not set specific requirement details or offer procedures that tell you how to perform this verification. It is up to the laboratory director, with the assistance of the technical consultant/supervisor, to establish appropriate procedures. The manufacturer can assist you by providing materials, procedures, and statistical analysis, but they may not perform the actual testing of the samples used in the verification process for you.

Note: *This LabGuide only addresses FDA-approved, unmodified tests. For tests that are not FDA-approved, or for tests that are FDA-approved but modified by the laboratory, you must* ***establish*** *performance specifications for the test to validate the method before reporting patients. Establishing performance specifications involves additional steps.*

**If the loaner instrument is the exact same make, model, and test method, and will be used to test the same analytes, this requirement can be reduced. You may verify comparable performance by testing and obtaining acceptable results on at least 2 levels of controls AND either previously tested PT samples or previously tested patient samples.*

***When relocating an instrument to a new location, only move it to locations that meet the manufacturer's requirements for environmental conditions. Consider the possibility that the new location has conditions that are different enough to impact instrument performance. For example, the new site may have different electrical circuitry, new potential interferences, and/or changes in temperature, humidity, and airflow. After relocation, if comparable results are obtained on previously tested specimens, QC results are in range, AND the laboratory determines that they have adequately demonstrated that performance specifications are not affected, a full new performance verification study would not be required.*

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DEFINITIONS OF PERFORMANCE SPECIFICATIONS TO BE VERIFIED

For unmodified, FDA-approved tests, you must verify accuracy, precision, reportable range, and reference ranges for quantitative tests (tests that give a numerical result). If the test is qualitative (a test that gives a negative or positive result), only verification of accuracy is applicable.

Accuracy: how close a test result is to the analyte's true value. You must verify that the test gives correct results in your laboratory. Accuracy can be verified by testing samples with known values and comparing the results you obtain. Acceptable limits must be established to define how close the results need to be. COLA recommends that you evaluate accuracy and precision using samples with values in the normal patient range and at one other level, depending on where you expect clinically significant patient test results to fall.

To confirm accuracy for qualitative tests, you must verify that the test correctly identifies the presence or absence of the analyte.

Precision: the degree to which repeated test results on the same sample agree. Samples should be repeated within the same run, run-to-run, and day-to-day. You must verify that results are reproducible in your lab, even when different testing personnel perform the test.

It may seem that precision could also be applicable to qualitative testing. You could obtain a sample known to be positive and test it multiple times. However if you do not obtain a positive result each time, it is the accuracy (ability to detect the analyte being measured) of the test that is in question, and the test may not be suitable for use in your clinical setting. Inconsistent results could also be the result of operator error, which emphasizes the need to have different personnel participate in the testing when assessing performance specifications. This enables you to detect test performance problems as well as training problems, and serves as a means to assess personnel competency.

Reportable range: the lowest and highest results that can be accurately measured and all the values in between. The manufacturer has established a reportable range for the test, which you must verify by testing known samples with values at the low and high end of that stated range. ***Your laboratory may only report results that fall within your verified range***. You must establish a policy for how you will report results that are higher or lower than the verified levels at each end of the range.

Reference range: the span of values for a particular test that represents the results you would expect to see in a healthy (normal) patient population. Reference ranges establish the normal values for the test and should reflect the medical decision limits for clinicians. Initially, you may use the manufacturer's suggested reference range. The laboratory is required to monitor the applicability of this range and make adjustments as necessary. If you know or suspect that your particular patient population varies from the norm (e.g., pediatrics, geriatrics, predominantly female, or predominantly male) you may need to adjust your reference range to more closely match your patient population.

When changing test methods, in addition to verifying the performance specifications for

When a change in test method occurs, the reference range and medical decision limits for the new test may be different. Though it is not required as part of the verification of performance specifications process, a method comparison is strongly encouraged. It is the best way to establish the relationship between the previous and new methods for the test.

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the new test, COLA encourages laboratories to perform a method comparison to determine the relationship between the normal ranges and medical decision limits of the previous method, and the normal ranges and medical decision limits of the new method.

Tips on Sample Selection

Samples with known values, such as calibrators, quality control materials, or proficiency testing (PT) samples have the advantage of having acceptable limits already defined by the manufacturer's package insert (for controls or calibrators) or PT summary report (for previously tested PT samples).

You may also use previously tested patient samples, but you will need to establish your own acceptable limits for variance prior to performing the verification study, taking good laboratory practice and patient care into consideration.

When selecting the samples to use, take into account the number of tests for which you need to perform the verification study. For instance, if you are adding a Chemistry analyzer that performs 20 different tests, each analyte will need to be verified. So, you will want to select samples that provide data for multiple analytes. Commercial samples, such as controls, calibrators, or standards, may be more efficient to use when verifying accuracy and precision in this situation. They have the additional advantage of representing the normal and abnormal values that you would expect to recover when testing patients. Additional samples with very high and very low values will need to be obtained to verify the reportable range for all 20 analytes. (You may be able to create your own low value samples by diluting controls or calibrators, but it is unlikely that an abnormal control will have a value high enough to verify the high end of the reportable range.)

The manufacturer cannot perform the actual testing for you, but may assist by providing samples to test, by providing procedural instructions, and/or by performing mathematical calculations and analysis.

Remember, the point is to verify that the test performs as intended when used in your laboratory environment by your personnel.

If you were adding a single test to an existing instrument, you might choose to select a single set of samples that would be suitable to use for the accuracy, precision, and reportable range. In this case a very low sample and a very high sample would be the absolute minimum, but adding a normal value sample would be preferable, and would still allow you to use only three different samples for your study.

Sometimes it can be difficult to find suitable samples with sufficient volume that would allow the laboratory to use the same samples throughout the study. Your laboratory director is responsible for determining the appropriate selection of samples to permit the laboratory to verify performance specifications and have confidence in test results, while being cognizant of the resources expended.

Methods to Verify Performance Specifications

The manufacturer cannot perform the actual testing for you, but may assist by providing samples to test, by providing procedural instructions, and/or by performing the mathematical calculations and analysis. Remember, the point is to verify that the test performs as intended when used in your laboratory environment, by your personnel. With careful planning, you can efficiently complete the process for quantitative tests within several days. For qualitative tests, the process is even simpler.

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Qualitative Tests

For qualitative tests (those that are reported as "positive" or "negative") you will need to verify accuracy by demonstrating that the test correctly identifies the presence or absence of the analyte. Precision, reportable range, and reference ranges do not apply to these tests.

The more samples and repetitions tested, the better the laboratory's data. A bare minimum for qualitative testing would be to test at least two negative and two positive samples, running at least two repetitions of each sample.

- Test at least two known negative samples in duplicate, on two different days, using a different operator each time if possible. (You will have four test results for each negative sample.)
- Test at least two known positive samples in duplicate, on two different days, using a different operator each time if possible. (You will have four test results for each positive sample.)

If you obtain a negative result for all repetitions of the known negative sample, and a positive result for all repetitions of the known positive sample, you have verified the accuracy of the test. If not, the test may not be suitable for use in your lab. Alternatively, the issue may be operator error, indicating the need for additional training or competency evaluation of your personnel.

Accuracy and precision should be performed concurrently using the same samples. In some situations, with careful sample selection, it may be possible to use these same samples to verify reportable range also.

Quantitative Tests

Accuracy and precision should be performed concurrently using the same samples. In some situations, with careful sample selection, it may be possible to use these same samples to verify reportable range; although, this may not always be the most efficient or practical route. (See "Tips on Sample Selection")

For statistical significance, obtaining 20 data points for a given measure is the standard; therefore, CMS and COLA encourage laboratories to follow this rule of 20 data points whenever possible. However, both organizations recognize that obtaining 20 data points may be prohibitive for some tests in some facilities.

For quantitative tests, a bare minimum would be to test at least two samples, running a minimum of five repetitions each. Laboratories are encouraged to use more samples and perform more repetitions whenever possible. The larger the number of samples and repetitions, the more meaningful the data becomes. Plan to test an adequate number of samples, as determined by the laboratory director, based on medical decision limits, laboratory test volume, and the number of tests or test systems to be verified.

Verification Procedure

Definitions, formulas, example calculations, and worksheets are provided at the end of this LabGuide.

Before beginning, the instrument must be properly installed in its permanent location and calibrated.

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1. Start with precision. Test at least five repetitions of at least two samples with different values. Do NOT use a zero value sample when verifying precision or accuracy.
   Test the samples:
   - In the same run (2 of the 5 repetitions);
   - In a different run on the same day (3rd repetition); and
   - On different days (4th and 5th repetitions performed on different days).

   If possible, have different staff perform the 3rd, 4th and 5th repetitions to check for operator variance. (This may not be critical if the test uses a fully automated test system that is not operator dependent.)

   To assess precision, calculate the mean, standard deviation (SD), and coefficient of variance (CV) for each set of results. Typically, you will find a different CV at different ranges for the analyte. Compare the CVs obtained to that stated in manufacturer's package insert section addressing performance specifications.

   The smaller the CV, the more precise the test.

2. The results obtained in the precision study can also be used to assess accuracy.
   - Compare the mean of the five repetitions for each sample to the known value of the sample;
   - Determine the difference between the two values, and compare to the limits of acceptability stated in the package insert for known samples, or use allowable Proficiency Testing scoring limits (found in the PT Summary Report).

   Another simple way to evaluate the results is to calculate a ratio of means for comparison. To do this, divide the mean you obtained by the known value and multiply by 100. The closer the result is to 100, the more accurate the test.

3. It is important to remember that reportable range defines the span of values for which the laboratory can report results. If you used samples with a very low value and a very high value for Step 1 (precision) and Step 2 (accuracy) and the results were acceptable, then you have already verified the reportable range. If however, the samples used do not cover the full range of values for which you expect to report results (for instance, if you used controls or calibrators for Step 1 & 2), you will need to test at least one repetition of two additional samples with known values - one at the low end and one at the high end of the stated reportable range.

   If a zero calibrator is available for the analyte, then you may extend the low end of your reportable range down to zero, provided you actually obtain a zero result when you test that sample.

   Use the same process you used to evaluate accuracy to evaluate your results for the reportable range.

   If you are using the same samples to verify accuracy, precision, and reportable range, COLA recommends that you include a third sample with a value in the middle of the range (where your normal patients are expected to fall). By doing this and obtaining

You can only report results that fall within the verified reportable range. You must establish a policy that addresses how results outside of this range will be handled and reported.

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acceptable results, you have also performed an initial calibration verification.

4. You may use the manufacturer's suggested reference range for the test when you first begin patient testing. The laboratory is required to monitor the applicability of this normal range and make adjustments as necessary. The goal is to use a reference range within which the majority of healthy individuals without the disease will fall.

   Perform this simple test to compare the manufacturer's reference range to data obtained when testing your normal patients:
   - Collect specimens from a minimum of ten normal patients;
   - Test each specimen only once, and spread the testing over a minimum of three days;
   - To calculate the normal patient mean: Add the values obtained for each sample, and divide by ten;
   - Calculate the standard deviation (SD)and determine the range of plus and minus two SD from the mean;
   - Compare this range to the manufacturer's range;

   Interpretation: If your lab's range falls within **THE** manufacturer's range, the reference range is comparable. Perform the test again when you have results for 20 to 30 normal patients to see if your laboratory's range still falls within the manufacturer's reference range with the larger data pool.

   Continue to perform this check periodically as part of your quality assessment program. In the event the ranges obtained above are not comparable, your lab will need to establish an appropriate reference range for your patient population.

Document all activities involved in verifying performance specifications. Retain this documentation for as long as the test method is in use and for two years after discontinuing the method.

Document all activities involved in verifying performance specifications.

Retain all records and documents for as long as the test method is in use and for at least two years after the method has been discontinued.

RESOURCES

- See LabGuide 12 for additional important information, if you are adding testing in a new specialty or subspecialty.
- See CLIA Facts 2 and CLIA Facts 16-B for details on the CLIA regulations for Verification of Performance Specifications.

CMS has published Brochure #2 called Verification of Performance Specifications that can be viewed on-line at: http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/CLIA_Brochures.html

Note for COLA laboratories

COLA labs must perform verification or establishment of performance specifications for all new nonwaived (moderate and high complexity) tests added to the test menu on or after June 1, 2007.

COLA labs should complete the following sections of the COLA Self-Assessment to ensure compliance with all the requirements associated with verification of performance specifications – VER 1-4, and VER 12-14.

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DEFINITIONS, FORMULAS, AND EXAMPLE CALCULATIONS

Definitions, formulas, and example calculations are provided below to assist you in completing the verification of performance specifications process.

Mean ($\bar{x}$): The mean is the average of the results. It is the sum of all results for the sample divided by the number (n) of results: $\bar{x} = (x_1 + x_2 + ...x_n) \div n$

Example:
You have 5 results for a sample:
5.0, 4.7, 5.1, 4.9, and 4.8
$\bar{x} = (5.0 + 4.7 + 5.1 + 4.9 + 4.8) \div 5$
$\bar{x} = 24.5 \div 5 = 4.9$
The mean is 4.9.

Standard Deviation (SD): Standard deviation (SD) is a predictable measure of variation around the mean. The less variation around the mean, the closer the SD is to the ideal of zero. Traditionally, 2 standard deviations from the mean (±2SD) is usually considered acceptable variation.

SD is the square root of the sum of the squared differences of each result from the mean, divided by n-1.

$$\sqrt{\Sigma (x-\bar{x})^2 / (n-1)}$$

To calculate the SD in our example, we first need to calculate $x-\bar{x}$ for each result (without regard to positive or negative), then square those results and total them.

$$\sqrt{(5.0–4.9)^2 + (4.7–4.9)^2 + (5.1–4.9)^2 + (4.9–4.9)^2 + (4.8 – 4.9)^2 / 5–1}$$

$5.0 – 4.9 = 0.1$	$0.1^2 = 0.01$
$4.7 – 4.9 = 0.2$	$0.2^2 = 0.04$
$5.1 – 4.9 = 0.2$	$0.2^2 = 0.04$
$4.9 – 4.9 = 0.0$	$0.0^2 = 0$
$4.8 – 4.9 = 0.1$	$0.1^2 = 0.01$

Next, divide the total (0.10) by 4 (n-1):

$(0.01 + 0.04 + 0.04 + 0.00 + 0.01) \div 4 = 0.10 \div 4 = 0.025$

Complete the calculation by determining the square root of 0.025: $\sqrt{0.025} = 0.158$

The SD for these values is 0.158.

To determine a 2 SD range, subtract the calculated SD twice from the mean to get the lower limit and add the calculated SD twice to the mean to get the upper limit:

$4.9 – 0.158 – 0.158 = 4.584$
$4.9 + 0.158 + 0.158 = 5.216$

Since the test is reported to one decimal place, we round off our calculations to arrive at a 2SD range of: 4.6 – 5.2.

Coefficient of Variation (CV): The coefficient of variation is the standard deviation of a set of results divided by the mean result. Since the smaller the CV, the better, CV results below 15% are generally considered good target values for most test methods. (Check the package insert for specifics for your test method.)

CV is expressed as a percentage or as a decimal fraction less than one. Multiply the decimal fraction by 100 to get the CV in percent.

For our example, $0.158 \div 4.9 = 0.0322 \times 100 = 3.2\%$.

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VERFICATION OF PEFORMANCE SPECIFICATIONS WORKSHEET

Note: Modify this worksheet as needed to test additional samples and/or repetitions as determined by your laboratory.

Date: _____ Analyte/Test Method: _____ Initials: _____

QUALITATIVE TEST

Day 1	Known Negative Samples	#1			#2		
	Known Positive Samples	#1			#2		
Day 2	Known Negative Samples	#1			#2		
	Known Positive Samples	#1			#2		

Conclusion _____

_____

Comments _____

QUANTITATIVE TEST

Sample #1 ID: _____ Known Value: _____

Sample #2 ID: _____ Known Value: _____

Precision

Day 1

Two repetitions of each sample in one run

#1 _____ _____

#2 _____ _____ Initials: _____

One repetition of each sample in an additional run

#1 _____

#2 _____ Initials: _____

Day 2: One repetition of each sample

#1 _____

#2 _____ Initials: _____

Day 3: One repetition of each sample

#1 _____

#2 _____ Initials: _____

Calculations		Results for Sample #1	Results for Sample #2
Precision	X_1		
Mean ($\overline{X}$) = $(x_1 + x_2 + \ldots x_n) \div n$	X_2		
(For this worksheet, n = 5.)	X_3		
	X_4		
	X_5		
	Sum		
	$\div n = \overline{X}$		

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Standard Deviation (SD)

$\sqrt{[(x_1-\bar{x})^2 + (x_2-\bar{x})^2 + (x_3-\bar{x})^2 + (x_4-\bar{x})^2 + (x_5-\bar{x})^2] \div (n-1)}$

Sample #1		Sample #2	
$(x_1-\bar{x})$ = _____	squared = _____	$(x_1-\bar{x})$ = _____	squared = _____
$(x_2-\bar{x})$ = _____	squared = _____	$(x_2-\bar{x})$ = _____	squared = _____
$(x_3-\bar{x})$ = _____	squared = _____	$(x_3-\bar{x})$ = _____	squared = _____
$(x_4-\bar{x})$ = _____	squared = _____	$(x_4-\bar{x})$ = _____	squared = _____
$(x_5-\bar{x})$ = _____	squared = _____	$(x_5-\bar{x})$ = _____	squared = _____
	Total = _____		Total = _____
	÷ (n-1)= _____		÷ (n-1)= _____
	Square root = _____ SD		Square root = _____ SD

Coefficient of Variation (CV)

Sample #1

SD _____ ÷ $\bar{x}$ _____ = CV _____

X 100 = _____ (CV as %)

Sample #2

SD _____ ÷ $\bar{x}$ _____ = CV _____

X 100 = _____ (CV as %)

CV compared to manufacturer's limits _____

Acceptability of results _____

Comments _____

_____

Accuracy

Compare the mean ($\bar{x}$) for each sample (see above) to the known value.

Sample #1 $\bar{x}$ = _____ Known value = _____ Difference = _____

Sample #2 $\bar{x}$ = _____ Known value = _____ Difference = _____

Known value is ☐ Manufacturer's limits ☐ PT limits ☐ Other, specify:

Or evaluate the ratio of means ($\bar{x}$ ÷ known value X 100). Goal is to be as close to 100 as possible.

Sample #1 _____ Sample #2 _____

Acceptability of results _____

Comments _____

_____

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Reportable Range

High sample result _____ Known value _____

Low sample result _____ Known value _____

Mid-range sample result (optional) _____ Known value _____

Zero calibrator result (if available) _____

Comments _____

_____

Verified reportable range is from _____ to _____

Develop a policy for how results that fall outside of this range will be handled and reported.

Reference Range

Minimum of 10 normal patients tested over at least three days to determine the normal patient mean.
(This worksheet has room to record up to 20 normal patients.)

Day 1	#1	_____	#2	_____	#3	_____	#4	_____	#5 _____
Day 2	#1	_____	#2	_____	#3	_____	#4	_____	#5 _____
Day 3	#1	_____	#2	_____	#3	_____	#4	_____	#5 _____
Day 4	#1	_____	#2	_____	#3	_____	#4	_____	#5 _____

Sum of the values = _____ ÷ the number of values = _____

Use this normal patient mean to calculate the Standard Deviation, using the method described above.

Standard Deviation (SD) = _____ ± 2 SD Range = _____

Does this range compare to the manufacturer's reference range? ☐ Yes ☐ No

Comments _____

_____

_____

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