COLA Clinical Laboratory Accreditation

COLA Primers Accreditation

COLA PRIMER 9

Split Specimen Analysis

● Overview ●

Split specimen analysis may be defined as the analysis of a specimen by a primary method (your laboratory method) and by a comparison method (method used by another CLIA-certified laboratory). This may be accomplished by the following processes:

- Splitting the specimen into two portions. One portion will be analyzed by your laboratory and the other is sent to another laboratory for analysis.

The other laboratory selected for Split specimen analysis must possess a valid CLIA certificate for the analytes, specialties, and subspecialties that are being tested. The laboratory selected should have the same instrument or method as your laboratory. If the laboratory selected is using a different instrument or method, or the same instrument but different reagents and/or method, your laboratory may want to determine the bias factor in order to obtain a more valid comparison.

Split specimen testing may be used:

- To determine the relative accuracy of a particular testing method
- To determine the linearity of an instrument compared to a reference instrument
- For troubleshooting questionable test results or test system problems
- When a laboratory has multiple instruments and/or methods for testing the same analyte
- As a means of verifying the accuracy of non-regulated (unregulated) analytes for which the laboratory does not participate in proficiency Testing (PT), which will be the focus of this COLA Primer

The laboratory must have an approved procedure for Split specimen analysis. This procedure should include:

- The list of non-regulated analytes to be tested
- The CLIA-certified laboratory(s) to be used
- The split specimen process to be utilized
- The number of specimens to be tested
- The frequency for split specimen analysis
- The limits of acceptability
- Actions to take when the limits of acceptability are not met.

● Method Bias ●

Bias refers to the amount of difference in results between two instruments analyzing the same specimen. It is caused by differences in instrument engineering and manufacturer, in methods used to perform testing, or in the reagents used to perform testing. It is important because the bias between some instruments and/or methods is great enough to affect the outcome of the comparison of the results. The laboratory may want to determine the bias factor in order to obtain a more valid comparison of the results.

Under ideal circumstances, the comparison of results between laboratories for an analyte should be performed on identical instruments using the same method and reagents. No bias factor would be needed in scoring the results.

Determining Instrument (Method) Bias

Your laboratory may wish to establish bias values with another CLIA-certified laboratory for the applicable analytes performed in your laboratory, in the event you choose to use split specimen analysis instead of PT for your non-regulated (unregulated) analytes.

Bias represents the difference between the average of a set of results for a particular analyte obtained by the primary method (your laboratory) and the average of the same set of results obtained by the comparison method (a CLIA-certified laboratory). The difference can be estimated by calculating the average value for the primary method (**Method A**) and the average value for the comparison method (**Method B**). Subtract (the average of A minus the average of B) to obtain the bias factor for that analyte, as shown in this example:

Method A		**Method B**	
Specimen #	Results (mg/dL)	Specimen #	Results (mg/dL)
1	75	1	70
2	80	2	86
3	110	3	115
4	130	4	128
5	65	5	59
6	100	6	107
7	73	7	80
8	98	8	103
9	68	9	70
10	74	10	78
TOTAL FOR A	**873**	TOTAL FOR B	**896**

AVERAGE FOR A is 873/10=**87.3** mg/dL AVERAGE FOR B is 896/10=**89.6** mg/dL
BIAS FACTOR = 87.3 – 89.6 = **-2.3 mg/dL**

This means, on average, your laboratory results (Method A) for this analyte will be 2.3 mg/dL **lower** than the comparison results (Method B). This bias of **-2.3 mg/dL** is only valid for the analyte, instruments, and methods compared by this study. The comparison and derived bias factor are no longer valid if either laboratory changes instruments, methods or reagents for the analyte.

● Specimens ●

Patient specimens are preferred for split specimen analysis. Specimens tested should cover the reportable range of the test in order to verify that the instruments compare favorably for normal values as well as high and low abnormal values. Artificial or spiked specimenscan be used when appropriate patient specimens are unavailable. In these situations, the specimens must be similar in composition to patient specimens.

Patient specimens may be collected over time (within the same day or over multiple days), as long as the specimen storage conditions for the analyte and method are met.

● Multiple Methods Within the Same Laboratory ●

When multiple non-waived instruments and/or methods are used to perform the same test, it is important for the laboratory and the practitioners it supports to understand the relationship between results produced by each method. This is most critical when tracking results on a specific individual over time.

If significant variances in results are present, they could potentially be interpreted as denoting changes in the patient's condition, when in fact they are merely the result of a bias among methods. This is easily done by performing split specimen analysis. COLA requires the laboratory to test five samples twice a year. If any bias is noted, it is important to reflect the difference in the reference ranges that are used on the test report. This requirement also includes backup instruments.

● Proficiency Testing●

CMS (Centers for Medicare and Medicaid Services) recognizes two kinds of analytes. **Regulated analytes** are specifically listed in the *Federal Register* and Proficiency Testing is required.

Non-Regulated analytes are all of the remaining analytes. Participation in PT for non-regulated analytes is not required, but the Laboratory must verify the accuracy of those tests at least twice a year using a minimum of five specimens. CLIA and COLA permit laboratories to use split specimen analysis or PT for the Non-regulated analytes.

● Regulated Analytes ●

The laboratory must enroll in PT for all regulated analytes. Split specimen analysis cannot be used by laboratories as a substitute for PT. If a lab is at cease testing for an analyte, split specimen testing cannot be used to re-instate testing of a regulated analyte. Re-instatement of a failed regulated analyte can only be accomplished through Proficiency Testing.

● Non-Regulated Analytes ●

If the laboratory does not enroll the non-regulated (unregulated) in PT, they must test five specimens twice a year through split specimen testing with another CLIA-certified laboratory. COLA strongly recommends the use of PT for unregulated analytes, when possible.

● Protocol for Split Specimen Analysis ●

- Select a comparison laboratory(s) with a valid CLIA Certificate that performs the tests you need to compare. Obtain a copy of their CLIA Certificate for your records.
- If the comparison laboratory is using instruments, methods and/or reagents different from your laboratory, establish bias values for those analytes.
- Collect or select five specimens for analysis. If possible, try to find specimens that will span the instrument/method reportable range.
- Choose your split specimen process (split the specimen into two portions or send out the same sample after your laboratory has performed the testing). Before splitting the specimen into 2 portions, mix well. Store and transport all specimens as per manufacturer's instructions.
- Test each specimen in the same manner as a patient specimen.
- Verify the quality control results for each analyte is acceptable prior to testing.
- Test the specimens in your laboratory and record the results.
- If you have chosen to send the specimens to a comparison laboratory after testing the sample in your lab, package the specimen and transport to the comparison laboratory.
- Record each referred specimen results from the comparison laboratory. Perform bias correction, if necessary.
- Compare the two sets of results for each analyte. Determine if the results agree within the acceptable performance range for the analyte.
- Repeat testing if the results are not within the acceptable limits and document corrective action.
- Forward the results to the laboratory director or qualified designee for review and approval.

● Acceptable Limits ●

The laboratory should establish their own acceptability limits. It is recommended that the results should vary no more than +/- 15 percent from the comparison laboratory's results after correcting for method bias, if applicable. Some labs may choose to have narrower limits, such as +/- 10 percent, which may be desirable for tests that are used to make patient treatment decisions.

● Grading Results ●

Your laboratory must have at least an 80 percent acceptable score for the five specimens tested for each analyte. This means that four out of five results must be within the acceptable limits established by the laboratory. Investigate all results outside of the acceptable limits and perform corrective action. Your laboratory is not required to report the results of split specimen analysis for non-regulated analytes to CLIA or COLA.

• Documentation •

Your laboratory must retain records of split specimen analysis for two years (three years in California). The documentation should include:

- The date the split sample testing was performed and by whom.
- Copies of the results from your laboratory including the instrument printouts or tapes.
- Copies of the results from the comparison CLIA-certified laboratory.
- Copies of documentation showing how the bias factor was calculated, if applicable.
- A summary of the comparison of both sets of results including the scoring and grading of the results.
- Any corrective action taken in response to a failure.

This documentation needs to be available for review during a CLIA or COLA on-site survey.

Split Specimen Analysis Report

**Enter your labs Acceptable limits (% Difference)

Date	Analyte	Sample #	Primary lab result	Comparison lab result	% Difference (+/- %)	Acceptable/ Unacceptable	Reviewed by
		1					
		2					
		3					
		4					
		5					
		1					
		2					
		3					
		4					
		5					
		1					
		2					
		3					
		4					
		5					
		1					
		2					
		3					
		4					
		5					

Corrective Action:

Approved By: _____ **Date:**_____