Split Specimen Analysis

Requirements for good laboratory practice and COLA Laboratory Accreditation programs are underlined.

INTRODUCTION

Split specimen analysis may be defined as the analysis of a specimen by a primary method (method used by your laboratory) and a comparison method (similar method used by a CLIA-certified laboratory). The analysis can be performed by:

- Actually splitting a specimen into two portions, one portion to be analyzed by the primary laboratory and the other portion to a CLIA-certified laboratory using a comparative method.
- Or analyzing the specimen in the primary laboratory and then submitting the same specimen for analysis to the other CLIA-certified laboratory.

Split specimen analysis may be used to determine the following:

- The relative accuracy of a particular testing method;
- The linearity of an instrument compared to a reference instrument; or,
- As a substitute for proficiency testing.
- As a means of verifying the accuracy of testing non-regulated analytes for which the laboratory does not participate in proficiency testing.

Laboratories are required by CLIA regulations to participate in proficiency testing for all regulated analytes. For non-regulated analytes, the lab must verify the accuracy of its test results at least twice a year. COLA permits laboratories to substitute split specimen testing for proficiency testing for unregulated analytes when they are not available from their primary provider. COLA strongly recommends that laboratories use proficiency testing rather than split specimen testing for the following reason: once enrollment fees are paid, the cost of testing unregulated analytes is generally cheaper and more accurate than doing split specimen analysis.

Split specimen analysis may be performed between satellite laboratories of a group practice, another local area physician office laboratory, any local area hospital laboratory, or a reference laboratory. The laboratory selected for split specimen analysis must possess a valid CLIA certificate for the analytes, specialties, and subspecialties being tested.

INSTRUMENT (METHOD) BIAS

Bias refers to the amount of difference in results between two instruments analyzing the same specimen. It is caused by different instrument engineering and manufacture, the use of different methods to perform the analysis, or different reagents. Bias is important in split specimen analysis because the bias between some instruments is great enough to affect the laboratory's score. Ideally, the comparison of results of an analyte between laboratories should be done on identical instruments using the same method and reagents.

If split specimen analysis is performed on an identical instrument using the same test method and reagents, no bias factor is needed in scoring the results.

If split specimen analysis is performed on a different instrument or the same instrument using a different method and/or reagents, a laboratory may want to determine the bias factor in order to validate the results for scoring.

DETERMINING INSTRUMENT (METHOD) BIAS

A bias study should be done prior to the use of split specimen analysis. A laboratory using another CLIA-certified laboratory may wish to establish bias values for the most critical analytes performed in the laboratory in the event they may choose to use split sample analysis.

Instrument bias represents the difference between the average of a set of results for a particular analyte obtained from the primary method (method used by the laboratory) and the average of a set of results obtained from the comparison method (method used by the reference or participant laboratory). The difference can be estimated by computing the average value for each of the two sets of results obtained from the primary method (A) and the comparison method (B). The average values for each set of results are then subtracted (A - B = bias factor) to give the bias factor for that particular analyte. See Figure 1 on the next page.

This means that, on average, the laboratory's results using its primary instrument for this analyte will be 6.3 mg/dl *lower than* the comparison instrument. This bias is valid only for the instruments and methods

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Table 1
Calculation of the Average of Split Specimen Results to Determine the Bias Factor

PRIMARY METHOD (A)		COMPARISON METHOD (B)	
Specimen #	Results(mg/dl)	Specimen #	Results (mg/dl)
1	65	1	72
2	93	2	98
3	58	3	55
4	100	4	102
5	150	5	163
6	50	6	63
7	75	7	82
8	100	8	104
9	83	9	88
10	60	10	70
TOTAL (A)	834	TOTAL(B)	897
AVERAGE (A)	834/10 = 83.4mg/dl	AVERAGE (B)	897/10 = 89.7mg/dl

A = 83.4 mg/dl B = 89.7mg/dl BIAS FACTOR = 83.4 - 89.7 = -6.3mg/dl

compared by the study. If either laboratory changes instruments, reagents, or the method of performing the analyte, the comparison is no longer valid.

NATURE OF SPLIT SAMPLE SPECIMENS

Only patient specimens are acceptable for split specimen analysis. As much as possible, specimens should represent the full range of values reported by the laboratory. Artificial specimens, such as calibrators or quality control material, are not acceptable for split specimen analysis.

Patient specimens may be accumulated over time (within the same day or during a couple of days), provided that the storage time of the specimens is appropriate for the analyte.

USING SPLIT SAMPLE ANALYSIS

For CLIA purposes, HCFA recognizes two kinds of analytes. "Regulated analytes" are specifically named in the *Federal Register* and PT participation is mandatory. "Non-regulated analytes" are all the remaining analytes, and, while PT participation is not mandatory, the laboratory must provide a comparison of its instrument/method with an outside laboratory at least twice a year. Split specimen analysis is one way of making this comparison.

REGULATED ANALYTES

Split specimen testing cannot be used by laboratories as a substitute for proficiency testing for regulated analytes. The laboratory must enroll in PT for all regulated analytes tested. Likewise, split specimen testing cannot be used to re-instate testing of a regulated analyte following repeated proficiency testing failures. Reinstatement of testing of a failed regulated analyte must be accomplished through proficiency testing.

NON-REGULATED ANALYTES

NUMBER OF SPLIT SAMPLE SPECIMENS FOR ANALYSIS

For non-regulated analytes not included in the laboratory's primary PT program, five specimens must be analyzed.

FREQUENCY OF USE OF SPLIT SAMPLE ANALYSIS

For non-regulated analytes not included in the laboratory's primary PT program, split specimen analysis should be performed two times a year.

GRADING CRITERIA

For non-regulated analytes not included in the laboratory's primary PT program, the laboratory's acceptable results should be no more than +/-15 percent from the comparison instrument after correcting for method bias, provided the laboratory has determined the bias between its instrument and the comparison instrument.

SCORING

For non-regulated analytes not included in the laboratory's primary proficiency testing program, the laboratory must have at least an 80 percent acceptable score for the five challenges per analyte. This means that at least four of the laboratory's five split specimens must be within +/-15 percent of the comparison instrument's results.

DOCUMENTATION

For non-regulated analytes not included in the laboratory's primary PT program, the laboratory must retain for a period of two years a record of split specimen analysis which includes the date, results of the in-house analysis (including copies of instrument tapes if they are available), and copies of the results from the other CLIA-certified laboratory. Any corrective actions taken in response to unsatisfactory performance must be documented. NOTE: The laboratory is not required to send this documentation to COLA, but should have it available for review during an on-site survey.

REPORTING TO HCFA

COLA is not required to report the results of split specimen analysis for non-regulated analytes to HCFA.

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SPLIT SPECIMEN ANALYSIS PROTOCOL

Pre-Analysis

1. Select participant or reference laboratory possessing a valid CLIA certificate for the analytes to be tested using the split specimen analysis method.
2. If the instrument, methods, or reagents are not the same as used by your laboratory, establish bias values for the analytes to be tested prior to split specimen analysis.
3. Choose appropriate split specimen process (split specimen into two portions or send out same specimen after it is tested by your laboratory). Make sure specimen is mixed well before splitting into two portions.
4. Collect five specimens for analysis.

Analysis

1. Treat each sample to be tested in the same manner as a patient sample.
2. Perform routine quality control on the instrument being used for testing and ensure that QC results are within the acceptable range.
3. Record each result to be compared against the referred sample result.
4. Record each referred sample result returned from the comparison laboratory.
5. Compare the two results from the primary method and the comparison method for each analyte to determine whether or not the results agree within the acceptable performance range for the particular analyte. Bias values may be used at this stage in the process, if applicable.

Post-Analysis

1. Document results and any corrective action which occurred during the split specimen process.
2. Documentation should include the date of analysis; results of the in-house analysis, including copies of instrument tape if it is available; and copies of the results from the reference or other participant laboratory.
3. Check for clerical errors before using results.

SPLIT SPECIMEN ANALYSIS REPORT

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					

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