Method Validation

Method validation studies confirm a manufacturer's claims for an analyte and are performed upon installation of a new analyzer. They may also be performed when a new test is added to an existing system or as required by a regulatory agency. Some regulatory agencies require the studies to be performed annually or biannually. Check with your regulatory agency for the requirements.

Each laboratory that introduces an unmodified, FDA cleared or approved test system must do the following before reporting patient test results:

1) Demonstrate that it can obtain performance specifications comparable to those established by the manufacturer for the following performance characteristics:
    a. Accuracy
    b. Precision
    c. Reportable range of test results for the test system.

2) Verify that the manufacturer's reference intervals (normal values) are appropriate for the laboratory's patient population.

The four performance tests listed above are required for CLIA compliance. Each laboratory should establish protocols and procedures in accordance with performance criteria appropriate for the patient population being tested and interpretation of regulations under which the laboratory operates. Complete details of CLIA guidelines can be found in the CLIA Brochure - Verification of Performance Specifications (PDF) https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/CLIA_Brochures.

Specific protocols for Method Validation studies should be based on local, state or federal requirements.

Precision

Precision is the reproducibility of an assay and is measured by running a specimen multiple times. Precision can be checked at a single level or across several levels. For a within-run precision study, a laboratory may choose to run 20 points on one level of control material or patient sample. A Tosoh Bioscience representative will perform a 20-point precision during instrument installation, unless the laboratory provides their own internal Method Validation protocol.

The specimens used to determine assay precision must be similar to routine patient specimens. To verify the manufacturer's claim, specimens should have values that are within the stated linear range on the analyte specification sheet.

Coefficient of variation is calculated using the following equation:

$$CV = (SD)\ (100) \div Mean$$

The %CV is dependent upon the sample concentration and is inversely proportional to the concentration. If the sample concentration is high, then the %CV can be low. If the sample concentration is low, then the %CV can be high. Each laboratory should determine the acceptable criteria for within-run precision.

AIA-System Within Run Precision

Account Name: _____ **TEST:** _____

Instrument: _____ **Test Cup Lot:** _____

Date: _____ **Expires:** _____

Tech: _____

Level:			
Range:			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
MEAN			
1 SD			
CV			

CV = (SD) (100) ÷ Mean

Reportable Range

Reportable range studies are required by CLIA regulations to substantiate the accuracy of the assay throughout the laboratory's reportable range. This study requires analysis of at least two replicates of three concentrations throughout the reportable range.

Tosoh Bioscience, Inc. manufactures Calibration Verification Material (CVM) for all 2-point sandwich assays. This material has approximately twice the analyte concentration of Calibrator 2 (P Calibrator) and is used to substantiate the reportable range for 2-point assays. Calibrators are used for 6-point assays.

A Tosoh Bioscience representative will perform the following protocol for the reportable range study during instrument installation unless the laboratory provides their own protocol.

For **2-point assays**, run the following three specimens:

1. Sample Diluting Solution (SDS) included in the CVM package or Calibrator 1
2. Calibrator 2 or a 50% dilution of the CVM
3. Calibration Verification Material

For **6-point assays**, run the following three specimens:

1. Calibrator 1
2. Calibrator 5 (or the calibrator that is closest to the midpoint of the assay range)
3. Calibrator 6 (or the calibrator that is closest to the upper limit of the assay range)

Instructions:

1. Analyze each specimen at least in duplicate.
2. Calculate the mean.
3. Calculate the percent recovery:
    - Divide the mean of the replicates by the expected mean x 100
    - Percent recovery should be between 90 -110%

The above procedure can be also used at six-month intervals for calibration verification requirements.

Other sources of material suitable for calibration verification include:

1. Previously tested patient specimens of at least three concentrations that cover the low, middle and upper end of the reportable range.
2. Calibration verification materials purchased from a vendor.

Each laboratory should establish protocols and procedures in accordance with performance criteria appropriate for the patient population being tested and interpretation of regulations under which the laboratory operates.

Please refer to CLIA Regulations "Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services (Appendix C), Subpart K, Part 1, D5439" for further information.

AIA-System Reportable Range Data

Account Name: _____ **TEST** _____

Instrument: _____ **Test Cup Lot:** _____

Date: _____

Tech: _____

Expected Value	Observed Value	Mean	% Recovery

Correlation

The ideal way to perform correlation studies is by analyzing the same samples on two instruments using the following criteria:

Specimen Requirements

Specimens should be fresh serum, not QC material or standards.
Previously frozen specimens should be simultaneously analyzed on both instruments. Analyte concentrations should cover the entire range of linearity.

AIA-System Correlation Data

Account Name: _____ **TEST:** _____

Instrument: _____ **Test Cup Lot:** _____

Date	Tech		Sample Number/ID	Reference (X)	Tosoh AIA System (Y)	Comments
		1				
		2				
		3				
		4				
		5				
		6				
		7				
		8				
		9				
		10				
		11				
		12				
		13				
		14				
		15				
		16				
		17				
		18				
		19				
		20				
		21				
		22				
		23				
		24				
		25				

Slope:		**Mean:**	
Intercept:		**Reference Range:**	
R:			