Verification of Method XXXXXX	
ISDH Laboratories	**Prepared by:**

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Purpose and Principle:

42 CFR Section 493.1253 (Standard: Establishment and verification of performance specifications) dictates that prior to implementing a new or revised method, the Indiana State Department of Health Laboratories (ISDHL) must demonstrate the capability of the method in terms of precision, accuracy, detection limits, linearity, interferences, adequacy, sensitivity, specificity, and predictive value.

Assays that are marketed by manufacturers already have established parameters prior to marketing. In these cases, the ISDHL only has to verify precision and accuracy for marketed assays prior to implementation of these assays.

Whenever the ISDHL must verify a method, the analysts will use this document as a template for generating the final report and data summary. The purpose and principle section is to contain the general background and overview of why the method was selected and verified in the ISDHL.

Precision Experiment(s)

Precision is a value measuring the extent of agreement of a set of replicate measurements. It can refer to either repeatability (within-run precision) or within-laboratory precision. Repeatability is a value indicating the disagreement among a set of replicate measurements when all measurements are made under identical conditions (or within a single run of a procedure). Within-laboratory precision is a value indicating the disagreement among replicate measurements over a longer time period when all known, major sources of measurement error within the laboratory are considered. Within-laboratory precision reflects the accumulation of various error sources, including repeatability.[1]

Precision can be reported either as a standard deviation (SD) or a coefficient of variation (CV %), which expresses the standard deviation as a percentage of the mean value of the replicate measurements. In either case, the mean value should be reported as well. When values of the SD or CV% increase, this indicates an increase in the imprecision. [1]

A. Experimental Design

1. Source of Specimens

   Materials to be used as samples for precision experiments should have analyte values near the concentrations the manufacturer used to establish the precision claims for the assay. Acceptable materials for precision experiments should use a matrix that mimics the patient sample as much as possible and may include the following:

   A. control samples other than those used to assess whether the assay is in control
   B. standards
   C. previously analyzed patient samples

A. Experimental Design (continued)

2. Range of concentrations/results to be tested
3. Time frame and run structure
   a) Analyze at least one run per day with at least three replicate samples at each of two concentrations daily for five days.
   b) If a run must be rejected because of quality control procedures or operating difficulties, discard the data, and conduct an additional run.
   c) Include the daily quality control samples normally used.
   d) Samples for the accuracy experiment(s) may be tested in the same runs.
   e) Calibrate as specified in the manufacturer's instructions for operators. If the manufacturer indicates in its claim that its precision data were generated over multiple calibration cycles, then the operator may choose to recalibrate during the experiment.

B. Data Summary

This is an example of a data table to use:

High Concentration/Value Replicates

	Run 1	Run 2	Run 3	Run 4	Run 5
Date					
Operator					
Replicate 1					
Replicate 2					
Replicate 3					
Run Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Run SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
2 x Run SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Grand Mean	#DIV/0!
Grand SD	#DIV/0!
2 x Grand SD	#DIV/0!

Low Concentration/Value Replicates

	Run 1	Run 2	Run 3	Run 4	Run 5
Date					
Operator					
Replicate 1					
Replicate 2					
Replicate 3					
Run Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Run SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
2 x Run SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Grand Mean	#DIV/0!
Grand SD	#DIV/0!
2 x Grand SD	#DIV/0!

C. Evaluation of data

Compare the data collected to one of the following expectations:

1. Refer to manufacturer claims if available, and compare the test assay precision results to the manufacturer claims.
2. If there are no manufacturer claims, use the mean of the test assay results +/- 2SD at the 95% confidence interval as the acceptable range.
3. If the assay provides only qualitative results, then the level of agreement that is required within each category of replicates is 90%.

D. Explanations of discrepancies/issues

If any discrepancies are revealed during the precision experiment(s), then an investigation must be conducted and the results recorded in this section. Also, if the method under evaluation does not meet the expected precision results, then explain any reasons here.

E. Comments

All other comments or suggestions are to be recorded in this section.

Accuracy Experiment(s)

Accuracy is conformance to a value, accepted standard, or expected value. For a test result, bias is a measure of accuracy; it is the difference between the test result and the accepted reference value for an analyte. For a measurement procedure, bias is expressed as the difference between the average result obtained by a procedure under specified conditions and an accepted reference value, perhaps from an accepted comparative procedure or a certified reference material.

A. Experimental Design

1. Source of specimens to test
   a) Run previously analyzed PT samples and compare the results with the appropriate PT group. Results of test assay are expected to be within the range provided by the PT.
   b) Run CAP survey or similarly verified PT materials and compare the results with the expected values. Results of the test assay are expected to be within the range provided by the survey vendor.
   c) Run commercial products with assigned values and compare the observed results with the assigned or expected results. Results of the test assay must lie within the range of expected results provided.
   d) Test fresh human specimens and compare the results to alternative methods which have been previously validated in our laboratory. Test assay results are expected to match the qualitative results of the comparator assay.
   e) Test human specimens that have previously been tested on verified/validated assay(s) and stored appropriately. Compare the results of the new assay with results from the historical assay(s). Test assay results are expected to match the qualitative results of the comparator assay.
2. Comparator selection
   Record which method was selected as a comparator method and provide the reason(s) why.

3. Range of concentrations/results to be tested
4. Time frame and run structure
   a) Obtain at least 20 specimens for testing that cover the entire range of results/concentrations, but do not exceed it. This means that testing is to at least include negatives, positives, and borderline specimens.
   b) Exclude samples that are known to contain substances that may be identified as interferents in either the test assay or the comparator assay(s).
   c) To reduce potential bias, the testing with each assay should happen as close in time to each other as possible.
   d) Analyze the samples on both the test and the comparator assays.
   e) Conclusions will be most reliable if the measurements are performed on five to seven samples/day for three to four days.
   f) This testing can be done concurrently with the precision experiment(s).
   g) If one isolated sample results differ more than what is observed with other samples, then that sample is to be repeated in duplicate for each assay. The discrepant data should be removed from the analysis and another sample added to maintain sample size.
   h) Follow all appropriate quality control steps for each assay. Any unacceptable performance based on failed quality control should be discarded and the run(s) should be repeated.

B. Data Summary

This is an example of a data table to use:

Specimen ID	Test Method Result	Comparator Method Result
Spec #1		
Spec #2		
Spec #3		
Spec #4		
Spec #5		
Spec #6		
Spec #7		
Spec #8		
Spec #9		
Spec #10		
Spec #11		
Spec #12		
Spec #13		
Spec #14		
Spec #15		
Spec #16		
Spec #17		
Spec #18		
Spec #19		
Spec #20		
% Match =	# of matched data points x 100%	
	20	

C. Evaluation of Data

If the test assay manufacturer provides claims on the accuracy of the assay compared to other assays, then use the manufacturer claims for accuracy if the ISDHL comparator assay is identical to what the manufacturer used in establishing their claims. If the assays provide quantitative results, then refer to CLSI Guidance document EP15 for calculations.

In all other cases, the expectation for test assay results should match 100% of the specimens tested via the comparator assay(s).

1) If the match does not equal 100%, an investigation must be conducted to discover potential reasons and solutions (i.e. discarding some data, stating particular testing criteria, etc.).
2) Once the investigation is complete, the assay must either be recommended for use or not.

D. Explanations of discrepancies/issues

If any discrepancies are revealed during the precision experiment(s), then an investigation must be conducted and the results recorded in this section. Also, if the method under evaluation does not meet the expected precision results, then explain any reasons here.

E. Comments

All other comments or suggestions are to be recorded in this section.

Reportable Ranges

Describe what all reportable results and ranges will be for this assay.

Proficiency/External QA

Provide a detailed description of what proficiency testing survey(s) will cover this method. Even if current surveys can cover, list those here. If current surveys do not cover, list any that could cover it.

Overall Evaluation of the Method

The overall evaluation is to include a brief description of the precision and accuracy experiments and their results, a brief explanation of discrepancies/issues, and an explanation of whether the method is to be implemented at the ISDHL or not.

References

1. CLSI. *User Verification of Performance: Precision and Trueness; Approved Guideline*. EP15-A2. Clinical Laboratory Standards Institute, 2008.

The following items are required for full validation of the assay (please insert these items before the Overall Evaluation of the Method):

Reference Range

A. Detection Limit:

The detection limit is defined as the smallest single result, which can be distinguished from a suitable blank. The limit may be a concentration or an amount and defines the point at which the analysis becomes just discernible. The detection limit is related to precision and also depends on the magnitude of blank readings.

B. Linearity:

Linearity is the measure of the degree to which a curve approximates a straight line. Linearity refers to overall system response rather than simply the instrument output. The linearity of the method is demonstrated during the method validation procedure and is monitored with each new calibration curve. The linearity is also monitored prior to and during a run. The degree to which the plotted curve conforms to a straight line is a measure of the method's linearity. Conversely, the deviation of the curve from the straight line is a measure of non-linearity. Not all assays in use within the laboratory can be evaluated in terms of linearity.

C. Normal values:

Normal values are what are expected when testing specimens from the expected population.

Sensitivity:

Sensitivity is a measure of the ability of an analytical method to detect small quantities of the measured component. "Sensitivity is defined as the ability to identify correctly those who have the disease." (1)

Specificity:

A. Overview

Specificity is the ability of an analytical method to determine solely the component(s) it purports to measure. "Specificity is defined as the ability to identify correctly those who do not have the disease." (1)

The relationships between sensitivity and specificity are shown below:

	Disease State:	
Result of Test	Disease:	No Disease:
Positive:	True positive (TP)	False positive (FP)
Negative:	False negative (FN)	True negative (TN)

Sensitivity = percentage of people with the disease who are detected by test. $\frac{TP}{TP + FN} \times 100$

Specificity = percentage of people without the disease who were correctly labeled by the test. $\frac{TP}{TP + FN} \times 100$

B. Interferences:

Interferences are those sample matrix components, which may either enhance or suppress the observed response. The analyst should be aware of those substances commonly known to interfere with each particular testing method in use and know how to detect and control for the effect of these substances upon the analysis.