Correlation Testing for Quantitative Assays

Purpose

Correlation testing is required to verify the comparability of quantitative laboratory results for analytes tested on different measurement systems.

Background Information:

Correlation or comparison testing is a method of measuring the relationship between two or more laboratory instruments testing the same analyte. Correlation means association - more precisely, it is a measure of the extent to which two variables are related.

The performance of correlation testing between two or more similar instruments is required by CAP, JCAHO and CLIA and it is part of good laboratory practice. Correlation must be performed between all instruments running the same assay in the same laboratory and between a primary laboratory and their back-up laboratory. It is vital for the purposes of patient care that physicians can be assured that all laboratory results released from an institution are equivalent. Correlation is required for all laboratories performing research funded by the NIH Division of AIDS.

This procedure assumes that for routine correlation testing, the instruments have been validated, appropriately calibrated and maintained and that internal QC is within acceptable limits.

Planning and Preparation

This method requires the laboratory to monitor and document the historic CV of the internal quality controls for each analyte. The tracking of the CV can be accomplished through the instrument manufacturer's system or the Laboratory Information System (LIS). If these two options are not available, please contact pSMILE for guidance on manually tracking historical CVs using internal quality control data.

Before starting the correlation study ensure that:

A. Appropriate personnel have been informed about the correlation testing, that they have been trained and know how to proceed once the samples are collected.
B. All instruments for the correlation study have:
    - Up-to-date maintenance and calibration
    - Validation (to include precision, accuracy, linearity, and reference range) completed.
    - Internal Quality Control (QC) results that are within acceptable range and that there are no biases observed.
    - Acceptable EQA performance on the primary instrument.

Sample Selection:

The use of fresh human samples (whole blood, serum, plasma, urine, etc.) is recommended. However, the use of EQA samples, linearity samples and/or commercial controls may be necessary to ensure that low, normal and high specimens are tested.

Before starting the correlation study ensure that:

A. The lab has access to appropriate samples for correlation.
B. The samples can be run on both instruments at the same time or within 2 hours (recommended).
C. If stored samples are used, ensure that the samples are stored appropriately and that the storage conditions are the same for samples run on both instruments.

Frequency:

The frequency and number of samples for correlation testing is at the discretion of laboratory director. Several factors should be taken into consideration when making this decision including:

- Impact of different results from different instruments on patient care
- Possibility of detecting insignificant error, such as that associated with sample handling versus not detecting significant error
- Time involved in acquiring, transporting, testing, evaluating and storing samples
- Cost of reagents and other material involved
- Availability of samples

If possible, given the availability of samples and reagents, pSMILE recommends performing correlation testing on a monthly basis using a minimum of one sample with a low abnormal assay value, one with a normal value and one with a high abnormal value. Once it is established that the instruments being correlated compare well, and the risk to patients from discrepant results is low, then testing can be performed less frequently using more specimens. At minimum, pSMILE recommends performing correlation testing every six months using six samples of varying assay levels (low, normal, high) each time. Whatever frequency and number of samples is decided upon, this should be documented in the relevant SOP and updated as changes in policy happen.

Special cause correlation testing may be necessary in the following cases:

- Failure of periodic monitoring of comparison testing
- EQA failure
- Internal QC failure
- After major instrument maintenance
- Clinician inquiry regarding the accuracy of results

Documentation:

Each laboratory should include details of the correlation testing in their Quality Manual and/or site SOPs. All documentation should be reviewed and approved by the Laboratory Director or Designee.

Procedure:

Please note that a spreadsheet tool is available on the Resources website that will perform these required calculations.

1. Select appropriate samples (numbers as defined by the laboratory). Ensure that this includes one sample with a low abnormal assay value, one with a normal value and one with a high abnormal value (see example in Table I below).
2. Run the samples on the first instrument in duplicate at minimum.
3. Run the samples on the second instrument, also in duplicate, as soon as possible, ideally within two hours.
4. Calculate the mean for each sample on both instruments.
5. Calculate the grand mean- the average of mean on instrument #1 and instrument #2 (see example in Table II below).
6. Calculate the difference between the mean of the first and second instruments.
7. Calculate percent difference by dividing the difference found in step #6) from the grand mean (see example in Equation I below) and multiply by 100.

Step-by-Step Examples

Table I

Glucose						
	Instrument #1			Instrument #2		
	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean
Sample #1	92	93	92.5	91	87	89
Sample #2	58	59	58.5	58	57	57.5
Sample #3	136	137	136.5	130	127	128.7
Sample #4	302	303	302.5	278	275	276.5
Sample #5	215	214	214.5	209	205	207

Table II

Glucose						
	Instrument #1			Instrument #2		
	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean
Sample#1	92	93	92.5	91	87	89

Grand Mean = (92.5+ 89)/2 = 90.75

Table III

Glucose						
	Instrument #1			Instrument #2		
	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean
Sample#1	92	93	92.5	91	87	89

Difference (Δ) = 92.5 – 89 = 3.5

Equation I

Calculate percent difference by dividing the Difference **(Δ)** by the Grand Mean.

% Difference = 3.5/90.75 x 100 = 3.85%

Evaluating Results

1. Obtain the cumulative CV of your QC level that is closest to the grand mean from step #5 in the procedure above.
    - This can usually be obtained from the instrumentation on which you run the control, or from your LIS. Contact pSMILE if you need more information on how to obtain your cumulative CV.
2. Divide the percent difference from step #7 above by your cumulative CV to obtain your correlation ratio.

Example:

$$\frac{\text{\% Difference (3.85\%)}}{\text{Cumulative CV (2.5\%)}} = 1.54$$

- This ratio can be calculated for each instrument pair and measures the percent difference in multiples of your cumulative CV. The cumulative CV is the percentage equivalent to 1SD of your QC system.
- Dividing the percent difference by the cumulative CV provides a ratio similar to a standard deviation index (SDI), which is the difference of a mean of values from one of those values, divided by 1 SD ((individual value – mean of values)/ 1SD) .

3. Determine the tolerance limit for your correlation ratio.
    - pSMILE recommends a tolerance limit of ≤ 3 when you begin monitoring correlation ratio. If dissimilar methods are compared, this limit may have to be increased.
    - Using ≤ 3 as a tolerance limit for your correlation ratio is equivalent to using ≤ 3SD in your QC evaluation. In other words, if your correlation ratio is equal to 3, the results from your instruments are more than 3SD apart from each other.
    - If you need assistance determining your correlation ratio tolerance limit, please contact your pSMILE representative.

    Table IV below shows an example of how to capture your correlation results using the acceptable tolerance limit of ≤ 3.

Table IV

Analyte	Instr. 1 Mean	Instr. 2 Mean	Grand Mean	Difference (Δ)	%Diff (%Δ)	Cumulative CV	%Diff/CV Ratio	Acceptable %Diff/CV Ratio	Pass/ Fail
Glucose	92.5	89	90.75	3.5	3.9	2.5	1.5	≤3	PASS
Glucose	58.5	57.5	58	1	1.7	2.5	0.7	≤3	PASS
Glucose	136.5	128.7	132.6	7.8	5.9	2.5	2.4	≤3	PASS
Glucose	302.5	276.5	289.5	26	9.0	2.2	3.6	≤3	FAIL
Glucose	214.5	207	210.75	7.5	3.6	2.2	1.4	≤3	PASS

Developing Acceptability Criteria

pSMILE recommends that guidelines for acceptability criteria be based on the capability of the instrument reflected in internal precision data, as outlined in this procedure. Only this option measures the accuracy of the results based on capability of the instrument. Other options are available, however, these acceptability criteria could potentially be so wide that while the correlation testing results could be acceptable the lab would miss an opportunity to address problems with the instrument performance while in reality the results were outside of the instrument accuracy.

Correlation coefficient should not be used as a method to evaluate the acceptability of your correlation testing. Correlation coefficient is a means to look for a relationship, not agreement between pairs. Two methods may have a perfect correlation throughout the measuring range but may not agree in value (i.e. one may be double the value of the other).

Troubleshooting

There are a variety of problems with instruments that could cause discrepant results when performing comparison testing. In general, any type of issue that would cause a malfunction in the instrument and reflect in bias, shifts or trends in your QC could cause a discrepancy when comparing to another instrument. It is not possible to cover troubleshooting of all types of issues within this SOP. However, when comparing instruments that are assumed to be in good working order as evidenced by good QC data, it is important to consider the differences between the instruments which might cause discrepant results. Such differences might be:

- Different methodologies
- Difference in calibration
- Difference in imprecision
- Difference in reagent lot or shipment (storage)
- Difference in lot of calibrators or assignment of values
- Difference in age of calibrators (date opened)
- Difference in reagent life on instrument
- Difference in instrument parameters (dilution ratios, incubation times, etc.)

If an explanation for the discrepant results still cannot be found, pSMILE recommends going through every function and parameter of the instruments being compared looking for any differences. Once the difference(s) are reconciled, re-run the correlation study to see if the discrepancy is resolved.

References

1. Clinical Laboratory Standards Institute (CLSI) *Verification of Comparability of Patient Results Within One Health Care System Implementation Guide. 1st ed.* CLSI EP31-ED1-IG. 2022.

2. Clinical Laboratory Standards Institute (CLSI) *Verification of Comparability of Patient Results Within One Health Care System Workbook. 1st ed.* CLSI workbook EP31-ED1-WB. 2022.

3. Clinical Laboratory Standards Institute (CLSI) *Measurement Procedure Comparison and Bias Estimation Using Patient Samples*. CLSI EP09c 3rd Ed. 2018.

4. College of American Pathologists (CAP) 2023. Commission on Laboratory Accreditation, Laboratory Accreditation Program; All Common Checklist

5. DAIDS GCLP. Version 4.1, 2021