

MTN Manual of Operational Procedures (MOP)

APPENDIX IV: Method Validation Policy

Prepared by	Date Adopted	Supersedes Procedure #
Adapted from HPTN policy		N/A

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1 SCOPE

This procedure applies to all Microbicide Trials Network (MTN) laboratories. Any time a new instrument or methodology is implemented or an existing instrument or method is changed within the laboratory, validation studies must be performed. Documentation of these studies must be maintained for the life of the instrument or methodology. Results of these studies must verify the performance specifications and claims of the manufacturer. This document is not a comprehensive explanation of method validation.

2 PURPOSE

The following describes assay validation studies suitable for manual and automated quantitative assays, such as for chemistry and hematology. If these procedures do not appear suitable for your assays, please contact the MTN Laboratory Center (LC) at mtnetworklab@mtnstopshiv.org for clarification. Results of assay validation studies must be sent to the LC for approval before that assay can be used in an MTN protocol.

3 VALIDATION PROCEDURES

Studies for quantitative assays that are U.S. Food and Drug Administration (FDA)-approved and unmodified contain the following elements:

Accuracy

Accuracy is the true value of a substance being measured. Verification of accuracy is the process of determining that the test system is producing correct, valid results. This is determined by:

- Assay materials with assigned values
- Comparing patient specimen results with a method of long-standing use
- Verifying results from inter-laboratory survey specimens
- Splitting specimens with another sufficiently accredited laboratory

Results must demonstrate that the system is accurate enough to provide clinically valid patient results. Limits of acceptability should be set by the Laboratory Director.

Precision

Precision is the reproducibility, the agreement of the measurements of replicate runs of the same sample.

Precision is the process of determining the range of random errors. The precision is measured in terms of coefficient of variation (CV) and standard deviation (SD). The smaller the CV and SD, the better the precision will be.

This can be determined by running a minimum of 20 replicates of a specimen or quality control (QC) material during a span of 10 to 20 days, if possible. The mean, CV and SD are calculated from the data obtained.

Precision data must demonstrate the assay performance, which is comparable to the performance specifications published by the manufacturer. When there are no specifications published, limits of acceptability must be set by the Laboratory Director.

Verification of Measurable Range (Linearity)

This is the range of test values over which there is a valid relationship between the instrument, kit or test systems measurement response. The response may not necessarily be linear.

- The laboratory must demonstrate a relationship between the actual and expected values of a test procedure.
- Verification must be run for assay validation and, at a minimum, annually.
- Verification determines both the lower and upper limit of reporting.
- Plot the expected values on the x-axis and the actual values on the y-axis.
- Manufacturer claims must be verified.
- If the reportable range study indicates a usable range outside the limits indicated by the manufacturer, the manufacturer-published reportable range must be used.
- If the reportable range study indicates a usable range smaller than the limits indicated by the manufacturer, the smaller range must be used.

- After verification of the measurable range, laboratories should establish their reportable range. This represents the highest and lowest values that may be reported. These may exceed the measurable range.

Reference Range Verification

Reference ranges are a measured set of values determined to occur in a healthy non-diseased population. Reference ranges can be chosen from documented literature, manufacturer-suggested ranges or existing laboratory ranges; or the laboratory may perform a full normal-value study to evaluate its own range. The laboratory must verify that their reference range is valid for their study population.

If a laboratory decides to use published ranges, these ranges must be verified. To validate or transfer this published range, the laboratory must analyze specimens from 20 healthy, non-diseased individuals for each subgroup. If two or fewer results fall outside the published range, it is validated. However, if more than two results fall outside the published range, a more extensive study should be conducted. The Laboratory Director ultimately decides which validation to use based on the study population.

Carryover Studies

Sample carryover may cause one high patient sample to affect the sample that follows it. Most of today's diagnostic analyzers take every possible precaution to avoid sample carryover. In spite of these efforts, a sample having a high result may affect one or more samples that follow it. The laboratory must show that neither its instruments nor its test system has any unacceptable carryover.

Carryover studies must be performed during assay validation, at least annually thereafter and when carryover is suspected. This can be completed in some cases using CAP panels. Follow manufacturer instructions for assessing carryover and acceptability limits.

Any deviation from the manufacturer recommendations will put that procedure into the modified category.

Studies for quantitative assays that are not FDA-approved, or are FDA-approved and have been modified, must also contain all of the previous items (one through five), as well as the following:

Analytical Sensitivity

This is the lowest measurable concentration that is distinguishable from zero. Successive dilutions of a previously analyzed patient specimen or control can be used.

Analytical Specificity

This is the ability to deal with interfering substances. At a minimum, run samples spiked with hemoglobin, bilirubin and lipids.

Any Other Applicable Performance Characteristics

Demonstration of carryover is one example.

4 ACCEPTABILITY CRITERIA

The Laboratory Director must set the limits for assay acceptability. In the absence of a Laboratory Director, a designated responsible individual from the site can set the criteria. LC staff may be able to offer guidance for setting limits.