**Validation Plan for Quantitative Method**

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| (Please fill in the table with your laboratory’s information  and details on the method being validated) | | |
| **Instrument/Method/Reagent to be validated:** |  | |
|  | Primary  Back-up | |
| (if applicable)  **Serial Number(s):** |  | |
| **Analyte(s):** |  | |
| **Kit Name:** |  | |
| **Sample Type(s):** |  | |
| **Reason for Validation:** | Initial Validation | Re-validation (choose one below)  Instrument move  Instrument modified  Method change  Other: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
| **Regulatory Status:**  (check all that apply) | FDA Approved  FDA Cleared  CE Marked  EUA  None | |

1. **Overview**
   1. This plan was written using “VAL 2001\_Quantitative Validation Guidelines” as a reference, please refer to this document if more details are needed.
   2. All raw data reports will be saved in (insert location details)
   3. The plan includes the following sections:

* Precision
* Accuracy
* Linearity
* Analytic Measurement Range (AMR) and Clinical Reportable Range (CRR)
* Analytical Sensitivity and Specificity
* Reference Ranges
* Method Approval
* (Insert/remove additional sections if needed-e.g. Carryover))

1. **Precision**
2. Precision is reproducibility - the agreement of the measurements of replicate runs of the same sample. It is the process of determining the range of random error. The precision is measured in terms of coefficient of variation (CV). Precision will be tested only on measured analytes, and not calculated analytes.
3. Short-term (within-run) and long-term (between-day) precision will be determined by running the normal and abnormal controls as follows:
   1. Short-term will be tested by running each control 20 times in one day.
   2. Long-Term will be tested by running each control (insert how you will be running theses samples ex. once per day for 20 days or 4 samples per day for 5 days).
4. The mean, standard deviation (SD) and CV will be calculated using the replicates.
5. Acceptability criteria: The CV% for each assay is expected to be equal to or less than the manufacturer’s performance specifications for precision. In the event that an assay does not perform as expected, the CV will be compared to the allowable random error (33% of pSMILE Total Allowable Error Limits for long-term and 25% of pSMILE Total Allowable Error Limits for short-term). Refer to pSMILE TEa Limits tables.
6. The manufacturer’s claims for precision testing and TEa limits for each analyte are listed below

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| Short-Term | | |
| Analyte | Mfg Precision | 25% of TEa |
| ALT | (00.00%) | (00.00%) |
| AST | (00.00%) | (00.00%) |
| Albumin | (00.00%) | (00.00%) |
| Long-Term | | |
| Analyte | Mfg Precision | 33% of TEa |
| ALT | (00.00%) | (00.00%) |
| AST | (00.00%) | (00.00%) |
| Albumin | (00.00%) | (00.00%) |

1. **Accuracy**
2. Accuracy is the true value of a substance being measured. Verification of accuracy is the process of determining that the test system is producing true, valid results. Accuracy will be tested on measured analytes only.
3. The ideal number of samples is 40, however a minimum of 20 samples that cover the reportable range of the method and include points near the medical decision points, if possible is acceptable.
4. Accuracy will be determined by one of the options below:
   1. Option A: A minimum of 20 samples, tested in duplicate. These will be primarily patient samples but may include commercial proficiency testing or control samples in order to provide material that covers the reportable range. The samples will be tested on (insert name of comparison instrument) located at (insert name and location of laboratory). The samples will be tested in duplicate on both instruments and duplicates will be averaged. Ideally testing will occur on both instruments within 2 hours.
   2. Option B: A minimum of 20 samples with known values, such as proficiency testing samples or External Quality assurance (EQA) samples, will be used as the reference method. (Insert name of proficiency panel(s)or standards) will be used and the results compared to the peer means or assayed values.
5. Acceptability criteria: Regression analysis will be used to determine if the methods are accurate within the TEa as follows:
   1. Correlation coefficient (R) must be >0.975
   2. A statistic called the “Error Index” will be used to measure the difference between the two methods as a ratio of the Total Allowable Error. This Error Index can be calculated by subtracting the reference method data point (X) from the method being validated data point (Y) and dividing by the Total Allowable Error (TEa). The equation is: (Y-X)/TEa
   3. The Error Index is measured for each X-Y pair, and must fall within -1 and 1. If more than 5% of the specimens have an Error Index of less than -1 or greater than 1, the accuracy experiment fails.
6. **Linearity**
7. A quantitative analytical method is said to be linear when measured results from a series of sample solutions are directly proportional to the concentration or activity in the test specimens. This means that a straight line can be used to characterize the relationship between measured results and the concentrations or activity levels of an analyte for some stated range of analyte values. Linearity will be tested on measured analytes only.
8. Linearity verification will be determined using the (insert provider and product name) samples.
9. At a minimum, samples will be run in duplicate.
10. Known values of the standards will be plotted on the X-axis and the mean of the measured values will be plotted on the Y-axis.
11. Slope and intercept will be calculated.
12. A predicted Y value will be calculated for each X value.
13. Predicted Y values will be plotted versus the corresponding known X values. A straight line will be drawn to connect the predicted Y points on the graph.
14. Measured Y values will be subtracted from the associated predicted Y value. This difference is the systematic error due to non-linearity.
15. Systematic error will be compared to 50% of the total error.
16. Acceptability criteria:
17. The method is linear if the difference between the predicted Y value and the measured Y value is less than the allowable error for each specimen point.
18. The systematic error must be less than 50% of the total error.
19. **Analytic Measurement Range and Clinical Reportable Range**
20. The **Analytic Measurement Range (AMR)** is the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process. AMR validation is the process of confirming that the assay system will correctly recover the concentration or activity of the analyte over the AMR. Reportable range will be verified on measured analytes only.
21. The **Clincal Reportable Range (CRR**) is the range of analyte values that a method can report as a quantitative result, allowing for specimen dilution, concentration or other pretreatment used to extend the direct AMR. The laboratory should establish a CRR that covers a range inclusive of Grade 4 Adverse Events on the DAIDS Toxicity Table.
22. Reportable range will be determined using the (insert proficiency provider and survey name) samples.
23. At a minimum, samples will be run in duplicate.
24. It may be necessary to dilute the lowest sample to verify the low end of Analytical Measurement Range (AMR).
25. The high end of the AMR will only be as high as the highest sample.
26. The Clinical Reportable Range (CRR) must extend the AMR in order to include grade 4 events of the Division of AIDS Toxicity Table.

* The lab will establish what dilutions are necessary to cover this range, bearing in mind that a minimum amount of dilution is ideal since accuracy decreases with increasing dilution.
* The laboratory will decide the maximum value of dilution that will be allowed. Any samples that do not give a numerical value beyond this allowed dilution should be reported as greater than the upper end of the CRR.

1. Acceptability criteria:
2. The reportable range must be within the manufacturer’s AMR.
3. The manufacturer’s upper limit will be accepted if the known sample is within percent TEa of the AMR upper limit.
4. The manufacturer’s lower limit will be accepted if the known sample is within the minimum detectable difference or percent TEa of the lower limit (whichever is greater).
5. **Analytical Sensitivity** is the lowest concentration of an analyte that can be measured (Lower Limit of Detection). **Analytical Specificity** is the determination of the effect of interfering substances.

* For an FDA approved, unmodified method the manufacturer’s stated analytical sensitivity and specificity will be used.
* For a non-FDA approved or modified method the laboratory must establish the lowest concentration that the method can accurately measure that is distinguishable from zero, and must determine the effect of interfering substances.

1. **Reference Ranges** 
   1. Reference ranges are a measured set of values determined to occur in a healthy non-diseased population. The laboratory must verify that their choice of reference ranges is valid for their study population. To verify or transfer a published range, the laboratory must analyze specimens from 20 healthy, non-diseased individuals, for each subgroup. If 2 or fewer results fall outside the published range, it is considered verified. If, however, more than 2 results fall outside the published range, a more extensive study should be conducted. In this case, 120 study participants are required per range subgroup to establish a new reference range.
   2. The reference range studies have been verified (or established) for the following populations:
      1. Adult reference ranges–describe the methods used to establish or verify reference ranges for each analyte. Include information on how “normal” subjects were screened, the total number of subjects included, and any other pertinent information.
      2. Pediatric reference ranges– describe the methods used to establish or verify reference ranges for each analyte. Include information on how “normal” subjects were screened, the total number of subjects included, and any other pertinent information. See example below:

“Reference ranges for pediatrics were adopted from Ugandan reference ranges in use at the XYZ Research lab in Kampala, Uganda. The ranges were evaluated by the ABC medical leadership team in consultation with local pediatricians and determined to be appropriate for the local Tanzania population. Adoption of these ranges was approved by the ABC Medical Director. All ranges will be verified and monitored over time as appropriate data becomes available.”

* 1. Acceptability criteria:
     1. Establishment: ranges will be determined using a non-parametric statistical method to determine the 95% reference limits. For most analytes the lower and upper reference limits are defined as the 2.5th and 97.5th percentiles, respectively.
     2. Verification: ranges will be considered verified if 90% of values fall within the proposed range
     3. Verification of pediatric ranges will be dependent on the ability to collect sufficient pediatric samples in each age category. Additional time may be required or fewer samples may be acceptable. The Medical Director will have final approval on the acceptability of pediatric reference range verification.

1. **Method Approval-** The final decision on method validation and acceptance is made after a careful review of all the studies performed as part of the complete method validation process. The Laboratory Director shall make the ultimate decision on method validation. Method acceptance is based on the results from the above studies plus an evaluation of the new method’s cost effectiveness, turn-around-time, laboratory staff training needs, and any other relevant operational considerations.
2. **Optional Sections-E.g. Carryover (May be applicable)**
3. Carryover is the determination of whether high concentration samples run immediately before low concentration samples causes falsely elevated results in the low sample.
4. Carryover studies can be performed by testing known high patient samples followed by known low patient samples to see if the results of the low-level material is affected. If carryover is detected, the laboratory must determine the analyte concentration above which subsequent samples may be affected and define this value in the procedure.
5. Carryover will be determined by testing the high-level commercial linearity samples provided by (insert provider) followed by known low level patient samples tested in the following configuration: (L1/L2/L3/H1/H2/L4/H3/H4/L5/L6/L7/L8/H5/H6/L9/H7/H8/L10/H9/H10/L11)
6. Acceptability criteria: Three times the lowest low SD. This is the SD that would be acceptable if no high samples were present.

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| **Prepared By:** |  |
| **Date:** |  |