Hepatitis Viral Load Quantitative Validation Guidelines (HCV RNA and HBV RNA)

Validation of a quantitative system consists of an established set of required experiments. Each laboratory should first design a validation plan describing how they will satisfy each of these requirements. The validation plan must also detail the acceptability criteria for each element. After completing all of the validation experiments, results should be compiled and filed in an organized manner. Package Inserts should be included as part the validation packet and submitted with your validation summary. All validation records should be retained for the life of the instrument. A validation summary should be prepared that contains a place for the Laboratory Director to sign, indicating the validation has been reviewed and approved. These guidelines have been created in collaboration with expertise from Johns Hopkins Molecular Microbiology Department and SmartSpot EQA Provider.

The following are the required components of validation for HCV and HBV Viral load using PCR or GeneXpert instrumentation:

1. **Precision** is reproducibility - the agreement of the measurements of replicate runs of the same sample. Replication experiments are performed to estimate the imprecision or random error of the analytical method.

    a. Sample Criteria

        - At least three patient samples spanning the Clinical Reportable Range (CRR).
        - Commercial Provider Panels available upon request from pSMILE with DAIDS approval.

    b. Testing and Results

        - Run each sample three times per day over a three-day span.
        - It is recommended that a sample be included that is 5-10 times the LOD (low end of detection) Ex. LOD=25 then pick sample= 125-250.

    c. Acceptability Criteria

        - Calculate the Mean and SD to determine acceptable ranges. The acceptability criteria between observed results is +/-0.5 log IU/mL. See example table below for 1 sample:

Days (Sample A)	Replicate	Observed Result (Log10)	Intra-Assay Precision (Std. Deviation)
1	1	2.70	0.09
	2	2.80	
	3	2.63	
2	1	2.70	0.06
	2	2.63	
	3	2.59	
3	1	2.75	0.09
	2	2.65	
	3	2.58	
	Inter-Assay Precision (Std. Deviation)	0.07	

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 testing is only required for measured analytes, and not required for calculated analytes. Consult the instrument user's manual to determine which analytes are measured and which are calculated.

    a. Determine the Reference Method

    - The ideal reference method is a similar instrument/method.
    - The reference method must be previously validated.
    - The reference method must currently be performing successfully on EQA.
    - Comparison to an in-house method is preferred if the in-house instrument meets the above criteria.

    b. Sample Criteria

    - The ideal number of samples is at least 20, if possible include >10 positive samples that cover the reportable range of the method starting at the LOD (linearity can be performed at the same time) to the high end of the Clinical Reportable Range (CRR).
    - Patient samples, quality control material, and external quality assurance (EQA) samples may be used. Commercial Provider Linearity/Accuracy Panels available upon request from pSMILE with DAIDS approval.

c. Testing and Results

- Accuracy and Linearity can be performed at the same time using the Provider Panels.

d. Acceptability Criteria

- Calculate the Mean and SD to determine acceptable ranges. The acceptability criteria is +/-0.5 log IU/mL from the known value.

3. **Linearity** for a quantitative analytical method is 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 a determined range of analyte values. Linearity testing is only required for measured analytes, and not required for calculated analytes. Consult the instrument user's manual to determine which analytes are measured and which are calculated. For Viral Load the linearity is represented by the AMR and LOD as shown below.

a. Sample Criteria

- A minimum of 5 samples that cover the reportable range of the method. Commercial Provider Linearity/Accuracy Panels available upon request from pSMILE with DAIDS approval.

b. Testing and Results

- At a minimum, run each sample in duplicate and average the results. This testing can be run at the same time as Accuracy.

c. Acceptability Criteria

- Calculate the Mean and SD to determine acceptable ranges. The acceptability criteria is +/-0.5 log IU/mL from the known value.

4. **Limit of Detection (LOD) and Analytical 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. The manufacturer defines the AMR but it is the laboratory's responsibility to verify it. AMR testing is only required for measured analytes, and not required for calculated analytes. Consult the instrument user's manual to determine which analytes are measured and which are calculated

If the test is FDA approved, only a verification is needed using at least 5 samples spanning the LOD and AMR range:

a. Sample Criteria

- Samples with known values, such as quality control, calibrators or commercial linearity standards should be used. Use high level standard and dilute out.
- It may be necessary to dilute the lowest sample to verify the low end of the AMR.
- The high end of the AMR will only be as high as the highest sample.

b. Testing and Results

- Run each sample in triplicate

c. Acceptability Criteria

i. Upper Limit Verification TEA is +/- 0.5 log

- The manufacturer's upper limit can be accepted if the known sample is within the percent TEa of your AMR upper limit.
- Your measured value must also be within TEa of the known sample.
- If a sample within TEa cannot be obtained, the highest known sample measured and within TEa should be used as the highest reportable undiluted value.

ii. Lower Limit Verification TEA is +/-0.5 log

- The manufacturer's lower limit can be accepted if the known sample is within the minimum detectable difference or percent TEa of the lower limit (whichever is greater).
- Your measured value must also be within TEa of the known sample.

Clinical Reportable Range (CRR) is the range of analyte values that a method can report as a quantitative result, allowing for specimen dilution, concentration or another pretreatment used to extend the AMR. The laboratory should establish a CRR that covers the range of Grade 4 Adverse Events on the DAIDS Toxicity Table without exceeding manufacturer's dilution guidelines. FDA cleared assay will have the range.

- The lab should 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 should decide the maximum value of dilution that will be allowed without exceeding the manufacturer's recommendations for dilution.
- 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.

5. **Analytical Sensitivity** is the lowest concentration of an analyte that can be measured (also called the Lower Limit of Detection).

    - For an FDA approved, unmodified method, the manufacturer's stated sensitivity 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.

    Analytical Specificity is the determination of the effect of interfering substances.

    - For an FDA approved, unmodified method, the manufacturer's stated specificity will be used.
    - For a non-FDA approved or modified method the laboratory must determine the effect of interfering substances.

6. **Reference Range- N/A**

7. **Method Approval**

    - The final decision on methodology 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.
    - There must be an approval with a signature from the Medical and/or Laboratory Director and preparer of validation documents with dates.

References

1. CLSI. *Preliminary Evaluation of Quantitative Medical Laboratory Measurement Procedures Implementation Guide- 1st Edition*. CLSI document EP10-ED3-IG. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.
2. Westgard, James O., Basic Method Validation: Training in Analytical Quality Management for Healthcare Laboratories, 4th edition, 2020 Madison, WI 53717.