HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank		
Document:	Departmental Policy and Procedure		
Title:	Test Method Validation		
Applies To:	All Laboratory and Blood Bank Staff		
Preparation Date:	January 01, 2025	**Index No:**	LB-DPP-009
Approval Date:	January 15, 2025	**Version :**	2
Effective Date:	February 15, 2025	**Replacement No.:**	LB-DPP-009 (1)
Review Date:	February 15, 2028	**No. of Pages:**	08

1. PURPOSE:

1.1 To ensure that all test methods are evaluated prior to reporting patient results.
1.2 To establish the guidelines for method selection and the procedures for verification of method performance.
1.3 To verify the claims of method manufacturers regarding analytical performance by following CBAHI standards, CLIA regulations and CLSI guidelines.

2. DEFINITON:

2.1 Method validation is a process to measure performance characteristics of a test, with the goal of determining whether the test is equivalent to the reference test for the intended conditions of use. "Equivalent" = the performance characteristics are statistically indistinguishable.
2.2 Verification is defined as an abbreviated validation process to demonstrate that a test performs as a substantial compliance to previously established claims.
2.3 Medical Decision Level is any concentration of the analyze that is critical for medical interpretation (diagnosis, monitoring or therapeutic decisions).
2.4 Random errors refer to fluctuations of measured values about their mean due to random factors (temperature, volume, electrical interferences, inconsistent handling by user, inconsistent environment conditions, etc).
2.5 Systematic errors are the changes that is always in one direction and will cause a shift in the mean value.

3. POLICY:

3.1 It is our laboratory policy to verify all test methods to ensure that all lab results are reliable and accurate. and in compliance with CBAHI standards.
3.2 Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.
3.2 Analytical methods validation is required in the following cases:
- 3.2.1 When placing a new system into service.
- 3.2.2 When existing methods are being enlarged by another purpose (e.g., another kind of biological material).
- 3.2.3 After major trouble shooting of the machine.
- 3.2.4 When a perennial problem is shown by quality control.
- 3.2.5 When a new (different) diagnostic Kit is being introduced.

3.3 Validation characteristics that should be considered include but are not limited to:
- 3.3.1 Accuracy: a concept related to the closeness of agreement between the average of one or more test results and an accepted reference value.
- 3.3.2 Precision (Repeatability): the closeness of agreement among test results obtained under prescribed conditions.
- 3.3.3 Sensitivity (lower detection limits): is the smallest concentration of an analyte that can be reliably measured by an analytical procedure.

3.3.4 Carryover.
3.3.5 Analytical Measurement Range (AMR).

4. PROCEDURE:

4.1 The laboratory must establish acceptance criteria as part of the validation/verification plan. Parameters for accuracy, precision, sensitivity and specificity should include a confidence level of at least 90%, or meet the claims of the manufacturer.

4.2 Qualitative methods include semi quantitative testing that use cut offs such as hepatitis testing in which no values/concentrations are included in the patient report. Test results are reported as positive/negative, normal/borderline/abnormal, reactive/nonreactive, detected/not detected, etc.

4.2.1 FDA cleared or approved methods establishment and verification of performance specifications states that each laboratory that introduces an unmodified, FDA-cleared or approved test system must demonstrate that it can obtain performance specifications comparable to those established by the manufacturer for the following performance characteristics before reporting patient test results: Accuracy, Precision, Reportable Range of the test results and verification that the manufacturer's reference intervals (normal values) are appropriate for the laboratory's patient population.

4.2.2 Accuracy: Demonstrates how close to the "true" value the new method can achieve, a method comparison experiment is used to estimate inaccuracy or systematic error:

4.2.2.1 Test material can include: calibrators/controls, reference material, proficiency testing material with known values, samples tested by another lab using the same or similar method, or by comparing results to an established comparative method. Test material matrix should match or be as close to the sample matrix as possible.

4.2.2.2 Most sources recommend comparing at least 40 patient specimens. CLIA current guidance suggests a minimum of 20 samples. A larger number has a better chance to detect interferences. Depending on the test system and test volume the number used can vary. The actual number is less important than the quality of the samples. The estimate of systematic errors will depend more on obtaining a wide range of test results than on a large number of samples.

4.2.2.3 A method comparison experiment for accuracy is recommended to be done over a minimum of 5 days. Continue for another 5 days if discrepancies are observed. If side-by side testing is done samples should be tested within 2 hours of each other to ensure that sample stability will not affect results. If this is not possible, refrigerating or freezing samples between testing may preserve the sample. Please take into account any freeze/thaw cycle limitations your method may have. If the laboratory cannot perform the experiment for the 5 days due to lack of samples, resources or other reasons, consult with your quality manager before proceeding.

4.2.2.4 Document the results of the new method comparing the known values from the reference sources, another certified laboratory's results or with results from the current method. It is preferable to include both reference and patient samples, but priority will be given to patient samples.

4.2.2.5 Calculate the percent of positive, negative and total accuracy by dividing observed results over known results multiplied by 100.

4.2.2.5.1 Example: New method =19 positives, 20 negatives
Current method or reference material with known values=20 positive, 20 negative

4.2.2.5.2 Percent positive accuracy 19/20 X 100 = 95%

4.2.2.5.3 Percent negative accuracy 20/20 X 100 = 100%

4.2.2.5.4 Total accuracy 39/40 X 100 = 98%

4.2.3 Precision also known as reproducibility can: Can the new method duplicate the same results? Use samples that have a matrix as close as possible to the real specimen. For clinical test patient samples are the first choice followed by control material and reference solutions.

4.2.3.1 Most sources agree that a minimum of 2 negative samples and 2 positive samples run in triplicate for 5 days will provide data for within-run and between-run

components to estimate precision. Having different operators perform the precision experiment must be done for methods that are operator dependent.

4.2.3.2 Calculate the percent within-run (intra), between-run (inter) and total precision by dividing observed results over known results multiplied by 100.

4.2.3.3 Example:

ID	Day 1			Day 2			Day 3		
Pos sample	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
Pos sample	Pos	Pos	Pos	**Neg**	Pos	Pos	Pos	Pos	Pos
Neg sample	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Neg sample	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Within run %	12/12/x 100 = 100%			11/12 x 100 = 92%			12/12/x 100 = 100%		

Total Precision: 58/60 x 100 = 96.7%

ID	Day 4			Day 5			Between run %
Pos sample	Pos	Pos	Pos	Pos	Pos	Pos	15/15x100 = 100%
Pos sample	Pos	Pos	Pos	Pos	Pos	Pos	14/15x100 =93%
Neg sample	Neg	Neg	Neg	Neg	Neg	**Pos**	14/15x100 =93%
Neg sample	Neg	Neg	Neg	Neg	Neg	Neg	15/15x100 = 100%
Within run %	12/12/x 100 = 100%			11/12 x 100 = 92%			

Total Precision: 58/60 x 100 = 96.7%

4.2.4 Reportable Range: CLIA defines this as the highest and lowest test values that can be analysed while maintaining accuracy. For tests without high or low values, define method criteria for a positive result.

4.2.4.1 To verify reportable range, test at least 3-5 low and high positive samples once. These samples can be combined with the accuracy/precision experiments include both weak and strong positive samples.

4.2.4.2 For methods depending on a cut off value to determine positive results, testing positive specimens near the cut off can serve as the cut off validation.

4.2.4.3 Qualitative cut off Phase III for qualitative tests that use a cut off value to distinguish positive from negative, the cut off value is established initially, and verified every 6 months thereafter.

4.2.5 Sensitivity & Specificity: CLIA does not require that these parameters to be verified.

4.2.6 Once the method experiments are complete, summarize the result in a method validation/verification summary. Clearly state the purpose of the verification, what platform/method and the number of samples for each experiment. Any discrepant results should be investigated and explained in the summary. Test results that show sample problems such as contamination degradation should not be used in the assessment but still listed with an explanation.

4.2.7 The summary should also contain a conclusion stating weather study that met the acceptance criteria or not and its suitability for us in the laboratory.

4.2.8 When parameters are just outside acceptance criteria, additional testing can be performed (add more samples to the study), but do not delete data. If the results show poor performance, check the instrument set-up, reagents, and procedures. Perform corrective actions and repeat the entire

validation/verification study. Any discrepant results should be investigated and explained in the summary.

4.2.9 If the study results fail to meet pre-established criteria, the test cannot be implemented for use in the laboratory.

4.3 Quantitative Methods: Includes laboratory methods that report numbers. QA will provide Validation software to assist in statistical analysis

4.3.1 Accuracy:

4.3.1.1 Demonstrates how close to the "true" value the new method can achieve. A method comparison experiment is used to estimate inaccuracy or systematic error. Test material can include: calibrators/controls, reference material, and proficiency testing material with known values, samples tested against a reference standard, high-quality method or another lab using the same method or by comparing results to an established in-house method.

4.3.1.2 In simple accuracy, the materials used have target ranges (not a single value) provided by the device or method manufacturer, and those ranges reflect the systematic errors (bias) and random errors (imprecision) introduced by the use of multiple reagent lots, multiple calibration events and multiple instruments.

4.3.1.3 A specimen test meets simple accuracy criteria if the measured concentration falls within the vendor defined target range of concentrations around the expected result.

4.3.1.4 The experiment requires at least two specimens, assayed in duplicate. Ideally, the lowest and highest specimen concentrations should challenge the limits of the reportable range. Verifying instrument performance across the reportable range in conjunction with the simple accuracy experiment is optional, but recommended.

4.3.1.5 Materials: It possible to assay recovery using single replicate but, on gets more reliable estimate when 2 to 4 replicates assayed better to use with known concentrations, i.e. QC's, calibrators, known sample materials, PT materials. Samples have to cover the Low-Med-High measurement range.

4.3.1.6 EP evaluator software program or other suitable software form will be used to calculate precision for every test evaluated..

4.3.1.7 Experiment:

4.3.1.7.1 Run each specimen 5 or 10 times, and calculate the % recovery

4.3.1.7.2 % Recovery= Target – Mean/ Target X 100%

4.3.1.7.3 Acceptance Criteria: If the % recovery is within 10%, then the method is accurate.

4.3.1.7.4 OR Run assayed control materials low & high in duplicate times, and then calculate the mean & SD. Acceptance Criteria: If the control mean result lie within + & -2SD, then the method is accurate.

4.3.2 Precision:

4.3.2.1 Precision is the ability to obtain the same result upon repeated measurement of a specimen. Simple Precision is the traditional precision analysis done in clinical laboratories.

4.3.2.2. EP evaluator software program or other suitable software form will be used to calculate precision for every test evaluated

4.3.2.3 Simple Precision computes the mean, standard deviation (SD), and coefficient of variation (CV). It also computes a 95% confidence interval for the SD. You can enter a precision goal (CLIA Total Allowable Random Error), and the program will report that the test "passes" if the SD is less than your Allowable Error.

4.3.2.4 The assessment of reproducibility is Within Run Precision, within a day, or over period of multiple days (complex precision), usually 5 days. Within run precision can be quickly evaluated by running pooled sera/blood or quality control material multiple times to establish reproducibility characteristics in terms of standard deviation (SD) and percent coefficient of variation (% CV)

4.3.2.5 If control materials are not available, pool patient samples to achieve comparable values.

4.3.2.6 Consider a minimum of 10 - 20 data points for each precision check. Precision of the method should be checked at 2 or 3 concentrations whenever possible. Concentration(s) as close as possible to the medical decision level(s) used in the laboratory should be used.

4.3.2.7 This module can identify and remove outliers. It does not use the SD to decide whether a result is an outlier. Instead, it uses Tukey's nonparametric method: Determine the Interquartile Range (IQR). The IQR is the distance from the 75th percentile to the 25th percentile. Points greater than P75 + 3 IQR or lower than P25 - 3 IQR are outliers.

4.3.2.8 Acceptance Criteria: Pass or Fail Test. The test passes if the computed SD does not exceed Allowable Random Error.

4.3.2.9 If the precision of the method fail, a problem exists which needs to be identified and corrected. The problem may originate with the equipment, reagents, supplies or the operators.

4.3.2.10 Document conclusion on the method Validation Summary Form.

4.3.3 Report Range: (Analytic measurement range= AMR), is the range of values that the method can directly measure without dilution or concentration .

4.3.3.1 Each section should have the AMR that provides acceptable results for the intended clinical use.

4.3.3.2 The sections must specify how to handle results that exceed the AMR.

4.3.3.3 If results outside this range are reported, the appropriate dilution or concentration protocols must be specified.

4.3.3.4 Material for AMR may be high control or calibrator or patient sample.

4.3.4 Carryover:

4.3.4.1 The error induced in the result of a specimen by contamination from the preceding one. It can be due to incomplete washing, new instrument or changed sample probe.

4.3.4.2 Sample carry-over: This may be measured by analyzing two identical specimens with a high concentration of analyte (recorded as al and a2) followed by two identical specimens with a low concentration (which are recorded as b1 and b2).

4.3.4.3 The carry-over (k) is usually expressed as: k = b1 - b2 / a2 – b2 x 100%.

4.3.4.4 Replicate measurements of k are made, and the mean result should be the same for high-low and low-high sequences.

4.3.4.5 With most automatic analyzers, carry-over is less than 1-2%, and usually this will not cause significant errors in routine analytical results.

4.3.4.6 Consequently if the precision, measured using different sequences of specimens, is satisfactory, carry-over is unlikely to be significant and need not normally be measured as part of the evaluation of an instrument. If, however, the precision is poor, it may be necessary to test whether this is due to excessive carry-over contamination of sample to sample run.

4.3.5 Approval of the method for clinical use :

4.3.5.1 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.

4.3.5.2 The laboratory medical director shall make the ultimate decision on method validation.

4.3.5.3 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.

5. MATERIALS AND EQUIPMENT:

5.1 Quality Control (QC) materials or pooled patient samples.

6. RESPONSIBILITIES:

6.1 Laboratory director

6.2 Sectional supervisors implements method verification and validation procedures in respective division.
6.3 Laboratory technologists adheres to written protocol for method performance verification, validation or modification.

7. APPENDICES:

7.1 CLIA Requirements for Analytical Quality

8. REFERENCES:

8.1 A Laboratory Guide to Method Validation, (Eurachem).
8.2 American National Standards Institute.
8.3 Equ35-B-01_Heme Val Plan Template v.1.0.
8.4 Westgard QC.
8.5 CLIA Requirements for Analytical Quality.

9. APPROVALS:

	Name	Title	Signature	Date
Prepared by:	Dr. Talal Abdelgawad	Clinical Pathologist		January 01, 2025
Reviewed by:	Dr. Kawther M. Abdou	Clinical Pathology Consultant		January 05, 2025
Reviewed by:	Ms. Noora Melfi Alanizi	Laboratory & Blood Bank Director		January 07, 2025
Reviewed by:	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 09, 2025
Reviewed by:	Dr. Tamer Mohamed Naguib	Medical Director		January 12, 2025
Approved by:	Mr. Fahad Hazam Alshammari	Hospital Director		January 15, 2025

Appendix 7.1 :

CLIA Requirements for Analytical Quality:

Routine Chemistry	
Test or Analyte	Acceptable Performance
Alanine aminotransferase	Target value ± 20%
Albumin	Target value ± 10%
Alkaline phosphatase	Target value ± 30%
Amylase	Target value ± 30%
Aspartate aminotransferase (AST)	Target value ± 20%
Bilirubin, total	Target value ± 20% (greater)
Blood gas p02	Target value ± 3 SD
Blood gas pCO2	Target value ± 8% (greater)
Blood gas pH	Target value ± 0.04
Calcium, total	Target value ± 10%
Chloride	Target value ± 5%
Cholesterol, total	Target value ± 10%
Cholesterol, high dens. lipoprotein	Target value ± 30%
Creatine kinase	Target value ± 30%
Creatine kinase isoenzymes	MB elevated (present or absent) or Target value ± 3 SD Creatinine
Creatinine	Target value ± 15% (greater)
Glucose	Target value 10% (greater)
Iron, total	Target value ± 20%
Lactate dehydrogenase (LDH)	Target value ± 20%
LDH isoenzymes	LDH1/LDH2 (+ or -) or Target value ± 30%
Magnesium	Target value ± 25%
Potassium	Target value ± 0.5 mmol/L
Sodium	Target value ± 4 mmol/L
Total protein	Target value ± 10%
Triglycerides	Target value ± 25%
Urea Nitrogen	Target value ± 9% (greater)
Uric acid	Target value ± 17%

Endocrinology	
Test or Analyte	Acceptable Performance
Cortisol	Target value ± 25%
Free thyroxine	Target value ± 3 SD
Human chorionic gonadotropin	Target value ± 3 SD or (positive or negative)
T3 uptake	Target value ± 3 SD by method
Triiodothyronine	Target value ± 3 SD
Thyroid stimulating hormone	Target value ± 3 SD
Thyroxine	Target value ± 20% or 1.0 mcg/dL (greater)

General immunology	
Test or Analyte	Acceptable Performance
Alpha-1 antitrypsin	Target value ± 3 SD
Alpha-fetoprotein	Target value ± 3 SD
Antinuclear antibody	Target value ± 2 dilution or (pos. or neg.)
Antistreptolysin O	Target value ± 2 dilution or (pos. or neg.)
Anti-Human Immunodeficiency virus	Reaction or nonreactive
Complement C3	Target value ± 3 SD
Complement C4	Target value ± 3 SD
Hepatitis (HBsAg, anti-HBc, HBeAg)	Reactive (positive) or nonreactive (negative)
IgA	Target value ± 3 SD
IgE	Target value ± 3 SD
IgG	Target value ± 25%
IgM	Target value ± 3 SD
Infectious mononucleosis	Target value ± 2 dilution or (pos. or neg.)
Rheumatoid factor	Target value ± 2 dilution or (pos. or neg.)
Rubella	Target value ± 2 dilution or (pos. or neg.)