1. PURPOSE

1.1. To conduct Method Validation & Verification of Biochemistry Scope of Testing. Validation and verification of measurement methods are procedures that aim to establish realistic expectations with the analyst and confidence with the end-user that the methods are fit for their intended purposes

2. SCOPE

2.1. This procedure is applicable to the Biochemistry Laboratory

3. REFERENCES

3.1. Equipment Manual
3.2. Test kit inserts
3.3. NABL 112
3.4. ISO 15189: 2012 Standard

4. DEFINITIONS

4.1. Diagnostic Sensitivity is the lowest concentration of an analyte that can be measured as defined by the manufacturer in test kit insert
4.2. Diagnostic Specificity is the determination of the effect of interfering substances. When unmodified method is used the manufacturer's stated specificity will be considered
4.3. Measurement precision is the closeness between indications or measured quantity values obtained by replicate measurements on the same or similar objects under the specified conditions of measurement. The quantitative expression of precision is the SD or CV/CV%

5. RESPONSIBILITY

5.1. Quality Manager
5.2. Technicians
5.3. Consultant Biochemist
5.4. Laboratory Director

6. METHOD VALIDATION PROCEDURE

6.1. Method Validation requirements

6.1.1. The method should be fully developed and optimized
6.1.2. A written standard operating procedure for the method is defined
6.1.3. Instruments are regularly technically controlled and well maintained
6.1.4. The persons performing the measurements have sufficient training and experience
6.1.5. Appropriate calibrators and two concentration levels are available for internal quality control purpose

6.2. Procedure for obtaining Repeatability Precision

6.2.1. A stable quality control is measured repeatedly on the same day
6.2.2. Replicate measurements for estimating repeatability precision is conducted in

a period of 8 working hours

6.3. Procedure for Intermediate measurement precision

6.3.1. Intermediate measurement precision is measured using stable control materials in two different concentrations that are measured daily

6.3.2. 2 Levels of controls are used for analyzing intermediate measurement precision

6.3.3. Common two-way analysis of variance is used to calculate the total SD and its components of SD within and between series of observations

6.4. Procedure for Measurement Reproducibility

6.4.1. Measurement reproducibility is conducted by split testing of samples by different operators and relevant comparison of results

6.5. Procedure to establish Limit of Detection (LOD)

6.5.1. The LOD is used to express the lowest concentration of an analyte that can be detectable by a specific instrument, method or sample

6.5.2. LOD is determined by spiking the blank solution by the order of different concentrations expected to have concentrations near to the LOD of the method

6.6. Measurement of Diagnostic Uncertainty

6.6.1. The diagnostic uncertainty is evaluated from internal and external quality control results

7. METHOD VERIFICATION PROCEDURE:

7.1. Method Verification is conducted by:

7.1.1. Comparison of methods experiments

7.1.2. Replication experiments

7.1.3. Linearity check

7.1.3.1. Linearity studies are performed as part of the procedure "Evaluation of Automated Test Methods" in order to determine linear reportable range. For each analyte, a set of linearity standards will be tested in the same manner as patient samples

7.1.3.2. Testing is performed in triplicate, and at a minimum, in duplicate, when performed within a single run. If one value deviates greatly from the others due to random error, it may be removed from the data analysis and repeated.

7.1.3.3. The test results will be graphed and statistically analyzed as described below under "Evaluation of Linearity Study Data."

7.1.3.4. Linearity study is been performed to determine the linear reportable range for a test method, it may be repeated as recommended by the manufacturer (i.e.: following relocation of the instrument or after major maintenance) or calibration verification may be performed in accordance with CLIA guidelines, to verify continued acceptable performance of calibration and stated reportable range of the analyzer or analyte.

7.1.3.5. Evaluation Of Linearity Study Data

a) The data from the linearity study are recorded on a linearity study sheet.
b) Values are plotted as observed values (Y axis) vs. expected values (X axis). Examine the raw data for obvious errors. If an analytical or technical problem is found, repeat the testing. Assessment will be made by evaluating the data and statistics using the following guidelines

7.1.3.6. Accuracy And Precision

a) Review the linearity data for acceptable accuracy and precision. Ideally, endpoint assays should be within 10% of the standard's stated value or peer group comparison value, but at a minimum, manufacturer's stated tolerance limits should be met.

b) Coefficient of Variation, which is a measure of precision, and is the standard deviation expressed as a percentage of the mean, ideally should also be less than 10%, or at a minimum, remain within the threshold of the manufacturer's stated acceptable performance.

c) It is ultimately the responsibility of the Laboratory Director & HOD – Biochemistry to determine acceptability of this data and the validity of analyzer results with respect to accuracy and precision.

d) Slope And Y-Intercept: Two key statistical values in determining linearity are:

e) Slope: Ideally, the slope is equal to 1.0.

- Acceptable Range: 0.9 - 1.1
- lf the slope is outside the acceptable range; examine the results of the highest standard first. It is possible that the test is nonlinear at its highest value.

f) Y-intercept:

- Ideally, the Y-intercept is equal to zero. For enzyme determinations and other assays with results in high numerical values, the Y—intercept may be much higher with no clinical significance. The Y—intercept for assays with low numerical values should be 0.0 + /— 1.0.

6.4. Reportable Range

6.4.1. A reportable range will be established for each analyte tested. The upper limit of the reportable range will be set at the concentration of the highest standard tested which exhibited acceptable results for linearity, accuracy and precision. This concentration, however, may not exceed the manufacturer's stated linear range

6.4.2. For analytes which have a lower limit of linearity, the lower limit of the reportable range will be set at the lowest standard tested which exhibits acceptable results, however, this concentration may not exceed the manufacturer's lower limit. Patient samples with · concentrations which exceed the reportable range will be diluted with the appropriate diluents and retested, when the analyzer provides this capability. Samples with concentrations which are lower than the reportable range will be reported as "Less than (the lower limit)".

6.5. Calibration Verification

6.5.1. Calibration verification is necessary to verify that an analyte's calibration is still valid, and confirms that testing provides continued accurate results throughout the previously established reportable range

6.5.2. If calibration of an analyte or test system is performed every six

months, utilizing three or more calibrators across a majority of the reportable range, then calibration verification is automatically met, and the laboratory does not need to perform further verification

6.5.3. For analyzers and analytes that are not calibrated with a minimum of three calibrators verifying the low, midpoint and high end of the reportable range, calibration verification must be performed to substantiate the continued accuracy of the monitors throughout the reportable range, after initial validation studies are performed with the setup of the analyzer.

6.5.4. Calibration verification is performed every six months, as stated in current CLIA regulations. Calibration verification should also be performed under the following conditions

6.5.4.1. Whenever major maintenance is performed or a critical component part of an analyzer has been replaced

6.5.4.2. Whenever reagent lots are completely changed (unless it has been stated and shown that these lot changes do not affect test results, as with manufacturer's instructions and guidelines in package inserts and analyzer specific manuals)

6.5.4.3. When control values are found to be continually unacceptable, as with shifts and trends in Levy-Jennings graphs over a period of time

6.5.5. To perform calibration verification, low, midpoint and high-level standards are tested in the same manner as patient samples. Evaluation of this analysis is achieved through use of slope, intercept, correlation coefficient or manufacturer established guidelines for acceptability criteria.

6.5.6. Each laboratory and its director should establish its own acceptance criteria for calibration verification. When acceptable performance is met, the calibration has been verified. If calibration verification is found to be unacceptable, the instrument must be recalibrated and all corrective action must be documented

6.6. Carry Over Testing

6.6.1. Carry Over Measurement is done as part of instrument evaluation and to establish instrument performance

6.6.2. Carry Over measurement is done by analyzing 3 identical specimens with a high concentration of analyte (recorded as H1, H2 & H3) followed by two identical specimens with a low concentration (which are recorded as L1, L2 and L3). The carry-over (k) is usually expressed as:

$$k = [(b1-b2)/(a1-a2)] \text{ X } 100\ \%$$

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

6.6.4. Carry-over (k) measured should be less than 1-2%, to ensure that there are no significant errors in routine analytical results

6.6.5. 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.

6.6.6. If, however, the precision is poor, it may be necessary to test whether this is due to excessive carry-over

6.6.7. However, it is important to verify that (k) is constant with time; if the correlation factor used differs from the true value at the time of analysis, errors will result

6.6.8. For abnormal (k) values sample contamination can be a major causative factor wherein there can be specimen cross-contamination arising from transfer of a portion of one specimen, via the sample probe, into the following one; and specimen-diluent contamination arising from contamination of a specimen by the diluent transferred from the probe of a sample-dilutor

8. ACCEPTANCE PROCESS

8.1.1. The final decision on methodology validation and verification acceptance is made after a careful review of all the reviews performed as part of the complete method validation process.

8.1.2. The Laboratory Director shall make the ultimate decision on method validation and verification

9. RECORDS

9.1. The following records are maintained as mentioned below:

S. No	Record	Responsibility	Review Period	Retention Period
1	Kit Insert File	In-charge	Every Year	1 Year
4	ILC / EQAS File	In-Charge	Every Month	1 Year
5	Staff Training Records File	In-Charge	Every Month	1 Year
6	Method Validation and Verification File	In-Charge	Every Year	Till life of the equipment
7	Test SOP File	In-Charge	Every Year	1 Year