## 1. PURPOSE

**1.1.** Linearity studies are performed to determine the linear reportable range for an analyte. The linearity for each analyte is assessed by checking the performance of recovery throughout the manufacturer's stated range of the testing system.

**1.2.** This is done using a set of standards containing varying levels of an analyte in high enough and low enough concentrations so as to span the entire range of the test system.

**1.3.** Linearities are performed whenever a new analyzer, analyte or method is introduced into the laboratory, or an analyzer is replaced.

**1.4.** Linearities may also be performed for troubleshooting purposes when quality control is unacceptable and deviations from acceptable data cannot be explained, or major analyzer repair or replacement of components has taken place.

## 2. SCOPE

**2.1.** This procedure is applicable to all the staff members and work areas where Laboratory testing process is conducted

## 3. REFERENCES

**3.1.** Quality System Procedures

**3.2.** Quality Manual

**3.3.** Test Analyte Literature

**3.4.** NABL 112

**3.5.** CLIA Guidelines

## 4. RESPONSIBILITY

**4.1.** Laboratory Director

**4.2.** HOD – Biochemistry

**4.3.** Quality Manager

**4.4.** Laboratory Technical In-Charge

**4.5.** IT Support In-Charge

## 5. PROCEDURE

**5.1.** Linearity studies will be 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

**5.2.** Testing should be 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.

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

**5.4.** Once a linearity study has 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.

## 6. EVALUATION OF LINEARITY STUDY DATA

**6.1.** The data from the linearity study will be recorded on a linearity study sheet.

**6.2.** 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.

### 6.3. Accuracy And Precision

**6.3.1.** 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.

**6.3.2.** 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.

**6.3.3.** 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.

**6.3.4. Slope And Y-Intercept:** Two key statistical values in determining linearity are:

6.3.4.1. **Slope:** Ideally, the slope is equal to 1.0.

6.3.4.1.1. Acceptable Range: 0.9 - 1.1

6.3.4.1.2. 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.

6.3.4.2. **Y-intercept:**

6.3.4.2.1. 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

## 7. RECORDS

The following records are maintained for refrigerator in the format mentioned below, for the period defined

| S. No | Record | Responsibility | Review Period | Retention Period |
|---|---|---|---|---|
| 1 | Linearity Testing Form | In-charge | Every Year | 1 Year |
| 2 | Calibration Verification Form | In-charge | Every Year | 1 Year |
| 3 | Carry Over Analysis Form | In-charge | Every Year | 1 Year |
| 4 | Machine Data Sheets | In-charge | Every day | 1 Year |
| 5 | Equipment Print Out Data | In-charge | Every day | 1 Year |