6.4 Calibrators and Controls

Calibrators and controls are reference standard solutions used to assess the performance of an analytical procedure.

6.4.1 Calibrators and controls shall be uniquely identified, separate solutions. They should be prepared from separate traceable reference materials and by two different individuals whenever possible. All drug stock solutions shall be prepared according to the Toxicology Prep Log Worksheets (see Appendix) which must be reviewed by the Forensic Toxicology supervisor or designee authorized to perform work in that discipline prior to use. Necessary deviations from standard drug stock solutions on the Toxicology Prep Log Worksheets shall be recorded and require verification of calculations by a third individual authorized to perform work in that discipline. The reviewer will verify that the stock solution was prepared according to the Toxicology method procedure policies. This verification shall be recorded.

6.4.2 When blood calibrators or controls are used, they shall be prepared using aliquots of human blood, from reliable sources such as the American Red Cross, Medic Regional Blood Center, Interstate Blood Bank, Innovative Research Inc., etc. Each lot received will be tested and verified to be negative. This verification may be done concurrently with sample analysis unless sample volume is limited. The verification shall be documented with the analysis of a negative control and be included in each standard packet where applicable.

6.4.3 Calibrators

A calibration curve shall be used for quantitative methods. This curve is a mathematical correlation accomplished by determining the range of analytical concentrations over which the method shall be used. Within this range, there will be a correlation between signal response (e.g., peak area ratio of analyte and internal standard) and analyte concentration in the sample.

6.4.3.1 Each examiner should evaluate which weighting model provides the best representation of their calibration data. Once the calibration curve is established, it shall not be changed during a given analytical run (e.g., one shall not switch from equal weighting to 1/X).

6.4.3.2 For linear calibration models, at least three non-zero concentration levels shall be used to establish the calibration curve and shall bracket the reported value of the unknown sample(s). For previously validated quadratic calibration models, at least five non-zero concentration levels shall be used to establish the calibration curve and shall bracket the reported value of the unknown sample(s).

6.4.3.3 For ethyl alcohol values of 0.05 gm% and higher, all calibration points used to establish the calibration curve are required to be within ±5% of the expected value. All other calibration points used in the Toxicology Unit are required to be within ±20% of the expected value when evaluated against the calibration curve.

6.4.4 Controls

Controls are reference standard solutions analyzed with unknown samples and are used to verify the calibration curve, for retention time verification, and for mass spectrum comparison.

6.4.4.1 General

6.4.4.1.1 At least one non-zero control standard shall be extracted/prepared and analyzed with every batch of unknown case samples. This positive control shall be processed in the same manner as the case sample(s) and is used to assure that a procedure is working within expected parameters (i.e., ability to detect a particular drug), and/or verify the calibration curve within its limits of quantitation.

6.4.4.1.2 At least one negative control standard shall be processed in the same manner as case samples. A negative shall be run immediately following each highest standard and immediately after the last case sample in an analytical batch, with the exception of the preliminary drug screen and alcohol analysis. This shall be used to verify that the extraction is free from contamination or analytes of interest and that no carryover is present following the highest calibrator. If more than one high standard is run within the batch, negative solvent blanks may be substituted for additional negative extracted blanks.

6.4.4.1.3 Calibrator and/or control standard(s) included within the analytical run shall be appropriate for the test being performed. In some circumstances drugs are detected in analyzed samples that were not included within the initial calibrators and/or control standard(s). In these cases, the sample(s) affected shall be repeated and analyzed concurrently with appropriate standards.

6.4.4.1.4 For all levels of methanol, acetone, and isopropanol, ethyl alcohol values less than 0.05 gm%, and toxicology analytes (i.e., drug testing), all control points are required to be within ±20% of its expected value when evaluated against the calibration curve.

6.4.4.1.5 For ethyl alcohol values 0.05 gm% and higher, all control points are required to be within ±5% of its expected value when evaluated against the calibration curve.

6.4.4.1.6 In some circumstances, the examiner may need to create separate calibration curves (i.e., low and high) to best fit the range of expected values. In these circumstances, more than one control may be run to verify each curve. If the control is not intended for use with that particular curve, it does not have to meet the above requirements. However, all controls bracketed by the utilized calibration curve must meet all acceptance criteria.

6.4.4.1.7 In some circumstances, a control may be included within a solution of other analytes that may be determined to be outside the limits of the acceptance criteria. In these circumstances, the examiner may need to prepare and analyze an additional

control at the same concentration. However, this shall be a planned activity and be documented in the standard packet where applicable.

6.4.4.1.8 Each examiner must carefully examine each analyzed negative control standard to determine that it is free of contamination from carryover or any other source. If for any reason a negative control standard(s) is contaminated, all samples within the run shall be repeated and the source of contamination shall be investigated.

6.4.4.1.9 If the quantitative result of a control standard is outside the acceptance criteria, the case sample(s) may either be reported qualitatively or shall be repeated if the quantitative results are necessary for interpretation in the case.

6.4.4.1.10 Cases may be reported if the retention times of drugs identified are within ±1% (±2% for acids and drugs analyzed on LC/MS/MS) of those of a calibrator or control standard of similar concentration and chromatography is consistent throughout the run.

6.4.4.1.11 Internal standard recoveries in case samples should not be less than 50% or greater than 200% of one of the following:

- the average of at least three representative calibrators and/or controls
- the recoveries of at least five representative cases within the batch

6.4.4.1.12 In instances where an analyte and the matching isotopically-labeled internal standard area recoveries appear to be suppressed, the affected cases shall be extracted a second time. If suppression is apparent in both the original and repeat extraction, then the sample may be evaluated on a case by case basis by the scientist or technical reviewer and documented in the case file.

6.4.4.1.13 If during any analytical run there is a power interruption, the instrument status shall be verified using the internal standard criteria above (**6.4.4.1.10, 6.4.4.1.11, & 6.4.4.1.12**).

6.4.4.2 Alcohols

6.4.4.2.1 An externally prepared, NIST traceable ethanol 0.08 gm% control, and an either internally or externally prepared 0.10 gm% control shall also be run within the initial series of standards. The 0.10 gm% shall contain a mixture of volatiles routinely analyzed to show adequate compound separation within the analytical run. If for any reason the first NIST standard or the ethyl alcohol concentration within the 0.10 gm% control is outside the acceptable quantitative range, all samples within the run shall be reanalyzed.

6.4.4.2.2 An externally prepared, NIST traceable 0.08 gm% control standard shall be run in every tenth position beginning with position 20 (20, 30, 40, etc.) and in the last position of the run. If the ethyl alcohol concentration in the control standard is outside the accepted quantitative range, all nine case samples before and after the control (if applicable) shall be repeated.

6.4.4.2.3 A negative control shall be run immediately following the highest standard.