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**Standard for a Quality Control Program in Forensic
Toxicology Laboratories**



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Standard for a Quality Control Program in Forensic Toxicology Laboratories

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Foreword

As part of a comprehensive quality assurance program, quality control is intended to verify that a method is operating within a predetermined set of criteria for a single analysis, as well to provide an evaluation of the analytical performance over time. After the method has been properly validated, quality control practices help ensure the validity of the test result and demonstrate a method's continued fitness for its intended use.

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All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

Keywords: *forensic toxicology, quality control, calibration*

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Standard for a Quality Control Program in Forensic Toxicology Laboratories

1 Scope

This document establishes minimum requirements for quality control practices in forensic toxicology laboratories. The document explains the importance of a quality control program, how to select and care for materials used to prepare quality control samples, proper preparation and use of calibrator and control samples, and requirements for their use in different types of assays. The document also provides direction for the review and monitoring of quality control data in forensic toxicology laboratories.

This standard applies to laboratories performing forensic toxicological analysis in the following sub-disciplines: postmortem forensic toxicology, human performance toxicology (e.g., drug-facilitated crimes and driving-under-the-influence of alcohol or drugs), non-regulated employment drug testing, court-ordered toxicology (e.g., probation and parole, drug courts, child services), and general forensic toxicology (non-lethal poisonings or intoxications). It is not intended for the area of breath alcohol toxicology.

2 Normative References

The following references are documents that are indispensable for the application of the standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*^a

ANSI/ASB Standard 017, *Standard Practices for Measurement Traceability in Forensic Toxicology*^a

3 Terms and Definitions

For purposes of this document, the following definitions and acronyms apply.

3.1

analytes of interest

Includes all targeted compounds in a screening assay, as well as compounds being quantitated and/or confirmed.

3.2

analytical run “batch”

A set of standards, controls, and/or case samples that are contemporaneously prepared and/or analyzed in a particular sequence.

3.3

blank matrix sample

A biological fluid or tissue (or synthetic substitute) without target analyte or internal standard.

^a Available from <http://www.asbstandardsboard.org/>.

3.4 calibration

Operation that, under specified conditions, establishes a relationship between the quantity value and corresponding indications.

3.4.1 historical calibration

A calibration that was performed and stored prior to the preparation and/or analysis of the case samples and quality control samples.

3.4.2 linear regression

Consists of finding the best-fit linear relationship between the instrument response or response ratio (Y) and the concentration of the analyte in the calibrator (X).

3.4.2.1 quadratic regression

A linear regression of polynomial degree 2.

3.4.2.2 simple linear regression

A straight line regression with only one predictor variable (polynomial degree of 1).

3.5 calibrator^b

Measurement standard used in calibration.

3.6 control

Material of known composition that is analyzed along with unknown sample(s) in order to evaluate the performance of an analytical procedure.

3.6.1 dilution control

A positive control that is diluted in the same manner as the diluted case sample(s).

3.6.2 matrix-matched control

A positive or negative control that is prepared in the same or similar matrix as the case sample(s) or material.

3.6.3 negative control

A test sample similar to the case sample(s) that does not contain the analyte(s) of interest at a reportable concentration. If an internal standard is used in the procedure, it shall be included in the negative control.

^b From ISO Guide 30:2015, <https://www.iso.org/standard/46209.html>

3.6.4

positive control

A test sample like the case sample(s) that contains the analyte(s) of interest at a known concentration.

3.6.5

process control

A control to test an analytical process such as hydrolysis or oxidation of an analyte.

3.7

decision point

Administratively defined cutoff concentration that is at or above the method's limit of detection or lower limit of quantitation and is used to discriminate between a negative and positive test result.

3.8

limit of detection

LOD

An estimate of the lowest concentration of an analyte in a sample that can be reliably differentiated from blank matrix and meets identification criteria for the analytical method.

3.9

lower limit of quantitation

LLOQ

An estimate of the lowest concentration of an analyte in a sample that can be reliably measured with acceptable bias and precision.

3.10

method of standard addition

MSA

A quantitative procedure by which known concentrations of target analyte are added to multiple aliquots of the case sample(s).

3.11

quality control program

A component of a quality assurance program that focuses on ensuring accuracy in laboratory test results through careful monitoring of test methods.

3.12

quality control materials

Materials used to prepare control samples including reference materials, certified reference materials, and blank matrix samples.

3.13

reference material

RM

Material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in a measurement or in examination of nominal properties.

3.13.1**Certified Reference Material^c
CRM**

Reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.

3.14**solvent blank**

A solvent without analyte(s) or internal standard(s) of interest.

3.15**upper limit of quantitation****ULOQ**

The highest concentration of an analyte in a sample that can be reliably measured with acceptable bias and precision.

4 Overview of the Quality Control Program**4.1 General**

Analytical methods shall be validated to meet the requirements of ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*. After this validation, the quality control program helps demonstrate that the method remains fit-for-purpose in its day-to-day use.

4.2 Quality Control Program Requirements

An individual (however named) who is familiar with the policies and procedures of the toxicology laboratory shall be designated to be responsible for the quality control program.

The laboratory shall have a comprehensive quality control program that includes the following.

- a) Pre-defined requirements for quality control materials to be used including:
 - 1) identification of the type, purity, and source;
 - 2) content and concentration;
 - 3) matrix requirements; and
 - 4) instructions for preparation and storage.
- b) Defined calibration models for all procedures, frequency of calibration, and criteria for the acceptance or rejection of calibrations.
- c) Control requirements for all procedures, to include frequency of use and criteria for acceptance or rejection of control results.

^c From ISO Guide 30:2015, <https://www.iso.org/standard/46209.html>

- d) Defined process for review of control results prior to the release of case results.
- e) Defined process to monitor and evaluate control results.

5 Sources, Verification, and Expiration of Quality Control Materials

5.1 General

The selection and care of materials used to prepare calibrators and controls are vital to an effective Quality Control Program. This section outlines the minimum requirements for materials used for the preparation of calibrators and controls. The form of a material shall be documented (e.g., free base, salt, gas, liquid).

5.2 Sources of Quality Control Materials

5.2.1 Blank Matrix Samples

Each lot or batch of blank matrix sample shall be evaluated for the absence of the target analyte(s) or interferences prior to or concurrent with use. The evaluation shall be with the analytical technique employed for that specific method.

EXAMPLE A negative ELISA screen of a whole blood sample is not sufficient to demonstrate that there are no interferences for a GC/MS method.

5.2.2 Reference Materials

The physical and chemical properties shall be determined for reference materials used to prepare calibrators and controls (see 5.3). When used for quantitative measurements, the purity shall also be determined.

5.2.3 Commercial Analytical Reference Materials

Commercial analytical reference materials may be powders or liquids, but more commonly are dilute standards of known concentrations.

NOTE These materials may meet the criteria for Certified Reference Materials.

5.2.4 Other Sources of Reference Materials

In the absence of conventional sources of reference materials, other sources (e.g., tablets, liquids, synthesized materials, chemicals and commercial products) may be used, once appropriately characterized.

Certain materials (e.g., tablets, commercial solvents) may only be suitable for qualitative identification of items and should only be used when no other reasonable options exist.

The laboratory shall document the use of these products, as well as the efforts pursued to obtain them from conventional sources.

5.3 Verification of the Quality of Reference Materials

5.3.1 Certified Reference Materials

If a reference material is used to establish measurement traceability, the requirements of ANSI/ASB Standard 017, *Standard Practices for Measurement Traceability in Forensic Toxicology* shall be followed. If the reference material is accompanied by an acceptable certificate of analysis, as defined in the ANSI/ASB Standard 017, the laboratory may use the material for qualitative and/or quantitative analyses without further verification. Certificates of analysis shall be maintained in accordance with the laboratory's records retention policy.

5.3.2 Non-Certified Reference Materials

For materials without an acceptable certificate of analysis, the laboratory shall characterize the target compound by a minimum of two structural elucidation techniques. This may be done within the laboratory itself or contracted to another laboratory. Techniques may include, but are not limited to:

- a) fourier transform infrared spectroscopy;
- b) mass spectrometry (electron impact full scan, chemical ionization full scan, high resolution or tandem);

NOTE Two different types of mass spectrometry do not meet the minimum criteria.

- c) nuclear magnetic resonance spectroscopy; and
- d) x-ray diffraction.

Results shall be compared to library spectra, published spectra, or the laboratory may elucidate the structure from the resulting spectra.

Records of the laboratory's characterization of the reference material shall be maintained in accordance with the laboratory's records retention policy.

5.3.3 Additional Requirements for Non-Certified Reference Materials Used for Quantitation

If the reference material is to be used quantitatively and certification of the purity or concentration is not available, then the laboratory shall establish the purity or concentration by a minimum of two techniques.

Techniques may include:

- a) gas chromatography-flame ionization detector;
- b) nuclear magnetic resonance spectroscopy;
- c) melting point;
- d) molar absorptivity; and

e) mass spectrometry.

NOTE Two different types of mass spectrometry do meet the minimum criteria.

Records of the laboratory's internal characterization of the reference material shall be maintained in accordance with the laboratory's records retention policy.

5.4 Storage and Expiration of Reference Materials

When provided, the manufacturer's recommended storage conditions for reference materials shall be followed. If storage conditions are not explicitly stated, the laboratory shall determine their appropriate storage based upon historical evaluation, literature references, or the storage of similar compounds.

If the reference material manufacturer provides an expiration date (sometimes referred to as the retest date), the laboratory shall adhere to that date unless the manufacturer has provided a written extension. In such instances, the new expiration date can be used.

If the manufacturer does not provide an expiration date, the laboratory shall assign an expiration date based upon historical evaluation, literature references, or the stability of similar compounds/solutions.

6 Preparation, Use, Storage, and Expiration of Calibrators and Controls

6.1 Preparation and Use of Calibrators and Controls

Matrix-matched samples shall be used as *calibrators* unless validation has justified the use of non-matrix-matched calibrators. When possible, matrix-matched *controls* shall be used.

NOTE Some manufacturer-specific procedures specify use of calibrators and/or controls that are not in the same matrix as case samples (e.g., synthetic matrix). In these instances, it is appropriate to use the manufacturer-recommended calibrators and controls, provided appropriate validation has confirmed their use with the matrix in use for the method.

Commercially-prepared calibrators and controls purchased from vendors that have acceptable certificates of analysis may be used without further verification. Commercial calibrators and controls that do not have acceptable certificates of analysis shall be verified to confirm the identity of the analytes of interest and to establish their target concentrations. Verification shall include meeting all quality control requirements established in the assay.

Materials used to prepare calibrators and controls in-house shall be obtained in the following preferential order to achieve the greatest level of independence:

- a) from different reputable manufacturers, or;
- b) from the same manufacturer, but from different lots, or;
- c) from the same manufacturer lot, but prepared by different analysts.

Calibrators and controls prepared in-house shall be verified as consistent with the target concentrations. Verification shall include meeting all quality control requirements established in the assay.

6.2 Storage Conditions of Calibrators and Controls

When provided, the manufacturer's recommended storage conditions of commercial calibrators and controls shall be followed. If storage conditions are not explicitly stated for commercial calibrators and controls, the laboratory shall determine their appropriate storage based upon historical evaluation, literature references, or the storage of similar compounds.

The storage conditions of in-house prepared quality control materials shall be established by the laboratory with consultation of information about the reference materials used to make the calibrators and controls (e.g., certificate of analysis).

6.3 Expiration of Calibrators and Controls

a) Commercially prepared calibrator or control.

If the manufacturer provides an expiration date for a commercially prepared calibrator or control, the laboratory shall adhere to that date unless the laboratory confirms the continued stability of the material with each subsequent use after the expiration date. This shall be done using a separate lot of non-expired reference material.

If the manufacturer does not provide an expiration date, the laboratory shall assign an expiration date based upon historical evaluation, literature references, or the stability of similar compounds or solutions.

b) In-house prepared calibrator or control.

For in-house prepared calibrators and controls (e.g., dilution of reference material), the laboratory shall assign an expiration date based upon historical evaluation (i.e., comparison with a separate lot of non-expired calibrator or control), literature references, or the stability of similar compounds or solutions. Alternatively, the expiration may be set to the shortest expiration date of its target components.

6.4 Documentation of Calibrators and Controls

The laboratory shall have documentation for the following elements of calibrator and quality control materials:

- a) content and concentration;
- b) date received, opened, prepared, and/or reconstituted by laboratory;
- c) expiration date;
- d) identification of the analyst who prepared the material (if applicable);
- e) lot number or unique identifier;

- f) name or identification of the material (e.g., laboratory name for the material identified in the SOP such as multi drug level 1, level 2, etc.); and
- g) storage requirements.

At a minimum, the storage container shall be labeled with the name/identification, lot number/other unique identifier, and expiration date.

7 Calibration Requirements for Quantitative Procedures

7.1 General

The calibration model and range for an assay shall be specified in the laboratory's analytical procedure and shall not deviate from the model and range documented in the method's validation. Unless using historical calibrations (Section 7.4), all procedures shall be calibrated daily or prior to a new analytical batch.

7.2 Calibration Models

For simple linear regression calibration models, the laboratory shall use a minimum of four calibrators and the curve fit shall not be forced through the origin.

For quadratic regression calibration models, the laboratory shall use a minimum of 6 calibrators and the curve fit shall not be forced through the origin.

The zero calibrator shall not be considered a calibration point in calibration curves.

NOTE Some manufacturer-specific techniques (e.g., CO-Oximetry) use a single calibrator to establish a simple linear regression calibration model by forcing a second calibration point through zero. In these instances, if a laboratory is following the manufacturer's method, it is acceptable to do so provided the method has been appropriately validated by the manufacturer and verified in-house to meet the bias and precision requirements expressed in ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*.

7.3 Dropping a Calibrator

If a laboratory desires to drop a calibrator from a calibration curve, the following must be met:

- a) The laboratory shall define the criteria used to drop a calibrator. A calibrator shall not be dropped solely to improve curve fit or control compliance.

NOTE Some acceptable reasons for dropping a calibrator may include poor extraction of a specific calibrator sample or a bad injection of a calibrator.

- b) The final number of calibrators shall not fall below the minimum number based on the regression model (Section 7.2).
- c) The reason for dropping a calibrator shall be documented in the record and the data supporting that decision shall be retained with the batch.
- d) If the lowest or highest calibration point is dropped, the reporting parameters shall be adjusted accordingly.

EXAMPLE If the lowest calibrator was declared as the method's LLOQ and is subsequently removed from the calibration curve, the next lowest calibrator shall become the LLOQ.

- e) The practice of dropping a calibrator shall be tracked to determine frequency, type of calibrator (concentration and analyte), and analyst.

7.4 Historical Calibrations

Acceptable use of historical calibrations for a quantitative assay shall be demonstrated through method validation before put into use. Afterwards, historical calibrations shall be checked with controls processed contemporaneously with the case samples.

New calibrations shall be performed after instrument maintenance or repair that may affect the calibration, preparation of new internal standard solutions, or control failures related to the calibration.

7.5 Calibration Acceptance Criteria

The laboratory shall define parameters for accepting calibrations and each calibration shall be evaluated against these predefined criteria.

At a minimum, the following criteria shall be used to accept the calibration.

- a) The criteria for curve fit acceptance will be a coefficient of determination (r^2 value) of 0.990 or better.
- b) Calibrator concentrations relative to the established calibration curve shall be evaluated against the maximum acceptable bias (as defined in the validation plan) *or* a maximum of $\pm 20\%$ of the target calibrator's concentration, whichever is less.

In the event that a calibration is not acceptable, the reason for the failure shall be documented and the batch rejected for the applicable analyte(s).

Each analyte shall be evaluated independently and the calibration failure for a single analyte does not invalidate calibrations of other analytes within that assay.

8 Control Requirements

8.1 General

Controls verify the assay performance on a routine basis. The selection of positive control levels shall be based upon decision points, forensic significance, and/or analytical criteria.

At a minimum, the following apply to the use of controls in all forensic toxicology analyses.

- a) All controls shall be tested and treated the same as case samples.
- b) Negative and positive controls shall be included with each analytical batch.
- c) Process controls shall be included when a procedure includes a technique such as hydrolysis or oxidation

- d) The laboratory shall define parameters for accepting or rejecting controls (Section 8.3). Each control sample shall be checked for acceptability using these predefined criteria.
- e) Case samples shall be collectively bracketed by controls during an instrumental run sequence.

8.2 Specific Control Requirements Based on Scope of Assay

8.2.1 General

The number and types of controls are dependent on the purpose of the assay. A laboratory may consider utilizing more controls than the minimum required by this standard. Consideration of the impact of a single control failure on a large batch may warrant more than the minimum number of controls specified below. For example, additional controls may allow for partial batch acceptance.

8.2.2 Non-targeted Screening Assays (to include immunoassays)

At a minimum, analytical runs involving non-targeted assays and immunoassays shall include the following.

- a) One negative control.
- b) One positive control containing representative analytes that challenges the assay performance by not exceeding three times the concentration of the decision point or LOD.
- c) One process control (as appropriate) that challenges the efficacy of the process for at least one representative analyte. The concentration of the analyte(s) in the process control shall be established by the laboratory.

Immunoassay kits may provide controls containing the assay's target analyte. In some situations, it may be desirable to use an analyte as a control that is less cross-reactive within the drug class than the immunoassay is designed to detect. If utilized, the laboratory shall document, validate, and define such use.

8.2.3 Targeted Screening Assays (to include instrumental and non-instrumental techniques)

At a minimum, analytical runs involving targeted assays (including instrumental and non-instrumental techniques such as color tests) shall include the following.

- a) One negative control.
- b) One positive control that challenges the detection limit of the assay for all target analytes by not exceeding three times the concentration of the decision point or LOD.
- c) One process control (as appropriate) that challenges the efficacy of the process for at least one representative analyte. The concentration of the analyte(s) in the process control shall be established by the laboratory.

8.2.4 Qualitative Confirmation/Identification Assays

At a minimum, analytical runs for qualitative confirmation/identification assays shall include the following.

- a) One negative control.
- b) One positive control that challenges the detection limit of the assay for each analyte of interest by not exceeding three times the concentration of the decision point or LOD.
- c) One process control (as appropriate) that challenges the efficacy of the process for at least one representative analyte. The concentration of the analyte(s) in the process control shall be established by the laboratory.

8.2.5 Quantitative Assays

At a minimum, analytical runs for quantitative assays shall include the following.

- a) One negative control.
- b) One low positive control that challenges the lower quantitation limit of the assay for each analyte of interest by not exceeding three times the concentration of the LLOQ.
- c) One high positive control that challenges the upper quantitation limit of the assay for each analyte of interest by not being less than 80% of the concentration of the highest calibrator.
- d) One process control (as appropriate) that challenges the efficacy of the process for each analyte of interest. The target concentration of the analytes in the process control should not be less than 80% of the concentration of the highest calibrator. When an appropriate process control is not reasonably available for all analytes of interest, a process control containing available and representative analytes of interest shall be included.

8.3 Control Acceptance Criteria Based on Scope of Assay

8.3.1 General

The laboratory shall define parameters for accepting controls and each control sample shall be evaluated against these predefined criteria. Each control shall be evaluated independently and the failure of a control for a single analyte does not invalidate the control of other analytes within that assay. In the event that a control is determined to have failed, the reason for the failure (if known) shall be documented. Acceptance criteria for the minimum number of controls must be met for all batches. Laboratories choosing to use more than the minimum number of controls shall have predefined criteria for batch or partial batch acceptance in the event of a control failure.

8.3.2 Acceptance and Evaluation Criteria for Controls in Screening and Qualitative Confirmation/Identification Assays

At a minimum, the following criteria shall be used to accept the controls in screen and qualitative assays.

- a) Negative controls shall not have a positive result for the analyte(s) of interest.

- b) Positive controls and process controls shall have positive results for the analyte(s) of interest.

The laboratory shall define requirements for the analyte of interest to be considered a positive result. These requirements should include relevant parameters such as color change, absorbance value difference, retention time, peak shape, instrument response (e.g., greater than 10% of the signal obtained from the lowest positive control or calibrator), signal-to-noise ratio, and/or mass spectrum data acceptance criteria.

8.3.3 Acceptance and Evaluation Criteria for Controls in Quantitative Assays

At a minimum, the following criteria shall be used to accept the controls in quantitative assays.

- a) Negative controls shall not have a positive result for the analyte(s) of interest.
- b) Positive controls and process controls shall have positive results for the analyte(s) of interest.

The laboratory shall define requirements for the analyte of interest to be considered a positive result. These requirements should include relevant parameters such as color change, absorbance value difference, retention time, peak shape, quantitative result greater than or equal to the LLOQ, signal-to-noise ratio, and/or mass spectrum data acceptance criteria.

- c) Positive controls and process controls shall be further evaluated against predefined control limits. Control limits for quantitative assays shall be established using one of the following approaches.
- 1) **Statistical Evaluation:** Warning limits ($\text{mean} \pm 2\text{SD}$) and control limits ($\text{mean} \pm 3\text{SD}$) shall be calculated based on historical control data. If the measured values for a positive control or process control are beyond the control limits, the control shall be rejected. If the measured values are outside of the warning limits, but within the control limits, the control shall be considered to be acceptable, but the control performance should be monitored for trends. If a trend is detected, it may be indicative of instrumental or procedural problems and should be addressed before control failures become a consistent problem.
 - 2) **Target-Based Control Limits:** Control limits shall be based on method validation requirements for bias (e.g., $\pm 20\%$ of the target or calculated mean value). Control limits shall not exceed the maximum allowable bias established in the validation plan for the assay. If the measured values for a positive control or process control are beyond the control limits, the control shall be rejected.

NOTE Stricter control limits may be expected for certain assays that require better accuracy such as blood alcohol measurements for legal proceedings (e.g., $\pm 10\%$ of the target or calculated mean value).

9 Other Considerations for Batch Evaluations

9.1 General

In addition to evaluating the calibration and control results, a laboratory shall evaluate other parameters that are important to the performance of a batch run of samples.

9.2 Instrument Performance

The laboratory shall have a procedure for evaluating the performance of calibrators, controls, and case samples throughout an analytical run. For example, chromatographic assays may include an evaluation of retention times and peak shape, while a mass spectral assay may include an evaluation of mass spectral ion ratios. The evaluation shall be sufficient to ensure the proper identification, detection, and, where appropriate, quantitation of the samples in a batch.

9.3 Internal Standard Recovery

The laboratory shall have a procedure for evaluating the recovery of internal standard throughout the analytical run. All calibrators, controls and case specimens in the batch shall be evaluated based on the laboratory's internal standard recovery criteria. At a minimum, the laboratories shall ensure the following.

- a) For qualitative assays, laboratories shall have minimum recovery requirements for the internal standard based on assay performance.
- b) For quantitative assays, laboratories shall establish minimum and maximum recovery criteria for the internal standard.

EXAMPLE The internal standard minimum and maximum recovery range may be based on comparison to the average of calibrators in an analytical run.

- c) If the internal standard recovery is outside the defined acceptability criteria, the laboratory shall take action to determine if the performance of the assay is negatively impacted for one or all samples within the analytical run.

9.4 Carryover

Unless fully characterized during the validation of the assay, carryover shall be evaluated, as appropriate. Results from negative controls or solvent blanks shall not contain the analyte of interest at a response meeting all reporting criteria (e.g., retention time, ion ratios, etc.).

If multiple consecutive negative controls, solvent blanks, or extracted blank matrix samples are used to evaluate carryover, the one immediately preceding the case sample, calibrator, or other quality controls shall meet the above acceptance criteria.

9.5 Dilutions

Should a laboratory use dilution techniques on case samples in quantitative assays, the concentration measured in the diluted sample shall be within the linear range of the calibration curve (i.e., between the lowest and highest calibrators). A dilution factor shall then be applied to calculate the concentration. Such calculations shall be checked by the use of a review procedure to verify appropriate math. Additionally, dilution controls may be included in each batch where case samples are diluted.

10 Method of Standard Addition

10.1 General

The method of standard addition (MSA) involves adding different known amounts of a target analyte to fixed amounts of a sample to compensate for a sample matrix effect that enhances or depresses the analyte signal. If used, the laboratory shall have a procedure describing how and when it will implement the MSA including the following.

10.2 MSA Calibration

MSA shall be performed using the unfortified case sample matrix (zero level) plus, at a minimum, three concentration levels of standard additions (three concentrations of fortified sample matrix).

The standards used should be approximately 50%, 100%, and 150% of the expected sample concentration.

NOTE Additional concentration levels may be added based upon the estimated sample concentration; however, it is critical that there is sufficient separation between the concentration of the unfortified sample and the concentration of the standard additions.

A simple linear regression analysis shall be applied to the minimum of four sample levels.

10.3 Dropping a Calibrator in MSA

See section 7.3 for requirements associated with dropping calibrators.

10.4 MSA Calibration Acceptance Criteria

The criteria for curve fit acceptance in MSA will be a coefficient of determination (r^2 value) of 0.990 or better.

10.5 MSA Quality Control

The calibration solution used to spike the samples and create the calibration curve shall be verified prior to or during use. The MSA is a self-controlling process; therefore, when it is employed, additional quality control samples are not required beyond verification of the calibration solution.

NOTE To verify the calibration solution, the laboratory may consider fortifying the sample matrix with a separate lot of drug solution as a quality check or, alternatively, the MSA may be conducted along with a regular batch of samples on the same day.

11 Quality Control Review

The laboratory shall have a procedure for the review of control results using the predefined performance criteria. This review shall be performed and documented by qualified laboratory personnel prior to the release of any results.

12 Monitoring of Quality Control Data

The laboratory shall have a procedure for monitoring and recording quality control data. At a minimum monitoring shall include the following elements.

- a) All control results shall be recorded and monitored, including those that fail.
- b) Where applicable, monitoring will include the statistical evaluation of data using a calculator, spreadsheets, or commercially-available products.
- c) Where applicable, control data shall be plotted in a manner that will allow for the detection and evaluation of trends.
- d) Any identified trends that could negatively impact the validity of the results shall be formally addressed and documented.

Annex A
(informative)

Bibliography

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