Quality Control for Toxicology Examinations

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1 SCOPE

This document serves as a general guideline for qualitative and quantitative toxicological analysis in the Chemistry Unit of the FBI Laboratory. These general criteria may be superseded by specific criteria for an individual technical procedure. This document applies to members of the Chemistry Unit authorized to perform and evaluate examinations in the toxicology discipline.

2 REFERENCES

- ANSI/ASB Standard 036, 1st ed., Standard Practices for Method Validation in Forensic Toxicology
- ANSI/ASB Standard 017, 1st ed., Standard Practices for Measurement Traceability in Forensic Toxicology
- ANSI/ASB Standard 054, 1st ed., Standard for a Quality Control Program in Toxicology Laboratories

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.

3.4 Calibrator

Measurement standard used in calibration.

3.5 Control

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

3.5.1 Dilution control

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

3.5.2 Matrix-matched control

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

3.5.3 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.

3.5.4 Positive control

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

3.5.5 Process control

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

3.5.6 Retention Time/Mass Spec Standard (RT/MS)

A standard that is used for retention time, mass spectrometry, and analyte response comparisons. It may or may not be in a matrix-matched to the items analyzed.

3.6 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.

3.7 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.

3.8 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.

3.9 Quality control materials

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

3.10 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.

3.10.1 Certified Reference Material (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.

3.10.2 Internal Standard

Reference material selected to monitor the performance of qualitative and quantitative procedures. Where possible, an internal standard with chemical and physical properties as similar to the analyte as possible should be used for chromatography-based quantitative assays. If the analyte is derivatized, the internal standard must form an analogous derivative.

3.11 Solvent blank

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

3.12 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

New analytical methods are validated to meet the requirements of LAB-100, CHEM-100 and 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

4.2.1 Toxicology Technical Leader (TL)

Designated to be ultimately responsible for the Quality Control Program.

4.2.2 Validation and Quality Control Manager (VQCM)

Designated to share responsibility for day-to-day management of quality control and validation operations. Quality control responsibilities include:

- Monitoring inventory, target concentration, and performance of control material
- Maintaining records in binders, spreadsheets, and databases

The Quality Control Program defines the following elements:

A. Pre-defined requirements for quality control (QC) materials, including:
    1. identification of the type, purity, and source
    2. content and concentration

3. matrix requirements
4. instructions for preparation and storage

These elements are listed in individual procedures as well as in CHEM-100.

B. Defined calibration models for all quantitative procedures, frequency of calibration, and criteria for the acceptance or rejection of calibrations.

General criteria for calibration acceptance and rejection are located in Section 7. Each quantitative procedure contains the calibration model parameters and specific criteria, as required.

C. Control requirements for all procedures, to include frequency of use and criteria for acceptance or rejection of control results.

General criteria for control acceptance and rejection are located in Section 8. Each procedure contains specific criteria, as required.

D. Defined process for review of control results prior to the release of case results.

General criteria for review of controls are located in Section 8.3. Each procedure contains specific criteria, as required.

E. Defined process to monitor and evaluate control results.

General criteria for review of controls are located in Section 11. Each procedure contains specific criteria, as required.

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.

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.

5.2.2 Reference Materials

The physical and chemical properties shall be determined for reference materials used to prepare calibrators and/or controls (see Section 5.3). When used for quantitative measurements, the purity of non-certified reference materials used to prepare controls 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.

Chemistry Unit personnel 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

See CHEM-100 for additional requirements related to 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* should be followed. If the reference material is accompanied by an acceptable certificate of analysis, as defined in the ANSI/ASB Standard 017, the material may be used for qualitative and/or quantitative analyses without further verification. Certificates of analysis shall be maintained in the *Chemicals and Materials System* (CAMS) database or available from the manufacturer's website.

5.3.2 Non-Certified Reference Materials

See CHEM-100 and ANSI/ASB 054 for additional guidance.

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

See CHEM-100 and ANSI/ASB 054 for additional guidance.

5.4 Storage and Expiration of Reference Materials

When provided, the manufacturer recommended storage conditions for reference materials shall be followed. If storage conditions are not explicitly stated, the appropriate storage shall be 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), that date shall be adhered to unless the manufacturer has provided written extension. In such instances, the new expiration date can be used.

If the manufacturer does not provide an expiration date, an expiration date shall be assigned 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. Matrix-matched controls are preferable. For the purposes of identification and/or estimating sample concentrations, unmatched controls or standards may 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 that meet the requirements to be deemed certified reference materials may be used without further verification. 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.

When possible, different pipettes are used to prepare calibrators and controls in-house. Additionally, different analysts will prepare control and calibrator working stocks when possible.

When developing new quantitative methods, the following should be considered for in-house prepared calibrators and controls:

A. Aliquots for day of use preparation should be normalized when possible.
B. Prepared control and calibrator samples or tubes (where matrix is added) may be made and frozen until use.
C. Sample automation may be used.

D. Positive displacement pipettes may be used to avoid solvent delivery and/or evaporation issues where possible.
E. New calibrator or control sets should be verified against existing ones when possible.

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 appropriate storage shall be established 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 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, that date shall be adhered to unless the continued stability of the material is confirmed 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, an expiration date shall be assigned 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 certified reference material), the expiration date shall be assigned 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 following elements of calibrator and quality control materials shall be documented:

A. content and concentration
B. date received, opened, prepared, and/or reconstituted

C. expiration date
D. identification of the analyst who prepared the material
E. lot number or unique identifier
F. name or identification of the material
G. storage requirements

At a minimum, the storage container shall be labeled with the name/identification, lot number/other unique identifier, and expiration date. The above elements are recorded in binders, CAMS, or provided by the manufacturer.

7 CALIBRATION REQUIREMENTS FOR QUANTITATIVE PROCEDURES

7.1 General

The calibration model and range for an assay shall be specified in technical procedures 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, a minimum of four calibrators are required and the curve fit shall not be forced through the origin. For quadratic regression calibration models, a minimum of six calibrators are required 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 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*. Similarly, specific methods may recommend or require that a zero calibrator be included as a calibration point (e.g., ICP-MS analyses); those methods shall specify the calibration model to be used.

7.2.1 Calibration Model Weighting

Weighting factors may include $1/x^0$ (no weighting), $1/x^{0.5}$, $1/x$, and $1/x^2$.

7.3 Dropping a Calibrator

In order to drop a calibrator from a calibration curve, the following must be met:

A. A calibrator shall not be dropped solely to improve curve fit or control compliance. Acceptable reasons for dropping a calibrator may include poor extraction of a specific calibrator sample, a bad injection of a calibrator, failure to add internal standard, etc.

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. The data supporting that decision shall be retained with the batch and within case notes by recording within the Toxicology Information Center database (ToxIC).
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.
The practice of dropping a calibrator shall be tracked to determine frequency, type of calibrator (concentration and analyte), and analyst. Quantitative batch review results are tracked within ToxIC.

7.4 Historical Calibrations

Acceptable use of historical calibrations for a quantitative assay shall be demonstrated through method validation before use. Afterwards, historical calibrations shall be checked with controls processed contemporaneously with the case samples. The interval from calibration to analysis may not exceed the interval established through stability studies.

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

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 calculated from the established calibration curve shall be within ± 20% of the target calibrator's concentration.

In the event that a calibration is not acceptable, the reason for the failure shall be documented in case notes and ToxIC, 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.

7.5.1 Calibration Curve Quick Reference

A.	Peak integrations across a batch should be consistently performed.
B.	Automated software integration is preferred.
C.	The use of manual integrations will be indicated.
D.	Coefficient of determination: ≥ 0.990.
E.	Calibrator response will bracket case specimen response.
F.	Calibrators calculated concentration: ± 20% of target.

G. Curve fit will not be forced through zero or include zero as a calibration point.
H. Linear regression models will have no fewer than four calibration points.
I. Quadratic regression models will have no fewer than six calibration points.
J. The regression model and weighting used may not deviate from those established during method validation.

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 parameters for accepting or rejecting controls are defined (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.
F. Solvent blanks, extracted blank matrix samples, or multiple negative controls may be analyzed as necessary to detect or prevent carryover into subsequent samples.

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 given batch may include 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

At a minimum, analytical runs involving non-targeted assays shall include the following:

A. One negative control.
B. One positive control containing representative analytes.
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 are defined for each procedure.
D. Include at least one positive control at or near the end of the batch.
E. Include a control mid-run if the batch contains 20 or more test samples.

F. Case samples shall be collectively bracketed by controls during an instrumental run sequence.

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 for target analyte(s). The positive control should challenge the detection limit of the assay for at least one analyte.
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 are defined in each procedure.
D. Case samples shall be collectively bracketed by controls during an instrumental run sequence.

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.
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 are defined in each procedure.
D. Case samples shall be collectively bracketed by controls during an instrumental run sequence.

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 (in duplicate). Ideally, this control 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 (in duplicate). Ideally, this control challenges the upper limit of quantitation 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. 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.
E. Case samples shall be collectively bracketed by positive controls during an instrumental run sequence.

F. A dilution control may be used to verify proper dilutions of case specimens. A dilution control can consist of a single replicate of a similarly diluted positive control.

8.3 Control Acceptance Criteria Based on Scope of Assay

8.3.1 General

Each control sample shall be evaluated against the predefined criteria documented in the applicable technical procedure(s). 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 within the batch and within case notes via recording in ToxIC. Acceptance criteria (as defined in a technical procedure) for the minimum number of controls must be met for all batches.

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. In the event that an analyte is detected in a negative control, no reporting for that analyte in that batch may be performed and the instance will be documented in the batch via recording in ToxIC.

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

The requirements for the analyte of interest to be considered a positive result are generally described for each procedure. These requirements may include relevant parameters such as color change, 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.

Generally, each procedure defines requirements for the analyte of interest to be considered a positive result. These requirements should include relevant parameters such as color change, 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 (mean ± 2SD) and control limits (mean ± 3SD) 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 failed but will be included in historical control tracking unless a reason for the failure (such as double spiking of the internal standard) is evident and demonstrates that the procedure was not followed properly. If the measured values are outside of the warning limits, but within the control limits, the control shall be considered 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 or predetermined requirements for bias (e.g., ± 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 failed but will be included in historical control tracking unless a reason for the failure (such as double spiking of the internal standard) is evident and demonstrates that the procedure was not followed properly.
    NOTE: Stricter control limits may be expected for assays that require better accuracy such as blood alcohol measurements for legal proceedings (e.g., ± 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, other parameters that are important to the performance of a batch run of samples shall be evaluated.

9.2 Instrument Performance

The performance of calibrators, controls, and case samples throughout an analytical run are evaluated during the batch review. This may include:

- An evaluation of retention times and peak shape
- An evaluation of mass spectral ion ratios
- An evaluation of instrument response

9.3 Internal Standard Recovery

All calibrators, controls, and case specimens in the batch shall be evaluated. Where internal standard recovery is substantially reduced, it may or may not indicate qualitative identification and/or quantitative inaccuracy depending on the appropriateness of the internal standard.

9.3.1 Qualitative Assays

A. All analytes used as internal standards should be recovered in the specimens analyzed.
B. Non-recovery may result in follow up actions that depend upon the individual assay, sample(s) analyzed, and case scenario.
C. If the internal standard recovery is outside acceptability criteria (where defined), action shall be taken to determine if the performance of the assay is negatively impacted for one or all samples within the analytical run. Actions will vary by procedure.
D. At least one internal standard shall be included in qualitative chromatographic assays.

9.3.2 Quantitative Assays:

A. All analytes used as internal standards should be recovered in the specimens analyzed.
B. Non-recovery may result in follow up actions that depend upon the individual assay, sample(s) analyzed, and case scenario.
C. At least one internal standard shall be included in quantitative chromatographic assays.
D. Calculate the average internal standard response for calibrators and controls.
E. Generate control limits by calculating 50% and 200% of the calculated average response.
F. Evaluate case samples against the control limits.
G. Perform any necessary actions (Section 10.3.1).

Individual method validation data may suggest that values other than the 50-200% range are more appropriate to evaluate a particular assay.

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).

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

If dilution techniques are used 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 technically reviewed to verify appropriate math, unless the calculation is performed by commercial or pre-approved software. Additionally, dilution controls may be included in each batch where case samples are diluted.

10 QUALITY CONTROL REVIEW

Control results are reviewed using predefined performance criteria. This review shall be performed and documented by authorized FBI Laboratory personnel prior to the release of any results.

10.1 Evaluation of Batches

Batches are reviewed prior to distribution of the data to case files. This review is performed by an authorized toxicology forensic examiner who did not perform the analysis. The review will include evaluation of records for accuracy and completeness, as well as review of relevant decision criteria. The review is recorded in batch specific files and ToxIC.

10.1.1 Partial or Incomplete Analysis

Occasionally an analytical batch may fail to complete the entire run, or an individual sample analysis may be incomplete or truncated. Typically, an analytical batch will be restarted at the beginning or part way through the batch to allow for complete data collection. Common causes for these scenarios include instrument communication error, data acquisition error, or power interruption. Assessment of the impact can vary by specific instance. Depending upon the affected data file(s) and procedure used, a batch or portions of a batch may or may not be useful for casework. Because of the variety of use-cases for a given batch, such a scenario is not necessarily a nonconformity but will be addressed by correcting the issue when possible.

The occurrence of partial/incomplete runs and any associated deviations will be recorded in batch records and/or ToxIC.

10.2 Retention of Batch Records

Batch specific records will be either retained in the individual case records, archived in toxicology files or stored within ToxIC and associated data files. This will vary depending upon the individual technical procedure. While data may not be discarded per se, in some instances a summary may be a more concise format to document particular types of quality control situations.

10.2.1 Minimum set of batch specific records retained for archive

A. A batch summary/cover sheet
B. Procedure notes

C. Instrument sequence
D. Calibrator and control (including process and dilution control) data, as applicable

10.2.2 Minimum set of batch specific records retained in case records

A. A copy of a batch summary/cover sheet
B. Procedure notes
C. Case sample specific data
D. Negative control (and preceding blank, as appropriate)
E. Positive control or calibrator (as appropriate)

10.2.3 Minimum data retained upon quality control failure or Incomplete Run

A. A quantitation summary (the entire calibration run may not be required)
B. A qualitative summary (for example, internal standard recovery reports)
C. Sequence log
D. Method information
E. Procedure notes
F. Relevant notes, to include reagent and material used (output from CAMS, etc.)
G. Documentation describing the nature of the quality control failure or incomplete acquisition
H. Documentation describing the affected data files and/or case samples

Sufficient data should be retained to aid in subsequent corrective actions should that be required.

10.2.4 Recording Rejected Data

The following elements will be recorded in batch/case records and/or ToxIC:

A. The reason for the rejection of data
B. Individual rejecting the data
C. Date of data rejection

Additionally, any retained data not used shall be marked to indicate as such. Methods of marking may include but are not limited to:

A. Hand-written notes
B. Electronically applied to a bound electronic file
C. Applied through watermark, header, or footer

10.3 Planned Action for Quality Control Failures

Depending upon the individual exam, responses to the different types of quality control failures that may occur will vary. In general, the following guidance lists action steps that may be appropriate:

10.3.1 Poor Recovery of Internal Standards

A. Reanalysis of the affected samples. Prior instrument maintenance may be required.
B. Re-extraction/reprocessing of the affected samples. Reagents or internal solution standards may need to be remade.
C. Not reporting qualitative results for the affected assay(s).
D. Not reporting quantitative results for the affected assay(s).
E. Depending upon frequency, method revalidation or retraining may be required.
F. Consideration of post-mortem sample degradation effects.

10.3.2 Poor Recovery of Positive Controls

A. Reanalysis of the affected samples. Prior instrument maintenance may be required.
B. Re-extraction/reprocessing of the affected samples. Reagents or positive control standards may need to be remade.
C. Not reporting qualitative results for the affected assay(s).
D. Not reporting quantitative results for the affected assay(s).
E. Depending upon frequency, method revalidation or retraining may be required.

10.3.3 Quantitative Control Values Out of Control

A. Reanalysis of the affected samples. Prior instrument maintenance may be required.
B. Re-extraction/reprocessing of the affected samples. Reagents, calibration, and/or control solutions may need to be remade.
C. Not reporting quantitative results for the affected assay(s).
D. Depending upon frequency, method revalidation or retraining may be required.

10.3.4 Quantitative Control Values within Warning Limits

A. Reagents, calibration, and/or control solutions may need to be remade.
B. Depending upon frequency, method revalidation or retraining may be required.
C. Investigation into the cause of the trend may be required.

10.3.5 Retention Time Shifts

For chromatographic methods, retention time shifts may be the result of analytical column degradation, or potentially other errors. Actions may include:

A. Verification of gas flows, column integrity, or mobile phase preparation
B. Instrument maintenance or column conditioning
C. Reanalysis of quality control and/or case samples

10.3.6 Instrument or Assay Performance Shifts

Changes in instrument or assay performance (e.g., response, mass accuracy) may adversely affect detection limits or identification parameters. Actions may include:

A. Verification of detector performance
B. Mass calibration verification
C. Instrument maintenance
D. Remaking of reagents or calibration/control solutions
E. Reanalysis of quality control and/or case samples

11 MONITORING OF QUANTITATIVE QUALITY CONTROL DATA

At a minimum, monitoring shall include the following elements.

A. All quantitative control results shall be evaluated.
    1. Analysts provide the control results to the VQCM for recording and monitoring.
    2. The VQCM can determine if a failed control result should be excluded from the data set due to a gross laboratory error (e.g., double-spiking a control with internal standard).
B. Where applicable, monitoring will include the statistical evaluation of data using a calculator, spreadsheets, or commercially available software.
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 by the TL or VQCM.
E. Quantitative control results are reviewed every three months by the TL or VQCM. The record of this review is recorded in a controlled Excel sheet used to record control values and evaluate trends.

12 PERFORMING THE ANALYSIS

12.1 Pipette and Balance Performance Checks for Quantitative Batches

Performance checks will be performed on the day of use for balances and pipettes that will be used for a significant measurement (i.e., a measurement that requires an estimation of measurement uncertainty). Pipettes are checked gravimetrically using aliquots of deionized water and a performance checked analytical balance. Procedures for daily performance checking of balances can be found in CHEM-100.

Pipette Type	Volumes	Material	Passing Criteria
Fixed	Duplicate	Deionized water	±2% of target
Adjustable	Duplicate, ends of pipette range, ≥ 15µL	Deionized water	±2% of full scale volume

Pipettor/Dilutor	Depending upon specific use-case, may be evaluated prior to use or evaluated as part of batch review as per individual technical procedure. (e.g., treat as fixed pipette and/or evaluate total batch precision)

If a pipette does not initially pass, the performance check may be repeated to account for any gross errors (e.g., loose tip). A pipette check may be repeated up to three times before the pipette will be taken out of service. A pipette that does not meet criteria is not used for quantitative or qualitative casework.

Pipettes not meeting quantitative criteria are marked appropriately.

Malfunctioning pipettes (e.g., due to breakage) are marked appropriately and removed from work until repaired.

Results will be recorded in a database on the day of the check, and the identifier for the performance checked pipette(s) will be recorded in casework/batch records.

Pipettes are calibrated at least once annually by an accredited calibration laboratory. Pipettes will be performance tested upon return to the FBI Laboratory from either repair or calibration services.

12.2 Calibration, Control and Case Materials

Calibration, control and case materials will be allowed to come to ambient temperature before aliquoting. This may take an hour for up to 10 mLs of solvent stored refrigerated, or multiple hours for items stored frozen.

12.3 Accessioning of Case Specimens

The accessioning of case specimens shall:

A. Ensure the correct sample is accessioned through the use of unique identifiers.
B. Ensure that a representative portion of the case specimen is obtained.
C. Use a minimum of one aliquot for qualitative analysis.
D. In general, use two aliquots for quantitative analysis. One aliquot may be used in limited sample situations or other case dependent scenarios.
E. Use dilutions of case samples as needed to provide on-curve quantitative measurements.
F. Matrix match the material used for dilutions where reasonable and appropriate. Information in technical procedures may supersede this guidance.
G. Use appropriate sample:diluent ratios for the selected exam(s) as specified in individual technical procedures.

12.4 Estimating the Level of Analyte in the Case Specimen

An estimate of the amount of analyte may be performed by analysis of the data generated and/or additional comparisons to standards or controls. This estimate can be useful in

determining whether additional quantitative analysis is warranted. The below table provides guidance as to which techniques or combinations of techniques is preferred.

Parameter	**Less Preferred <----------> Most Preferred**		
Matrix	None (sample response only)	Solvent standard	Matrix-matched control
Internal Standard	None	Present	Present, deuterated analog
Points of Comparison	1 point comparison	2-3 point comparison (semi-quantitative)	Full calibration
Response	Color change	Instrument response	Instrument response 'within range' of sample response

The choice of techniques used may vary by individual technical procedure or case scenario. Estimates derived from non-quantitative analysis are inherently limited. Calculations used to perform estimates will be a part of the batch or case record and will be checked as part of the technical review process unless they are performed by a software package.

12.5 Labeling of Consumables

Where appropriate, analysts are encouraged to reduce the possibility of error by labeling all consumables, including tubes, extraction/filtration media, autosampler vials, etc.

13 REVISION HISTORY

Revision	Issued	Changes
14	12/08/2023	1 – Updated wording to “document” rather than “procedure”. 2 – Added ABFT Checklist and clarified edition of ASB standards. 3 – Removed definitions for calibration (and sub-definitions), LOD, LOI and MSA. 4.1 – Clarified wording. 4.2.2.C – Fixed a typo. 5.2.4 and 5.4 - Clarified wording. Removed much of Section 5.3 and referred to CHEM-100 and ANSI/ASB 054. 5.5 – Clarified thermometer locations and monitoring schedule. 6.1 – Clarified preference for different pipettes and/or different staff to make calibrator and controls. Added considerations for preparation of in house calibrators and controls. 7.5.1.J, 8.2.1, 8.2.2 and 8.2.3 - Clarified wording. 8.3.1 – Clarified wording. 8.3.1 and 8.3.2 – Provided guidance for documentation of batch treatment when control(s) fail. 8.3.3.C – Clarified what to do when controls fail, and removed “rejected”. Removed Section 10 (Method of Standard Addition). Clarified new Section 10.1.1 New 11A – specified *quantitative* controls. Removed old Section 13 (Reporting Results). New 12.1 – Required recording on pipette check on day of check and that pipettes will be performance tested upon return from repair or calibration and updated wording throughout. New 12.2 – Added requirement for materials to come to room temperature before aliquoting. New 12.3.G – Clarified that sample:diluent ratios will be included in individual technical procedures.
15	03/15/2024	Minor grammatical edits and removed reference to ABFT throughout document. 5.5 – Removed section on temperature monitoring 8.3.1, 8.3.2, 10.1.1 - clarified action steps for control failures or partial or incomplete analysis