Sections in This Document

1. Purpose

This procedure describes the monitoring activities in a laboratory quality control (QC) program to ensure the quality of test results.

2. Scope

This procedure is applicable to Office of Regulatory Science (ORS) laboratories performing regulatory testing.

3. Responsibility

A. Laboratory Management

1. Establish a laboratory quality control program.
2. Ensure that quality control is performed and review quality control data for acceptability and trends.

B. Analysts

1. Conduct quality control analyses in accordance with the laboratory quality control program.

C. Quality System Managers

1. Monitor quality control data for non-conformances and trends.

4. Background

None

5. References

A. Taylor, J. K. (1993). Handbook for SRM users. Gaithersburg, MD: National Institute of Standards and Technology

B. ISO/IEC 17025:2017, General requirements for the competence of testing and calibration laboratories, Section 7.7

C. AOAC International Guidelines for Laboratories Performing Microbiological and Chemical Analysis of Food, Dietary Supplements, and Pharmaceuticals. An Aid to Interpretation of ISO/IEC 17025:2017; August 2018.

6. Procedure

6.1. Laboratory Quality Control Program

Laboratory quality control (QC) is an essential aspect of ensuring that data released is fit for the purpose determined by the quality objectives (i.e. accuracy and precision). The dual foundations of the laboratory quality control program are its internal quality control, composed of day-to-day and sample-set to sample-set monitoring of analytical performance, and its external QC, based on the laboratory's performance in proficiency testing programs.

When properly executed, quality control samples monitor the various aspects of data quality on a routine basis. In instances where QC falls outside acceptable limits, the sample data produced may be questionable and, after investigation, a determination is made as to its validity. With professional experience and a common-sense approach quality control is the principal recourse available for ensuring that only defensible data is released.

6.1.1. Quality Control Materials

A quality control material may include, but is not limited to, Certified Reference Materials (CRMs), Reference Materials (RMs), replicate analysis, positive/negative control samples, laboratory control samples (LCS), blanks, and matrix spikes.

In the absence of any CRM or RM, the laboratory shall do its best to obtain a material with some limited consensus of accuracy (e.g. by subjecting material to multiple methods or analyses in-house, sharing material with another laboratory to determine an average result, etc.)

The suitability of the quality control material used shall be justified by the laboratory.

For enumeration assays, a quantified quality control material shall be used.

When testing for pathogens or select agents, a quality control material that contains a surrogate analyte may be used.

6.1.2. Internal Quality Control

QC is used to measure accuracy, precision, contamination, and matrix effects. QC material shall be used with each batch of samples analyzed. Generally, QCs are run per batch or set of samples at a frequency of 5% or one every twenty samples. If the laboratory is not able to meet this guideline, the batch must be defined and justified in the laboratory's documents. The batch shall begin and end with QC material.

The laboratory determines, where feasible, the accuracy and precision of all analyses performed.

Internal QC is run with each analytical batch to verify continuous system suitability specifications established are met, as well as demonstrates accuracy and precision or other parameters determined for the method type.

Method precision is periodically evaluated by the laboratory using the QC schemes described below.

6.1.2.1. Chemistry QC schemes

A. QC schemes required for chemistry consist of:

1. Meet system suitability requirements: system suitability is intended to determine whether the 'system' including instruments, analysts, etc. is capable of performing a particular process, test, or assay.
2. Blanks, either matrix or reagent, to determine and measure contamination and interferences - The results of blanks should be compared with the sample analyzed per analysis to determine whether the source of any analyte present is due to sample or laboratory contamination, interferences, the sample matrix, or the actual analyte in the samples. Blanks should be below the method detection limit where possible. Blank results are evaluated and corrected where possible. If blank results are consistently above the method detection level (MDL) established, the MDL should be re-established. High blank results may also indicate contamination either from the solvent, laboratory equipment or laboratory environment.
3. Matrix spikes and/or reagent spike- Matrix spikes measure the effects the sample matrix may have on the analytical method, usually the analyte recovery. Method accuracy is recorded and controlled based on the percent recovery of matrix spikes for quantitative analysis and the positive response of the analyte for qualitative analysis.
4. Duplicate samples or matrix spike duplicates - Duplicate sample or matrix spike duplicates measure precision of the analytical process. Duplicate analysis usually involves a replicate sample, sub-sampled in the laboratory, but for some methods it is in the form of a matrix spike duplicate. Method precision is recorded and controlled based on the relative percent difference (RPD) or the positive response for qualitative analysis.
5. Quality control samples - Quality control samples (QCS) measure method performance. The matrix of the QCS should match the matrix of the samples being analyzed and should pass through the entire sample preparation process. The QCS, therefore, measures both the sample preparation process and the analytical process.
6. Standards - Calibration check standards referred to as initial calibration verification (ICV) and continuing calibration verification (CCV) are used to determine whether an analytical procedure is in control and stays within control. They are used to detect analytical method errors from procedural or operator errors or contamination from laboratory sources.
7. Accuracy and precision control charts

B. The chemistry laboratories shall define the acceptance criteria for each test method for the following items (when included in the test method): system suitability, calibration curves, calibration checks, ICV, CCV, QCS, blanks, matrix spikes, and duplicates.

C. The accuracy expressed as percent recovery should be 80% to 120% unless otherwise specified, i.e. by in-house statistical analysis.

D. The precision expressed as relative percent difference (RPD) should be < 20% unless otherwise specified, i.e. in-house statistical analysis. In procedures where multiple determinations or subs are analyzed, one can be chosen as the duplicate quality control sample.

E. Analyze calibration check standards/samples. Each Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV) should have a percent recovery of 90% to 110% unless otherwise specified, i.e. by in-house statistical analysis.

F. The chemistry laboratories shall have a procedure or policy that provides guidance and/or criteria for the reprocessing and/or reintegrating of analytical data.

G. Drug Chemistry must meet USP system suitability requirements when performing a USP method. Requirements are stated in USP monographs.

6.1.2.2. Microbiology QC Schemes

A. QC schemes required for microbiology include running QC controls concurrently with each sample batch or set. They are:

1. Positive and negative culture controls - positive and negative controls give correct response,
2. System controls
3. Collector controls,
4. Applicable kit controls (positive and negative),
5. Media quality - the culture controls additionally verify the acceptability of the media,
6. Un-inoculated media – un-inoculated media control reveals no visible growth, and
7. Accuracy and precision control charts.

B. Sample Duplicates or Spiked Samples– For quantitative methods, sample duplicates (for samples with expected values > 25 cfu/g) or spiked samples will be run for each batch of samples.

NOTE: The use of microbiological controls for sample analysis of virology samples and pharmaceutical analysis samples should follow virology SOPs, designated USP chapters and ORA-LAB.001 (Microbiological Controls for Sample Analysis) or SOP-000288 (Microbiological Controls for Medical Product Sample Analysis)

C. Media lot testing for suitability and acceptance for use must occur prior to sample testing (i.e. no concurrent studies).

6.1.2.3. QC Schemes for Other Testing

A. For other testing (e.g. radionuclides, microscopy, metrology, etc.) the required QC schemes are defined by the laboratory. The following performance parameters should be included, if applicable:

1. Meet system suitability requirements: System suitability is intended to determine whether the 'system,' including instruments, analysts, etc., is capable of performing a particular process or test.
2. Accuracy – a known or reference sample,
3. Precision – a duplicate sample,
4. Blank – a system control, reagent blank to check for environmental or laboratory contamination, and
5. If applicable, calibration check standards analyzed periodically in the analytical batch for quantitative analyses.

6.1.3. External Quality Control Program

A. Participation in proficiency testing is an important means of quality control and assessing laboratory performance. Accrediting agencies require laboratories to participate in programs relevant to the laboratory's scope of testing.

B. Each laboratory maintains a documented proficiency testing (PT) plan that includes:

1. How the laboratory will cover its entire Biological and Chemical scopes annually.

   Proficiency testing for all Chemical scope methods may not be necessary if the laboratory can provide evidence, showing similarity

of tests on the Chemical scope. The justification showing the similarities must be included on the PT plan.

2. The scheduled PT for 4 years.

C. When no PT or interlaboratory comparison is available, the laboratory shall develop and justify an alternative plan for monitoring data.

D. The ORS laboratories participate in the AOAC - International Laboratory Proficiency Testing Program (LPTP), the FDA National Check Sample Program (NCSP) for foods, medical products, colors, metals, nutrition, sulfites, and filth, and other available proficiency tests identified by the laboratory or program. The Office of Regulatory Science (ORS) has the principal responsibility for managing the NCSP.

E. Proficiency testing and interlaboratory comparisons are used as a tool to assist in the identification of laboratory problems that may exist and that have eluded the internal quality control program. They are not intended to represent individuals in the laboratory, unless this represents the normal mode of operation in which only one person is involved in the analysis. It is essential that proficiency testing samples are treated as routine samples to the extent possible.

F. Analyses of proficiency samples should not be repeated, unless it is necessary to repeat the entire procedure or the data on those specific samples exceed the method's linearity.

G. Proficiency testing is quality control for method analytical data. It is not necessary to perform a check sample where normally done as a Program requirement.

H. Samples should be run in duplicate only in those procedures where samples are normally analyzed in duplicate.

I. Proficiency testing shall be rotated among qualified analysts

J. Management review all PT data and ensures proficiency and/or interlaboratory comparison data are submitted to the issuing authority by the date(s) defined when issued. Copies of all proficiency studies are retained in the laboratory. The final reports from the issuing authority are reviewed by the testing area management and the Quality System Manager upon receipt. All unacceptable results are investigated in accordance with ORA-LAB.4.9 Control of Nonconforming Work.

K. Most external PT providers issue acceptability limits and criteria; when issued, the laboratory shall use that criteria. If the PT provider does not issue acceptability criteria or the laboratory is performing proficiency

testing by alternative means, then they shall have procedures that define the acceptability of the results.

6.1.4. Other Quality Control Monitoring Activities

Other QC procedures that may be used include:

A. Replicate testing - Replicate testing may be performed on samples which are found to be violative. The original sample results are verified by using an alternative method or by rechecking results by the same method. A violative chemistry result may be verified by a second instrument, another method, a second analyst or repeated by the same analyst. A violative microbiology result by a rapid screening method is verified by a culture method.

B. Retesting of retained items - Retained samples can be re-introduced into the workload as regular samples in order to assess laboratory performance.

C. Correlation - Checking for correlation means evaluating the interrelated characteristics of the sample. By comparing results from different analyses on the same test item, one checks for reasonableness (i.e. Does the data make sense or correspond as anticipated?). Certain characteristics within the sample will maintain an analogous relationship to one another with regard to the type of test being performed. If one characteristic is dependent on or at all indicative of another characteristic, they should be compared for consistency. The designated reviewer should be able to anticipate and recognize an analogous relationship with different characteristics of the same sample. Any deviation such as the absence of expected primary characteristics or the sudden appearance of previously unobserved characteristics of the sample, signals the probability of error.

6.1.5. Evaluation of Quality Control Data

All worksheets are submitted to the supervisor or designee for review. The QC range of each quality control data is evaluated for acceptability. Data that fall inside established control limits are judged to be acceptable, while data lying outside of the control interval are considered suspect. Control limits established by the laboratory are not to be exceeded except as resolved under a recorded corrective action process. This planned action includes the checking of results for calculation or transcription errors, preparation or use of new standards, recalibration of instrument, reanalysis of all samples with new controls or reagents, use of alternate system, and repeating analysis.

6.1.6. Quality Control Charts

The control chart is a graphical presentation of QC efficiency. If the procedure is in-control, the results will almost always be within established control limits.

Accuracy and precision control charts are used to determine if the measurement system process is in control and whether the results generated by the measurement system are acceptable. The control chart provides the tool for distinguishing the pattern of indeterminate (random) variation from the determinate (assignable cause) variation. This technique displays the test data from a process or method in a form which graphically compares the variability of all test results with the average or expected variability of small groups of data, in effect, a graphical analysis of variance.

The average or mean value is calculated and the spread (dispersion or range) is established. Common practice sets the warning limits at ± 2 standard deviations while control limits are set at ± 3 standard deviations on each side of the mean. Since the distribution of averages exhibits a normal form, the probability of results exceeding the control limits is readily calculated.

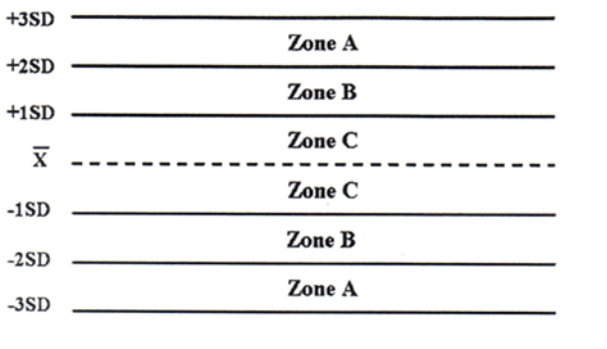

Figure 1: Example of a Control Chart

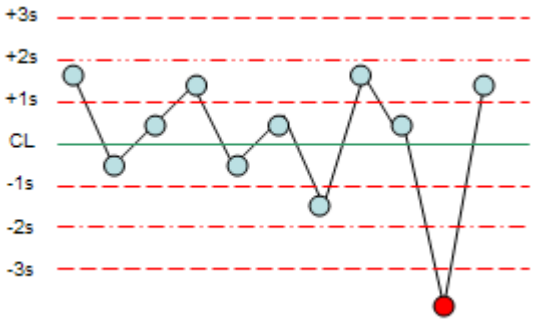

Figure 2: A Control Chart showing a 3SD outlier

Further, the chart will disclose trends and shifts from assignable causes which can be corrected. A trend will show a tendency or movement in a particular

direction. If a series of consecutive data points move steadily either upward or downward, a trend is indicated. If a series of consecutive data points fall either above or below the center line, a shift is indicated. When a trend or shift is detected, it is annotated as such on the chart and reviewed to the extent possible to identify if a significant concern is indicated. If the review indicates a significant concern, follow the nonconforming work procedure.

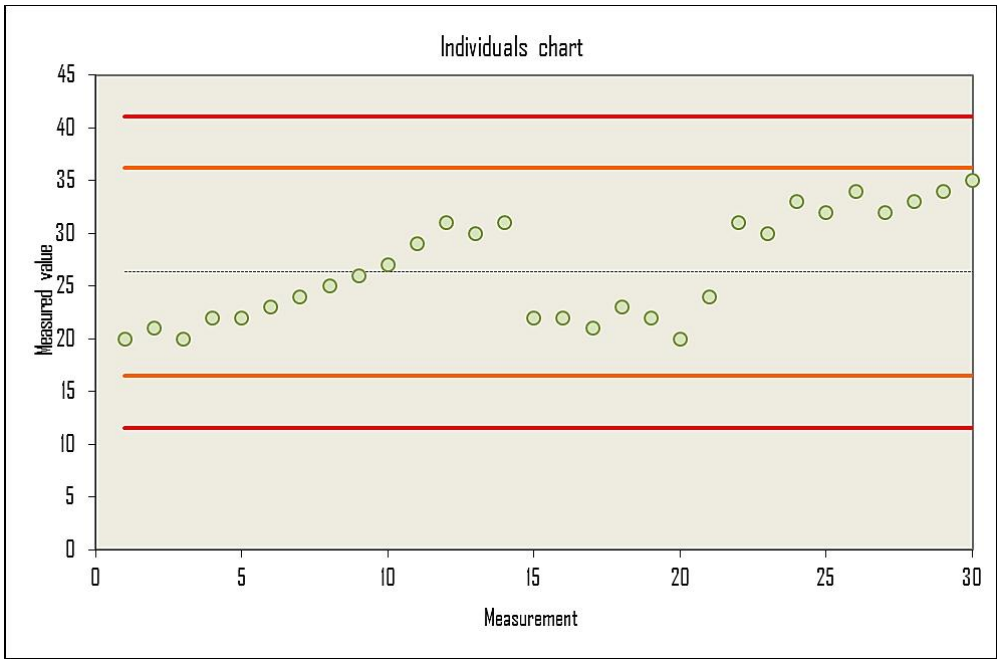

Figure 3: Control Chart showing a trend

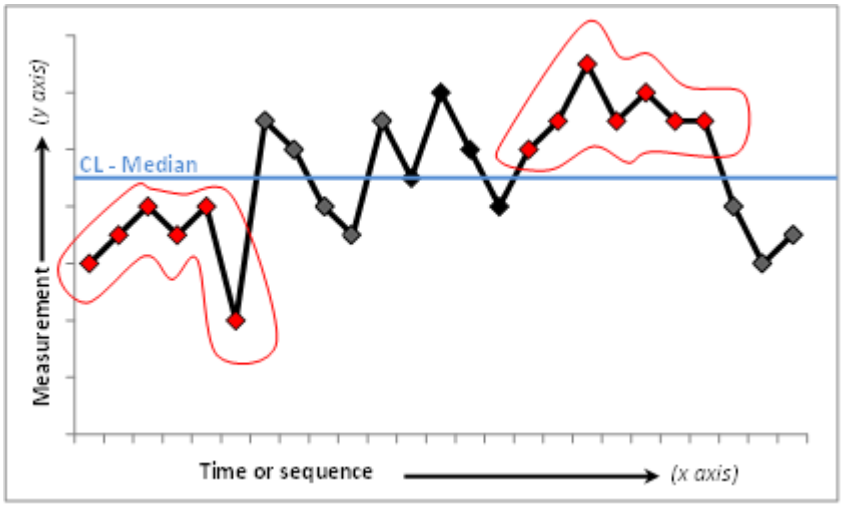

Figure 4: Control Chart showing a Shift

It is emphasized that there is absolutely no substitute for sound judgment based on an appreciation of the analytical system, the technique, the quality control materials utilized, and the analytical interpretation of the data generated by the procedure.

Accuracy charts (other names are Mean Chart, Levy (Levey)-Jennings or Shewhart Control Chart) - The data from a series of analytical tests are plotted with the vertical scale in units such as percent (percent recovery), and the horizontal scale in units of batch number or time. The mean and standard deviation is calculated on the data. Upper and lower control limits are established at the mean $\pm$ 3X the calculated standard deviation. Upper and lower warning limits are established at the mean $\pm$ 2X the calculated standard deviation.

Precision charts (other names are Range Chart or R-chart) – The data from duplicates are plotted with the vertical scale in units such as percent (RPD), and the horizontal scale in units of batch number or time. The mean and standard deviation is calculated on the data. The upper control limit is established at the mean X 3.27 and the upper warning limit is established at the mean X 2.51. Precision control charts do not have a lower warning and control limit.

6.1.7. Statistical Process Control

A. Statistical limits are determined at the 99% confidence interval. The evaluation of control limits is made after no less than seven to ten points are accumulated from different conditions, i.e. separate runs/batches, different days, different analysts, etc.

B. Accuracy is expressed as percent recovery of spiked samples.

C. Percent recovery is calculated as follows for spikes in solvent or standard spikes:

$$\% \text{ Recovery} = 100 \times \frac{X}{K}$$

where:

X = observed value

K = known value

D. Accuracy is calculated for spikes into natural matrices as follows:

$$\text{Recovery} = 100 \times \frac{X_s - X_u}{K}$$

where:

X_s = measured value for spiked sample

X_u = measured value for unspiked sample

K = known value of the spike in the sample

E. For accuracy, this interval is computed as:

Accuracy Interval = Mean Recovery ± 3 X S

where: S = Standard deviation for individual values

$$S = \sqrt{\frac{\sum (X_i - X)^2}{N-1}}$$

where:

X_i = value of individual measurement

X = arithmetic mean of the measurements

N = number of the measurements

F. For qualitative analysis, accuracy is expressed as positive (presence) or negative (absence).

G. Precision is expressed as relative standard deviation (RSD) or relative percent difference (RPD) of duplicate samples.

H. RSD is calculated from standard deviation and mean recovery, when the standard deviation is derived from multiple recovery results as follows:

$$RSD = CV = 100 \times \frac{\sigma}{X}$$

where:

RSD = relative standard deviation

CV = coefficient of variation

σ = standard deviation

X = arithmetic mean of the measurements

I. RPD is calculated when only two sample results are available as follows:

$$RPD = \frac{|R_1 - R_2|}{R} \times 100$$

where:

| R1 - R2 | = Absolute difference between the determinations

R = arithmetic mean of the two values

J. For precision based on RPD of duplicate samples this limit is computed as:

Upper Control Limit = 3.27 X RPD

where: RPD = mean relative percent difference

K. For qualitative analysis, precision is expressed as true and false positive rates; and true and false negative rates.

$$\% \text{ false positive} = \frac{\#\, false positives}{total \#\, known negatives} \times 100$$

$$\% \text{ false negative} = \frac{\#\, false negatives}{total \#\, known positives} \times 100$$

6.1.8. Treatment of Outliers and Trends

A. An outlier is a datum that is different from the main data pattern, and/or is not representative of the data set. Outliers are extreme cases of one variable, or a combination of variables, which have a strong influence on the calculation or statistics. The principal safeguards against obtaining or using an outlier are vigilance during all operations and visual inspection of data before performing statistical analyses. Each suspected outlier is evaluated and rejected if found to be unrepresentative, or to have a high probability of being unrepresentative. Rejection for a reason is referred to as rejection for assignable cause.

B. A plot outside of the control limits may be an indication of an assignable cause. If a quality control result falls above or below the control limits (3 SD) of the control chart, the value is investigated. The investigation is a planned action to correct the problem and to prevent the reporting of incorrect results. Sometimes the investigation will reveal a recording or computational mistake that can be revised to obtain the correct value. If the investigation reveals an assignable cause, i.e. deterioration of reagents, improperly prepared reagents, inadequate storage of reagents or standards, the analysis is repeated. When outliers are found, all analytical results for that analytical batch are inspected to ensure that erroneous results are not reported.

C. Quality control data outside of the control limits (3SD) rejected due to assignable cause remain in the permanent records of the laboratory, for

example, on QC charts. However, a datum so determined to be an outlier will be flagged as such and is excluded from the data set before statistical calculations are made. Control limits calculated from data sets containing outliers are not valid.

7. Glossary/ Definitions

A. Accuracy – Accuracy is the nearness of a measurement or the mean of a set of measurements to the true value. Accuracy is assessed in terms of percent recovery for quality control check samples and matrix spikes.

B. Analytical solution – An analytical solution is the sample in the form as introduced to an instrument. The analytical solution is the end result of the sample preparation, extraction, and digestion procedures.

C. Analytical spike – An analytical spike is a sample made by spiking an analytical solution after the sample preparation or digestion process.

D. Batch - A batch is the basic unit of measure by which the number of quality control samples needed is determined. The analytical batch is those samples, sample extracts, or sample digestates that are analyzed together with the same method sequence, the same lots of reagents, and manipulations common to each sample within the same time period or in continuous sequential time periods. The extraction batch is those samples extracted or digested using the same techniques. Samples in each analytical or extraction batch should be of similar composition.

E. Calibration blank – A calibration blank is usually an organic or aqueous solution that is as free of analyte as possible and prepared with the same volume of chemical reagents used in the preparation of the calibration standards and diluted to the same volume with the same solvent (water or organic) used in the preparation of the calibration standard. The calibration blank is used to give the null reading for the calibration curve. For methods in which the calibration solutions receive the full sample preparation treatment, the calibration blank is identical to, and becomes referred to as, the method blank.

F. Continuing Calibration Verification (CCV) – A CCV is a standard solution used to verify freedom of excessive instrument drift. The CCV is a periodic check of the calibration.

G. Control charts – This is a chart consisting of an expected value (typically the mean) and an acceptable range of occurrences expressed as control limits. The values obtained from measurements versus the time sequence of entries are plotted to produce control charts.

H. Duplicate samples – Duplicate samples are two separate samples taken from the same source (i.e. samples in separate containers and analyzed independently).

I. Initial Calibration Verification (ICV) – This is an independent standard solution used to verify the calibration standard level. An independent standard solution is defined as a standard solution composed of the analyte of interest from a separate (different) source, a different lot or a separately prepared set of two primary standards may be used.

J. Laboratory Control Sample (LCS) – A well characterized sample of known analytes and concentration, or a stable artifact, that is measured over time to monitor the stability of the measurement process.

K. Matrix spike sample – A matrix spike sample is prepared by adding a predetermined quantity of stock solution of representative analytes to an actual sample matrix (as opposed to an ideal matrix, e.g. reagent water, or site blanks, etc.) prior to sample extraction/digestion and analysis. The matrix spike is used to measure accuracy of the method in the sample matrix.

L. Matrix spike duplicate analysis - Equal and predetermined quantities of stock solutions of certain analytes are added to each of two aliquots of a sample prior to extraction or digestion and analysis. Matrix spike duplicates can be used to measure precision.

M. Method detection limit (MDL) – The MDL is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. It is determined from the analysis of replicates of a sample containing the analyte at very low concentration.

N. Monitor – To monitor is to observe and record activity to measure compliance with a specific standard of performance; routine and ongoing collection of data about the indicator.

O. Precision – Precision is the agreement between a set of replicate measurements without assumption or knowledge of the true value. Analytical precision is assessed by means of laboratory duplicate or replicate or duplicate matrix spike analysis. The most commonly used estimates of precision are the relative standard deviation (RSD) or the coefficient of variation (CV).

P. Quality Assurance – Quality assurance is an integrated system of management activities involving planning, implementation, assessment, reporting, and quality improvement to ensure that a process, item, or service is the type and quality needed and expected by the client.

8. Records

A. Control charts for accuracy and precision

B. Proficiency test results

C. Nonconforming work and corrective action records

9. Supporting Documents

A. ORA Laboratory Manual, Volume II, ORA-LAB.4.9 Control of Nonconforming Work

B. ORA Laboratory Manual, Volume II, ORA-LAB.4.11 Corrective Action

C. ORS.002 Standard Operating Procedures National Check Sample Program

D. ORA-LAB.001 Standard Operating Procedure (SOP): Microbiological Controls for Sample Analysis

E. SOP-000288 Microbiological Controls for Medical Product Sample Analysis

10. Document History

Revision #	Status* (D, I, R)	Date	Author Name and Title	Approving Official Name and Title
1.2	R	12/06/06	LMEB	LMEB
1.3	R	04/03/07	LMEB	LMEB
1.4	R	11/15/07	LMEB	LMEB
1.5	R	09/14/10	LMEB	LMEB
1.6	R	01-22-13	LMEB	LMEB
02	R	08/13/2019	LMEB	LMEB

* - D: Draft, I: Initial, R: Revision

11. Change History

Revision #	Change
02	Revisions made as needed to align this procedure with the revised ISO/IEC 17025:2017 and AOAC requirements. Revision to formatting and policy clarifications were also made. Images of control chart examples were included.

12. Attachments

None