SCOPE: This procedure applies to employees who are responsible for laboratory operations on site.

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

RESPONSIBILITY: It is the responsibility of the management to ensure that the following procedures are adhered to and understood by all relevant personnel and the personnel follow State or local health department requirements.
Company is responsible for establishing a laboratory quality control program.
Laboratory Supervisors are responsible for ensuring that quality control is performed and for reviewing quality control data for acceptability. Analysts are responsible for conducting quality control analyses in accordance with the laboratory quality control program.

DEFINITIONS

- **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.
- **Analytical batch** – An analytical batch is the basic unit of measure by which the number of quality control samples needed is determined. The analytical batch is those samples 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. For analyses involving extractions or digestions, the analytical batch is those samples extracted or digested together on the same day. Samples in each analytical batch should be of similar composition.
- **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.
- **Analytical spike** – An analytical spike is a sample made by spiking an analytical solution after the sample preparation or digestion process.
- **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.

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

- **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).
- **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.

INSTRUCTIONS:

Laboratory Quality Control Program

Laboratory quality control is an essential aspect of ensuring that data released is fit for the purpose determined by the quality objectives (i.e. accuracy and precision)

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

1. Internal quality control:
   QCs are used to measure accuracy, precision, contamination, and matrix effects. Generally, QCs are run per batch or set of samples at a frequency of 5% or one every twenty samples. This level sufficiently demonstrates the validity of results. The laboratory determines, where feasible, the accuracy and precision of all analyses performed.

a) QC schemes utilized for chemistry consist of:
   - *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.

- *Matrix spikes* - Matrix spikes measure the effects the sample matrix may have on the analytical method, usually the analyte recovery. Method accuracy is documented and controlled based on the percent recovery of matrix spikes for quantitative analysis and the positive response of the analyte for qualitative analysis.
- *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 documented and controlled based on the relative percent difference (RPD) or the positive response for qualitative analysis.
- *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.
- *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.
- *Accuracy and precision control charts*

b) QC schemes utilized for microbiology include running QC controls concurrently with each sample batch or set. They are:
    - *positive and negative culture controls* - positive and negative controls give correct response,
    - *system and collector controls,*
    - *applicable kit controls* (positive and negative),

- *media quality* - the culture controls additionally verify the acceptability of the media,
- *un-inoculated media* – un-inoculated media control reveals no visible growth, and
- *accuracy and precision control charts*.

c) QC schemes utilized for miscellaneous testing (i.e. microscopic) includes QC samples for:

External Quality Control Program

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. Proficiency surveys are used as a tool to assist personnel in the identification of laboratory problems that may exist and that have eluded the internal quality control program. Therefore, it is essential that proficiency testing samples are treated as routine samples to the extent possible. 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. Samples should be run in duplicate *only* in those procedures where samples are normally analyzed in duplicate. Supervisors are individually responsible to ensure that the performance and evaluation of proficiency samples are submitted within the designated time frame. A corrective action is initiated if necessary according to the laboratory's corrective action process. All unacceptable results are investigated and the cause or causes identified for the unacceptable performance, and corrective action implemented.

Other Quality Control Monitoring Activities

There are other QC procedures that may be occasionally used. These are:

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 (analytes) 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 supervisor or 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.

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 be exceeded except as resolved under a documented 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, repeating analysis.

Quality Control Charts

1. 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. The control chart is actually

a graphical presentation of QC efficiency. If the procedure is *in-control,* the results will almost always be within established control limits. Further, the chart will disclose trends and cycles from assignable causes which can be corrected. 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.

a) 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 ±3X the calculated standard deviation. Upper and lower warning limits are established at the mean ± 2X the calculated standard deviation.
b) 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.

Statistical Process Control

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.

1. Accuracy is expressed as percent recovery of spiked samples.
    a. Percent recovery is calculated as follows for spikes in solvent or standard spikes: % Recovery = 100 X *KX* where:
    X = observed value
    K = known value
    b. Accuracy is calculated for spikes into natural matrices as follows: Recovery = 100 X *KXXus*− where:
    X_s = measured value for spiked sample
    X_u = measured value for unspiked sample
    K = known value of the spike in the sample
2. Precision is expressed as relative standard deviation (RSD) or relative percent difference (RPD) of duplicate samples.

a. RSD is calculated from standard deviation and mean recovery, when the standard deviation is derived from multiple recovery results as follows:
   RSD = CV = 100 X $X\sigma$ where:
   RSD = relative standard deviation
   CV = coefficient of variation
   σ = standard deviation
   X = arithmetic mean of the measurements
b. RPD is calculated when only two sample results are available as follows:
   RPD = $RRRs-1$ X 100 where:
   $| R_1 - R_2 |$ = absolute difference between the determinations
   R = arithmetic mean of the two values

3. 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 plotting 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.

d. 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 plottings move steadily either upward or downward, a trend is indicated. If a series of consecutive plottings 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, a corrective action is initiated to determine the cause.

WORKING AREA

General characteristics with which the areas must comply:

a) A biological control laboratory should be designed according to the technical requirements which will facilitate an adequate flow of staff, material, equipment, samples, other resources necessary for the work and waste, and also complying with the minimum safety requirements to allow the management of potentially dangerous substances and the appropriate use of laboratory animals when required, as well as the evacuation of staff if necessary.
b) The lighting and ventilation should correspond to the needs of each working area, according to the specific requirements of the activity carried out. The surfaces of the work benches should be smooth, easy to clean and made of material resistant to chemicals.
c) The hot and cold water, treated water, vacuum, gas, steam and electricity installations should be arranged so that they guarantee adequate use during the work and also facilitate maintenance and repair operations. The sewage system should be constructed of a material which ensures its integrity in view of the characteristics of the effluent.
d) The installations should take into account biosafety standards.

The following working areas are defined:

- Sample reception area
- Area for physicochemical analysis
- Area for microbiological analysis
- Area for biological assays

- Experimental animal area
- Instrumentation area
- Area for washing, preparation and sterilization of materials
- Administrative area
- Storage
- Disposal of contaminating chemical and biological residues
- General services.

Physicochemical analysis laboratory

1. There should be areas effectively separated for the performance of tests which require the use of dangerous solvents or radioactive substances or which cause the emission of toxic vapours or gases or release heat, as well as for the preparation of reagents and solutions.
2. The work surfaces in the area should be sanitary, with the necessary ventilation and protection against direct sunlight.
3. There should be extraction hoods and the necessary safety equipment (masks, goggles, aprons, acid-resistant gloves).

Microbiological analysis laboratory

1. There should be an area for the preparation and distribution of culture media or a service for the supply of these.
2. The walls, floors and ceilings should be smooth and easily cleaned. The joins between walls, between walls and floor and between walls and ceilings should have sanitary finishes.
3. Where necessary there should be an area for the maintenance and growth of test microorganisms and a room for incubators.
4. There should be a sterile area for the performance of the sterility test, with a laminar flow cabinet.

Area for biological assays

The design and atmospheric conditions will depend on the assay to be performed and the risk involved in the work. There should be the following working areas:

- Area for the control of bacterial vaccines: a Class II safety cabinet is required at least.
- Area for the control of viral vaccines: a Class II safety cabinet is required at least.

- Area for the control of human rabies vaccine: a Class II B3 safety cabinet is required at least.

To be able to effectively work with light sensitive organisms, these safety cabinets should allow for working under low light conditions.
If other biological products are controlled within the laboratory (blood derivatives, cytokines, hormones, biotechnology products), there should be areas for these assays.

Instrumentation area
There should be a specific centralized area for the installation and use of specialized analytical instruments, with controlled relative humidity and temperature and voltage stabilizer.

Area for washing, preparation and sterilization
All the conditions necessary for performing the activities of washing, preparation and sterilization of materials should be met. There should be autoclaves, ovens, and adequate air exhaust systems.

Area for documentation archiving and control
The processing and archiving of the documentation (SOPs, manuals, instruction sheets, registrations) should be carried out, ensuring their confidentiality and allowing their periodic revision and distribution.

Storage
Reagents, culture media and other materials should be stored in areas separate from the testing laboratory, taking special care with inflammable, toxic and radioactive fluids and solids. Air exhaust systems and protection against vectors should be installed and temperature and relative humidity should be controlled in required areas.

Disposal of chemical and biological contaminating residues
There should be a specific area for chemical waste, isolated from the working areas with containers according to the type of solvents to be disposed of (corrosive, volatile, radioactive, mixtures) or based on their physicochemical properties.
There should be special containers for biological waste (animals' litter, test animals, culture media, swabs, gauze, needles and sharps).

Equipment and instruments

1. The laboratory should have the necessary equipment and instruments for the correct performance of the tests. New instruments and equipment should be installed and calibrated by the distributor who should leave a written report of the visit as part of the dossier. The system is established to ensure correct functioning and to maintain the service record.
2. The laboratory should have a list of equipment and instruments which should include:
    - the name,
    - brand,
    - inventory number,
    - serial number,
    - model and year,
    - location,
    - cost,
    - date of purchase,
    - date of first use.

3. A file should be opened for the equipment or instrument which must contain the general data and registration, and preventive or corrective maintenance, calibration and checking reports should be annexed.
4. Each piece of equipment or instrument should have its operating manual in the local language. The operating instructions should describe in a general manner the steps to follow for the use of the equipment and should be kept in a visible place near the equipment.
5. Each piece of equipment should have its registration of use and control card kept close by.
6. Specific preventive maintenance programmes should be established for each piece of equipment, as well as instrument calibration or checking programmes to ensure that they operate so that the measurements made are traceable (where the concept is relevant) in relation to national measuring standards and if feasible to those specified by the National Weights and Measures Committee. If the equipment is out of specification, staff should carry out the corresponding corrective actions and in the meantime put up an “out of service” sign. In the case of instruments, it should

be demonstrated, through calibration that they are in a satisfactory condition to operate again.

7. When equipment is in operation it should undergo in-service checking between periodic calibrations.

MONITORING:

1. A designated employee will inspect that each employee is following this SOP.

CORRECTIVE ACTION:

1. Any employee found not following the procedures in this SOP to be retrained.

VERIFICATION AND RECORD KEEPING:

1. The manager will verify that employees are following this SOP by visually observing the employees during all hours of operation.

DOCUMENTATION RETENTION:

The records applied to this procedure are to be kept on file for a minimum of 3 years.