Commonwealth of Kentucky

Wastewater Laboratory Certification Manual

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APPENDICES

I. INTRODUCTION

This manual is incorporated by reference in 401 KAR 5:320 and establishes technical procedures and minimum requirements of the Kentucky Wastewater Laboratory Certification Program.

A copy of this manual may be found on the following website:

http://water.ky.gov

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II. GENERAL LABORATORY REQUIREMENTS

This section establishes requirements for all laboratories. Information regarding specific disciplines (e.g., Chemistry, Microbiology, and Whole Effluent Toxicity can be found in the appropriate critical elements sections of this manual.

All certified wastewater laboratories are responsible for meeting the requirements specified in this section and any specific discipline section for which they are certified (e.g. Chemistry, Microbiology, and Whole Effluent Toxicity.

1.0 Personnel

The laboratory shall have sufficient supervisory, quality assurance and technical staff with the necessary education, training, technical knowledge, and experience for their assigned functions.

1.1 Changes in Key Laboratory Personnel

Key laboratory personnel changes (as identified in 401 KAR 5:320) shall be communicated to DOW in writing within 30 days of the change.

1.2 Personnel Records

Personnel records shall be maintained for laboratory supervisors, consultants, and analyst/technician for a minimum of five (5) years. For the new analyst/technician, academic background and continuing education shall be documented and updated annually, as necessary. A copy of the results of the proficiency test (PT) samples, blind samples, and successful completion of the training courses, where applicable, shall be on file.

2.0 Training Records

Training records shall be maintained by a certified wastewater laboratory for laboratory personnel for a minimum of five (5) years. These records shall include job-related formal education and training courses taken by the analyst/technician that pertain to his or her responsibilities, including analytical methodology, laboratory safety, sampling, quality assurance, data analysis, etc.

Analyst/technician training records shall include all method detection limit (MDL) studies, initial demonstrations of capability (IDC) and on-going demonstration of capability (ODC) summary results and all associated raw data. This information shall be available during an on-site audit or by request by DOW.

3.0 Quality Assurance Plan

A laboratory analyzing wastewater compliance samples shall adhere to quality control procedures established in the analytical methods in 40 C.F.R. 136. Each laboratory shall prepare, implement, and maintain a written Quality Assurance Plan (QAP). The QAP shall be kept current by conducting an annual review and making necessary revisions. Laboratory personnel shall be familiar with the contents of the QAP. If the annual review results in substantive updates or revisions, the amended QAP shall be submitted to the cabinet with the application for renewal of certification.

The laboratory QAP shall be a stand-alone document. However, it may reference other documents such as Standard Operating Procedures (SOP), published methods, or other published literature.

The following items shall be addressed in the laboratory QAP:

3.1 Laboratory Organization and Responsibility

3.1.1 Include a chart or table showing the laboratory organization and lines of responsibility, including QA managers.

3.1.2 List the key individuals who are responsible for ensuring the production of valid measurements and the routine assessment of measurement systems for precision and accuracy (e.g., who is responsible for internal audits and reviews of the implementation of the plan and its requirements).

3.1.3 Reference the job descriptions of the personnel and describe training to keep personnel updated on regulations and methodology, and document that laboratory personnel have demonstrated proficiency for the methods they perform (e.g. MDL Study and IDC).

3.2 Process Used to Identify Clients' Data Quality Objectives (DQO).

3.3 SOPs with Dates of Last Revision

3.3.1 The laboratory shall maintain SOPs that accurately reflect all phases of the current laboratory activities.

3.3.2 Keep a list of SOPs and their effective dates.

3.3.3 Ensure that current copies of SOPs are in the laboratory and in the QA manager's files.

3.3.4 Ensure that SOPs are reviewed annually and revised as changes to the procedure are made.

3.3.5 Ensure that SOPs have signature pages and revision dates.

3.3.6 Ensure that SOPs are read, understood, and used by applicable laboratory personnel.

3.4 Field Sampling Procedures

3.4.1 Describe the process used to specify and document the following; sample collectors, sampling procedures and locations, required preservatives, proper containers, correct sample container cleaning procedures, sample holding times from collection to analysis, and sample shipping and storage conditions.

3.4.2 Ensure that appropriate forms are legibly completed in indelible ink (electronic records are acceptable);

3.4.3 Ensure that sampling protocol is written and available to sampling personnel.

3.5 Laboratory Sample Receipt and Handling Procedures

3.5.1 Laboratory notebooks shall be filled out in indelible ink, entries dated and signed (electronic records are acceptable).

3.5.2 Samples shall be stored at the proper temperature, isolated from laboratory contaminants, standards, and highly contaminated samples. Holding times shall not be exceeded.

3.5.3 Integrity of all samples shall be maintained (e.g. by tracking samples from receipt by laboratory through analysis to disposal – internal sample tracking document).

3.5.4 Require Chain-of-Custody (COC) procedures for all compliance samples.

3.5.5 Criteria for flagging or rejection of samples that do not meet shipping, holding time, volume of sample or preservation requirements and procedures for notification of sample originators shall be specified (client/customer or field sampling contractor)

3.5.6 Describe how samples are checked and how checks are documented when samples arrive. Checks shall include verification of proper containers, temperature, other preservation, and custody seals when applicable.

3.6 Instrument Calibration Procedures (may reference an SOP)

3.6.1 Specify type of calibration used for each method and frequency of use.

3.6.2 Describe calibration standard's source, age, storage, and labeling (including date received, date opened and initials of analyst, and expiration date).

3.6.3 Perform data comparability checks.

3.6.4 Use control charts (or other trend analyses of quality control results) for calibration check standards to monitor for trends and ensure acceptance criteria are met.

3.7 Analytical Procedures (may reference an SOP)

3.7.1 Cite complete reference method using nomenclature found in 40 CFR 136.

3.7.2 Describe quality control procedures required by the methods that shall be followed.

3.7.3 All analytical procedures, observations, results (including quality control) must be documented using either a bound notebook, bench sheets, or electronic medium.

3.8 Data Reduction, Validation, Reporting and Verification (may reference an SOP)

3.8.1 Describe the data reduction process and method of conversion of raw data to required reporting limits.

3.8.2 Describe the data validation process and frequency of review.

3.8.3 Describe the reporting procedures; include procedures and format(s).

3.8.4 Describe the data verification process and frequency of review.

3.8.5 Describe the procedure for data corrections.

3.9 Quality Control Checks and Frequency of Use (may reference an SOP)

3.9.1 Parameters for chemistry and radiochemistry shall include or reference:

3.9.1.1 Instrument performance check standards;

3.9.1.2 Frequency and acceptability of method detection limit (MDL) calculations;

3.9.1.3 Frequency and acceptability of demonstration of low level capability;

3.9.1.4 Calibration, internal and surrogate standards;

3.9.1.5 Laboratory reagent blank, method blank (MB), field reagent blank and trip blank;

3.9.1.6 Field and laboratory matrix replicates;

3.9.1.7 Quality control and proficiency test (PT) samples;

3.9.1.8 Laboratory fortified blank and laboratory fortified sample matrix replicates;

3.9.1.9 Initial demonstration of method capability;

3.9.1.10 On-going demonstration of method capability;

3.9.1.11 Use of control charts or trend analysis; and

3.9.1.12 Qualitative identification/confirmation of contaminants.

3.9.2 Parameters for microbiology shall include or reference:

3.9.2.1 Positive and negative culture controls;

3.9.2.2 Sterility controls; and

3.9.2.3 PT samples and quality control samples.

3.10 List Schedules of Internal and External System Reviews and Data Quality Audits and Inter-Laboratory Comparisons (may reference an SOP).

3.11 Preventive Maintenance Procedures and Schedules

3.11.1 Describe location of instrument manuals and schedules and documentation of routine equipment maintenance.

3.11.2 List any maintenance contracts in place.

3.11.3 Describe laboratory procedure to ensure sample holding times are met (e.g. redundant instrumentation, contracts with other laboratory facilities, etc).

3.12 Corrective Action Contingencies

3.12.1 Describe response to obtaining unacceptable results from analysis of PT samples and from internal QC checks.

3.12.2 Position titles of individuals responsible for the various corrective actions.

3.12.3 Describe how corrective actions taken are documented.

3.13 Record Keeping Procedures

3.13.1 Describe procedures and documentation of those procedures.

3.13.2 Describe security policy of electronic databases.

3.13.3 Describe records retention policy including backup of electronic file procedures.

4.0 Field Sampling and Analysis Procedures

4.1 Field Sampling Activities Performed by Laboratory Personnel

Field sampling procedures are an integral part in the quality of analytical data. Sampling activities are not addressed within the scope of the wastewater laboratory certification program but are established utilizing a standard operating procedure (or a quality assurance project plan) that is consistent with the compliance program's DQO.

The certified laboratory is responsible for comparing the chain-of-custody (COC) that accompanies compliance samples to the laboratory to the actual samples received. The laboratory shall verify that the information on the COC is correct. Anomalies with the samples or the COC shall be communicated to the client, documented, and a corrective action agreed upon between the laboratory and the client. If errors in the COC, samples, or sampling procedures do not meet the requirements of the method, then the samples shall be rejected and the client shall be notified.

Refer to the critical elements for chemistry, microbiology, or whole effluent toxicity for specific requirements in Chapters III, IV, and V respectively.

4.2 Field Analysis Activities Performed by Laboratory Personnel

An employee of a Certified Laboratory may perform the following field analyses utilizing the requirements specified in Chapter II Section 8.0:

- Dissolved oxygen (DO)
- Residual chlorine
- pH
- Temperature
- Conductivity
- Turbidity

Results of field activity and analyses performed must be documented in a field notebook (or equivalent). At a minimum the following must be recorded:

- Date and time
- KY Laboratory ID number
- Analyst (i.e., qualified field personnel)
- Location (include name and GPS coordinates if available)
- Weather conditions (if applicable)
- Provide explanation of any pertinent field activity (e.g., sample collection, addition of preservatives, preparation of shipping containers, etc.)

- Document instrument calibration and verification (including lot numbers and expiration dates)
- Acceptability of calibration and field quality control (e.g., sample duplicates)
- Sample results including units

4.3 Field Analysis Quality Control (also refer to table below)

4.3.1 The following quality control measures apply to a certified laboratory performing residual chlorine, pH, conductivity, and turbidity analyses in the field:

4.3.1.1 Calibration of instruments shall be performed daily (when in use), using a primary standard;

4.3.1.2 A calibration verification (CV) shall be performed daily (when in use). This may be accomplished using a secondary standard;

4.3.1.3 A method blank shall be analyzed daily (when in use, excluding pH and conductivity); and

4.3.1.4 A quality control sample (QCS) shall be analyzed at least quarterly. A purchased second source standard in 4.3.1.2 meets this requirement and no additional QCS is needed. If a laboratory performs an analysis less frequent than quarterly, a successful QCS must be performed with each analysis prior to the analysis of compliance samples.

4.3.2 The following quality control measures apply to a certified laboratory performing dissolved oxygen, residual chlorine, pH, temperature, conductivity, and turbidity analyses in the field:

4.3.2.1 Duplicate analysis of samples, reanalysis and evaluation for precision of a compliance sample (see definition of laboratory duplicate in Appendix B), shall be performed once per batch of twenty samples or once per quarter, whichever is more frequent. Sample batches may extend beyond one day. Duplicate analysis of a sample may be utilized to demonstrate precision (both results must be documented, first analysis for compliance the second for QC). The calculation for duplicate analysis can be found in Section 7.3 of Chapter III.

4.3.3 The following quality control measures apply to a certified laboratory performing in-line or continuous monitoring:

4.3.3.1 Analysis of a grab sample shall be performed utilizing an off-line meter once per month, at a minimum. This sample shall be evaluated as a duplicate analysis; and

4.3.3.2 The meter utilized for duplicate analysis shall meet the requirements established in 4.3.1 above.

QC								
	MB		CV		QCS		Duplicate	
	Frequency	Acceptance Criteria (1)	Frequency	Acceptance Criteria (1)	Frequency	Acceptance Criteria (1)	Frequency	Acceptance Criteria
pH	N/A	N/A	Daily	±0.1 SU	Quarterly	5%	1/20	< 20% RPD
Temperature	N/A	N/A	N/A	N/A	N/A	N/A	1/20	< 20% RPD
Conductivity	N/A	N/A	Daily	10%	Quarterly	10%	1/20	< 20% RPD
TRC	Daily	< RL	Daily	10%	Quarterly (2)	10%	1/20	< 20% RPD
Turbidity	Daily	< RL	Daily	10%	Quarterly (2)	10%	1/20	< 20% RPD
DO	N/A	N/A	N/A	N/A	N/A	N/A	1/20	< 20% RPD

(1) May utilize acceptance criteria off of the manufacture's certificate of analysis.
(2) Secondary standards (e.g., gel, etc.) are not allowed for QCS determination.

5.0 Proficiency Testing Requirements and Frequency

5.1 Laboratory

At least annually, a certified wastewater laboratory shall satisfactorily analyze a PT sample for each method-analyte pair for which it is certified. Discharge Monitoring Report- Quality Assurance (DMR-QA) Program participation is an acceptable alternative for a PT Study. PT samples shall be analyzed in the same manner as routine samples. A certified laboratory shall purchase PT sample(s) from a provider approved by the American Association for Laboratory Accreditation.

5.2 Laboratory Only Performing Field Analyses

A certified laboratory performing only field analyses shall satisfactorily analyze a PT sample annually for the following parameters (as applicable): residual chlorine, pH, conductivity, and turbidity.

5.3 Proficiency Testing Submission

A certified laboratory shall make arrangements with its PT providers to have the results submitted directly to DOW. PT study results shall be sent to:

By Mail:

Kentucky Department for Environmental Protection
Division of Water
Wastewater Laboratory Certification Officer
300 Sower Boulevard; 3rd Floor
Frankfort, KY 40601

By Email:
dowptresults@ky.gov

5.4 Drinking Water Studies

Participation in a drinking water Proficiency Test study (e.g. WS study) shall not be substituted for a wastewater study.

5.5 Proficiency Testing Failure

If the analysis results from the certified laboratory do not fall within the acceptance limits specified by the PT provider, then re-analysis of a separate PT sample shall be conducted until meeting PT acceptance limits within the calendar year. If a certified laboratory fails to successfully analyze a specific contaminant (by a specific method) or receives unacceptable results for two consecutive PT samples for the same contaminant, then the laboratory's certification status will be reviewed by DOW and appropriate action will be taken. Appropriate action includes: 1) provide technical assistance to the laboratory; 2) downgrading of laboratory's certification from certified to provisionally certified; or 3) revocation of the laboratory's certification.

5.5.1 If a certified laboratory receives two (2) consecutive unacceptable results for a PT Study for one or more contaminants, the laboratory shall submit a corrective action plan to DOW. The corrective action plan shall address what actions the laboratory is planning to: 1) identify the cause of the missed analyses; and 2) correct any issues identified. After completing the corrective action plan, the laboratory shall successfully perform a PT study for all previously unacceptable contaminants.

5.5.2 The repeat PT study shall be performed within 90 days of notice of an unacceptable result for each affected method-analyte pair.

5.5.3 If a laboratory participates in multiple PT studies for the same method-analyte pair, the final PT study must be acceptable.

5.6 Proficiency Testing Required for Each Method

If a laboratory wishes to be certified for a contaminant by more than one method, the laboratory shall analyze a PT sample by each of the methods for which it wishes to be certified. The methods listed on the laboratory's certification certificate shall be the methods by which the PT samples were analyzed.

5.7 Documentation and Record Keeping

The laboratory shall maintain all PT sample analysis documentation, including: analysis date, analyst/technician performing the analysis, and all associated raw data for a period of five (5) years. These documents will be requested during an on-site audit and shall be available for review. Documents may also be requested as part of any technical assistance provided to the laboratory.

6.0 Report Requirements and Record Keeping

The following are required for a certified wastewater laboratory analyzing wastewater compliance samples:

6.1 Legal Defensibility

Compliance monitoring data shall be made legally defensible by keeping thorough and accurate records. The QAP or SOPs shall describe the policies and procedures used by the facility for record integrity, retention, and storage. Chain of custody procedures shall be utilized. The use of arrow downs or ditto marks shall not be utilized when recording observations.

6.2 Laboratory Analytical Reports

To reproduce data, a certified laboratory shall include the following on laboratory reports of compliance samples, at a minimum:

6.2.1 Report Date

- Reference Number – unique number referring to Chain-of-Custody (C-O-C)
- Date of Report

6.2.2 Laboratory Information

- Laboratory Name
- Laboratory Address
- Kentucky Laboratory Identification Number
- Contact Information (phone, fax, email, webpage, etc.)

6.2.3 Client Information

- Permitted Facility Name
- Address
- KPDES Number

6.2.4 Sample Collection Information

- Date Sampled
- Time Sampled

6.2.5 Sample Identification

- Location Name
- Outfall Number
- Laboratory Sample Identification Number
- Matrix Type

6.2.6 Report of Analysis

- Parameter Name
- Reference Method
- Sample Results
- Minimum Reporting Level and Units
- Method Detection Limit (MDL) and Units
- Data Qualifier(s) – Flag(s) (if applicable)
- Analyst
- Preparation Date and Time (if applicable)
- Analysis Date and Time

6.2.7 Conclusion

- Data Qualifier(s) (Flag) Definitions – if used in report
- Identify any sub-contracted laboratory analysis, include KY laboratory ID number

6.3 Maintenance of Records

A certified laboratory shall maintain records for five (5) years or until the next on-site audit, whichever is longer. All references in this manual to a five (5) year records retention period shall include "or until the next on-site audit, whichever is longer." A change in ownership, mergers, or closure of laboratory does not eliminate this requirement. Records include all raw data, calculations, and quality control data. These data files may be hard copy, or electronic. If the laboratory changes its computer hardware or software, it shall make provisions for transferring historic data to the new system so that it remains retrievable.

6.4 Sample Records

Data shall be recorded in indelible ink with any changes lined through once, such that the original entry is visible. Changes shall be initialed and dated. Data may also be kept electronically. The following information shall be readily available:

6.4.1 Date, location, time of sampling, name, organization & phone number of the sampler, and analyses required;

6.4.2 Sample type (grab or composite);

6.4.3 Date and time of receipt of the sample at laboratory;

6.4.4 Container size, container type, preservation (e.g., pH, temperature, etc.), hold time, and condition upon receipt;

6.4.5 Results of field measurements, including field laboratory ID number, such as pH & DO; and

6.4.6 Transportation or delivery means of sample.

6.5 Analytical Records

Data shall be recorded in indelible ink with any changes lined through such that the original entry is visible. Changes shall be initialed and dated. All data may also be kept electronically. The following information shall be readily available:

6.5.1 Laboratory and persons responsible for performing analysis;

6.5.2 Analytical techniques/methods used;

6.5.3 Date and time of preparation and analysis;

6.5.4. Results of sample, including units, and quality control analysis;

6.5.5 Calibration and standards information; and

6.5.6 Analyst/technician Initial Demonstration of Capability documentation shall be kept on file as well as results of proficiency testing.

6.6 Reconstruction of Data

Adequate information shall be available to allow the auditor to reconstruct the final results for compliance and PT samples, including:

6.6.1 Audit reports, corrective action plans, and all follow-up documentation;

6.6.2 Analyst training records;

6.6.3 QAP and SOPs, current and all previous versions for the past five (5) years;

6.6.4 Purchasing documentation regarding chemical, supplies, and/or equipment;

6.6.5 All compliance sample results, summary sheets, bench sheets, instrument raw data, notebooks and any other record(s) deemed necessary to reconstruct the reported result; and

6.6.6 PT sample results and all associated raw data.

6.7 Computer Programs

Computer programs shall be verified initially and be available for inspection. Access to computer programs and electronic data shall be limited to authorized personnel. Laboratories shall protect programs against unwanted editing capability in order to maintain accuracy of results.

6.8 Sub-Contracting

Any DOW- certified wastewater laboratory may sub-contract wastewater compliance samples to another DOW-certified wastewater laboratory for analysis. The initial (primary) laboratory is responsible for ensuring that any wastewater compliance sample analysis is sub-contracted to a laboratory that is certified by DOW for the specific method-analyte pairing that has been requested. If the primary laboratory reports analytical results generated by a sub-contract laboratory, they must clearly identify that result on the report.

7.0 Requirements for Maintaining Certification

7.1 Proficiency Test (PT) Samples

At least annually (calendar year January 1 to December 31), a wastewater laboratory certified for specific method-analyte pairs shall satisfactorily analyze a PT sample to maintain certification in accordance with section 5.0 of this chapter.

7.2 Methodology

A certified laboratory shall use the methodologies and procedures approved by the US EPA and established in 40 C.F.R. 136 unless the methodology is specified in the KY permit

7.3 On-site Evaluation

DOW shall verify that a laboratory is maintaining the required standard of quality for certification. DOW may use a combination of information to determine a laboratory's quality, including but not limited to:

- On-site evaluation;
- PT Sample results;
- QC Results
- Compliance reports; and
- Review of requested documentation.

7.4 Notification to DOW of Major Changes

A certified laboratory shall notify DOW, in writing, within 30 days of major changes in personnel, equipment, or laboratory location. A major change in personnel is defined as the loss or replacement of the laboratory supervisor, manager (quality or production), director or a primary analyst that is no longer available to analyze a particular contaminant for which the laboratory is certified. If the certified laboratory can no longer produce valid data based on major changes at the laboratory, certification status shall be modified by DOW if necessary.

8.0 Equipment and Supplies

8.1 pH Meter

8.1.1 Accuracy and display increments shall be at least ±0.1 S.U.

8.1.2 pH buffer aliquots shall be used only once.

8.1.3 Electrodes shall be maintained according to the manufacturer's recommendations.

8.1.4 pH meters shall be standardized before each use period using a combination of pH 4, pH 7, and pH 10 buffers. These buffers shall be used in accordance with manufacturer's specification or expected range of use with pH 7 and either pH 4 or 10 standard buffers, whichever range covers the desired pH of the media or reagent. The date and buffers used shall be recorded in a log book, with the analyst's initials.

8.1.5 Record pH meter slope after each calibration, if provided by the meter.

8.1.5.1 If the pH meter does not have a feature to automatically calculate the slope, but can provide the pH in millivolts (mV), use the following formula to calculate the slope: Slope (as %) = |mV at pH 7 - mV at pH 4| x 100/177.

8.1.5.2 Slope shall be between 95% and 105%.

8.1.6 Commercial buffer solution containers shall be dated upon receipt and when opened. Buffers shall be discarded by the expiration date. A pH calibration verification solution that brackets the sample shall be analyzed and read within ± 0.1 S.U. of the true value. If criteria are not met, the meter shall be recalibrated using appropriate standards.

8.2 Balance

8.2.1 Balance range must be appropriate for the application for which it is to be used.

8.2.2 Top load or pan balances shall have readability of 0.1 g.

8.2.3 Analytical balances shall have a readability of 0.0001 g.

8.2.4 Balances shall provide a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less.

8.2.5 Balances must be operated in accordance with the manufacturer's recommendations. Balances must be on a stable and level surface. Laboratory personnel must ensure that the balance is level prior to each day of use.

8.2.6 Balances shall be verified each day of use using ASTM traceable Class 1, 2, or 3 weights (minimum of two traceable weights that bracket laboratory weighing needs) (ASTM, 1916 Race St., Philadelphia, PA 19103). Verifications shall be recorded in a logbook with the initials of the individual performing the check along with the date and time the check was performed. All measurements checks shall have limits established by the laboratory. These limits may be obtained from the balance vendor performing annual verification and maintenance of the analytical balance. Non-reference weights shall be calibrated annually with reference weights. Correction values shall be on file and used. A reference weight shall be re-certified every five years. Damaged or corroded weights shall be replaced.

8.2.7 Service contracts or internal maintenance protocols and maintenance records shall be available. Maintenance, calibration, and cleaning shall be conducted at least annually by a qualified technician.

8.3 Temperature Monitoring Device

8.3.1 Dial thermometers that cannot be calibrated shall not be used. The fluid column in glass thermometers shall not be separated. Digital thermometers are acceptable provided they meet the requirements within this manual.

8.3.2 The verification of glass and electronic thermometers shall be checked annually, and dial thermometers quarterly, at the temperature used, against a National

Institute of Standards and Technology (NIST, formerly National Bureau of Standards {NBS}) reference thermometer or one that meets the requirements of NBS Monograph SP 250-23. The correction factor and date of expiration (or verification) shall be indicated on the thermometer. In addition, the laboratory shall record in the thermometer QC record book the following information:

- date of check
- temperature of laboratory traceable thermometer
- serial number of laboratory traceable thermometer
- temperature of NIST reference thermometer (or equivalent)
- serial number of NIST reference thermometer (or equivalent)
- correction factor
- analyst's initials

8.3.3 If a laboratory thermometer differs by more than 1°C from the reference thermometer (or the smallest measurable unit on the thermometer, whichever is greatest), it shall be discarded. Reference thermometers (NIST or equivalent) shall be re-verified at least every five years. Reference thermometer verification documentation shall be maintained.

8.3.4 Continuous recording devices that are used to monitor incubator temperatures shall be re-verified at least annually. A reference thermometer that meets the specifications described in paragraph 8.3.2 shall be used for verification.

8.3.5 Infrared (IR) thermometers that are used to check the temperature of incoming samples are verified annually by comparing the reading against a NIST reference thermometer placed in a bottle of refrigerated water. The IR thermometer shall read within 0.5°C of the reference thermometer.

8.4 Refrigerator

8.4.1 Refrigerators shall maintain a temperature of $\leq$ 6 °C but not freezing. Verified thermometers shall be graduated in at least 1°C increments and the thermometer bulb immersed and suspended in liquid.

8.4.2 On days the refrigerator is in use, and the laboratory is staffed, the corrected temperature shall be recorded at least once per day in a refrigerator temperature log. If temperatures exceed the limits specified in 8.4.1 of this section, corrective action must be taken if temperatures remain outside limits. All refrigerators shall be assigned a unique identifier.

8.4.3 Refrigerator temperature logs must contain the following; date and time of temperature reading, observed temperature after correction factor is applied, analyst name or initials, and any corrective action taken due to temperature exceedances.

8.5 Pipets

8.5.1 All pipets shall meet the requirements of their applicable use.

8.5.2 Multi-volume dispensing devices shall have each dispensing head calibrated at a minimum of three volume settings each. The volumes shall bracket range of use and include at least one volume within the expected range.

8.5.3 Glass and plastic pipets shall have legible markings and shall not be chipped or etched.

8.5.4 Pipets delivering volumes of 10 mL or less shall be accurate to within a precision of 2.5%.

8.5.5 All auto dispensing devices shall be checked quarterly and adjusted or replaced if the precision or accuracy is greater than 2.5%.

8.6 Glassware and Plasticware

8.6.1 Glassware shall be borosilicate glass or other corrosion-resistant glass and free of chips and cracks that are an obvious safety hazard or pose detriment to analysis results or accuracy. Markings on graduated cylinders shall be legible.

8.6.2 Graduated cylinders for measurement of sample volumes shall be accurate to within a precision of 2.5%.

8.6.3 Glassware cleaning requirements specified in the methods must be followed. If no specifications are listed, then glassware should be washed in a warm detergent solution and thoroughly rinsed first with tap water and then with reagent water. This cleaning procedure is sufficient for general analytical needs.

8.7 Color Standards

Wavelength settings on spectrophotometers shall be verified at least annually with color standards. The specific checks and their frequency shall be as prescribed in the laboratory's quality assurance (QA) documents. A record of these checks shall be kept as prescribed in the laboratory's QA documents and be available for inspection.

8.8 Traceability of Calibration/Verifications

All calibrations or verifications of measurement devices shall be traceable to national standards if applicable.

9.0 Quality Assurance / Quality Control

9.1 Quality Assurance Plan

A certified laboratory shall prepare and maintain a QAP. All laboratory activities including sampling, test methods, instrument operation, data generation, data management, and corrective action procedures shall be described in the QAP. The QAP shall be read and understood by all laboratory personnel involved in the collection, analysis, quality control, or reporting of wastewater compliance samples.

9.2 Standard Methods Quality Control

A certified laboratory performing measurements using any of the approved Standard Methods shall comply with the quality control procedures specified in the EPA approved editions of Standard Methods. If the method lacks quality assurance/quality control (QA/QC) procedures, the laboratory has the following options to comply with the QA/QC requirements:

9.2.1 Refer to and follow the QA/QC published in the "equivalent" EPA method for that parameter that contains the required QA/QC procedures;

9.2.2 Refer to the appropriate QA/QC section(s) of an approved 40 C.F.R. 136 method from a consensus organization compendium; or

9.2.3 Incorporate the following twelve quality control (QC) elements, where applicable, into the laboratory's documented standard operating procedure (SOP) for performing compliance analyses when using an approved 40 C.F.R. 136 method when the method lacks applicable QA/QC procedures. One or more of the twelve QC elements may not apply to a given method and may be omitted if a written rationale is provided indicating why the QC element is inappropriate for a specific method.

9.2.3.1 Demonstration of Capability (DOC); including initial and on-going;

9.2.3.2 Method Detection Limit (MDL);

9.2.3.3 Laboratory blank(s), including reagent blank (LRB) and method blank (MB);

9.2.3.4 Laboratory fortified blank (LFB), also referred to as a spiked blank, or laboratory control sample (LCS);

9.2.3.5 Matrix spike (MS) and matrix spike duplicate (MSD), or laboratory fortified matrix (LFM) and LFM duplicate, may be used for suspected matrix interference problems to assess precision. Duplicate analysis of a

sample may be utilized to demonstrate precision (both results must be documented, first analysis for compliance the second for QC);

9.2.3.6 Internal standards (for Mass Spectrometry detector analyses), surrogate standards (for organic analysis), or tracers (for radiochemistry);

9.2.3.7 Calibration (initial and continuing), also referred to as initial calibration verification (ICV) and continuing calibration verification (CCV);

9.2.3.8 Control charts (or other trend analyses of quality control results);

9.2.3.9 Corrective action (root cause analysis);

9.2.3.10 QC acceptance criteria;

9.2.3.11 Definitions of preparation and analytical batches that may drive QC frequencies (shall not exceed 20 samples); and

9.2.3.12 Minimum frequency for conducting all applicable QC elements.

9.3 Documenting Quality Control

These twelve quality control elements shall be clearly documented in the written standard operating procedure for each analytical method not containing all of these QA/QC procedures, where applicable.

Refer to Chapters III, IV, V, and VI of this manual for specific quality assurance and quality control requirements.

10.0 General Methodology Requirements

A certified wastewater laboratory shall perform analytical methods in accordance with 40 C.F.R. 136. The most current version of 40 C.F.R. 136 shall be used by all laboratories to determine the applicability of a particular methodology.

The electronic version of 40 C.F.R. 136 may be found at the following website: www.ecfr.gov

11.0 Sample Management

Sample management is a key element of quality assurance and shall be documented in the QAP and SOP. The certified laboratory is responsible for the elements of sample management for which it has direct control. The process that results in evidence that the integrity of samples has been maintained from the time of sampling until the analyses are completed shall be documented in the QAP or SOP. The documentation shall include

sample preservation, storage, and a complete chain-of-custody (including internal laboratory sample tracking documentation).

12.0 Data Management

The goal of a certified laboratory is to produce analytical results of a known quality that is both accurate and legally defensible. Results must also meet the expectations of the data user, analytical program, or compliance program for which they are intended. Laboratory data that are used to generate results shall be managed properly. Adequate analysis data and information shall be available to allow for the reconstruction of the final results for compliance and PT samples.

12.1 The following shall be performed to ensure the defensibility and usability of laboratory data:

12.1.1 All observations shall be recorded in a laboratory logbook or suitable bench sheet (electronic records are acceptable);

12.1.2 A permanent record of all analysts' names, initials, and signatures shall be maintained;

12.1.3 Logbook entries shall be dated and initialed;

12.1.4 Indelible ink (black or blue) shall be used. Pencils are not acceptable;

12.1.5 Deletions and corrections shall be crossed-out with a single line so that the original entry is still visible, accompanied with the date and initials of the person making the deletion or correction. Correction fluid / tape shall not be used;

12.1.6 Records of standard / reagent preparation shall be maintained. All stock standard solutions, intermediate standard solutions, and working standard solutions shall be documented. Requirements for the recorded information are:

12.1.6.1 All pertinent information shall be recorded, including constituents of the solution, vendor, vendor lot number, purity, concentration, amount used, date opened, and expiration date (if applicable). Equations showing how calculations were made shall be recorded or referenced;

12.1.6.2 All solution information, including name, final volume, solvent used, and final concentration shall be recorded. Vendor, lot number, and grade of solvents used shall be included. Expiration date for the solution shall be recorded if applicable;

12.1.6.3 Certificate of Analysis (C of A) shall be maintained as part of the permanent record, if provided by the supplier; and

12.1.6.4 The date that the solution is prepared and the initials of the person preparing the standard shall be recorded;

12.1.6.5 The use of expired reagents is not allowed.

12.1.7 Records of sample receipt shall be maintained for all samples, including PT samples. Requirements for sample information are:

12.1.7.1 Pertinent sample information available to the laboratory shall be recorded in the sample logbook (electronic records are acceptable). The laboratory shall record the sampling date, sample identification (location), type of sample (e.g. grab or composite), matrix, container type, container size (volume), and the requested analyses. A unique laboratory sample identification number shall be assigned to the sample;

12.1.7.2 The date and time of sample receipt at the laboratory shall be recorded with the name of the person receiving and relinquishing the samples;

12.1.7.3 Temperature of the samples shall be recorded upon receipt at the laboratory. Also, the condition of the sample containers shall be noted in the sample logbook;

12.1.8 Records of analytical procedures, including:

12.1.8.1 Laboratory and person(s) responsible for performing analysis;

12.1.8.2 Analytical techniques/methods used;

12.1.8.3 Instrument ID if applicable;

12.1.8.4 Results of sample and quality control analyses;

12.1.8.5 Calibration results; and

12.1.8.6 Analyst/technician initial demonstration of capability / on-going demonstration of capability (IDC/ODC), method detection limit study (MDL) and PT Study results; and

12.1.9 Laboratory Information Management Systems (LIMS) / Electronic Data Maintenance:

12.1.9.1 Archival of electronic records shall meet the retention requirements of the compliance program;

12.1.9.2 SOP shall be in place and address:

12.1.9.2.1 System security;

12.1.9.2.2 Data entry, analysis, processing, storage, retrieval, backup and recovery;

12.1.9.2.3 Interpretation of system error codes (if applicable) and their corresponding corrective actions;

12.1.9.2.4 Procedure for making authorized changes to correct errors in data entry;

12.1.9.2.5 Maintenance of system hardware; and

12.1.9.2.6 Electronic reporting of data.

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III. CRITICAL ELEMENTS FOR CHEMISTRY

In addition to the General Laboratory Practices, chemistry laboratories shall adhere to all of the critical elements specified below.

1.0 Chemistry Methodology

A certified laboratory submitting compliance sample results to Kentucky shall be certified for each contaminant and the analytical method used for analysis. Only results from a certified wastewater laboratory shall be accepted. Methods shall be in accordance with 40 C.F.R. 136.

1.1 Alternate Test Procedure Methods

If a laboratory chooses to apply for and use an alternate test procedure (ATP) method as specified in the most recent version of 40 C.F.R. 136, the laboratory shall utilize the proper procedure for applying for an ATP and a signed copy of the ATP from EPA shall be maintained by the laboratory.

1.2 Method Modification

If a laboratory chooses to utilize a method modification as specified in the most recent version of 40 C.F.R. 136, the laboratory shall utilize the proper procedure for documentation of the modification that shall be maintained by the laboratory.

2.0 Laboratory Facility and Safety

A certified wastewater chemistry laboratory shall have adequate space to conduct all analyses for which the laboratory is certified. Fume hoods shall be operational and used when necessary. The sample storage area shall be isolated from all potential sources of contamination. Standards requiring refrigeration shall be stored separately from field samples. Incompatible acids, bases, and reagents shall be stored separately.

3.0 Laboratory Equipment and Instrumentation

The laboratory shall have all of the proper instrumentation and equipment necessary to perform the approved methods for which wastewater certification is requested. All instruments shall be properly maintained and calibrated prior to use.

Maintenance books shall be utilized by the laboratory for each applicable instrument. Requirements for maintenance logs shall be included in the laboratory's Quality Assurance Plan.

4.0 General Chemistry Laboratory Practices

The following is a brief overview of the general laboratory practices for the chemistry laboratory.

4.1 General

Chemicals/reagents: Chemicals and reagents used shall meet any requirements specified in the approved methods. If not specified, then Analytical Reagent Grade (AR) or American Chemical Society (ACS) grade chemicals or better shall be used for analyses in wastewater certified laboratories. The current promulgated edition of Standard Methods for the Examination of Water and Wastewater, Part 1080, may be consulted for more detailed information on reagent grades. Expired Chemicals/reagents shall not be used for analysis.

4.2 Inorganic Contaminants

Reagent water: The laboratory shall have a source of reagent water having a resistance value of at least 0.5 megohms-cm (conductivity less than 2.0 micromhos/cm) at 25^{o}C when required by the approved method. High quality water meeting such specifications may be purchased from commercial suppliers or produced by the laboratory. Quality checks to meet specifications shall be made on a routine basis depending on the water source and documented in a logbook. The laboratory shall meet any individual analytical method's specifications or additional requirements for the reagent water. Inorganic methods require distilled or deionized water free of the analyte(s) of interest and trace metal methods require ASTM Type 1 water (see Appendix C for specifications). Some methods may have special specifications for water; they shall be followed as appropriate.

Glassware preparation: Glassware cleaning requirements specified in the methods shall be followed. The laboratory Quality Assurance Plan or SOP shall specify the glassware preparation procedures.

4.3 Organic Contaminants

Reagent water: Reagent water for organic analysis shall adhere to any requirements and quality control criteria specified in the methods.

Glassware preparation: Glassware cleaning requirements specified in the approved methods shall be followed. The laboratory Quality Assurance Plan shall specify the glassware preparation procedures.

5.0 Sample Collection, Handling and Preservation

The manner in which samples are collected and handled is critical to obtaining valid data. It is important that a written sampling protocol with specific sampling instructions be

available to, and used by, sample collectors and available for inspection during an on-site audit.

5.1 Rejection of Samples

The laboratory shall have a sample rejection criteria specified in its Quality Assurance Plan or in a Standard Operating Procedure. The laboratory shall consistently follow its criteria and reject any field sample(s) taken for compliance purposes that does not meet the laboratory's acceptance criteria. This shall be documented.

The criteria specified in Section 5.2 through 5.7 shall be included in the laboratory sample rejection criteria, at a minimum.

5.2 Sample Containers and Preservation

The type of sample container and the required preservative for each contaminant are provided in 40 C.F.R. 136. The laboratory shall verify the preservative upon receipt of the sample(s), including temperature and pH if applicable. The laboratory shall record the results of preservation verification in the sample receiving log. Commercial freezer packs ('Blue ice') shall not be used. For all samples required to be preserved at $\leq 6^{o}C$ (without freezing), but not received under those conditions the laboratory shall reject the sample and arrangements shall be made for a replacement sample unless the sample is received within 2 hours of collection and is used for testing upon arrival or stored immediately at $\leq 6^{o}C$ (without freezing).

5.3 Maximum Holding Times

Samples shall be prepared and analyzed within the maximum holding times required by the method. Holding times are also listed in 40 C.F.R. 136. Samples that are prepared and analyzed outside of the maximum holding time shall not be submitted to Kentucky DOW for compliance purposes.

If the laboratory is not able to analyze the sample(s) within the required maximum holding time, arrangements shall be made for sample recollection with the client or shipment of samples to another certified wastewater laboratory in a timely manner with the client's documented permission.

5.4 Sample Collection and Transport

There shall be strict adherence to correct sampling procedures, sample handling, complete identification of the sample, and prompt transfer of the sample to the laboratory when required by the method. When the laboratory is not responsible for sample collection and transport, it shall verify that the paperwork, preservatives,

containers, and holding times are correct as required by the methods or notify the client, in writing, that the sample(s) are not valid for compliance purposes.

5.5 Sample Collector (if provided by the certified laboratory)

The sample collector shall have the proper training in sampling procedures and have suitable sampling instructions for each type of sample to be collected. Sample collectors shall be able to demonstrate proper sampling technique.

Field measurements made by the sample collector shall be recorded in the field logbook and follow the proper test procedures, including instrument calibration and maintenance. The sample collector shall be trained to test for the following parameters (as applicable): conductivity, pH, residual chlorine, dissolved oxygen, temperature, and turbidity. Training shall include the proper calibration, use, and storage of field instruments. Training records shall be maintained and available upon request.

5.6 Chain of Custody

The chain of custody shall contain, at a minimum, the identification, location, date and time of collection, collector's name, preservative added and shipping requirements, container type, container volume, sample type, analysis, and any special remarks concerning the sample. Indelible ink shall be used.

Field measurements performed by a certified field only laboratory must be recorded in a field notebook and transferred to the COC. Refer to Section 4.2 of Chapter II for specific requirements.

5.7 Sample Compositing

Compositing of field samples shall be performed in accordance with an approved procedure (e.g. QAP, discharge permit, etc.). Use of an automated field composite sampler shall be performed according to the manufacturer's specifications and meet the requirements of the specific program.

6.0 Quality Assurance / Quality Control

Quality assurance and quality control are fundamental concepts in the certification process. A certified laboratory shall properly document procedures and observations to ensure the quality of the reported results.

7.0 Quality Control Acceptance Criteria and Calculations

A certified laboratory shall establish acceptance criteria for all applicable quality control samples. These criteria shall follow the criteria established in Chapter II, Section 9.0.

These quality control requirements shall be included in the QAP or SOP with any applicable calculations (see Section 7.3).

7.1 Demonstration of Capability

An environmental testing laboratory submitting wastewater compliance or Kentucky Pollutant Discharge Elimination System (KPDES) permit effluent sample data to DOW shall use an EPA-approved wastewater method established in 40 C.F.R. 136. The laboratory shall demonstrate the capability to report the results of wastewater analyses at or below the required reporting limit (RRL) as established by either the Commonwealth of Kentucky or the EPA.

To effectively demonstrate method capability, the laboratory shall: 1) perform an initial demonstration of capability (IDC); 2) perform an on-going demonstration of capability (ODC) annually; 3) perform a method detection limit study (MDL) annually using 40 C.F.R. 136 Appendix B guideline; and 4) analyze a known standard at or below the RRL, and meet acceptance criteria, as part of their calibration/verification procedure.

7.1.1 IDC / ODC

An IDC and ODC are used to demonstrate that the laboratory and analyst are capable of performing analysis with acceptable precision, accuracy, sensitivity, and specificity pertaining to that particular method. This is done by analyzing four (4) mid-range concentration laboratory fortified blanks (LFB) on the same or different day, and then calculating the percent recovery for each. The percent recovery is used to verify that:

- each of the four IDCs (or ODCs) are within 80 to 120% of the mean value; and
- the calculated percent relative standard deviation (%RSD) is at or below 15%;
- Other limits may apply as specified in the method or individually approved by DOW based on method performance.

An IDC shall be performed initially by each analyst.

An ODC shall be performed annually by the primary analyst/technician and once per 5 year audit cycle for all back up analysts.

The Percent Relative Standard Deviation (%RSD) shall be calculated as follows:

$$\%RSD = [s / \bar{x}] \times 100\%$$

where s = standard deviation (n-1)

$\bar{x}$ = mean of 4 replicates

7.1.2 MDL

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the target analyte concentration is greater than zero. The MDL is determined from analysis of a sample in a given matrix containing the target analyte and is established using the EPA technique specified in 40 C.F.R. 136 Appendix B.

Laboratories that do not analyze an MDL per each instrument shall demonstrate that the Minimum Reporting Level (MRL) is achievable on all instruments that the MDL study is applied. For reference methods where the MDL is not applicable, the laboratory must document the procedure utilized for the determination of the MRL.

7.1.3 Reporting Limit Standard

In order to demonstrate the laboratory's capability to report down to the required reporting limit, the laboratory shall analyze a known standard at or below the required reporting limit. The reporting limit standard (RLS) shall be analyzed as either the lowest concentration calibration standard or as a stand-alone verification standard.

If the RLS is used as the lowest concentration calibration standard, it shall also be used to determine the acceptance of the calibration curve. The RLS shall meet the acceptance criteria set by the laboratory. The lowest value of the calibration standard cannot be ignored or not considered during any future verification of the calibration curve.

If the RLS is used as a stand-alone standard it shall meet the acceptance criteria set by the laboratory. The RLS shall be analyzed with each calibration curve but at least quarterly for all methods.

7.1.4 Summary of Demonstration of Capability Requirements, Acceptance Criteria, and Frequency (if other values are indicated in the method, they shall be utilized instead of the following table).

Demonstration	Parameter	Requirement	Frequency
IDC/ODC	IDC/ODC Replicates (4 mid-range LCS)	• Each shall be within 80 to 120% of the mean value; • %RSD shall be ≤15%.	Initial and Annual
MDL	See 40 CFR 136 Appendix B	• \ • Calculated MDL shall be less than the MRL •	See 40 CFR 136 Appendix B
RLS	Verification Standard	• Concentration shall be ≤ MRL. • Percent recovery shall be 70-130% unless demonstrated and documented to be unachievable.	Performed with each calibration, but at least quarterly

7.2 Minimum Quality Control and Acceptance Criteria

Laboratory quality control samples that are analyzed in accordance with the QAP or the individual methods shall have an associated frequency and acceptance limit criteria established by the laboratory.

7.3 Quality Control Calculations

General quality control calculations are provided below as guidance for certified laboratories:

Relative Percent Difference (RPD) - for duplicate measurements (precision) using:

$$RPD = \frac{|Dup_1 - Dup_2|}{(Dup_1 + Dup_2)/2} \times 100$$

Where Dup_1 = concentration of sample
Dup_2 = concentration of duplicate of sample

Relative Standard Deviation (RSD) – is used to express the precision and repeatability of a particular test method.

$$\%RSD = [s / \bar{x}] \times 100\%$$

where s = standard deviation (n-1)
$\bar{x}$ = mean

Percent recovery (%Rec) - for fortified samples (accuracy) using:

$$\%\ Rec = \frac{(A - B)}{C} \times 100$$

Where A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample
C = fortification concentration

7.4 Instrument Calibration

All instruments and equipment used within the laboratory shall be routinely calibrated by a laboratory analyst in accordance with the Quality Assurance Plan or Standard Operating Procedure (SOP). Many small instruments and measurement devices are also annually calibrated by a third-party calibration/verification service. Detailed calibration and continuing instrument calibration verification procedures shall be described in laboratory's QAP or SOPs.

Primary calibration standards used for calibration shall be purchased from a reputable dealer or prepared at the laboratory using appropriate grade material. All purchased primary standards shall be certified by the vendor for purity and identity and when available are NIST traceable. Vendor supplied Certificates of Analysis shall be requested and retained by the laboratory for a minimum of five (5) years.

Calibration standards (working standards) are dilutions or mixtures of stock standards used to calibrate an instrument. These standards are prepared or re-standardized according to the QAP or SOP. Second source standards are routinely used to validate primary calibration standards, technique, and methodology and when available are in the same matrix as the samples being analyzed. They are purchased or prepared from a different source than that used in the preparation of primary standards for use in the standard curve and are analyzed immediately following calibration. NIST traceable reference materials are used when available. Certificates of analysis shall be requested and retained by the laboratory for a minimum of five (5) years.

The calibration range defines how results are reported and samples are processed. Results below the low calibration standard shall be reported as less than (<) the Minimum Reporting Limit (MRL) (or appropriately qualified according to laboratories QAP or SOP). Results above the high calibration standard shall be diluted and reanalyzed so that the instrument reading is within the calibration range. Diluted sample results must be clearly identified to the laboratory user along with proper adjustments to both the MRL and MDL values.

Linear calibration curve (linear regression) shall be calculated as follows:

1. Plot all x,y pairs;
2. y = mx + b where: m=slope
   x = concentration
   y = instrument response
   b = y intercept

Other calibration options may be acceptable such as Quadratic (minimum of five calibration standards), Relative Standard Error, etc. If utilizing a calibration option other than linear regression, the laboratory shall ensure it is appropriate for the method in which it is being utilized and calculated following the method requirements.

An outlier calibration standard (other than a high point or a low point) may be eliminated only after an investigation has been performed and the reasons for the problem have been documented (e.g., statistical test, or review of standard preparation logs). At no time shall calibration point(s) be eliminated solely to meet or improve performance relative to calibration curve acceptance criteria. If a low calibration standard is removed, the laboratory MRL must be adjusted accordingly. If a high calibration standard is removed, the laboratory must use the next highest calibration standard as their standard for determining if sample reanalysis is required at a dilution.

8.0 Sample Management

Sample management is a key element in maintaining a high level of quality assurance and shall be documented in the laboratory's QAP or SOP. The certified laboratory shall be responsible for those elements of sample management over which it has control. The process that results in evidence that the integrity of samples has been maintained from the time of sampling until the analyses are completed shall be documented in the QAP. The documentation shall include sample preservation and storage and a complete chain-of-custody, including internal laboratory sample tracking.

9.0 Data Management

Data management requirements can be found in Chapter II, Sections 6.0 & 12.0.

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IV. CRITICAL ELEMENTS FOR MICROBIOLOGY

1.0 Analytical Methodology

The laboratory shall demonstrate that the filtration equipment, filters, sample containers, media, reagents, and other equipment (i.e., sealer) have not been contaminated through improper handling or preparation, inadequate sterilization, or environmental exposure.

1.1 General

1.1.1 For compliance samples, a laboratory shall use only an analytical methodology specified in 40 C.F.R. 136.

1.1.2 A laboratory shall be certified for all analytical methods that it uses for compliance purposes. At a minimum, the laboratory shall be certified for one *E. coli* method or fecal coliform, depending on which group of organisms the facility chooses to analyze.

1.1.3 Water samples shall be shaken before analyzing.

1.1.4 If dilution buffer or distilled water is used, check the accuracy of the buffer volume in one dilution bottle in each batch or lot. For a 90-mL or 99-mL volume, the tolerance shall be ±2 mL.

1.1.5 Sample volume analyzed for *E. coli* or fecal coliforms in wastewater shall report results per 100 mL. To assure accuracy and consistency within methods and between methods, it is important that the laboratory obtain precise measurement of the volume of sample to be analyzed. To ensure that the required volume of 100 mL is reported, no matter which of the approved methods the laboratory will be employing for analysis, a calibrated measuring vessel shall be used for measurement of the sample volume. It is inappropriate for a portion of the sample to be poured to waste in order to meet the required sample volume. If sample volume is adjusted, it shall be done utilizing sterile procedures (e.g., pipet, etc.).

1.1.6 Media (or defined substrate)

1.1.6.1 Dehydrated media shall be stored in a cool, dry location (unless specified otherwise by the manufacturer) and discarded by the manufacturer's expiration date. Caked or discolored media shall be discarded.

1.1.6.2 For media prepared in the laboratory, the date of preparation, type of medium, lot number, sterilization time and temperature, final pH, media expiration date, amount prepared, and the technician's initials shall be recorded.

1.1.6.3 For media prepared commercially, the date received, type of medium, manufacturer, lot number, pH verification (compare with manufacturer or

method pH), amount received, and expiration date for each lot shall be recorded. Media shall be stored according to manufacturer's instructions. Media shall be discarded by the manufacturer's expiration date.

1.1.6.4 The performance of each new lot of dehydrated or prepared commercial medium and each batch of laboratory-prepared medium shall be checked before use for sterility and with positive and negative culture controls. Record results and initial.

1.1.6.4.1 Laboratories shall perform positive and negative control tests on commercial and laboratory prepared media as well as verification and confirmation tests quarterly on all media with a shelf life of greater than 90 days, for the method(s) for which they are certified.

1.1.6.4.2 This may be accomplished by preparing 24-48 hour slant or broth culture of appropriate organisms. For methods designed to yield quantitative data, serial dilutions shall be prepared to obtain a reasonable number of viable organisms (approximately 20-80 colonies) with membrane filtration (MF) techniques and determinate values for multiple tube fermentation (MTF)/Quanti-Tray methods.

1.1.6.4.3 Control organisms (total coliforms, *E. coli*, and non-total coliforms) can be stock cultures or commercially available disks/swabs impregnated with the organism. Results shall be recorded. The analyst shall document ATCC strains used to QC each new lot/batch of media as well as monthly QC of the method. Commercial disks, ampules, or swabs containing control organisms shall be discarded by the manufacturer's expiration date. Laboratories that maintain stock cultures (agar plates/slants/broth) shall transfer cultures monthly and check for purity quarterly using MacConkey agar. However, no more than 5 transfers of a stock culture will be permitted. The laboratory shall start with new stock (commercial disk, ampule, swab) once the control organism has been transferred 5 times. Transfers/purity check of stock cultures shall be documented.

1.1.6.4.4 Each new batch (tube/plate) of laboratory prepared and each lot of commercially prepared media shall be checked for sterility by placing one/batch or lot in a 35°C±0.5ºC for 24 hrs. If growth occurs the entire lot/batch shall be discarded. This shall be performed prior to first use of medium.

1.1.6.5 If prepared medium is stored after sterilization, it shall be maintained in the dark, avoiding moisture loss, as per Table IV-1. Prepared plates may be stored in sealed plastic bags or containers. For either broth or agar media, each bag or container shall include the date prepared or an expiration date. If the medium is stored in a refrigerator, it shall be warmed to room temperature before use; tubes or plates that show growth and/or bubbles shall be discarded.

Liquid media shall be discarded if evaporation exceeds 10% of the original volume.

1.1.6.6 Enzyme substrate media shall not be prepared from basic ingredients, but rather purchased from a commercially available source.

1.1.6.7 Enzyme substrate media shall be protected from light.

1.1.6.8 Some lots of enzyme substrate media have been known to fluoresce. Therefore, each lot of medium shall be checked before use with a 365-366 nm ultraviolet light with a 6-watt bulb. For checking Colilert or Colilert-18 media, a packet of medium shall be dissolved in sterile water in a non-fluorescing vessel. If the medium exhibits faint fluorescence, the laboratory shall use another lot that does not fluoresce.

1.1.6.9 If the samples plus enzyme substrate media exhibit an inappropriate color change before incubation, the analysis shall be discarded and another lot of medium used.

Table IV-1
Maximum Holding Times and Temperatures for Laboratory Prepared Media

Container	Maximum storage temperature	Maximum storage time
Poured agar plates	1-5°C	2 weeks
Broth in tubes, bottles, or flasks with loose-fitting closures	1-30°C	2 weeks*
Broth in tightly closed screw-cap tubes, bottles or flasks	1-30°C	3 months

*M-Endo Broth – 96 hours after preparation when stored at 1-5°C

2.0 Laboratory Facility

The laboratory shall maintain effective separation between areas where activities are incompatible, minimize traffic flow, and ensure that contamination does not adversely affect data quality.

3.0 Laboratory Equipment and Supplies

The laboratory shall have the equipment and supplies needed to perform the approved methods for which certification has been requested.

3.1 Temperature Monitoring Device

3.1.1 For 35°C incubators, 41±0.5°C, and 44.5°C water baths (or incubator), glass, dial or electronic thermometers shall be graduated in 0.1°C increments, except as noted for hot air ovens (3.4.1) and refrigerators (Chapter II Section 8.4). Dial thermometers that cannot be calibrated shall not be used. The fluid column in glass thermometers shall not be separated.

3.1.2 Continuous recording devices that are used to monitor incubator temperatures shall be recalibrated at least annually.

3.2 Incubator Unit

3.2.1 Incubator units shall have an internal temperature monitoring device and maintain the temperature specified by the method used, usually 35°±0.5°C, 41°±0.5°C, or 44.5°±0.2°C.

3.2.2 Calibration-corrected temperature shall be recorded for each thermometer being used at least twice per day during each day the incubator is in use, with readings separated by at least 4 hours. Documentation shall include the date and time of reading, temperature, and technician's initials.

3.3 Autoclave

3.3.1 Pressure cookers shall not be used.

3.3.2 The autoclave shall have an internal heat source, a temperature gauge with a sensor on the exhaust, a pressure gauge, and an operational safety valve. The autoclave shall maintain a sterilization temperature of 119º-124°C during the sterilizing 121º C cycle and complete an entire cycle (i.e., time between starting autoclave and removing items from autoclave) within 45 minutes when a 12-15 minute sterilization period for media is used. The autoclave shall depressurize slowly enough to ensure that media will not boil over and bubbles will not form in inverted tubes.

3.3.3 The date, contents, sterilization time and temperature, total time in autoclave, and analyst's initials shall be recorded each time the autoclave is used. Copies of the service contract or internal maintenance protocol and maintenance records shall be kept. Maintenance shall be conducted at least annually and shall include a pressure check and calibration of temperature device. A record of the most recent service performed shall be on file and available for inspection.

3.3.4 A maximum-registering-temperature thermometer (MRT), electronic temperature read-out device, or continuous recording device shall be used during each autoclave cycle to ensure that the proper temperature was reached, and the temperature recorded (print out acceptable). The MRT shall be placed in chamber

of autoclave in an upright position in a flask (or beaker) with water. Once the sterilization cycle has been completed, remove MRT and place at room temperature (upright position in empty flask), for 5 minutes. Read and record results in autoclave log. Overcrowding shall be avoided.

3.3.4.1 Spore strips or spore ampules shall be used monthly as bioindicators to confirm sterilization and results, and lot number of bioindicator shall be recorded and initialed by analyst. Diacks, temperature sensitive tape, or equivalent indicator system may be used as a routine sterilization check, but shall not be used for monthly QC check of autoclave, nor do they replace the use of the MRT thermometer or its equivalent.

3.3.4.2 The calibrated corrected temperature (55º-60°C) for the Bioindicator incubator thermometer shall be recorded for each day of use.

3.3.5 Automatic timing mechanisms shall be checked quarterly for sterilization time (15 min) using the liquid cycle as well as total cycle time (45 min) with a stopwatch or other accurate timepiece or time signal, and the results recorded and initialed.

3.3.6 Autoclave door seals shall be clean and free of caramelized media.

3.3.7 All microbiological waste (e.g., positive enzyme substrate tests [MPN], MF tests, stock cultures, etc.) shall be decontaminated in an autoclave [119°-124ºC] for a minimum of 30-45 minutes or decontaminated by an approved commercial vendor that is licensed by the state of Kentucky or appropriate federal agency. The lab shall maintain a log documenting items that were decontaminated.

3.4 Hot Air Oven

3.4.1 The oven shall maintain a stable sterilization temperature of 170°-180°C for at least two hours. Overcrowding shall be avoided.

3.4.2 The date, contents, sterilization time and temperature, and analyst's initials shall be recorded.

3.4.3 Spore strips shall be used monthly to confirm sterilization.

3.5 Conductivity Meter

3.5.1 Meters shall be suitable for checking laboratory reagent-grade water and readable in units of either µmho/cm or µS/cm.

3.5.2 The meter shall be calibrated at least monthly, following the manufacturer’s recommendations and using an appropriate certified and traceable low-level standard. The analyst shall record the lot number of the standard used for

calibration. If the meter cannot be calibrated with a commercial standard, the cell constant shall be determined at monthly intervals, using an approved method.

3.5.3 If an in-line unit cannot be calibrated or the calibration verified, it shall not be used to check reagent-grade water.

3.6 Inoculating Equipment

3.6.1 Sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipet tips shall be used. If wood applicator sticks are used, they shall be sterilized by dry heat. The metal inoculating loops and/or needles shall be made of nickel alloy or platinum. (When performing an oxidase test, nickel alloy loops shall not be used).

3.7 Membrane Filtration Equipment

3.7.1 MF units shall be stainless steel, glass, or autoclavable plastic, not scratched or corroded, and shall not leak.

3.7.2 If graduation marks on clear glass or plastic funnels are used to measure sample volume, their accuracy shall be checked with a Class B graduated cylinder or better (or other Class B glassware), and record of this calibration check retained. Tolerance shall be ≤ 2.5%.

3.7.3 A 10X to 15X stereo microscope with a fluorescent light source shall be used to count the target colonies (e.g., sheen colonies on M-Endo broth or Endo LES media).

3.7.4 Membrane filters shall be approved by the manufacturer for fecal coliform/*E. coli* wastewater analysis. Approval is based on data from tests for toxicity, recovery, retention, and absence of growth-promoting substances. Filters shall be cellulose ester, white, grid marked, 47 mm diameter, and 0.45μm pore size, or alternate pore sizes if the manufacturer provides performance data equal to or better than the 0.45μm pore size. Membrane filters and pads shall be purchased presterilized or autoclaved for 10 minutes at 121°C before use. Each new lot shall be checked for sterility by placing one filter per lot into sterile tryptic soy broth and incubating at 35°±0.5°C for 24 hrs. Note: The single strength tryptic soy broth (TSB) used to perform the sterility check of each new lot of membrane filters shall be listed on the quality control log along with the date sterility check was performed, date results were checked, date batch was prepared or lot number (if commercially prepared), and the analyst's initials.

3.7.5 The lot number for membrane filters and the date received shall be recorded. Ensure that membrane filters are not brittle or distorted, and that manufacturer's specification/certification sheet is available.

3.7.6 Forceps used shall be blunt and smooth-tipped without corrugations on the inner sides of tips.

3.7.7 At least one membrane filter and filtration unit sterility check shall be conducted at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter and testing for growth. If the control indicates contamination, all data from affected samples shall be rejected and an immediate resampling shall be requested within 24 hours of rejection. A filtration series ends when 30 minutes or more elapse between sample filtrations.

3.7.8 Each filtration funnel shall be rinsed after each sample filtration with two or three 20-30 mL portions of sterile rinse water to ensure that entire sample is rinsed off the funnel before the filter is removed. After the filter is removed, the funnel may be rinsed again with two or three 20-30 mL portions of sterile rinse water or exposed to UV light with a 254-nm wavelength for at least two minutes to prevent carry-over between samples, especially for surface water samples.

3.8 Culture Dishes (loose or tight lids)

3.8.1 Presterilized plastic or sterilizable glass culture dishes shall be used. To maintain sterility of glass cultured dishes, use stainless steel or aluminum canisters, or a wrap of heavy aluminum foil or char-resistant paper.

3.8.2 Loose-lid Petri dishes shall be incubated in a tight-fitting container, e.g., plastic vegetable crisper containing a moistened paper towel to prevent dehydration of membrane filter and medium.

3.8.3 Opened packs of disposable culture dishes shall be resealed between use periods.

3.8.4 For membrane filter methods, culture dishes shall be of an appropriate size to allow for the transfer of a single membrane per plate.

3.9 Pipets

3.9.1 To sterilize and maintain sterility of glass pipets, stainless steel or aluminum canisters shall be used, or individual pipets shall be wrapped in char-resistant paper or aluminum foil.

3.9.2 Opened packs of disposable sterile pipets shall be resealed between use periods.

3.9.3 Calibrated micropipettes may be used if tips are sterile.

3.10 Glassware and Plasticware

3.10.1 Glassware shall be borosilicate glass or other corrosion-resistant glass and free of chips and cracks. Markings on graduated cylinders and pipets shall be legible. Plastic items shall be clear and non-toxic to microorganisms.

3.11 Sample Containers

3.11.1 Sample containers shall be wide-mouth plastic or non-corrosive glass bottles with non-leaking ground glass stoppers or caps with non-toxic liners that shall withstand repeated sterilization, or sterile plastic bags containing sodium thiosulfate. Other appropriate sample containers may be used.

3.11.2 Glass stoppers shall be covered with aluminum foil or char-resistant paper for sterilization.

3.11.3 Glass and plastic bottles that have not been presterilized shall be sterilized by autoclaving. Glass bottles may also be sterilized by dry heat.

3.11.4 If chlorinated water is to be analyzed, sufficient sodium thiosulfate ($Na_2S_2O_3$) shall be added to the sample bottle before sterilization to neutralize any residual chlorine in the water sample.

3.12 Ultraviolet Lamp (shortwave and longwave, if used)

3.12.1 The shortwave germicidal unit (254nm) shall be disconnected monthly and lamps cleaned by wiping with a soft cloth moistened with ethanol. The longwave unit (365-366nm), used for fluorometric tests, shall also be kept clean in the same manner.

3.12.2 If a shortwave germicidal UV unit (254nm) is used for sanitization, the unit shall be tested quarterly with a UV light meter or agar spread plate. The lamp shall be replaced if it emits less than 70% of its initial output or if an agar spread plate containing 200 to 250 microorganisms, exposed to the UV light for two minutes, does not show a count reduction of 99%.

3.12.3 A longwave UV lamp (365-366nm) with 6 watt bulb shall be used with enzyme substrate media for the detection of *E. coli* (Colilert, EC/MUG, or NA/MUG).

3.12.4 If a longwave UV lamp is used to determine fluorescence, the lamp shall be tested quarterly with a longwave UV meter or a positive control (Colilert, EC/MUG or NA/MUG). The lamp shall be replaced when the emission of the lamp is less than 70% of its initial output or when the fluorescence of the positive control is significantly diminished.

3.13 Tray Sealer

3.13.1 The sealer shall be checked monthly by adding a dye to the water. If dye is observed outside the wells, either perform maintenance or use another sealer.

4.0 General Laboratory Practices

4.1 Sterilization Procedures

4.1.1 Autoclaving times at 121°C are listed in Table IV-2 below. Except for membrane filters and pads and carbohydrate-containing media, indicated times are minimum times and may necessitate adjustment depending upon volumes, containers, and loads. Carbohydrate-based media shall not be over-sterilized.

Table IV-2 Autoclaving Times

Item	Time (min)
Membrane filters & pads	10
Carbohydrate containing media	12-15[1]
Contaminated test materials	30[2]
Membrane filter assemblies	15
Sample collection bottles	15
Individual glassware	15
Dilution water blank	15
Rinse Water (0.5-1L)	15-30[2]

1 except when otherwise specified by the manufacturer
2 time depends upon water volume per container and autoclave load

4.1.2 Autoclaved membrane filters and pads and all media shall be removed immediately after completion of the sterilization cycle.

4.1.3 Membrane filter equipment shall be autoclaved before the beginning of a filtration series. A filtration series ends when 30 minutes or longer elapses after a sample is filtered.

4.1.4 Ultraviolet light (254 nm) may be used to sanitize equipment (after initial autoclaving for sterilization), if all supplies are presterilized. Ultraviolet light may be used to reduce bacterial carry-over between samples during a filtration series.

4.2 Sample Containers

4.2.1 At least one sample container shall be selected at random from each batch or lot of sterile sample bottles or other containers, and the sterility confirmed by adding approximately 25 mL of a sterile non-selective broth (e.g., tryptic soy, trypticase soy, or tryptone broth). The broth shall be incubated at 35°±0.5°C, and checked

after 24 and 48 hours for growth. Record results. Re-sterilize entire batch if growth is detected.

4.2.2 A blank tray/bottle (with sterile water and media) shall be processed at least once daily to ensure the sterility of the trays, bottles, media, and sealer.

4.3 Reagent-Grade Water

4.3.1 Only satisfactorily tested reagent water from distillation or deionization units shall be used to prepare media, reagents, and dilution/rinse water for performing microbial analyses.

4.3.2 The quality of the reagent water shall be tested and shall meet the following criteria:

TABLE IV-3

PARAMETER	LIMITS	FREQUENCY
Conductivity	<2 microhms/cm (microsiemens/cm) at 25°C or >0.5 megohms resistance	Monthly[4]
Pb, Cd, Cr, Cu, Ni, Zn	Not greater than 0.05mg/L per contaminant. Collectively, not greater than 0.1mg/L	Annually
Total Chlorine Residual[1]	<0.1mg/L	Monthly
Heterotrophic Plate Count[2]	<500 CFU/mL[5]	Monthly
Bacteriological Quality of Reagent Water[3]	Ratio of growth rate 0.8 to 3.0	Annually

1DPD Method shall be used. Not required if source water is not chlorinated.
2 Pour Plate Method or SimPlate MethodSee *Standard Methods*.
3 See *Standard Methods*.
4 Monthly, if meter is in-line or has a resistivity indicator light; otherwise, with each new batch of reagent water.
5 CFU means colony-forming units (same as colonies, but is a more precise term).

4.4 Dilution/Rinse Water

4.4.1 Stock buffer solution or peptone water shall be prepared, as specified in an approved method.

4.4.2 Stock buffers shall be autoclaved or filter-sterilized, and containers shall be labeled and dated. Stock buffers shall be refrigerated. Stored stock buffers shall be free from turbidity.

4.4.3 Each batch (or lot, if commercially prepared) of dilution/rinse water or distilled water shall be checked for sterility by adding 50 mL of water to 50 mL of a double strength non-selective broth (e.g., tryptic soy, trypticase soy or tryptose

broth). Incubate at 35°±0.5°C, and check for growth after 24 and 48 hours. Record results. Discard batch if growth is detected.

4.5 Glassware Washing

4.5.1 Distilled or deionized water shall be used for final rinse.

4.5.2 A glassware inhibitory residue test shall be performed before the initial use of a washing compound and whenever a different formulation of washing compound, or washing procedure, is used. Record results.

4.5.3 Prior to the initial use of a washing compound and whenever a different formulation of washing compound, or washing procedure is utilized, check dry glassware for pH reaction. Use 0.04% bromothymol blue (or equivalent pH indicator) and observe color reaction. Clean glassware without an alkali or acid residual shall have a neutral color reaction (blue-green for bromthymol blue). Record results.

5.0 Sample Collection, Handling, and Preservation

Sections 5.1 is applicable to those laboratories that collect samples; all laboratories are responsible for sections 5.2 and 5.3.

5.1 Sampling

5.1.1 Each laboratory shall have a procedure manual that includes all the SOPs used for its self-monitoring program. The facility's SOPs shall cover sampling, equipment calibration and maintenance, analytical methods, quality control activities, and laboratory data handling and reporting. Facility SOPs shall include enough detail to use this document as a training manual for new employees.

- Samples shall be representative of the sample site.
- The sample volume shall be sufficient to perform all the tests required.
- Sample collection and analysis schedules for parameters specified in permit and/or other tests not covered in permit, but are used to determine plant performance.
- Sample collection locations.
- Sample types such as grab, composite or flow proportioned composites including instructions on sampler setup.
- Sample handling requirements such as sampling containers, preservatives (e.g. acid, thiosulfate, refrigeration etc.), and holding time.

5.2 Sample Icing

5.2.1 Samplers shall maintain samples at <10°C during transit to the laboratory unless the time since sample collection has been less than two hours. Laboratories shall reject samples that have been frozen.

5.2.2 Sample temperature upon receipt shall be recorded. A sample that has a temperature upon receipt of >10°C, shall be flagged unless the time since sample collection has been less than two hours.

5.3 Sample Holding/Travel Time

5.3.1 Per 40 C.F.R. 136, the time from collection to placement into an incubator shall not exceed 8 hours.

6.0 Quality Assurance

6.1 Proficiency Testing

A microbiology laboratory shall successfully analyze at least one PT sample annually for each method which they are certified. Each PT shall be used only with a single analytical method. The PT sample shall not be split in any manner. Discharge Monitoring Report- Quality Assurance (DMR-QA) Program participation is an acceptable alternative for a PT Study.

6.2 Proficiency Testing Required for Each Method

Unless otherwise specified, each method is considered an independent method.

7.0 Records and Data Reporting

See Chapter II Section 6.0 & 12.0

8.0 Calculation and Reporting of Results

8.1 Discharge Monitoring Reports and TNTC/CNFG Results

Results that are TNTC or CNFG are not appropriate for wastewater microbial data analysis, and cannot be entered into the discharge monitoring report (DMR). They shall not be utilized on a report. A result of TNTC shall be reported as 60,000 cfu/100mL. A result of CNFG shall require a replacement sample be collected within 24 hours of the results.

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V. CRITICAL ELEMENTS FOR WHOLE EFFLUENT TOXICITY

1.0 Effluent/Receiving Water Sampling and Sample Handling

1.1 Introduction

The purpose of this section is to provide requirements for the handling of effluent and receiving water samples that will ensure the proper completion of permit-required toxicity tests. It is understood that all of the conditions and requirements described herein may not be appropriate for all KPDES-permitted discharge situations. Therefore, deviations from such conditions may be allowed if approved by DOW prior to use. These deviations shall be provided to the laboratory. Sampling requirements stated in this section are the responsibility of those individuals taking the samples.

Sampling activities are not covered within the scope of the certification program but are established utilizing a standard operating procedure or a quality assurance project plan and be consistent with the compliance program's DQO.

1.2 Sample Handling, Preservation, and Shipping

1.2.1 Effluent Sample Holding Time

1.2.1.1 The following holding times shall apply to all effluent samples. Sample holding time begins when a grab sample is collected or when a composite sampling period is completed.

1.2.1.2 Maximum holding time prior to the initial use of an effluent sample for toxicity testing shall be 36 hours after the completion of sample collection. Time of "initial use" is defined as the point in time when organisms have been introduced into test chambers for all tests.

1.2.2 Receiving Water Sample Holding Time

1.2.2.1 The following holding times shall apply to all effluent samples. Sample holding time begins when a grab sample is collected or when a composite sampling period is completed.

1.2.2.2 Maximum holding time prior to the initial use of an effluent sample for toxicity testing shall be 36 hours after the completion of sample collection. Time of "initial use" is defined as the point in time when organisms have been introduced into test chambers for all tests.

1.2.2.3 There is no maximum holding time prior to final use for receiving water samples. However, samples shall be stored at $\leq 6^{o}C$ (without freezing), in the dark until used and when not in use. A receiving water sample may be used

throughout the test as long as the sample is first used within the 36-hour holding time specified above.

1.2.3 Sample Shipping

1.2.3.1 Effluent samples shall be shipped with ice or under other refrigerated conditions to the performing laboratory. Commercial freezer packs ('Blue ice') shall not be used.

1.2.3.1.1 Every effort shall be made to maintain a sample temperature of $\leq$ 6ºC (without freezing), which may require samples to be pre-chilled in a refrigerator prior to shipment. At a minimum, adequate refrigeration (ice or mechanically chilled) shall be provided with the sample in the shipping container to ensure that the sample temperature does not exceed 6ºC (without freezing) upon arrival at the performing laboratory. It is acceptable to place the sample containers in plastic bags to preserve sample and label integrity.

1.2.4 Sample Receiving

1.2.4.1 Upon receiving effluent or receiving water samples in the laboratory, the temperature and pH of the sample, date and time of receipt, and the initials of the laboratory personnel receiving the sample shall be recorded

1.2.4.2 All samples shall be stored at $\leq$ 6ºC (without freezing), in the dark until used and when not in use.

1.2.4.3 Any sample shall be rejected and arrangements made for a replacement if the sample is not received at $\leq$ 6ºC (without freezing), unless the sample is received within 2 hours of the end of the collection period and the sample is used for testing upon arrival or stored immediately in the dark at $\leq$ 6ºC (without freezing).

2.0 Reference Documents

Unless otherwise specified in this manual, laboratories shall follow the guidelines of these documents:

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th Edition.

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Edition.

3.0 Test Report Requirements

3.1 See Chapter II Sections 6.0 & 12.0

VI. CONTACT INFORMATION

A complete application for certification shall be sent to:

Kentucky Department for Environmental Protection
Division of Water
Attn: Laboratory Certification
300 Sower Boulevard; 3rd Floor
Frankfort, KY 40601

Checks shall be made payable to:

Kentucky State Treasurer

Mail to:
Kentucky Department for Environmental Protection
Division of Water
Attn: Laboratory Certification
300 Sower Boulevard; 3rd Floor
Frankfort, KY 40601

To speak to Laboratory Certification personnel call (502) 564 – 3410.

For more information go to our website:

http://water.ky.gov

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Appendix A
Glossary of Acronyms

ASTM	American Society of Testing and Materials
CAL	Calibration Standard
CCC	Continuing Calibration Check Standard
CCV	Continuing Calibration Verification
C.F.R.	Code of Federal Regulations
CFU	Colony Forming Units
CNFG	Confluent Growth
CO	Certification Officer
COC	Chain-of-Custody
CPM	Certification Program Manager
CV	Coefficient of Variation
CWA	Clean Water Act
DMR	Discharge Monitoring Report
DO	Dissolved Oxygen
DOW	Kentucky Division of Water
DQO	Data Quality Objectives
EPA	United States Environmental Protection Agency
FD	Field Duplicate
FRB	Field Reagent Blank
ICAL	Initial Calibration Standard
ICV	Initial Calibration Verification Standard
IDC	Initial Demonstration of Capability

IPC	Initial Performance Check
IS	Internal Standard
ISO	International Organization for Standardization
KPDES	Kentucky Pollutant Discharge Elimination System
LCS	Laboratory Control Sample
LD	Laboratory Duplicate
LDR	Linear Dynamic Range
LFB	Laboratory Fortified Blank
LFD	Laboratory Fortified Matrix Duplicate
LFM	Laboratory Fortified Sample Matrix
LMB	Laboratory Method Blank
LPC	Laboratory Performance Check Solution
LRB	Laboratory Reagent Blank
LTB	Laboratory Trip Blank
MDL	Method Detection Limit
MF	Membrane Filtration
MPN	Most Probable Number
MRL	Minimum Reporting Level
MRT	Maximum Registering Temperature Thermometer
MS	Matrix Spike
MSD	Matrix Spike Duplicate
MSDS	Material Safety Data Sheet
MTF	Multiple Tube Fermentation

NIST	National Institute of Standard Technology
OCW	Office of Clean Water
ODC	Ongoing Demonstration of Capability
OGWDW	U.S. EPA Office of Ground Water and Drinking Water
ORD	U.S. EPA Office of Research and Development
PDS	Primary Dilution Standard Solution
PT	Proficiency Test Sample
QA	Quality Assurance
QAM	Quality Assurance Manager
QAP	Quality Assurance Plan
QC	Quality Control
QCS	Quality Control Sample
RLS	Reporting Limit Standard
RRL	Required Reporting Limit
SD	Standard Deviation
SSCV	Second Source Calibration Verification
SOP	Standard Operating Procedure
SSS	Stock Standard Solution
SUR	Surrogate
TNTC	Too Numerous to Count
TNI	The NELAC Institute
TSB	Tryptic Soy Broth
WET	Whole Effluent Toxicity

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Appendix B
Definitions and Laboratory Terms

Accuracy: A measure of the closeness of an individual measurement or the average of a number of measurements to the true value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations. Refer to *Standard Methods*, Data Quality Section for a more detailed explanation.

Acute: Lethality or other harmful effect sustained by either an indigenous aquatic organism or a representative indicator organism used in a toxicity test, due to a short-term exposure, of ninety-six (96) hours or less, to a specific toxic substance or mixture of toxic substances.

Aliquot: Contained an exact number of times in something; a division or part. In this manual it is used to mean a portion of the whole sample.

Ambient: The conditions of the environment into which an effluent is received, as it would be in its natural, unaltered state.

Assessment: An all-inclusive term used to denote any of the following: audit, performance evaluation, management systems review, peer review, inspection, or surveillance.

Batch: A group of samples that are prepared and analyzed as a group.

Bias: The systematic or persistent distortion of a measurement process which causes errors in one direction.

Biased Sampling: A collection scheme based on a very limited or prejudiced non-random collection of specimens.

Blank / Blank Sample: A specimen that is intended to contain none of the analytes of interest and which is subjected to the usual analytical or measurement process to establish a zero baseline or background value. Some examples are trip, bottle, equipment, instrument, reagent, and method blanks..

Calibration: Comparison of a measurement standard, instrument, or item with a standard or instrument of higher accuracy to detect and quantify inaccuracies and to report or eliminate those inaccuracies by adjustments.

Calibration Blank: A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to auto-zero the AA instrument.

Calibration Standard (CAL): A solution prepared from the primary dilution standard solution or stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

Chain-of-custody (COC): An unbroken trail of accountability that ensures the physical security of samples, data, and records.

Chronic: Lethality, reduced growth or reproduction, or other harmful effect sustained by either indigenous aquatic organisms or representative indicator organisms used in toxicity tests due to long-term exposures, relative to the life span of the organisms or a significant portion of their life span, to toxic substances or mixtures of toxic substances.

Coefficient of Variation (CV): The standard deviation divided by the mean; a unit-free measure of variability. Also known as Relative Standard Deviation.

Compliance sample: Any sample submitted to DOW under a permitting program to fulfill requirements of a permit.

Composite sample: A combination of individual samples of equal volume taken at approximately equal intervals not exceeding one hour over a specified period of time.

Confirmation: Verification of the presence of a component through the use of an analytical technique based on a different scientific principle from the original method (e.g., second column, alternate wavelength or detector, etc.).

Continuing Calibration Check Standard (CCC): A standard containing one or more of the target analytes that is prepared from the same standards used to calibrate the instrument. This standard is used to verify the calibration curve at the beginning of each analytical sequence. It may also be analyzed throughout and at the end of the sequence. The concentration of continuing standards may be varied, when prescribed by the reference method, so that the range of the calibration curve is verified on a regular basis.

Continuing Calibration Standard(s) (CCAL): A standard containing one or more of the target analytes that is prepared from the same standards used to calibrate the instrument. This standard is used to verify the calibration curve at the beginning of each analytical sequence. It may also be analyzed throughout and at the end of the sequence. The concentration of continuing standards may be varied, when prescribed by the reference method, so that the range of the calibration curve is verified on a regular basis.

Continuing Calibration Verification (CCV): See Continuing Calibration Check Standard (CCC).

Control Chart: A graphic representation of the variability in a measurement process generally plotted in order over time. A chart with upper and lower control limits on which values of some statistical measure for a series of samples or subgroups are plotted.

Control Treatment: An exposure of the test organisms to dilution water with no effluent added; used as a standard of comparison in judging toxic effects and the validity of data.

Corrective Action: Any measures taken to rectify conditions adverse to quality or to eliminate the causes of an existing nonconformity, defect, or other undesirable situation in order to prevent reoccurrence.

Corrective Action Plan: A scheme for amending a measurement or management process.

Data Audit: A qualitative and quantitative evaluation of the documentation and procedures associated with measurements to verify that the resulting data are acceptable.

Data Reduction: The process of transforming the number of data items by arithmetic or statistical calculations, standard curves, concentration factors, etc. and collation into a more useful form. Data reduction is irreversible and generally results in the loss of detail.

Data Quality Objectives (DQO): Qualitative and quantitative specifications used to design a study that will limit uncertainty to an acceptable level.

Data Validation: An analyte and sample specific process that extends the evaluation of data beyond method, procedural, or contractual compliance (i.e. data verification) to determine the analytical quality of a specific data set.

Data Verification: The process of evaluating the completeness, correctness, and conformance/compliance of a specific data set against the method, procedural, or contractual requirements.

Deficiency: An unauthorized deviation from acceptable procedures or practices, or a defect in an item that may render it as unacceptable or indeterminate; nonfulfillment of a specification or standard.

Detection: Any concentration of an analyte which equals or exceeds the laboratory's detection limit.

Duplicate: Repeat; usually provided because it permits easier viewing and counting of test organisms, avoids possible violations of loading limits, and ensures against the invalidation of the test which might result from accidental loss of a test vessel, where all of the test organisms for a given treatment are in a single chamber.

Field Duplicates (FD1 and FD2): Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

Field Reagent Blank (FRB): An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

Good Laboratory Practices: The established guidelines to ensure reporting of high quality and reliable data to support the research or marketing efforts for experimentation or product manufacturing processes regulated by governmental agencies

Grab sample: A single sample taken at one moment of time or a combination of several smaller samples of equal volume taken in less than a two-minute period.

Holding time: The allowed time from when a sample was taken (or extracted) until it shall be analyzed.

Initial Calibration Standard (ICAL): A solution prepared from the primary dilution standard solution or stock standard solutions and diluted as needed to prepare an initial calibration curve.

Initial Calibration Verification Standard (ICV): See Continuing Calibration Check Standard (CCC).

Initial Demonstration of Capability (IDC): Demonstration of acceptable precision, accuracy, sensitivity, and specificity for the method to be used performed prior to analyzing compliance samples.

Initial Performance Check (IPC): See Initial Calibration Check Standard (ICCS).

Initial Use: The point in time when organisms have been introduced into test chambers for all tests to begin the permit required exposure period, and end the required initial holding time of the sample.

Instrument Performance Check Solution (IPC): A solution of method analytes, used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

Internal Standard (IS): A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution, and is not an analyte that is not a sample component..

Laboratory Control Sample (LCS): See Laboratory Fortified Blank.

Laboratory Duplicates (LD1 and LD2): Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

Laboratory Fortified Blank (LFB): (Spike) is an aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

Laboratory Fortified Matrix Duplicate (LFD): A sample aliquot taken from the same field sample source as the Laboratory Fortified Sample Matrix (LFM) to which known quantities of the analytes of interest are added in the laboratory. The LFD is analyzed exactly the same as the field samples. Analysis of the LFD provides a measure of the precision of the laboratory procedures in a specific matrix.

Laboratory Fortified Sample Matrix (LFM): An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix shall be determined in a separate aliquot and measured values in the LFM corrected for background concentrations.

Laboratory Method Blank (LMB): See Laboratory Reagent Blank.

Laboratory Performance Check Solution (LPC): A solution of one or more compounds (analytes, surrogates, internal standards, or other test compounds) used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

Laboratory Qualifier: Code (flag) applied to the data by the contract analytical laboratory to indicate a verifiable or potential data deficiency or bias.

Laboratory Reagent Blank (LRB): (Method blank) is an aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

Laboratory Trip Blank (LTB): A sample of laboratory reagent water placed in a sample container in the laboratory and treated as a field sample, including storage, preservation, and all analytical procedures. The LTB container follows the collection bottles to and from the collection site, but the LTB is not opened at any time during the trip. LTB is not exposed to site conditions or pumping and collection equipment. The LTB is primarily a travel blank used to verify that the samples were not contaminated during shipment.

Linear Dynamic Range (LDR): The concentration range over which the instrument response to an analyte is linear.

Material Safety Data Sheet (MSDS): A product fact sheet that explains certain characteristics about the product such as reactivity, corrosiveness, and flammability and may contain product toxicity data.

Matrix: The material of which the sample is composed or the substrate containing the analyte of interest, such as drinking water, wastewater, air, soil/sediment, biological material, etc. Also called medium or media.

Matrix Spike Duplicate Sample (MSD): See Laboratory Fortified Matrix Duplicate (LFD).

Matrix Spike Sample (MS): See Laboratory Fortified Sample Matrix (LFM).

Method Detection Limit (MDL): The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The MDL is determined from analysis of a sample in a given matrix containing this analyte. See 40 C.F.R. 136 Appendix B.

Minimum Reporting Level (MRL): The lowest concentration of an analyte that can be reliably quantified that is greater than the method detection limit, of sufficient accuracy and precision to meet the intended purpose, and acceptable quality control criteria for the analyte at this concentration are met. This defined concentration can be no lower than the concentration of the lowest calibration standard for that analyte or, in non-calibrated methods, the limitations defined by the equipment and volumes utilized.

Mixing Zone: a mixing zone is an area where an effluent discharge undergoes initial dilution and is extended to cover the secondary mixing in the ambient water body. A mixing zone is an allocated impact zone where water quality criteria can be exceeded as long as acutely toxic conditions are prevented.

Ongoing Demonstration of Capability (ODC): Demonstration of acceptable precision, accuracy, sensitivity, and specificity for the method to be used, performed on an annual basis.

Permit effluent sample: A sample submitted under a permitting program that relates directly to a permitted discharge.

Positive Control Sample: A prepared standard that undergoes an analytical procedure to provide comparison with an unknown specimen thereby monitoring recovery to assure that a test and/or its components are working properly and producing correct or expected results

Precision: The closeness of repeated measurements on the same parameter within a sample.

Primary Control: An exposure of the test organisms to dilution water with no effluent added; used as a standard of comparison in judging toxic effects and the validity of data.

Primary Dilution Standard Solution (PDS): A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

Proficiency Testing Samples (PTs): A sample provided to a laboratory for the purpose of demonstrating that the laboratory can successfully analyze the sample within specified acceptance limits specified in the regulations. The qualitative and/or quantitative composition of the reference material is unknown to the laboratory at the time of the analysis. Also known as a Performance Evaluation Sample.

Quality: The totality of features and characteristics of a product or service that bear on its ability to meet the stated or implied needs and expectations of the user.

Quality Assurance (QA): An integrated system of management activities involving planning, quality control, quality assessment, reporting, and quality improvement to ensure that a product or service meets defined standards of quality with a stated level of confidence.

Quality Assurance Manager (QAM): The individual designated as the principal manager within the organization having management oversight and responsibilities for planning, documenting, coordinating, and assessing the effectiveness of the quality system for the organization.

Quality Assurance Plan (QAP): A comprehensive plan detailing the aspects of quality assurance needed to adequately fulfill the data needs of a program.

Quality Control (QC): The overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it meets the needs of the users; operational techniques and activities that are used to fulfill requirements for quality.

Quality Control Sample (QCS): A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards ('second source'). The QCS is used to check laboratory performance with externally prepared test materials.

Raw Data: The documentation generated during sampling and analysis that includes, field notes, hardcopies of electronic data, disks, untabulated sample results, QC sample results, printouts of chromatograms, instrument outputs, and handwritten notes.

Recovery: The act of determining whether or not the methodology measures all of the analyte contained in a sample, often expressed in percent recovered.

Reporting Limit Standard (RLS): A procedural standard that is analyzed to evaluate instrument performance at or below the minimum reporting limit.

Representative Sample: A subset of a population that reflects what should occur under normal operating conditions.

Required Reporting Limit (RRL): The minimum limit that can be reported and meet the limits established within the KPDES Permit.

Sample: A single item or specimen selected from a larger population, such as any subset of a population of any medium (air, water, soil, etc.) used to characterize or make inferences regarding that population.

Sample Batch: See Batch.

Second Source Calibration Verification (SSCV): See Quality Control Sample (QCS).

Sensitivity: The capability of a method or instrument to discriminate between measurement responses representing different levels of a variable of interest.

Slug loads: Random doses of a chemical or compound in an influent that is not normally present at such concentrations.

Spike: A known quantity of an analyte added to a sample for the purpose of determining recovery or efficiency (analyst spikes), or for quality control (blind spikes). See matrix spike sample and laboratory fortified sample matrix.

Split/Split Sample: Two or more representative portions taken from one specimen in the field or in the laboratory and analyzed by different analysts, methods, or laboratories.

Standard Operating Procedure (SOP): A written document that details the method of an operation, analysis, or action whose techniques and procedures are thoroughly prescribed and that is officially approved as the method for performing certain routine or repetitive tasks.

Stock Standard Solution (SSS): A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

Surrogate (SUR): A pure analyte(s), that is extremely unlikely to be found in any sample, and that is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the surrogate is to monitor method performance with each sample.

The NELAC Institute (TNI): Formally NELAC, a voluntary organization of State, Federal, and other groups to establish mutually acceptable standards for accrediting environmental laboratories.

Toxicity test: A test that measures the degree of response of an exposed test organism to a specific chemical or effluent or other waters.

Traceability: The ability to follow the history, application, or location of an item or activity by means of recorded identifications; for calibration, this relates measuring equipment to national or international standards, primary standards, basic physical constants or properties, or reference materials, and, for data collection processes, this relates to calculations and data generated throughout the project back to the requirements for the quality of the project.

Validation: Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled. In design and development, validation concerns the process of examining a product or result to determine conformance to user needs.

Whole Effluent Toxicity (WET): The aggregate toxic effect of an effluent as measured by a toxicity test.

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Appendix C
ASTM Type I, II, III, and IV Water Specifications

Parameter	Units	Type I	Type II	Type III	Type IV
Conductivity (maximum)	µS/cm	0.056	1.0	4.0	5.0
Resistivity (minimum)	megohm-cm	18	1.0	0.25	0.2
pH	S.U.	*	*	*	5.0 to 8.0
Total Organic Carbon (maximum)	µg/L	50	50	200	No Limit
Sodium (maximum)	µg/L	1	5	10	50
Chlorides (maximum)	µg/L	1	5	10	50
Total silica (maximum)	µg/L	3	3	500	No Limit

* pH is not a significant indicator when comparing ionic contamination.

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