Required Documentation for PA State (Chapter 252) Accreditation

Disclaimer: The information in this guidance document does not supplant the provisions of the Environmental Laboratory Accreditation Regulations, 25 Pa Code, Chapter 252 ("Chapter 252"). This document is a tool to help laboratories to comply with the requirements of Chapter 252. If there is any discrepancy between the contents of this document and Chapter 252, the regulations shall prevail. The documentation requirements for Radiochemistry and Toxicity testing were intentionally omitted from this document. The specialized requirements for Radiochemistry and Toxicity testing are given in §252.405 and §252.403, respectively. The examples given in this document are for illustrative purposes only, meant to aide individuals in visualizing applications of the regulatory requirements. These examples do not represent all method or regulatory requirements.

1. Quality Manual (§252.401): See *Writing a Quality Manual for PA State (Chapter 252) Accreditation* for information regarding the Quality Manual ("QM") requirements.

2. Personnel Training Records (§252.304. b, §252.307.j, and §252.401.e): Laboratories must document the training and competency of laboratory employees through personnel records. More information on documenting the training and competency of laboratory personnel may be found in *Writing a Quality Manual for PA State (Chapter 252) Accreditation*. The following information must be maintained for each laboratory employee. Many laboratories choose to create a separate folder for each employee that contains the following information:

   2.1. Information required for each lab employee:

      2.1.1. Resumes, transcripts, etc., demonstrating that the employee meets the qualification requirements for his/her position.

         NOTE: The laboratory management establishes the minimum qualifications, education and experience requirements for all analytical positions in the lab. The only position with State-mandated education and experience qualifications is the laboratory supervisor. See §252.302 for required lab supervisor qualifications.

      2.1.2. A signed statement indicating that the employee has read, understood and is using the latest version of the laboratory QM.

      2.1.3. A signed statement indicating that the employee has read, understood and is using the latest version of analytical SOPs.

      2.1.4. Documentation of the employee's technical and analytical training and training on lab procedures. Documentation of technical training does not require labs to send personnel to formal training sessions. This documentation may simply be a summary of training provided in-house and on the job. Such documentation should include the dates of training and material covered during each training date.

      2.1.5. Documentation of the employee's Lab Ethics and legal responsibility training. Documentation of ethics training does not require labs to send personnel to formal training sessions. This training may be provided in-house, as long as the employee's ethical and legal responsibilities are clearly outlined.

2.1.6. A signed statement indicating that the employee acknowledges and understands his/her personal ethical and legal responsibilities, including potential punishments for unlawful or unethical behavior.

2.1.7. An Initial Demonstration of Capability (IDC) (§252.307.j)

2.1.7.1. Every analyst must perform an IDC for each analysis they perform. The IDC must be performed initially, before a new analyst begins to process real environmental samples, and the IDC must be repeated when changes are made to the method, instrument used or personnel performing the method. See *Procedures for Performing a Demonstration of Capability for Microbiology* (Procedure P3a) for information on performing IDCs for Microbiological methods. Follow the instructions given in Section 11.6.3 of this document to prepare Quality Control Samples for Microbiology IDCs.

2.1.7.2. Labs must retain all raw data associated with the IDC so that the entire procedure and all associated lab activities can be reconstructed from the lab's records. These records include but are not limited to calibration data, QC (i.e., blanks, continuing calibration verification standards), temperature logs (when a method is temperature sensitive, such as BOD/CBOD), calculations, standard preparation logs, etc.

2.1.8. Annual Continued Demonstration of Capability (DOC)

2.1.8.1. Every analyst must perform a DOC for each analysis they perform. The DOC must be performed every 12 months for each analysis. Labs can choose to fulfill the annual DOC requirement by performing any one of the options listed at §252.304.b.3.vii.A-E.

2.1.8.2. Labs must retain all raw data associated with the DOC so that the entire procedure and all associated lab activities can be reconstructed from the lab's records. These records include but are not limited to calibration data, QC (i.e., blanks, continuing calibration verification standards), temperature logs (when a method is temperature sensitive, such as BOD/CBOD), calculations, standard preparation logs, etc

2.2. Laboratories must also keep a list of the dates of employment for each lab employee, an example of each lab employee's signature and initials, and a list of which persons are authorized to approved or release data. The lab may choose to keep this information in each employee's training file along with the required documentation listed above. However, some laboratories find it more convenient to have this information in a single list stored separately from the information specified above. The Department does not mandate the format in which these records are kept. The Department only requires that the information be maintained such that lab personnel may easily retrieve the necessary information upon request.

3. Equipment Records (§252.306.b): Laboratories must have equipment records for each piece of equipment in the laboratory. The equipment records are required to include:

3.1. The name of the equipment, type, manufacturer name, identification & serial number, condition when received (new, used, reconditioned, etc.).

3.2. The location in laboratory, date received, date placed into service (if information is available).

3.3. A manufacturer's reference manual and/or operating instructions, or state the location of the reference manual.

3.4. Dates and details of any maintenance, including routine preventative maintenance.

3.5. Dates and details of any damage, malfunction, modification, or repair.

3.6. Dates and results of calibration or calibration verifications.

Note: Many labs choose to keep a separate lab notebook for each piece of equipment. Labs typically keep the information described in items 3.1-3.5 above in this notebook, and keep the calibration records (Item 3.6) separately. Other methods of equipment record keeping, such as loose-leaf notebooks, etc., are acceptable. The Department does not mandate the format in which these records are kept. The Department only requires that the information be maintained such that lab personnel may easily retrieve the necessary information upon request.

4. Equipment Records for specified pieces of laboratory equipment (§252.306.f): Chapter 252 lists specific documentation and records requirements for certain pieces of laboratory equipment. Labs must also follow the equipment requirements described in Section 3 of this document, in addition to those given below.

4.1. NIST thermometer (&252.306.f.1):

4.1.1. NIST thermometers must be recalibrated every 5 years to NIST standards, and documentation of the calibration must be retained.

4.1.2. The laboratory must keep a calibration certificate demonstrating traceability to NIST standards.

4.2. Working thermometers (&252.306.f.2):

4.2.1. Working thermometers must be calibrated against a NIST thermometer every 12 months, at the temperature used. Records of the annual calibration must be kept. The calibration record must include the date of calibration, the NIST thermometer identification, the working thermometer identification, the NIST thermometer reading, the working thermometer reading, the correction factor (if any), and the initials of person performing the calibration.

4.2.2. Working thermometers must be labeled with a unique identifier, the date of last calibration, and the correction factor from calibration. Usually, thermometers come from the manufacturer with an identification number etched into the glass. The date of last calibration and correction factor may be designated on the temperature log sheet for that thermometer, provided that the thermometer's unique identifier appears on the thermometer itself **and** the temperature log. Some labs make a flag for each thermometer using lab tape that indicates the last date of calibration and the correction factor.

4.3. Certified reference weights, ASTM Type 1,2 or 3 (Class S or S-1) (§252.306.f.3):

4.3.1. Reference weights must be re-certified at least every 5 years, and documentation of re-certification must be retained.

4.3.2. The laboratory must keep a calibration certificate demonstrating traceability to NIST standards.

4.4. Pan or Analytical balance (§252.306.f.4):

4.4.1. Balances must be calibrated and serviced by a qualified person at least annually. A record of annual service & calibration performed must be retained, and the date of last service must be recorded on the balance (sticker, tape, etc.).

4.4.2. Laboratories must verify the calibration of balances daily, or before each use, whichever is less frequent, using at least 3 certified reference weights that bracket the range of use. Records of the balance verification must be retained and must include the balance identification, the date of verification, the reference weights used, the correction factor (if any), and the initials of the person performing the verification.

4.5. pH meter (§252.306.f.5):

4.5.1. Laboratories must standardize pH meters daily, or before each use, whichever is less frequent, and records of the standardization must be retained. The standardization records must include the date of standardization, the standard buffers used, and the initials of person performing the standardization.

4.6. Conductivity meter (§252.306.f.6):

4.6.1. Laboratories must calibrate a conductivity meter daily, or before each use, whichever is less frequent, and records of the calibration must be retained. The calibration records must include the date of calibration, the standards used, the results of calibration or cell constant determined, and the initials of the person performing the calibration

4.7. Refrigeration and freezer equipment (§252.306.f.7):

4.7.1. Laboratories must record temperatures daily, every day of use.

4.7.2. The temperature record must include the refrigerator or freezer identification, the date, the calibration corrected temperature (the thermometer reading is adjusted according to any correction factor that is assigned at the time the working thermometer is calibrated against the NIST thermometer, see Section 4.2), and the initials of the person reading the thermometer.

4.8. Incubators, water baths and heating blocks (§252.306.f.8):

4.8.1. Laboratories must record temperatures daily, every day of use. When incubators, water baths or heat blocks are used in Microbiological testing, laboratories must record temperatures twice per day, with readings separated by at least 4 hours, for every day of use.

4.8.2. The temperature records must include the incubator, water bath or heat-block identification, the date, the time, the calibration corrected temperature (the thermometer reading is adjusted according to any correction factor that is assigned at the time the working thermometer is calibrated against the NIST thermometer, see Section 4.2), and the initials of the person reading the thermometer.

4.9. Volumetric dispensing devices such as burettes, auto-pipettors, dilutors, and re-pipettes, except Class A glassware (§252.306.f.9):

4.9.1. Laboratories must check the accuracy of volumetric dispensing devices at least once every 3 months, using a gravimetric method. See Appendix A, Section A.1, for an example procedure.

4.9.2. Records of the accuracy check must be retained and include the date, the apparatus identification, the type of liquid used in the check, the volume and density (aka specific gravity) of the liquid used, the initial weight of the beaker (unless tared), the weight of the beaker with liquid, the measured weight of the dispensed liquid, the calculated weight of the dispensed liquid, the accuracy of the delivery volume, the correction factor (if any), and the initials of the person performing the check.

4.10. Graduated sample containers such as funnels and sample bottles, except Class A glassware (§252.306.f.10):

4.10.1. When the graduations on sample containers, funnels or sample bottles are used to measure sample volume for analysis, laboratories must check the accuracy of the container's graduations. The accuracy check must be performed for each lot number of bottle, or at least once per year, whichever is more frequent. See Appendix A, Section A.2, for an example procedure.

4.10.2. Records of the accuracy check must be retained and include the apparatus identification, the lot number of bottles tested, the date, the volume of liquid measured in the test apparatus, the volume of sample measured with Class A glassware, the accuracy of the tested container, the correction factor (if any), and the initials of the person performing the check.

4.11. Spectrophotometer or Colorimeter (§252.306.f.11):

4.11.1. Laboratories calibrate or check spectrophotometers and colorimeters according to the manufacturer's specifications, test methods or §252.402.c-f.

4.11.2. A record of the calibration must be maintained.

5. Reagent, Standards & Support Service Records (§252.306.g-h): Laboratories must maintain records for all reference materials, reagents, and support services utilized by the laboratory for testing or analysis.

5.1. Purchased Chemicals (§252.306.h.4): Laboratories must label purchased standards, chemicals, solutions, or reagents with the date of receipt and the date opened. Purchased standards, chemicals, solutions, and reagents without an expiration date given by the manufacturer must be discarded after 10 years from the date of receipt.

5.2. Prepared Chemicals (§252.306.h.3): Laboratories must label the containers of reagents, standards & solutions that are prepared or diluted in-house with the identification of the compound/solution, the concentration of the compound/solution, the date prepared, the expiration date, and the initials of the person preparing the solution.

5.3. Laboratories must keep records (reagent logs) of all standard, reagent & solution preparation (§252.306.h.2.). The records must include the identification of the compound/solution, the concentration of the compound/solution, the date prepared, the expiration date, and the initials of the person that prepared the solution.

6. Analytical SOPs (§252.307.d): Laboratories must have analytical SOPs for all fields of accreditation. For information on writing analytical SOPs to comply with Chapter 252, see *Writing an Analytical SOP for PA State (Chapter 252) Accreditation*.

7. Range of Quantitation and Detection Limit Studies (§252.307.h, §252.402.k): Laboratories must perform range of quantitation and detection limit studies for each analyte reported, where such procedures are applicable to the method.

7.1. Detection limit (DL): The lowest concentration or amount of the target analyte that can be identified, measured and reported with confidence that the analyte concentration is not zero. See 40 CFR 136, Appendix B, or *Standard Methods for the Examination of Water and Wastewater* (20th ed.), Section 1020.B.3 for information on performing a detection limit study.

Note: Detection limit studies are not required for any analyte for which spiking solutions or quality control samples are not available (i.e. temperature) or where results are logarithmic (i.e. pH) or expressed as presence/absence. Detection limit studies are not required for gravimetric methods or Microbiological testing.

7.1.1. Labs must retain all raw data associated with the study so that the entire procedure and all associated lab activities can be reconstructed from the lab's records. These records include but are not limited to calibration data, QC (i.e., blanks, continuing calibration verification standards), temperature logs (when a method is temperature sensitive), calculations, standard preparation logs, etc.

7.2. Range of Quantitation: The concentration range within which a laboratory reports quantitative results. The range of quantitation is defined by a low concentration standard and a high concentration standard and is usually the same as the calibration range for a method. The lower limit of the quantitation range may not be lower than the concentration of the lowest calibration standard analyzed. Likewise, the high limit of the quantitation range may not be higher than the concentration of the highest calibration standard analyzed. In addition, the lowest standard of the calibration curve and the quantitation limit (QL) may not be lower than the detection limit of the method (See Section 7.1). The range of quantitation of the method should be defined in each analytical SOP.

8. Validation Studies (§252.307.i): Laboratories must perform any method validation studies that are required by the reference method, before reporting any sample results by that method.

8.1. Examples of validation procedures are Method Detection Limit (MDL) studies, Initial Demonstrations of Capability (IDC), On-going or Continuing Demonstrations of Capability (DOC), and comparison studies. Data from the validation study must be kept on file for the duration of the method's use and for at least 5 years after the method is no longer used.

8.2. Performance of a method validation study required by the reference method may also fulfill Chapter 252 requirements for performance of an IDC or MDL. Likewise, performance of an IDC or MDL for compliance with Chapter 252 may fulfill the reference method's requirement for a method validation study. This depends on the specific requirements of the method validation study. Be sure to read the method validation study procedure and the Chapter 252 requirements carefully before choosing to use one study or procedure to fulfill multiple requirements.

9. Chemistry QC and data record retention (§252.402):

9.1. Initial calibration (§252.402.c, §252.402.d, §252.402.e):

9.1.1. Labs must retain all raw data necessary to reconstruct the initial instrument calibrations. Calibration records must include the concentration and number of standards used, the instrument response for each standard, the calibration curve generated, and the results of the curve based on the method's acceptance criteria. For example, if a method requires that calibration curves have a slope of –57mV ± 3mV to be considered acceptable. Lab personnel must record the slope of the curve generated by initial calibration so that, upon examination of the lab's calibration records, it may be determined whether a certain sample set is associated with an acceptable calibration curve.

9.1.2. The initial calibration procedures, including calculations, integrations, acceptance criteria, and associated statistics, must be documented in the lab's SOP.

9.2. Calibration verification (§252.402.f):

9.2.1. Labs must retain all raw data necessary to reconstruct the calibration verifications. Calibration verification records must include the number and concentration of the standards used, the instrument response to each standard, any calculations performed to generate results for the verification, and evaluation of the calibration verification standards against the method's acceptance criteria. The calibration verification records must demonstrate that sample results are bracketed by acceptable calibration verification.

9.2.2. The calibration verification procedures, including calculations, integrations, acceptance criteria, and associated statistics, must be documented in the lab's SOP.

9.3. Raw sample data (§252.706.b.a-e):

9.3.1. Labs must retain the raw data records necessary to reconstruct all lab activities associated with sample analysis, including but not limited to the original handwritten data or observations, instrument printouts, and calculations used in analysis and

reporting of samples and QC samples. The required Chemistry QC is described in Chapter 252, §252.402.

9.3.1.1. Example – Calculation for sample reporting: For biological oxygen demand (BOD), dissolved oxygen uptake (DO uptake) is calculated by the formula:

DO Uptake = initial DO - final DO

Data records must include the initial DO and the final DO readings, and not simply the result for DO uptake.

9.3.1.2. Example – QC Calculation: Relative percent difference (RPD) is used for comparing analyses of duplicate samples. Data records must include the values used in the RPD equation, and not simply the calculated RPD result.

9.3.2. Labs must have a written procedure for reporting sample results (§252.401.j) and a written plan that details how records will be maintained (§252.706.e). See *Writing a Quality Manual for PA State (Chapter 252) Accreditation* for information regarding these policies.

9.4. Corrective actions (§252.401.i):

9.4.1. Labs must document and keep a record of all corrective actions taken when quality control measurements do not meet established acceptance criteria. See *Writing a Quality Manual for PA State (Chapter 252) Accreditation* for more information on corrective actions.

9.5. In-house established acceptance criteria (§252.402):

9.5.1. Labs must document the procedures used to define acceptance criteria developed in-house for the evaluation of QC measures (i.e., surrogate recoveries, laboratory control samples, duplicate samples, method blanks, etc.) in methods where no established criteria exists. Typically, acceptance criterion development procedures are contained as a policy in the Quality Manual. Many laboratories evaluate past data from an analysis to generate appropriate and reasonable acceptance criteria. For instance, a lab may consider whether it's comfortable with duplicate sample results from an analysis that show more than 10% relative percent difference. If a greater than 10% RPD value for duplicates usually indicates a problem for this analysis, then the acceptance criteria should be ± 10%. If the lab is never able to achieve a 10% RPD, then the lab may set wider limits for this analysis (± 20% or ± 30%).

10. Microbiology Equipment Records (§252.404.c): Chapter 252 lists specific documentation and records requirements for laboratory equipment used in Microbiological testing. Labs must also follow the equipment requirements described in Sections 3 & 4 of this document.

10.1. Autoclave (§252.404.c.1):

10.1.1. Labs must check the performance and functional properties of the autoclave prior to its first use. The performance and functionality check must be documented. Many labs choose to document the performance and functionality check in the autoclave's equipment log. Establishing the functionality of the autoclave is dependent on how

the autoclave will be used in each laboratory. The autoclave's performance and functionality may be established by the following:

10.1.1.1. Comparing temperature readings from the maximum/minimum thermometer in the front of the autoclave versus the back of the autoclave. This comparison establishes that there is no temperature variation based on where items are placed in the autoclave. Such variations in temperature could impact the sterilizing capability of the autoclave.

10.1.1.2. Using biological indicators to demonstrate that the autoclave effectively kills microbial organisms.

10.1.1.3. Determining whether sterility is achieved based on load capacity. Is the same sterility achieved whether the autoclave is fully loaded or whether only a few items are place inside?

10.1.2. Labs must test the sterilization capacity of the autoclave monthly using a biological indicator, and documentation of the test must be retained. The records of the sterilization capacity check must include the autoclave identification, the date, the incubation time and temperature, the results of the test, and the initials of the individual performing the test.

10.1.3. If a mechanical timing device is used during autoclave cycles, the device must be verified every 3 months, and records of the check must be retained. Records of the timing check must include the autoclave identification, the date, the time recorded by the mechanical timing device, the actual time, the correction factor, and the initials of the individual performing test.

10.1.4. Labs must service the autoclave annually, which must include a pressure check and a calibration of the temperature device. Records of the servicing must be retained, and the date of the service must be recorded on the autoclave (sticker or tape).

10.1.5. Every autoclave batch must be documented. Batch records must include the date, contents, sterilization time & temperature, total cycle time (recorded as time in and out), and the initials of the responsible person.

10.1.6. Corrective actions must be taken and documented if the autoclave cycle fails to meet any requirement. See *Writing a Quality Manual for PA State (Chapter 252) Accreditation* for more information about documenting corrective actions.

10.2. Hot air oven (§252.404.c.2):

10.2.1. The performance and functional properties must be checked prior to first use of the hot air oven, and the check must be documented. See Section 10.1.1 of this document for more information.

10.2.2. Labs must test the sterilization capacity of the oven monthly using a biological indicator, and documentation of the test must be retained. The records must include the oven identification, the date, the incubation time and temperature, the results of the test, and the initials of the individual performing the test.

10.2.3. Each oven use must be documented. Batch records must include the date, contents, sterilization time & temperature, total cycle time (recorded as time in and out), and the initials of the responsible person.

10.3. Membrane filtration equipment (§252.404.c.5):

10.3.1. Labs must maintain records of membrane filters. The records must include the type of filter, the lot number, the date received and date opened, and the manufacturer's specification/ certification sheet for each lot. A sterility check must be performed on each lot of membrane filters. See Section 11.5.5 for more information.

10.4. Ultraviolet lamp (§252.404.c.5.vi):

10.4.1. When a laboratory uses a UV lamp to sanitize filtration funnels, the lamp effectiveness must be tested every 3 months, and records of the test must be retained. The records must include the lamp identification and the reading from the UV light meter or plate count results (depending on method used). The lamp bulbs must be replaced if output is less than 70% of the original light output, or if count reduction is less than 99% for a plate containing 200-300 organisms.

10.5. Quanti-Tray™ Sealer (§252.404.c.12):

10.5.1. A monthly tray sealer check must be performed. The tray sealer check record must list the sealer identification, date, results, and initials of the person performing the test.

11. Microbiology QC and record retention (§252.404): Chapter 252 lists specific documentation and record keeping requirements for analytical testing in the Microbiology category. Labs must also follow the record keeping requirements of §252.706. See Section 9.3 and 13 of this document.

11.1. Lab ware washing procedure (§252.404.c.10):

11.1.1. Labs must check each lot of detergent used to wash lab ware used in Microbiological testing for inhibitory residue, and records of the test must be retained. Records of the Inhibitory Residue Test (*Standard Methods for the Examination of Water and Wastewater*, 20th ed., Section 9020 B.4.a.2) must include the detergent identification and lot number, date, calculations, results, and initials of the person performing the test. Many detergent manufacturers perform this test prior to distributing their product. Labs can request the documentation of this test from the manufacturer or repeat the procedure on each lot of detergent purchased.

11.1.2. Labs must check washed lab ware for acid/alkaline residue monthly, and records of test must be retained. The test must be performed on at least one piece of washed lab ware with a suitable pH indicator, such as 0.04% bromothymol blue (*Standard Methods for the Examination of Water and Wastewater*, 20th ed., Section 9020 B.4.a.1). Records of the pH check should include the date, the piece of lab ware tested, the color change reaction observed, and the initials of the person performing the test.

11.2. Reagent Water (§252.404.d):

11.2.1. Records of monthly and annual reagent water analysis must include the date, type of test, results, and initials of the responsible person. See Chapter 252, §252.404.d.2-6 for more information on the required monthly and annual tests.

11.3. Dilution/Rinse Water (§252.404.e):

11.3.1. Containers of stock buffers must be labeled and dated.

11.3.2. Preparation of stock buffer and dilution/rinse water must be documented in a reagent preparation log. The preparation record must include the date prepared, the lot number or laboratory identification of solutions used, the amounts of reagents measured, the final pH, and the initials of the responsible individual. A sterility check must be performed on each lot of commercially prepared dilution/rinse water or each batch of in-house prepared dilution/rinse water. See Section 11.5.4 of this document for more information.

11.4. Media (§252.404.f):

11.4.1. Purchased media: Each lot of commercially prepared media must be documented. The record must include the date received, the type of media, the lot number, and the result of pH verification. A sterility check must be performed on each lot of commercially prepared media. See Section 11.5.2 for more information.

11.4.2. Prepared media: If a lab prepares media from dehydrated stock, the preparation of each batch of prepared media must be documented. The preparation record must include the date of preparation, the type of media, the lot number, the amounts of each component measured, the sterilization time and temperature, the final pH, and the initials of the person preparing the media. After sterilization, each bag, container or rack of broth or agar media must be labeled with the date prepared or expiration date. A sterility check must be performed on each batch of laboratory prepared media. See Section 11.5.2 for more information.

11.5. Sterility blanks (§252.404.g):

11.5.1. Filtration Series: Sterility blanks must be prepared and analyzed at the beginning of each filtration series, after every ten samples, and at the end of the series for each membrane filtration unit used during a filtration series. The results of these blanks must be recorded. For laboratories using a single filtration unit (funnel/base combination) for testing all samples, the sterility blank must be performed using the same filtration unit as used for routine samples. For laboratories using multiple filtration units or a filtration manifold for testing, a sterility blank must be performed on each unit or port at the beginning and end of the filtration series and after every ten samples, unless each port is only used for one sample, and the sterility of the autoclave batch has been verified. See Appendix A, Section A.3, for an example procedure. If a lab only filters one sample in the filtration series, then only the beginning sterility blank is required. If a lab is using disposable one-use funnels, then a sterility check is only required on each lot number of disposable funnels.

11.5.2. Media: Sterility checks must be performed on each lot number of purchased or lab-prepared media prior to the first use of the media. The record must include the media identification, the lot number, the date, the results, and the initials of the person performing the check. See Appendix A, Section A.4 for an example procedure.

11.5.3. Sample Containers: Sterility checks must be performed on each lot of purchased, pre-sterilized sample containers using non-selective growth media, such as tryptic soy broth, nutrient broth, plate count agar, m-HPC, R2A agar, or NWRI agar. Sterility checks must also be performed on sample containers sterilized by the laboratory, one per sterilization batch. The record must include the sample container identification, the date, the results, and the initials of the person performing the check. See Appendix A, Section A.5, for an example procedure.

11.5.4. Dilution/Rinse Water: Sterility checks must be performed on each batch of lab prepared dilution/rinse water and each lot number of commercially prepared dilution/rinse water using non-selective growth media, such as tryptic soy broth, nutrient broth, plate count agar, m-HPC, R2A agar, or NWRI agar. The concentration of the media must be single strength after the addition of dilution water. The record must include the dilution/rinse water identification, the date, the results, and the initials of the person performing the check. See Appendix A, Section A.6, for an example procedure.

11.5.5. Membrane Filters: Sterility checks must be performed on each lot number of membrane filters. The records must include the membrane filter identification (manufacturer, lot number, or other identifying information), the date, the results, and the initials of the person performing the check. See Appendix A, Section A.7, for an example procedure.

11.6. Positive and negative culture control checks (§252.404.h):

11.6.1. Positive and negative culture control checks must be performed and documented for each lot or batch of media. The record must include the date, the media lot or batch number, the type of media, the identification of the positive culture control organism, the identification of the negative culture control organism, the results for each type of organism, and the initials of the person performing the testing. See Appendix A, Section A.8, for an example procedure.

11.6.2. The lab must document the traceability of its stock positive and negative culture control organisms to a recognized National collection.

11.6.3. The positive and negative culture controls must be prepared as follows (§252.404.i.3-4):

11.6.3.1. Organism density methods: Labs must prepare the positive control sample targeting 20-80 viable organisms per the usual volume of liquid analyzed. The positive control must be processed through all steps of the method, and the density of the control determined and documented.

11.6.3.2. Presence/Absence methods: Labs may inoculate the control sample by transferring a portion of the stock culture to 100mL reagent or dilution water. The

positive control must be processed through all the steps of the method, and the result documented.

11.7. Test variability/reproducibility (§252.404.i.):

11.7.1. Colony counting methods: Labs must perform duplicate counts on one positive sample monthly for each month that the test is performed. If the lab has two or more analysts, two different analysts must count the same positive test plate, and the counts are compared. Counts may not differ by more than 10%. If the lab has one analyst, the analyst must count the same positive plate twice, and the counts are compared. Counts may not differ by more than 5%.

11.7.2. All methods: Labs must ensure that one positive sample is analyzed monthly, if the method protocol does not require a positive culture control during sample analysis. The monthly positive sample is not required to be from a known positive stock. Positive results from a real environmental sample may fulfill this requirement.

12. PT Studies (§252.501):

12.1. The lab must investigate failed PT studies and implement corrective actions. The investigation and corrective action must be documented. See *Writing a Quality Manual for PA State (Chapter 252) Accreditation* for more information about documenting corrective actions.

12.2. All raw data associated with a PT study must be retained for at least 5 years. The retained data must be complete enough to permit reconstruction of the analysis and all lab activities associated with the PT study. Records must include original hand written data or observations, instrument printouts, associated calibrations and continuing calibration verifications, associated QC measures, calculations, preparation logs (standard, reagent, media), etc.

13. Record keeping requirements (§252.706):

13.1. The laboratory must maintain records for a minimum of 5 years that allow the historical reconstruction of ***all*** laboratory activities associated with the testing of samples.

Appendix A:

A.1 Mechanical Volumetric Glassware Verification (§252.306.f.9)

1. Verify the balance with 3 certified reference weights, bracketing the range of use. See Section 4.4 of this document.
2. Tare or weigh a clean dry beaker. Do not handle the beaker with bare hands once the beaker is tared or weighed. Handle the weighed or tared beaker using gloves or lab tissue. Record the weight of beaker, unless tared.
3. Dispense pre-set volume of liquid from mechanical volumetric device into the beaker. Record the volume measurement from the device and the type of liquid dispensed.
4. Weigh beaker with liquid, and record the weight.
5. Determine the measured weight of the liquid ($W_{[liquid]}$). If the beaker was tared in Step #1:

$$W[liquid] = W[beaker + liquid]$$

If the beaker was weighed in Step #1:

$$W[liquid] = W[beaker + liquid] - W[beaker]$$

6. Determine the calculated weight of the liquid ($Wcal_{[liquid]}$) based on the Density (aka specific gravity) of the liquid dispensed. Density is a physical property and is unique for each liquid. Labs must find the density of the liquid that is used in this test. The density of purchased chemicals should be printed on the container's label or the MSDS sheet. However, if the lab dilutes the purchased chemical to a working concentration, the density is changed, and the density listed on the manufacturer's label should not be used. Labs may use reagent water to perform the verification ($D_{[H20]}$ = 1.0 g/mL).

$$Wcal[liquid](g) = V(mL) \times D(g/mL)$$
$$V = volume \cdot dispensed(mL)$$
$$D = density(g/mL)$$

Note: Be careful with units. The above equation will not work correctly if values are not in the units specified above.

7. Compare the measured weight of the liquid ($W_{[liquid]}$) to the calculated weight of the liquid ($Wcal_{[liquid]}$).

$$\%Diff = \frac{Wcal[liquid] - W[liquid]}{Wcal[liquid]} \times 100\%$$

Note: All weights should be in grams.

8. Labs should compare the results of the verification to in-house developed acceptance criteria, such as ± 0.3mL or ± 2.5%.
9. See Section 4.9 of this document for required documentation.

A.2 Graduated Sample Container Verification (§252.306.f.10)

1. Fill the sample container or funnel to the graduation mark used for sample measurement with liquid. Record the equipment type and identification (i.e., Pre-sterilized sample containers & lot number), the date and the graduation mark used.
2. Pour into a Class A graduated cylinder. Record the apparatus used and the measurement.
3. Compare the sample container measurement to the graduated cylinder measurement:

$$\%Diff = \frac{V[container] - V[ClassA]}{V[ClassA]} \times 100\%$$

Note: All volumes should be in the same units (i.e., mL)

4. Labs should compare the results of the verification to in-house developed acceptance criteria, such as ± 0.3mL or ± 2.5%.
5. See Section 4.10 of this document for required documentation.

A.3 Sterility Checks – Filtration Series (§252.404.g.2)

One funnel/filter base combination used for entire filtration series:

1. Using clean, sterile equipment, membrane filtration funnel & base, filter 100mL (or other specified volume) sterile dilution/rinse water, according to the method protocol.
2. Treat the sterility blank as a real environmental sample. Break vacuum, remove filter and place onto media, according to method protocol. The samples and the sterility blanks should be filtered through the same funnel/base combination.
3. Ten real environmental samples may be filtered through the unit. Rinse the funnel thoroughly between samples with at least 3 successive 20mL portions of sterile dilution water.
4. After the 10th sample, another sterility blank must be filtered.
5. Up to 10 more samples may be filtered before another sterility blank must be prepared. Repeat sterility blanks after every 10th sample or until 30 minutes elapses from the start of the filtration series.
6. When all samples have been filtered or 30 minutes has elapsed since the start of the filtration series (§252.404.c.5.ii), an ending sterility blank must be filtered.
7. Incubate samples and sterility blanks under the same conditions, according to method protocol.
8. Remove from incubator and observe growth. There should be no bacterial growth on the sterility check plate. If growth is observed on the sterility check plate, all data preceding the failed sterility check in the filtration series up to the last acceptable sterility check, must be appropriately qualified.

Multiple funnel/filter base combinations or Filtration manifold:

1. Using clean, sterile equipment, membrane filtration funnels & bases, filter 100mL (or other specified volume) sterile dilution/rinse water through **each** funnel/base combination and manifold port to be used to analyze samples, according to the method protocol. The beginning sterility blanks must be filtered before real environmental samples are processed.
2. Treat the sterility blanks as real environmental samples. Break vacuum, remove filters and place onto media, according to method protocol.

3. Ten real environmental samples may be filtered through each manifold port or funnel/base combination. For example if the manifold has 5 ports, 50 samples may be filtered (10 through each port). Rinse the funnels thoroughly between samples with at least 3 successive 20mL portions of sterile dilution water.
4. After the 10th set of samples is filtered, or 30 minutes has elapsed since the start of the filtration series (§252.404.c.5.ii), another set of sterility blanks must be filtered through each manifold port.
5. Up to 10 more sets of samples may be filtered through each manifold port or funnel/base combination before another set of sterility blanks must be prepared and filtered.
6. When all samples have been filtered, an ending set of sterility blanks must be filtered through each manifold port or funnel/base combination.
7. Incubate samples and sterility blanks under the same conditions, according to method protocol.
8. Remove from incubator and observe growth. There should be no bacterial growth on the sterility check plates. If growth is observed on any sterility checks, all data preceding the failed sterility check in the filtration series, until the last acceptable sterility check must be appropriately qualified for the samples filtered on the funnel/base combination or manifold port with the failed sterility check. If the sterility checks from other funnel/base combination or manifold ports are acceptable then the samples filtered on those locations should not be qualified.
9. The sterility blanks should be documented with the associated sample data. See Section 9.3 of this document for record keeping requirements.

Note: If the filtration series contains only one sample (or one sample dilution), only the beginning sterility blank is required. If pre-sterilized, disposable funnel/filter base combinations are used, a sterility blank is only required for one unit per lot of funnel/filter base combinations.

A.4 Sterility Checks – Media (Example for a membrane filtration method) (§252.404.g.1)

1. Prior to first use, one aliquot, ampoule, or plate from each lot number of commercially prepared media or each batch of in-house prepared media must be tested for sterility.
2. Using clean, sterile equipment, membrane filtration funnels & bases, filter 100mL sterile dilution/rinse water, according to method protocol.
3. Break vacuum and place onto a plate containing a new lot number of pre-prepared, ready-to-use medium or new batch of in-house prepared medium.
4. Incubate according to method protocol.
5. Remove from incubator and observe growth. There should be no bacterial growth on the plate. If growth is observed, the source of contamination must be investigated, and the lot or batch of media may not be used.
6. See Section 11.5.2 of this document for required documentation.

A.5 Sterility Checks – Sterilized Sample Containers* (§252.404.g.4)

1. Prior to first use, one container shall be selected from each lot number of purchased, pre-sterilized sample containers or each autoclaved batch of in-house sterilized containers to be checked for sterility.
2. Prepare tryptic soy broth, according to manufacturer's instructions or *Standard Methods for the Examination of Water and Wastewater* (20th ed.), Section 9211 D.1.b.2, and autoclave for 15 minutes at 121ºC, or according to manufacturer's instructions.

3. Pour approximately 50mL of media into selected pre-sterilized sample container, close, and swirl so that media touches all inside surfaces of the container.
4. Incubate at 35ºC for 24 h.
5. Remove from incubator. Cloudiness, turbidity, discoloration, or fuzziness in the media indicates bacterial growth. If growth is observed, the source of contamination must be investigated, and the lot of containers may not be used. If the results are inconclusive, incubate for an additional 24 h, and observe growth.
6. See Section 11.5.3 of this document for required documentation.

A.6 Sterility Checks – Dilution/Rinse Water* (§252.404.g.5)

1. Prior to first use, each batch of in-house prepared dilution/rinse water or each lot number of commercially prepared dilution/rinse water must be tested for sterility.
2. Prepare buffered dilution/rinse water in accordance with *Standard Methods for the Examination of Water and Wastewater* (20th ed.), Section 9050 C.1.
3. Prepare double strength tryptic soy broth according to manufacturer's instructions or *Standard Methods for the Examination of Water and Wastewater* (20th ed.), Section 9211 D.1.b.2. However, double the quantity of all dry ingredients per the volume of water required by the recipe for single strength media. Autoclave for 15 minutes at 121ºC, or according to manufacturer's instructions.
4. Measure and pour 50mL of double strength tryptic soy broth into a pre-sterilized sample container.
5. Add 50mL of dilution/rinse water from Step #1 into the same sample container, and gently swirl to mix.
6. Incubate at 35ºC for 24 h.
7. Remove from incubator. Cloudiness, turbidity, discoloration, or fuzziness in the media indicates bacterial growth. If growth is observed, the source of contamination must be investigated, and the lot (or batch) of dilution/rinse may not be used. If the results are inconclusive, incubate for an additional 24 h, and observe growth.
8. See Section 11.5.4 of this document for required documentation.

A.7 Sterility Checks – Membrane Filters*

1. Prior to first use, one membrane filter shall be selected from each lot number of filters and checked for sterility.
2. Prepare tryptic soy broth, according to manufacturer's instructions or *Standard Methods for the Examination of Water and Wastewater* (20th ed.), Section 9211 D.1.b.2, and autoclave for 15 minutes at 121ºC, or according to manufacturer's instructions.
3. Pour approximately 50mL of tryptic soy broth into a pre-sterilized sample container.
4. Place a sterile membrane filter into sample container with sterile forceps, making certain that the filter is placed down into the liquid media.
5. Incubate at 35ºC for 24 h.
6. Remove from incubator. Cloudiness, turbidity, discoloration, or fuzziness in the media indicates bacterial growth. If growth is observed, the source of contamination must be investigated, and the lot of filters may not be used. If the results are inconclusive, incubate for an additional 24 h, and observe growth.
7. See Section 11.5.5 of this document for required documentation.

*Note: The sterility checks described in Sections A.5, A.6 and A.7, may be combined into a singe test by placing 50mL of double strength media into a pre-sterilized container from a new lot number, with 50mL of dilution/rinse water from a new lot number or batch, and a sterilized membrane filter from a new lot number. Incubate at 35ºC for 24 h, and observe growth as indicated above. If no growth is observed, the sterility has been checked for the pre-sterilized containers, dilution/rinse water and membrane filters with a single test. However, if contamination is present, it cannot be determined which variable (container, dilution/rinse water, or membrane filter) presents the source of contamination, and the sterility checks described in Sections A.5, A.6 and A.7, must be preformed separately to investigate.

A.8 Positive & Negative Culture Controls (§252.404.h)

Positive Culture Control (Example for a membrane filtration method) (§252.404.h.1):

1. Prior to first use, each lot number of commercially prepared medium or each batch of in-house prepared medium must be tested with at least one pure culture of a known positive reaction for the organisms under test. Therefore, choose a positive organism based on the test method and type of media. *Standard Methods for the Examination of Water and Wastewater* (20th ed.), Section 9020 B, Table 9020:V, lists appropriate organisms for selective tests. For non-selective tests, such as Heterotrophic Plate Count, any pure bacterial culture may be used.
2. Pure control cultures may be purchased commercially. The cultures are dehydrated and lyophilized and contained in single-use ampoules. Prepare culture according to manufacturer's instructions and targeting the appropriate number of organisms. See Section 11.6.3 of this document.
3. Using a 100mL aliquot of the pure positive culture, filter and incubate according to method protocol.
4. Remove from incubator and observe growth. Growth must be observed for the positive culture control. If no growth is observed for the positive culture control, the lot (or batch) of media may not be used.

Negative Culture Control for Selective Media (Example for a membrane filtration method) (§252.404.h.2):

1. Prior to first use, each lot number of commercially prepared ***selective*** medium or each batch of in-house prepared ***selective*** medium must be tested with at least one pure culture of a know negative reaction for the organisms under test. Therefore, choose a negative organism based on the test method and type of media. *Standard Methods for the Examination of Water and Wastewater* (20th ed.), Section 9020 B, Table 9020:V, lists appropriate organisms for selective tests.
2. Pure control cultures may be purchased commercially. The cultures are dehydrated and lyophilized and contained in single-use ampoules. Prepare culture according to manufacturer's instructions and targeting the appropriate number of organisms. See Section 11.6.3 of this document.
3. Using a 100mL aliquot of the pure negative culture, filter and incubate according to the method protocol.
4. Remove from incubator and observe growth. No-growth must be observed for the negative culture control. If growth is observed for the negative culture control, the lot (or batch) of media may not be used.