Microbiology Laboratory Analyst Training

Kentucky Division of Water

Laboratory Certification Program

To Protect and Enhance Kentucky's Environment

General Laboratory Practices

Personnel Requirements | Laboratory Facilities | Sample Receiving | Lab Equipment and Supplies

Personnel Requirements

DWCM Chapter V; Section 1[1]

Analyst

- High school education
- 30 days of training under the direct supervision of a KYDOW approved analyst
- 60 additional days of bench experience, with final review oversight in a microbiology laboratory
- Successfully complete a set of microbiology proficiency test (PT) samples for all methods the lab will be using
- Participation in a formal drinking water microbiology workshop

Supervisor/ Consultant

- Must meet all requirements for Analyst
- Have a Bachelor's degree in microbiology, biology or equivalent (If degree is not in microbiology, supervisor must have at least 1 college-level microbiology course)

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- Two (2) weeks of training at a federal or state agency, or academic institution in microbiological analysis

or

- 80 hours of on-the-job training in water microbiology at a certified laboratory

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Laboratory Facilities

DWCM Chapter V; Various Sections[1]

- Facility should be clean, temperature and humidity controlled with adequate lighting
- Drinking water and wastewater analysis should be kept separate
- A written QA plan should be prepared and followed
- PT sets must be performed annually and released directly to KYDOW from PT provider
- Service contracts and calibrations must be documented

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Receiving Samples at Laboratory

DWCM Chapter V; Sections 6 & 8[1]

- A sample should be invalidated for:
  - Time between sample collection and receipt by laboratory has been exceeded
    - Drinking water for Total Coliforms: **30 hour hold time**
    - Surface water: **8 hour hold time**
  - Presence of disinfectant in sample is noticed
  - Use of a container not approved by the lab for the purpose intended
  - Insufficient sample volume (<100mL)
  - Presence of interfering contaminants noticed
  - Evidence of freezing
  - Sample temperature exceeds the maximum allowable

- The following should be recorded on a sample identification form or chain of custody:
  - Name of system
  - Sample identification
  - Sample site location
  - Sample type
  - Collection date and time
  - Requested analysis
  - Disinfectant residual
  - Name of Sampler
  - Any other observations

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Laboratory Equipment and Supplies

Laboratory must document receipt, quantity, expiration of all laboratory supplies used for compliance samples and quality control.

Sample Containers

DWCM Chapter V; Sections 3 & 4[1]

- Sample containers should be sterilized and able to hold 100 mL of sample with enough headspace to thoroughly mix sample+media.
- Sodium thiosulfate must be added to the sample bottle prior to sampling in order to neutralize any residual chlorine in the sample.

Quality Control:

- Select **one container from every batch**/ lot to check for sterility.
  - Add 25mL of sterile TSB; incubate at 35° ± 0.5°C for 48 hours. If growth is detected, use a different lot of containers.
  - Record date check was performed, date results were read, lot/ batch number of TSB, and analysts initials
- Select **one container from every batch**/ lot to check for auto fluorescence.
  - If bottle fluoresces, use different lot of containers

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Reagent Grade Water

DWCM Chapter V; 4.3[1]

- Only satisfactorily tested reagent water from stills or deionization units may be used to prepare media, reagents, and dilution water for microbial testing
- The following criteria must be met for reagent water:
  - **Conductivity (monthly):** >0.5 megohms resistance or <2 micromhos/cm at 25°C
  - **Pb, Cd, Cr, Cu, Ni, Zn (annually):** <0.05 mg/L per contaminant and <0.1 mg/L collectively
  - **Total Chlorine Residual (monthly):** <0.1 mg/L
  - **Heterotrophic plate count (monthly):** ≤500 CFU/ml
  - **Bacteriological quality of reagent water [BST] (annually):** Ratio of growth rate between 0.8 and 3.0

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Dilution/ Rinse/ Sterile Water

DWCM Chapter V; 4.4[1]

- Stock buffer solution should be prepared by SM 9050C or purchased
- Stock buffers should be sterilized and then refrigerated.
  - Discard if solution becomes turbid
  - Containers should be labeled and dated

Quality Control:

- **Each** lot/ batch should be checked for sterility.
  - Add 50mL sterile water to 50mL double strength TSB; incubate at 35° ± 0.5°C for 48 hours. Discard if growth is detected.
  - Record date check was performed, date results were read, lot/ batch number of TSB, lot/ batch of water, and analysts initials.

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Culture Dishes

DWCM Chapter V; 3.12[1]

- Pre-sterilized plastic or sterilizable glass culture dishes should be used.
  - Sterility should be maintained by wrapping dishes in heavy aluminum foil, char-resistance paper, or stainless steel/ aluminum canisters.
- Loose-lid petri dishes should be incubated in a tight fitting container, with a moistened paper towel to prevent media dehydration.
- Opened packs of disposable dishes should be resealed between uses.
- Culture dishes should be appropriate size if used for membrane filter methods.

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Pipets

DWCM Chapter V; 3.13[1]

- Calibrated micropipetters may be used.
  - Pipet tips must be sterile
  - Micropipetters should be calibrated annually, and adjusted or replaced if accuracy is not within 2.5%.
- Glass pipets should be sterilized, then sterility should be maintained by wrapping pipets in heavy aluminum foil char-resistance paper, or stainless steel/ aluminum canisters.
- Pipets should have legible markings and should not be chipped or etched.
- Opened packs of disposable pipets should be resealed between uses.
- Accuracy must be within 2.5%

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Inoculating Equipment

DWCM Chapter V; 3.10[1]

- Acceptable materials: Sterile metal loops, disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipet tips

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Glassware and Plasticware

DWCM Chapter V; 3.14 & 4.5[1]

- Glassware should be made of borosilicate glass or other corrosion-resistant glass and free from chips and cracks.
- Plastic items must be clear and nontoxic to microorganisms.
- Markings should be legible.
- Graduated cylinders (or precalibrated containers) must be accurate within 2.5%.

Washing:

- Distilled or deionized water should be used for the final rinse.
- Glassware should be washed with a detergent designed for laboratory use.
- A glassware inhibitory residue test should be performed **before use** of a washing compound.
  - SM 9020B
  - Record results
- **Each batch** of washed glassware should be checked for pH reaction using 0.04% bromthymol blue.
  - pH should be neutral
  - Record results

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Autoclave

DWCM Chapter V; 3.5[1]

- Must have an internal heat source, temperature gauge with a sensor on the exhaust, pressure gauge, and an operational safety valve.
- Must maintain sterilization temperature during cycle and **complete entire cycle** (from start to removing items) in **45 minutes** for a 12-15 sterilization period.
- Door seals should be clean and free from caramelized media.
- Drain screens should be cleaned frequently and debris removed.
- Avoid overcrowding the autoclave.

- Maintain records of **each** autoclave use including:
  - Date
  - Contents
  - Sterilization time
  - Sterilization temperature
  - Total time in the autoclave
  - Analyst's initials

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Autoclave.. Quality Control

DWCM Chapter V; 3.5[1]

- Maintenance should be conducted **annually**.
  - Maintenance records should be kept and available for inspection.
- A maximum-temperature-registering (MRT) thermometer, electronic temperature readout device, or continuous recording device should be used during **every** use.
  - Ensure proper temperature was reached and record results
- Spore strips or ampules should be used **monthly** as bioindicators to confirm sterilization.
  - Record ampule incubator temperature and results of bioindicator test.
- Automatic timing mechanisms should be checked **quarterly** with a stopwatch.
  - Record results.

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Sterilization Procedures

DWCM Chapter V; 4.1[1]

Autoclaving times at 121°C for specific media:	
Membrane filters and pads	10 minutes[3]
Carbohydrate containing media	12-15 minutes[1,3]
Contaminated test materials	30 minutes[2]
Membrane filter assemblies	15 minutes
Sample collection bottles	15 minutes
Glassware	15 minutes
Dilution water blank	15 minutes
Rinse water (0.5-1 L)	15-30 minutes[2]

[1] Except where otherwise specified by the manufacturer
[2] Time depends upon water volume per container and autoclave load
[3] Do not exceed time and remove immediately after completion of sterilization cycle.

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Hot Air Oven

DWCM Chapter V; 3.6[1]

- Oven should maintain sterilization temperature of 170°-180°C for two hours.
- Thermometer should be graduated in at least 10° increments and bulb placed in sand during use.
- Avoid overcrowding the oven.
- Maintain records of **each** oven use including:
  - Date
  - Contents
  - Sterilization time
  - Sterilization temperature
  - Analyst's initials

Quality Control

- Spore strips should be used **monthly** to confirm sterilization.

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pH Meter

DWCM Chapter V; 3.1[1]

- Accuracy and scale graduations within ±0.1 S.U.
- Use buffer aliquots only once.
- Purchased buffer solutions should be dated and initialed when received
- Buffer solutions should be discarded by expiration date.

Quality Control

- Standardize meter before **every use** with pH 7.0 and either pH 4.0 or pH 10.0 buffers.
  - Slope should be 95-105%
  - Perform electrode maintenance or replacement if outside acceptable range
  - Record date, buffer lot #, slope, and analyst initials

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Conductivity Meter

DWCM Chapter V; 3.8[1]

- Should read in micromhos/cm or microsiemens/cm

Quality Control

- Calibrate meter at least **monthly**, following manufacturer's recommendations.
  - Use certified and traceable low-level standard
  - If meter cannot be calibrated with a standard, the **cell constant should be determined monthly** using a method in SM 2510.
  - If an in-line unit cannot be calibrated, it should not be used for monthly reagent grade water check.

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Balance

DWCM Chapter V; 3.2[1]

- Balance should have readability of 0.1g.
  - Should provide sensitivity of 0.1g for a load of 150g and 1mg for a load of 10g or less.

Quality Control

- Calibrated **monthly** with three (3) ASTM Class 1, 2, or 3 weights.
  - Record results
- Non-reference weights should be calibrated **every six months** against reference weights.
  - Correction values should be kept on file and used.
- Reference weights should be replaced or recertified **every five (5) years**.
  - Replace when damaged or corroded
- Maintenance and cleaning should be conducted **annually** by a certified technician.
  - Service contract records should be kept on file.

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Temperature Monitoring Device

DWCM Chapter V; 3.3[1]

- Thermometers must be graduated in 0.5°C increments or less for 35°C incubators and 0.2°C increments for 44.5°C incubators
  - **Kentucky certified labs are <u>strongly</u> suggested to have 35°C incubator thermometers graduated in 0.1°C increments**
  - For refrigerators: can be graduated in 1°C increments
- Glass thermometers should have no column separation.
- Discard thermometer if calibration differs by more than 1°C from reference thermometer
- In QC record book, record:
  - Serial # of laboratory thermometer
  - Serial # of NIST-traceable thermometer
  - Temperature of laboratory thermometer
  - Temperature of NIST-traceable thermometer
  - Correction factor
  - Date of check
  - Analyst's initials

Quality Control

- Calibration should be performed **annually** (dial thermometers- **quarterly**) against a NIST-traceable reference thermometer.
  - Calibration factor and date should be indicated on thermometer and used.
- Reference thermometers (NIST) should be recalibrated **every five years**
- Infrared thermometers must be verified every six (6) months against a NIST-traceable thermometer.
- Continuous recording devices should be recalibrated **annually** against a NIST-traceable thermometer.

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Refrigerator

DWCM Chapter V; 3.9[1]

- Refrigerator should maintain a temperature of 1-5°C.
  - Thermometer should be graduated in at least 1°C increments and the bulb immersed in liquid.

Quality Control

- On laboratory operation days, the calibrated-corrected temperature should be recorded **once per day**.
  - If not within limits, unit should be monitored and corrective action taken.

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Incubator

DWCM Chapter V; 3.4[1]

- Must have internal temperature monitoring device and maintain method specified temperature.
  - 35.0 ± 0.5°C or 44.5 ± 0.2°C
- Thermometers should be placed on top and bottom shelved of use area and bulbs immersed in liquid.
- For 44.5 ± 0.2°C incubation, a circulating water bath with gable cover can be used.

- Quality control:
- On laboratory operation days, the calibrated-corrected temperature should be recorded **twice per day, separated by at least four hours**.
  - Every thermometer in incubator should be read and temperature recorded.
  - If not within limits, unit should be monitored and corrective action taken.

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Sealer & MPN Trays Quality Control

Sealer

- Leak check should be conducted **monthly**.
  - Adding dye and water to tray, run through sealer. If dye is observed outside of well, perform maintenance or use a different sealer
  - Record results

Quanti-Tray or Quanti-Tray/2000

- Select **one tray from every batch**/ lot to check for sterility.
  - Add 100mL of sterile TSB; incubate at 35° ± 0.5°C for 24 hours. If growth is detected, use a different lot of trays.
  - Record results.
- Select **one tray from every batch**/ lot to check for auto fluorescence.
  - If tray fluoresces, use different lot of containers
  - Record Results

Ultraviolet lamps

DWCM Chapter V; 3.16[1]
WWCM Chapter IV; 3.12.4[2]

- For fluorometric tests, a longwave unit (365-366 nm) is used.
- For sterilization and sanitation, a germicidal shortwave unit (254 nm) is used.
- All units must be kept clean

Quality Control

- Unit output should be tested **quarterly.**
  - A UV light meter or testing against a positive control may be used to evaluate output.
  - UV lamps should be replaced when output emits less than 70% of original output, or fluorescence of positive control is significantly diminished.
  - Record results

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)
2 – Commonwealth of Kentucky Wastewater Laboratory Certification Manual (March 2018)