



Guidelines for Performing Quality Control Procedures

Water Microbiology Laboratories

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**Illinois Department of Public Health - Division of Laboratories
Quality Assurance Section**

PREFACE

This guideline is intended to provide assistance to laboratories seeking certification and laboratories currently certified for water microbiology methods that need clarification performing some quality control (QC) procedures. This publication is to be used as a guideline only. If a laboratory uses a quality control method not found in this guideline, that method will be reviewed by the certification officers to determine that it meets the requirements of Illinois Department of Public Health: 77 Ill. Adm. Code 465: Certification of Environmental Laboratories. Any method, which meets the above referenced requirements, may be used for quality control purposes. Where a specific method for performing a quality control procedure is cited in the Illinois rules for certification of environmental laboratories, an alternate procedure cannot be used.

Blank QC forms were developed to help assure that the laboratory will include all information required in the rules for certification. All forms are available on the IDPH website at <https://dph.illinois.gov/topics-services/lab-testing-services/environmental-water-lab-certification#forms> or by e-mail. Forms that have calculations in Excel are available by contacting your Laboratory Certification Officer. It is not mandatory that laboratories use these forms. However, the forms used must include all information on the original form and be in a format that can be readily interpreted without further explanation.

Forms are furnished to assist the laboratory in meeting the requirements, and to assure that all laboratories are using the forms in a uniform manner. Uniformity within the program helps to assure that all analyses conducted by certified laboratories throughout the state will provide valid results. Forms that have calculations automatically determined by the computer programs are completed with printed entries.

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EQUIPMENT CALIBRATION

Balance Calibration

Testing Frequency: Monthly

1. Calibrate balances using NIST Echelon I or II, or equivalent ASTM 1, 2, or 3 weights. Use a minimum of three weights, which bracket the weighing requirements of the laboratory.
2. Correction data shall accompany each ASTM (or NIST) weight. Have the weights recertified every five years.
3. Keep weights in a protective enclosure when not in use. Only handle the weights with the forceps provided by the supplier. Replace any weights that show any visible signs of damage. Keep the weights free from chemical spills.
4. Determine the weight of each standard weight and record.
5. Record the corrected weight as stated on the certificate of calibration for the weight.
6. Balances used for weighings of 2 g or more must be sensitive to 100 mg at a 150-gram load. Balances used for weighings of less than 2 g must be sensitive to 1 mg at a 10 gram load.
7. If the balance is not measuring accurately, contact a qualified maintenance representative. Do not use the balance until it is properly calibrated.

Conductivity Meter Calibration

Testing Frequency: Daily (when used)

1. Prior to use, calibrate the meter with a conductivity standard, according to manufacturer's directions. Use a certified and traceable low-level standard of 20 micromhos or less. The meter reading must be within 2% of the value of the standard. If the reading is not within the acceptable range, determine the cause, and then document corrective action taken.
2. Do not use the meter unless it has been properly calibrated.

Note: $1\mu\text{S}/\text{cm} = 1\mu\text{mho}/\text{cm}$

pH Meter Calibration

Testing Frequency: Daily (when used)

1. Follow manufacturer's instructions for calibration of the pH meter and for preparation and storage of the electrodes.
2. Use buffers only once and then discard.
3. Do not use buffers past their expiration date.
4. Remove the electrodes from the storage solution, rinse with laboratory pure water, blot protective shield dry with a soft tissue (per manufacturer's recommendation), and place in pH 7.00 buffer. Take a reading of the 7.0 buffer per manufacturer's directions.
5. Remove the electrode from the buffer, rinse with laboratory pure water, blot protective shield

dry with a soft tissue (per manufacturer's recommendation), and place in pH 4.00 and/or 10.00 buffer. Follow manufacturer's directions to adjust meter to this buffer.

6. Record the pH meter calculated percent slope reading. No manual calculations. Slope must be between 95-105%.
7. If the calibration readings are not acceptable, determine the cause, then take and document corrective action.
8. Do not use the pH meter until it is properly calibrated.

Thermometer Calibration

Testing Frequency: Annually

NIST Thermometer Ice Point Determination

1. Crush frozen laboratory pure water by a procedure that will prevent contamination of the ice.
2. Fill a wide-mouth container with the crushed ice and add enough laboratory pure water to make icy slush. Remove excess water and add more crushed ice as necessary during the temperature determination to maintain a slush consistency.
3. Determine immersion point based on the type of thermometer (total or partial immersion).
4. Insert the thermometer(s) into the slush for approximately 5 minutes, and then record the thermometer(s) temperature.
5. Reposition thermometer(s) in slush, wait one minute, and take a second reading.
6. Compare the average temperature and compare with the true temperature (0.0°) to obtain the correction factor.
7. If the correction factor has not changed, the calibration is still correct.

Test Thermometer Calibration

1. Calibrate each test thermometer at temperature of use.
2. Do not use any thermometer that has a correction factor greater than $\pm 1^{\circ}\text{C}$.
3. Tag all thermometers with date tested, correction factor, and analyst's initials, and maintain calibration records in the laboratory log.
4. Indicate + or – for the correction factor on the thermometer tag and on the thermometer calibration record.

Option A: Point of Use

1. Place the NIST thermometer and the test thermometer next to each other in a water bath. For use in other types of equipment, such as incubators or refrigerators, place thermometers in a liquid filled container prior to placing in the equipment.
2. Allow thermometer temperature to stabilize. Allow a minimum of five minutes for water baths. For all other thermometers, leave thermometers in the unopened piece of equipment overnight to allow adequate temperature stabilization.
3. If a full immersion NIST thermometer is placed inside a piece of equipment, determine the stem factor correction. Compare the test thermometer temperature against the true

temperature as determined by the NIST thermometer (by applying the correction factor stated on the NIST thermometer certificate that is closest to the temperature of the thermometer being calibrated) and record the correction factor.

Option B: Agitated liquid bath

1. Place the NIST and test thermometers in the bath at the correct level based on thermometer type (partial or total immersion).
2. Allow at least five minutes for the temperature to stabilize, and then obtain the thermometer temperatures.
3. Compare the test thermometer temperature against the true temperature as determined by the NIST thermometer (by applying the correction factor stated on the NIST thermometer certificate that is closest to the temperature of the thermometer being calibrated) and record the correction factor.

EQUIPMENT PERFORMANCE

Autoclave Sterility Check

Testing Frequency: Monthly

1. Biological indicators are composed of a standardized population of heat-resistant bacterial spores, such as *Geobacillus stearothermophilus*, most commonly in the form of ampules or strips. They are used to determine if the sterilization cycle parameters were sufficient to kill the test microorganisms.
2. Place the ampules or strips in the front and back, or top and bottom of the autoclave; determine position by the size and shape of the autoclave. For very small autoclaves, one ampule or spore strip is sufficient.
3. Perform the test with a full load during a typical sterilization cycle.
4. Indicate what material was sterilized with the spores.
5. Remove the ampules or strips from the autoclave after the cycle is completed.
6. Place an ampule or strip that has not been autoclaved with the others.
7. If strips are used, aseptically place them in tryptic soy broth.
8. Incubate ampules or strips at the time and temperature stated on the package insert.
9. Record results. Expected results following incubation:
Autoclaved spores = No Growth
Non-Autoclaved spores = Growth
10. If results are not acceptable, take and document corrective action. Repeat the test as soon as possible to demonstrate that the corrective action was effective.

Autoclave Timer Accuracy Check

Testing Frequency: Quarterly

1. Check autoclaves with automatic timers for accuracy using a stopwatch. Begin timing at the start of the sterilization cycle and conclude timing at the end of the cycle. For a 15-minute sterilization period, the time must be within 60 seconds.
2. If results are not acceptable, take and document corrective action. Repeat the test as soon as possible to demonstrate that the corrective action was effective.
3. An exception to this rule would be a data logger or an autoclave that has a printout of time that has been calibrated annually by an outside service.

Oven Sterility Check Using Spore Strips

Testing Frequency: Monthly

1. Use strips impregnated with *Bacillus atrophaeus* spores to determine whether the oven has reached sterilization temperature for a sufficient period to sterilize the contents. Do not use ampules because they can explode at the temperature reached during the sterilization cycle.
2. Place a strip in the front and the back, or the top and the bottom of the oven; determine the location by the size and shape of the oven. Perform this test with a full load during a typical sterilization cycle. Indicate in the records what material was sterilized with the spores.
3. Remove the strips from the oven after the cycle is completed.
4. Incubate at the time and temperature stated on the package insert.
5. Record results. Expected results following incubation:
Autoclaved spores = No Growth
Non-Autoclaved spores = Growth
6. If results are not acceptable, take and document corrective action. Repeat the test as soon as possible to demonstrate that the corrective action was effective.

Media Dispenser Accuracy Check

Test Frequency: Beginning of run, each volume change, and periodically throughout extended runs

1. Indicate the Brand/Model of dispenser used.
2. Record "Set Volume" as the desired volume to be dispensed.
3. Record acceptable volume range.
4. Calibrate dispenser.
5. After completion of calibration, dispense into a Class A graduated cylinder and record the "Cylinder Volume." Repeat twice and record the three volumes.
6. Dispense into the cylinder after completing run. Record the final measurement.
7. The cylinder volume must be within 2.5% of the "Set Volume."

Quanti-Tray/Quanti-Tray 2000 Sealer Test

Testing Frequency: Monthly

1. Add a dye (e.g., bromcresol purple) to 100 mL water.
2. Add water to tray and operate sealer.
3. If dye is observed outside the wells, do not use sealer.
4. Record results. If results are not acceptable document corrective action taken. Repeat the test as soon as possible to demonstrate that the corrective action was effective.

Temperature Monitoring Records

1. Indicate in the records when an adjustment is made to the thermostat.
2. When possible, provide an explanation of why the temperature is out of tolerance and, if applicable, the corrective action taken (e.g., power failure; defective thermostat, replaced 11/29/22; and air conditioner malfunctioned, repairs made 10/9/22).
3. If the temperature is out of tolerance for an extended period of time, record all affected sample results as "laboratory accident." Contact an LCO for additional information.
4. Record corrected temperature. Example: If the correction factor is determined to be +0.1°C and the thermometer reading is 44.7°C, then record the temperature as 44.8°C.
5. Temperatures in incubation equipment shall be recorded twice daily (times separated by at least 4 hours). Documentation shall include date and time of reading, temperature and initials of analyst.

Agar Tempering Water Bath

Frequency: Daily or when in use

Tolerance: $45 \pm 1^{\circ}\text{C}$

Refrigerator

Frequency: Daily

Tolerance: $1 - 5^{\circ}\text{C}$

Spore Incubator

Frequency: Daily or when in use

Tolerance: Follow spore manufacturer's written instructions. Maintain a copy of the instructions in the quality assurance manual.

Fecal Water Bath

Frequency: Twice daily

Tolerance: $44.5 \pm 0.2^{\circ}\text{C}$

Incubator

Frequency: Twice daily

Tolerance: $35 \pm 0.5^{\circ}\text{C}$

Ultraviolet Lamp Efficiency Tests

Testing Frequency: Quarterly

Option A - Percent Killed:

1. Prepare a previously assayed coliform culture in buffered dilution water to yield a count of 200 to 250 organisms per mL.
2. Add 1 mL of the diluent to each of two petri dishes.
3. Expose one dish to ultraviolet light for two minutes. Do not expose the other dish.
4. Add 10 to 15 mL of heterotrophic plate count agar tempered to $45 \pm 1^\circ\text{C}$ to each dish and mix thoroughly.
5. Incubate both dishes at $35 \pm 0.5^\circ\text{C}$ for 48 ± 3 hours.
6. Count each plate and record.
7. Determine the percent killed by dividing the exposed count by the unexposed count, multiplying by 100, then subtracting the result from 100.

Example:

$$\text{Exposed count} = 2$$

$$\text{Unexposed count} = 248$$

$$2 \div 248 = 0.008$$

$$0.008 \times 100 = 0.800$$

$$100 - 0.800 = 99.2\%$$

8. The percent killed must be equal or greater than 99%.
9. If the results are not acceptable, determine the cause, document corrective action, and as soon as possible repeat the test to demonstrate that the actions taken are acceptable. Do not use the light unless it has been demonstrated that it is performing within acceptable limits.

Option B - Ultraviolet Light Meter:

1. Determine the lamps output with an ultraviolet light meter when the lamps are first put into service.
2. Quarterly, check the output.
3. Determine the percentage of quarterly output compared with initial output. Determine percentage by dividing current output by initial output, then multiplying by 100. Example:
Initial output = $1500 \mu\text{W}/\text{cm}^2$
Current output = $1100 \mu\text{W}/\text{cm}^2$
 $1100 \div 1500 = 0.733$
 $0.733 \times 100 = 73.3\%$
4. Replace the lamps if current output is less than 70% of initial output.

MEDIA AND SUPPLIES

Membrane Filtration Funnel Calibration

Test Frequency: Before first use

1. If graduation marks on clear glass, stainless steel, or plastic funnels are used to measure the filtered sample volume, check the calibration of each membrane filtration funnel.
2. Place the liner from a membrane filter in the funnel to prevent water from leaving the funnel.
3. Add water to the calibration mark.
4. Carefully pour contents into a Class A graduated cylinder. If the volume is above the 100 mL graduation, determine the overage by using a Class A graduated pipet.
5. Record results.
6. Tolerance: $\pm 2.5\%$.
7. Do not use the graduation mark on any funnel that is not within the accepted limits. If a funnel that is out of tolerance is used for filtration, clearly indicate on the funnel that it is not within tolerance and measure sample volumes with a graduated cylinder demonstrated to be within tolerance.
8. Maintain this record for a minimum of five years after the last funnel entered on the form is no longer in use.

Sample Containers

Sterility Check

Testing Frequency: Each Batch Prepared or Each Lot of Containers Purchased

1. Check at least one container per batch (or per lot if purchased) for sterility by adding approximately 25 mL of sterile non-selective broth (e.g., tryptic soy, trypticase soy, nutrient broth or tryptone broth) to each container.
2. Cap and rotate the container in such a manner that the broth comes in contact with all surfaces.
3. Incubate at $35 \pm 0.5^\circ\text{C}$.
4. Check for growth at 24 and 48 hours. Turbidity = growth.
5. No growth should be detected. Do not use containers if growth is present. Take and document corrective action.

Sodium Thiosulfate Check

Testing Frequency: Each Batch Prepared or Each Lot of Containers Purchased

1. Check at least one container per batch (or per lot if purchased) for the presence of a sufficient amount of sodium thiosulfate to neutralize the chlorine present in the samples routinely received in the laboratory.

- i. Collect a potable sample at the laboratory tap.
 - ii. Check the sample for residual chlorine using the DPD Method sited in “Standard Methods for the Examination of Water and Wastewater”¹.
2. Determine that the test reagent is properly performing by testing a sample of chlorinated tap water that has not been exposed to a dechlorinating agent.
 - i. Collect a potable sample at the laboratory tap, collecting it in a container without sodium thiosulfate.
 - ii. Check the sample for residual chlorine using the DPD Method sited in “Standard Methods for the Examination of Water and Wastewater”¹.
3. Take and document corrective action if there is any residual chlorine detected in the sample collected in the container(s) with sodium thiosulfate or if the test reagent does not detect chlorine in the sample collected in the container without sodium thiosulfate. Notify suppliers of purchased containers if the containers do not meet the requirements. Do not use containers that do not meet the requirements.

Volume Check of Pre-calibrated Containers

Test Frequency: Each new lot of containers

1. Before first use, check the pre-calibrated volume of the containers by measuring the volume of one container per lot with a class “A” graduated cylinder.
2. Do not use containers if the volume is not within $\pm 2.5\%$ of the specified volume. Take and document corrective action.

Fluorescence Check of Pre-calibrated Containers

Test Frequency: Each new lot of containers

1. Check each lot of containers before use with a 365-366 nm ultraviolet light with a 6-watt bulb placed within 5 inches of container.
2. Record results.
3. If the container exhibits faint fluorescence, return to manufacturer.

Alkalinity/Acidity Test (Bromothymol Blue Test)

Testing Frequency: Each batch washed

1. Determine that the bromothymol blue solution is neutral before initial use with a pH meter. Recheck the solution if a color change is noted during the period of use.
2. Add a few drops of bromothymol blue indicator to test for residual alkaline or acid residue to a representative piece of each type of clean and dried glassware or plastic ware from each batch prepared. The indicator is green when neutral, blue when alkaline, and yellow when acidic. The indicator is green when the glassware is properly washed and rinsed.
3. If the results of the indicator test are not neutral (green), take corrective action by re-rinsing

all of the glassware/plastic ware and air-drying. Retest a representative piece. Document that the corrective action taken resulted in acceptable results.

4. Do not use any glassware from a batch that has not passed the bromothymol test.

Inhibitory Residue Test

Testing Frequency: Initially, if different formulation or washing procedure used

Option A:

1. Wash and rinse six glass Petri dishes according to the usual procedure. Designate as Group A.
2. Prepare six glass petri dishes as above, rinse 12 times with successive portions of laboratory pure water. Designate as Group B.
3. Rinse six petri dishes with detergent wash water (in use concentration), dry without further rinsing, and designate as Group C.
4. Sterilize the three groups by the usual procedure.
5. Designate six sterile plastic petri dishes as Group D.
6. Add 1 mL of a suspension of *E. aerogenes* (which has previously been assayed) to yield 50 to 150 colony forming units to three of the six plates from each group. Add 0.1 mL to the remaining three plates of each group to help assure that the counts will be within the acceptable range.
7. Following the requirements for the Heterotrophic Plate Count, Pour Plate Method 9215 B, "Standard Methods for the Examination of Water and Wastewater"¹, add 10-12 mL of standard plate count agar to each plate, plate controls, and incubate at 35°C for 48 ± 3 hours.

Option B:

1. Group A = Wash and rinse six culture tubes or dilution bottles following the usual cleaning procedure.
2. Group B = Prepare a set of tubes or dilution bottles as in step 1, and then rinse with twelve successive rinses of laboratory pure water.
3. Group C = Wash six culture tubes or dilution bottles with detergent wash water (in-use concentration) and drain dry without rinsing.
4. Group D = Designate six sterile plastic petri dishes.
5. Sterilize Groups A, B, and C. Dispense 20 mL of nutrient broth in each set of tubes or dilution bottles, and into the Group D petri dishes.
6. Inoculate each tube, bottle, or dish with 1 mL of *E. aerogenes*.
7. Incubate all tubes or dilution bottle cultures at 35°C for 24 hours.
8. Prepare appropriate dilutions of triplicate plates using standard plate count agar to yield a count of 50 to 150 colony-forming units and incubate for 48 ± 3 hours at 35 ± 0.5°C, following the requirements for the Heterotrophic Plate Count, Pour Plate Method 9215 B, "Standard Methods for the Examination of Water and Wastewater"¹. Follow all the requirements of this method.

Interpretation of Results:

1. Differences in averaged counts on plates in Groups A, B, and C must be less than 15%, if there are no toxic or inhibitory effects.
2. Differences in averaged counts of less than 15% between Groups A and B and greater than 15% between Groups A and C indicate that the detergent has inhibitory properties that are eliminated during routine washing.
3. If results indicate that inhibitory properties are present that are not eliminated during routine washing, take and document corrective action.

Cap Toxicity Test

Testing Frequency: Each batch purchased

Determine that the caps contain no toxic substances before placing them into use, whenever a new shipment of plastic caps for either dilution or sample bottles is received.

1. Remove 10-15 caps from the shipment and autoclave them in sterile buffered rinse water (dilution water). Prepare buffered water according to "Standard Methods for the Examination of Water and Wastewater"¹, 9050.C.1.a
2. Save the water from this autoclaving run and prepare a 99 mL dilution blank using this water. Autoclave this dilution blank for 15 minutes and allow it to cool to room temperature.
3. Inoculate the dilution blank with a previously assayed suspension of *E. aerogenes* that will provide colony-forming units within the countable range (30 to 300 colony forming units) throughout the period of the test.
4. Shake the dilution blank vigorously and plate immediately (0 minutes) with Standard Plate Count Agar.
5. Allow the dilution blank to stand at room temperature for 15 minutes, shake the sample vigorously and plate again (15-minute count).
6. Repeat two more times until you have plates that have been plated at 0, 15, 30, and 45 minutes.
7. Analyze all samples (Repeat Steps 4 - 6, including controls, as required in the Heterotrophic Plate Count, Pour Plate Method 9215 B, "Standard Methods for the Examination of Water and Wastewater"¹).
8. Immediately after the agar has solidified (no longer than 20 minutes after plating) incubate the plates at $35^{\circ}\text{C} \pm 0.5^{\circ}$ for 48 ± 3 hours.
9. Compare the counts from each time interval. If the 45-minute count is at least 20% less than the 0-minute count, the caps contain a toxic substance and must be treated before use.
10. Should it be determined that the caps contain a toxic substance, autoclave **all** of the caps in **several changes** of sterile buffered rinse water and repeat the above procedure until it is determined that the caps are no longer toxic. Document that corrective action was taken.

Laboratory Pure Water

Chlorine Residual

Testing Frequency: Monthly

1. Test laboratory pure water as specified in “Standard Methods for the Examination of Water and Wastewater”¹.
2. Determine that the test is performing properly by testing a sample of chlorinated tap water that has not been exposed to a dechlorinating agent (positive control).

Do not use the laboratory pure water if any chlorine residual is detected. Take corrective action (if purchased, obtain a new supply; if prepared in-house, maintain distillation, deionization, filtration, and/or reverse osmosis system). Document corrective action taken and repeat the test as soon as possible. Determine that there is no chlorine residual before resuming use of the water.

Conductivity

Testing Frequency: Monthly

Conductivity must be less than 2 micromhos/cm (resistivity greater than 0.5 megohms-cm). Use a properly calibrated conductivity meter (using a low-level standard of 20 micromhos or less). Use manufacturer’s instructions on proper calibration of meter. Meter reading shall be within 2% of the value of the standard. Obtain the conductivity of a freshly prepared (or if purchased, initially opened) sample of laboratory pure water. If results are unacceptable, immediately determine and correct the cause. Obtain a sample after the problem has been corrected and test to determine that the conductivity is acceptable. If acceptable results cannot be obtained, another source of acceptable laboratory pure water must be acquired. Do not prepare media or reagents from water that is not within the acceptable range.

Heterotrophic Plate Count

Testing Frequency: Monthly

1. Determine the heterotrophic plate count of the laboratory pure water with the Pour Plate Method, “Standard Methods for the Examination of Water and Wastewater”¹, [section 9215B] or the SimPlate method. Plate duplicate 1.0 mL and 0.1 mL portions for the pour plate method.
2. An acceptable count is < 500/mL.
3. Do not use any water that has not yielded acceptable results.

Suitability Test

Testing Frequency: Annually, when the source of laboratory pure water changes, or when modifications are made to the treatment system that could affect the quality of the water.

Refer to “Standard Methods for the Examination of Water and Wastewater”¹ [section 9020 B.5.f.1]. This test is not required for medium quality water or better as defined in Standard Methods section 1080C and section 9020B.4d (conductivity <1 µmho/cm @ 25°C).

Dilution and Rinse Water Sterility Check

Testing Frequency: Each batch prepared or each lot purchased

1. Check each batch of prepared or each lot of commercial dilution/rinse water for sterility by adding 50 mL of dilution/rinse water to 50 mL of double strength non-selective broth (e.g., tryptic soy, trypticase soy, nutrient broth or tryptose broth).
2. Incubate at $35 \pm 0.5^\circ\text{C}$.
3. Check for growth at 24 and 48 hours.
4. Discard if growth is detected. Document corrective action taken and prepare or obtain a new supply.

Dilution Water Volume Check

Testing Frequency: Each batch prepared or each lot purchased

1. Check 1 of 25 dilution blanks per batch of prepared or lot of commercial dilution water blanks for volume using a Class A graduated cylinder.
2. Discard the entire batch or lot if the volume of any of the blanks tested is not $99 \text{ mL} \pm 2 \text{ mL}$.

Note: It may be necessary to add 101 mL to 102 mL dilution water initially, to allow for water loss during autoclaving. Determine the initial volume that must be added to obtain $99 \text{ mL} \pm 2 \text{ mL}$ after autoclaving and include in the quality assurance manual.

Note: For “Dilution Water pH Determination” see page 16.

Membrane Filter Check

Testing Frequency: Each lot

1. Check new lot of membranes by performing a positive control with a count of 20 to 80 colonies per plate.
2. Record positive culture used and positive result.
3. Examine membrane for any gridline inhibition and record results.
4. Any gridline inhibition is recorded as unacceptable, and membranes returned to vendor.

Media Performance Checks

Depending upon the strain, organisms may give either typical or atypical results. For example, *Klebsiella pneumoniae* may form either typical or atypical colonies and may or may not be thermo tolerant. Some *Escherichia coli*, including serotype O157:H7, are neither thermo tolerant nor fluoresce in the presence of MUG. When using an organism for quality control purposes, document with references the characteristics of the strain used.

Enzyme Substrate Quality Control

Testing Frequency: Each lot of medium

Media Performance Check:

1. Label four sterile water bottles A, B, C, and D. Adding one pre-measured (by the manufacturer) aliquot of medium to each of four 100 mL sterile water. Inoculate the containers as follows:
 - A = MUG-positive *E. coli* strain
 - B = MUG-negative coliform
 - C = Non-fluorescent, non-coliform
 - D = Not inoculated/incubated. This container is checked for fluorescence and pH.
2. Incubate and interpret results for each sample. Record results. Expected results:
 - A = Positive color and fluorescence
 - B = Positive color only (no fluorescence)
 - C = No color or fluorescence
3. If acceptable results are not obtained, do not use the lot of media. Document corrective action taken.

Fluorescence Check

1. Check each lot of medium before use with a 365-366nm ultraviolet light with a 6-watt bulb placed within 5 inches of container. Check fluorescence immediately after adding medium into sterile water.
2. Record results.
3. If the medium exhibits faint fluorescence, discard or return to manufacturer. Document corrective action taken.

m-Endo Broth/Agar Media Performance Check

Testing Frequency: Each batch prepared or each lot purchased

1. Set up two plates and filter a positive and a negative control.
2. Incubate at $35 \pm 0.5^{\circ}\text{C}$ for 22-24 hours.
3. Record results: appearance of colonies present or no growth.
4. Typical results of positive cultures:
 - i. *E. coli* = colonies with golden green sheen
 - ii. *Enterobacter aerogenes* = colonies with golden green sheen
5. Typical results of negative cultures:
 - iii. *Pseudomonas aeruginosa* = clear colonies or no growth
 - iv. *Proteus mirabilis* = clear colonies
 - v. *Enterococcus faecalis* = no growth
 - vi. *Staphylococcus aureus* = no growth

m-FC Broth/Agar Performance Check

Testing Frequency: Each batch prepared or each lot purchased

1. Set up two plates and filter a positive and a negative control.
2. Incubate at $44.5 \pm 0.2^{\circ}\text{C}$ for 24 ± 2 hours.
3. Record results: appearance of colonies present or no growth.
4. Typical results of positive cultures:
 - i. *Escherichia coli* = blue
5. Typical results of negative cultures:
 - i. *Enterobacter aerogenes* = no growth
 - ii. *Pseudomonas aeruginosa* = no growth
 - iii. *Proteus mirabilis* = no growth
 - iv. *Enterococcus faecalis* = no growth
 - v. *Staphylococcus aureus* = no growth

Lauryl Tryptose Broth Performance Check

Testing Frequency: Each batch prepared, or each lot purchased

1. Set up two tubes and inoculate the first with a negative control and the second with a positive control.
2. Incubate at $35 \pm 0.5^{\circ}\text{C}$ for 24 to 48 hours.
3. Record results: growth with gas, growth without gas, or no growth.
4. Typical results of positive cultures:
 - i. *E. coli* = growth with gas
 - ii. *Enterobacter aerogenes* = growth with gas
5. Typical results of negative cultures:
 - i. *Proteus mirabilis* = no growth
 - ii. *Enterococcus faecalis* = no growth
 - iii. *Staphylococcus aureus* = no growth

Brilliant Green Bile

Testing Frequency: Each batch prepared or each lot purchased

1. Set up two tubes and inoculate the first with a negative control and the second with a positive control.
2. Incubate at $35 \pm 0.5^{\circ}\text{C}$ for 24 to 48 hours.
3. Record results: growth with gas, growth without gas, or no growth.
4. Typical results of positive cultures:
 - i. *E. coli* = growth with gas
 - ii. *Enterobacter aerogenes* = growth with gas
5. Typical results of negative cultures:
 - i. *Proteus mirabilis* = no growth
 - ii. *Enterococcus faecalis* = no growth
 - iii. *Staphylococcus aureus* = no growth

EC Broth

Testing Frequency: Each batch prepared or each lot purchased

1. Set up two tubes and inoculate the first with a negative control and the second with a positive control.
2. Incubate at $44.5 \pm 0.2^{\circ}\text{C}$ for 24 ± 2 hours.
3. Record results: growth with gas, growth without gas, or no growth.
4. Typical results of positive cultures:
 - i. *E. coli* = growth with gas
5. Typical results of negative cultures:
 - i. *Proteus mirabilis* = no growth
 - ii. *Enterococcus faecalis* = no growth
 - iii. *Staphylococcus aureus* = no growth

EC Broth + MUG

Testing Frequency: Each batch prepared or each lot purchased

1. Set up two tubes and inoculate the first with a negative control and the second with a positive control.
2. Incubate at $44.5 \pm 0.2^{\circ}\text{C}$ for 24 ± 2 hours.
3. Record results: growth with fluorescence or no growth.
4. Typical results of positive cultures:
 - i. *E. coli* = growth with fluorescence
5. Typical results of negative cultures:
 - ii. *Proteus mirabilis* = no growth
 - iii. *Enterococcus faecalis* = no growth
 - iv. *Staphylococcus aureus* = no growth

Standard Plate Count Agar

Testing Frequency: Each batch prepared or each lot purchased

1. Set up a positive and negative (no inoculum) control plate
2. Incubate at $35 \pm 0.5^{\circ}\text{C}$ for 48 ± 3 hours.
3. Record results = growth or no growth
4. The following cultures will typically grow on standard plate count agar: *E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus faecalis*, *Staphylococcus aureus*

SimPlate

Testing Frequency: Each lot purchased

1. Set up a positive and negative (no inoculum) control plate
2. Incubate at $35 \pm 0.5^{\circ}\text{C}$ for 48 ± 3 hours.
3. Record results: number of fluorescence wells or no growth
4. The following cultures will typically grow on standard plate count agar: *E.coli*, *Enterobacter aerogenes*, *Pseudomonal aeruginosa*, *Proteus mirabilis*, *Enterococcus faecalis*, *Staphylococcus aureus*

Dilution Water, Media and Buffer pH Determination

Testing Frequency: Each batch prepared or each lot purchased

1. After determining that the pH meter is properly calibrated, follow manufacturer's directions to determine the pH of each batch of prepared media (or lot of purchased media). The media must be at room temperature for the result to be valid.
2. Record pH.
3. If the pH is not within the limits shown below, determine the cause.
4. Do not use any media that does not have an acceptable pH value. Document corrective action taken.

<u>Media</u>	<u>pH range</u>
m-Endo Broth/Agar	7.2 ± 0.2
m-FC Broth/Agar	7.4 ± 0.2
Lauryl Tryptose Broth (single strength)	6.8 ± 0.2
Lauryl Tryptose Broth (double strength)	6.8 ± 0.2
Lauryl Tryptose Broth (triple strength)	6.8 ± 0.2
Brilliant Green Bile, 2%	7.2 ± 0.2
EC Broth	6.9 ± 0.2
EC Broth + MUG	6.9 ± 0.2
Standard Plate Count Agar	7.0 ± 0.2
Stock Phosphate Buffer	7.2 ± 0.5
Dilution Water	7.2 ± 0.2
Nutrient Agar + MUG	6.8 ± 0.2
SimPlate	7.0 ± 0.3
Colilert	7.3 ± 0.3
Colilert-18	7.3 ± 0.3
Colisure	7.3 ± 0.3
Colilert	7.3 ± 0.3
E*Colite	6.9 ± 0.2
Readycult	6.8 ± 0.2
Modified Colitag	6.8 ± 0.2

m-Colibblue 24	7.0 ± 0.2
MI agar	6.95 ± 0.2
MI Broth	7.05 ± 0.2
Non-selective broth	Per manufacturer's instructions

Media Inventory

1. Document that all media is used before the expiration date, and that the media is used on a first in, first out basis.
2. Use the media inventory form in conjunction with the other media quality control forms, to demonstrate that all media received was satisfactorily determined to meet performance criteria before use.
3. Record the following information for each container of media received:
 - a. Type of media (e.g., lauryl tryptose broth, m-Endo broth, and Colilert)
 - b. Brand
 - c. Lot number
 - d. Date Received
 - e. Date Opened
 - f. Expiration Date
 - g. Date Discarded

STERILIZATION RECORDS NON-MEDIA

Autoclave

Frequency: Each cycle

1. Autoclave items listed below at $121 \pm 1^\circ\text{C}$ for the minimum duration given, with the exception of membrane filters and pads and carbohydrate-containing media. Exceeding the time stated below can compromise these items.

<u>Item</u>	<u>Duration of Autoclaving at $121 \pm 1^\circ\text{C}$</u>
Membrane filters and pads	10 minutes
Carbohydrate-containing media	12-15 minutes
Contaminated materials and discarded tests	30 minutes
Membrane filter assemblies	15 minutes
Sample collection bottles	15 minutes
Individual glassware	15 minutes
Rinse water volumes of 500 to 1000 mL	45 minutes
Rinse water volumes in excess of 1000 mL	Time adjusted for volume
Dilution water blanks	15 minutes

2. The maximum elapsed time for exposure of carbohydrate-containing media to any heat (from the time of closing the loaded autoclave to unloading) is 45 minutes.

3. Record contents, date, time in autoclave, duration (time sterilization temperature is reached to time sterilization cycle turns off), time removed from autoclave, total time in autoclave, temperature of autoclaving, and initials.

Oven

Frequency: Each cycle

1. Sterilize dried glassware at $175^{\circ} \pm 5^{\circ}\text{C}$ for at least two hours.
2. Record item, date, time placed in oven, duration (time sterilization temperature is reached to time oven is turned off), time removed from oven, sterilization temperature, and initials.

QUALITY CONTROLS FOR TESTING PROCEDURES

Agar Weight Loss Determination for the Heterotrophic Plate Count Procedure

Testing Frequency: Quarterly

1. Separately weigh three empty petri dishes (top and bottom). Record weights.
2. Pour 10-12 mL of Standard Plate Count Agar into each plate and allow to solidify.
3. Weigh each plate again (including the agar). Record weights.
4. Determine the weight of the agar in each plate by subtracting the result of step 1 from the result of step 3. Record results.
5. Incubate the plates for 48 ± 3 hours at $35 \pm 0.5^{\circ}\text{C}$.
6. Weigh each plate again (including the agar). Record weights.
7. Determine the amount of weight lost from the agar by subtracting the result of step 6 from step 3. Record results.
8. Determine the percentage of agar weight loss by dividing the result of step 7 from the result of step 4. Record results.
9. The average percent weight loss of the agar should not exceed 15% of its original weight during a normal incubation period (48 ± 3 hours) at the proper temperature ($35 \pm 0.5^{\circ}\text{C}$).
10. If the weight loss exceeds 15%, document corrective action taken. Repeat the test to demonstrate that the corrective action was satisfactory.

Heterotrophic Plate Count Controls

Maintain records on laboratory bench sheets

Agar: per bottle poured
Dilution Water Blank: per plating series (check each batch/lot used during series)
Pipets: per plating series (check each container used during series)
Air Density: per plating series

1. Microbial Density of Air:
 - i. After labeling all plates and before pipetting the first sample, pour approximately 15 mL of agar into a petri dish.
 - ii. Immediately remove the petri dish lid and set it inside down on the counter next to the plate. Locate the air plate in the plating area, not off to the side.
 - iii. Expose the agar in the plate for 15 minutes; set a timer.
 - iv. Incubate 48 ± 3 hours at $35 \pm 0.5^\circ\text{C}$.
 - v. Count plate and record results. Acceptable results: less than 15 colonies.
 - vi. When 15 or more colonies appear on the exposed plate, take corrective action and document.
2. Dilution Water Blank:
 - i. Pour approximately 1 mL from a dilution water blank into a petri dish.
 - ii. Add agar and gently mix.
 - iii. Incubate 48 ± 3 hours at $35 \pm 0.5^\circ\text{C}$.
 - iv. If any growth appears after 48 ± 3 hours of incubation at $35 \pm 0.5^\circ\text{C}$, take corrective action and document.
3. Pipets:
 - i. After pouring the dilution water blank control, pipet 1 mL of dilution water into a petri dish. If a dilution blank is not used in a series, pour approximately 15 mL of agar into a plate, then using the pipet to be tested, withdraw 1 mL of agar, dispensing it back into the petri plate.
 - ii. Gently swirl to mix.
 - iii. Incubate 48 ± 3 hours at $35 \pm 0.5^\circ\text{C}$.
 - iv. If any growth appears, take corrective action and document.
4. Agar:
 - i. Pour approximately 15 mL of agar into a petri dish and gently swirl. This control is the last sample poured per each container of agar.
 - ii. Incubate 48 ± 3 hours at $35 \pm 0.5^\circ\text{C}$.
 - iii. If any growth appears, take corrective action and document.
5. If the agar control is contaminated, and the petri dishes are the suspected source of contamination, conduct further testing to verify the source of contamination. Pour 10-12 mL of agar into a petri dish, and then incubate the rest of the agar in its original container. If growth appears in the petri dish alone, the petri dish is contaminated.
6. Sample results are invalid if any controls exceed limits. The results cannot be reported.

Membrane Filtration Procedures

Maintain records on laboratory bench sheets.

Testing Frequency: Each funnel used in each filtration series

1. Beginning Rinse: At the beginning of a series, direct approximately 20 mL of rinse water around the interior of the filtration funnel while the vacuum is running. After all of the water has passed through the membrane, repeat the process. Place the membrane in a plate containing medium. Incubate with the other plates in the series.
2. Intermediate Rinses: It is recommended that the procedure described in "1" above be repeated after every ten samples to avoid rejecting all of the samples within a series when contamination occurs during the filtration series.
3. Final Rinse: Perform the procedure described in "1" above at the conclusion of the filtration series.
4. If the rinse controls are positive, do not report positive results affected and request replacement samples. Negative sample results are valid and, if possible, can be used to determine the point at which contamination occurred.

Analyst Verifications and Comparisons

Total and Fecal Coliforms by Membrane Filter for SWTR

Testing Frequency: Monthly

1. FC Verification: Each analyst approved for the fecal coliform procedure by the membrane filter technique must verify fecal coliform analyses. Pick ten isolated colonies from membranes containing typical blue colonies and transfer to lauryl tryptose broth and EC medium. Incubate the lauryl tryptose broth at $35^{\circ} \pm 0.5^{\circ}\text{C}$ for 24 to 48 hours. Incubate the EC medium at $44.5^{\circ} \pm 0.2^{\circ}\text{C}$ for 24 hours. Turbid growth with gas production indicates a positive result.
2. Comparison Counts: If there is more than one analyst in the laboratory approved for a procedure, each analyst must count the same heterotrophic plate count, total coliform membrane, and fecal coliform membrane. Total and Fecal Coliform for source water samples (SWTR) only. Colony counts between analysts must agree within ten percent.
3. If analyst results are not acceptable, determine cause, and take and document corrective action.

Chemical Treatment of Biological/Infectious Waste Solutions with Bleach (Sodium Hypochlorite)

This treatment is for liquids with cultures in suspension.

1. Add household bleach to achieve 15% bleach solution, volume per volume
 - i. 17.5 mL bleach per 100 mL culture
 - ii. 87.5 mL bleach per 500 mL culture
 - iii. 175 mL bleach per 1 L culture (about $\frac{3}{4}$ cup bleach)
 - iv. 1.75 L bleach per 10 L culture (about $\frac{1}{2}$ gallon bleach)
 - v. 17.5 L bleach per 100 L culture (about 4.5 gallons bleach)
2. Mix and treat for a minimum of twenty (20) minutes.
3. Optional: If desired, water may be dechlorinated following treatment. Sodium thiosulfate may be added at the rate of 20 grams per 1 L of solution, mixed and allowed to react for 2 hours.
4. Dispose treated liquid into the sink with copious amounts of water.

Comment: The specific solutions stated in the rule are percent (%) solutions of household bleach, not percent solutions of the active ingredient: sodium hypochlorite. The hypochlorite concentration of household bleaches range from 3.00 – 5.25 %. The resulting hypochlorite concentration of the treatment solution ranges from 0.45-0.79 % (or 4500 – 7875 ppm). If a laboratory strength (concentrated) solution of sodium hypochlorite is used, the volume should be adjusted accordingly to achieve the same final concentration of 4500 to 7800 ppm hypochlorite.

Method to generate colony forming unit counts

Option A

1. Start with a 24-hour culture of the organism in TSB (Trypticase Soy Broth) that you are studying.
2. Take the tube and vortex for approximately 10 seconds.
3. Take a 0.5 mL portion of the 24-hour culture and transfer to a 99 mL dilution blank. This is dilution A. Shake 25 times in 7 seconds in a one-foot arc.
4. Take 1 mL of dilution A and transfer to a 99 mL dilution blank. This is dilution B. Again shake 25 times in 7 seconds in a one-foot arc.
5. Take 1 mL of dilution B and transfer to a 99 mL dilution blank. This is dilution C. Again shake 25 times in 7 seconds in a one-foot arc.
6. A 0.1 mL portion of dilution C will contain between 30 - 50 CFUs of the organism of interest.

This procedure will work with most organisms. Caution should be noted if you culture your organisms in a more inhibitory broth than TSB, your numbers will be lower.

Option B

To obtain counts of 60-120 CFUs. Purchase a set of McFarland Equivalence Turbidity standards tubes. They range from 0.5 to 4 on a turbidity scale. ATCC lyophilized disks of the organism you want can be used. Grow the culture on agar slants or plates. Swab the plate with growth and put it into a small test tube with about 7 mL of sterile water. Move the swab up and down to create turbidity. Compare the

tube between 1-2 turbidity tube. Add more water if too strong or add more cultures if weak. Once the culture tube has a turbidity between 1 and 2, add 1 mL into a 99 mL dilution blank. Shake well. From the first dilution blank add 1 mL into a second dilution blank. Shake well. From the second dilution blank add 1 mL into a third dilution blank. From the third dilution blank add 0.2 mL, 0.3 mL, and 0.4 mL into three separate dilution blanks. Filter the three dilution blanks to get your countable plates. HPC can also be run from the dilution blanks.

REFERENCES

¹ "Standard Methods for the Examination of Water and Wastewater" 21st, 22nd, 23rd Edition American Public Health Association, 800 I Street, NW, Washington DC 20001-3710

² "Manual for the Certification of Laboratories Analyzing Drinking Water," EPA 815-R-05-004, 5th Edition (January 2005)