**Section 465.400 Quality Assurance for Media, Equipment and Supplies**

The following minimum requirements shall apply to quality assurance checks of laboratory media, equipment, and supplies:

a) The pH meter or meters shall be standardized before each use period with pH 7.0 and either pH 4.0 or pH 10.0 standard buffers, whichever range covers the desired pH of the media or reagent. A record of the standardization, including the percent slope, shall be maintained. Percent slope shall be 95 to 105%. Each buffer aliquot shall be used only once. Commercial buffer solutions shall be dated and shall not be used past the expiration date. Electrodes shall be maintained according to manufacturer's recommendations.

b) Balances shall be calibrated monthly using NIST standardized Echelon I or II, or equivalent ASTM 1, 2, or 3 weights. A minimum of three weights that bracket the weighing requirements of the laboratory shall be used, and these weights shall be recertified every five years. A certificate shall accompany the weights. A certificate shall state either that the weights are compliant with the requirements of ASTM E1617-13 class 1, 2 or 3 tolerances or that they are compliant with the NIST Handbook 150-2G. The certificate shall list corrective data. Electronic balances shall be calibrated annually by a qualified service representative who is not affiliated with the laboratory. A certificate of calibration from the service representative shall be available for inspection.

c) Glass and electronic thermometers and temperature-recording devices including data loggers shall be calibrated annually at temperature of use against an NIST certified thermometer to within ± 1.0° C. Mercury NIST-certified thermometers shall be checked at the ice point annually and recalibrated at least every five years at each temperature of use. Digital NIST-certified thermometers shall be checked at the ice point annually and recalibrated at least every five years to demonstrate linearity. If datalogger or autoclave digital temperature record is used, datalogger or autoclave digital temperature record shall be calibrated annually by a qualified service representative who is not affiliated with the laboratory. A certificate of calibration from the service representative shall be available for inspection. Digital thermometer probe and meter shall be calibrated as a unit. The calibration factor, date calibrated, temperature of calibration, and analyst's initials shall be tagged on each thermometer. In addition, the laboratory shall record the following information in a Quality Assurance (QA) record book:

1) Serial number or unique identifier of laboratory thermometer;

2) Serial number of NIST-traceable thermometer;

3) Temperature of laboratory thermometer;

4) Temperature of NIST-traceable thermometer;

5) Correction (or calibration) factor;

6) Date of calibration; and

7) Analyst's initials.

d) Temperature in incubation equipment shall be recorded continuously by a temperature-recording device or recorded twice daily (at times separated by at least four hours) from in-place thermometers immersed in liquid unless otherwise specified by the manufacturer of a temperature monitoring system and placed on the top and bottom shelves of the use area. Documentation shall include the date and time of reading, temperature (as determined using the correction factor of the thermometer in use), and analyst's initials. Temperature readings from walk-in incubators with a continuous temperature reading device shall be supplemented by readings from in-place thermometers placed on various shelves other than where the recorder probe is located.

e) Date, contents, sterilization time and temperature, total time in autoclave, and analyst's initials shall be recorded each time the autoclave is used. Charts, if used, are to accompany written records.

f) Hot air ovens shall be equipped with a thermometer registering up to at least 180º C, or with a temperature-recording device. The oven thermometer shall be graduated in 10º C increments or less, with the bulb placed in sand during use. Date, contents, sterilization time and temperature, total time in oven, and analyst's initials shall be recorded each time the hot air oven is used.

g) Only membrane filters recommended for water analysis by the manufacturer shall be used. Manufacturer data sheets containing information as to lot number, ink toxicity (gridline inhibition), recovery, retention, and absence of growth-promoting substances for membrane filters shall be entered into the laboratory's record system. The lot numbers of membrane filters and date received shall be recorded. Membrane filters shall not be brittle or distorted, and the manufacturer's specification/certification sheet shall be available. Positive control shall be run on each new lot of membrane filters. Any gridline inhibition shall be recorded as unacceptable. Unacceptable membranes shall not be used.

h) Washing processes shall provide clean glassware with no stains or spotting. Distilled or deionized water shall be used for final rinse. Laboratory glassware shall be washed with a detergent designed for laboratory use. A glassware inhibitory residue test (Standard Methods, Section 9020B, under Laboratory Supplies) shall be performed, and acceptable results obtained, before the initial use of a detergent and whenever a different formulation or washing procedure is used. Results shall be recorded and maintained until specific formulation of detergent is no longer in use.

i) A representative piece of each type of glassware or plastic ware from each batch of clean, dried glassware or plastic ware shall be tested for residual alkaline or acid residue using bromothymol blue indicator. If the result of the indicator test is not green, corrective action shall be taken by re-rinsing, then air drying and retesting.

j) At least one bottle per lot or batch of sterilized sample bottles, whirlpaks or QuantiTray envelopes shall be checked before first use for sterility by adding approximately 25 ml of sterile non-selective broth media to each bottle or whirlpak and 100 ml to Quantitray envelope. The bottle shall be capped and rotated so that the broth comes in contact with all surfaces and shall be incubated at 35º + 0.5º C and checked after 24 and 48 hours for growth. Sample bottles shall not be used unless satisfactory results are obtained.

k) At least one bottle per lot or batch of sterilized sample bottles prepared with sodium thiosulfate shall be checked for a sufficient amount of the dechlorinating reagent by collecting a potable sample at the laboratory tap, then checking for residual chlorine. Corrective action shall be taken if there is any residual chlorine, and bottles checked shall not be used until corrective action has been completed.

l) At least one bottle per lot of precalibrated sample containers shall be checked before first use by measuring the volume with a Class A graduated cylinder. Tolerance shall be ± 2.5%.

m) Current service contracts or in-house protocols shall be maintained on balances, autoclaves, hot-air sterilization ovens, water stills, deionizers, reverse osmosis apparatuses, water baths, incubators, etc. Service records on the equipment shall include the date, name of the servicing person, and a description of the service provided.

n) Records shall be available for inspection on all batches of sterilized media showing the type of medium, lot numbers, date, sterilization time and temperatures, final pH, and the names/unique initials of the persons responsible for all or any part of the recorded data. The final pH of the medium at 25° C shall be:

|  |  |  |
| --- | --- | --- |
| Media |  | pH |
|  |  |  |
| M-Endo broth |  | 7.2 ± 0.2 |
| M-Endo agar |  | 7.2 ± 0.2 |
| M-Endo LES agar |  | 7.2 ± 0.2 |
| brilliant green |  | 7.2 ± 0.2 |
| lactose bile broth |  |  |
|  |  |  |
| EC Medium |  | 6.9 ± 0.2 |
| EC-MUG |  | 6.9 ± 0.2 |
| plate count agar |  | 7.0 ± 0.2 |
| M-FC broth/agar |  | 7.4 ± 0.2 |
| lauryl tryptose broth |  |  |
| single strength |  | 6.8 ± 0.2 |
| double strength |  | 6.8 ± 0.2 |
| triple strength |  | 6.8 ± 0.2 |
| Nutrient agar with 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 |
| E\*Colite |  | 6.9 ± 0.2 |
| Readycult |  | 6.8 ± 0.2 |
| Modified Colitag |  | 6.8 ± 0.2 |
| m-Coliblue 24 |  | 7.0 ± 0.2 |
| MI agar |  | 6.95 ± 0.2 |
| MI broth |  | 7.05 ± 0.2 |
| Non-selective broth |  | Per manufacturers instructions |

o) The laboratory using commercially manufactured prepared media shall record the date received, type of medium, lot number, sample performance when checked against cultures known to give positive and negative results, and pH verification per subsection (n). Media shall be used or discarded by the manufacturer's expiration date.

p) Each new lot of prepared commercial medium and each batch of laboratory prepared medium shall be checked before use with positive and negative culture controls. Additionally, each batch of prepared media (whether commercially prepared or laboratory prepared) shall be checked for sterility. Control organisms (e.g., total coliform, fecal coliform, and E. coli) shall be either known stock cultures (periodically checked for purity) or commercially available cultures impregnated with the organism. Results shall be recorded. The following table identifies a few positive and negative culture controls that laboratories might consider.

|  |  |  |
| --- | --- | --- |
| **Group** | **Positive Culture Control** | **Negative Culture Control** |
| Total Coliforms | Escherichia coliKlebsiella aerogenes | Staphylococcus aureusProteus vulgarisPseudomonas aeruginosa |
| Fecal Coliforms | Escherichia coliKlebsiella pneumoniae(thermotolerant) | Klebsiella aerogenes |
| E. coli | Escherichia coli(MUG-positive strain) | Klebsiella aerogenesKlebsiella pneumoniae(thermotolerant) |
| Enterococci | Enterococcus faecalisEnterococcus faecium | Staphylococcus aureusEscherichia coliSerratia marcesens |

q) Examples of appropriate American Type Culture Collection (ATCC) strains include the following:

Enterococcus faecalis ATCC 11700

Enterococcus faecium ATCC 6057

Klebsiella aerogenes ATCC 13048

Escherichia coli ATCC 8739 or 25922

Klebsiella pneumoniae (thermotolerant) ATCC 13883

Proteus vulgaris ATCC 29905

Pseudomonas aeruginosa ATCC 27853

Serratia marcesenes ATCC 14756

Staphylococcus aureus ATCC 6538

r) Lactose broth may be used in lieu of LTB if the laboratory conducts at least 25 parallel tests between this medium and LTB using water normally tested and this comparison demonstrates that the false-positive rate and false-negative rate for total coliforms, using lactose broth, is less than 10%.

s) A maximum registering thermometer or data logger shall be used during each autoclave and hot air oven cycle to verify sterilization temperatures. An exception to this rule would be an autoclave that has a printout of temperature that has been calibrated annually by an outside service with a NIST thermometer. The oven maximum registering thermometer shall be placed in sand. The autoclave maximum registering thermometer or data logger shall be placed in a container of water, unless otherwise specified by manufacturer’s instructions. Spore strips or ampules shall be used monthly, when in use, to confirm sterilization of the autoclave. Spore strips shall be used monthly to confirm sterilization for the hot air oven. Ampules shall not be used in the hot air oven because they may explode or melt. Strips or ampules that have not been placed in the autoclave or hot air oven shall be used as positive controls each time the sterilization is checked. A record of these results shall be maintained to include the date, material sterilized, and the initials of the analyst involved. Automatic timing mechanisms on autoclaves shall be checked quarterly with a stopwatch. For a 15-minute sterilization period, the autoclave time shall be within 60 seconds of the stopwatch time. 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.

t) When a media-dispensing apparatus is used, the media preparer shall check and maintain a record of the accuracy of the dispenser with a graduated cylinder at the start of each volume change and periodically throughout extended runs.

u) Micropipettors shall be calibrated annually and replaced if the precision or accuracy is greater than 2.5% tolerance. Micropipettors shall be calibrated with 10 consecutive weighings annually (using a separate tip for each weighing), and the average of all 10 weighings shall be ± 2.5% of specified delivery volume. For volumes ≥ 1.0 mL, volume shall be checked by using a Class A graduated cylinder.

v) The refrigerator temperature shall be determined daily by an accurate thermometer (thermometer immersed in liquid, data logger, or other automated approved method) placed on the top shelf. The refrigerator unit shall be visibly clean. Outdated materials in the refrigerator and freezer compartments shall be discarded.

w) Ultraviolet sterilization lamps shall be tested quarterly by exposing agar spread plates containing 200 to 250 microorganisms to the light for two minutes. If the irradiation does not reduce the count of control plates by 99 percent, the lamps shall be replaced. Alternatively, lamps shall be replaced if they emit less than 70% of the initial output. Cleaning of ultraviolet sterilization lamps shall be cleaned at least monthly by disconnecting the unit and cleaning the lamps with a soft cloth moistened with ethanol. Protective eye wear shall be used when checking the operation of a 254 nm lamp.

x) Water baths shall be cleaned at least monthly. The use of distilled or deionized water for water baths is recommended.

y) Media shall be used on a first in, first out basis. Records shall be kept of the kind, amount, date received, and date opened for media. The date opened and the date received shall be written on each bottle/container/box as appropriate. Dehydrated media shall be used within six months after opening, except media stored in a desiccator which shall be used by the manufacturer's expiration date. All media shall be discarded if visible deterioration is observed (e.g., clumping, color change). It is recommended that media be ordered in quantities to last no longer than one year, and that media be ordered in quarter pound multiples rather than one pound bottles to keep the supply sealed and protected as long as possible. Any media that have passed the manufacturer's expiration date shall be discarded.

z) The conductivity meter shall be calibrated at least monthly, following the manufacturer's recommendations, using a certified and traceable low level standard of 20 micromhos or less. The meter reading shall be within 2% of the value of the standard. If an in-line unit cannot be calibrated, it shall not be used to check reagent-grade water.

aa) A spectrophotometer or colorimeter (if used) shall have wavelengths in the visible range. A calibration standard and method specific blank shall be analyzed every day that the instrument is used prior to sample analysis. The calibration standard shall give a reading in the desired absorbance range and shall be obtained from an outside source.

bb) Each batch of prepared or each lot of commercial dilution/rinse water shall be checked for sterility by adding 50 mL of water to 50 mL of double-strength, nonselective broth. The batch shall be incubated at 35° ± 0.5° C and checked for growth after 24 and 48 hours. The batch shall be discarded if growth is detected.

cc) Each batch of prepared or each lot of commercial dilution water blanks shall be checked for pH; pH shall be 7.2 ± 0.2.

dd) The accuracy of dilution blank volumes shall be verified by checking one bottle for every 25 prepared or purchased using a Class A graduated cylinder. Volume shall be 99 mL ± 2 mL. Purchased dilution blanks shall be used by manufacturer's expiration date.

ee) Each lot of purchased single use membrane filtration equipment shall be checked before use with a Class A graduated cylinder, and a record shall be maintained. Tolerance shall be ± 2.5%. Sterility check shall be performed before and after analysis per Section 465.360(k)(2).

ff) Membrane filter equipment calibration shall be checked before first use with Class A graduated cylinders, and a record shall be maintained. Tolerance shall be ± 2.5%.

(Source: Amended at 46 Ill. Reg. 19150, effective November 17, 2022)