4740.2110 QUALITY CONTROL CRITERIA FOR BACTERIOLOGY.

Subpart 1. **Scope.** This part applies to laboratories performing tests under the bacteriological test category unless otherwise indicated. All requirements in this part must be incorporated into the laboratory's procedures unless otherwise directed by the approved method. The quality control requirements specified by the laboratory's standard operating procedures manual must be followed. All quality control measures must be assessed and evaluated on an ongoing basis and quality control acceptance criteria must be used to determine the validity of the data.

Subp. 2. **Sterility checks and blanks.**

A. A blank must be analyzed for each lot of preprepared, ready-to-use media, including chromofluorogenic reagent, and for each lot of media prepared in the laboratory. The analysis must be done before first use of each lot of media.

B. For filtration technique, a laboratory must conduct one beginning and one ending sterility check for each laboratory-sterilized filtration unit used in a filtration series. The filtration series may include single or multiple filtration units that have been sterilized before beginning the series. For presterilized single-use funnels purchased, a sterility check must be performed on one funnel per lot. The filtration series is considered ended when more than 30 minutes elapse between successive filtrations. During a filtration series, filter funnels must be rinsed with three 20 to 30 milliliter portions of sterile rinse water after each sample filtration. In addition, laboratories must insert a sterility blank after every ten samples per filtration unit or sanitize filtration units by ultraviolet light after each sample filtration.

C. For pour-plate technique, sterility blanks of the media must be made by pouring, at a minimum, one uninoculated plate for each lot of preprepared, ready-to-use media and one for each lot of media prepared in the laboratory.

D. Sterility checks on sample containers must be performed on at least one container for each lot of purchased, presterilized containers. For containers sterilized in the laboratory, a sterility check must be performed on one container per sterilized batch using nonselective growth media.

E. A sterility check must be performed on each batch of dilution water prepared in the laboratory and on each batch of preprepared, ready-to-use dilution water using nonselective growth media.

F. At least one filter from each new lot of membrane filters must be checked for sterility using nonselective growth media.

Subp. 3. **Positive controls.** Each preprepared, ready-to-use lot of media, including chromofluorogenic reagent, and each lot of media prepared in the laboratory must be tested

with at least one pure culture of a microorganism known to elicit a positive reaction. This must be done before first use of each lot of media.

Subp. 4. **Negative controls.** Each preprepared, ready-to-use lot of selective media, including chromofluorogenic reagent, and each lot of selective media prepared in the laboratory must be analyzed with one or more known negative culture controls, that is, nontarget microorganisms that should not grow on the test media, as appropriate to the method. This must be done before first use of each lot of media.

Subp. 5. **Test variability.** For test methods that specify colony counts, such as methods using membrane filters or plated media, duplicate counts must be performed monthly on at least one positive sample for each month that the test is performed. With respect to this test for variability, if the laboratory has two or more analysts, each analyst must count typical colonies on the same plate and counts must be within ten percent difference between analysts to be acceptable. In a laboratory with only one microbiology analyst, the same plate must be counted twice by the analyst, with no more than five percent difference between the counts.

Subp. 6. **Method evaluation.** A laboratory must demonstrate proficiency with the test method before first use, by comparison to a method already approved for use in the laboratory, by analyzing a minimum of ten spiked samples whose matrix is representative of those normally submitted to the laboratory, or by analyzing and passing one proficiency test series provided by an approved proficiency sample provider. The laboratory must maintain documentation of the proficiency demonstration as long as the method is in use and for at least five years after the date of last use.

Subp. 7. **Test performance.** To ensure that analytical results are accurate, a laboratory must confirm a target organism specified in the method.

Subp. 8. **Quality of standards, reagents, and media.**

A. Culture media may be prepared from commercial dehydrated powders or may be purchased ready to use, unless otherwise indicated in the approved method. Media may be prepared by the laboratory from basic ingredients when commercial media are not available or when it can be demonstrated that commercial media do not provide adequate results. Media prepared by the laboratory from basic ingredients must be tested for performance, such as for selectivity, sensitivity, sterility, growth promotion, and growth inhibition, before first use. Detailed testing criteria information must be defined in the laboratory's standard operating procedures manual or quality assurance manual.

B. Reagents, commercial dehydrated powders, and media must be used within the shelf life of the product. The specifications of the reagent, powder, or media must be documented according to the laboratory's quality assurance manual.

C. Distilled water, deionized water, or reverse-osmosis produced water that is free from bactericidal and inhibitory substances must be used in the preparation of media, solutions, and buffers. The quality of the water must be monitored for chlorine residual, specific conductance, and heterotrophic bacteria plate count monthly, when in use; when maintenance is performed on the water treatment system; or at startup after a period of disuse longer than one month. Analysis for metals and the bacteriological water quality test, to determine the presence of toxic agents or growth promoting substances, must be performed annually. Results of these analyses must meet the specifications of the required method and records of analyses must be maintained for five years. Laboratories that can supply documentation to show that their water source meets the criteria, as specified by the method, for ASTM or NCCL Type I or Type II reagent water and is free of bacteria that can grow under these test conditions are exempt from performing the bacteriological water quality test.

D. Media, solutions, and reagents must be prepared, used, and stored according to a documented procedure following the manufacturer's instructions or the test method. Documentation for media prepared in the laboratory must include the date of preparation, preparer's initials, type and amount of media prepared, manufacturer and lot number, final pH of the media, and expiration date.

E. Documentation for media purchased preprepared and ready-to-use must include the manufacturer, lot number, type and amount of media received, date of receipt, expiration date of the media, and the verification pH of the liquid.

Subp. 9. **Selectivity.**

A. To ensure identity and traceability, reference cultures used for positive and negative controls must be obtained from a recognized national collection or organization.

B. Microorganisms may be single-use preparations or cultures maintained by documented procedures that demonstrate the continued purity and viability of the organism.

C. Reference cultures may be revived, if freeze-dried, or transferred from slants and subcultured once to provide reference stocks. The reference stocks must be preserved by a technique that maintains the characteristics of the strains. Reference stocks must be used to prepare working stocks for routine work. If reference stocks have been thawed, they must not be refrozen and reused.

D. Working stocks must not be cultured sequentially more than five times and must not be subcultured to replace reference stocks.

Subp. 10. **Temperature measuring devices.** Temperature measuring devices such as liquid-in-glass thermometers, thermocouples, and platinum resistance thermometers used in incubators, autoclaves, and other equipment must be of the appropriate quality to meet specifications in the test method. The gradation of the temperature measuring devices must

be appropriate for the required accuracy of measurement and the devices must be calibrated to national or international standards for temperature. All measurements must be recorded.

Subp. 11. **Autoclaves.**

A. The performance of each autoclave must be evaluated initially by establishing its functional properties and performance, for example heat distribution characteristics with respect to typical uses. Autoclaves must meet specified temperature tolerances. Pressure cookers must not be used for sterilization of growth media.

B. Demonstration of sterilization temperature must be provided by use of a continuous temperature recording device or by use of a maximum registering thermometer with every cycle. Appropriate biological indicators must be used once per month to determine effective sterilization. Temperature-sensitive tape must be used with the contents of each autoclave run to indicate that the autoclave contents have been processed.

C. Records of autoclave operations must be maintained for every cycle. Records must include: date, contents, maximum temperature reached, pressure, time in sterilization mode, total run time, which may be recorded as time in and time out, and operator's initials.

D. Autoclave maintenance, either internally or by service contract, must be performed annually and must include a pressure check and calibration of the temperature device. Records of the maintenance must be maintained in equipment logs.

E. The autoclave's mechanical timing device must be checked quarterly against a stopwatch and the actual time elapsed must be documented.

Subp. 12. **Ultraviolet instruments.** Ultraviolet (UV) instruments used for sanitization must be tested quarterly for effectiveness with an appropriate UV light meter or by plate counts on agar spread plates. Bulbs must be replaced if output is less than 70 percent of original for light tests or if count reduction is less than 99 percent for a plate containing 200 to 300 organisms.

Subp. 13. **Incubators, water baths, ovens.**

A. The stability and uniformity of temperature distribution and the time required after test sample addition to reestablish equilibrium conditions in incubators and water baths must be documented. Temperature of incubators and water baths must be documented twice daily, at least four hours apart, on each day of use.

B. Ovens used for sterilization must be checked for sterilization effectiveness monthly with appropriate biological indicators. Records must be maintained for each cycle that include the date, cycle time, temperature, contents, and analyst's initials.

Subp. 14. **Procedure for washing labware.**

A. A laboratory must have a documented procedure for washing labware, if applicable. Detergents designed for laboratory use must be used.

B. Glassware must be made of borosilicate or other noncorrosive material, free of chips and cracks, and have readable measurement marks.

C. Labware that is washed and reused must be tested for possible presence of residues that may inhibit or promote growth of microorganisms by performing the inhibitory residue test annually and each time the laboratory changes the lot of detergent or washing procedures.

D. Washed labware must be tested at least once daily, each day of washing, for possible acid or alkaline residue by testing at least one piece of labware with a suitable pH indicator such as bromothymol blue. Records of tests must be maintained.

Statutory Authority: *MS s 144.97; 144.98*

History: *31 SR 446*

Published Electronically: *September 26, 2013*