**Section 465.350 General Laboratory Practices**

a) The following requirements shall apply to sterilization procedures:

1) Autoclaving of the following items shall be carried out at 121º ± 1º C for the durations specified below:

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| Item |  | Minimum duration of  autoclaving at 121° ± 1° C |
| Membrane filters and pads |  | 10 minutes |
|  |  |  |
| Carbohydrate-containing media (lauryl tryptose, brilliant green lactose bile broth, etc.) |  | 12-15 minutes |
|  |  |  |
| Contaminated materials and discarded tests |  | 30 minutes |
|  |  |  |
| Membrane filter assemblies (wrapped), sample collection bottles (empty), and individual glassware items |  | 15 minutes |
|  |  |  |
| Rinse water volumes of 500 mL to 1000 mL |  | 45 minutes |
|  |  |  |
| Rinse water volumes in excess of 1000 mL |  | Time adjusted for volume; check for sterility |
|  |  |  |
| Dilution water blanks |  | 15 minutes |

2) Membrane filters and pads and all media shall be removed from the autoclave immediately after completion of the sterilization cycle.

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

4) Membrane filter assemblies shall be autoclaved between each sample filtration series. A UV sterilizer or boiling water may be used on membrane filter assemblies for at least two minutes to prevent bacterial carryover between sample filtrations, but shall not be used as a substitute for autoclaving between sample filtration series.

5) Dried glassware to be sterilized in a hot-air sterilizing oven shall be kept at 175º ± 5º C for at least 2 hours.

6) Empty sample containers shall be moistened with several drops of distilled water before autoclaving to prevent an "airlock" sterilization failure.

b) Laboratory pure water, which may be distilled or deionized, or other processed water shall meet the standards set forth in Section 465.380. Only water determined to be laboratory pure water shall be used for performing bacteriological analyses.

c) Rinse and dilution water shall be prepared in the following manner:

1) A stock phosphate buffer solution of potassium dihydrogen phosphate (KH2PO4) and a magnesium chloride solution shall be prepared as specified in "Standard Methods for the Examination of Water and Wastewater." The pH of stock phosphate buffer solution is 7.2 ± 0.5.

2) The phosphate buffer solution and magnesium chloride solution shall be autoclaved or filter sterilized, labeled, dated, and stored at 1º to 5º C.

3) The stored stock phosphate buffer solution and magnesium chloride solution shall be free of turbidity.

4) Rinse and dilution water shall be prepared by adding 1.25 mL of stock phosphate buffer solution and 5.0 mL of magnesium chloride solution per liter of laboratory pure water.

5) Alternatively, commercially prepared phosphate buffer and magnesium chloride solution may be used when preparing rinse and dilution water. The date received, expiration date, proof of sterility, and pH of phosphate buffer shall be recorded.

d) The following minimum requirements shall be met for storing and preparing media:

1) Laboratories shall use commercial dehydrated media or commercially manufactured prepared media for routine bacteriological procedures.

2) All media shall be prepared according to the media specifications of "Standard Methods for the Examination of Water and Wastewater."

3) Dehydrated media containers shall be kept tightly closed and stored in a cool, dry location. Discolored or caked dehydrated media shall not be used.

4) All water used shall be laboratory pure water.

5) Dissolution of the media shall be completed before dispensing to culture tubes or bottles.

6) Multiple Tube Fermentation (MTF) media, when prepared in tubes with loose-fitting caps, shall be used within one week after preparation. If MTF media are refrigerated after sterilization, they shall be incubated overnight at 35º C to confirm usability. Tubes of MTF media showing growth or gas bubbles shall be discarded. Refrigerated M Endo agar shall be used within two weeks after refrigeration.

7) MTF media in screw cap containers may be held up to three months, provided that the media are stored in the dark and evaporation does not exceed 1.0 mL per 10 mL total volume.

(Source: Amended at 38 Ill. Reg. 16240, effective July 15, 2014)