7.1.5 INSTRUCTIONS FOR MEDIA PREPARATION

1. **m-Endo media for total coliform analysis.**
   a. Empty the vial containing 4.8 g dehydrated m-Endo media into a 250-mL beaker or flask and add 100 mL of a 2-percent ethanol solution.
   b. Stir the mixture well for several minutes to break up clumps and prevent agar from adhering to the flask.
   c. Place the flask in a heated water bath or on a hot plate and heat slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. **Do not autoclave.**
   d. When medium reaches the boiling point, promptly remove from heat. **Do not boil.**
   e. Cool the medium to a temperature of about 50°C and pour 6 to 7 mL in 50-mm petri dish bottoms. Quickly place petri dish tops loosely on petri dish bottoms.
   f. When the medium has solidified, close petri dishes by pressing on tops firmly. The dishes are suitable for use after the medium has solidified. About 15 to 20 petri dishes can be filled from 100 mL of media.
   g. Plates not used immediately after preparation should be placed upside down in small plastic bags to prevent drying and stored in darkness in a refrigerator for a maximum of 5 days.

2. **NA-MUG media for confirmation of *Escherichia coli* after primary culturing of total coliform bacteria with m-Endo medium.**
   a. Add 2.3 g of NA-MUG media to 100 mL of deionized or distilled reagent grade water in a 250-mL flask or beaker.
   b. Stir the mixture well for several minutes to break up clumps and prevent media from adhering to the flask.
   c. Place the flask in a heated water bath or on a hot plate and heat slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching.
   d. Autoclave at 121°C and 15 lb/in^2 for 15 minutes. Allow to cool to 44 to 46°C, or when the flask is cool enough to pick up bare handed.
   e. Pour 6 to 7 mL of medium into 50-mm petri dish bottoms.

About 15 to 20 petri dishes can be filled from 100 mL NA-MUG media. Quickly place petri dish tops loosely on petri dish bottoms.

f. When the medium has solidified, close petri dishes by pressing on tops firmly. The dishes are suitable for use after the medium has solidified. Prepared dishes, sealed in small plastic bags to prevent drying, can be stored in a refrigerator up to 2 weeks.

3. **m-FC media for fecal coliform bacteria analysis.**

a. Prepare a rosolic acid solution by adding 10 mL of 0.2 N sodium hydroxide to 0.1 g rosolic acid crystals. Shake the mixture to dissolve crystals. **Do not heat.** The crystals will dissolve in 15 minutes. Prepare a new solution for each analysis.

b. Empty the vial containing 5.2 g dehydrated media into 100 mL of deionized or distilled water in a 250-mL flask.

c. Stir the mixture well for several minutes to break up clumps and prevent media from adhering to the flask.

d. Place the flask in a heated water bath or on a hot plate and heat slowly to 90°C. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. **Do not autoclave.**

e. With a clean pipet, add 1 mL rosolic acid solution per 100 mL of media when the medium reaches 90°C. Continue heating until boiling begins. Remove from heat.

f. Cool the medium to a temperature of about 45 to 50°C and pour 6 to 7 mL in 50-mm petri dish bottoms. Place petri dish tops loosely on petri dish bottoms to allow condensation to escape.

g. When the media has solidified (about 10 minutes), close petri dishes by pressing on tops firmly. The petri dishes are suitable for use after the medium has solidified.

h. Petri dishes not used immediately after preparation should be placed upside down in small plastic bags to prevent drying and can be stored in a refrigerator for no more than 72 hours.

4. **KF media for fecal streptococci analysis.**

a. Empty the vial containing 7.64 g dehydrated media into 100 mL of distilled or deionized water in a 250-mL beaker or flask.

b. Stir the mixture well for several minutes to break up clumps and prevent media from adhering to the flask.

c. Add 10 mL of deionized water to the 0.015 g TTC (triphenyltetrazolium chloride crystals).

d. Place the flask in a heated water bath or on a hot plate and heat slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. After boiling begins, simmer at this temperature for 5 minutes.

e. Remove media solution from heat and cool to 50 to 60°C. Sterilize the TTC solution by aseptically filtering through a disposable sterile membrane filter (0.22 mm). Add 1 mL of sterile TTC solution to 100 mL of media and stir. Prepare fresh TCC solution each time media is prepared.

f. Cool the medium to a temperature of about 50°C and pour 6 to 7 mL in 50-mm petri dish bottoms. Place the petri dish tops loosely on the dish bottoms to allow condensation to escape.

g. When the medium has solidified, close the petri dishes tightly by pressing firmly on the tops. Petri dishes are suitable for use after the medium has solidified.

h. Petri dishes not used immediately after preparation should be placed in small plastic bags to prevent drying and can be stored in the refrigerator for up to 2 weeks if sterile TTC was used. If not, the medium should be used within 24 hours.

5. **m-TEC media for *E. coli* analysis.**

a. Empty the vial containing 4.53 g dehydrated m-TEC media into 100 mL of distilled or deionized water in a 250-mL beaker or flask.

b. Stir this mixture for several minutes to break up clumps. Make sure that none of the media adheres to the bottom or side of the beaker.

c. Place the beaker containing the media solution in a heated water bath or on a hot plate and begin heating slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. After boiling begins, remove the beaker from the hot plate or boiling water bath and autoclave at 121°C and 15 lb/in^2 for 15 minutes. Allow to cool to 45 to 50°C, or when the beaker is cool enough to pick up bare handed.

d. Pour 6 to 7 mL of the medium into 50-mm petri dish bottoms. Place the petri dish tops loosely on dish bottoms to allow condensation to escape.

e. When the medium has solidified (in about 10 minutes) close the petri dishes tightly by pressing firmly on the tops. The petri dishes are suitable for use after the medium has solidified.

f. Petri dishes not used immediately after preparation should be placed in small plastic bags to prevent drying and stored in the refrigerator (at about 5°C). The dishes can be stored up to 2 weeks.

6. **Urea-phenol broth for confirmation of *E. coli*.**

a. **Confirmation of *E. coli* by urea-phenol broth media is required.** After sample incubation, prepare the urea-phenol red broth by adding 100 mL of sterile distilled or deionized water to 2.0 g urea and 0.01 g phenol red crystals in a 250-mL beaker and mix thoroughly.

b. When preparing the filter pads for counting colonies, use a clean pipet or dropper and add 2.0 mL of the solution to each absorbent pad before placing the filter on the pad. Before transferring the filters from m-TEC to urea-phenol broth, carefully drain excess solution from each pad by tilting the plate against a clean lab wipe.

7. **m-E media for enterococci bacteria analysis.**

a. Pour 5 to 10 mL distilled or deionized water into a bottle containing 0.015 g TTC crystals. Cap and shake the TTC to dissolve the crystals. If using a QWSU kit, retain the remaining 90 mL of distilled water. **Do not heat.**

b. Empty the vial containing 7.12 g m-E medium into the remainder of the distilled water (approximately 90 mL) in a 250-mL flask or beaker.

c. Stir this mixture for several minutes to break up clumps. It is important that none of the media adheres to the bottom or the side of the beaker.

d. Prepare the nalidixic acid solution by adding 10 mL of 0.2 N NaOH solution to 0.25 g nalidixic acid crystals. Shake the mixture to dissolve crystals. **Do not heat.** Make sure all the crystals dissolve.

e. Place the beaker containing the media solution in a heated water bath or on a hot plate and stir the solution constantly, or use a stir bar and magnetic stirring hot plate to prevent scorching. After ingredients dissolve, autoclave at 121°C and 15 lb/in^2 for 15 minutes. Allow to cool enough to pick up bare handed.

f. The dissolved reagents can now be added. Using a sterile pipet, add 1.3 mL of the nalidixic acid solution. If the medium is intended to be used within 24 hours, add all the TTC solution directly from the bottle. If the prepared medium will be stored for a longer period before use, the TTC solution should

be sterilized by passage through a 0.22-mm membrane filter in an aseptic manner.

g. Pour 6 to 7 mL of the medium into 50-mm petri dish bottoms. Place the petri dish tops loosely on the dish bottoms to allow condensation to escape.

h. When the medium has solidified (about 10 minutes), close the petri dishes by pressing firmly on the tops. The plates are suitable for use after the medium has solidified. Prepared petri dishes, sealed in small plastic bags to prevent drying, can be stored in a refrigerator for up to 2 weeks if sterile TTC was used. If not, the medium should be used within 24 hours.

8. **EIA media for confirmation of enterococci bacteria.**

a. **Confirmation of enterococci bacteria by EIA is required.** If using the QWSU kit, the second bottle of 100 mL distilled water is used to prepare the EIA medium. This medium is used for confirmation of enterococci colonies after primary incubation of the filters on m-E medium.

b. Empty the vial containing 1.65 g EIA media into 133 mL of distilled or deionized water in a 250-mL flask or beaker.

c. Stir this mixture for several minutes to break up clumps. Make sure that none of the media adheres to the bottom or the side of the beaker.

d. Place the beaker containing the media solution in a heated water bath or on a hot plate and stir the solution constantly, or use a stir bar and magnetic stirring hot plate to prevent scorching.

e. After ingredients dissolve, autoclave at 121°C and 15 lb/in^2 for 15 minutes. Allow beaker to cool enough to pick up bare handed.

f. Pour the 6 to 7 mL of EIA medium into 50-mm petri dish bottoms. Place the petri dish tops loosely on dish bottoms to allow condensation to escape.

g. When the medium has solidified (in about 10 minutes) close the petri dishes tightly by pressing on the dish tops. The petri dishes are suitable for use after the medium has solidified.

h. Prepared petri dishes, sealed in small plastic bags to prevent drying, can be stored in a refrigerator for up to 2 weeks.