APPENDIX J
OHIO WATER MICROBIOLOGY LABORATORY
MEDIA QUALITY-CONTROL PROCEDURES

For membrane filtration, QC procedures are followed when a new batch of media or buffer is made. For Colilert, QC procedures are followed with every 20th sample. Some QC procedures are also performed by field personnel (as specified by project work plans) to ensure the integrity of the agar and (or) the procedure is correctly performed.

Pure culture tests

1. Identify the lot number of the media or buffer by the date made.

2. Transfer a loopful of a pure culture to a fresh nutrient agar (NA) slant and incubate at room temperature.

   Positive controls.
   All in-house prepared media are tested using the following positive controls. These controls may also be used by field personnel.
   - For MI, mTEC, Colilert, and modified mTEC, use *E. coli* as positive controls for total coliforms and (or) *E. coli.*
   - For mEI, use *Enterococcus faecalis* as a positive control for enterococci

   Field personnel may also analyze the following positive control:
   - For MI, additionally use *Serratia marcescens* as a non-*E. coli* positive control for total coliforms.

   Negative controls.
   - For Colilert, use *Pseudmonas sp* ATCC 10145 as a negative control for total coliforms and *E. coli*
   - For modified mTEC, use two negative control cultures (optional):
     - *Proteus mirabilis* ATCC 25933 is able to grow on modified mTEC but does not produce the characteristic pink color
     - *Pseudomonas sp* ATCC 10145 is unable to grow on modified mTEC
   - For MI, use *Pseudmonas sp* ATCC 10145 as a negative control for total coliforms and *E. coli* (does not grow on MI).
   - For mEI and mFC, use *Enterobacter cloacae* (does not grow on mFC or mEI).

   Field personnel may also analyze the following negative control:
   - For MI, additionally use *Providencia alcalifaciens* (grows on MI but will not fluoresce) as a negative control for total coliforms.

   The slants are transferred once a month.

3. After 18-24 hours incubation, transfer a loopful of bacteria into a sterile test tube containing 10 mL phosphate-buffered dilution water (PB). Vortex the mixture for 15 seconds. Transfer 1 mL of the mixture to 99 mL PB water (this is the 10^{-2} dilution).

4. Make further dilutions, if required. Pipet 1 mL of the 10^{-2} dilution to 99 mL PB water (10^{-4} dilution) and then 1 mL of the 10^{-4} dilution to another bottle containing 99 mL PB water (10^{-6} dilution). Always use a new pipet while making each subsequent dilution.

- For ***E. coli***, plate an equipment blank, 1-, 3-, and 10-mL volumes of the 10^{-6} dilution on the membrane-filtration medium you are testing. For the Colilert Quantitray/2000, use 100 mL of the 10^{-8} dilution. Incubate and read as specified in the method.

- For ***Serratia marcescens,*** plate an equipment blank, 1-, 3-, and 10-mL volumes of the 10^{-6} dilution on the MI medium you are testing.

- For **enterococci**, plate an equipment blank, 1- and 3-mL volumes of the 10^{-4} dilution on the medium you are testing. Incubate and read as specified in the method.

- For ***Pseudmonas***, use 100 mL of the 10^{-8} dilution for the Colilert Quantitray/2000. For membrane filtration, use 1-, 3-, and 10-mL volumes of the 10^{-6} dilution. Also, to ensure these dilutions contain viable test organisms, streak plate 0.1 mL onto a non-selective medium such as TSA.

- For ***Providencia alcalifaciens,*** use 1-, 3-, and 10-mL volumes of the 10^{-6} dilution on the MI medium you are testing.

- For ***Proteus mirabilis*** ATCC 25933, use 3 ml of the 10^{-2} dilution.

5. Record results in the QC media notebook. Use the ideal-count plate (20-80 colonies for total coliforms and *E. coli*; 20-60 for enterococci and negative controls) and record the number of the colonies, the volume, and the dilution. For Colilert, use the most-probable number table provided by the manufacturer.

QC for MI agar plates using a sewage sample (optional)

If requested by the project chief, QC is also done for a new batch of MI agar plates poured. Tests are done using a sewage sample to evaluate the effectiveness of cefsulodin (an antibiotic added at the time of plate preparation) and the selectivity of the agar.

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1. Identify the sublot number of the media by the date poured. This date is written on the bottom of each plate.

2. Transfer 1 mL of raw sewage from the refrigerator to 99 mL of PB water (this is a 10^{-2} dilution). Transfer 1 mL of the 10^{-2} dilution to 99 mL of PB water (10^{-4} dilution). Always use a new pipet when making each subsequent dilution.

3. Plate, from the 10^{-4} dilution, an equipment blank, 1-, 3-, 10- and 30-mL volumes. Incubate and read as specified in the method.

4. Record the results in the QC media notebook. Record the number of the colonies, the volume, and the dilution.

QC for *Clostridium perfringens* on mCP agar

QC for *Clostridium* is done:

- When a new lot of media is made.
- Every 20th sample or when a new analyst is processing or reading plates.

1. Use an untreated sewage sample that is less than 2 weeks old. Remove the larger solids by decanting the liquid portion, if necessary.

2. Make a 10^{-2} dilution of the decanted sewage sample. Plate 10- and 30-mL volumes of the 10^{-2} dilution. Incubate and read as specified in the method.

3. Record results in the QC media notebook as described above.