Quality Assurance for E. coli Analysis

Laboratory Equipment and Instrumentation

- Thermometers – 9020B.4.a
  - Annually check accuracy of all working temperature-sensing devices… against a certified NIST thermometer or one traceable to NIST and conforming to NIST specifications.
  - Record calibration results, along with the date and the technician's signature, in a quality control logbook.
  - Mark the necessary calibration correction factor on each temperature measuring device so that only calibrated-corrected temperature values are recorded.
  - Verify accuracy of the reference certified thermometer as specified on the certificate of calibration or at least every 5 years.
  - For general purposes use thermometers graduated in increments of 0.5°C or less.
- Autoclave – 9020B.4.h
  - For routine use, verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121°C has been reached.
  - Test monthly for sterilization efficacy (with *Geobacillus stearothermophilus*)
  - Verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121°C has been reached.
- Refrigerator – 9020B.4.i
  - Maintain temperature at 2-8°C
  - Check and record temperature daily
- Membrane filtration equipment (if MF procedure is used) – 9020B.4.k
  - Wash and rinse filtration assemblies thoroughly after use, wrap in nontoxic paper or foil, and sterilize.
  - UV sterilize or boil funnels between samples
    - If using boiling water, make sure membrane filtration equipment is cool before adding next sample
- Membrane filters and pads (if MF procedure is used) – 9020B.5.i.3
  - Check filters for brittleness if lot is held for one or more years
- Ultraviolet lamps (if used) – 9020B.4.l
  - When used, disconnect lamps monthly and clean bulbs with a soft cloth moistened with ethanol
- Incubator – 9020 B.4.o
  - During usage periods check and record calibration-corrected temperature twice daily (morning and afternoon, separated by at least 4 hours) on each shelf in use to ensure temperature consistency throughout unit.

Laboratory Supplies

- Glassware – 9020 B.5.a
  - 1) pH check - To test clean glassware for alkaline or acid residue add a few drops of 0.04% bromthymol blue (BTB) or other pH indicator and observe the color reaction.
  - BTB should be blue-green (in the acceptable neutral range).
- Dilution water bottles – 9020 B.5.c
  - Dilution waters available commercially are acceptable.

    - Check one per lot for pH and volume (99 ± 2 mL) and examine bottles for a precipitate
    - Discard by expiration date
    - Before use of each batch or lot conduct sterility (one bottle per lot or quarter with that same lot number, whichever is more frequent)
        - Sterility Checks – 9020B.9.d
            - Check each new batch (or lot) of buffered water for sterility before first use by adding 50 mL of water to 50 mL of a double-strength broth (e.g. tryptic soy, trypticase soy or tryptose broth).
            - Alternatively, aseptically pass 100 mL of dilution water through a membrane filter and place filter on nonselective medium.
            - Incubate at 35±0.5°C for 24 hours and observe for growth.
            - For membrane filter tests, check the sterility of the entire process by using sterile reagent or dilution water as the sample at the beginning and end of each filtration series of samples and test for growth
- Sample bottles – 9020 B.5.d.
    - Check accuracy of 100 mL mark, one per lot and record results.
- Multi-well trays and sealers – 9020 B.5.e
    - Evaluate sealing performance of heat sealer unit monthly by adding one to two drops of food-color dye to 100 mL deionized water sample, run through sealer and visually check each well for leakage.
    - **Real people language – analyze a method blank once per lot (of sterile water, media, bottles and trays) or once per quarter, whichever is more frequent, to demonstrate sterility.**
    - **As a monthly check of a sealer efficacy, perform and document a visual check that trays are properly sealed. If all sample wells are positive for total coliform and sufficient contrast, visually examine the tray cells for leakage and document the check. If insufficient color contrast is present us food-color dye as previously recommended by method.**

General QC Requirements

- Coliforms – Total and E. coli Hach Method 10029 – m-ColiBlue24®
    - Blank – daily
        - Run at least one membrane filter blank at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter, placing in a petri dish with mColiBlue broth and testing for growth.
    - Positive and Negative Controls – Check certified control cultures with each lot of media ***and*** petri dishes with pads OR once a quarter, whichever is more frequent.
        - *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control.
    - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.
- Enzyme Substrate Test SM 9223 B, 22nd Edition (2004) – Colilert Method
    - Quality Control

        - Test each lot of media or quarterly (whichever is more frequent) purchased for performance by inoculation with two certified control bacteria: *Escherichia coli* and a noncoliform.
        - Also add a sterile water control. If a sterile water control exhibits faint fluorescence or faint positive coliform, discard use and use a new batch of substrate.
        - Incubate these controls at 35±0.5°C as indicated above.
    - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.

Bibliography

American Public Health Association (APHA), American Waterworks Association (AWWA), and Water Environment Federation (WEF). 2012. *Standard Methods for the Examination of Water and Wastewater*. 22nd ed. American Public Health Association, Washington, D.C.