# Procedure for the Preparation and Analysis of Spiked Reference and Matrix Samples

**DEPARTMENT OF ENVIRONMENTAL PROTECTION LABORATORY**
**BIOLOGY PROGRAM**
**TALLAHASSEE, FLORIDA**


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## 1. SCOPE AND APPLICATION

1.1. The analysis of a clean sample matrix, fortified with a known quantity of the target organism from a manufactured standard such as a Quality Control (QC) standard or a Certified Reference Material (CRM), permits the monitoring of initial and ongoing method performance, as long as manufacturer instructions for use are followed. It is also useful as a demonstration of capability for analysts. The laboratory will assess performance for accuracy with the established acceptance criteria in the COA.

## 2. SUMMARY OF METHOD

2.1. Four spiked reference samples of either *Enterococcus faecalis* (*E. faecalis*), *Escherichia coli* (*E. coli*), or *Klebsiella variicola,* are prepared using vials of phosphate buffer, or Autoclaved DI water, and analyzed according to the respective method.

2.2. Interferences

2.2.1. Temperature of the reference bacteria pellet can affect its activity. Analysis should begin when the capped pellet has acclimated to room temperature (15-30°C).

2.2.2. 2.2.2 Time of inoculation can affect bacterial activity. Analyze spiked sample within 30 minutes of reconstitution.

2.2.3. Aseptic techniques must be used to ensure that there is no contamination of the sample via the media, reagents, supplies, or the analyst.

2.2.4. Mix contents thoroughly after each inoculation and again before processing by vigorously agitating the vials. This breaks up clumps of bacteria and homogenizes the sample.

2.3. Definitions

2.3.1. ***Enterococcus faecalis*** **–** Gram-positive cocci bacteria. One of several members of the genus *Enterococcus* that can be found in the feces of humans and other warm-blooded animals. Formerly of the Group D fecal streptococci group.

2.3.2. ***Escherichia coli*** **–** Gram-negative, non-spore forming, rod-shaped bacteria. One of several bacteria species known as fecal coliforms. Fecal coliforms are commonly found in the feces of humans and warm-blooded animals.

2.3.3. ***Klebsiella variicola*** **–** Gram-negative, non-spore forming, rod-shaped bacteria. One of several members of the family *Enterobacteriaceae.*


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2.3.4. TSA - Tryptic Soy Agar. A non-selective soybean and casein enriched agar medium used for the growth of aerobic and facultative anaerobic bacteria

## 3. EQUIPMENT AND SUPPLIES

3.1. Sterile gloves, latex or nitrile

3.2. Inoculating loops, sterile

3.3. Glass serological disposable pipettes (1 mL), sterile and individually wrapped, with pipette pump; calibration checked on each new lot per SOP MB-03.3.

3.4. Plastic serological disposable pipettes (50 mL) sterile and individually wrapped, with pipette pump; calibration checked on each new lot per SOP MB-03.3.

3.5. Glass serological disposable pipettes (10 mL) sterile and individually wrapped, with pipette pump; calibration checked on each new lot per SOP MB-03.3.

3.6. Thermo Fisher Scientific Heratherm IMH 60 Model 51028066(S/N 42511812) or Fisher Scientific Isotemp Incubator Model 650F (S/N 303N0034) maintained at 35 ± 0.5 °C

3.7. Water baths TSWB 4 (S/N 300471596) and TSWB 5 (S/N 300472033) maintained at 35.0 ± 0.5 °C. Water baths TSWB 3 (S/N 300311499) and TSWB 7 (S/N 300483022) maintained at 41.0 ± 0.5 °C, and TSWB 8 (S/N 300536247 ) maintained at 44.5 ±0.2 °C.

3.8. Colilert-18 snap pack media (for the detection of E. coli and T. coli)

3.9. Enterolert snap pack media (for the detection of enterococci)

3.10. IDEXX Shrink-Banded Vessels without Sodium Thiosulfate

3.11. Antifoam dropper bottle

3.12. Quanti-Tray/2000 trays

3.13. UV light 365 nm, 6 watts

3.14. Colilert-18 Quanti-Tray/2000 comparators for E.coli and T.coli

3.15. Quanti-Tray rubber insert for Quanti-Tray/2000

3.16. Quanti-Tray Sealer Model Plus (S/N QTP 13215001064 or QTP 13181503550)

3.17. Vacuum Filtration system (See Figure 14.1)

3.18. Membrane filtration unit, sterile wrapped in foil


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3.19. UV Sterilizer unit

3.20. Forceps, sterile, straight or curved with smooth tip

3.21. Membrane filters, sterile, white gridded, 47 mm diameter, 0.45±0.02 µm pore size

3.22. Bunsen burner

3.23. Beaker, 30 mL

## 4. REAGENTS AND STANDARDS

4.1. Microbiological Reference Materials:

4.1.1. Certified Reference Material (CRM)

4.1.2. *Escherichia coli NCTC 9001*

4.1.3. *Klebsiella variicola* ATCC 31488

4.1.4. *Enterococcus faecalis* NCTC 775

4.1.5. *Pseudomonas aeruginosa* NCTC 12951

4.1.6. *Streptococcus bovis* NCTC 8177

4.2. Microbiologics Kwik-Stik™ Reference Culture Microorganisms

4.2.1. *Pseudomonas aeruginosa* ATCC® 10145

4.2.2. *Aerococcus viridans* ATCC 10400

4.2.3. *Serratia marcesens* ATCC 43862

Note: Kwik-Stik cultures are prepared according to SOP MB-03.1

4.3. Dilu-Lok bottle with 99 mL Phosphate Buffer with $MgCl_2$ for membrane filtration method.

4.4. Autoclaved DI Water for Quanti- Tray

4.5. Tryptic Soy agar (TSA) - Prepare per SOP MB-02.33. Dry plates at room temperature for 24 hours prior to use.

## 5. SAMPLE PREPARATION PROCEDURE


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5.1. Retrieve four vials of the target organism from the freezer and allow the capped vials to equilibrate to room temperature (15-30°C). Do not open vial until equilibration is complete. This should take about 15 minutes.

5.2. Once the sample pellet is at room temperature, open the vial and aseptically transfer the pellet to the hydration fluid. Shake vigorously to dissolve.

5.3. Analyze spiked samples according to method requirements

5.4. POSITIVE CONTROLS

5.4.1. For the detection of E. coli and T. coli, use the Universal Microbe CRM, or a single pure E. coli pellet, and a single pure *K. variicola,* respectively. For the detection of enterococci, use a single pure E. *faecalis* pellet. Membrane filtration analysis requires four Dilu-Lok bottles spiked with the target bacteria and analyzed according to SOP MB-01.1. Quanti-Tray requires four spiked IDEXX vessels, containing Autoclaved DI Water, with the target bacteria and analyzed according to SOP MB-01.15

5.5. NEGATIVE CONTROLS

5.5.1. For E. coli and T. coli use *Pseudomonas aeruginosa*. Either pellet or plated working cultures can be used for spiking.

5.5.2. For enterococci use *Streptococcus bovis* and *E. coli* pellet or Universal Microbe CRM. Plated working cultures *Aerococcus viridans* and *Serratia marcesens,* can also be used as negative controls.

5.5.3. For membrane filtration, spike one Dilu-Lok bottle with a negative control microbe, and filter 0.1 mL

5.5.4. For Quanti-Tray, spike one 100 mL vessel, containing Autoclaved DI Water with a negative control microbe, and analyze

## 6. SAMPLE ANALYSIS

6.1. For initial method performance (IPR), ongoing method performance (OPR), and demonstration of capability (DOC) for analysts, prepare and analyze four Reference Samples by fortifying the corresponding hydration fluid with the target organism.

6.2. For membrane filtration, spike four Dilu-Lok bottles with a positive control microbe and analyze according to SOP MB-01.1. Filter 6.0, 7.0 and 8.0-mL volume, if using the Universal water microbe cocktail; or 10, 15, and 20-mL, if using a single pure E. coli culture. For the negative control, spike one Dilu-Lok bottle with the target organism and filter 0.1 mL


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For Quanti-Tray analysis, spike four 100 mL sample vessels, containing Autoclaved DI water, with a positive control microbe and analyze according to SOP MB-01.15. For negative controls, spike one additional vessel with a negative culture for E.coli, and two vessels with the negative cultures for Enterolert.

6.3 Analyze blanks along with the spiked samples.

## 7. RECOVERY AND PRECISION

7.1 Retrieve the Certificate of Analysis (COA) for the reference culture located in the following folder: \\Fldep1\dear\Labs\Lab5\Biology\BenchCommon\COA\Micro supplies\cultures and check that the test results fall within the appropriate Acceptable Range specified. See SOP MB-01.1 for membrane filtration calculations or SOP MB-01.15- for Quanti- Tray.

7.2 Check precision, log range value and mean range, by comparing results against the microbiology Control Charts All Tests 032514 excel file, found in the following folder: \\Fldep1\dear\Labs\Lab5\Biology\BenchCommon\Micro.

## 8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Always autoclave any expired or unused bacterial cultures for 45 minutes before discarding in the trash.

8.2. Expired or unused IDEXX media is collected by hazardous waste

8.3. Discard any unused melted agar in hazardous waste container. Do not reuse media that has re-solidified.

8.4. Refer to Bureau of Laboratories Health and Safety Plan for safety equipment, use of laboratory hoods, and chemical storage policies.

8.5. Read MSDS information for reagent handling and disposal.

## 9. REFERENCES

9.1. EPA Method 1600 (Sept 2014)

9.2. Standard Methods 9020 (2017)


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9.3. Standard Methods 9222 (2006)

9.4. NELAC Quality Systems Manual

9.5. Difco™ & BBL™ Manual \\Fldep1\dear\Labs\Lab5\Biology\Bench Common\Micro

9.6. FDEP Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

9.7. FDEP Laboratory Health and Safety Plan

https://floridadep.sharepoint.com/dear/Shared%20Documents/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202021.pdf

9.8. DEP SOP MB-02.33, *Preparation of Dehydrated Culture Media*

9.9. DEP SOP MB-03.1, *Maintenance of Bacterial Cultures*

9.10. DEP SOP MB-03.3, *Calibration and Use of Mechanical and Disposable Pipettes*

9.11. IDEXX Quanti-Tray/2000 Colilert-18 method instructions

9.12. IDEXX Quanti-Tray/2000 Enterolert method instructions

9.13. IDEXX Quanti-Tray Sealer PLUS Manual

9.14. DEP SOP MB 01.1, *Membrane Filter Method for Fecal Coliforms*

9.15. DEP SOP MB 01.15, *Quanti-Tray/2000 Colilert-18 Method for Escherichia coli, Total coliforms, and Enterolert Method for Enterococci*

## Appendix of Changes

| | |
|---|---|
| 02/19/2008 | Added SOPs to references section and updated safety and hazardous waste management section. |
| 02/11/2010 | Added correct version of referenced method. |
| 02/02/2011 | Updated references to include new versions of Quality Manual and Health and Safety Plan. |
| 01/05/2012 | Removed procedure for ADB broth and added reference to procedure in media SOP. |
| 06/20/2016 | Added Summary of Method, including Interferences and Definitions. In Equipment and Supplies, included calibration SOPs and Incubation Model and Serial Number. In Reagents and Standards, detailed preparation and use of TSA and included *Escherichia coli*. (Also included *E. coli* throughout the SOP.) In |


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| | |
|---|---|
| | Sub-section Sample Spiking, included Quanti-Tray procedure. Added Difco™ & BBL™ Manual and Pipette Calibration SOP to References. |
| 12/18/2017 | Modified sentence structure in the following areas: Summary of Method, Interferences, Undiluted spiking suspension (USS) and Serial dilution of USS. Added one significant figure to volume amounts and added the words Dilu-lok, species name, and Sample Spiking to figure 8.1. |
| 01/03/2019 | Updated references to match current versions. |
| 12/19/2019 | Added new equipment references used for incubation and storage purposes. Updated Flowchart of Spiking Suspension Procedure for specific DOC tests. |
| 12/17/2020 | Updated equipment references for incubators in use. Updated links for Quality Manual and Health and Safety Plan. |
| 12/28/2021 | Updated link for the Difco™ & BBL™ Manual. Added a note to section 5.4.1, and updated reference to the flowchart. |
| 12/21/2022 | Updated SOP by adding the new protocol to determine initial method performance (IPR), ongoing method performance (OPR), and demonstration of capability (DOC) for analysts. Former procedure remains in the SOP, unedited, to serve as an alternative method. Updated FDEP Laboratory Health and Safety Plan. |


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## 10. APPENDIX

10.1. Former Procedure for the Preparation and Analysis of Spiked Reference and Matrix samples

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## 11. SCOPE AND APPLICATION

11.1. The evaluation of Spiked Reference Samples permits the monitoring of initial and ongoing method performance. It is also useful as a demonstration of capability for analysts. Sample matrix spikes make it possible to evaluate the effect of specific matrices on method recovery.

## 12. SUMMARY OF METHOD

12.1. A serial dilution of an undiluted spiking suspension (USS) of either *Enterococcus faecalis* (*E. faecalis*) or *Escherichia coli* (*E. coli*) is performed in vials of phosphate buffer water until it reaches a $10^{-6}$ dilution. Each dilution is then plated onto prepared TSA plates using 0.1 mL and 1.0 mL aliquots. Each volume is plated in triplicate. The plates are inverted and incubated for 20 ± 4 hours. At the end of the incubation period, count and record number of colonies per plate. The ideal colony count should be between 30 and 300 colonies for at least one dilution.

12.2. Interferences

12.2.1. TSA is not selective and can support the growth of many types of bacteria. Aseptic techniques must be used to ensure that there is no contamination of the sample via the media, reagents, supplies, or the analyst.

12.2.2. TSA should be tempered to 44-46 °C after melting to prevent it from re-solidifying before it is poured. Once TSA re-solidifies, it is no longer usable and should be disposed of properly.

12.2.3. TSA plates should be prepared 24 hours prior to use. Sample will not be absorbed properly if the media has not sufficiently solidified and bacteria will not grow properly if media has been allowed to over-dry.

12.2.4. Mix contents thoroughly after each inoculation and, again, before plating by vigorously agitating the vials. This breaks up clumps of bacteria and homogenizes the sample.

12.3. Definitions


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12.3.1. **TSA -** Tryptic Soy Agar. A non-selective soybean and casein enriched agar medium used for the growth of aerobic and facultative anaerobic bacteria.

12.3.2. ***Enterococcus faecalis* –** Gram-positive cocci bacteria. One of several members of the genus *Enterococcus* that can be found in the feces of humans and other warm-blooded animals. Formerly of the Group D fecal streptococci group.

12.3.3. ***Escherichia coli* –** Gram-negative, non-spore forming, rod-shaped bacteria. One of several bacteria species known as fecal coliforms. Fecal coliforms are commonly found in the feces of humans and warm-blooded animals.

13. EQUIPMENT AND SUPPLIES

13.1. Sterile gloves, latex or nitrile

13.2. Inoculating loops, sterile

13.3. Glass serological disposable pipettes (1 mL), sterile and individually wrapped, with pipette pump; calibration checked on each new lot per SOP MB-03.3.

13.4. Calibrated pipette (per SOP MB-03.3) for dispensing 0.1 and 1.0 mL

13.5. Thermo Fisher Scientific Heratherm IMH 60 Model 51028066(S/N 42511812) or Fisher Scientific Isotemp Incubator Model 650F (S/N 303N0034) maintained at 35 ± 0.5 °C

14. REAGENTS AND STANDARDS

14.1. *Enterococcus faecalis* (*E. faecalis*) ATCC #19433, working stock (See SOP MB-03.1.)

14.2. *Escherichia coli* (*E. coli*) ATCC #11775, working stock (See SOP MB-03.1.)

14.3. Dilu-Lok bottle with 99 mL Phosphate Buffer with $MgCl_2$, 5 bottles per bacteria species.

14.4. Tryptic Soy agar (TSA) - Prepare per SOP MB-02.33. Dry plates at room temperature for 24 hours prior to use.

14.4.1. Media can be poured into plates immediately after preparation, or it can be stored in milk dilution bottles in the Biology Walk-in Refrigerator or the Thermo Scientific Isotemp Refrigerator S/N 0162177801170112 located in B212 for up to 3 months and melted just before use. Melted prepared media can be tempered in a water bath at 44-46 °C for up to 3 hours. Appropriately discard any re-solidified media.

14.4.2. Each bottle of prepared media can pour up to 5 plates, including controls.


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14.4.3. Number of plates needed per bacteria species: 9 plates for each volume being plated, plus 1 control plate for each bottle of media used.

14.5. Azide Dextrose broth (ADB) – Prepare per SOP MB-02.33.

14.6. Lauryl Tryptose Broth (LTB)—Prepare per SOP MB-02.33.

15. SPIKING SUSPENSION PROCEDURE (See Flowchart 9.1)

15.1. Undiluted Spiking Suspension (USS)

15.1.1. For *E. faecalis* USS, add 1.0 mL of ADB to a 99 mL phosphate buffer bottle. For *E. coli* USS, add 1.0 mL of LTB to a 99 mL phosphate buffer bottle.

15.1.2. Add a loopful of *E. faecalis* to the ADB phosphate buffer bottle and shake vigorously. Similarly, add a loopful of *E. coli* to the LTB phosphate buffer bottle and shake vigorously.

15.1.3. Incubate at 35 ± 0.5 °C for 20 ± 4 hours.

15.2. Serial dilution of USS

15.2.1. After incubation, shake the USS vigorously and proceed to add 1.0 mL of the USS to a phosphate buffer bottle labeled with the species and the letter “A”.

15.2.2. Thoroughly mix dilution “A”. Withdraw 1.0 mL of sample “A” and add it to a second bottle of phosphate buffer labeled with the species and the letter “B”.

15.2.3. Thoroughly mix dilution “B”. Withdraw 11.0 mL of sample “B” and add to a phosphate buffer bottle labeled with the species and the letter “C”.

15.2.4. Thoroughly mix dilution “C”. Withdraw 11.0 mL of sample “C” and add it to a phosphate buffer bottle labeled with the species and the letter “D”.

15.2.5. Thoroughly mix dilution “D”.

15.3. Spread plates of spiking suspensions

15.3.1. Shake dilution “B” bottle a minimum of 25 times. Pipet 0.1 mL of the suspension onto a TSA plate labeled with the species and the volume. Use a bent inoculating needle to spread the suspension evenly over the surface, without tearing into the agar. Repeat for two more plates. Make triplicate plates in the same manner for dilutions “C” and “D”.

15.3.2. For IPRs, OPRs, and DOCs, also make triplicate plates using 1 mL of the suspension, following the method in 5.3.1.


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15.3.3. Allow the suspensions to absorb into the agar. Then, incubate inverted plates at 35 ± 0.5 °C for 20 ± 4 hours.

15.3.4. After incubation, count and record the number of colonies per plate.

15.4. Sample spiking

15.4.1. A Reference Sample is prepared by adding 3.0 mL of dilution "D" to a 99 mL phosphate buffer bottle and mixing thoroughly. Analyze a 5.0- and 10.0-mL volume of the Reference Sample by membrane filtration on appropriate media. Analyze 20.0 mL dilutions using the appropriate Quanti-Tray methods.

NOTE: For analysts' DOCs, use 0.5 mL of dilution "B" of Enterococcus faecalis (E. faecalis) spike for Enterococcus Quanti-Tray test, and 0.5 mL of dilution "C" of E. coli spike for Colilert-18 Quanti-Tray and Membrane Filtration.

15.4.2. For initial method performance (IPR), prepare and analyze four Reference Samples.

15.4.3. For ongoing method performance (OPR), prepare and analyze one Reference Sample.

15.4.4. As demonstration of capability (DOC) for analysts, prepare and analyze four Reference Samples.

15.5. Matrix Spike (MS)

15.5.1. To prepare a matrix spike sample, add 3.0 mL of dilution "D" to 100 mL of sample being tested and mix thoroughly. Analyze appropriate volume of spiked sample by membrane filtration or Quanti-Tray using appropriate media.

15.5.2. Analyze the unspiked sample along with the spiked sample to obtain background concentration of sample for MS recovery calculations.

16. RECOVERY AND PRECISION CALCULATIONS

16.1. Concentration of USS (Use TSA plate results from Section 4.3 above.)

$$Enterococci_{USS} = \left(CFU_1 + CFU_2 + \mathsf{K} + CFU_n\right)/\left(V_1 + V_2 + \mathsf{K} + V_n\right)$$

Where,

$Enterococci_{USS}$ = CFU/mL of enterococci in undiluted spiking suspension (USS).


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CFU = Use all counts from TSA plates that were between 30-300 CFU per plate.

V = Volume of undiluted sample on each TSA plate that had counts between 30-300 CFU. For example, a TSA plate from the "D" dilution has a volume of $10^{-7}$ mL.

n = Number of plates with counts within 30-300 CFU.

16.2. Concentration of spiked sample (From Section 4.4 and 4.5.)

$$T_{spiked} = (Enterococci_{USS}) \times (V_{spiked})$$

Where,

$T_{spiked}$ = True concentration (CFU/100mL) of spiked enterococci in sample.

$Enterococci_{USS}$ = CFU/mL of enterococci in undiluted spiking suspension (USS). (From Section 5.1.)

$V_{spiked}$ = Volume in mL of USS per 100 mL of sample. For example, the Reference Sample in Section 4.4.1 has a USS volume of 3.0 X $10^{-6}$ mL.

16.3. Percent Recovery of Spiked sample

$$R = 100 \times \frac{(N_S - N_U)}{T}$$

Where,

R = Percent recovery

$N_S$ = Enterococci (CFU/100 mL) result from analysis of the spiked sample.

$N_U$ = Enterococci (CFU/100 mL) result from analysis of the unspiked sample. (For matrix spikes.)

T = True concentration of enterococci in spiked sample. (From Section 5.2.)

16.4. Precision (Used for IPR and DOC)

$$RSD = \frac{SD}{A_R} \times 100$$

Where,


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RSD = Precision as relative standard deviation

SD = Standard Deviation

$A_R$ = Mean percent recovery

## 17. SAFETY/HAZARDOUS WASTE MANAGEMENT

17.1. Always autoclave any expired or unused bacterial cultures for 45 minutes before discarding in the trash.

17.2. Discard any unused melted agar in hazardous waste container. Do not reuse media that has re-solidified.

17.3. Refer to Bureau of Laboratories Health and Safety Plan for safety equipment, use of laboratory hoods, and chemical storage policies.

17.4. Read MSDS information for reagent handling and disposal.

## 18. REFERENCES

18.1. EPA Method 1600 (Sept 2014)

18.2. Standard Methods 9020 (2017)

18.3. Standard Methods 9222 (2006)

18.4. NELAC Quality Systems Manual

18.5. Difco™ & BBL™ Manual \\Fldep1\dear\Labs\Lab5\Biology\Bench Common\Micro

18.6. FDEP Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

18.7. FDEP Laboratory Health and Safety Plan http://publicfiles.dep.state.fl.us/DEAR/DEAR%20Safety/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202016.pdf

18.8. DEP SOP MB-02.33, *Preparation of Dehydrated Culture Media*

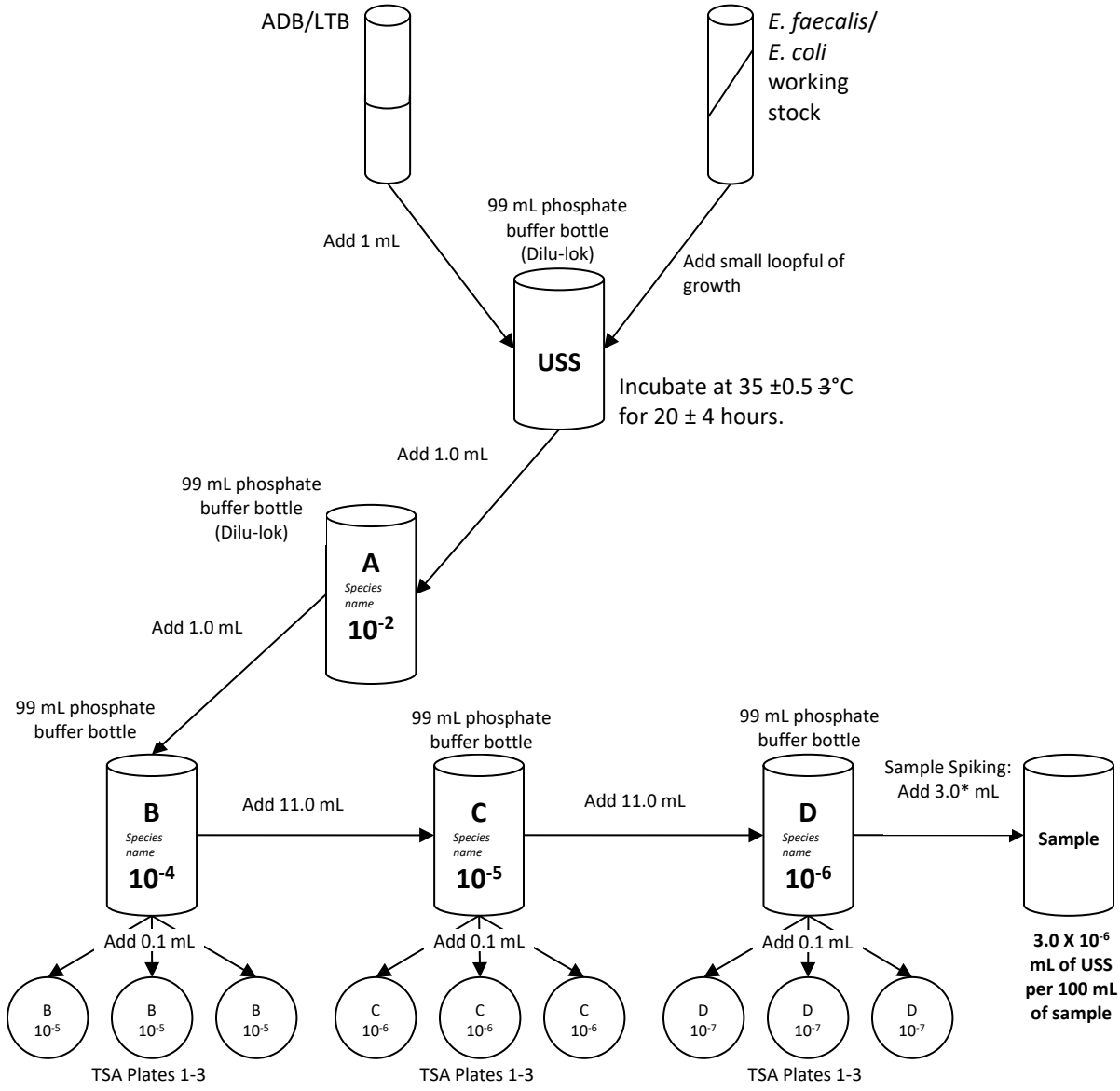
18.9. DEP SOP MB-03.1, *Maintenance of Bacterial Cultures*

18.10. DEP SOP MB-03.3, *Calibration and Use of Mechanical and Disposable Pipettes*

## 19. TABLES, DIAGRAMS


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Figure 9.1 – Flowchart of Spiking Suspension Procedure




⁎For analysts' DOCs, we take 0.5 ml of "B" of Enterococcus faecalis (E. faecalis) spike for Enterococcus Quanti-Tray test and 0.5 ml of "C" of E. coli spike for Colilert-18 Quanti-Tray and MF.

## Appendix of Changes

02/19/2008 Added SOPs to references section and updated safety and hazardous waste management section.

02/11/2010 Added correct version of referenced method.


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02/02/2011 Updated references to include new versions of Quality Manual and Health and Safety Plan.

01/05/2012 Removed procedure for ADB broth and added reference to procedure in media SOP.

06/20/2016 Added Summary of Method, including Interferences and Definitions. In Equipment and Supplies, included calibration SOPs and Incubation Model and Serial Number. In Reagents and Standards, detailed preparation and use of TSA and included *Escherichia coli*. (Also included *E. coli* throughout the SOP.) In Sub-section Sample Spiking, included Quanti-Tray procedure. Added Difco™ & BBL™ Manual and Pipette Calibration SOP to References.

12/18/2017 Modified sentence structure in the following areas: Summary of Method, Interferences, Undiluted spiking suspension (USS) and Serial dilution of USS. Added one significant figure to volume amounts and added the words Dilu-lok, species name, and Sample Spiking to figure 8.1.

01/03/2019 Updated references to match current versions.

12/19/2019 Added new equipment references used for incubation and storage purposes. Updated Flowchart of Spiking Suspension Procedure for specific DOC tests.

12/17/2020 Updated equipment references for incubators in use. Updated links for Quality Manual and Health and Safety Plan.

12/28/2021 Updated link for the Difco™ & BBL™ Manual. Added a note to section 5.4.1, and updated reference to the flowchart.


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