Preparation of Buffered Dilution Water and Stock Components

1. SCOPE AND APPLICATION

1.1. Buffered dilution water is used during the filtration step of the microbiology analysis process. It is used to ensure the even dispersal of the sample aliquot while passing through the filter, and to rinse the funnels clean of any sample residue. It also helps maintain a stable pH during incubation. Buffered dilution water is also used for the method blanks.

2. EQUIPMENT AND SUPPLIES

2.1. Supplies for stock solutions

2.1.1. 1 L Class A volumetric flask

2.1.2. 2 L Erlenmeyer flask or 2 L screwcap bottle

2.1.3. Milk dilution bottles (20)

2.2. Supplies for Buffered Dilution Water

2.2.1. 1 L Class A volumetric flask or graduated cylinder

2.2.2. 2 L Erlenmeyer flasks

2.2.3. 2 L Glass storage bottles

2.2.4. 6 L Erlenmeyer flask

2.2.5. Disposable 50 mL pipette – Check calibration and use per SOP MB-03.3

2.2.6. Disposable 10 mL pipette – Check calibration and use per SOP MB-03.3

2.2.7. Aluminum foil

2.2.8. Autoclave tape

2.2.9. Disposable transfer pipette

2.2.10. Sir Bar (One per 6 L Erlenmeyer flask)

2.3. Equipment

2.3.1. Oakton PC2700 pH and Conductivity Meter (S/N CP354757) calibrated per DEP

2.3.2. SOP MB-03.5 Oakton PC2700 pH and Conductivity Meter Calibration and Use.

2.3.3. Mettler Toledo PB403-S/FACT balance (S/N 1128150043)

2.3.3.1. Calibrate and use per SOP TA-6.38.

2.3.4. Mettler Toledo MS303S balance (S/N B247544008)

2.3.4.1. Calibrate and use per SOP BB-35.

2.3.5. Steris Amsco Model #250LS (S/N 031761851) autoclave

2.3.5.1. Calibrate and use per SOP BG-08.

2.3.6. Heratherm S/N 42511812 maintained at 35 ± 0.5 °C

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2.3.7. Isotemp 60L S/N 43315553 maintained at 35 ± 0.5 °C

2.3.8. Isotemp Refrigerator/Freezer S/N 0162177801170112 maintained at 1-5 °C

2.3.9. Hot plate with stirrer

3. REAGENTS AND STANDARDS

3.1. KH_2PO_4 (potassium dihydrogen phosphate)

3.2. $MgCl_2•6H_2O$ (magnesium chloride)

3.3. Deionized (DI) water

3.4. 1 N NaOH (sodium hydroxide)

3.5. 0.2 N NaOH (sodium hydroxide)

3.6. *Escherichia coli* (ATCC 11775)

3.6.1. Prepare per SOP MB-03.1

3.7. Double strength Tryptic Soy Broth (TSB 2X) for quality control check

3.7.1. Prepare per SOP MB-02.33.

4. PREPARATION

4.1. Stock KH_2PO_4

4.1.1. In a 1 L Class A volumetric flask, dissolve 34.00 ± 0.01 g ACS-certified potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of DI water. Swirl to completely dissolve the reagent. Dilute to 1 L with DI water.

4.1.1.1. Make sure to record balance use (including proper calibration and reagent mass) in the appropriate balance log.

4.1.2. Adjust the pH to 7.2 ± 0.5 with 1 N NaOH.

4.1.3. Fill 10 milk dilution bottles and cap loosely. Autoclave for a 15-minute media cycle.

4.1.3.1. Record autoclave use (including total autoclave cycle time, max pressure, and max temperature) in LIMS

Laboratory/Technical/Laboratory Logbook *Autoclave_Log*.

4.2. Stock $MgCl_2$

4.2.1. In an Erlenmeyer flask, dissolve 81.10 ± 0.01 g ACS-certified magnesium chloride ($MgCl_2•6H_2O$) in 1 L DI water. Swirl to completely dissolve the reagent.

4.2.1.1. Make sure to record balance use (including proper calibration and reagent mass) in the appropriate balance log.

4.2.2. Fill 10 milk dilution bottles and cap loosely. Autoclave for a 15-minute media cycle.

4.2.2.1. Record autoclave use (including total autoclave cycle time, max pressure, and max temperature) in LIMS

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Laboratory/Technical/Laboratory Logbook *Autoclave_Log*.

4.3. Buffered Dilution Water

4.3.1. Using a 1 L Class A volumetric flask or a graduated cylinder, fill two 6 L Erlenmeyer flasks with 6 L DI water each. To each flask, using a 10-mL pipette, add 7.5 mL of stock KH_2PO_4. Using a 50-mL pipette, add 30 mL of stock $MgCl_2$ to each flask.

4.3.2. Place each 6 L flask onto a hot plate (heat off) with a stir bar and turn on the stirrer. Take the pH of the buffered water and adjust the pH to 7.2 ± 0.1 with 0.2 N NaOH using a disposable transfer pipette.

Note: Small amounts of NaOH adjust the pH greatly. Do not add more than 5-10 drops at a time when first adjusting the pH and wait until the pH has stabilized before adding more. As the pH nears 7.2 ± 0.1 reduce the number of drops to 1-2 drops at a time.

4.3.3. Once the pH is stable, pour approximately 1 L of the prepared buffered water into six 2 L Erlenmeyer flasks and six 2 L glass storage bottles. Cover each flask with foil and loosely cap the glass bottles. Place a small piece of autoclave tape on the foil and on the orange caps of each flask and bottle.

4.3.4. Autoclave for a 15-minute media cycle.

4.3.4.1. Record autoclave use (including total autoclave cycle time, max pressure, max temperature, and if the autoclave tape passed or failed) in LIMS.

Laboratory/Technical/Laboratory Logbook *Autoclave_Log*.

4.3.5. Immediately after removing flasks from autoclave pour the contents of the flasks into the glass bottles so that each bottle contains 2 L of buffered water. Measure final pH of buffered water once cooled to room temperature.

Note: Autoclaved water is superheated and extremely hot after the autoclaving process. When transferring water between bottles, **exercise extreme caution** to prevent burns or injury. Always handle with care, using appropriate personal protective equipment (PPE), and ensure bottles are securely positioned to avoid spills or splashes.

4.4. Documentation and Storage

4.4.1. Record each preparation in LIMS **Laboratory/Technical/Standard Preparation Tracker**. Record the final pH of stock KH_2PO_4 in Standard Preparation Tracker. Print labels for stocks and buffered water and attach to dilution bottles and flasks when cool.

4.4.2. Store stock solutions in Isotemp Refrigerator/Freezer for up to 12 months. If either KH_2PO_4 or $MgCl_2$ becomes turbid before 12 months, discard. Discarded or used up stock solutions and buffered water should also be discarded in LIMS Standard Preparation Tracker. Store buffered water on the cart at the micro filter station for up to 6 months. Refrigerate after opening and discard if turbidity develops.

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5. QUALITY CONTROL

5.1. Buffered Water QC

5.1.1. Before use, run a quality control check on the buffered water. In 2 beakers, make a 1:1 (approx. 25 mL) TSB 2X and buffered water solution. Add *E. coli* to one of the beakers. This is the positive control. The other beaker is the negative control. Incubate the beakers at 35˚C ± 0.5˚C for 24 hours.

5.1.1.1. After incubation, if growth occurs (turbidity) in the negative control, repeat the quality control check. If the repeat test fails, discard the prepared buffered water and make a new batch.

5.1.2. Enter all appropriate QC information into a LIMS Micro-QC worksheet using **Prep Worksheet Manager**.

5.1.3. Record the QC check(s) in LIMS **Standard Preparation Tracker**. Use the **Verify Standard** button to document buffered water QC results. In the "Comments" box, link the MICRO-QC worksheet associated with buffered water.

Note: Do not re-autoclave buffered water! This may result in excessive deterioration or decomposition of components.

6. SAFETY/HAZARDOUS WASTE MANAGEMENT

6.1. Refer to the DEP Laboratory Health and Safety Plan.

6.2. Read MSDS information for reagent handling and disposal.

7. REFERENCES

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

7.2. DEP Laboratory Health and Safety Plan, https://www.floridadep.net/resources/health-safety-manual

7.3. Standard Methods Online Edition (2017), Section 9050C

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

7.5. DEP SOP MB-03.5, Oakton PC2700 pH and Conductivity Meter Calibration and Use

7.6. DEP SOP PCR-2.1, *Calibration, Use and Maintenance of Mettler Toledo MS303S Balance*

7.7. DEP SOP TA-06.18, *AMSCO Renaissance Series 3021 Autoclave Calibration and Use*

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

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

Appendix of Changes

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08/20/2008	Updated to Standard methods 20th Edition and added a references section.
08/27/2009	Updated QC and Documentation sections and holding times.
08/13/2010	Updated to new version of Health and Safety Plan
08/12/2011	Updated to Bureau quality manual and added specific incubators used.
06/15/2012	Updated to new SOP format and corrected interpretation of Diack control results.
06/03/2013	Updated to reflect use of new balance and added serial numbers.
05/23/2014	Updated to reflect use of new pH meter & added serial number.
09/22/2017	Updated to remove old autoclave and incubator. Added new incubator.
09/21/2018	Updated link to latest Quality Manual. Removed SOP MB 3.2.
09/18/2019	Adjusted Isotemp Refrigerator/Freezer temperature range. Removed Diack control information from section 2.2, 4.3, and 5.2. Updated autoclave sterilization cycle time. Added refrigeration for open buffered water to 4.3.5. Updated to Standard Methods (2017).
09/18/2020	Updated QC recording procedures.
09/30/2021	Updated SOP references and equipment serial numbers in Section 2.3. Updated DEP Laboratory Quality Manual and DEP Laboratory Health and Safety Plan in References Section.
09/08/2022	Updated autoclave use procedures. Reformatted SOP to make information easier to access.
08/24/2023	Updated buffered dilution water preparation procedures. Updated buffered water QC procedures. Updated supplies and equipment. Reformatted Preparation section.
12/03/2024	Updated supplies and equipment. Note added to section 4.3.5. Removed archived SOP MB-03.4 from section 7.
09/30/2025	Updated equipment and supplies with the addition of Mettler Toledo PB403-S/FACT balance.
10/15/2026	Added Stir Bars to the supplies list. Added 0.2 N NaOH to Reagents and Standards. Specified to use 0.2 N NaOH and added a note under 4.3.2 detailing how 0.2 NaOH should be used to adjust the pH of the Buffered Dilution Water. Specified in 4.3.3 to add autoclave tape to each bottle and flask. Specified to record if autoclave tape passed or failed in 4.3.4.

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