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Standard Operating Procedure for Preparation of Buffered Dilution Water, Sodium Hydroxide, Phosphate Buffer, Magnesium Chloride and Sodium Thiosulfate Solutions

Access to this SOP shall be available within the laboratory for reference purposes; the official copy of this SOP resides on the official Georgia EPD website at https://epd.georgia.gov/about-us/epd-laboratory-operations. Printed copies of this SOP will contain a watermark indicating the copy is an uncontrolled copy.

1 Scope and Application

The Bacteriology Laboratory prepares buffered dilution water for use in the Multiple Tube Method, Heterotrophic Plate Count, MMO-MUG Method, and Membrane Filtration Method. Commercially prepared buffered dilution water may be used if known to give equivalent results. Sodium Hydroxide, Magnesium Chloride, and Phosphate Buffer are used in the preparation of the buffered dilution water. Sodium Thiosulfate is used as a dechlorinating agent and placed in sample field bottles. When preparing dilution water and reagents, follow manufacturer's directions for preparation and sterilization.

2 Definitions

2.1 Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions. (SOP Reference 12.3)

3 Interferences

3.1 Method interferences may be caused by contaminants in reagents, bottles, or glassware. To abstain from interferences, all reagents, glassware, and bottles are sterilized and tested for sterility prior to use.

3.2 When preparing dilution water and reagents, use only distilled or deionized reagent-grade water that has been tested and found free from traces of dissolved metals or inhibitory compounds.

3.3 All glassware must be washed, sterilized, and put in the hot air oven at 180ºC for 2 hours. A pH check is performed on all batches of glassware using a 0.04% solution of bromothymol blue. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants.

4 Safety

4.1 Refer to EPD Laboratory Chemical Hygiene Plan and Fire Safety Plan, online revision. (SOP Reference 13.5)

5 Apparatus and Equipment

5.1 Automatic Pipetting Machine
5.2 Autoclave capable of sterilizing at 121°C
5.3 Refrigerator, 2-8°C
5.4 Pan Balance
5.5 pH Meter
5.6 Set of certified ASTM Class 1 or better weights
5.7 Stirrer/Hot Plate
5.8 ½ Gallon plastic milk jugs
5.9 250 mL glass bottles w/caps
5.10 5 gallon glass solution bottle
5.11 Glass milk dilution bottles w/rubber plugs (graduated, 160ml), or plastic screw caps.
5.12 2 or 4 Liter glass aspirator bottles
5.13 Large foam plugs – 34-45 mm
5.14 Weigh boats – various sizes
5.15 Spatulas – various sizes
5.16 Graduated cylinder – 1000ml and/or 2000ml
5.17 Beaker – 1000ml and/or 2000ml
5.18 Kraft paper
5.19 Wire Baskets
5.20 String
5.21 Stirring rods – various sizes

6 Reagents

6.1 1 *N* Sodium Hydroxide (NaOH) made in Laboratory or commercially obtained.
6.2 Potassium Dihydrogen Phosphate (KH_2PO_4)
6.3 Distilled or Deionized Water
6.4 Sodium Thiosulfate ($Na_2S_2O_{3,\ anhydrous}$ or $Na_2S_2O_3 \cdot 5H_2O$)
6.5 Magnesium Chloride ($MgCl_2 \cdot 6H_2O$) solution made in Laboratory or commercially obtained.

7 Sample Collection

7.1 Not applicable to preparation of reagents and this SOP.

8 Calibration

8.1 Annually, calibration of the balance should be made by a certified vendor. Before each use, verify calibration by using two weights. Bracket calibration using one weight above and one weight below the target weight. A full range of weights should be checked monthly.
8.2 Weights should be recertified every three years.
8.3 pH meters should be calibrated before each use using 7.0 and 4.0 pH buffers. The thermometer probe in the pH meter should be calibrated yearly against a NIST certified thermometer.
8.4 Maintain sterility with equipment, reagents, and technique.

9 Quality Control

9.1 Refer to Section 10 Procedures of this SOP.

10 Procedure

General

10.1 Before daily use, standardize the pH meter with 4.0 and 7.0 buffers. Record results in pH logbook.

10.2 Before daily use, verify balance calibration by checking the weights of at least two different weights (one above and below the actual weight needed) and record results in the balance logbook. Handle each weight with tweezers or glove only (no bare hands). Check balance "bubble" to make sure it is centered and therefore the balance is leveled. Make sure the balance is clean and free from dust or debris.

10.3 When making reagents, record all pertinent information in the media and Reagent Preparation Logbook, such as batch number, lot number, pH, final concentrations, date, initials, etc. Record dilution water information in the Dilution Water Sterility Check Book.

10.4 Carefully weigh the appropriate amount of reagent on the balance using a weighing boat and spatula.

10.5 Weigh out the appropriate amount of each reagent as specified below in sections 10.8, 10.9, 10.10, or 10.11, mix each as required.

10.6 Place appropriate sized stirrers inside the beaker. Place beaker on Stirrer/Hot Plate and stir solution to completely dissolve. Remove stirrer prior to final reagent dilution when a final volume of 1L is required.

10.7 After dissolution, take pH of reagent by dispensing a small amount into a clean beaker. Always record pH in logbooks. If the pH is inaccurate, the reagent must be remade.
NOTE: Magnesium chloride pH check is not necessary.

Media Preparation-Reagents

10.8 **Sodium Thiosulfate Solution** –

10% Solution	$Na_2S_2O_{3,\ anhydrous}$	100g to 1L
10% Solution	$Na_2S_2O_3 \cdot 5H_2O$	152.1g to 1L

Add appropriate amount of sodium thiosulfate from table in one liter of distilled water. Pour into a ½ gallon plastic milk jug. Shake till dissolved. The pH should be 6.9 ± 0.2. Label bottle appropriately. Store in refrigerator.
Note: Do Not Autoclave.

10.9 **1 *N* Sodium Hydroxide (NaOH)** – Dissolve 40 g of Sodium Hydroxide granules in approximately 200ml of distilled water and cool. Once cool dilute to 1 L. Pour into a ½ gallon plastic milk jug. The pH should be 13.1 ± 0.2. Label bottle appropriately. Store in refrigerator.
Note: Do Not Autoclave.

10.10 **Phosphate Buffer Solution**– Dissolve 34.0 g of Potassium Phosphate monobase in 500 mls of distilled water. Stir until dissolved. Adjust pH to 7.2 ± 0.5 using 1 *N* NaOH (see 10.9 above). Then bring total concentration up to 1 Liter by using distilled water. Dispense 150 ml – 200 ml into 250 ml glass bottles (one liter makes approximately 5 bottles). Place caps loosely on bottles. Autoclave at 121°C for 15 minutes. After buffer has cooled, pour a small amount into a beaker and take the final pH. Record results in logbook. If the

pH is inaccurate, then reagent must be remade. Tighten caps, label appropriately and refrigerate. Discard if turbidity develops.

10.11 **Magnesium Chloride Solution-** Dissolve 81.1g of magnesium chloride ($MgCl_2 \cdot 6H_2O$) in 1 Liter of distilled water. Stir until dissolved. Dispense 150ml – 200ml into 250ml glass bottles (1 Liter makes approximately 5 bottles). Place caps loosely on bottles. Autoclave at 121°C for 15 minutes. After magnesium chloride solution has cooled, tighten caps. Label appropriately and refrigerate. Discard if turbidity develops.

Media Preparation-Buffered Dilution Water

10.12 To prepare 20 Liters of **Buffered Dilution Water**: Dispense 20 Liters of distilled water into a 5 gallon glass solution bottle. Add 25ml phosphate buffer solution; add 100ml magnesium chloride solution.

10.12.1 For small dilution bottles: Dispense 99 ± 2 mL of dilution water in milk bottles using the pipette machine. Cap bottles with rubber plugs or plastic screw caps. Cover each basket of bottles with Kraft paper. Place autoclave tape on each basket of buffered dilution water. Sterilize dilution water in an autoclave at 121°C for 30 minutes. Each basket should also be labeled with a batch number for quality control purposes.
NOTE: When using plastic screw caps place loosely prior to autoclaving.

10.12.2 For large jugs: Dispense 2 or 4 Liters into aspirator bottles. Cap with large yellow plugs and wrap top with Kraft paper tying with string. Sterilize at 121°C for 60 minutes.

10.13 Take the final pH. Final pH should be 7.2 ± 0.1. Record results in logbook. If the pH is inaccurate, then water must be remade.
NOTE: pH values will change with time.

Reagent Storage

10.14 Store dehydrated reagents (granules) in tightly closed bottles at less than 30°C in an atmosphere of low humidity. Do not use them if they discolor or become caked and lose the character of free-flowing granules.

10.15 Store prepared reagents in tightly closed screw-cap bottles at 4°C for up to 3 months.

10.16 Dilution water can be stored at less than 30°C for up to two weeks.

10.17 Label all prepared reagents and buffered dilution water correctly before storage. Include name of reagent, date reagent/water was prepared, expiration date, and initials of preparer.

10.18 Discard any expired reagent promptly.

10.19 Dilution water must be used prior to its expiration date and pass all quality control measures prior to use.

11 Calculations

11.1 The calculations involved in this method include measuring the appropriate amount of reagent and distilled water.

12 Waste Management

12.1 See GA EPD SOP – EPD Laboratory Waste Management Standard Operating Procedures (SOP Reference 13.4)

13 References

13.1 Standard Methods for the Examination of Water and Wastewater, 9050C, 9060A, 2006, American Public Health Association: Washington, D.C.
13.2 Manual for the Certification of Laboratories Analyzing Drinking Water, EPA/815-R-05-004, January 2005.
13.3 GA EPD Laboratory Quality Assurance Plan, online revision.
13.4 GA EPD Laboratory SOP – EPD Laboratory Waste Management SOP, SOP 6-015, online revision.
13.5 GA EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.

14 Practical Quantitation Limits (PQLs) Precision and Accuracy Criteria, and Quality Control Approach

14.1 No PQLs associated with this method. Final pH measurements and sterility must be maintained.

Updates to Previous Version:
Updated online revision.