



**US Environmental Protection Agency
Office of Pesticide Programs**

**Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD**

**Standard Operating Procedure for
Quality Assurance of Purified Water**

SOP Number: QC-01-05

Date Revised: 10-25-11

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

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for
Quality Assurance of Purified Water

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1.0 SCOPE AND APPLICATION:

1.1 This protocol outlines the procedures for monitoring the quality of the deionized water used in the laboratory to make media, reagents and disinfectants.

2.0 DEFINITIONS: None.

3.0 HEALTH AND SAFETY:

3.1 Several of the tests to be performed on the purified water require that the sample water be preserved with sulfuric or nitric acid. To protect against possible chemical burns, the laboratory worker must wear a lab coat, gloves, and protective eyewear (e.g., glasses or goggles) while filling water sample bottles containing sulfuric or nitric acid.

4.0 CAUTIONS:

4.1 Let the water run for approximately 30 seconds prior to filling sample collection bottles.

4.2 The water collected for the heterotrophic plate counts must be analyzed by QC Laboratories within 30 hours of collection and must be kept cold during transit by packing the container with ice packs. The samples should be collected on the same day, and as close to the FedEx pickup time as practicable.

4.3 To maintain evidence of chain of custody and to ensure that the testing laboratory performs the analyses requested by the ESC/OPP Microbiology Laboratory, the vendor chain of custody forms and the sample identification labels must be legibly completed.

4.4 Ship water samples to vendors on Mondays, Tuesdays, and Wednesdays only to ensure that the samples arrive prior to a weekend.

5.0 INTERFERENCES:

5.1 Each new lot of the DPD Powder Pillows reagent (i.e., DPD [salt of N,N-Diethyl-p-Phenylenediamine Potassium Iodide Sodium Phosphate, Dibasic] Total Chlorine Reagent Powder Pillows) should be checked for reagent accuracy. If the DPD Powder Pillows do not demonstrate reagent accuracy, the total chlorine residual test may be jeopardized. See section 10.13 for a description of the method to check reagent accuracy.

5.2 Discard expired DPD Total Chlorine Reagent Powder Pillows.

- 5.3 All test parameters will be conducted at the monitoring frequency indicated in Table 1. Monitoring Frequency and Acceptable Limits for Water Quality Parameters (section 10.2).
- 5.4 Individual test parameters will be tested more frequently than described in Table 1. Monitoring Frequency and Acceptable Limits for Water Quality Parameters (section 10.2) if a problem with water quality is identified (see 11.0 and 14.0). The laboratory may opt to test the water whenever the source of the water is changed, there is an upgrade, or there is a known failure of the building's deionized water system.

6.0 PERSONNEL QUALIFICATIONS:

- 6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

- 7.1 The deionized water port on the north wall of room B206 is outfitted with a Barnstead B-pure Pressure Cartridge System. The Barnstead B-pure Pressure Cartridge System contains cartridges which raise the quality of the deionized water. Refer to the Barnstead B-pure Pressure Cartridge System Operation Manual and Parts List, Series 583 for operation and maintenance of the equipment and instructions on changing filters (see ref. 15.1).
- 7.2 Vendor-supplied test request/chain of custody forms and water collection bottles. Contact vendor to obtain additional forms and bottles.
- 7.3 Styrofoam shipping container
- 7.4 Refrigerant packs
- 7.5 Hach Total Chlorine Test Kit, 0-3.5 mg/L (Hach catalog number 2231-03)
- 7.6 Isopropyl alcohol
- 7.7 Soft cloth (e.g., Texwipe TX409 absorbent wipers)
- 7.8 Chlorine Standard Solution, 50-75 mg/L, 2-mL PourRite ampule (Hach catalog number 14268-20)

7.9 Nalgene 16 oz. leak-proof environmental sampling bottles (Nalgene part number 332189-0016).

8.0 INSTRUMENT OR METHOD CALIBRATION: Not applicable

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Refer to section 4.0 for sample handling and storage conditions.

10.0 PROCEDURE AND ANALYSIS:

10.1 The water used in the laboratory to make media and reagents and to dilute disinfectants during efficacy testing is taken from the Barnstead B-pure Pressure Cartridge System filtration unit on the north wall of room B206. Consequently, this is the source of the water collected for quality assurance testing (see 4.1).

10.2 The water is checked for specific heavy metals (cadmium [Cd], lead [Pb], nickel [Ni], zinc [Zn], copper [Cu], and chromium [Cr]), total heavy metals (cumulative value of the six specific heavy metals), total organic carbon, conductivity, total chlorine residual, heterotrophic plate counts, and water quality/suitability. The frequency of testing and accepted limits of water quality as specified in “Standard Methods”, Table 9020: II (see ref. 15.2) are presented in Table 1 below:

Table 1. Monitoring frequency and acceptable limits for water quality parameters.

Test	Monitoring Frequency	Accepted Limits
Heavy Metals, Total	Annually	<0.10 mg/L*
Heavy Metals, Single (Cd, Cr, Cu, Ni, Pb, and Zn)	Annually	< 0.05 mg/L
Water Quality (or Water Suitability) Test	Annually	0.8 to 3.0 ratio
Total Organic Carbon	Monthly	< 1.0 mg/L
Conductivity	Monthly	< 2 umhos/cm at 25EC
Total Chlorine Residual	Monthly	<0.1 mg/L**
Heterotrophic Plate Counts	Monthly	< 500 Colony Forming Units/mL (CFU/mL)

*The accepted limit for total heavy metals is determined by finding the cumulative value of the six specific heavy metals. If the testing result for the specific heavy metals are “not detected”, determine the cumulative value of the reportable limits (RL). This value must be below 0.10 mg/L.

**As detected by Hach Total Chlorine Test Kit (0.0-3.5 mg/L)

- 10.2.1 The Barnstead B-pure Pressure Cartridge System filtration unit in room B206 has an in-line resistivity meter which continuously reads and displays the resistivity of the water (in megohms resistance, inverse of umhos/cm). When using water for any purpose, the lab staff must monitor the resistivity meter, while the water is flowing, to ensure that the water demonstrates > 0.5 megohms resistance (same as < 2 umhos/cm). The resistance reading must be recorded once for every day of use in the Purified Water Resistivity Record (see 16.4). If the resistivity falls below 0.5 megohms, see 14.0 for suggested corrective actions or inform the analyst currently responsible for verifying the quality of the deionized water.
- 10.2.2 The Use test specified in Table 9020:II (see reference 15.2 page 9-7) is performed only when a new source of reagent grade water is used (i.e., new source or lot of bottled water). This test is not applicable to the laboratory.
- 10.3 Monitoring of total heavy metals, specific heavy metals, total organic carbon, conductivity, heterotrophic plate counts, and water suitability is performed by a competent vendor.
- 10.4 When sampling each month, legibly, in ink, complete the vendors test request/chain of custody form.
- 10.5 Collect the water for analysis from the Barnstead B-Pure filtration unit on the north wall of room B206 by filling the appropriate sample collection bottle as indicated in Table 2 below:

Table 2. Collection specifications for water quality parameters.

Test	Type of Collection Bottle/ Preservative
Heavy Metals, Single (Cd, Cr, Cu, Ni, Pb, and Zn)	One Pint/Nitric Acid
Total Organic Carbon	One Pint/Sulfuric Acid*
Conductivity	One Pint/No Preservative
Heterotrophic Plate Counts	Sterile Bottle/No Preservative
Water Quality (or Water Suitability Test)	Square, Glass Bottle/No Preservative

*Note: Collect the water for analysis by filling one Nalgene 16 oz. leak-proof environmental sampling bottle. Close the sample bottle and transport it to the fume hood in B203. Remove the cap from the sample bottle. While wearing nitrile gloves, use a glass Pasteur pipet to add 4-6

drops of sulfuric acid to the water sample. Remove the bulb and place the Pasteur pipet, tip down, in a sterile beaker. Put the cap back on the sample bottle and invert the sample bottle several times. Turn on the tap water. Pour out the excess sulfuric acid from the beaker into the sink and rinse out the beaker with water. Set it aside to be washed. Place the Pasteur pipets under gently-running water and rinse out the excess acid. Dispose of the pipets in a broken glass box.

- 10.6 As each individual sample collection bottle is filled, legibly write the collection time (in military time) on the appropriate vendor sample identification label and on the test request/chain of custody form.
 - 10.6.1 After filling the heterotrophic plate count sample collection bottle, place the bottle in the refrigerator until all other bottles have been filled, labeled, and placed in the shipping container.
- 10.7 Water Suitability Test: Collect deionized water in the bottle supplied by the vendor. Place the glass bottle (loosely capped) on a hotplate and bring the water slowly to a boil over medium-high heat. Do not turn the hotplate dial to “high” as the rapid heating process may cause the glass bottle to break. After the water has boiled for 2 minutes, remove the bottle from the hotplate and let cool on the bench top. Once cool, tighten the cap and package the bottle for shipment.
- 10.8 Once all sample collection bottles have been filled and the collection time recorded on the vendor sample identification labels and the test request/chain of custody form, place the bottles in a styrofoam shipping container. Remove the heterotrophic plate count sample collection bottle from the refrigerator, place it in a plastic bag with one to two ice packs, and tape or tie the bag closed. Place the bag in the shipping container.
- 10.9 Add packing material to the sample collection bottles to prevent the bottles from moving during shipment.
- 10.10 Complete the vendor chain of custody form and prepare for shipment.
 - 10.10.1 Place the vendor test request/chain of custody form in a plastic bag and tape or tie it closed. Place the bag in the shipping container and tape the container closed.
- 10.11 Ship the package by priority overnight shipping.
- 10.12 Chlorine Residual Test: Monitor the total chlorine residual of the water each month using the Hach Total Chlorine Test Kit, 0-3.5 mg/L (Hach catalog number 2231-03).
 - 10.12.1 Clean a beaker and the kit’s plastic viewing tubes and caps with

isopropyl alcohol or a non-abrasive detergent prior to commencing the test. Rinse several times with the sample deionized water (from the Barnstead B-Pure filtration unit on the north wall of room B206). Use a soft cloth for wiping or drying the plastic viewing tubes. Do not use paper towels or tissue as this may scratch the plastic.

- 10.12.2 Collect sample water from the Barnstead B-Pure filtration unit on the north wall of room B206 in the beaker. Pour water from the beaker into one plastic viewing tube until the water level reaches the first line (bottom edge of the frosted area=equals 5 mL). This is the blank.
- 10.12.3 Place the blank in the top left opening of the color comparator.
- 10.12.4 Fill the second plastic viewing tube to the first line (bottom edge of the frosted area) with sample water from the beaker.
- 10.12.5 Add the contents of one DPD (salt of N,N-Diethyl-p-Phenylenediamine Potassium Iodide Sodium Phosphate, Dibasic) Total Chlorine Reagent Powder Pillow to the second tube. To open the powder pillow, tap the bottom of the packet on a hard surface, tear open the packet along the dashed line, open the packet and form a spout by squeezing the side edges, and pour the contents into the sample. (Note: Check reagent accuracy of each new lot of DPD Total Chlorine Reagent Powder Pillows. See 10.13).
- 10.12.6 Cap the second tube and swirl to mix. Accuracy of the test is not affected by undissolved powder.
- 10.12.7 Wait 3 minutes. The result of the test must be read within 6 minutes of the addition of the powder.
- 10.12.8 Place the second tube in the top right opening of the color comparator. Hold the comparator up to a light source such as a window or lamp. Look through the openings in the front of the comparator.
- 10.12.9 Rotate the color disc until the color matches in the two openings.
- 10.12.10 Read the mg/L total chlorine in the scale window. Record the mg/L total chlorine on the Total Chlorine Residual of Purified

Water form (see 16.2).

- 10.12.11 Rinse the viewing tubes several times with de-ionized water and allow to dry before putting them back in the kit.
- 10.12.12 Return the color disc to its plastic storage envelope.
- 10.13 Accuracy check of each new lot of DPD. -When a new lot of DPD Powder Pillows is received, conduct the following test prior to using the DPD Powder Pillows to monitor the total chlorine residual of the laboratory's deionized water (see 10.12).
 - 10.13.1 Clean two beakers and the kit's plastic viewing tubes and caps with isopropyl alcohol or a non-abrasive detergent prior to commencing the test. Rinse several times with deionized water (from the Barnstead B-Pure filtration unit on the north wall of room B206). Use a soft cloth for wiping or drying the plastic viewing tubes. Do not use paper towels or tissue as this may scratch the plastic.
 - 10.13.2 In a beaker, collect approximately 100 mL sample water from the Barnstead B-Pure filtration unit on the north wall of room B206. Pour water from the beaker into one plastic viewing tube until the water level reaches the first line (bottom edge of the frosted area-equals 5 mL). This is the blank.
 - 10.13.3 Cap the blank and place it in the top left opening of the color comparator (containing color disc).
 - 10.13.4 Snap open an ampule of the Chlorine Standard Solution, (50-75 mg/L, 2-mL PourRite ampule). Note the amount of free chlorine at the time that the ampules were filled. This value can be found on the first page of the instructions accompanying the chlorine standard.
 - 10.13.5 Prepare a dilution of the standard to yield anywhere from 1 to 3 mg/L free chlorine. For example, if the amount of free chlorine in the standard is 64.2 mg/L, adding 1 mL of the chlorine standard to 49 mL water will yield a water sample with approximately 1.3 mg/L chlorine (calculation: $[64.2 \text{ mg/L}][1 \text{ mL}] = [X \text{ mg/L}][50 \text{ mL}]$; solving for X yields $X=1.28 \text{ mg/L}$). Using a sterile 25 mL pipet, pipet 49 mL of water from the beaker and add it to a second, empty beaker. Using a sterile 1 mL pipet, add 1 mL of the chlorine

standard to the 49 mL of water. Swirl to mix.

- 10.13.6 Fill the second plastic viewing tube to the first line (bottom edge of the frosted area) with chlorinated water from the second beaker.
- 10.13.7 Add the contents of one DPD Total Chlorine Reagent Powder Pillow to the second tube. To open the powder pillow, tap the bottom of the packet on a hard surface, tear open the packet along the dashed line, open the packet and form a spout by squeezing the side edges, and pour the contents into the sample.
- 10.13.8 Cap the second tube and swirl to mix. Accuracy of the test is not affected by undissolved powder.
- 10.13.9 Wait 3 minutes. The result of the test must be read within 6 minutes of the addition of the powder.
- 10.13.10 Place the second tube in the top right opening of the color comparator. Hold the comparator up to a light source such as a window or lamp. Look through the openings in the front of the comparator.
- 10.13.11 Rotate the color disc until the color matches in the two openings.
- 10.13.12 Read the mg/L total chlorine in the scale window. The mg/L total chlorine read from the color comparator must approximate the chlorine concentration (mg/L) in the prepared dilution in order for the reagents to be used in the total chlorine residual test.
- 10.13.13 Record the results in the DPD Reagent Accuracy Form (see 16.1).
- 10.13.14 Discard the remaining chlorine standard in the ampule by pouring it down the sink. Rinse the ampule with water and place it in a box for broken glass.
- 10.13.15 Rinse the viewing tubes several times with deionized water and allow to dry before putting them back in the kit.
- 10.13.16 Return the color disc to its plastic storage envelope.

11.0 DATA ANALYSIS/CALCULATIONS:

- 11.1 When the report of water sample analysis is received from the vendor laboratory,

record the testing results legibly and in indelible ink under the “Test Results” column of form 16.3, Quality Assurance of Purified Water Form. Compare the testing results with the “Accepted Limits”. For each parameter, if the testing results for that parameter fall within the accepted limits, indicate in form 16.3 that the water quality is acceptable. If the testing results for that parameter fall outside of the accepted limits, indicate that the water quality is not acceptable, and record the corrective action taken (see section 14.0).

- 11.2 Record the results of the total chlorine residual test in the Quality Assurance of Purified Water Form (see 16.3). Compare the testing results with the “Accepted Limits”. If the testing results fall within the accepted limits, indicate in form 16.3 that the water quality is acceptable. If the testing results fall outside of the accepted limits, indicate that the water quality is not acceptable, and record the corrective action taken (see section 14.0).
- 11.3 The water quality is acceptable only if the results for all parameters fall within the accepted limits.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

- 12.1 The reports of water sample analysis must be placed in the Quality Assurance of Purified Water Record Book along with the carbon copy of the vendor chain of custody form.
- 12.2 Data will be recorded promptly, legibly, and in indelible ink on all forms (see 16.0). The forms will be kept in the Quality Assurance of Purified Water Record Book. The Record Book is kept in a secured file cabinet in the file room D217. Archived data is subject to OPP’s official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL: None

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

- 14.1 If the water quality, including residual chlorine, falls outside of acceptable limits take corrective actions and retest the water to demonstrate that the water quality parameter(s) that previously fell outside of acceptable limits is/are now within acceptable limits.
- 14.2 Disinfectant efficacy tests may have been performed between the time the water quality fell outside of acceptable limits (i.e., potentially one day after the date of the previous sampling for that test parameter) and the time that results of successful retesting are received. If the media and reagent controls associated

with each test indicate that the media and reagents perform(ed) acceptably, the test data are considered valid.

- 14.3 Refer to Table 3 below for examples of corrective measures to take if water quality parameters fall outside of the acceptable limits:

Table 3. Water quality parameters and suggested corrective measures.

Parameter	Suggested Corrective Measures
Heavy Metals, Total	Replace the cartridges in the B-pure Pressure Cartridge System. See ref. 15.1
Heavy Metals, Single (Cd, Cr, Cu, Ni, Pb, and Zn)	Replace cartridges in the B-pure Pressure Cartridge System. See ref. 15.1
Total Organic Carbon	Replace the cartridges in the B-pure Pressure Cartridge System. See ref. 15.1
Conductivity/Resistivity	Replace the cartridges in the B-pure Pressure Cartridge System. See ref. 15.1
Heterotrophic Plate Counts	Replace the final filter in the B-pure Pressure Cartridge System. See ref. 15.1
Water Quality (or Water Suitability) Test	Replace the cartridges in the B-pure Pressure Cartridge System. See ref. 15.1

- 14.4 If the corrective measures in 14.3 have been taken and retesting demonstrates that the water quality parameter(s) that previously fell outside of acceptable limits continue to fall outside of acceptable limits, take one or more of the following courses of action:

14.4.1 Discuss the problem with the Facility Operation Branch Mechanical Engineer (410-305-4357). The problem may stem from the Environmental Science Center's deionized water system, which is the feedwater for the Barnstead B-pure Pressure Cartridge System filtration unit. Retest the water following any corrective measures taken by the Facility Operation Branch Mechanical Engineer.

14.4.2 Consult with Region III scientists to determine if they have experienced a similar problem. Retest the water following any corrective measures taken.

14.4.3 Call the vendor laboratory and seek advice from technical staff. Retest the water following any corrective measures taken.

15.0 REFERENCES:

- 15.1 Barnstead B-pure Pressure Cartridge System Operation Manual and Parts List,

Series 583.

- 15.2 Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E. eds. Standard Methods for the Examination of Water and Wastewater, Online Edition. American Public Health Association, American Water Works Association, Water Environment Federation, Section 9020 approved 2005.
- 15.3 U.S. EPA Region III and OPPTS Environmental Science Center Specifications, Volume 2, November 7, 1996.
- 16.0 FORMS AND DATA SHEETS:
 - 16.1 DPD Reagent Accuracy Form
 - 16.2 Total Chlorine Residual of Purified Water Form
 - 16.3 Quality Assurance of Purified Water Form
 - 16.4 Purified Water Resistivity Record Form

16.3 Quality Assurance of Purified Water Form
 OPP Microbiology Laboratory

QUALITY ASSURANCE OF PURIFIED WATER FORM					
Month/Year			Initials		
Parameters**	Accepted Limits	Test Results	Acceptable?		Corrective Action Taken
			Yes	No	
Heavy Metals, Total	< 0.10 mg/L				
Heavy Metals, Single: Chromium	< 0.05 mg/L				
Copper	< 0.05 mg/L				
Nickel	< 0.05 mg/L				
Zinc	< 0.05 mg/L				
Cadmium	< 0.05 mg/L				
Lead	< 0.05 mg/L				
Total Organic Carbon	<1.0 mg/L				
Conductivity	<2 umhos/cm at 25EC				
Total Chlorine Residual	<0.1 mg/L*				
Heterotrophic Plate Counts	< 500 CFU/mL				
Water Quality (or Water Suitability) Test	0.8 to 3.0 ratio				

*As detected by Hach Total Chlorine Test Kit (0.0-3.5 mg/L)

**Heavy metals analyses and the Water Quality Test are performed annually.

