

SOP Number	QC-01-09
Title	Quality Assurance of Purified Water
Revisions Made	<ul style="list-style-type: none"><li>• Minor editorial changes for clarification purposes.</li></ul>

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Title	Quality Assurance of Purified Water
Scope	This protocol outlines the procedures for monitoring the quality of the deionized water used in the laboratory to make media.
Application	Verifying water quality is an important component of a laboratory's internal quality control program. Poor water quality can significantly affect the outcome of procedures.

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TABLE OF CONTENTS

<u>Contents</u>	<u>Page Number</u>
1. DEFINITIONS	3
2. HEALTH AND SAFETY	3
3. PERSONNEL QUALIFICATIONS AND TRAINING	3
4. INSTRUMENT CALIBRATION	3
5. SAMPLE HANDLING AND STORAGE	3
6. QUALITY CONTROL	3
7. INTERFERENCES	3
8. NON-CONFORMING DATA	3
9. DATA MANAGEMENT	4
10. CAUTIONS	4
11. SPECIAL APPARATUS AND MATERIALS	4
12. PROCEDURE AND ANALYSIS	4
13. DATA ANALYSIS/CALCULATIONS	8
14. FORMS AND DATA SHEETS	8
15. REFERENCES	8

<b>1. Definitions</b>	Abbreviations/definitions are provided in the text.
<b>2. Health and Safety</b>	Follow procedures specified in SOP MB-01, Laboratory Biosafety. Consult the Safety Data Sheet for specific hazards associated with products.
<b>3. Personnel Qualifications and Training</b>	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
<b>4. Instrument Calibration</b>	Not Applicable.
<b>5. Sample Handling and Storage</b>	Refer to section 12 for sample handling and storage conditions.
<b>6. Quality Control</b>	None
<b>7. Interferences</b>	<ol style="list-style-type: none"> <li>1. Check 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) for reagent accuracy. If the DPD Powder Pillows do not demonstrate reagent accuracy, the total chlorine residual test may be jeopardized. See section 12.4 for a description of the method to check reagent accuracy.</li> <li>2. Discard expired DPD Total Chlorine Reagent Powder Pillows.</li> <li>3. Conduct all test parameters at the monitoring frequency indicated in Table 1: Monitoring Frequency and Acceptable Limits for Water Quality Parameters (section 12.1).</li> <li>4. Test individual test parameters more frequently than described in Table 1 if a problem with water quality is identified (see section 8, Non-Conforming Data and section 13, Data Analysis and Calculations). The laboratory may opt to test the water whenever the source of the water is changed, if there is an upgrade, or there is a known failure of the building's deionized water system.</li> </ol>
<b>8. Non-conforming Data</b>	<ol style="list-style-type: none"> <li>1. Management of non-conforming data will be consistent with SOP ADM-07, Non-Conformance Reports.</li> <li>2. If the water quality, including residual chlorine, falls outside of acceptable limits (Table 1), consult with the Facility Engineer 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.</li> </ol>

	<ol style="list-style-type: none"> <li>3. The water may have been used to prepare media or reagents 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 (e.g., performance and/or sterility testing) indicate that the media and reagents are acceptable, the media and reagents are considered valid.</li> </ol>
<b>9. Data Management</b>	<ol style="list-style-type: none"> <li>1. Archive data consistent with SOP ADM-03, Records and Archives.</li> <li>2. Place reports in the Quality Assurance of Purified Water Record Book.</li> </ol>
<b>10. Cautions</b>	<ol style="list-style-type: none"> <li>1. Let the water run for approximately 30 seconds prior to filling sample collection bottles.</li> <li>2. Legibly complete any required chain of custody forms and sample identification labels to maintain evidence of chain of custody and to ensure that the testing laboratory performs the analyses requested by the ESC/OPP Microbiology Laboratory.</li> </ol>
<b>11. Special Apparatus and Materials</b>	<ol style="list-style-type: none"> <li>1. <i>Building deionized water spigots.</i> Located in each laboratory sink. There are two in B206.</li> <li>2. <i>Hach Total Chlorine Test Kit.</i> 0-3.5 mg/L (Hach catalog number 2231-03), for determination of chlorine concentration in the water sample.</li> <li>3. <i>Chlorine Standard Solution.</i> 50-75 mg/L, 2-mL PourRite ampule (Hach catalog number 14268-20), for calibration of the Hach Total Chlorine Kit.</li> </ol>
<b>12. Procedure and Analysis</b>	<ol style="list-style-type: none"> <li>1. The EPA Region 3 Laboratory and Technical Services Branch (LTSB) Laboratory Analysis Section will perform the following tests: 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), and total organic carbon.</li> <li>2. Water conductivity is monitored daily by the facility engineers. If the parameters fall outside of acceptable limits the facility will be notified not to use the deionized water.</li> <li>3. Follow the LTSB sample submission SOP for sample collection and chain of custody requirements.</li> <li>4. MLB will perform the total chlorine residual test and heterotrophic plate counts.</li> </ol>

<p><b>12.1 Frequency of Analysis and Acceptable Limits</b></p>	<p>Table 1. Monitoring frequency and acceptable limits for water quality parameters.</p> <table border="1" data-bbox="480 390 1437 831"> <thead> <tr> <th>Test</th> <th>Monitoring Frequency</th> <th>Accepted Limits</th> </tr> </thead> <tbody> <tr> <td>Heavy Metals, Total</td> <td>Annually</td> <td>&lt;0.10 mg/L*</td> </tr> <tr> <td>Heavy Metals, Single (Cd, Cr, Cu, Ni, Pb, and Zn)</td> <td>Annually</td> <td>&lt; 0.05 mg/L</td> </tr> <tr> <td>Total Organic Carbon</td> <td>Annually</td> <td>&lt; 3.0 mg/L</td> </tr> <tr> <td>Conductivity</td> <td>***Daily</td> <td>&lt; 2 umhos/cm at 25°C</td> </tr> <tr> <td>Total Chlorine Residual</td> <td>Twice a year</td> <td>&lt;0.1 mg/L**</td> </tr> <tr> <td>Heterotrophic Plate Counts</td> <td>Twice a year</td> <td>&lt; 500 Colony Forming Units/mL (CFU/mL)</td> </tr> </tbody> </table> <p>*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.</p> <p>**As detected by Hach Total Chlorine Test Kit (0.0-3.5 mg/L)</p> <p>***This is monitored daily by the ESC engineering staff. These records are maintained by the ESC engineering staff and are available upon request.</p>	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	Total Organic Carbon	Annually	< 3.0 mg/L	Conductivity	***Daily	< 2 umhos/cm at 25°C	Total Chlorine Residual	Twice a year	<0.1 mg/L**	Heterotrophic Plate Counts	Twice a year	< 500 Colony Forming Units/mL (CFU/mL)
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<p><b>12.2 Collection and Preparation of Water</b></p>	<ol style="list-style-type: none"> <li>1. Run the water from a deionized water spigot in B206 for approximately 30 seconds and then collect 1 liter of deionized water in a sterile plastic bottle and submit it to the LTSB laboratory.</li> <li>2. Complete any required test request/chain of custody forms and sample identification labels as needed.</li> </ol>																					
<p><b>12.3 Chlorine Residual Test</b></p>	<ol style="list-style-type: none"> <li>a. Monitor the total chlorine residual of the water twice a year using the Hach Total Chlorine Test Kit, 0-3.5 mg/L (Hach catalog number 2231-03).</li> <li>b. 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 deionized water. 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.</li> <li>c. Collect sample water from a deionized water spigot in B206 into one plastic viewing tube until the water level reaches the first line (bottom edge of the frosted area). This is the blank.</li> <li>d. Place the blank in the top left opening of the color comparator.</li> <li>e. Fill the second plastic viewing tube from the same deionized</li> </ol>																					

	<p>water spigot to the first line (bottom edge of the frosted area).</p> <ol style="list-style-type: none"> <li>f. Add the contents of one DPD Total Chlorine Reagent Powder Pillow to the second tube. (Note: Check reagent accuracy of each new lot of DPD Total Chlorine Reagent Powder Pillows. See section 12.4.)</li> <li>g. Cap the second tube and swirl to mix. Accuracy of the test is not affected by undissolved powder.</li> <li>h. Wait 3 minutes. The result of the test must be read within 6 minutes of the addition of the powder.</li> <li>i. 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.</li> <li>j. Rotate the color disc until the color matches in the two openings.</li> <li>k. Read the mg/L total chlorine in the scale window and record the results on the Total Chlorine Residual of Purified Water Form.</li> <li>l. Rinse the viewing tubes several times with de-ionized water and allow to dry before putting them back in the kit.</li> </ol>
<p><b>12.4 Accuracy check of a new lot of DPD Powder Pillows</b></p>	<ol style="list-style-type: none"> <li>a. When a new lot of DPD Total Chlorine Reagent Powder Pillows is received, conduct the following test prior to using the DPD Total Chlorine Reagent Powder Pillows to monitor the total chlorine residual of the laboratory's deionized water.</li> <li>b. 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 a deionized water spigot in 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.</li> <li>c. Collect sample water from a deionized water spigot in B206 into one plastic viewing tube until the water level reaches the first line (bottom edge of the frosted area). This is the blank.</li> <li>d. Cap the blank and place it in the top left opening of the color comparator (containing color disc).</li> <li>e. 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</li> </ol>

	<p>standard.</p> <ol style="list-style-type: none"> <li>f. Prepare a dilution of the standard to yield 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: <math>[64.2 \text{ mg/L}][1 \text{ mL}] = [X \text{ mg/L}][50 \text{ mL}]</math>; solving for X yields <math>X=1.28 \text{ mg/L}</math>). Using a sterile serological 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.</li> <li>g. Fill the second plastic viewing tube to the first line (bottom edge of the frosted area) with chlorinated water from the second beaker.</li> <li>h. Add the contents of one DPD Total Chlorine Reagent Powder Pillow to the second tube.</li> <li>i. Cap the second tube and swirl to mix. Accuracy of the test is not affected by undissolved powder.</li> <li>j. Wait 3 minutes. The result of the test must be read within 6 minutes of the addition of the powder.</li> <li>k. 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.</li> <li>l. Rotate the color disc until the color matches in the two openings.</li> <li>m. 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.</li> <li>n. Record the results in the DPD Reagent Accuracy Form (see section 14).</li> <li>o. 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.</li> <li>p. Rinse the viewing tubes several times with deionized water and allow to dry before putting them back in the kit.</li> </ol>
<p><b>12.5 Heterotrophic Plate Count</b></p>	<ol style="list-style-type: none"> <li>a. Perform the heterotrophic plate count twice a year.</li> <li>b. Run the water from a deionized water spigot in B206 for</li> </ol>



	<p>approximately 30 seconds and then collect about 100 mL of the water in a sterile container.</p> <p>c. Filter the water through a 0.2 µm filter and place the filter on a trypticase soy agar (TSA) or blood agar plate and incubate at 36° C for 2-3 days.</p> <p>d. Record the results on the Quality Assurance of Purified Water Form (see section 14). The expected outcome is &lt;500 colony forming units/mL.</p>						
<p><b>13. Data Analysis/ Calculations</b></p>	<p>1. When the report of water sample analysis is received from the LTSB laboratory, record the testing results legibly and in indelible ink under the “Test Results” column of the Quality Assurance of Purified Water Form. Compare the testing results with the “Accepted Limits” (see Table 1). For each parameter, if the testing results for that parameter fall within the accepted limits, indicate in the form 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.</p> <p>2. Record the results of the total chlorine residual test in the Quality Assurance of Purified Water Form. Compare the testing results with the “Accepted Limits” (see Table 1). If the testing results fall within the accepted limits, indicate in the form 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. The water quality is acceptable only if the results for all parameters fall within the accepted limits.</p>						
<p><b>14. Forms and Data Sheets</b></p>	<p>Test Sheets. Test sheets are stored separately from the SOP under the following file names:</p> <table data-bbox="537 1423 1414 1604"> <tr> <td>DPD Reagent Accuracy Form</td> <td>QC-01-09_F1.docx</td> </tr> <tr> <td>Total Chlorine Residual of Purified Water Form</td> <td>QC-01-09_F2.docx</td> </tr> <tr> <td>Quality Assurance of Purified Water Form</td> <td>QC-01-09_F3.docx</td> </tr> </table>	DPD Reagent Accuracy Form	QC-01-09_F1.docx	Total Chlorine Residual of Purified Water Form	QC-01-09_F2.docx	Quality Assurance of Purified Water Form	QC-01-09_F3.docx
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<p><b>15. References</b></p>	<p>1. Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E. eds. Standard Methods for the Examination of Water and Wastewater, Online Edition. Section 9020 (2017).</p> <p>2. U.S. EPA Region III and OCSPP Environmental Science Center Specifications, Volume 2, November 7, 1996.</p>						