AED LABORATORY OPERATING PROCEDURES

Instructions for using the LOP template (found on page 2). Questions concerning the Job Hazard Analysis, call SHEMP (3009).

BACKGROUND AED will follow the NHEERL Quality Management Plan, Section 2.4 to address operating procedures. LOPs must be written for any routine technical procedure used in data/sample collection, use or analysis.

The LOP process may also be used to document those administrative procedures that have not risen to the level of significance deemed necessary for an official AED-SOP (as defined by the Union-Management Partnership Council).

LOP authors are responsible for keeping them current. Initial review and approval for use is indicated via the signatures of the QAM, SHEM and the author. The author's signature indicates the LOP has received a technical review by at least one other staff member familiar with the procedure. NHEERL requires biennial review of LOPs for continued adequacy. This occurrence will be indicated by the author's initialing and dating the front page underneath their original signature.

Published literature references may be used to supplement LOPs, but they do not replace LOPs.

HEADER Descriptive title to fill area under "AED Laboratory Operating Procedure", three lines maximum. Instruction requirements will appear throughout this document. If you wish to remove them from the screen, toggle off the "¶" button on the toolbar along the top of the page. These comments will not print out with your document.

FOOTER LOP-AED/XXX/YY/14-01-001 January 5, 2015

This document control language is a unique identifier for the LOP and links it to the author. **XXX** pertains to your branch abbreviation (e.g., IDB), **YY** needs to be changed to your initials, **95** is the last two digits of the year in which the LOP was written, **01** indicates this was the first LOP originated by this author in the indicated year, 1995; **001 -** indicates that this is the first revision of this LOP (the original would be 000); and **March 20, 1996** is date of most recent revision

POINT OF CONTACT Add your name above the local contact information.

OBJECTIVES The objective, the first section, is a general statement of what the LOP is intended to achieve. It should inform the user of the scope and application and identify those sample matrices, characteristics, physical properties, chemicals or classes of chemicals to which this LOP applies. Sufficient detail should be included to ensure against improper usage.

MATERIALS AND EQUIPMENT List all reagents and materials used including manufacturer and lot numbers. List the specific equipment/apparatus used or needed for the procedure. Provide explicit explanations as needed.

PROCEDURE - NOTE: FOR EACH STEP WITH AN IDENTIFIABLE HAZARD A CORRESPONDING ENTRY MUST BE COMPLETED IN THE JOB HAZARD ANALYSIS FORM AT THE END OF THIS DOCUMENT.

Detailed explanations of the steps or activities necessary to accomplish the LOP in a consistent manner. In the following format:

3.1 Title of Section 3.1....
3.1.1
3.2 Title of Section 3.2....
3.2.1 Title of Subsection 3.2.1.....

Also include the following:

Definitions (identifying any acronyms, abbreviations, or specialized terms used),

Health & Safety Warnings (indicating operations that could result in personal injury or loss of life and explaining what will happen if the procedure is not followed or is followed incorrectly; listed here and at the critical steps in the procedure),

Complete the Job Hazard Analysis

Cautions (indicating activities that could result in equipment damage, degradation of sample, or possible invalidation of result, or potential **environmental contamination (along with steps to minimize the potential**); listed here and at the critical steps in the procedure),

Interferences (describing any component of the process that may interfere with the accuracy of the final product)

QA/QC

Discuss what steps have been taken to ensure the quality of the data being generated with this LOP (sample handling, control samples, sample replicates, analytical replicates, SRM requirements, data reviews, replicate field observations, etc.)

Discuss training and the criteria used to demonstrate proper training of, and acceptable performance by, each analyst using this LOP.

This section also includes data validation which is the process by which data are filtered and accepted or rejected based on previously set criteria. This is the final step before the release of the data and should include checks for proper identification, transmittal errors, internal variability, and temporal and spatial variability (if possible). The validity of the samples and the measurement process used should be documented. A statistical estimation of the limits of uncertainty, if possible, should also be made.

TROUBLE SHOOTING This section is to provide the method user information and background on knowledge gained through experience or the method development process.

REFERENCES As appropriate

DETERMINATION OF SPECIFIC UV ABSORBANCE AT 254 NM IN SOURCE WATER AND DRINKING WATER IN SUPPORT OF TMDL DEVELOPMENT

POINT OF CONTACT:

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DISCLAIMER This procedure was written to meet the needs of the research program at the U.S. EPA-Atlantic Ecology Division. It is not a U.S. EPA Standard Method and must not be referred to as such. Mention of Trade names or commercial products does not constitute endorsement or recommendation for use.

1.0 OBJECTIVES

This LOP is part of a technical assistance agreement between the EPA Atlantic Ecology Division and the RI Department of Environmental Management (RIDEM). This LOP is written in support of development of Total Daily Maximum Limits (TMDLs) by RIDEM to address nutrient related water quality impairments in the nine water supply reservoirs operated by the City of Newport, Rhode Island.

For this project, the specific objective is to:

Use the procedures in EPA Method 415.3 Rev 1.2: **Determination of Total Organic Carbon and Specific UV Absorbance at 254 nm in Source Water and Drinking Water** (Potter and Wimsatt, 2005) to provide information on UV light absorbance (UVA) of surface water collected from the nine freshwater reservoirs and ponds of the Newport Water Supply.

In general, the UVA procedure requires that the water sample be passed through a 0.45-µm filter and transferred to a quartz cell. The cell is then placed in a spectrophotometer to measure the UV absorbance at 254 nm and reported in $cm^{-1.}$ This information, when combined with dissolved organic carbon (DOC) data, can be used to calculate Specific UV Absorbance (SUVA) values for these water bodies. The SUVA value is a measure of the dissolved organic carbon (DOC) concentration available to likely form disinfection by-products when chlorinated and to determine compliance with the Disinfection/Disinfection By-Products Rule requirements for removing natural organic matter (Hach, 2011). For this method, DOC is operationally defined as organic matter contained in a water sample that is soluble and/or colloidal that can pass through a 0.45µm filter.

2.0 MATERIALS AND EQUIPMENT

0.45 µm filters, GE Healthcare GEH7141154EA
glass fiber "prefilters" – if necessary
glass funnel

Perkin-Elmer Lambda 35 UV Spectrophotometer
Quartz cells
Filter apparatus
1-L Boston round glass bottles (certified to meet EPA OSWER Directive # 9240.0-05A "Specifications and Guidance for Contaminant-Free Sample Containers 12/92)
Erlenmyer flasks
Laboratory reagent water (LRW = UVA $\leq$ 0.01 cm^{-1}, best performance UVA$\leq$0.0045 cm^{-1})
Methanol
IN-SPEC™ optical standard and background solution for a 254 nm spectrophotometric check (UV-3, #8303)
Muffle Furnace (capable of heating to 550 °C)
Vacuum source

3.0 PROCEDURE - NOTE: FOR EACH STEP WITH AN IDENTIFIABLE HAZARD A CORRESPONDING ENTRY MUST BE COMPLETED IN THE JOB HAZARD ANALYSIS FORM AT THE END OF THIS DOCUMENT.

3.0.1 GLASSWARE PREP

All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water followed by LRW. Non-volumetric glassware is then heated in a muffle furnace at 450°C for 8 hours to eliminate interferences.

3.1 IN THE FIELD

3.1.1 Sample Collection

Water samples to determine ultraviolet absorption (UVA) are collected using 1-L Boston round glass bottles (Eagle-Picher TOC Certified, Cat. No. 112-01A/C TOC, Eagle-Picher Technologies, LLC).

Samples are collected in clean glass bottles by filling the bottle almost to the top. Amber bottles are preferred, but clear glass bottles may be used if care is taken to protect samples from light.

Field duplicate samples are collection at one sample site for every ten samples collected.

At the time of collection, the samples are **NOT preserved with acid** and are delivered as soon as possible to the laboratory. Samples should arrive packed in ice or frozen gel packs. Upon arrival at the laboratory, samples are stored at < 6 °C, until analysis for up to 48 hours from the time of collection.

If there is no visible ice or the gel packs are completely thawed, the laboratory should report these conditions to Brian Zalewsky, RIDEM (401-222-4700 x7145), immediately. Samples shipped that are improperly preserved, and/or do not arrive at the laboratory within 48 hrs, cannot be used to meet compliance monitoring requirements under the Safe Drinking Water Act (SDWA).

3.2 IN THE LABORATORY:

3.2.1 Filter Preparation – Wash Volume Determination

Due to the possibility of lot-to-lot variations in the levels of contamination or adsorption, for each filter lot, the amount of LRW needed to wash the filters and the amount of sample that needs to be filtered and discarded prior to collection of filtrate (filter-to-waste volume) will be determined. A minimum of three

filters from each new lot are cleaned and checked for desorption/adsorption prior to using filters from the lot for actual samples.

Glass-fiber filters (~0.7µm), without organic binders, may be used as pre-filters, if it is anticipated that the sample will clog the 0.45 µm filter before enough filtrated is collected.

Initially, pre-filters are cleaned by heating to 450°C for eight hours and cooling to room temperature.

Choose three pre-filters (if used) and three filters (0.45µm) from each lot to make this volume determination. The averages of the final volumes determined for each type of filter will then be used generally for washing all filters from the associated lots.

(A) Pre-Filter (0.7µm) and Filter (0.45µm) Preparation to remove UV absorbing materials and DOC –

Filter membranes (along with the filtering apparatus) selected for UVA measurements must be cleaned to avoid significant UV absorbing materials. UV-absorbing materials are removed from the filter and filter apparatus by passing LRW through the filter.

Step 1) Place a filter on the filter apparatus.

Step 2) Use three successive rinses of 10 mL of LRW, each collected separately for a total of 30 mL, to remove UV-absorbing materials that could leach from the filter and apparatus. (The volume of each rinse is determined by the volumes necessary to conduct subsequent analyses. For instance, 10ml is used here because this is the UVA sample size

Step 3) Demonstrate acceptable cleaning by analyzing each sequential filtrate separately as a filter blank and meeting quality control criteria of absorbance < 0.01 cm^{-1} UVA. (Section 4.3). The volume of LRW used to achieve acceptable blanks can then be used for rinsing all filters of the same lot.

For example, three successive filter rinses of 10 mL LRW, upon analysis yield UV absorbance readings of 0.03, 0.01, and 0.01cm^{-1}. Therefore, the volume of LRW necessary for filter cleaning to remove absorbing materials that may leach off the filter is 20mL (the criterion was met in the second 10ml filtrate). Note: EPA Standard Method 415.3 indicates three successive rinses of 250ml are necessary to remove UV absorbing materials from pre-filters, indicating the possibility that rinses may require more LRW than the 10ml rinses mentioned above.

(B) Pre-Filter (0.7µm) and Filter (0.45µm) Preparation to inhibit adsorption of UV absorbing materials and–

For this determination, a low-turbidity water sample needs to be selected. A series of at least three filtrates are collected in separate (appropriately prepared) containers. The volume of each filtrate is determined based on the minimum volume required to make an analytical determination. For UVA the volume is 10ml.

Each resulting filtrate is analyzed according to this method in the order collected, the results being compared with the analysis of an unfiltered sample. When the concentration of the filtrate is within ±15% of the concentration of that measured in the unfiltered sample, then the recommended filter-to-waste volume is the sum of the volumes of that filtrate and any previous filtrates in the series.

For example, if the unfiltered sample has a UV absorbance at 254 nm of 0.20 cm^{-1} and the filtrate series (each filtrate = 10 mL) have absorbances of 0.12 cm^{-1}, 0.17 cm^{-1}, and 0.19 cm^{-1}, then a minimum of 20 mL of sample should be filtered-to-waste prior to collecting filtrate for the actual UVA analysis.

Step 1) Analyze an unfiltered sample.

Step 2) Using a filter already prepared via 3.2.2(A) filter a series of three sample aliquots (using the analytical sample size), each collected separately.

Step 3) Analyze the filtrates in the order collected. When the concentration of the filtrate is within ±15% of the concentration of that measured in the unfiltered sample, then the recommended filter-to-waste volume is the sum of the volumes of that filtrate and any previous filtrates in the series. Average the filter-to-waste volumes for the three filters from the lot to obtain the sample volume for rinsing all other filters from that lot.

3.2.2 Pre-Analysis

Remove the UVA sample(s) from cold storage and allow them to come to room temperature

3.2.3 Day-to-Day Spectrophotometer Performance Check

The day-to-day performance of the spectrophotometer is checked using the IN-SPEC™ optical standard prior to analyzing any UVA samples using the procedure described below.

Step 1) Using a 40 ml beaker and small funnel, fill a quartz cell with the IN-SPEC™ background solution. Place in the reference cell holder and close the cover.

Step 2) Click **Start** the instrument autozeros and a blank sample is requested by the instrument.

Step 3) Place a second quartz cell, also containing the IN-SPEC™ background solution, in the "sample cell holder", close cover.

Step 4) Click **OK.** The background correction is performed and then the next sample is requested.

Step 5) Remove the quartz cell from the "sample cell holder" position. Using a 40 ml beaker and small funnel, fill a third quartz cell with the IN-SPEC™ optical standard and place into the "sample cell holder." Click **OK** to read the UVA of the standard. The reading must be within 10% of the expected absorbance value, which will be ±10% of 0.165 cm^{-1} (0.1485 – 0.1815 cm^{-1}) at 254 nm (for UV3 #8303, absorbance).

Step 6) Record the absorbance of standard in the spectrophotometer instrument logbook.

Step 7) Remove cells from the "sample cell holder" and clean with LRW, rinse with methanol, and air dry. At this time, the cell with background solution remains in the instrument's reference cell holder.

3.2.4 UVA-Filter Blanks

A Filter Blank (UVA-FB) will be run at a minimum rate of one every twenty samples or one each day of instrument running (whichever is shorter), with a new filter blank being run with each new lot of filters.

Using a clean filter apparatus and filter(s) (prepared in Section 3.2.1), filter an aliquot (30mL) of LRW and transfer from the flask into a quartz cell (usning a small beaker and funnel) for UVA analysis. UVA-FB volume must be the same as the sample volume collected in Section 3.2.5.

The UVA-FB's maximum allowable background absorbance is 0.01 cm^{-1} UVA. If 0.01 cm^{-1} UVA for the UVA-FB is exceeded, the cause must be identified and any determined source of contamination must be eliminated. The spectrophotometer performance must then be rechecked (via Section 4.2).

3.2.5 Filtration Procedure

Step1) Assemble the filtering apparatus with pre-filter (if no pre-filter used, start at Step 7) and wash with LRW using volume determined in Section 3.2.1(A).

Step 2) Apply vacuum until no visible LRW remains on the filter.

Step 3) Remove the vacuum and swirl the LRW filtrate in the flask and discard.

Step 4) Re-assemble the filtering apparatus. Wash pre-filter with field sample using volume determined in Section 3.2.1(B).

Step 5) Remove the vacuum and swirl the field sample filtrate in the flask and discard.

Step 6) Reassemble the filtering apparatus and pre-filter the appropriate volume of sample necessary for UVA analysis. Remove vacuum and set aside the pre-filtered filtrate.

Step 7) (Re)assemble the filtering apparatus with 0.45µm filter and wash with LRW using volume determined in Section 3.2.1(A).

Step 8) Apply vacuum until no visible LRW remains on the filter.

Step 9) Remove the vacuum and swirl the LRW filtrate in the Erlenmyer flask and discard.

Step 11) Reassemble the filtering apparatus and wash the 0.45µm filter with field sample using volume determined in Section 3.2.1(B).

Step 12) Remove the vacuum and swirl the sample filtrate in the Erlenmyer flask and discard

Step 13) Reassemble the filter apparatus with the same flask and pour 30 mL of the pre-filtered filtrate (if prefilters were used) or raw field sample into the top of the filter apparatus.

Step 14) Attach the vacuum, filter the sample into the Erlenmyer flask, and retain the filtrate for UVA analysis.

3.2.6 Spectrophotometer Analysis of Ultraviolet Absorbance

Refer to UV Winlab Reference Manual

Step 1) On the PC, start **UV Winlab** which opens the **Methods** Window

Step 2) In the **Method Editor** window, enter details to create a method to analyze from a Start wavelength at 250 nm to an End wavelength at 300 nm at 1 nm intervals.

Step 3) Enter the sample name (for instance, NewportPond1 and sample collection date) under **Sample Identity** in the Methods window and save from the File menu.

Step 4) Select "**Autozero on start**" in the UV WinLab software program to automatically zero the spectrophotometer

Step 5) Select Use next **autoinc.filename** to automatically update the filename of future samples.

Step 6) Place a quartz cell containing one of the UVA-Filter Blanks in the reference cell holder and close cover. Click **Start**, the instrument autozeros and a blank sample is requested.

Step 7) Place the other quartz cell, containing the second UVA-Filter Blank, in the sample cell holder, close cover.

Step 8) Click **OK.** The background correction is performed and then the field sample is requested.

Step 9) Discard the second UVA-Filter Blank

Step 10) Rinse the sample cell with a small amount of the field sample by directly either pipetting or pouring the sample into the quartz cell. Discard the rinse.

Step 11) Refill the sample cell with field sample by directly either pipetting or pouring the sample into the quartz cell. Carefully clean the cell window.

Step 12) Place the sample cell back in the holder and close the spectrophotometer cover.

Step 13) Click **OK** to perform the analysis of the field sample.

For field duplicates, the FD1 (field duplicate sample 1) & FD2 (field duplicate sample 2) filtrates are also read and recorded for QA analysis.

Step 14) Select **Save** from the File menu to save the measurements of UVA absorption to file.

Step 15) Enter **Sample** information as the **Filename** and click **OK** to save the data.

4.0 QA/QC

QC requirements for UVA analysis include: the initial check of spectrophotometer capability for UVA determination (IDC), the regular analysis of spectrophotometer check solutions (SCS), filter blanks, and field duplicates (Potter and Wimsatt, 2005).

4.1 Initial Check Of Spectrophotometer Performance

The UV Spectrophotometer will be checked for calibration and certified annually per Service Maintenance Agreement with Perkin – Elmer Corp. The results will be recorded or kept in the instrument log book.

4.2 Day-to-Day Spectrophotometer Performance Check

The day-to-day performance of the spectrophotometer is checked using the IN-SPEC^{TM} optical standard and background solution prior to analyzing any UVA samples as described in Section 3.2.3.

The reading must be within 10% of the expected absorbance value, which will be ±10% of 0.165 cm^{-1} (0.1485 – 0.1815 cm^{-1}) at 254 nm (for UV3 #8303, absorbance). Record the absorbance of standard in the spectrophotometer instrument logbook.

4.3 Filter Blanks (equivalent to LRW blanks)

Acceptable filter cleaning is demonstrated by analyzing filter blanks which must have an absorbance of < 0.01 cm^{-1} UVA. To determine filter blank absorbance, the filter blank will be treated exactly the same as a UVA sample. The volume used in this determination will be the same as the sample volume. If criterion exceeded, the cause must be identified and any determined source of contamination must be eliminated. Follow Section 3.2.4.

4.4 Field Duplicates

Duplicate samples analyses serve as a check on sampling and laboratory precision.

Within each analysis batch, a minimum of one set of field duplicates must be analyzed (FD1 and FD2). Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2) using the equation:

$$RPD = \frac{|FD1 - FD2|}{(FD1 + FD2)/2} * 100$$

The RPD for field duplicates for UVA readings should be < 10% RPD.

5.0 TROUBLE SHOOTING

6.0 REFERENCES

Hach, 2011. Application Note: UV Transmission and UV Absorbance Methods. techhelp@hach.com

Potter, B.B. and J.C. Wimsatt, 2005. EPA Method 415.3: Determination of Total Organic Carbon and Specific UV Absorbance at 254 nm in Source Water and Drinking Water. EPA Document #: EPA/600/R-05/055, Revision 1.1. February. 56 pg.

Atlantic Ecology Division (AED)

JOB HAZARD ANALYSIS

Hazard Types (HT)

Job Task:

1. Toxic Chemic
2. Flammable Chemicals
3. Corrosive Chemicals
4. Environmental
5. Explosion (Chemical Reaction)
6. Explosion (Over pressurization)
7. Mechanical / Vibration
8. Electrical (Shock, Short Circuit)
9. Electrical (Fire)
10. Electrical (Static, ESD)
11. Electrical (Loss of Power)
12. Ergonomic (Overexertion)
13. Ergonomic (Human Error)
14. Vibration
15. Fall (Slips / Trips)
16 Fall (To a Different Level)
17. Excavation (Collapse)
18. Fire, Heat, Thermal, Cold
19. Noise
20. Radiation (Ionizing / Nonionizing)
21. Visibility
22. Weather
23. Caught (In, On, Between)
24. Struck (by, against)

Personal Protective Equipment:

Chemicals In Use:

CRITICAL TO SAFETY (CTS)

Risk Estimation Matrix

Probability of Occurrence of Harm	SEVERITY OF HARM			
	Catastrophic	**Serious**	**Moderate**	**Minor**
VERY LIKELY	High	High	High	Medium
LIKELY	High	High	Medium	Low
UNLIKELY	Medium	Medium	Low	Negligible
REMOTE	Low	Low	Negligible	Negligible

* High = CTS tasks should receive engineering controls prior to assigning administrative or PPE controls.

Step #	Procedures (LOP procedure step)	Potential Hazards	HT	Check CTS	Recommended Safe Practice
	Field Collection	Fall (Slips / Trips)	15	Negligible	Field Awareness
	Filter Cleaning	Ergonomic (Human Error)	13	Negligible	Careful lab practices
	Creating the UVA-Filter Blanks	Ergonomic (Human Error)	13	Negligible	Careful lab practices
	Spectrophotometer Analysis of Ultraviolet Absorption	Ergonomic (Human Error), Toxic Chemical (methanol rinses)	1,13	Negligible	Careful lab practices

Personal Protective Equipment Recommendations			
Where engineering and administrative controls aren't feasible or sufficient for controlling hazards, PPE must be used to protect workers. The following PPE are recommended for the noted tasks:			
Eye and Face Protection			
X	Safety glasses with side shields		Reflective goggles/face shield
	Chemical splash goggles		Cutting/braising/welding eye protection
	Face shield		Other:
Head Protection			
	Hard hat, bump cap		Helmet, cowl, hood
	Welding helmet/ mask		Other:
Foot Protection			
	Safety shoes/ boot	x	Other: Close toed shoes
	Chemical-resistant boots		
Body Protection			
x	Apron (splash, work)		Head-reflective garments
x	Lab Coat		Sleeves (cut-resistant)
	Coveralls (work, chemical resistant) Type chemical: Type coverall:		Other:
Respiratory Protection			
	Respirator		Type of respirator:
Hand Protection			
	Rubber insulating gloves		Rubber insulating sleeves
	Rubber insulating hoods	X	Other: Nitrile gloves

PPE Hazard Assessment Form

HEALTH AND SAFETY HAZARDS		
Chemical Hazards		
x	Vapors/Gases	Methanol
	Dusts/Mists/Fumes	
x	Liquid Splash	Methanol
	Other	
Comments:		
Physical Hazards		
	Impact-Flying dust, particles, chips	
	Penetration/Punctures	
	Cuts/Lacerations	
	Compressions–Pinch, crush, rollover	
	Heat-Sparks, molten splash, high temperatures	
	Cold-Cyrogens, cold temperatures	
	Light (optical radiation)	
	Electrical Shock	
	Fire	
	Radiation-Ionizing, Non-ionizing	
Comments:		
Biological Hazards		
	Bloodborne Pathogens	
	Animals (Zoonotic)	
	Other	
Comments:		

DISCLAIMER

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