

## STANDARD OPERATING PROCEDURE Procedure for Determining Total Residual Oxidants (TRO) in Water

**Compiled By -** **Name:** Tom Markee  
**Title:** GSI Chemist  
**Date:** July 7, 2009

**Approved By -** **Name:** Nicole Mays  
**Title:** GSI Senior Quality Systems Officer  
**Date:** July 7, 2009

**Cleared For Issue By -** **Name:** Allegra Cangelosi  
**Title:** GSI Principal Investigator and Project Manager  
**Date:** July 7, 2009

### RECORD OF REVISIONS:

No.	Date	Type	No.	Date	Type
1	06/09/2010	Added chlorine standard solution ampule to "Equipment List". Revised ¶3 and ¶4 to reflect use of chlorine standard solution (certified standard) to create standard curve rather than bleach. Added note to ¶8. Added chlorine reference standard information to "Quality Assurance/Quality Control" section.	7		
2	09/17/2010	Removed 1 mL – 5 mL pipettor from "Equipment List". Added "Spectrophotometer Set-Up and Operation" section. Added "Standard Preparation and Analysis" to "Sample Analysis" section, and added text throughout section to describe the standard curve procedure. Added Appendix 1.	8		
3			9		
4			10		
5			11		
6			12		

## **STANDARD OPERATING PROCEDURE**

### **Procedure for Determining Total Residual Oxidants (TRO) in Water**

#### **BACKGROUND**

The Great Ships Initiative (GSI) is a regional effort devoted to ending the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System and globally. In support of that goal, the GSI has established superlative freshwater ballast treatment evaluation capabilities at three scales—bench, land-based, and on board ship. Each scale is dedicated to addressing specific evaluation objectives. These include:

#### *GSI Bench-Scale Tests*

- Range finding for effective doses under a range of ambient conditions;
- Chemical degradation over time under a range of ambient conditions;
- Detection of any residual toxicity under a range of ambient conditions; and
- Confirmation of treatment process.

#### *GSI Land-Based Tests*

- Detection of scale-up, mechanical operation issues;
- Effectiveness of a dose with respect to the full range of ambient organisms; and
- Detection of any whole water effluent toxicity.

#### *GSI Shipboard Tests*

- Confirmation of biological and operational performance as expected in the ship environment; and
- Confirmation of performance as expected under a broad range of ambient conditions.

The GSI awards its independent status-testing services to developers of ballast treatment systems and processes determined to be promising. GSI status-testing is performed at the scale appropriate to the state of development of the target treatment system, with the goal of facilitating the rapid progression of meritorious ballast treatment systems through the research and development and approval processes to a market-ready condition.

GSI has no involvement, intellectual or financial, in the mechanics, design or market success of the actual treatment systems it tests. To ensure that GSI tests are uncompromised by any real or perceived individual or team bias relative to test outcomes, GSI test activities are subject to rigorous quality assurance/quality control (QAQC) procedures and documentation. This attention to QAQC assures high quality and credible evaluation of GSI and its findings.

## INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes the procedure used to determine total residual oxidants (TRO) in water—an important analysis parameter for many prospective ballast treatment systems. In this procedure, a Hach *N,N*-diethyl-*p*-phenylenediamine (DPD) Total Chlorine Reagent powder pillow (Hach Company, Loveland, CO, USA) is added to the water sample. If TROs are present, the sample will develop a red color which is proportional to the TRO concentration. A calibration curve developed using chlorine standards reacted with the Hach DPD reagent can then be used to determine the concentration of TRO (as chlorine) in the sample.

## DEFINITIONS

**Brackish Water (BW):** Synthetic water created from laboratory water (LW) with the addition of commercially prepared salts, such as Instant Ocean®, to obtain a salinity of 16 parts per thousand (as measured by a refractometer).

**High Organic Content Laboratory Water (HOC-LW):** Synthetic water created from LW amended with organics and used as a surrogate in place of Duluth-Superior Harbor water.

**Laboratory Water (LW):** City of Superior, Wisconsin municipal water that has been dechlorinated by passage through an activated carbon filter. Sodium sulfite may be added to remove remaining traces of chlorine. Note: Based on data from previous testing, background levels of chlorine from below the limit of detection (i.e.,  $\leq 3 \mu\text{g/L}$ ) to  $10 \mu\text{g/L}$  are expected in dechlorinated LW.

**Prospective Ballast Treatment System (BTS):** A system containing an active substance and/or component that mechanically, physically, chemically, or biologically serves to remove, render harmless, or avoid the uptake or discharge of potentially invasive organisms within ballast water (IMO, 2005).

**Salt Water (SW):** Synthetic water created from LW with the addition of commercially prepared salts, such as Instant Ocean®, to obtain a salinity of 32 parts per thousand (as measured by a refractometer).

## EQUIPMENT LIST

- Spectrophotometer capable of analysis at 515 nm.
- Cuvettes, 1 cm.
- Kimwipes.
- Wash bottle with deionized water.
- Volumetric flasks (10 mL).
- 10-100  $\mu\text{L}$  pipettor with disposable tips.
- 100-1000  $\mu\text{L}$  pipettor with disposable tips.
- Containers for sample collection and analysis.

- Hach DPD Total Chlorine Reagent Powder Pillows (Hach Cat. No. 21056-69).
- Hach Chlorine Standard Solution as Cl<sub>2</sub> (Hach Cat. No. 1426810).

## PROCEDURE

### Spectrophotometer Set-up and Operation

1. Turn the spectrophotometer on and allow to warm up (10 minutes minimum). Use the “Mode” control to set the instrument in the transmittance mode. Adjust the knob on the left (Power/Zero Control) so that the instrument reads 0.0 % transmittance (no cuvette in the sample compartment).
2. Rinse and fill several cuvettes with deionized water and wipe the outside of the cuvettes with a Kimwipe to remove any moisture, fingerprints, dust, etc. Note: Handle cuvettes only by the upper portion of the cuvette. Be sure to wipe cuvettes each time before placing them into the spectrophotometer. The cuvettes need only be filled about two-thirds full.
3. Adjust the top knob (Wavelength Control) to a wavelength of 515 nm.
4. Place a cuvette into the spectrophotometer so that the mark on the cuvette is aligned with the raised line on the top of the cuvette chamber. Close the sample chamber door.
5. Adjust the right knob (Transmittance/Absorbance Control) so that instrument display reads 100.0 % transmittance.
6. Repeat step 4 with additional cuvettes and read the % transmittance (%T). Continue this process until two cuvettes are found that have the same or very similar %T readings (i.e., %T  $\pm$ 0.4). Use two cuvettes with the same or similar %T readings for the remainder of the experiment. Adjust the cuvette with the higher %T reading to read 100 %T. The cuvette with the higher %T reading will be labeled the “blank” cuvette and the other will be the “sample” cuvette. The cuvettes will be labeled this way for the remainder of the experiment.
7. Keep the blank cuvette filled with the deionized water. The sample cuvette will be used for the reagent blank, standards, and samples.
8. Use the “Mode” control to set the instrument in the absorbance mode.

### Sample Collection

1. Collect sample water in beakers or sample bottles from the appropriate location(s), depending on the type of test being conducted.
2. Minimize sample agitation to avoid loss of oxidants due to off-gassing from the sample.
3. Collect a minimum of 10 % of samples in duplicate.

Note: Samples containing more than 300 mg/L alkalinity or 150 mg/L acidity as CaCO<sub>3</sub> may not develop the full amount of color, or it may instantly fade. These interferences can be eliminated by neutralizing the sample to a pH of 6 to 7 with either 1 N sulfuric acid or 1 N sodium hydroxide.

**Standard Preparation and Standard and Sample Analysis**

1. Open the Hach Chlorine Standard Solution ampule (50 – 75 mg/L Cl<sub>2</sub>).
2. Based on the certified chlorine concentration of the Hach Chlorine Standard Solution, calculate the amount of stock solution (V<sub>1</sub>) to add to each of the volumetric flasks to obtain the concentrations indicated in Table 1. All dilutions are made with deionized water. The calculation to use for determining the amount of standard needed for each dilution is:

$$C_1 \times V_1 = C_2 \times V_2$$

where:

- C<sub>1</sub> = chlorine conc. of stock
- V<sub>1</sub> = the volume of stock used
- C<sub>2</sub> = chlorine conc. of standard
- V<sub>2</sub> = the volume of standard

**Table 1. Chlorine Working Standards.**

Standard Concentration (mg/L TRO as Cl <sub>2</sub> )	Final Volume (mL)
0.0	10
0.5	10
1.0	10
2.0	10
3.0	10

3. Starting with the reagent blank and proceeding with one solution at a time, transfer 10 mL of the reagent blank and each standard into a 30 mL beaker. Add the contents of one Hach DPD Total Chlorine Reagent Powder Pillow to each solution. Swirl the beaker to help dissolve the reagent. Allow the color to develop for 3 minutes. The absorbance of the solution should be read within 6 minutes of when the DPD powder pillow was added to the liquid.
4. Rinse the sample cuvette with several aliquots of each solution to be measured, and discard the rinses into a waste beaker. After the rinsing procedure, fill the sample cuvette at least two-thirds full with the solution to be analyzed. Wipe the outside of the cuvette with a Kimwipe to remove any moisture or finger prints. Check to see that the cuvette walls are clean and dry.

5. Adjust the instrument to read 0.000 absorbance (A) while the blank cuvette containing deionized water is in the sample compartment by use of the Transmittance/Absorbance Control.
6. Remove the blank cuvette and place the sample cuvette into the instrument, read and record the A.
7. Repeat steps 4, 5, and 6 for each of the standards.
8. Collect the samples to be analyzed in pre-cleaned beakers or other sample collection containers. Immediately transfer 10 mL of the sample, or a smaller aliquot for high concentration samples (i.e., samples with expected concentrations above 3 mg/L) into a 30 mL beaker to which the contents of a Hach DPD Total Chlorine Reagent Powder Pillow has been added. This is especially true of samples that have substances (i.e. organic compounds and reduced forms of iron and manganese) present with which the oxidants readily react. If an aliquot of sample less than 10 mL is used, dilute the sample volume to 10 mL with deionized water. Allow the sample to react with the reagent for at least 3 minutes. Read the absorbance of the solution within 6 minutes of adding the sample to the reagent. Note: If the water samples are colored or turbid, an aliquot of each water sample that has not been treated with the Hach DPD Total Chlorine Reagent should be used as a sample specific blank. Calculate corrected absorbance values for the colored or turbid water samples by subtracting the absorbance of the sample specific blank from the associated treated sample.
9. Repeat step 8 for each sample being analyzed.
10. After having read the A of the blank, standards and all samples, turn the instrument off by turning the Power/Zero Control counter-clockwise until it clicks.
11. Prepare a calibration curve using the concentrations of the standards as the “x” values and the associated corrected absorbance values as the “y” values. Calculate the corrected absorbance values by subtracting the absorbance of the 0.0 mg/L standard (reagent blank) from each of the other standards and samples. The calibration curve (2<sup>nd</sup> order polynomial) should be prepared in Microsoft Excel.
12. Use the TRO Data Analysis spreadsheet (uses 2<sup>nd</sup> order polynomial fit, see example spreadsheet in Appendix 1) to determine the TRO concentration (as chlorine) of the samples. After the data for the calibration curve has been entered into the spreadsheet, the data will be plotted and the equation for the line will be displayed on the graph. The coefficients (A and B) of the  $x^2$  and  $x$  terms of the polynomial equation need to be entered into the appropriate cells of the spreadsheet. When this has been done the spreadsheet will calculate the experimental values obtained for the standards. Check to see that these agree well with the theoretical concentrations of the standards. If they do not agree well, determine where the problem exists before proceeding. Enter the absorbance values for the samples. The spreadsheet will calculate their concentrations. Note: If it is desired to report the TRO values as bromine, the TRO value as chlorine is multiplied by a factor of 2.25. This may be more appropriate for saltwater samples.

## **QUALITY ASSURANCE/QUALITY CONTROL**

1. Conduct all QAQC procedures according to *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2010)* or *GSI/QAQC/QAPP/BS/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale Tests (2010)*.
2. Analyze data to ensure that all applicable data quality criteria are met.
3. Collect and analyze in duplicate at least 10 % of the samples to document sampling and analytical variability. Whenever possible, spike at least 10 % of the samples with a spiking solution containing chlorine. A chlorine containing reference standard can be used to check the accuracy of the TRO analysis. The result for the reference standard should be reported as TRO as chlorine.
4. Follow all procedures outlined in this SOP. Any deviations known ahead of time must be approved by the GSI Lead Investigator for Bench-Scale Studies and communicated to the GSI Senior QAQC Officer. Any deviations made during the experiment must be recorded and also approved by the GSI Lead Investigator for Bench-Scale Studies as soon as practicable.
5. Record data on data collection forms or in specific laboratory notebooks. All instrument data output) and data forms must be stored in a project-specific three-ring binder. Ensure hard copies of instrument data output and data collection forms are scanned and stored electronically.

## **DATA STORAGE AND ARCHIVING**

1. Store and archive data according to *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2010)* or *GSI/QAQC/QAPP/BS/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale Tests (2010)*.
2. Archive all hard- and electronic-copies of data and records generated for a period of five years.

## **REFERENCES AND RELATED DOCUMENTS**

Chlorine, Total, DPD Method. Hach Water Analysis Handbook, 3<sup>rd</sup> Ed., 1997, pp. 379-386.

Eaton AD, Clesceri LS, Rice EW & Greenberg AE (2005). DPD Colorimetric Method. Standard Methods for the Examination of Water and Wastewater: Method 4500-C1 G, 4-67 to 4-68.

*GSI/QAQC/QMP/1 – Great Ships Initiative Quality Management Plan (2010)*.

*GSI/QAQC/QAPP/BS/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale Tests (2010).*

*GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2010).*

*GSI/SOP/G/RA/SC/3- Procedure for Labeling Samples collected at the GSI Land-Based RDTE Facility.*

*GSI/SOP/G/RA/SC/4 – Procedure for Labeling GSI Bench-Scale Samples.*

Great Ships Initiative website: [www.greatshipsinitiative.org](http://www.greatshipsinitiative.org); Standard Operating Protocols/Procedures: <http://www.nemw.org/GSI/protocols.htm>.

International Maritime Organization (IMO) (2005). Guidelines for Approval of Ballast Water Management Systems (G8) Adopted by Resolution MEPC.125 (53). London, England.

# **APPENDIX 1**

## **Total Residual Oxidants (TRO) Example Spreadsheet**

**TRO Data Analysis Sheet**

Analyst: \_\_\_\_\_  
 Project: \_\_\_\_\_

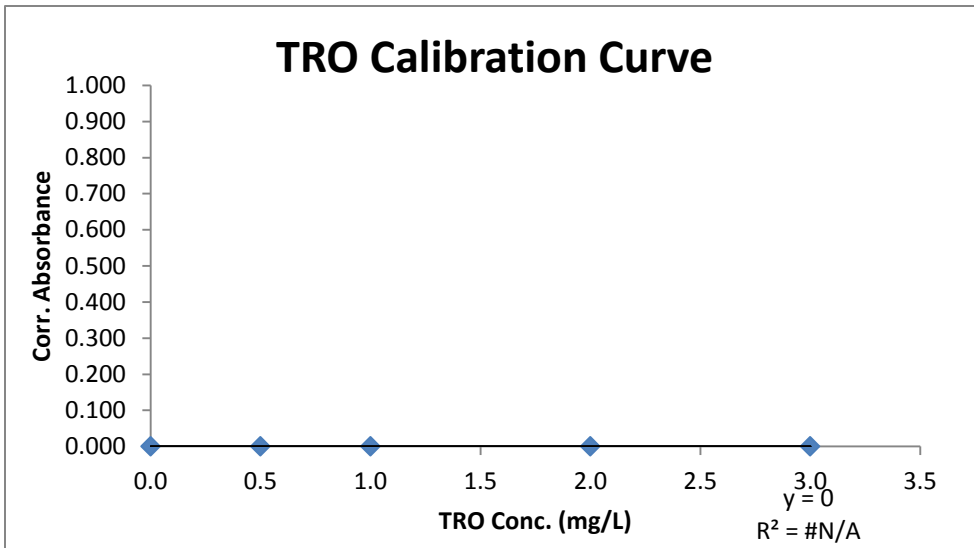
Date: \_\_\_\_\_  
 Spec. 20 #: \_\_\_\_\_

Calc.

Conc (mg/L)	A	Corr. A	Conc (mg/L)
DI H2O			
0.0		0.000	#DIV/0!
0.5		0.000	#DIV/0!
1.0		0.000	#DIV/0!
2.0		0.000	#DIV/0!
3.0		0.000	#DIV/0!

Ax<sup>2</sup> + Bx

A=	
B=	



Sample ID	Corr.		Calc.	Mean	Spk	RPD
	A	A	Conc (mg/L)	± Std. Dev.	Rec(%)	(%)
		0.000	#DIV/0!			
		0.000	#DIV/0!			

