

## STANDARD OPERATING PROCEDURE

### Procedure for Microbial Sample Collection

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#### RECORD OF AMENDMENTS:

No.	Date	Type	No.	Date	Type
1	05/20/2011	Updated § "Background". Added text to § "Introduction". Divided § "Equipment List" into list of equipment and list of supplies. Updated text throughout the § "Procedure" to reflect current sample collection method. Added Appendix 1, 2, and 3.	7		
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## **STANDARD OPERATING PROCEDURE**

### **Procedure for Microbial Sample Collection**

#### **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 treatment dose against diverse freshwater taxa and water quality conditions;
- Generation of freshwater relevant chemical degradation curves; and
- Estimation of residual toxicity given diverse freshwater taxa and water quality conditions.

##### *GSI Land-Based Tests*

- Pre-certification testing, i.e., operational and biological performance (including residual toxicity) status-testing given scale-up and a range of challenge conditions; and
- Certification/verification testing, i.e., formal assessment of performance against international and other discharge standards.

##### *GSI Shipboard Tests*

- Confirmation of biological and operational treatment performance as expected in the ship environment;
- U.S. Coast Guard Shipboard Technology Evaluation Program (STEP) testing;
- Shipboard type approval testing;
- Ship discharge monitoring; and
- Methods development.

GSI awards its independent status-testing services to candidate systems only if technical and programmatic criteria are met. Decisions are based on third party technical assessments as well as GSI Advisory Committee programmatic input. Testing services are currently offered at no cost to the developer with the exception of transportation and system installation/removal costs. Instead, tests are supported by general project funds which derive from federal and state agency grants, Great Lakes port contributions, and in-kind contributions by local governments and universities.

GSI has no involvement, intellectual or financial, in the mechanics, design or market success of the actual treatment systems it tests. To ensure GSI remains completely independent and is uncompromised by any real or perceived individual or project bias, GSI subjects itself to rigorous quality management policies and procedures. In addition, GSI test activities are subject to rigorous QAQC procedures and documentation. This attention to quality management and QAQC assures the high quality and credible evaluation of both GSI and its findings.

## INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes collection of microbial samples at the GSI Land-Based RDTE Facility located on the Duluth-Superior Harbor of Lake Superior (Superior, WI). One sample is collected for multiple analyses. The types of analyses conducted are dependent upon the ballast water treatment system being evaluated, and are specified in the Test/Quality Assurance Project Plan (TQAP) or other planning documentation. Typically, samples are analyzed for the following bacteria:

- Total heterotrophic bacteria according to *GSI/SOP/BS/RA/MA/1 – Procedure for Conducting Heterotrophic Plate Counts (HPCs) Using IDEXX’s SimPlate® for HPC Method* and/or *GSI/SOP/BS/RA/MA/7 – Procedure for Enumerating Culturable Heterotrophic Bacteria Using the Spread Plate Method*.
- *Enterococcus spp.* according to *GSI/SOP/BS/RA/MA/3 – Procedure for the Detection and Enumeration of Enterococcus Using Enterolert®*.
- Total coliform bacteria/*Escherichia coli* according to *GSI/SOP/BS/RA/MA/4 – Procedure for the Detection and Enumeration of Total Coliforms and E. coli Using IDEXX’s Colilert®*.

Additional microbial analyses involving bacteriophage MS2 (*GSI/SOP/BS/RA/MA/5 – Procedure for the Detection and Enumeration of Male-Specific (F+) Coliphage Using Double Agar Layer Technique*) and *Vibrio cholerae* (*GSI/SOP/BS/RA/MA/6 – Procedure for Colony Blot Preparation for the Enumeration of Culturable Vibrio cholerae and Presence of ctxA Gene*) may also be included, depending upon the objectives of the ballast water treatment system evaluation.

## EQUIPMENT

- Autoclave
- Six 3.8 m<sup>3</sup> sample collection tubs with attached yardsticks, plankton net hooks and flexible drainspout hose with quick-connect fittings.
- One 3.0 m<sup>3</sup> stripping tank with attached yardstick, plankton net hook and removable flexible drainspout hose with quick-connect fittings.
- One removable drainspout nozzle with quick-connect fitting.

## SUPPLIES

- 1 L sterile polypropylene sample bottles
- Autoclave tape
- Chain of custody form(s); only required for samples that are analyzed in an outside laboratory (see Appendix 1)
- Cooler with ice packs (for storage and transport) Laboratory notebook and/or pre-printed datasheets
- Neutralizing agent (e.g., sodium thiosulfate; required only if the treated water contains residual chlorine or other halogens)
- Preprinted sample bottle labels Protective eyeglasses/goggles and gloves (required only if a chemical treatment is to be tested)

- Sample collection datasheets (see Appendix 2 for intake; see Appendix 3 for discharge).

## PROCEDURE

### Sample Collection Preparation

1. Add a neutralizing agent (i.e., sodium thiosulfate) to the sample bottles if the treated water contains residual chlorine or other halogens.
2. Autoclave one, 1 L sample bottle per sample being collected using the following method:
  - a. Place a piece of autoclave tape on each sample bottle.
  - b. Autoclave sample bottles at 121 °C for 15 minutes, according to the procedure outlined in the Yamato Sterilizer Instruction Manual (Yamato Scientific America, Inc. 2008).
  - c. Record the date, contents autoclaved, times and temperatures, and autoclave maintenance and performance in the Autoclave Log Book located near the autoclave.
3. Label the sample bottles with the relevant sample identification nomenclature (See *GSI/SOP/G/RA/SC/2 – Procedure for Labeling Samples Collected at the GSI Land-Based RDTE Facility*) and store adjacent to each sampling location (collection tub) prior to sampling. Leave cover on bottle until immediately before sampling to avoid contamination.
4. Prepare all data recording sheets (see Appendix 2 and 3) and attach to clipboards.
5. Prepare to collect microbial samples immediately after algae/small protozoa sample collection but before zooplankton sample collection (see *GSI/SOP/LB/RA/SC/1 – Procedure for Collecting Biological Samples via In-Line Sample Ports*).

### Sample Collection

1. Prepare sterile, polypropylene sample bottle.
2. Keep the sample bottle closed until it is to be filled. Immediately prior to filling, remove the cap. Ensure that the inner surface of the cap and neck of the bottle do not come in contact with any non-sterile surfaces.
3. Do not rinse the sterile microbial sample bottles. For each sample collection procedure specified in the Test/Quality Assurance Plan (TQAP) or other planning documentation, collect approximately 1 L of sample water (i.e., 900 mL to 975 mL), leaving at least 2.5 cm of headspace to facilitate mixing by shaking prior to analysis (Eaton et al., 2005).
4. Cap the sample bottle immediately.

5. Record the time of sample collection from each tub in a field notebook or on a pre-printed datasheet using indelible ink (use Appendix 2 for intake samples and Appendix 3 for discharge samples).

### **Sample Preservation and Storage**

1. Begin sample analysis as soon as possible after collection. Record the time of sample receipt in a laboratory notebook or on a pre-printed datasheet (see various analysis SOPs).
2. Ensure that the samples are held at the GSI Land-Based RDTE Facility in insulated coolers with ice packs (2 °C to 8 °C), and transported to the LSRI microbial laboratory within 1-2 hours of sample collection.

Refrigerate samples upon receipt at the laboratory and process within six hours. Record the start time for sample processing and analysis in a laboratory notebook or on a pre-printed datasheet (see various analysis SOPs).

### **QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)**

1. Conduct all quality assurance/quality control procedures according to the *GSI/QAQC/QAPP/LB/1 – Great Ships Initiative (GSI) Quality Assurance Project Plan (QAPP) for Land-Based Tests* (2011).
2. Follow all procedures outlined in this SOP. Any SOP amendments must be approved by the GSI Principal Investigator or one of the two Lead On-Site Investigators. Any deviations made during the experiment must be recorded and also approved by the GSI Principal Investigator or one of the two Lead On-Site Investigators as soon as practicable.
3. Provide copies of the sample collection records to the GSI QAQC staff at the end of the sampling day by scanning the sample collection datasheets and posting them to the internal GSI SharePoint Website.

### **DATA STORAGE AND ARCHIVING**

1. Store and archive data according to *GSI/QAQC/QAPP/LB/1 – Great Ships Initiative (GSI) Quality Assurance Project Plan (QAPP) for Land-Based Biological Tests* (2011).
2. Archive all hard- and electronic-copies of data and records generated for a period of at least seven years. Ensure that electronic-copies of data are retrievable for the length of the storage period.

## REFERENCES AND RELATED DOCUMENTS

Eaton AD, Clesceri LS, Rice EW & Greenberg AE, Eds. (2005). Method 9060: Microbiological Examination Sample Collection. Standard Methods for the Examination of Water & Wastewater, 21<sup>st</sup> edition, 9-20 to 9-22.

Great Ships Initiative website: [www.greatshipsinitiative.org](http://www.greatshipsinitiative.org).

Great Ships Initiative Standard Operating Protocols: <http://www.nemw.org/GSI/protocols.htm>.

GSI/QAQC/QMP/1 – Great Ships Initiative Quality Management Plan (2011).

GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2011).

GSI/SOP/G/RA/SC/2 – Procedure for Labeling Samples Collected at the GSI Land-Based RDTE Facility.

GSI/SOP/LB/RA/SC/1 – Procedure for Collecting Biological Samples via In-Line Sample Ports.

GSI/SOP/LB/RA/SC/3 - Procedure for Algae/Small Protozoa Sample Collection.

GSI/SOP/BS/RA/MA/1 - Procedure for Quantifying Heterotrophic Plate Counts Using IDEXX's SimPlate for HPC Method.

GSI/SOP/BS/RA/MA/3 – Procedure for the Detection and Enumeration of Enterococcus Using Enterolert™.

GSI/SOP/BS/RA/MA/4 – Procedure for the Detection and Enumeration of Total Coliforms and *E. coli* using Colilert®.

Yamato Scientific America, Inc. 2008. Instruction Manual for Sterilizer Model SN200/210/300/310/500/510, SQ500/510, Third Edition.

# **APPENDIX 1**

## **Great Ships Initiative (GSI) Chain of Custody (COC) Form**



## **APPENDIX 2**

**GSI/FORM/LB/C/1: Sample Collection Form for Intake Samples Collected  
from the Sample Collection Tubs**

### Ballast Treatment System Test Sample Collection Form (GSI/FORM/LB/C/1)

Sample Collection Tub Intake (F) Samples

System Name: \_\_\_\_\_

Date				Test #			
Duration	Days			Hours			
Collection Team							

Sample Collection Scenario - Fill Control and Treatment Tanks at same time

Note which tanks are being filled C1 T1 C2 T2

Collect water in up to 6 tubs Harbor ZP Pre-Trial

Sample Point and Port	SP3A	SP3B	SP3C	SP2A	SP2B	SP2C
Tub #	4	5	6	1	2	3

Time start line flush		Time valves operated manually		Time end fill	12456	3
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Time Tubs start fill						
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Total amount of water in tank (US Gal)	Treatment		Control	
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Height of water placed in Sampling Tub	SP3A	SP3B	SP3C	SP2A	SP2B	SP2C
	4 (PT)	5 (PT)	6 (T)	1	2	3

Total amount of water placed in Sampling Tub (US Gal)						
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Collect phytoplankton, microbial, chemistry and zooplankton samples as directed by workplan; record sonde data on Sonde Datasheet

Time phytoplankton (PP) collected						
Phyto discard/sample volume (l)						

Time microbes (MA) collected						
Microbial sample volume (l)						

Time zooplankton (ZP) collected						
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Weather:	Time:	Sky:
	Air Temp:	Wind: @ kts.
	Water Temp:	Water Level: in. Above LWD

Notes:

# **APPENDIX 3**

**GSI/FORM/LB/C/2: Sample Collection Form for Discharge Samples  
Collected from the Sample Collection Tubs**

