

STANDARD OPERATING PROCEDURE Procedure for Microbial Sample Collection

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STANDARD OPERATING PROCEDURE

Procedure for Microbial Sample Collection

BACKGROUND

The [Great Ships Initiative](#) (GSI) is a collaborative effort to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System through independent research and demonstration of environmental technology, financial incentives and consistent basin-wide harbor monitoring. To that end, GSI has established research capabilities at three scales—bench, land-based, and shipboard. Each scale is dedicated to addressing specific evaluation objectives, with protocols as consistent with IMO and federal requirements as practicable. Developers of ballast treatment systems apply for GSI research services [online](#), and awards are offered based on an objective review process. GSI incubation/testing will allow meritorious ballast treatment systems to progress as rapidly as possible to an approval-ready and market-ready condition.

The GSI's Land-Based Research, Development and Technology Evaluation (RDTE) Facility in Superior, Wisconsin is used to conduct full-scale biological evaluations of prospective ballast treatments suitable to Seaway-sized vessels. The facility draws raw intake water and entrained organisms from Duluth-Superior Harbor at up to 680 m³/hr. After initial transport through 16 inch HDPE line to the facility, a carefully designed “Y-split” in the intake piping simultaneously channels one half of the flow (up to 340 m³/hr) to a treatment track and one half (up to 340 m³/hr) to a matched control track (figure 1). Water in the treatment track passes through the experimental ballast treatment system and into one of the 200 m³ cylindrical treatment retention tanks (test tank #1 or #2; figure 1). Water in the control track by-passes the treatment system and is channeled directly into a matched control retention tank (control tank #1 or #2; figure 1). After storage (duration dependent on test requirements), the water is discharged sequentially from the treatment and control retention tanks at up to 340 m³/hr. Depending on the test scenario, the water is either discharged to the harbor or sewer system, into an alternate retention tank, or through the treatment system again for discharge or retention.

Treatment and control intake and discharge water is sampled at pressure/flow controlled in-line sample points (SPs). Intake samples are collected concurrently on the control and treatment tracks respectively (using SP2 and SP3, figure 2). Discharge samples are collected from one of two discharge sample points (SP9 or SP10; figure 2), with sequential sampling of control and treatment water. At each of these SPs there are three replicate sample ports with a center-located 3.8 cm internal diameter (ID) elbow-shaped pitot tube (figure 3) connected to a 3.8 cm ID PVC transfer pipe that carries the sample water to one of six collection tubs located at a centralized sampling station (figure 2). Other SPs shown on figure 5, with one port per SP, are used for calibration testing the facility itself and not typically used for sample collection during a treatment system evaluation.

A mobile field laboratory provides bench-scale facilities to support time-sensitive assays associated with tests conducted at the GSI Land-Based RDTE Facility. The laboratory is located

at the facility during testing but may be moved to other sites in the Great Lakes-St. Lawrence Seaway System to support GSI shipboard tests when required. It is climate-controlled, and has enough desk and counter space to allow for simultaneous microscopic and analytical analysis of zooplankton, phytoplankton and bacteria samples. In addition, laboratories of the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI) and the University of Minnesota-Duluth's Natural Resources Research Institute provide non-time sensitive analysis of samples from the land-based tests. Since both facilities are only a few miles from the facility, samples can be easily transported for rapid analysis.

Figure 1. Simplified Schematic of the GSI Land-Based RDTE Facility.

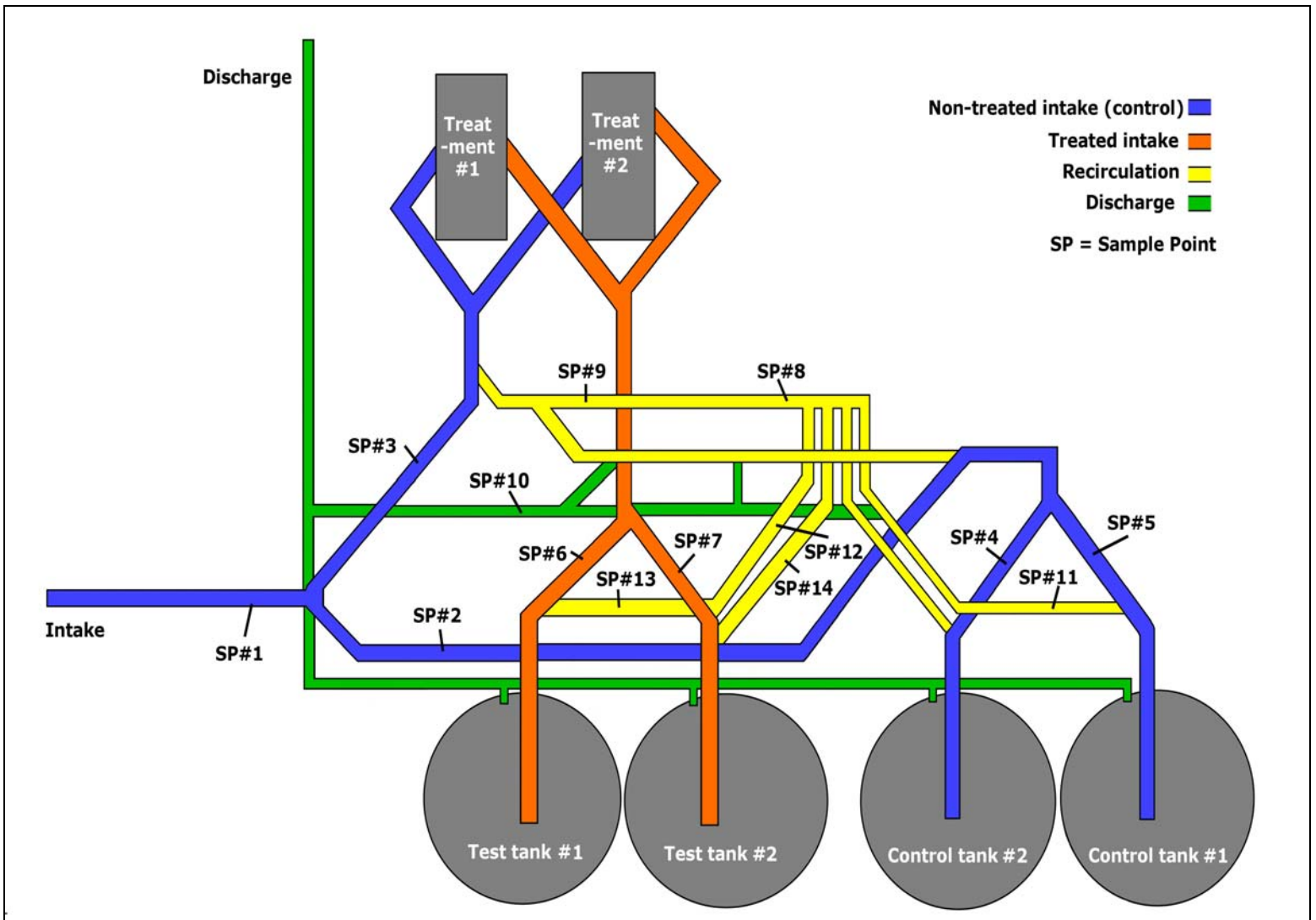


Figure 2. Schematic of the GSI Land-Based RDTE Facility Showing the Location of the Intake and Discharge Sample Points (SPs), Sample Ports, and Corresponding Sample Collection Tubs.

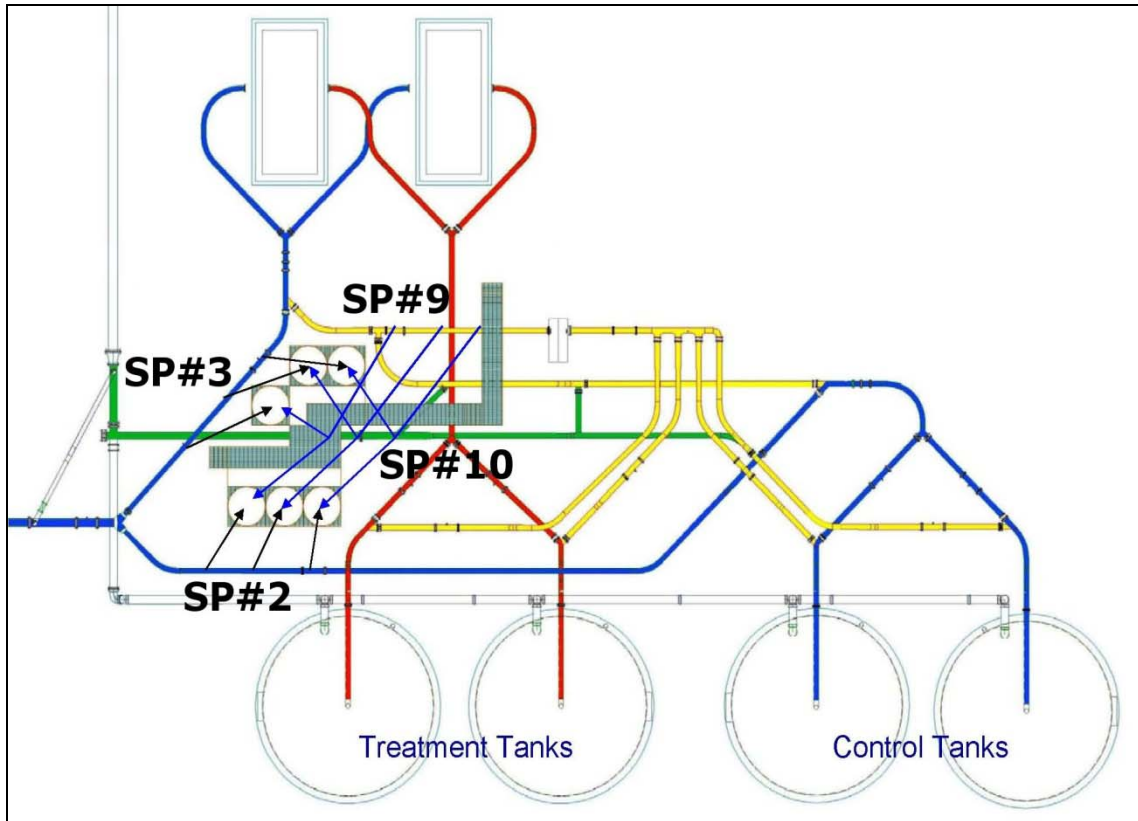
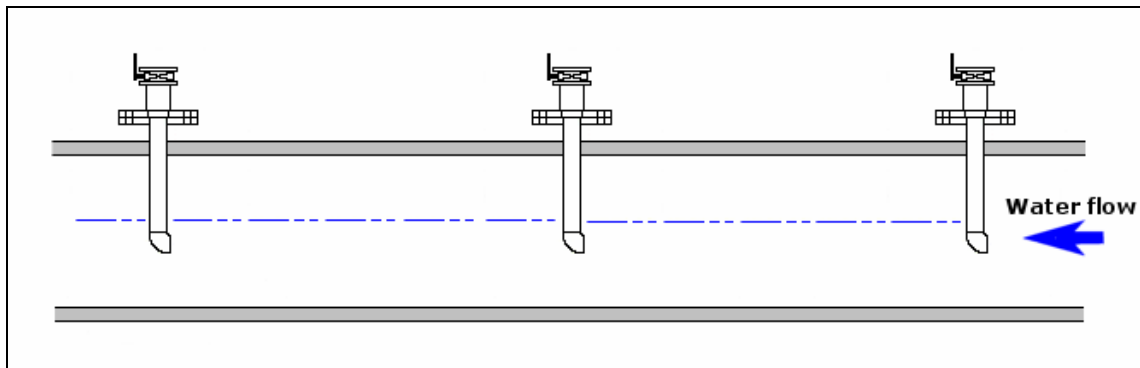


Figure 3. Schematic of a Sample Point (SP) Showing the Design of the Three Sample Port Pitots.



INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes collection of microbial samples at the GSI Land-Based RDTE Facility. Each sample collected will be enumerated for heterotrophic bacteria, enterococci, total coliforms, and *E.coli*. Additional microbial tests involving MS2 and *vibrio cholera* may also be included.

EQUIPMENT LIST

- Six 3.8m³ sample collection tubs with attached yardsticks, plankton net hooks and flexible drainspout hose with quick-connect fittings.
- One 3.0m³ 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.
- One 1-Liter sterile sample bottle per sample to be collected.
- Two 5-Liter discard buckets; one for control water, one for treated water (washed and re-used).
- Protective eyeglasses/goggles and gloves (if a chemical treatment is to be tested).
- Colored tape (various colors).
- Preprinted sample bottle labels.
- One clipboard, data sheet and pen for each sampling event.
- One field notebook.
- Cooler with ice packs (for storage and transport).
- Chain of custody form.

PROCEDURE

1. Autoclave one 1-Liter sample bottle per sample being collected. Check that container has autoclave tape to verify it has been sterilized.
2. 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*) from the first pair of treatment/control tubs to be drained for zooplankton sampling and the stripping tank.
3. Label the discard buckets and 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 and chain of custody forms, and attach to clipboards.

Sample Collection

1. Do not rinse sterile microbial sample bottles. Otherwise use the same sample collection process as for algae/small protozoa sample collection (see *GSI/SOP/LB/RA/SC/3 - Procedure for Algae/Small Protozoa Sample Collection*). The bacteria samples should have a small amount of headspace in the sample bottles.
2. Collect a 1-liter microbial sample from each relevant sample collection tub (one control, one treatment, and/or the stripping tank).
3. Fill out chain of custody forms to be transported with samples.
4. Transport samples in an insulated container or refrigerator at 2 – 8 °C for analysis at the LSRI laboratories (see *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™*; and *GSI/SOP/BS/RA/MA/4 – Procedure for the Detection and Enumeration of Total Coliforms and E. coli using Colilert®*).

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

1. Conduct all quality assurance/quality control procedures according to the GSI/QAPP/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Bench-Scale and Land-Based Biological Tests (2009).
2. Follow all procedures outlined in this SOP. Any deviations known ahead of time 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. Collect one field duplicate per treatment system being tested (or a minimum of 10 % of total samples collected).
4. Provide copies of the daily log to Quality Assurance Manager at the end of the sampling day along with the sample collection records.

DATA STORAGE AND ARCHIVING

1. Store and archive data according to GSI/QAPP/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Bench-Scale and Land-Based Biological Tests (2009).
2. Archive all hard- and electronic-copies of data and records generated for a period of five years.

REFERENCES AND RELATED DOCUMENTS

Cangelosi AA (2006). RDTE Facility for the Great Ships Initiative (GSI) (OAR-SG-2006-20000364). Project Proposal to the National Oceanic and Atmospheric Administration/U.S. Fish and Wildlife Service. Northeast-Midwest Institute, Washington D.C.

Eaton AD, Clesceri LS, Rice EW & Greenberg AE, Eds. (2005). Standard Methods for the Examination of Water & Wastewater.

Great Ships Initiative website: www.greatshipsinitiative.org.

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

GSI/QAPP/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale and Land-Based Biological Tests (2009).

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®.