Great Ships Initiative

STANDARD OPERATING PROCEDURE:
General Microbiology Preparation Procedures

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RECORD OF AMENDMENTS:

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STANDARD OPERATING PROCEDURE
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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 general microbiology procedures used in GSI bench-scale and land-based tests. Procedures include the preparation of media, stock cultures, and a dilution series, etc.

STERILE/ASEPTIC TECHNIQUE

Aseptic technique is used to prevent microbial contamination of the self, environment, or sample. Because contaminating bacteria and other microbes are found everywhere, such as on fingertips, on the dust particles in the air, and bench tops, etc., it is important to minimize contact with these contaminating surfaces. Inoculation loops, the tips of the pipettors, and other equipment in use should not be touched or placed onto contaminating surfaces. While working in a chemical hood, a movable glass panel or sash should be used to cover the face area and act as a physical barrier. This and/or the air flow present in a laminar flow hood or under the "umbrella" of warm air around a Bunsen burner flame helps maintain a particulate free environment.

- Prior to preparing media, making transfers, dilutions, etc. spray laboratory bench and hood surfaces with 70 % ethanol or a 1 % bleach solution and wipe dry.

When working in a hood:

- Let the air flow for 2-3 minutes to filter and establish air flow patterns.
- Avoid rapid movements that will disrupt laminar air flow.
- Spray all equipment to be taken into the cabinet such as media bottles, pipette tip boxes, and gloves with 70 % ethanol.

When working around a Bunsen burner flame:

Note: The purpose of using a flame is not necessarily to sterilize but to create warm air convection currents up and away from the container or equipment you are working with.

- Work in close proximity to the flame and be cautious of yourself and surroundings.
- Pull back and secure long hair.
- ALWAYS flame the lip of container whenever liquid is to be poured from it (e.g., pouring plates).
- For liquid transfers using a micropipette, begin by loosening the cap of the liquid's container and the receiving container. When using sterile pipette tips, insert the micro-pipette into the tip, taking good care that the tip does not touch any surface. Aspirate the appropriate amount and loosely set the cap back on the liquid container. Again, ensure that the tip does not touch any other surface, open the receiving container and release the liquid. Discard the tip.
- When working with cultures in test tubes, work rapidly yet with careful technique. Keep the tubes open a minimum amount of time, hold them at a 45 degree angle so that dust cannot fall into the open tube, and hold the tubes away from the face while transferring.

Hold the tube in your less dominant hand and the transferring instrument in the dominant hand. Remove and grasp cap with the little finger of your dominant hand, flame the tube, carefully make the transfer and replace cap.

- Petri dish lids prevent dust from falling directly onto plates but allow diffusion of air around the edges. There are no direct air currents into the plate, and to enter, dust particles would have to rise vertically more than a centimeter. This does not often occur because of the density of the particles. Whenever the lid is removed, it should be held over the plate as a shield. Do not place the lid on the bench top. Do not leave plates uncovered. Do not walk around the room with an open plate.

Always clean all work areas and wash your hands thoroughly before leaving the laboratory!

Note: These are guidelines. You may find a set of techniques that best suit your working style. This is fine as long as you adhere to the basic concepts of good sterile technique.

LABELING

1. Label media bottles/plates with the following data:
   a. Name of media, including concentration if applicable.
   b. Date Prepared
   c. Initials of preparer
   d. Recommended holding time.
   e. Any special storage conditions or other pertinent information.

LIQUID MEDIA PREPARATION

Equipment

- Balance
- Spatula
- Weighing dishes
- Pyrex 1-4L beaker for heating media
- Hot plate
- pH meter and reagents to adjust pH
- Autoclavable media bottle 30-50 % larger than volume of media preparing
- Autoclave tape
- Autoclave
- 1 % bleach solution or 70 % ethanol for disinfecting bench tops
- Bunsen burner
- Gloves
- Goggles
- Lab coat
- Labeling tape and permanent marker

Procedure

1. Tare balance and weigh solid media into a clean weighing dish.

2. Measure desired volume of reagent grade water with a graduated cylinder and pour into a beaker with a magnetic stir bar in the bottom.

3. Place the beaker on a stirring hot plate and add the solid ingredients.

4. Stir until the media is dissolved. If recommended in media instructions, heat gently while stirring.

5. If needed, adjust the pH as per the specific media instructions.

6. Pour the media into a labeled, autoclave-safe bottle. Fill no more than 2/3 full to prevent spillover.

7. Apply autoclave tape to bottle, loosen cap completely, and autoclave for 15 minutes at 121 °C or as recommended in media instructions.

8. If adding antibiotics, allow media to cool to 48 °C and then add the appropriate volume of antibiotics.

9. Once media has cooled completely, tighten cap and store in refrigerator or as specified in media instructions.

Note: Sterile technique should be used for all of the following procedures described in this document!

PLATE PREPARATION

Equipment

- Balance
- Spatula
- Weighing dishes
- Pyrex 1-4L beaker for heating media
- Hot plate
- pH meter and reagents to adjust pH
- Autoclavable media bottle 30-50 % larger than volume of media preparing
- Autoclave tape
- Autoclave
- 1 % bleach solution or 70 % ethanol for disinfecting bench tops
- Bunsen burner
- Sterile Disposable Petri Dishes
- Water bath capable of maintaining 45 ºC – 48 ºC
- Gloves
- Goggles
- Lab coat
- Labeling tape and permanent marker
- Reagent-Grade Water

Procedure

1. Tare balance and weigh solid media into a clean weighing dish.

2. Measure reagent grade water with a graduated cylinder and pour into a beaker with a magnetic stir bar in the bottom.

3. Place the beaker on a stirring hot plate and add the solid ingredients.

4. Heat gently and stir, until the media is dissolved.

5. If needed, adjust the pH as per the instructions in the recipe.

6. Pour the media into an autoclave-safe bottle, no more than 2/3 full to prevent spillover.

7. Loosen the cap, apply autoclave tape, and autoclave for 15 minutes at 121°C or as recommended on media bottle.

8. Disinfect the work space and light the flames for sterile conditions.

9. Label empty plates with media type, date and initials and place them on the counter with the bottom side down.

10. Allow media to cool to approximately 45 °C - 50 °C. A water bath set at 45 °C - 48 °C will prevent further cooling and setting of agar.

10. If adding antibiotics, add the appropriate volume at this time.

11. Open the bottle of agar, flame the lip of the bottle, and lift the lid of the Petri dish enough to pour approximately18 - 20 mL into the bottom of each plate. Hold the lid directly over the dish while pouring and work quickly to prevent contamination.

12. Once the media has been poured, but before it sets, pass the flame over the surface of the agar to pop any bubbles.

13. Leave plates on the counter to dry overnight, or dry them in a laminar flow hood with lids cracked open for 10-30 minutes if needed immediately.

14. Store plates inverted at 4° C to 8°C in a sealed plastic container labeled with media type, date prepared, initials, and recommended holding time.

PREPARING BACTERIAL STOCK CULTURES

Equipment

- 1 % bleach solution or 70 % ethanol for disinfecting bench tops
- Bunsen burner
- Pure log phase culture of desired bacteria
- 50 % sterile glycerol
- Freezer vials
- Gloves
- Goggles
- Lab coat
- Labeling tape and permanent marker or labels

Procedure

1. Obtain a pure host culture from American Type Culture Collection (ATCC; Manassas, VA; http://www.atcc.org/) or use a previously frozen culture.

2. Prepare a log phase culture of the host.

3. Mix 400 µL sterile 50 % sterile glycerol with 400 µL log-phase culture (25 % glycerol final concentration) in a 1.5 mL labeled freezer vial. Store at ≤-80 ^{o} C.

4. Bacterial stock cultures can be retained for up to one year when stored at ≤-80° C.

PREPARIING STREAK PLATES - used to isolate individual organisms in a culture.

Procedure

1. Disinfect the work area and light the Bunsen burner to create a sterile work space.

2. Using a sterile loop or stick, dip the tip into the liquid culture.

3. Gently touch loop to agar in zig-zagging motion across one third of a sterile streak plate (Figure A)

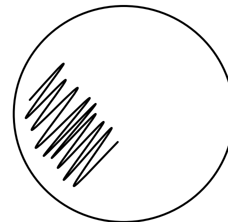

Figure A

4. Flame the loop, or using another sterile disposable loop or stick, drag sterile loop across the first set of lines, being careful not to puncture the agar, and streak a second set of lines across another third of the plate (Figure B).

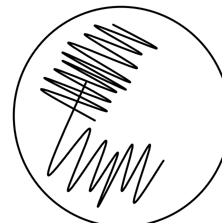

Figure B

5. Repeat step four, pulling the sterile loop across the second set of lines, and streak a third line across the remaining third of the plate (Figure C).

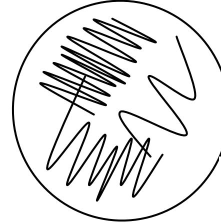

Figure C

6. Invert the plates and incubate 16 – 24 hours at 36 ^{o} C (±1° C).

7. Individual colonies should appear in third section of plate.

PREPARING AN OVERNIGHT CULTURE

Equipment

- 1 % bleach solution or 70 % ethanol for disinfecting bench tops
- Bunsen burner
- Sterile graduated cylinder
- Water bath capable of maintaining 37 °C ± 1.0 °C and capable of shaking 100-150 rpm
- Stock culture of bacteria
- Sterile loops or sticks for inoculations
- 125 mL sterile flask with foil cover, slip cap or plug
- Gloves
- Goggles
- Lab coat
- Labeling tape and permanent marker

Procedure

1. Disinfect the counter and light the Bunsen burner to create a sterile work space.

2. Aseptically add 25 mL of desired broth to a labeled sterile flask. (Volume may be adjusted.)

3. With a sterile loop, dip the loop into a liquid bacterial culture or pull a single colony from a streak plate.

4. Dip the loop into the sterile broth, inoculating it.

5. Label flask as described above.

6. Place the flask into a shaking (100-150 rpm) water bath at 37 °C ± 1°C for 16 - 24 hours.

PREPARING A LOG PHASE CULTURE

Equipment

- 1 % bleach solution or 70 % ethanol for disinfecting bench tops
- Bunsen burner
- Sterile graduated cylinder
- Water bath capable of maintaining 37 °C ± 1.0 °C and capable of shaking 100-150 rpm.
- Incubator at 36 °C ± 1.0 °C
- Stock culture, or streak plate of desired bacteria
- Sterile loops or sticks for inoculations.
- 125mL sterile flasks with foil caps, slip caps or plugs.
- Gloves
- Goggles
- Lab coat
- Labeling tape and permanent marker

Procedure

1. Disinfect the counter and light the Bunsen burner to create a sterile work space.

2. Aseptically add 25mL of recommended broth to a sterile flask. (Volume may be adjusted if necessary.)

3. Dip a sterile loop into a liquid bacterial culture or pull a single colony from a streak plate (See Preparing Streak Plates above). **Note: a log phase culture may be prepared directly from a frozen stock culture but it will take longer to reach the log phase than an overnight (16-24 hour old) culture.**

4. Dip the loop into the sterile broth, inoculating it.

5. Place the flask into a shaking (100-150 rpm) water bath at 37 °C ± 1°C for 2-4 hours.
6. After the culture has grown for about 2 hours, measure its absorbance. See Measuring Absorbance below. An absorbance of 0.1 – 0.5 at 520nm indicates it has entered log phase.
7. If the sample is has not reached log phase yet, return to water bath and check the absorbance again after 30 minutes.
8. When the culture has reached log phase, use immediately or put culture on ice, or in refrigerator until ready to use (up to 48 hours, but best if used within 6 hours).

MEASURING ABSORBANCE/ OPTICAL DENSITY (OD)

Equipment

- 1% bleach solution or 70% ethanol for disinfecting bench tops
- Bunsen burner

- Blank (sterile media same media of culture)
- Spectrophotometer capable of holding 13 x 100mm cuvettes or equivalent.
- Cuvettes-1cm light path
- KimWipes or lint-free tissues
- Gloves.
- Goggles.
- Lab coat.
- Labeling tape and permanent marker
- Pure bacteria culture. `

Procedure

1. Turn on the spectrophotometer to allow it to warm up for a minimum of 15 min.

2. Set wavelength to 520 nm (or desired wavelength) using dial on top right of spectrophotometer.

3. Zero the spectrophotometer by placing approximately 4 mL broth (or other Blank media which matches that of the sample) in a cuvette.

4. Wipe cuvette gently with a Kimwipe and place in the spectrophotometer.

5. Set absorbance to zero using the dial on front right. **Note that the cuvette need not be sterile. The same cuvette should be used for the blank and the sample to avoid differences which may occur among the cuvettes.**

6. Measure the absorbance of sample.
   a. Transfer ~4 mL of sample to the cuvette. Use sterile technique to avoid contamination of the sample/culture being measured.
   b. Wipe cuvette with a Kimwipe and place in the spectrophotometer.
   c. Record the absorbance.

7. Rinse cuvette with DI water and place in rack to dry.

8. Turn off the spectrophotometer and replace the protective cover.

Pure bacterial suspensions with **cell counts greater than 1.0E+07** can be estimated using the turbidimetric method. Absorbance increases in a linear fashion as the cell number increases. Once a curve, (absorbance vs. cell counts) has been created for a specific media, organism, wavelength, and spectrophotometer, the cell counts for a suspension can be estimated, by measuring absorbance, as long as those particular parameters remain the same. More can be found on this procedure at http://science.kennesaw.edu/~jhendrix/btec4200/calibrated.pdf or http://www.microbiol.org/white.papers/WP.OD.htm.

PREPARING A DILUTION SERIES

Note: These instructions are for making a series of simple 10^{-1} dilutions in 13mm sterile test tubes with sterile caps. A total volume of 5mL is achieved by using 0.5mL of sample (agent of interest) to be diluted and 4.5mL of the desired diluent. The diluent is the inactive ingredient added to a sample to lessen the concentration of the agent of interest. The diluent may be the broth that a culture was grown in, buffered or non-buffered water, or other recommended solution. The volumes in this section may be adjusted **proportionately** depending on the concentration and volume of sample available and final volumes required etc.

Equipment

- 1% bleach solution or 70% ethanol for disinfecting bench tops
- Bunsen burner
- Diluent of choice
- Sample to be diluted
- Sterile dilution tubes with caps (16 x 100mm) and test tube rack to accommodate.
- Pipettors and associated sterile pipette tips capable of delivering between 0.1 mL and 10 mL volumes
- Vortex mixer
- Gloves
- Goggles
- Lab coat
- Labeling tape and permanent marker

Procedure

1. Using sterile technique, transfer 4.5mL of desired diluent to four (or more, if greater dilution is desired) sterile 13mm tubes with caps.

2. Label the tubes 10^{-1}, 10^{-2}, 10^{-3},10^{-4}.

3. Label an additional tube (without diluent) “undiluted” and a sample ID if applicable. Transfer 5 mL of sample/stock to this tube.

4. Add 0.5 ml of undiluted stock (or original sample/culture) to the tube labeled “10^{-1}”. Cap and vortex for 5 seconds to mix well.

5. Transfer 0.5 ml from the tube labeled “10^{-1}” to the tube labeled “10^{-2}”. Mix well.

6. Transfer 0.5 ml from the tube labeled “10^{-2}” to the tube labeled “10^{-3}”. Mix well.

7. Transfer 0.5 ml from the tube labeled “10^{-3}” to the tube labeled “10^{-4}”. Mix well.

8. Repeat until the desired dilution is reached.

Final Dilution factors

Tube /Dilution	Final volume of undiluted sample per mL
undiluted sample	1
10^{-1}	0.1
10^{-2}	0.01
10^{-3}	0.001
10^{-4}	0.0001
10^{-5}	0.00001
10^{-6}	0.000001
10^{-7}	0.0000001
10^{-8}	0.00000001

REMINDER: Sterile technique should be used for all of the following procedures

TITERING A BACTERIOPHAGE STOCK CULTURE/SPIKING SUSPENSION USING THE DOUBLE AGAR LAYER (DAL) PROCEDURE.

Equipment

- 1% bleach solution or 70% ethanol for disinfecting bench tops
- Bunsen burner
- Sterile Disposable Petri Dishes with recommend media
- Water baths capable of maintaining 36 °C ± 1.0 °C and 45°C to 48°C and capable of shaking 100-150rpm.
- Incubator at 36 °C ± 1.0 °C
- Stock culture of phage
- Sterile tubes, with appropriate soft agar, with caps (16 x 100mm) and test tube rack to accommodate. For soft agar
- Pipettors and associated sterile pipette tips capable of delivering volumes between 0.1 mL and 10mL volumes.
- Light box or equivalent
- Gloves.
- Goggles.
- Lab coat.
- Permanent marker

Procedure

1. Obtain desired coliphage stock. Stocks may be purchased from ATCC or equivalent company (e.g., MS2 coliphage=ATCC #15597-B1) and propagated, as described in the following section, and stored for future use. Phage stocks should be retitered monthly when testing.

2. Prepare bottom layer plates with 1.5% TSA or recommended medium for bottom agar layer.
   a. Label with dilution of coliphage or blank, bacterial host, coliphage type, and date.
   b. Warm in incubator ($36^{o}C \pm 1°C$) just before procedure.

3. Before opening the phage specimen, prepare a log-phase broth culture of the recommended host. (*E. coli* F_{amp} for MS2).

4. Prepare top (soft) agar tubes (0.7 % TSA with ampicillin/streptomycin for MS2 but other media may be recommended for other hosts). Hold tubes in 44-48 ^{o} C water bath to keep molten.

5. Aseptically rehydrate the freeze-dried phage with 5 ml of tryptic soy broth (TSB) or recommended medium. Stock phage suspension from freezer may also be thawed and used.

6. Using TSB (or recommended broth), prepare a dilution series of the rehydrated phage suspension (or stock culture) as described in Preparing a Dilution Series section above. MS2 coliphage stock may require a 10^{-6} dilution or more.

Double agar layer procedure

Note: For this part of the assay, two double-layer bottom agar plates (duplicates) are required for each of the coliphage dilutions, including the blank and undiluted sample. DO NOT add host and coliphage until ready to plate!

1. With top agar still in water bath, aseptically inoculate each top agar tube with 100 µl of log-phase host culture.

2. Immediately add 500 µL of undiluted coliphage to each of two tubes. Mix the inoculum by rolling each tube briefly in the palm of hand. Try to not make bubbles.

3. Pour contents of each tube into appropriately labeled bottom agar plates.

4. Repeat steps 1- 3 for each dilution.

5. For the blank, add host but do not add coliphage. Instead, add 500 µl of TSB. Mix and pour into bottom agar plate labeled "Blank".

6. Allow overlay to harden, cover, invert plates and incubate for 16-24 hours at 36 ^{o}C ± 1 °C.

7. Store the remaining undiluted coliphage at $2^{o}C$ - $8^{o}C$ for use in preparing coliphage stocks or for experiments.

8. After incubation time, count and record the number of plaques (zones of clearing). Use of a light table is recommended.

9. Using the plates which yield zero to 300 PFU per plate, calculate the concentration of stock/spiking solution.
   a. Undiluted spiking suspension PFU/mL =(PFU_1 +PFU_2....PFU_n)/(V_1+V_2....V)
      i. PFU= number of plaque forming units from plates of all countable sample dilutions.
      ii. V=volume of undiluted sample in all plates with countable plaques.
      iii. n=number of useable counts.

EXAMPLE CALCULATION OF SPIKING SOLUTION CONCENTRATION

Dilution	PFU/plate (for each duplicate plate)	Volume of *undiluted* sample (mL)	*PFU*/mL (from below)
1:10000	TNT, TNTC	0.00005	882
1:100000	45,48	0.000005	88.2
1:1000000	0,4	0.0000005	8.82
1:10000000	0,0	0.00000005	0.882

(45+48+0+4)/(0.000005+0.000005+0.0000005+0.0000005) = 97/0.000011= 8818182 PFU/mL

Undiluted spiking suspension contains = 8818182 PFU/mL.

PROPAGATION OF BACTERIOPHAGE STOCK CULTURE

Equipment

- Bottom agar layer plates with host's recommended media
- Top agar layer tubes with host's recommended media
- 1% bleach solution or 70% ethanol for disinfecting bench tops
- Bunsen burner
- Water bath capable of maintaining 45°C to 48°C
- Sterile loops or sticks for removing soft agar.
- 125mL sterile flasks with slip caps or plugs.
- Pipettors and associated sterile pipette tips capable of delivering volumes between 0.1 mL and 10mL volumes.
- 50 % sterile glycerol
- Freezer vials
- Tryptic Soy Broth (TSB) or other recommended media for eluding phage
- Sterile centrifuge tubes.
- Syringe
- Syringe filters 0.22 µm
- Gloves
- Goggles
- Lab coat
- Labeling tape and permanent marker

Procedure

1. Obtain desired coliphage stock. Stocks may be purchased from ATCC or equivalent company (e.g., MS2 coliphage=ATCC #15597-B1).

2. Before opening the phage specimen, prepare a log-phase broth culture of the recommended host. (*E. coli* F_{amp} for MS2).

3. Prepare two bottom layer plates and two tubes of soft agar.

4. Using undiluted phage prepare two plates as described in the double agar layer procedure.

5. After incubation, scrape the soft agar off the surface of the double agar plates using a sterile "hockey stick" or loop and place in sterile 50mL centrifuge tubes.

6. Add 5 mL of TSB, mix well and allow to bacteriophage to elude from agar in refrigerator for 1-4 hours.

7. Centrifuge tubes for 25 minutes at about 1000 rpm to sediment cellular debris and agar.

8. Remove and pass the supernatant through a 0.22 μm filter to remove the bacteria.

9. Transfer 1-5 mL aliquots to sterile freezer vials.

10. Store filtrate at 2 ^{o}C - 8°C for up to a year.

11. Titer filtrates prior to using.

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

1. Verify the accuracy of the pipettors to be used at least once per every treatment technology being tested.

2. Verify the temperatures of the incubators at least once per day when they are being actively used.

3. 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).*

4. Follow procedures as 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.

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

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

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.

GSI/SOP/LB/G/O/1 - *Procedure for Operating the GSI Land-Based RDTE Facility.*

GSI/SOP/LB/RA/SC/4 – Procedure for Microbial Sample Collection.

Great Ships Initiative website: www.greatshipsinitiative.org; Standard Operating Protocols/Procedures: http://www.nemw.org/GSI/protocols.htm.

U.S. EPA method 1601: Male –specific (F^{+}) and Somatic Coliphage in Water by Two-step Enrichment Procedure, April 2001

http://science.kennesaw.edu/~jhendrix/btec4200/calibrated.pdf

http://www.microbiol.org/white.papers/WP.OD.htm