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3 **DRAFT**  
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5 **US Environmental Protection Agency**  
6 **Environmental Technology Verification Program**  
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8 **Generic Protocol for the Verification of Ballast**  
9 **Water Treatment Technologies**  
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11 **For Stakeholder Review Only**  
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23 In Cooperation with:

24  
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26 Environmental Standards Division (CG-3PSO-4)  
27 2100 2nd Street, S.W.  
28 Washington, DC 20593  
29

30 and

31  
32 U.S. Navy – Naval Research Laboratory  
33 Key West, FL  
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## NOTICE

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## FOREWORD

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

This document represents a substantial effort by many individuals. The original draft was prepared by Battelle, Duxbury, MA, while significant input in later drafts was provided in a multi-year effort by the Naval Research Laboratory, Key West, FL. Much input was also provided by the biological sub-group of the Ballast Water Treatment Protocol Technical Panel, as well as the Technical Panel in whole.

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### Revised Protocol Authors

Edward Lemieux	Naval Research Laboratory
Jonathan Grant	Battenkill Technologies, Inc.
Timothy Wier	EXCET, Inc.
Dr. Lisa Drake	Science Applications International Corporation
Stephanie Robbins	Science Applications International Corporation
Kevin Burns	Science Applications International Corporation

### NRL Technical Staff

Scott Riley	Science Applications International Corporation
Luke Davis	Science Applications International Corporation
Wayne Hyland	Azimuth Technical Consultants, Inc.
Bruce Nelson	Battenkill Technologies, Inc.
Tiffanee Donowick	Science and Engineering Technologies, Inc.
Robert Brown	Azimuth Technical Consultants, Inc.

### Original Protocol Writers

Deborah Tanis	Battelle
Carlton D. Hunt	Battelle

### NSF Staff

Thomas Stevens	Project Manager, ETV Water Quality Protection Center
----------------	--

### EPA Staff

Raymond Frederick	EPA ETV Source Water Protection Pilot Manager
Carolyn Esposito	ORD-NRMRL, Urban Watershed Management Branch

### ETV Water Quality Protection Center – Technical Panel Participants for Current and Past Versions

Donald Anderson	Woods Hole Oceanographic Institute
Allegra Cangelosi	Northeast-Midwest Institute
Dorn Carlson	National Oceanic and Atmospheric Administration
Fred Dobbs	Old Dominion University
Richard Everett	U.S. Coast Guard
Maurya Falkner	California State Lands Commission
Richard Fredricks	Maritime Solutions, Inc.
Stephan Gollasch	GoConsult and Chairman, ICES/IOC/IMO Working Group

Frank Hamons  
Richard Harkins  
Penny Herring  
Russell Herwig  
Brian Howes  
James Hurley  
Thomas Mackey  
Lucie Maranda  
Kathy Metcalf  
Richard Mueller  
Gail Roderick  
Andrew Rogerson  
Terri Sutherland  
Mario Tamburri  
Fred Tsao  
Thomas Waite  
Nick Welschmeyer

on Ballast and Other Ship Vectors (WGBOSV)  
American Association of Port Authorities  
Formerly with the Lake Carriers Association, currently  
with Keystone Shipping Company  
U.S. Coast Guard R&D Center  
University of Washington  
University of Massachusetts  
U.S. Coast Guard R&D Center  
Hyde Marine, Inc.  
University of Rhode Island  
Chamber of Shipping of America  
Northeast Technical Services Company, Inc.  
U.S. Coast Guard R&D Center  
California State University, Fresno  
Fisheries and Oceans Canada  
University of Maryland  
U.S. Navy  
Florida Institute of Technology  
Moss Landing Marine Laboratories

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## 1 GLOSSARY OF TERMS

2  
3 **Accuracy:** The degree of agreement between an observed value and an accepted reference value,  
4 including a combination of random error (precision) and systematic error (bias) components that  
5 are due to sampling and analytical operations (EPA, 1992).

6 **Ambient Populations:** The biological organisms, including bacteria, protists, and zooplankton  
7 that are naturally occurring in the water at the TF location.

8 **Ballast Water Treatment System (or System):** Prefabricated, commercial-ready, treatment  
9 systems designed to remove, kill or inactivate (prior to discharge) organisms in ballast water. The  
10 entirety of a Vendor's ballast water treatment product will be used to achieve the Vendor claims  
11 for treatment efficacy or operational performance, and includes all components, in an integrated  
12 fashion.

13  
14 **Bias:** The systematic or persistent distortion of a measurement process that causes errors in one  
15 direction.

16 **Challenge Water:** Water supplied to a treatment system under test. Challenge water must meet  
17 specified ranges for living organism densities and water quality parameters and is used to assess  
18 the efficacy of the treatment equipment under full-scale operational conditions.

19 **Comparability:** The measure of the confidence with which one data set can be compared to  
20 another.

21 **Completeness:** The amount of data collected as compared to the amount needed to ensure that  
22 the uncertainty or error is within acceptable limits.

23 **Core Parameters:** The measurements that are required as part of the ETV verification.

24 **Cyst:** The dormant cell or resting stage of microalgae, heterotrophic protists, and metazoans,  
25 including but not limited to cysts of dinoflagellates, spores of diatoms, cysts of heterotrophic  
26 protists, and cysts of rotifers.

27  
28 **Effluent:** The treated discharge water produced by a ballast water treatment system.

29  
30 **Equipment:** The ballast water treatment system, defined as either a package or a modular system,  
31 which is tested in the Verification Testing Program.

32 **ETV Testing:** Testing of a technology following provisions of this protocol, with the final  
33 outcome being development of a Verification report, which summarizes all findings of the testing,  
34 and a Verification statement, which is signed by the US EPA and the Verification Organization  
35 (VO).

36 **In-Line Treatment:** A treatment system or technology used to treat ballast water during normal  
37 flow of ballast during uplift or discharge.

- 1 **In-Tank Treatment:** A treatment system or technology used to treat ballast water during the  
2 time that it resides in the ballast tanks. This may involve treatment steps during uptake.
- 3 **Mean Time Between Failure (MTBF):** The predicted elapsed time between inherent failures of  
4 a system during operation. MTBF can be calculated as the arithmetic mean (average) time  
5 between failures of a system. The MTBF is typically part of a model that assumes the failed  
6 system is immediately repaired (zero elapsed time), as a part of a renewal process. This is in  
7 contrast to the mean time to failure (MTTF), which measures average time between failure with  
8 the modeling assumption that the failed system is not repaired.  
9
- 10 **Normally distributed data:** Data that meet the following criteria: the data forms a bell shaped  
11 curve when plotted as a graph, the mean is at the center of the distribution on the graph, the curve  
12 is symmetrical about the mean, the mean equals the median, and the data are clustered around the  
13 middle of the curve with very few values more than three standard deviations away from the mean  
14 on either side.
- 15 **Owner:** The owner of a test site used for verification testing of a ballast water treatment system.
- 16 **Performance Data:** Removal efficacy and effluent concentration data for core and supplemental  
17 parameters for a given set of Challenge conditions.
- 18 **Precision:** The degree to which a set of observations or measurements of the same property,  
19 obtained under similar conditions, conform to themselves. Precision is usually expressed as  
20 standard deviation, variance, or range, in either absolute or relative terms (NELAC, 1998).
- 21 **Protocol:** A written document that clearly states the objectives, goals, scope, and procedures for  
22 the study. A protocol shall be used for reference during Vendor participation in the verification  
23 testing program.
- 24 **Proxy Measurement:** A parameter used in lieu of another measurement (i.e., chlorophyll *a* as a  
25 bulk measure of phytoplankton).
- 26 **Quality Assurance Project Plan (QAPP):** A written document that describes the  
27 implementation of quality assurance and quality control activities during the life cycle of the  
28 project (also see Test/quality assurance plan).
- 29 **Representativeness:** The degree to which data accurately and precisely represent a characteristic  
30 of a population.
- 31 **Sensitivity:** The capability of a test method or instrument to discriminate between different levels  
32 (e.g., concentrations) of a variable of interest.
- 33 **Stakeholder Advisory Group (SAG):** A group overseen by a Verification Organization (VO)  
34 consisting of representatives from verification customer groups, technology developers and  
35 vendors, the consulting engineer sector, the finance and export communities, and government  
36 permittees and regulators.

- 1 **Standard Operating Procedure (SOP):** A written document containing specific instructions and  
2 protocols to ensure that quality assurance requirements are maintained.
- 3 **Standard Test Organisms:** Biological organisms of known types and abundance added to the  
4 challenge water during testing of ballast water treatment technologies.
- 5 **Start-Up:** The period between the time the ballast water treatment system is activated and when  
6 stable operating conditions are achieved.
- 7 **Stable Operation:** The time interval following a start-up period that the ballast water treatment  
8 system performs consistently within the range of Vendor-specified operating conditions.
- 9 **Supplemental Parameters:** A measurement taken that is specific to a particular treatment and  
10 augments the results of the core parameter measurements.
- 11 **Technical Panel:** A group comprised of a subset of stakeholders and other individuals with  
12 technical expertise in ballast water issues, such as scientists, engineers, and ship architects.
- 13 **Test Cycle:** One fill/discharge cycle (including appropriate holding periods) designed to gather  
14 data on treatment efficiency.
- 15 **Test Facility:** A site that provides the necessary infrastructure, systems and personnel to  
16 complete the verification testing described in this protocol. The facility may be part of the Testing  
17 Organization or may be independent from the Testing Organization, but in any case shall be  
18 totally independent from technology Vendors testing at their site.
- 19 **Test/Quality Assurance Plan (TQAP):** Also called a Quality Assurance Project Plan (QAPP),  
20 this is a written document that describes the procedures for conducting a test or study according to  
21 the verification protocol requirements for the application of a ballast water treatment system at a  
22 particular site. At a minimum, the TQAP shall include detailed instructions for sample and data  
23 collection, sample handling and preservation, precision, accuracy, goals, and quality assurance  
24 and quality control requirements relevant to the particular site.
- 25 **Testing Organization (TO):** An organization qualified to conduct studies and testing of ballast  
26 water treatment technologies in accordance with protocols and TQAPs.
- 27 **Upset Conditions:** Deviation or exception from normal or Vendor defined operating conditions,  
28 for example, system faults or hardware failures.
- 29 **Vendor:** A business that manufactures, assembles, or sells ballast water treatment technologies.
- 30 **Verification:** The establishment of evidence on the performance of a ballast water treatment  
31 system under specific conditions, following a predetermined study protocol(s) and TQAP(s).
- 32 **Verification Organization (VO):** The party responsible for overseeing TQAP development,  
33 overseeing testing activities in conjunction with the Testing Organization, and overseeing the

1 development and approval of the Verification Report and Verification Statement for the ballast  
2 water treatment system.

3 **Verification Report:** A written document, typically prepared by the TO, containing all raw and  
4 analyzed data, all quality assurance and quality control (QA/QC) data sheets, descriptions of all  
5 collected data, a detailed description of all procedures and methods used in the verification testing,  
6 and all QA/QC results.

7 **Verification Statement:** A written document, approved by the U.S. Environmental Protection  
8 Agency (EPA), prepared for a verification test conducted under the Environmental Technology  
9 Verification (ETV) Water Quality Protection Center and summarizing the content of the  
10 Verification Report.

11 **Verification Test:** A complete test of a treatment system, which includes enumeration of ambient  
12 and test populations in the challenge water and other defined locations to determine the efficacy of  
13 the technology.

14 **Viable:** According to the IMO G8 guidelines, ‘organisms and any life stages thereof that are  
15 living’.

16

## Abbreviations and Acronyms

1		
2		
3	<b>ATP</b>	Adenosine triphosphate
4		
5	<b>BE</b>	Biological efficacy
6		
7	<b>BWTS</b>	Ballast water treatment system(s)
8		
9	<b>CT</b>	Concentration-time relationship (curve) demonstrating the relationship between
10		concentration and time that achieves desired treatment effect.
11		
12	<b>m<sup>3</sup></b>	Cubic meter
13		
14	<b>CFR</b>	Code of Federal Regulations
15		
16	<b>DOC</b>	Dissolved organic carbon
17		
18	<b>DOM</b>	Dissolved organic matter
19		
20	<b>EPA</b>	U.S. Environmental Protection Agency
21		
22	<b>ETV</b>	Environmental Technology Verification
23		
24	<b>FRU</b>	Field replaceable unit
25		
26	<b>µg/L</b>	Micrograms per liter
27		
28	<b>mgd</b>	Million gallons per day
29		
30	<b>mg/L</b>	Milligrams per liter
31		
32	<b>MAWP</b>	Maximum allowable working pressure
33		
34	<b>MM</b>	Mineral matter
35		
36	<b>MOA</b>	Memorandum of agreement
37		
38	<b>MSDS</b>	Material safety data sheets
39		
40	<b>MTBF</b>	Mean time between failures
41		
42	<b>NRL</b>	U.S. Naval Research Laboratory
43		
44	<b>NSF</b>	NSF International (formerly National Sanitation Foundation)
45		

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1	<b>NTU</b>	Nephelometric turbidity unit
2		
3	<b>O&amp;M</b>	Operations and maintenance
4		
5	<b>OSHA</b>	Occupational Safety and Health Administration
6		
7	<b>Owner</b>	TF owner, if different from the Testing Organization (TO)
8		
9	<b>POM</b>	Particulate organic material
10		
11	<b>PSU</b>	Practical salinity units
12		
13	<b>QA</b>	Quality assurance
14		
15	<b>QAPP</b>	Quality assurance project plan
16		
17	<b>QC</b>	Quality control
18		
19	<b>QMP</b>	Quality management plan
20		
21	<b>SAG</b>	Stakeholder Advisory Group
22		
23	<b>SOP</b>	Standard operating procedure
24		
25	<b>STO</b>	Standard test organism
26		
27	<b>TF</b>	Test Facility
28		
29	<b>TO</b>	Testing Organization
30		
31	<b>TQAP</b>	Test/quality assurance plan
32		
33	<b>TSS</b>	Total suspended solids
34		
35	<b>USCG</b>	U.S. Coast Guard
36		
37	<b>VO</b>	Verification Organization
38		
39	<b>WQPC</b>	Water Quality Protection Center
40		

# Chapter 1

## Introduction

### 1.1 The ETV Program

The U.S. Environmental Protection Agency (EPA) established the Environmental Technology Verification (ETV) Program in 1995. The goal of the ETV Program is to promote environmental protection by accelerating the development and commercialization of improved and more cost-efficient environmental technologies through third-party verification, performance reporting, and information dissemination. The ETV Program neither certifies nor endorses environmental technologies, but rather provides objective, high-quality, peer-reviewed performance data that can be utilized by customer groups and regulators when selecting or permitting use of an environmental technology. The ETV program consists of six Centers focusing on multiple areas of environmental concern. The Water Quality Protection Center (WQPC) develops protocols to verify technologies that protect ground and surface water quality by preventing or reducing contamination.

Through a formal Memorandum of Agreement (MOA), the U.S. Coast Guard (USCG) and EPA formed a partnership between the USCG and the ETV Program to facilitate the development of protocols for evaluating the capabilities of ballast water treatment systems (BWTSs or System) and to provide a pathway to begin the development of technical procedures for approving BWTSs for installation on ships (Fact Sheet dated June 11, 2001 Ballast Water Agreement with the U.S. Coast Guard).

### 1.2 Objectives of Verification Testing

The objective of ETV ballast water treatment verification testing is to verify the performance characteristics of commercial-ready treatment technologies with regard to specific verification factors, including biological treatment performance, predictability/reliability, cost, environmental acceptability, and safety. Given the variety of ship and ballast tank types, treatment technologies, and treatment configurations, this protocol addresses the use of a land-based testing facility (TF) – rather than shipboard testing – to provide comparable conditions for verifying treatment performance. Land-based ETV BWTS verification testing will be conducted in a manner providing information that is comparable, to the maximum practical extent, to use conditions to ensure consumers and other stakeholders can make informed choices in selecting a treatment option.

### 1.3 Purpose and Scope of the Protocol

The parties involved with ETV testing, including vendors, testing organizations, testing site owners, and verification organizations, can use the information provided in this protocol as guidance for BWTS verification testing. This protocol provides guidance on the following necessary elements of verification testing:

- 1       ▪ Acceptability for the program;
- 2       ▪ Vendor provided specification and information; and
- 3       ▪ Test/quality assurance plan (TQAP) development and content.

4  
5 This protocol is intended for verification testing of entire BWTSs, not individual component  
6 technologies that could be combined to form a system. The systems addressed by the protocol  
7 could be in many configurations, such as treatment on uplift or discharge, treatment in-transit  
8 (in-tank), or combinations of these options.

9  
10 Periodic review and revision of protocols is a critical aspect of the ETV Program. As such, this  
11 protocol will be reviewed and revised as necessary following the initial round of testing and  
12 periodically following the first review. These efforts will keep the protocol scientifically and  
13 functionally up to date.

14 **1.4 Verification Testing Process**

15 Verification testing is a three-step process, consisting of planning, verification, and data  
16 assessment/reporting phases. The planning phase includes development of standardized  
17 challenge conditions and the specific experimental design as it will be applied to the testing of  
18 the Vendor’s BWTS. A site- and treatment system-specific TQAP are prepared during the  
19 planning phase in accordance with the guidance provided in Chapter 4 of this protocol. The  
20 BWTS Vendor, Testing Organization (TO), and Verification Organization (VO) collaborate on  
21 the planning phase documents. The verification phase involves the testing of the BWTS by the  
22 TO under the conditions and standard operating procedures specified in the TQAP. In the data  
23 assessment and reporting phase, data are processed and analyzed by the TO, who prepares the  
24 draft Verification Report and Verification Statement. The VO is responsible for QA review of  
25 the data generated during the testing and coordination of the finalization of the Verification  
26 Report and Statement.

27 **1.5 Policies and Program Specifications and Guidelines**

28 Treatment system verification testing will be conducted in accordance with the TQAP and with  
29 the policies, specifications, and guidelines set forth by the ETV Quality Management Plan (for  
30 test-specific activities) as well as the ETV WQPC Quality Management Plan for testing  
31 activities.

32

## Chapter 2

### Responsibilities of Involved Organizations

Verification testing will involve several organizations with responsibilities divided among them. These organizations may include the Vendor of the treatment system, the TO (TO), the Test Facility (TF) owner, the Verification Organization (VO), EPA, and sometimes the Technology Panel and Stakeholder Advisory Group.

#### 2.1 Vendor

The Vendor of the ballast water treatment system will apply to ETV for verification testing. The Vendor must provide the VO and TO verification testing objectives and any existing relevant performance data, along with the information required in Chapter 3. This information will be considered during the development of the TQAP, which will be reviewed and approved by the Vendor. The Vendor will provide a complete System along with any relevant operation and maintenance manuals. Additionally, the Vendor will be responsible for assuring proper installation and set up of the equipment at the test site, training of TO personnel on BWTS operation, and confirmation of the System's proper operation prior to commissioning and commencement of maintenance or treatment efficacy testing. The Vendor will be available for logistical and technical support as required during the planning and verification phases, but will not be directly involved in the testing. The Vendor will also be responsible for reviewing the Verification Report and Statement generated from the TO.

#### 2.2 Testing Organization (TO)

The TO is responsible for preparing the TQAP and working with the Vendor and VO to gain EPA approval of the TQAP, conducting the verification testing and all aspects of test data management, and may be responsible for preparing the final Report and Verification Statement. The TO is also responsible for coordinating all personnel and testing activities, operating the Vendor's equipment as specified in the equipment operations and maintenance manual(s), and evaluating and reporting on the performance of the equipment. Maintaining security for testing activities and site safety for all personnel is also the responsibility of the TO.

#### 2.3 TF Owner (Owner)

If different from the TO, the Owner of the verification TF may provide logistical and technical support during planning and verification phases, as agreed upon by the TO, Vendor, and Owner. The Owner must notify the TO of any logistical or operational developments that may affect the verification testing process and results.

#### 2.4 Verification Organization (VO)

The VO is responsible for overseeing the development and approval of the TQAP, which details study objectives, specific test procedures, and assurance requirements. In addition, the VO will collaborate with the TO to administer testing activities at the TF. The VO is also responsible for

1 review and gaining EPA approval for the Verification Report, which will contain all raw  
2 analytical data, data descriptions, details of procedures and methods, and all QA/QC data sheets  
3 and results. The VO is also responsible for review and gaining EPA approval of the Verification  
4 Statement, which summarizes the contents of the Verification Report. The Report and Statement  
5 are typically drafted by the TO, but they may be drafted by the VO or a contractor to the VO.  
6 The VO is also responsible for initiating and coordinating periodic review and revision of this  
7 protocol.

## 8 **2.5 Environmental Protection Agency (EPA)**

9 The EPA Office of Research and Development (ORD), through the National Risk Management  
10 Research Laboratory (NRMRL) in Cincinnati, Ohio oversees the ETV Program. The EPA Center  
11 Manager for the WQPC, operating from the Urban Watershed Management Branch (UWMB) in  
12 Edison, New Jersey, will be responsible for review and approval of TQAPs for BWTS  
13 verification testing, the Verification Report and Statement generated from the testing, and for  
14 assuring that the Report and Statement are posted on the EPA/ETV web site. EPA is also  
15 responsible for coordinating review and approval of revisions that may be proposed to this  
16 protocol.

## 17 **2.6 Stakeholder Advisory Group (SAG)**

18 Stakeholder Advisory Groups (SAGs) are established in each of the ETV Program's six Centers,  
19 and consist of representatives from verification customer groups, such as buyers and users of  
20 technology, developers and vendors, the consulting engineering sector, the finance and export  
21 communities, and government regulators. The SAGs support generic verification protocol  
22 development, prioritizing the types of technologies to be verified, and defining and conducting  
23 outreach activities appropriate to the technology area and customer groups. In addition, the  
24 SAGs may review WQPC-specific procedures and selected ETV verification reports emerging  
25 from the ETV WQPC and serve as information conduits to the particular constituencies that each  
26 member represents. The Ballast Water SAG, of the WQPC, is charged with addressing ballast  
27 water treatment technologies.

## 28 **2.7 Technology Panel**

29 The Technology Panel is comprised of a subset of stakeholders and other individuals with  
30 technical expertise in ballast water and environmental technology issues. Scientists, engineers,  
31 technology vendors, naval architects, and regulators supported the development of this  
32 Verification Test Protocol. In the future, the Technology Panel may be responsible for reviewing  
33 the technology specific TQAPs and Verification Reports and Statements. The Panel will also  
34 play a key role in working with the VO in reviewing and revising this protocol as needed.

35

# Chapter 3

## Ballast Water Treatment System Capabilities and Description

### 3.1 Ballast Water Treatment System Definition

For the purposes of this verification testing program, ballast water treatment systems (BWTS) are defined as:

*Prefabricated, commercial-ready, treatment systems designed to remove, kill or inactivate (prior to discharge) organisms in ballast water. This includes all components, in an integrated fashion, required for shipboard operation.*

Note that it is understood that many of the proposed regulatory discharge standards, and in fact the desired effect of BWTSs, is that these technologies should render organisms unviable or incapable of reproduction. In other words, to “kill, remove or inactivate” is technically unnecessary when the objective is to eliminate the organism’s capability for reproduction. However, as the introduction of “viability” as a measure of efficacy significantly complicates the Protocol and test methods, and since “kill, remove or inactivate” is a conservative approach, the latter has been adopted as the measure of biological efficacy in this Protocol.

This definition includes both in-line (systems that treat the flow of ballast water either on uplift or discharge) and in-tank systems (systems that treat ballast water during the time it resides in the ballast tanks). Typically, BWTSs treat an average design flow between 1.4 – 17 m<sup>3</sup> per minute (370 – 4,490 gpm) or a total tank volume within a range of 20 – 14,500 m<sup>3</sup> (5,280 – 3,830,000 US gal).

Systems that will be tested under this program will be capable of treating the entire discharge or ballast water volume for biological organisms, either through a one-step treatment process or through multi-step treatment processes, and will be capable of treating a wide range of source water typical of ballast uplifted from fresh, coastal, estuarine and/or marine origins. These technologies may be biological, physical, or chemical in nature or a combination of any or all of the technologies. Treatment systems, or components of systems, that provide only partial treatment of the discharge are excluded from verification testing.

### 3.2 Technology or Treatment Performance Claims

The Vendor will supply a statement of treatment performance claims for the treatment or technology. Discharge water quality specifications should reference current EPA regulations or recommendations for shore discharge standards. The statement should include, as a minimum:

- Quantitative measures of biological treatment efficacy expressed as a concentration upon discharge for a range of biological size groups as defined in Section 5.2.2; minimum reporting parameters are specifically detailed in Section 5.4.6;
- Quantitative measures of operational performance requirements to achieve the biological treatment performance stated above; these should include, as a minimum, the allowable

1 and treatable flow rate range and water quality (dissolved and particulate matter  
2 concentration and size range, salinity, water temperature, turbidity and dissolved oxygen  
3 content);

- 4 ■ Treatment capabilities over the anticipated range of maritime environmental conditions  
5 must be identified by the Vendor; the effects of extremes in temperature, turbidity,  
6 biomass density, or other environmental conditions that may impact the treatment system  
7 must be noted where these may cause variations in Vendor performance specifications;
- 8 ■ Quantify the concentration of disinfection residuals, by-products and toxicity for relevant  
9 systems;
- 10 ■ The required operational and maintenance conditions (operator time, power requirements,  
11 chemical consumption requirements, reliability, etc.) to achieve the biological  
12 performance under a range of source water conditions typical to fresh, coastal, estuarine,  
13 and marine ballast water (water conditions are detailed in Section 5.2.1); and
- 14 ■ The projected mean-time between failure (MTBF) for the technology given the operation  
15 and maintenance schedules provided for the technology.

### 16 3.3 Acceptability for Testing

17 The System must meet the definition of a BWTS, meet all existing environmental regulatory  
18 requirements for operation and treatment byproduct discharge (including EPA Registration under  
19 FIFRA for any antimicrobial chemical used in the System), and must be safe to operate for the  
20 crew and vessel. Only complete treatment systems will be accepted for verification testing.

21  
22 The VO has the right to reject a proposed System that does not satisfy the definition of a BWTS  
23 in Section 3.1. A proposed treatment system may also be denied acceptance to the verification  
24 testing program if, for technical or logistical reasons, it cannot be accommodated at the TF or its  
25 use will result in non-compliance with the discharge requirements for the TF.

### 26 3.4 Test BWTS Requirements

27 All piping, valves and fittings shall comply with regulations and marine industry standards as  
28 contained in applicable sections of 46 CFR Part. Pressure piping shall be fitted with relief valves  
29 set not to exceed maximum allowable working pressure (MAWP).

30  
31 Electrical and electronic components in alternating current (AC) systems must be capable of  
32 operating satisfactorily under normally occurring variations in voltage and frequency. Unless  
33 otherwise stated, the variations from the rated value may be taken from Table 1. Direct current  
34 (DC) system devices must be capable of operating satisfactorily at minus 15% voltage.  
35 Conductors, power supply, and over-current protection shall be provided in accordance with 46  
36 CFR Subchapter J and appropriate marine industry standards.

37  
38 **Table 1. Acceptable Variations for Frequency and Voltage**

<b>Quantity in Operations</b>	<b>Permanent Variation</b>	<b>Transient Variation</b>
Frequency	±5%	±10% (5 s)
Voltage	+6%, -10%	±20% (1.5 s)

1 Operating conditions and tolerances for TO supplies of water pressure and flow, power  
2 conditions, air pressure and flow, or any other requirements specific to the BWTS must be  
3 clearly identified in System documentation.

4  
5 System design should provide for appropriate lift and/or hoist points during installation. Center  
6 of gravity, no step areas and other installation specific information should be clearly identified.  
7 Any areas presenting a hazard to personnel during installation, checkout, and operation should be  
8 visibly marked.

9  
10 Recommendations to ensure post-installation operator access to maintenance ports, access  
11 panels, and field replaceable units (FRUs) should be clearly identified in an installation guide  
12 with appropriate layout diagrams.

### 13 **3.5 Operating and Maintenance (O&M) Evaluation**

14 The BWTS will be evaluated during the testing to determine if the System is:

- 15
- 16     ▪ Designed and constructed to ensure that user access is restricted to essential controls for  
17 normal operation of the system;
- 18     ▪ If access beyond these controls is available for emergency maintenance and temporary  
19 repair, and requires the breaking of security (lockout) seals or activation of another  
20 device indicating an entry to the equipment;
- 21     ▪ Provides capability for efficient maintenance and repair operations and provides a high  
22 mean-time between failures (MTBF);
- 23     ▪ If minor and major maintenance schedules, pre-requisite training, level of effort, and  
24 recommended spares/supplies are detailed in the appropriate sections of the O&M  
25 Manual;
- 26     ▪ If adequate documentation, including drawings, diagrams and instructions necessary for  
27 routine maintenance, troubleshooting, and repairs, are provided.
- 28     ▪ Designed to ensure any potential exposure to hazards or hazardous materials that are  
29 involved in the maintenance or operation of the equipment are minimized;
- 30     ▪ If explicit warning labels identifying the hazard are installed in accordance with OSHA  
31 and/or other appropriate federal regulations;
- 32     ▪ If procedures for working with stated hazards are clearly identified in the operating  
33 instructions;
- 34     ▪ If by-product, disposable component, or field replaceable unit (FRU) that presents a  
35 safety or environmental hazard are explicitly identified, along with procedures for  
36 material handling and disposal according to relevant regulations; and
- 37     ▪ If the **VENDOR** provides technical support for this system via phone and internet,  
38 including contact information for both methods.
- 39

40 The BWTS means for its operation and control will be evaluated during the testing to determine  
41 if the:

- 1     ▪ Control system ensures that services needed for the proper operation of the BWTS are  
2     provided through automatic arrangements and operators are promptly alerted when  
3     conditions warrant human intervention;
- 4     ▪ Operator is able to control all BWTS functions through a single Control Unit;
- 5     ▪ Control Unit automatically monitors and adjusts optimal treatment dosages or intensities,  
6     or other aspects of the BWTS, and/or provides control signals to the ballast water system  
7     of the vessel to properly provide the necessary treatment;
- 8     ▪ Control Unit provides a continuous self-monitoring function when the BWTS is in  
9     operation;
- 10    ▪ Control Unit includes a tamper-proof or tamper-evident recording device, located in a  
11    position easily accessible to the person in charge of the BWTS, that provides the operator  
12    the parameters listed below during ballast water treatment while continuously logging the  
13    data:
  - 14
  - 15    ○ Proper functioning and health status of all the services needed for the proper  
16    operation of the BWTS;
  - 17    ○ All parameters necessary to ensure the proper operation of the BWTS;
  - 18    ○ Status of the valves present in the BWTS, including those leading to overboard  
19    discharge;
  - 20    ○ Total quantity of ballast water treated;
  - 21    ○ Ballast water treatment rates;
  - 22    ○ Alarm conditions;
  - 23    ○ Date and time of start and end of the treatment operation;
  - 24    ○ Ballast operation monitored (upload, discharge);
  - 25    ○ Calibration and maintenance events;
  - 26    ○ Other system events of interest;
  - 27    ○ Relevant and necessary measurement information required for control and monitoring  
28    operation of the BWTS;
  - 29    ○ Meter and sensor accuracy to measure the suite of parameters appropriate and  
30    necessary for control of the BWTS, representing the actual value of the parameters  
31    being monitored within 10% despite the presence of contaminants normally expected  
32    in ballast water and the operational environment of the BWTS;
  - 33    ○ Diagnostics to enable the local operator to check the functioning of the electrical and  
34    electronic circuitry, as well as the calibration of meters and sensors according to the  
35    manufacturer's specifications;
  - 36    ○ An emergency manual override function to be used in the event of failure of the  
37    Control Unit;
  - 38    ○ Audio and visual alarms and a recording in the event there is discharge of any  
39    effluent or a component failure whenever the Control Unit is not fully operational;  
40    and
  - 41    ○ A means to print reports and logged data, as applicable, or stored electronically with  
42    printout capability, upon the following events:
    - 43    – the BWTS is started
    - 44    – the BWTS is stopped
    - 45    – an alarm condition develops
    - 46    – normal conditions are restored

- 1           – manual override is engaged
- 2       ▪ In case of a single failure compromising the proper operation of the BWTS, audible and
- 3       visual alarm signals are given in all stations from which ballast water operations are
- 4       controlled, including, but not limited to, the following conditions:
- 5
- 6           ○ Power failure to the BWTS or any subsystem;
- 7           ○ Failure of any sensor, meter, or recording device;
- 8           ○ Hazardous condition detected by control system; and
- 9           ○ Operation outside set points of the BWTS for proper treatment.

### 10   **3.6 Calibration and Test Requirements**

11   The BWTS will be evaluated during the testing to determine if the System provides:

- 12
- 13       ▪ Diagnostic routines and procedures to maintain accuracy of measured process
- 14       parameters, including:
  - 15           ○ The degree to which diagnostics are automated;
  - 16           ○ If self test routines are incorporated as part of the control unit;
  - 17           ○ If the manufacturer specified appropriate diagnostic intervals; and
  - 18           ○ If the diagnostics confirm that parameters are within specifications or that
  - 19           calibration is required.
- 20       ▪ Diagnostics for fault checking, system maintenance and repair;
- 21       ▪ Automated diagnostics that also may be manually initiated by the operator;
- 22       ▪ Diagnostics that isolate faults down to field replaceable units (FRUs);
- 23       ▪ If the accuracy of the System components that take measurements are verifiable
- 24       according to the manufacturer’s instructions; and
- 25       ▪ If only the manufacturer or persons authorized by the manufacturer do the accuracy
- 26       checks.

### 27   **3.7 System Documentation Evaluation**

28   The documentation provided for the BWTS will be evaluated during verification to determine if

29   the specifications provide detailed requirements and tolerances for the following System

30   parameters:

- 31
- 32       ▪ Ballast water turbidity, pressure, temperature and flow rate ranges (include any other
- 33       applicable criteria);
- 34       ▪ Electrical power requirements;
- 35       ▪ Air/pneumatic pressure and flow rate ranges;
- 36       ▪ Weight;
- 37       ▪ Dimensions;
- 38       ▪ Environmental limitations (e.g., ambient temperature);
- 39       ▪ Treatment limitations;
- 40       ▪ Safety hazards; and
- 41       ▪ The **VENDOR** provided list of procedures for unpacking and verifying contents of shipped
- 42       items.
- 43

1 The documentation of the installation procedures and requirements in the installation guide will  
2 be evaluated to determine if:

- 3
- 4     ▪ All areas of mechanical, electrical, hydraulic, pneumatic, and any other interface
- 5     requirements are addressed;
- 6     ▪ Time estimates in man-hours provided for installation procedures are appropriate;
- 7     ▪ If applicable standards are referenced and special precautions and hazards identified; and
- 8     ▪ Appropriate diagrams, photographs and/or assembly drawings detail footprints,
- 9     attachment points, interfaces, and any referenced components or subassemblies.

10

11 The adequacy of the O&M Manual(s) provided with the system will be evaluated during the  
12 verification. If not included in the O&M Manual, ancillary documentation provided with the  
13 BWTS will be evaluated for the detail provided for the following items:

- 14
- 15     ▪ Piping and instrumentation diagrams;
- 16     ▪ Electrical schematics and wiring diagrams;
- 17     ▪ Photographs;
- 18     ▪ Guides for diagnostics and troubleshooting;
- 19     ▪ Parts lists; and
- 20     ▪ Operator training – minimal additional special training required to operate the System
- 21     (identifying and supplied).

### 22 **3.8 Technical Data Package Submission**

23 A technical data package must be submitted to the TO by the **VENDOR** of a BWTS to be  
24 considered for verification. Vendor-specific performance claims should be identified along with  
25 relevant existing performance data.

26

27 The information in the technical data package should demonstrate that the treatment processes  
28 are well characterized and the equipment is designed to meet specific ballast water treatment  
29 performance criteria at the intended operational scale. Photographs with appropriate reference  
30 scales should be included. The data package shall also document operational and maintenance  
31 requirements and conditions. At a minimum, the technical documentation provided by the  
32 Vendor should address the items identified in the format outline in Section 3.9.

33

34 Much of the required information will likely be available in the Vendor O&M Manual(s), which  
35 are part of the required documentation. The information presented in an O&M Manual will,  
36 however, vary by Vendor. To be considered for a Challenge test under this Protocol, Vendors are  
37 required to submit a technical documentation package. This allows each Vendor the opportunity  
38 to incorporate those data most appropriate to the content topic. In addition to the Technical Data  
39 Package and the O&M Manual(s), Vendors may also provide ancillary reference information  
40 through any combination of manuals, product literature, and electronic files. Any ancillary  
41 information or proprietary information must be clearly identified as such, and the intended  
42 purpose/relevance of providing the information must be clearly stated.

43

1 While not required for Verification, but likely to be part of a submittal for regulatory  
2 compliance, the manufacturer may provide certifications or quality assurance documentation for  
3 all QA/QC and factory testing that occurs during the manufacture of the equipment. If provided,  
4 relevant standards traceability data should also be provided.

### 5 **3.9 Format for the BWTS Technical Data Package**

6 A. Cover Page

7 B. Table of Contents

8 C. General Description & Capabilities (Marketing and technical specifications, and other items  
9 below)

10 C.1 System volume, weight, power & mechanical interface requirements

11 C.1 Vendor performance objectives (Vendor should describe primary and non-primary  
12 objectives of ETV testing, i.e., verification testing, or full scale evaluations)

13 D. Target operating environments and conditions

14 D.1 General Features

15 D.1 Permitting and Certifications

16 D.1 Scalability (no specified requirement – please address range of applicable ballast system  
17 volumes and rates for the described treatment system)

18 E. Installation Requirements and Instructions

19 E.1 Hydraulic and mechanical connections

20 E.1 Electrical connections to mains

21 E.1 Hazard locations

22 E.1 Other special installation criteria / handling

23 E.1 Considerations for maintenance / consumables / repair

24 E.1 Shipping and delivery considerations (no specified requirement – Vendor should  
25 describe ability / methods to transport treatment system)

26 E.1 Interfacing for performance monitoring, alarms & controls (no specified requirement –  
27 Vendor should describe available options)

28 F. Operating and Maintenance Instructions

29 F.1 Operating and Maintenance Manual (may provide as standalone document(s), but – any  
30 references in the text of the Technical Data Package to the separate O&M Manual  
31 must/should be specific to page and paragraph.)

32 F.1 Training Materials

33 F.1 Repairs and Troubleshooting

34 F.1 Recommended Spares (and sources)

35 F.1 Safety Precautions and Issues

36 F.1 Environmental Hazards and Issues, Including By-Products

37 F.1 Expendables, Materials Handling, and Waste Disposal

38 F.1 Technical Support contact information

39 G. System Performance Specifications

40 G.1 Discharge water quality

41 G.1 Treatment capabilities vs. environmental conditions

42 G.1 Control features and capabilities

- 1 G.1 Factory testing criteria and procedures
- 2 G.1 Human operator requirements
- 3 G.1 Data Storage
- 4 G.1 Automated capabilities
- 5 G.1 Alarms and safety capabilities
- 6 H. Calibration and System Test Procedures
- 7 H.1 Diagnostics
- 8 H.2 Quality assurance during operation
- 9 H.3 Calibration schedules and procedures
- 10 I. Detailed Description of System Operation
- 11 I.1 Theory, processing and principles of operation (no specified performance requirement –
- 12 Vendor should provide background on how and why treatment system works, including
- 13 explanation of any environmental limiting factors)
- 14 I.2 Selection of materials used in fabrication
- 15 I.3 Design considerations for marine applications
- 16 I.4 Ancillary Documentation Package (this section is for documentation not referenced
- 17 elsewhere)
- 18 I.5 Reference drawings and photographs
- 19 I.6 Materials / parts lists
- 20 I.7 Certifications
- 21 I.8 Test Results / Qualification Data (no specified requirements – this should be results of
- 22 Vendor and/or independent testing of system performance)
- 23

# Chapter 4

## Treatment Verification TQAP Development

### 4.1 Description of Ballast Water Treatment System

Each ballast water treatment verification test will be completed following a written TQAP. From the Vendor-supplied treatment system documentation submitted as outlined in Chapter 3, the TQAP should include those materials, data, and information that are necessary to describe the treatment system's principle of operation, physical properties, installation and commissioning, startup and operation, data collection, required actions during upset conditions and required consumables. These may include, but are not limited to:

- Vendor treatment and operation claims as identified in Section 3.2
- Engineering description
- Process description including performance ranges and expectations
- Discharge characteristics
- Footprint
- Photographs
- TO physical and electrical interfaces
- Safety and Environmental Hazards and Precautions

### 4.2 Required Elements of the TQAP

The TQAP will detail test objectives, specific test procedures (including sample and data collection, sample handling, analysis and preservation) and quality control and assurance requirements (including measures of precision, accuracy, comparability, and representativeness). The experimental approach for the ballast water treatment test, treatment system start-up, and verification procedures will be presented in the TQAP. The TQAP will include a summary description of the standardized water quality and biological challenge conditions established by the experimental configuration as described in Section 5.3. The Plan will summarize how the challenge conditions will be implemented at the TF relative to the ballast water treatment system being tested. Any modifications or supplements to the treatment verification protocols will be defined and discussed in the Plan. The TQAP will also address quality assurance/quality control (QA/QC) requirements, data handling and presentation, and environmental, health, and safety issues.

The TO, with input from the Vendor, is responsible for preparing the TQAP. If the Vendor desires data from ETV testing to be made available for Type Approval or other regulatory purposes, the data required should be clearly identified in the TQAP. The VO shall review and coordinate the approval of the TQAP prior to the start of verification testing.

The TQAP shall include:

- 1     ▪ Title page/approval page with all project participants
- 2     ▪ Table of contents
- 3     ▪ Project description and treatment performance objectives
- 4     ▪ Project organization and personnel responsibilities
- 5     ▪ TF description
- 6     ▪ Treatment system description
- 7     ▪ Experimental design (including installation/start-up plan)
- 8     ▪ Challenge water conditions and preparation (including TF Standard Operating Procedures
- 9       (SOPs) for preparation)
- 10    ▪ Sampling and analysis plan including sampling and analytical procedures
- 11    ▪ Data management, analysis and reporting
- 12    ▪ Environmental, health and safety plan
- 13    ▪ References
- 14    ▪ Appendices
  - 15       ○ Quality Assurance Project Plan (QAPP)
  - 16       ○ Vendor operation and maintenance manual

17

18 Content requirements for the QAPP are discussed in more detail in Appendix A.

19

# Chapter 5

## Experimental Design

The primary purpose of ETV verification of BWTSSs is to verify the biological treatment performance claims made by the BWTSS Vendor. Other factors pertinent to the treatment system's performance will also be evaluated, including engineering and environmental metrics. To ensure that consumers and other stakeholders can make informed choices in selecting a treatment system, land-based (which could include testing on a barge/fixed platform designed to meet the requirements of this protocol) verification testing conducted in accordance with this protocol is intended to provide comparable data sets for each technology or system to the maximum practical extent. One way to ensure this is to use a standard set of challenge conditions when testing each treatment system. Standardized challenge conditions included in this protocol address both water quality conditions and the biological organism concentrations and distribution used to evaluate treatment performance. Key water quality challenge conditions are standardized under this protocol because the effectiveness of many treatments may be influenced by certain water quality characteristics (e.g., salinity, turbidity, color, etc.). Moreover, the natural environment has a large range of conditions, which may or may not provide adequate information on a system's ability to perform in accordance with the Vendor's claims under non-ideal water quality conditions. Therefore, non-ideal water quality conditions form the basis for challenging the treatment systems. To this end, the protocol also includes the analysis of ambient species and a set of standard test organisms to measure treatment efficacy. Standard test organisms are included to better evaluate the effectiveness of treatments on organism life stages known to be resilient under test conditions and to provide a means of comparing results among technologies and tests conducted at different locations or on different dates.

The general objectives of the verification testing are to:

- Provide a comprehensive set of water quality and biological challenge conditions against which treatment effectiveness can be quantitatively evaluated.
- Develop adequate data to document system performance against the verification factors.

The requirements for testing are described in the following sections, which provide guidance on the four key elements of the protocol: 1) test verification factors, 2) water quality and biological challenge conditions, 3) the TF experimental configuration, and 4) verification testing, including commissioning of the equipment and the measurement programs required under this protocol. Variations in the protocol for specific treatment system types (e.g., in-line treatment versus in-tank treatments) are also described.

### 5.1 Test Verification Factors

All treatment systems will be verified according to the following factors:

- Biological treatment efficacy

- 1       ▪ Operation and maintenance
- 2       ▪ Reliability
- 3       ▪ Cost factors
- 4       ▪ Environmental acceptability
- 5       ▪ Safety

### 6   **5.1.1 Biological Treatment Efficacy**

7 Biological treatment efficacy is defined as the removal, inactivation, or death of organisms and  
8 will be measured in terms of the concentration of selected organism size classes in the treated  
9 discharge. Additional measures of efficacy may include measurements in terms of removal  
10 efficiency (e.g., a percentage reduction of organisms present at uptake), against a threshold (e.g.,  
11 a water quality standard), or in relation of treatment vs. control discharge concentrations. The  
12 measurement program required by the protocol evaluates the primary Treatment Efficacy criteria  
13 by measuring the quantity of living organisms in both the challenge water and the treated  
14 discharge.

### 15   **5.1.2 Operation and Maintenance**

16 Operation and maintenance includes the labor expertise, equipment, and consumables required to  
17 operate the system to achieve the stated performance goals and objectives. The quantitative  
18 indicators to be considered during verification are described in detail in Section 5.4.9.1.

### 19   **5.1.3 Reliability**

20 Reliability is a statistical measure of the number of failures (either qualitative or quantitative) per  
21 known quantity of test cycles. This is described in greater detail in Section 5.4.9.8.

### 22   **5.1.4 Cost Factors**

23 Cost factors include only those factors that can be verified, such as labor hours to operate and  
24 maintain the system, expendable material, such as filter cartridges, and pounds or gallons of  
25 chemicals consumed by the treatment system. Data is collected in units, to which unit prices,  
26 which are likely to vary from location to location, can be applied to determine costs. These are  
27 discussed further in Section 5.4.9.2.9.

### 28   **5.1.5 Environmental Acceptability**

29 Environmental acceptability assesses ballast water quality following treatment for factors other  
30 than the abundance and viability of organisms. For example, this will determine if the treated  
31 water meets acceptable water quality characteristics for such measures as dissolved oxygen,  
32 temperature, treatment residuals, pH, etc. This is discussed in further detail in Section 5.4.4.

### 33   **5.1.6 Safety Factors**

34 Safety factors include any treatment-specific considerations that may pose a threat to the safety  
35 of the operator or shipboard operations. These are not intended to be comprehensive in nature,  
36 which is best evaluated by Classification Societies, such as the American Bureau of Shipping,  
37 but are included as observations that can be made during the verification testing. Further  
38 discussion of these observations are discussed in Section 5.4.9.2.11.

39

1 Performance test results will be reported using standard ETV formats to make certain the  
2 reported information among treatment technologies tested is comparable. Flexibility is  
3 permissible to ensure reporting for a specific treatment system type is appropriate and accurate.  
4

5 Some information supplied by Vendors may not be verified under the protocol. Nonetheless,  
6 such information can be included in the Verification Test Report as non-verified information.  
7 This information may include shipboard compatibility (e.g., corrosion resistance, system weight,  
8 system volume including clearances needed to perform maintenance and replace vital  
9 components, and compatibility with other common shipboard systems such as operational flow  
10 rates). Submission and reporting requirements for non-verified, Vendor-supplied information is  
11 included under Chapter 3.

## 12 **5.2 Challenge Conditions**

13 This protocol recognizes that land-based testing cannot fully replicate actual treatment system  
14 performance onboard ship. However, land-based verification testing can provide sufficient  
15 information to verify the expected performance of treatment in the shipboard environment. It is  
16 understood that all treatment technologies will face a range of physical/chemical water quality  
17 conditions and biological organisms when operated onboard a ship. Therefore, each treatment  
18 system's performance will be verified using a set of standard challenge conditions. This protocol  
19 defines the following objectives for the challenge conditions:  
20

- 21 ▪ To verify a treatment system's performance using a set of challenging, but not rare, water  
22 quality conditions representative of the natural environment.
- 23 ▪ To verify removal or kill of bacteria, protists, and zooplankton using ambient and  
24 standard test organisms as defined by size classes and analytical techniques that identify  
25 living quantities for these organisms.  
26

27 The standard challenge conditions are thus specified using two groups of factors that must be  
28 addressed to properly challenge treatment technologies: water quality and living organisms. The  
29 requirements for each group are presented in the following sections.

### 30 **5.2.1 Challenge Water – Water Quality Characteristics**

31 Since water quality conditions in ports and harbors around the world vary greatly, treatment  
32 systems may encounter a wide range of conditions. Also, certain water quality conditions are  
33 known to interfere with the ability of some treatment processes. It is therefore critical to evaluate  
34 the effectiveness of a treatment system under water quality conditions that are challenging to the  
35 technology being tested. Simulating all potential water quality conditions in a land-based testing  
36 design would be prohibitively expensive<sup>1</sup> and not essential for verifying the performance of a  
37 treatment system. Because water quality parameters that can interfere with various treatments  
38 are generally understood and few in number (e.g., salinity, turbidity, organic matter either as  
39 dissolved or particulate forms), the number of water quality metrics that must be explicitly  
40 included in the protocol can be limited. Thus, this protocol defines three possible challenge

---

<sup>1</sup> Similarly, shipboard testing of all potential water quality conditions will require extensive logistics to move a treatment system to a matrix of natural conditions, as well as investment in methods and protocols by which the treatment effectiveness is established using natural populations of organisms.

1 conditions that represent some of the more challenging, natural conditions that may be  
2 encountered by ballast water treatment systems. Challenge water quality characteristics to be  
3 used during testing events are presented in Table 2.  
4

5 **Table 2. Water Quality Challenge Matrix for Verification Testing**

<b>Water Types</b>	<b>Minimum Water Characteristics</b>
Fresh (Salinity <1 PSU)	DOM: 6 mg/L as DOC POM: 4 mg/L as POC
Brackish (Salinity 10-20 PSU)	MM: 20 mg/L TSS = POM + MM: 24 mg/L
Marine (Salinity 28-36 PSU)	Temperature: 4 – 35 °C

6  
7  
8 Another basic premise in the design for this protocol is that ballast water treatment systems are  
9 designed to function effectively in the full range of water quality characteristics that will be  
10 encountered under operational conditions. By challenging the treatment systems with these  
11 conditions, it is assumed treatment will be effective under less demanding conditions. Challenge  
12 waters have been tailored to a minimum set of water quality conditions that may be achieved  
13 either through naturally occurring conditions or through augmentation, if appropriate, and  
14 validated by the TF. The challenge conditions are specified for three possible levels of salinity,  
15 <1, 10 to 20, and 28 to 36 PSU (Practical Salinity Units), and water quality characteristics  
16 problematic for the range of technologies being developed to treat ballast water, namely,  
17 suspended solids and dissolved organic matter (DOM).<sup>2</sup>  
18

19 In nature, solid material that interferes with treatment effectiveness is composed of several types  
20 of particles, which can be of biological origin or of mineral origin, specifically clay and silt. The  
21 water quality challenge conditions defined by the solids content of the matrix include particulate  
22 organic matter (POM) and mineral matter (MM). These two types of particles are both present  
23 in natural waters at a range of concentrations and size distributions. Therefore, both forms are  
24 included in the challenge conditions to address issues of particulate removal and turbidity, which  
25 can interfere with transmission of light or other treatment actions.  
26

27 Various forms of dissolved chemicals and compounds, particularly organic material, can directly  
28 affect the efficiency of some treatment processes. Dissolved organic matter (DOM) and  
29 dissolved organic carbon (DOC) are two terms used to describe this component of natural water.

<sup>2</sup> The protocol does not explicitly call for verification at a series of temperatures, even though some treatments may have strong temperature dependence or may include temperature manipulation as part of the treatment procedure. Rather than include temperature as a controlled water quality condition, which can have significant cost implications for the TF, accurate and continuous monitoring of the source and treated water temperatures is required for all test cycles. If temperature manipulations are to be included, the Test Plan will include protocols for these manipulations. Temperature challenges should be addressed in shipboard testing, and in bench-scale tests of treatment process.

1 DOM/DOC often contains many chromophores that contribute substantially to the color of the  
2 water, another potential interference for treatments. Thus, the color of a water and DOM/DOC  
3 concentration are often interrelated.

4  
5 The measurement methods for evaluating the status of the challenge water quality conditions are  
6 described in Section 5.4.6. They include standard analytical methods to document the  
7 concentration of total suspended solids, particulate organic matter or dissolved organic matter,  
8 and methods that indirectly measure these parameters (e.g., turbidity measured by  
9 electronic/optical measurement such as nephelometry (NTUs) or transmissometry (beam  
10 attenuation) or fluorescence (color /DOM)).

11  
12 Standardization of the water quality conditions for the verification testing requires a consistent  
13 set of source water (e.g., fresh or marine water), as well as the use of well-characterized organic  
14 matter and mineral matter. The TF will be responsible for providing these materials and  
15 ensuring the water quality conditions are as described under this protocol. The water quality test  
16 conditions will be standardized for salinity, particulate organic matter, mineral matter, and  
17 dissolved organic matter as described in the following sections:

#### 18 *5.2.1.1 Salinity*

19 Natural water of less than 1 PSU will be used for fresh water conditions, while natural seawater  
20 will be used for marine conditions. Testing at multiple salinities at a given TF should only be  
21 conducted if there are natural water sources with the differing salinities (e.g., fresh and brackish  
22 waters). Artificial modification of the salinity of the waters should be used only if it can be  
23 demonstrated that the concentrations, diversity and condition of organism populations required in  
24 Section 5.2.2 will not be impacted by adjustment of the salinity.

#### 25 *5.2.1.2 Particulate and Dissolved Organic Matter*

26 In the case of POM, if the natural waters have insufficient concentrations, the TO may augment  
27 them through the addition of humic material (e.g., Micromate humates [Mesa Verde Resources,  
28 Placitas, New Mexico]). Other sources include particulate carbon from sources such as ground  
29 up seaweed or plankton detritus. DOM can be very difficult to adjust or augment if the natural  
30 waters have insufficient content. There has been some success using *Camellia sinensis*  
31 (decaffeinated iced tea mix) to augment natural DOM content, but a TF must assess the effect of  
32 additives on the ambient and test organisms (if used) before using.

#### 33 *5.2.1.3 Mineral Matter (MM) - Clays and Silts*

34 Mineral particles in the size range typically found in coastal and estuarine waters are readily  
35 obtained from commercial sources and will be used as the source of the mineral matter. A study  
36 of sediment size in ballast tanks suggests that particles are mostly fine grained (less than 63 µm)  
37 and most vessels contain <10% sand (F. Dobbs, Pers. Com.). Thus, addition of the commercially  
38 available clay minerals (with a majority of particles in the 10 to 50 µm size) addresses the  
39 objective of having a prescribed level of non-biological particles as part of the water quality  
40 challenge conditions. Specifically, ISO 12103-1, A3 MEDIUM TEST DUST and ISO 12103-1,  
41 A4 COARSE TEST DUST can be used for this purpose. As these particles will tend to settle out  
42 over time within the augmentation storage vessels, the test protocols must include a means of

1 maintaining any sediments in a homogeneous suspension prior to addition of the challenge water  
2 (e.g., continuous mixing of the sediment augmentation tank).

3  
4 The TO should verify that, whatever source of augmentation or delivery system is used, the  
5 addition of that material should minimize to the extent possible biocidal or growth stimulant  
6 response to either the ambient or standard test organisms (if used). The TF will be responsible  
7 for preparing the challenge water, documenting the challenge conditions, and validating that the  
8 conditions are maintained at the treatment system or control entry point. Challenge waters will  
9 be prepared under Standard Operating Procedures developed by the TF. The TQAP will include  
10 these SOPs and describe any planned deviations from the SOPs.

#### 11 *5.2.1.4 Challenge Water – Water Quality Deviations*

12 In some cases, a specific ballast water treatment system may be unable to operate with all of the  
13 prescribed challenge water quality conditions as specified in Table 2. This may be either due to  
14 mechanistic limitations of the technology (e.g., electrolytic chlorination (without brine addition)  
15 is inoperable in fresh water) or by design (e.g., scale). In such cases, deviations may be  
16 permitted provided that significantly challenging and realistic conditions are identified and  
17 justified by the TO, and that the VO approves the deviation. In no case, however, shall the total  
18 number of test cycles be reduced. All deviations will be specified in the verification report as  
19 limiting conditions of the technology.

### 20 **5.2.2 Challenge Water - Biological Organism Conditions**

21 The death or removal of living organisms is central to the need to treat ballast water. To ensure  
22 proper evaluation of a BWTS's performance, the effects on biological organisms living in the  
23 challenge water will be measured for each treatment system tested. Biological efficacy will be  
24 evaluated as function of a System's ability to kill or remove organisms that are naturally  
25 occurring at the Test site, as well as standard test organisms (STOs) if used.

#### 26 *5.2.2.1 Total Organism Concentrations*

27 A minimum total input concentration of living organisms, by size class, is defined in Table 3.  
28 Ambient populations of each size classes will comprise a minimum of 75% of the total  
29 concentration. Note that concentration of ambient organism may be performed to reach the  
30 target concentrations. STOs (if used) may make up not more than 25% of the total per size class.  
31 Each size class must contain at least 5 different species from at least 3 phyla/divisions. Challenge  
32 water meeting these criteria shall be demonstrated for each test cycle at 1) the influent point of  
33 the control tank and 2) immediately prior to the point of treatment for systems that treat upon  
34 uptake or at the treatment tank influent point for systems that treat either wholly in-tank or upon  
35 discharge.

1 **Table 3. Minimum Criteria for Challenge Water Total Living Populations**

Organism Size Class	Total Concentration	Ambient Concentration (Minimum)	STO Concentration <sup>1</sup> (Minimum)	Diversity
≥ 50 μm	10 <sup>5</sup> organisms/m <sup>3</sup>	7.5 x 10 <sup>4</sup> organisms/m <sup>3</sup>	5 x 10 <sup>3</sup> organisms/m <sup>3</sup>	5 species across 3 phyla
≥ 10 μm and < 50 μm	10 <sup>3</sup> organisms/mL	7.5 x 10 <sup>2</sup> organisms/mL	50 organisms/mL	5 species across 3 phyla
<10 μm	10 <sup>3</sup> /mL as culturable aerobic heterotrophic bacteria. <sup>2</sup>	7.5 x 10 <sup>2</sup> organisms/mL	50 organisms/mL	5 species across 3 phyla

2 <sup>1</sup> If used.

3 <sup>2</sup> Note it is assumed that the effects on culturable aerobic heterotrophic bacteria will be indicative of the effects on  
4 all bacteria.  
5

6 **5.2.2.2 Standard Test Organism Populations<sup>3</sup>**

7 Standard test organisms are included for several reasons. The incorporation of STOs would  
8 provide each test and test site with a standardized minimum level of biological resistance to  
9 treatment, or robustness in organism, over a wide range of potential treatments. The use of cysts  
10 or spores as STOs would greatly increase the resistance, and would provide increased inter-site  
11 and intra-test comparability. STOs likewise may serve as a quality assurance check for the TF  
12 since the known input concentration of STOs can be compared with the discharge concentration.  
13 The use of STOs is not, however, intended to achieve minimum required population densities for  
14 valid tests. Recommended STOs are identified in Table 4, along with the recommended  
15 densities to be added to the challenge water.

16  
17 It is recognized that the use of STOs at a TF could be problematic as they may not be native to  
18 the TF's ambient organism populations and/or the effort required to use them may be  
19 impractical. If the STOs identified in Table 4 are unsuitable for use by the TF, alternatives may  
20 be considered and utilized with completion of validation experimentation and the concurrence of  
21 the VO. Facilities wishing to replace any of the recommended STOs with ambient or other  
22 organisms should conduct sufficient experimentation and provide evidence indicating a broad  
23 resistance to treatments. Collaborative research by Anderson et al. (2008) identified the  
24 recommended standard test organisms as a function of biological functional group and salinity,  
25 and similar methods should be used by the TF to determine site-specific replacements for those  
26 STOs.  
27  
28

<sup>3</sup> The practicality of using specific suite of organisms at all ETV test facilities is uncertain at this time, due in part to local restrictions on the use of non-native organisms, and in part due to the uncertainties and expense involved in preparing the necessary cultures. Test facilities should explore the potential for using STOs, as recommended in this section, and report the findings to the VO as part of the process for becoming a recognized TO.

1

**Table 4. Recommended Standard Test Organisms**

<b>Size Class</b>	<b>Marine/Brackish Water</b>	<b>Fresh Water</b>	<b>Minimum Concentration</b>
Zooplankton	<i>Artemia franciscana</i>	<i>Ostracod</i>	5 x 10 <sup>3</sup> organisms/m <sup>3</sup>
Protists	<i>Tetraselmis sp.</i>	<i>Green microalgae</i>	50 organisms/mL
Bacteria	<i>Geobacillus sp.</i>	<i>Geobacillus sp.</i>	50 organisms/mL

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The addition of STOs is not intended to achieve minimum required population densities. Where used, the test organism density shall not be less than 5% of the minimum living organism criteria for any given class size established in Table 3. These concentrations are to be achieved at the point of treatment or entry into the control tank. As such, the means or methods used to add these organisms to the challenge water should be validated on an independent basis to verify the TF capability to achieve the required concentrations when system or component effects on mortality unrelated to the treatment system are included.

Although the use of STOs can be a direct measure of treatment efficacy, facility-to-facility and test-to-test comparisons and internal QA/QC, use of inert objects, such as stained, killed organisms or microbeads of appropriate size, may be used for these purposes with concurrence of the VO.

Where STOs are used in the testing, safeguards and protocols to prevent the accidental release to the environment of any non-ambient organisms must be used in the test process. Further discussion of this is included in Chapter 9, Environmental, Health, and Safety Plan. Where STOs are used in the testing, safeguards and protocols to prevent the accidental release to the environment of any non-ambient organisms must be used in the test process. Further discussion of this is included in Chapter 9, Environmental, Health, and Safety Plan.

23

### **5.2.3 Challenge Water – Flow Rates and Volumes**

24

#### *5.2.3.1 Flow Rate*

25

26

27

28

29

Treatment tests will evaluate equipment at operational flow rates defined by the Vendor’s O&M manual. The TF shall be capable of providing flow rates of at least 200 m<sup>3</sup> per hour (880 gallons per minute) and an available volume per test cycle of at least 400 m<sup>3</sup>. The TF shall provide sufficient challenge water volume to meet these requirements, and the TQAP will identify the rates that will be tested.

30

#### *5.2.3.2 Volume*

31

32

33

34

A minimum of 200 m<sup>3</sup> shall be processed in each BE test cycle. The recommended minimum volume for in-tank testing is 200 m<sup>3</sup> (~52,800 gallons). The TF shall provide test and control ballast tank configurations of at least 200 m<sup>3</sup>. Larger volumes may be used depending on Vendor specifications and availability of tanks at the TF.

## 1 5.3 Test Facility Physical Configuration

### 2 5.3.1 Overall experimental configuration

3 As a minimum, the TF should encompass four components: (1) fluid delivery capacity, (2) a  
4 control tank and piping system, (3) a treatment tank and piping system, and (4) a discharge  
5 collection tank and post-test treatment system. The fluid delivery systems include pumps,  
6 piping, flow distribution controls, flow rate controls and relevant instrumentation to support the  
7 challenge water requirements described in Section 5.2. The control tank shall be utilized to hold  
8 untreated challenge water for each biological efficacy test cycle. The treatment tank will be  
9 utilized to hold all test water subject to the BWTS during the test cycle. Both tanks shall be a  
10 minimum of 200 m<sup>3</sup> and suitably constructed to hold such volumes for at least one day. The tank  
11 drains shall be located, to the extent possible, to minimize the retention of water following  
12 discharge. Tank intake and discharge piping, fittings and relative configurations shall be  
13 identical or the equivalent as validated by the TO. Finally, the discharge tank may be necessary  
14 if the TF is required to post-treat on-site the control and treated challenge or test waters to  
15 remove added inorganic and organic matter, disinfection by-products, or other regulated  
16 substances prior to discharge back to the environment. The discharge tank should be of  
17 sufficient volume to store at least 200 m<sup>3</sup>, but preferably large enough to store the cumulative  
18 volume of the control and treatment tank.

19  
20 There are multiple potential locations of ballast water treatment systems when used onboard  
21 vessels. The TF must be arranged to support testing of systems, which operate at uptake,  
22 discharge, in-tank or a combination of these. Examples of the arrangement for in-tank and in-  
23 line treatment are shown in Figures 1 and 2. As shown, the test configuration includes a flow-  
24 splitter such that challenge or test water is provided to both the control and treated legs  
25 simultaneously. Note that in such an arrangement, the fluid pumping capacity of the TF would  
26 be a minimum of 400 m<sup>3</sup>/hr. A sequential fill configuration may also be allowable, in which the  
27 treatment and control are filled or drained sequentially. The latter may result in reduced pump  
28 capacity needs (but still requires a minimum pump capacity of 200 m<sup>3</sup> per hour), less overall  
29 logistic complexity, and reduced piping through the dual use of sampling apparatus, feed and  
30 discharge plumbing, instrumentation and so on. In either case, the TF shall validate, to the  
31 satisfaction of the VO, that significant differences between treatment and control lines in  
32 biological and physical responses are minimal, and that there is no cross contamination by dual  
33 use of the site infrastructure.

### 34 5.3.2 Sampling Methodology

35 Several types of samples are to be acquired during the verification testing of a ballast water  
36 treatment system. During biological efficacy tests, discrete samples for water quality and  
37 biological enumeration shall be acquired over the course of the test on a time averaged basis. A  
38 minimum volume of 3 m<sup>3</sup> shall be collected per location. *In situ* instrumentation to monitor water  
39 quality and physiochemical parameters are also included. All sampling is assumed to be in-line,  
40 whether discrete or *in situ*. No instantaneous or grab samples are identified in this document  
41 either from system piping or from ballast tanks. Characterization of ambient waters may require  
42 these types of sampling.

1 **5.3.2.1 Sampling Locations**

2 Required sample locations for various treatment scenarios are shown in Figures 1 and 2; samples  
 3 should be collected according to one of these test designs, unless otherwise accepted by the VO  
 4 in the TQAP. Samples (data) from the challenge water must be obtained, in accordance with the  
 5 guidance in Section 5.3.2.4, immediately prior to water entry to the Control tank, and  
 6 immediately before entry to the BWTS (in-line treatment) or the ballast tank in the case of in-  
 7 tank treatments (if demonstrated as representative of the control and challenge water, a single  
 8 sample collected ahead of the splitter shown in Figures 1 and 2). For in-line BWTSs, samples of  
 9 treated water must be collected (1) immediately following the treatment system and (2)  
 10 following the holding tank at the end of the one-day hold time. For in-tank treatments, samples  
 11 of treated water must be collected from the ballast tank discharge following the Vendor defined  
 12 contact period. Further definition of hold times is described in Section 5.4.5. Systems that  
 13 incorporate treatment at multiple locations (e.g., upon uptake and discharge) will only require  
 14 sampling after the final stage of treatment. Sampling locations for the control tanks and BWTS  
 15 must exactly mimic the treatment tanks and System. Finally, in-tank sampling via plankton net  
 16 tows shall not be utilized for the purposes of verifying biological efficacy, as this method may  
 17 not result in representative samples. The exact locations, frequency, and methods to be used to  
 18 collect the samples must be defined in the TQAP.

19 **5.3.2.2 Sample Collection Requirements - Frequency**

20 Continuously recording *in situ* sensors (as available and feasible) may be used to measure water  
 21 quality and proxy parameters during Verification Testing. Description of the sensors, how they  
 22 operate, and how they are calibrated shall be included in the TQAP. Minimum instrument  
 23 performance requirements are provided in Table 5. Discrete samples for water quality  
 24 characterization will also be obtained during Verification Testing as discussed above, and they  
 25 will be collected at the time biological verification samples are collected. A higher frequency of  
 26 collection for discrete samples may be used if additional samples for calibrating the sensors are  
 27 necessary. The sample collection requirements and frequency of obtaining samples from the  
 28 control tanks and piping system will identically match those of the treatment tanks and system.  
 29 The appropriate frequency of discrete sample collections made in lieu of *in situ* sensing shall be  
 30 described in the TQAP.

32 **Table 5. Accuracy and Precision Requirements for Potential Sensors**

Sensor	Reporting Units	Range	Accuracy	Precision
Temperature	°C	0 to 30	0.1	0.01
Conductivity (salinity)	MS/cm	0.5 to 65	0.1	0.01
Transmissometer (20-cm)	per m	0 to 40	0.20	0.01
Dissolved oxygen	mg/L	0 to 20	0.10	0.05
Fluorometer	µg/L	0.03 to 75	50% of reading <sup>1</sup>	0.01

33 <sup>1</sup> When compared to wet chemistry results.

1 *5.3.2.3 Sample Replication*

2 Verification Testing will include replication only in analysis. Sample collection replicates are  
3 based on the time integrated sample volumes collected during the test cycle (see examples shown  
4 in Figures 1 and 2. These sample volumes form the minimum sample collection replication  
5 required during each Test cycle. Each of the integrated sample tanks will be sub sampled for the  
6 Core parameters, which are discussed later. The TQAP will describe each type of analytical  
7 replication planned, including acceptable ranges of variability.

8 *5.3.2.4 In-line Sampling for Biological Efficacy*

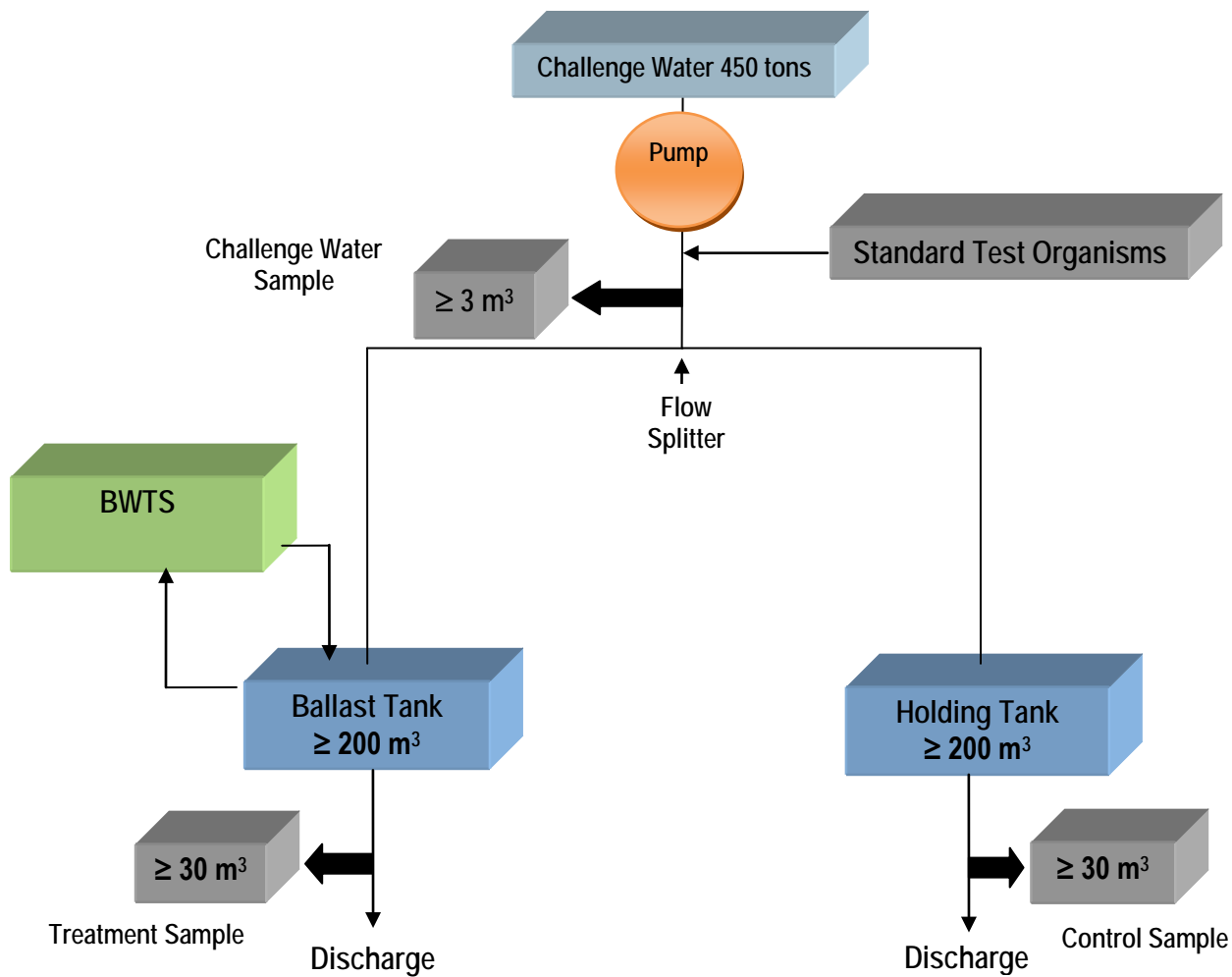
9 To obtain an accurate measurement of the organism concentration at the sample location, the  
10 installation of an isokinetic sampling facility at each of these locations is recommended.  
11 Isokinetic sampling is primarily intended for the sampling of water mixtures with secondary  
12 immiscible phases (i.e., sand or oil) in which there are substantial density differentials. In such  
13 conditions, convergence and divergence from sampling ports is of significant concern. Since  
14 most organisms are relatively neutrally buoyant, true isokinetic sampling is likely unnecessary  
15 for testing ballast water treatment systems. Nonetheless, the mathematics related to isokinetic  
16 sampling are deemed useful for describing and specifying appropriate sampling geometries.  
17 Isokinetic sampling is necessary to ensure that a sample contains the same proportions of the  
18 various flowing constituents as the flow stream being sampled. During isokinetic sampling the  
19 sampling device does not alter the profile or velocity of the flowing stream at the moment or  
20 point at which the sample is separated from the main flow stream. To achieve isokinetic  
21 sampling conditions, a sampler is designed to separate a subsection of the total flow-stream in a  
22 manner that does not encourage or discourage water entry other than that which is otherwise in  
23 the cross-section of the sampler opening. In other words, flow streams in the main flow of the  
24 pipe should not diverge or converge as they approach the opening of the sampler.

25  
26 Recommendations for the design and installation of appropriate sampling facilities are given  
27 below. In any case, validation of the Test Facilities configuration should include verification that  
28 the chosen sampling design, geometry and installation result in representative samples and  
29 minimize organism mortality as a result of sample acquisition.

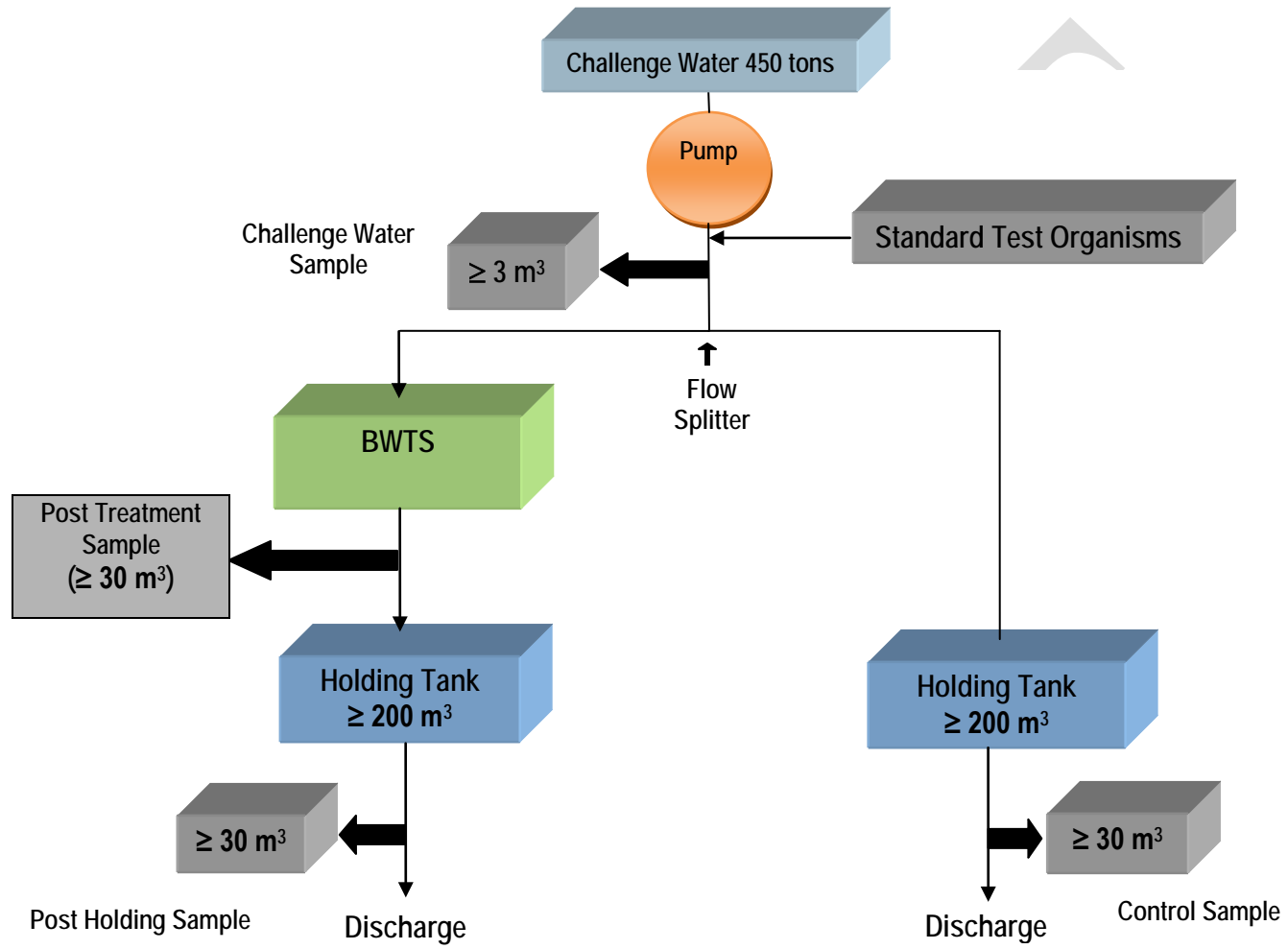
30 *5.3.2.5 Design of In-line Sampling Apparatus*

31 Through computational fluid dynamics modeling, it has been shown that the isokinetic diameter  
32 calculation can provide guidance for sizing of sample ports for sampling of organisms (Richard  
33 et al., 2008). Simulations showed that flow transitions from the main stream were best for sample  
34 port diameters between 1.5 and 2.0 times the isokinetic diameter. Ports sized in this range had  
35 smooth transitions and pressure profiles that allowed for direct sampling without the need of a  
36 pump to induce sample collection. The isokinetic sample port diameter should therefore be  
37 determined generally according to the equation:

38  
39 
$$D_{iso} = D_m \sqrt{\frac{Q_{iso}}{Q_m}}$$



**Figure 1. Sampling design example for in-tank treatment.**



**Figure 2. Sampling design example for in-line treatment.**

1 where  $D_{iso}$  and  $D_m$  are the diameters of the sample port opening and the main flow in the line to  
2 be sampled, respectively; and  $Q_{iso}$  and  $Q_m$  represent the respective volumetric flow rates through  
3 the two pipes. It is recommended that sample port size be based on the combination of maximum  
4 sample flow rate and minimum ballast flow rate that yields the largest isokinetic diameter.

5  
6 The opening of the sampling pipe should be chamfered to provide a smooth and gradual  
7 transition between the inside and outside pipe diameters. The length of the straight sample pipe  
8 facing into the flow can vary, but it should not usually be less than one diameter of the sampling  
9 pipe. The sampling port should be oriented such that the opening is facing upstream and its  
10 entrance leg flow is parallel to the direction of main pipe flow and concentric to the larger pipe,  
11 which may require sampling pipes to be “L” shaped with an upstream facing leg, if installed  
12 along a straight section of discharge pipe.

13  
14 The need to be able to service the sample pipe is important and should be considered, taking  
15 safety into consideration. Therefore, the sampling pipe should be retrievable either manually or  
16 mechanically, or it must be in a system that can be isolated. Because of the potential for the  
17 opening and interior of the sample pipe to become occluded by biological or inorganic fouling, it  
18 is recommended that samplers be designed to be closable at the opening, removed between  
19 sampling intervals, or be easily cleaned prior to sampling.

20  
21 The sample pipe and all associated parts of the sampler that come into contact or near proximity  
22 with the system piping should be constructed of galvanically compatible materials and generally  
23 corrosion resistant. Any corrosion of the sampling system will affect sample flow rates and  
24 potentially sample representativeness.

25  
26 If flow control of the sample flow rate is required, ball, gate, and butterfly valve types should be  
27 avoided as they may cause significant shear forces, which may result in organism mortality. For  
28 flow control, it is recommended that diaphragm valves or similar valve types be used to  
29 minimize sharp velocity transitions. For flow distribution, ball valves may be utilized only if  
30 they are either fully open or fully closed

31  
32 When sampling is conducted on the discharge of a tank through the use of a pump (i.e., a non-  
33 gravity drain) and the sample port is located upstream of the pump, it may not be possible to  
34 draw an adequately sized sample since the line will be under suction with a variable hydrostatic  
35 pressure head. Therefore, maintenance of a time-averaged sample flow requires the sample to be  
36 drawn from the discharge utilizing a pump. In such cases, a diaphragm pump is recommended to  
37 minimize pump-induced organism mortality during sampling.

#### 38 *5.3.2.6 Installation of an In-line Sample Point*

39 The sample taken should be removed from the main pipeline at a location where the flowing  
40 stream at the sample point is representative of the contents of the stream. The sample port  
41 entrance should be placed at a point where the flow in the main pipe is fully mixed and fully  
42 developed.

43  
44 The sampling point should be installed in a straight part of the system piping and the sampling  
45 fixture should be positioned such that a representative sample of ballast water is taken. It is

1 recommended that the position of the sample point be determined using methods such as  
2 computational fluid dynamics.

### 3 *5.3.2.7 Operation of an In-line Sample Points*

4 In-line biological samples will be collected on a time-integrated basis such that a composite  
5 sample of the entire period of uptake or discharge is acquired. The sample flow rate should be  
6 appropriately controlled to maintain an even distribution of samples acquisition over that time  
7 period.

## 8 **5.3.3 Test Organism & Water Quality Augmentation**

9 Where the addition of test organisms (either augmentation of ambient organisms or standard test  
10 organisms) is required for Biological Efficacy testing, a method for the injection or addition of  
11 test organisms to the challenge water must be provided. Similarly, water quality parameters that  
12 require adjustment from the ambient conditions to the requisite challenge water properties will  
13 require some type of injection process. In the case of test organisms, various means are available  
14 to inject or add organisms to the challenge water, for instance, by a batch method to a large,  
15 discrete source volume or by injection into the flow stream. In any case, the following  
16 requirements are applicable:

- 17
- 18     ▪ Any test organism addition or injection method must minimize, to the extent possible,  
19     organism mortality as a result of its addition/injection mechanism.
- 20     ▪ The method must result in a well-mixed and uniform distribution, spatially and  
21     temporally, of standard test organisms within the challenge water and at its introduction  
22     at the point of treatment or tank intake.
- 23     ▪ The concentration of living test (if used) organisms at the point of treatment or tank  
24     intake must conform to the requirements given in Section 5.2.2.
- 25     ▪ The point of addition or injection must be situated such that the flow is well mixed at the  
26     subsequent point from which the first discrete sample is acquired to ensure a  
27     representative sample is obtained; inclusion of substantial pipe lengths and/or a static  
28     mixer may need to be considered.
- 29     ▪ All methods for the injection or addition of test organisms must be validated by the TF to  
30     meet the above requirements.
- 31

32 For water quality additions, for example sediments or dissolved organics, the addition should  
33 occur far enough upstream from the point of water quality sampling to ensure that the sample is  
34 well mixed. Furthermore, the apparatus used for addition should minimize the system related  
35 mortality on ambient and test organisms, to the extent possible.

## 36 **5.3.4 Control & Instrumentation**

37 The testing described throughout this protocol is complex and logistically challenging.  
38 Moreover, since these tests are designed to provide a repeatable and accurate verification of a  
39 Vendor's claims, it is of the utmost importance to ensure that each phase and measurement is  
40 conducted with a high degree of reliability, repeatability, and accuracy. Accomplishing such  
41 ends is further complicated by the inclusion of biological organisms and related measurements,  
42 which result in a variety of design and timing complexities. As a result, it is recommended that  
43 the TF include a typical supervisory control and data acquisition (SCADA) system to support the

1 many operations and data acquisition tasks associated with this testing. A typical SCADA  
2 system provides the TF with the ability to: 1) provide automatic control of pumps, valves and  
3 sub-systems to maintain operational set points; 2) acquire and archive all events, data and  
4 conditions; 3) provide controllable process control algorithms which improve system efficiency,  
5 safety and repeatability; and 4) provide facility- and treatment-system diagnostics during  
6 commissioning, testing, and upset conditions. Instituting such a system can be expected to  
7 improve measurements, quality assurance, standardized reporting, and reduce labor and analysis  
8 time.

9  
10 Whether a SCADA system is utilized or not, the TF should include within its QAPP a discussion  
11 of how the instrumentation associated with TF operation, process control (either manual or  
12 automatic), and condition monitoring of the verification tests shall be operated, maintained and  
13 calibrated. Also, as a minimum, the TF shall include sufficient instrumentation and condition  
14 monitoring such that a substantive record is established which verifies that 1) challenge  
15 conditions were obtained and maintained, 2) the treatment system was operated in accordance  
16 with the Vendor's requirements and 3) no system or environmental effects occurred to perturb  
17 the verification test or treatment system operation. The test instrumentation and test operating  
18 procedures shall be documented in the TQAP.

#### 19 **5.4 Verification Testing**

20 Verification testing will be separated into three distinct phases, 1) Treatment System  
21 Commissioning, 2) Biological Efficacy (BE) Tests, and 3) Operating and Maintenance (O&M)  
22 tests. Commissioning tests are intended to validate, prior to the commencement of either BE or  
23 O&M tests, that the treatment system is installed correctly and operating in accordance with the  
24 Vendor's requirements. A minimum of three BE tests shall be completed at each of two  
25 salinities selected by the Vendor (the Vendor may complete testing at all three salinities if  
26 desired) and with all of the challenge conditions specified in Section 5.2 to assess and verify the  
27 biological efficacy of the treatment system under pre-established conditions. O&M testing shall  
28 be conducted with ambient source water conditions, with the intention of operating the system  
29 with realistic physical conditions, to assess the systems engineering performance.

30  
31 Ballast water treatment system performance, operating conditions, and certain O&M criteria will  
32 be recorded and monitored during verification testing by the TO. Results will be presented in the  
33 Verification Report, described in Chapter 6. The factors to be verified during ballast water  
34 treatment system verification testing include: biological treatment performance, operation and  
35 maintenance, predictability/reliability, cost factors, environmental acceptability, and safety.

36  
37 Any of several treatment sequences may be used by a particular treatment system (see Table 6),  
38 including in-line treatment (during ballasting or deballasting), in-tank treatment, or a  
39 combination of the two. The stage in the ballasting cycle at which treatment is applied may also  
40 vary. This verification testing protocol accounts for these through flexibility in the TF and  
41 Verification TQAP. The guidance in the following section provides the basic test requirements  
42 and rationale for inclusion in the TQAP that will provide details specific to the treatment system  
43 and its operation.

1 **Table 6. Likely Treatment Sequences and Applications Inherent to Ballast Operations**

Sequence Number	Ballast Operation Application
1	Treatment applied during ballasting/ No treatment during deballasting.
2	Treatment applied during ballasting/ Treatment applied during deballasting.
3	No treatment applied during ballasting/ Treatment applied during deballasting.
4	No treatment applied during ballasting/ Treatment applied during transit/ No treatment during deballasting.
5	No treatment applied during ballasting/ Treatment applied during transit/ treatment during deballasting.
6	Treatment applied during ballasting/ Treatment applied during transit/ No treatment applied during deballasting.
7	Treatment applied during ballasting/ Treatment applied during transit/ Treatment applied during deballasting.

2

3

4 The over-arching objectives of the verification testing (including all phases) are to:

5

- 6 ■ Evaluate the treatment performance of the ballast water treatment system relative to the removal or kill of ambient and test organisms (if used), operating under Vendor-specified conditions;
- 9 ■ Evaluate the treatment system O&M criteria;
- 10 ■ Determine and record cost factor data; and
- 11 ■ Record and document test conditions, observations, and results.

12

13 Other testing objectives may be defined by the Vendor and included in the TQAP. The requirements for Verification Testing are described in the following sections and must be addressed in the TQAP.

14

#### 16 **5.4.1 Treatment System Commissioning**

17 The TQAP shall describe all the tests and start-up requirements required to validate that the treatment system is installed correctly and operating in accordance with the Vendor’s requirements. The objectives of the Commissioning are to:

18

- 21 ■ Install and start the ballast water treatment system in accordance with the Vendor O&M manual
- 23 ■ Reach stable operating conditions
- 24 ■ Make modifications as needed to ensure stable operations under TF condition
- 25 ■ Record and document all installation and start-up conditions, observations and results

1  
2 The treatment system shall be installed at the TF according to the Vendor instructions included  
3 in the TQAP. Ideally, this phase of the verification will include close coordination between the  
4 Vendor and TO to quickly resolve discrepancies or malfunctions. Commissioning tests may  
5 include small-scale tests of various Vendor sub-systems or components, validation of treatment  
6 system integration into the TF (e.g., a leak test or communication tests), or any other Vendor-  
7 required installation tests that may be expected during a shipboard installation. However, at least  
8 one valid, full-scale verification test cycle, meeting all of the requisite challenge conditions,  
9 should be conducted successfully by the TO without Vendor assistance. While the challenge  
10 conditions are to be employed during Commissioning Tests, it is not necessary to conduct a  
11 complete suite of analytical measurements to assess biological treatment efficacy.  
12

13 A successful Commissioning is defined as one in which (1) all TF requirements and conditions  
14 defined by the challenge conditions were met and (2) all components of the Treatment system  
15 operated in accordance with the Vendor requirements. Subsequent BE and O&M verification  
16 testing cannot commence until Commissioning is successfully completed and agreed upon by the  
17 TO and Vendor. The Verification Report should document all of the small-scale, component-  
18 level tests conducted and their results, any treatment system or TF deficiencies or failures, and  
19 their successful resolution. Finally, the Verification Report should document in detail the  
20 challenge conditions during the full-scale Commissioning verification test cycle.

#### 21 **5.4.2 Operation and Maintenance (O&M) Manual**

22 The O&M manual shall be incorporated into the TQAP and will be essential to the development  
23 of the monitoring and maintenance plan incorporated in the TQAP. The Vendor shall identify  
24 factors that affect the operation of the BWTS, including any warm up or other requirements that  
25 must be completed to achieve operational stability. The Vendor's O&M manual shall specify  
26 what constitutes stable operating conditions for the BWTS, factors that may affect operating  
27 conditions, and any adjustments required to reach or to maintain a stable operating condition.  
28 Adjustments made in the operating conditions will be presented in the final Verification Report.

#### 29 **5.4.3 Vendor and TO Requirements**

30 An installation/start-up work plan shall be prepared and included as part of the TQAP. The TO  
31 shall conduct start-up procedures for the BWTS in accordance with this installation/start-up plan  
32 and with the Vendor O&M manual. At the end of the start-up period, the TO will assess whether  
33 the BWTS is in a stable operating state, as specified in the O&M manual, and the Vendor will  
34 certify in writing that the System is installed and operating as intended. If the operation is stable,  
35 the verification testing can begin. If not, start-up procedures will be repeated no more than two  
36 additional times. If the BWTS does not achieve stable operating conditions after three start-up  
37 cycles, the TO, in conjunction with the Vendor, will review the start-up work plan for  
38 applicability and determine where adjustments and modifications are required. In any case, the  
39 TO will have the option of concluding or postponing further testing at the conclusion of three  
40 failed start-up cycles.  
41

42 The Vendor will identify any additional equipment, System maintenance, changes to operating  
43 conditions, or other modifications needed to ensure effective BWTS operation and to attain or  
44 maintain stable operational conditions.

1 **5.4.4 Toxicity Testing for Biocide Treatments**

2 The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, requires  
3 registration by the U.S. Environmental Protection Agency (EPA) of pesticides sold or used in the  
4 United States, which includes biocide products that might be used in BWTSs. The Vendor is  
5 required to provide information regarding FIFRA registration of any biocide to be used in their  
6 BWTS.

7  
8 The residual toxicity in the discharge from BWTSs employing a biocide is of concern to the TF  
9 (as part of the TF's NPDES permit requirements), as well as for the environmental acceptability  
10 of the treated ballast water from the BWTS in use. Toxicity testing of the water following  
11 treatment shall be conducted during the Commissioning phase of the verification testing  
12 according to the toxicity methods cited in Section 5.4.7.5. If the post-treatment effluent passes  
13 the toxicity tests, then verification testing can proceed. If, however, the effluent fails the toxicity  
14 test, verification testing shall not be initiated, and further toxicity tests shall be required. The  
15 Vendor shall be allowed no more than two additional attempts to pass the toxicity tests within 30  
16 days of the initial test. This may require modifications to the approach for verifying the  
17 technology in the TQAP or other investigations to understand the toxicity response. In the event  
18 a TF's NPDES permit requires a toxicity evaluation of the treated waters at the end of each test  
19 with the addition of a biocide, or if the Vendor requests additional toxicity testing during the  
20 verification, the TQAP shall address the additional testing.

21 **5.4.5 BE and O&M Verification Strategy: Test Duration and Coordination**

22 A minimum of three valid BE tests, described in detail below, are required for each salinity  
23 regime (defined in Section 5.2.1) under which the treatment system is verified. At a minimum,  
24 testing at two salinity conditions shall be conducted. In addition, O&M testing of the treatment  
25 system shall distribute testing of a minimum treated volume of 10,000 m<sup>3</sup> amongst the BE test  
26 cycles. These O&M test cycles are equivalent to ~50 hours of operation at 200 m<sup>3</sup> per hr (or  
27 ~65 hours of operation at 150 m<sup>3</sup> per hr). Upon completion of the Commissioning verification  
28 tests, the next verification test shall be a BE test cycle. This sequence allows the testing to  
29 validate operation of a new unit prior to substantial operational testing. For example, for the case  
30 in which 6 BE test cycles will be conducted, each BE test cycle should be separated by 2,000 m<sup>3</sup>  
31 in O&M testing. This approach also involves a substantial duration for the testing period and  
32 associated range of ambient water conditions over this time.

33  
34 During actual shipboard operation, ballasting procedures may occur over time periods ranging  
35 from minutes to hours. For each in-line treatment BE or O&M verification cycle, a minimum  
36 operational period of one (1) hour is required, although this may be extended if flow rates are  
37 reduced from 200 m<sup>3</sup>/hr.

38  
39 In addition to the uptake time, a minimum 1-day holding time within both the treatment and  
40 control tank is required for each BE test cycle to simulate the time that water would reside in a  
41 ballast tank. Thus, the duration of each test cycle will be defined by the operational approach  
42 used by the treatment system. The holding time of the required BE test cycles may be extended  
43 if the Vendor requires such time as part of the BWTS or process.

1 The holding time included in this protocol is intended to provide a conservative assessment of  
 2 the BWTS's ability to treat ballast water according to the Vendor's claims. For in-tank treatment  
 3 with additional in-line treatment during ballast water discharge, the duration will be equal to in-  
 4 tank treatment requirements and the deballasting time. Regardless, subsequent to the 1-day tank  
 5 holding time, the control tank discharge must exhibit a minimum concentration of living  
 6 organisms, as defined in Table 7. These criteria are necessary, in addition to the input Challenge  
 7 conditions, to constitute a valid BE test cycle. These control tank discharge concentrations are  
 8 intended to make certain that treatment efficacy measurements attributed to the BWTS in any  
 9 given BE test cycle are not the result of natural or non-treatment system related effects.

11 **Table 7. Criteria for Concentrations of Living Organisms in Control Tank Discharge**

Organism Size Class	Minimum Concentration
$\geq 50 \mu\text{m}$	100 organisms/m <sup>3</sup>
$\geq 10 \mu\text{m}$ and $< 50\mu\text{m}$	100 organisms/mL
$< 10 \mu\text{m}$	5 x 10 <sup>2</sup> /mL as culturable aerobic heterotrophic bacteria

12  
 13  
 14 Shorter or longer tank hold times may be utilized but must be justified in the TQAP.  
 15 Justifications for shorter tank hold times may include an inability to sustain organism  
 16 populations in the control tank to achieve the requirements in Table 7 because of natural  
 17 mortality. In such cases, tank hold times may be shortened, as appropriate and agreed upon, such  
 18 that an adequate assessment of the BWTS treatment efficacy may be made.

19  
 20 For in-tank treatments, test duration will include the minimum contact time the Vendor  
 21 prescribes for effective treatment, but not less than a cumulative 1-day holding time for each of  
 22 the required BE test cycles. As with the in-line approach, testing of the BWTS without active  
 23 ingredients may be run in parallel with the challenge test to reduce the overall duration of the  
 24 verification test. Modifications may be made according to Vendor-specified requirements for  
 25 treatment, but they must be justified in the TQAP. For example, if holding water for a specified  
 26 time after the treatment's minimum contact time is required by the Vendor, that time interval  
 27 would be added to each verification test incorporating challenge organisms. For combinations of  
 28 in-tank and in-line treatment, test duration will be equal to treatment time (in-line plus in-tank).

29  
 30 The O&M test cycles will provide data on the system's operation and support the assessment of  
 31 non-biological verification factors. In the case of in-tank treatment approaches, particularly  
 32 those using biocides or other chemical/physical means of achieving treatment, the TQAP may  
 33 elect to operate the BWTS during O&M cycle either eliminating or reducing dosage of the active  
 34 agent (i.e., to verify the electro/mechanical aspects of the BWTS). In some cases, it may be  
 35 possible to use inert substances in place of treatment chemicals to reduce the need for  
 36 conditioning prior to discharge back to the environment. Any such substitution must mimic the  
 37 operation of the BWTS when using treatment chemicals and must be agreed to by the VO.

1 **5.4.6 Biological Efficacy (BE) Verification Testing**

2 As discussed above, a minimum of three BE test cycles per salinity regime will be conducted;  
3 each having a minimum tank holding time of 1-day and having input challenge conditions as  
4 described in Section 5.2. The BE verification test cycles are intended to measure the efficacy  
5 with which the treatment system removes or kills organisms under challenging conditions. The  
6 remainder of this section provides the detailed description of test parameters, measurements, and  
7 analyses related to assessing biological efficacy and monitoring challenge water conditions. Due  
8 to the nature of the verification tests, a set of Core and Auxiliary measurement parameters will  
9 apply to each BE Verification Test. Core and Auxiliary parameters, sampling location, and  
10 sample/measurement approach are shown in Table 8. Core parameters are those that are required  
11 during each BE test cycle and are the minimum measurements required to verify the Vendor’s  
12 claims regarding treatment efficacy and the validity of the BE test cycle. Auxiliary parameters  
13 are: (1) useful indicators of core parameters, (2) required by the Vendor or VO, or (3) otherwise  
14 advisable to assess test validity or treatment efficacy. Guidance on sampling methods, sample  
15 volumes, sample container type, preservation method, and maximum holding time for each  
16 parameter is shown in Table 9.

17  
18 The TO, in conjunction with the TF and the Vendor, will assess the use of continuous, *in situ*  
19 (inline) biological or other process measurements during verification testing. Any selected  
20 methods must be described and justified in the TQAP and approved for use by the VO.

21  
22 The TO shall present a detailed schedule for Verification Test sample collection and analytical  
23 methods in the TQAP. At a minimum, the TQAP shall contain the scheduled sample collection  
24 times (expressed as time from start of test), parameters for testing, number of replicates, and  
25 number of control samples.

26 **5.4.6.1 Water Quality Parameters & Analysis**

27 Water quality samples shall be collected as described in Section 5.3.2.1 and defined in the  
28 TQAP, with the volumes described in Table 9. Note that some analyses, following methods  
29 described in Table 10, must be performed within 6 hours of the sample collection. In cases where  
30 water quality samples can be stored for appropriate time periods, TO logistics may warrant  
31 outsourcing of water quality analyses to an independent, qualified laboratory, with agreement by  
32 the VO. Reliable, continuously recording *in situ* sensors are available for temperature, salinity,  
33 dissolved oxygen, chlorophyll *a*, and turbidity. Such sensors may, with VO approval, be used to  
34 measure water quality parameters during verification testing. Discrete analytical samples shall  
35 be collected to provide test-specific verification or calibration of the sensor data and to allow  
36 comparison of sensor data to Vendor-supplied information as appropriate. Sensor maintenance  
37 and calibration shall be described in the test site operating procedures and the TQAP. Data  
38 quality objectives for quality control and quality assurance purposes are provided in Table 11.  
39 These data quality objectives and the related QA/QC measures should be discussed in the QAPP  
40 of the TQAP as described in Appendix A.

1

**Table 8. Core and Potential Auxiliary Parameter and Measurement Techniques**

Parameter	Measurement Class	Sample Location and Approach <sup>1,2</sup>	
		Challenge Water	Post Treatment
Temperature	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Salinity	Core	<i>In situ</i> or Discrete grab	<i>In situ</i> or Discrete grab
Total suspended solids	Core	Discrete grab	Discrete grab
Particulate organic matter	Core	Discrete grab	Discrete grab
Dissolved organic matter	Core	<i>In situ</i> , continuous, discrete	<i>In situ</i> , continuous, discrete
Dissolved oxygen	Core	<i>In situ</i> , discrete	<i>In situ</i> , discrete
pH	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Ambient Organism Concentration	Core	Discrete	Discrete
Standard Test Organism Concentration (if used)	Core	Discrete, continuous	Discrete
Ballast System Flow Rate	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Ballast System Pressure	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Sampling Flow Rates	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Chlorophyll <i>a</i> (biomass)	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Dissolved nutrients (N, P, Si)	Auxiliary	NA	Discrete
Turbidity	Auxiliary	<i>In situ</i> , continuous	<i>In situ</i> , continuous
ATP (living material)	Auxiliary	Continuous as available	Continuous as available

2

3

4

5

6

7

8

<sup>1</sup> *In Situ* = in-line or in-tank measurements, Discrete Grab = an acquired sample for analysis at a specific place and time, Continuous = measurement is continuous throughout the period of operation at some defined rate.

<sup>2</sup> The frequency and means for calibrating and validating performance of *in situ* monitoring devices must be addressed in the TQAP.

1 Discrete samples for determination of total suspended solids, particulate organic matter (as  
2 carbon, POM), total dissolved organic matter (as carbon, DOC), and nutrient concentrations shall  
3 be collected appropriate to the tests being conducted. The concentration of mineral matter may  
4 be determined as the difference between the total suspended solids and the particulate organic  
5 matter concentration (mass per liter basis). In addition, when appropriate, samples should be  
6 acquired or measurements made *in situ* to measure residual toxicity or the concentration of  
7 chemical residuals or disinfection by-products. Guidance for such measurements and sample  
8 collection are highly dependent on the chemicals of interest or in use; a qualified laboratory  
9 should be consulted for the appropriate handling and measurement methods.

10 The analytical methods must be applied within defined holding times (Table 9) after appropriate  
11 preservation, per industry standard procedures. Where available, US EPA, *Standard Methods* or  
12 other methods (i.e., ASTM) approved by the VO will be used to quantify each parameter. If  
13 standardized methods are not available, the sampling and analytical methods to be used shall be  
14 documented in the TQAP. These methods will follow accepted scientific practices and be  
15 accepted by the VO.

16 *5.4.6.2 Biological Parameters*

17 Biological samples will be collected using methods and techniques appropriate to the size class  
18 and anticipated concentration being measured. The samples for biological analyses will be  
19 acquired from each of the time integrated sample volumes acquired during the test cycle. The TO  
20 will ensure that the contents of the integrated sample collection tanks have been thoroughly  
21 mixed to ensure homogeneity prior to sub-sampling.

22  
23 The abundance of living ambient and test organisms (if used) will be quantified in (1) the uptake  
24 challenge water just prior to treatment and entry into control tank, (2) the discharge of the control  
25 tank after the appropriate hold time, (3) the discharge following an in-line BWTS and (4) the  
26 discharge from the holding tank (for both types of treatment) of treated water after the  
27 appropriate holding time. In the case of the control and treated discharge, biological samples  
28 will be retrieved from a point upstream of any pumps or significant components which could be  
29 expected to affect mortality or sample representativeness.

30 *5.4.6.2.1 Sample Volumes & Data Quality*

31 Samples from the discharges of successful treatments will likely have low concentrations of  
32 organisms. Enumeration of the organisms from these samples (determined from 20 one-mL  
33 subsamples from a concentrated whole water sample, as described below) is represented by the  
34 Poisson distribution, and therefore the cumulative or total count is the key test statistic (Lemieux  
35 et al., 2008b). Further, a chi-square transformation can be utilized to approximate the confidence  
36 intervals.

37

**Table 9. Sample Volumes, Containers and Processing**

	Parameter	Min. Sample Volume (mL) <sup>1</sup>	Sample Containers <sup>3</sup>	Processing/ Preservation <sup>3</sup>	Maximum Holding Time
Core Parameters	Electronic <i>in situ</i> data (Temperature, pH, Salinity, etc.) <sup>2</sup>	NA	NA	Maintain digital archive.	NA
	Total suspended solids	100	250 mL HDPE or glass	Process immediately or store at 4°C.	1 week
	Dissolved organic carbon	25	40-mL glass vial	Pass sample through a GF/F; freeze filtrate until analysis.	28 days
	Particulate organic carbon	500	Whatman GF/F in foil	Pass through a GF/F; freeze filter until analysis.	28 days
	Dissolved oxygen	300	300 mL glass BOD bottle	Fix per Oudot et al. (1988); titrate 2-24 h later.	24 hours
	Phytoplankton Enumeration (Live/Dead Analysis) <sup>4</sup>	3 m <sup>3</sup> concentrated to 1000 mL	Dark 1000-mL HDPE bottle	No preservation; stain with Fluorescein Diacetate and CMFDA as described in the protocol	Process immediately
	Zooplankton Enumeration (Live/Dead Analysis) Low Concentration/Discharge	3 m <sup>3</sup> concentrated to 1000 mL	1 L flask	No preservation; Sub-sample into well plate (20 1-mL wells observed). Observe with dissecting microscope and probe organisms to determine live/dead status. Fix with Lugol's solution for total counts.	Process immediately
Bacteria	1000	1 L sterile HDPE	Plate on appropriate media.	Process immediately.	
Auxiliary Parameters	Dissolved inorganic nutrients	40	60-mL polyethylene bottle	Pass through a Nuclepore™ (Whatman Inc., Piscataway, NJ) membrane filter; freeze filtrate until analysis.	28 days
	Chlorophyll <i>a</i> and phaeopigments	400	Whatman GF/F in foil	Pass through GF/F; fix with a saturated MgCO <sub>3</sub> solution; freeze filter until analysis.	4 weeks

GF/F: pre-ashed glass fiber filter.

<sup>1</sup> Volume processed for analysis; volumes are quantitative.

<sup>2</sup> Conductivity, temperature, pressure, dissolved oxygen, chlorophyll *a* fluorescence, transmissometry.

<sup>3</sup> Name brand items (e.g., Nuclepore™, Whatman™) may be substituted with comparable items from a different manufacturer.

<sup>4</sup> Dinoflagellate methods are under development.

**Table 10. Recommendation for Water Quality Sample Analysis Methods**

<b>Parameter</b>	<b>Units</b>	<b>Instrument</b>	<b>Method/Reference</b>
<b>Dissolved ammonium</b>	μM	Autoanalyzer	APHA Standard Method No. 4500-NH3 / 20 <sup>th</sup> edition EPA Method No. 349.0 <a href="http://www.epa.gov/nerlcwww/m349_0.pdf">http://www.epa.gov/nerlcwww/m349_0.pdf</a>
<b>Dissolved inorganic nitrate and inorganic nitrite</b>	μM	Autoanalyzer	ESS Method No. 220.3 <a href="http://www.epa.gov/glnpo/lmmb/methods/methd220.pdf">http://www.epa.gov/glnpo/lmmb/methods/methd220.pdf</a> APHA Standard Method Nos. 4500-NO2-B and 4500-NO3-F, 19 <sup>th</sup> edition EPA Method No. 353.4 <a href="http://www.epa.gov/nerlcwww/m353_4.pdf">http://www.epa.gov/nerlcwww/m353_4.pdf</a>
<b>Dissolved inorganic phosphate</b>	μM	Autoanalyzer	ESS Method No. 310.1 <a href="http://www.epa.gov/glnpo/lmmb/methods/methd310.pdf">http://www.epa.gov/glnpo/lmmb/methods/methd310.pdf</a> EPA Method No. 365.5 <a href="http://www.epa.gov/nerlcwww/m365_5.pdf">http://www.epa.gov/nerlcwww/m365_5.pdf</a>
<b>Dissolved inorganic silicate</b>	μM	Autoanalyzer	EPA Method 366.0 <a href="http://www.epa.gov/nerlcwww/m366_0.pdf">http://www.epa.gov/nerlcwww/m366_0.pdf</a>
<b>Dissolved organic carbon</b>	μM	Carbon Analyzer	APHA Standard Method No. 5310-C, 20 <sup>th</sup> edition ASTM Method Nos. D6317, D2579, D4129, D4839, D513-02 and D5790  LMMB Method No. 096 <a href="http://www.epa.gov/glnpo/lmmb/methods/docanal2.pdf">http://www.epa.gov/glnpo/lmmb/methods/docanal2.pdf</a>  LMMB Method No. 014 <a href="http://www.epa.gov/glnpo/lmmb/methods/pocdoc2.pdf">http://www.epa.gov/glnpo/lmmb/methods/pocdoc2.pdf</a> EPA Method No. 440.0 <a href="http://www.epa.gov/nerlcwww/m440_0.pdf">http://www.epa.gov/nerlcwww/m440_0.pdf</a>
<b>Particulate organic matter</b>	μM	Carbon analyzer or CHN Analyzer	LMMB Method No. 097 <a href="http://www.epa.gov/glnpo/lmmb/methods/pocanal2.pdf">http://www.epa.gov/glnpo/lmmb/methods/pocanal2.pdf</a> APHA Standard Method No. 5310-C, 20 <sup>th</sup> edition LMMB Method No. 014 <a href="http://www.epa.gov/glnpo/lmmb/methods/pocdoc2.pdf">http://www.epa.gov/glnpo/lmmb/methods/pocdoc2.pdf</a> EPA Method No. 440.0 <a href="http://www.epa.gov/nerlcwww/m440_0.pdf">http://www.epa.gov/nerlcwww/m440_0.pdf</a>
<b>Chlorophyll <i>a</i>/phaeopigments</b>	μg/L	Fluorometer	EPA Method 445.0 <a href="http://www.epa.gov/nerlcwww/m445_0.pdf">http://www.epa.gov/nerlcwww/m445_0.pdf</a> EPA Method No. 446.0 <a href="http://www.epa.gov/nerlcwww/m446_0.pdf">http://www.epa.gov/nerlcwww/m446_0.pdf</a> EPA Method 447.0 <a href="http://www.epa.gov/nerlcwww/m447_0.pdf">http://www.epa.gov/nerlcwww/m447_0.pdf</a> ASTM Method No. 3731-87 (1998)
<b>Total suspended solids</b>	Mg/L	5-place balance	ESS Method No. 340.2 (LMMB Method No. 065) <a href="http://www.epa.gov/glnpo/lmmb/methods/methd340.pdf">http://www.epa.gov/glnpo/lmmb/methods/methd340.pdf</a> APHA Standard Method No. 2540D (1998) EPA Method 160.2 <a href="http://www.epa.gov/region09/qa/pdfs/160_2.pdf">http://www.epa.gov/region09/qa/pdfs/160_2.pdf</a>
<b>Dissolved oxygen</b>	Mg/L	Radiometer TitraLab	EPA Method No. 360.1 (Probe Method) APHA Standard Method No. 4500-OG (Probe Method)

**Table 11. Data Quality Objectives for Water Quality Samples**

<b>Core Parameter</b>	<b>Frequency of QC Sample Collection</b>	<b>Method Detection Limit</b>	<b>Data Quality Indicator Type/Acceptance Criteria</b>
Dissolved nutrients	<u>Procedural blank</u> Two (2) per treatment cycle <u>Sample replicates</u> Three (3) sample replicates per treatment cycle	Ammonia and silica 0.02 µM Nitrate, nitrite, phosphate 0.01 µM	<u>Procedural blank</u> <5 times MDL <sup>1</sup> <u>Sample replicates</u> ≤2% PD <sup>2</sup>
Total suspended solids (DI water and seawater)	<u>Procedural blank</u> Two (2) per treatment cycle <u>Sample replicates</u> Three (3) sample replicates per treatment cycle	0.1 mg/L	<u>Procedural blank</u> <5 times MDL <u>Sample replicates</u> <10% RPD <sup>3</sup>
DOC	<u>Procedural blank</u> Two (2) per treatment cycle <u>Sample replicates</u> Three (3) sample replicates per treatment cycle	20 µM	<u>Procedural blank</u> ≤15% PD <u>Sample replicates</u> ≤10% RPD
POM	<u>Procedural blank</u> Two (2) per treatment cycle <u>Sample replicates</u> Three (3) sample replicates per treatment cycle	5.5 µM	<u>Procedural blank</u> ≤15% PD <u>Sample replicates</u> ≤10% RPD
Chlorophyll <i>a</i> /phaeophytin	<u>Procedural blank</u> Two (2) per treatment cycle <u>Sample replicates</u> Three (3) sample replicates per treatment cycle	0.02 µg/L	<u>Procedural blank</u> ≤5% PD <u>Sample replicates</u> ≤15% RPD
Dissolved oxygen	<u>Procedural blank</u> NA <u>Sample replicates</u> Three (3) sample replicates per treatment cycle		<u>Procedural blank</u> NA <u>Sample replicates</u> ≤5% CV <sup>4</sup>

<sup>1</sup> MDL = method detection limit.

<sup>2</sup> Percent Difference (PD) = [(true concentration – measured concentration)/true concentration] × 100%.

<sup>3</sup> Relative Percent Difference (RPD) = {[absolute value (replicate 1 - replicate 2)]/[(replicate 1 + replicate 2)/2]} × 100.

<sup>4</sup> Filter blanks used for QC purposes only.

1 Assuming, for organisms  $\geq 50 \mu\text{m}$ , that the desired minimum precision is that the upper bound of  
2 the chi-square statistic should not exceed twice the observed mean (this corresponds to a  
3 coefficient of variation of 40%), a count of 6 organisms is required. The volume required to  
4 successfully count 6 organisms is dependent on the whole water sample volume, concentration  
5 factor, number of sub-samples counted, and the target concentration. Table 12 provides the  
6 resultant upper bounds, based on the Poisson distribution for a 95% confidence interval from the  
7 chi-square transformation for a variety of sample volumes at a concentration factor of 3000 ( $3 \text{ m}^3$   
8 concentrated to 1 L) assuming 20 subsamples of 1 mL. Given these assumptions,  $30 \text{ m}^3$  must be  
9 sampled to enumerate  $10 \text{ organisms/m}^3$ , with the desired level of precision given above. If the  
10 concentration factor is increased or the quantity of subsamples increased, the total sample  
11 volume may be likewise reduced. As discussed previously, sample replication is unnecessary as  
12 the Poisson distribution pools the data to improve the measurement precision and assumes the  
13 organisms to be randomly distributed. **Note that this approach would not be appropriate if**  
14 **samples are not continuously acquired on a time-averaged basis.**  
15

16 In any case, sample size should be selected relative to the targeted concentration and to provide  
17 the level of precision required to supply a 95% upper confidence limit which is (1) no more than  
18 twice the observed mean and (2) does not exceed the targeted concentration or as otherwise  
19 defined by the TO. Examples are provided in Table 13 for standards for organisms larger than  
20  $50 \mu\text{m}$  compared to standards that are currently proposed or considered domestically and  
21 internationally. The chart provides the volume of sample required assuming that the entire  
22 sample is concentrated to 1 L and 6 organisms are counted. N is the number of samples  
23 analyzed, with each sample dispensed into a well plate and having 20 one-mL subsamples  
24 observed.  
25

26 A similar approach for organisms  $\geq 10 \mu\text{m}$  and  $< 50 \mu\text{m}$  may be applied; however, the targeted  
27 concentrations are considerably more dense, and the anticipated total counts can be expected to  
28 be higher. The Poisson distribution assumption still applies, and a more stringent level of  
29 precision may be applied. Specifically, if the desired level of precision is set at a coefficient of  
30 variation of 10% or the upper confidence limit not more than approximately 20% of the  
31 estimated density, then the volumes given in Table 14 result. These volumes are the required  
32 whole water sample volume to be concentrated to 1 L as a function of the number of 1 mL sub-  
33 samples (N).  
34

#### 35 *5.4.6.2.2 Zooplankton Enumeration*

36 Time integrated in-line sample volumes should be concentrated at the time of sampling using  $35$   
37  $\mu\text{m}$  mesh plankton nets ( $50 \mu\text{m}$  in the diagonal). The concentrated contents of the cod-end  
38 should be rinsed into a flask. The volume capacity of the flask will be dependent on the organism  
39 density of the sample but typically requires a range of 1 to 4 L capacity. Fresh, artificial  
40 seawater, filtered seawater, or freshwater, as appropriate, should be added to maintain oxygen  
41 levels for the living organisms to be counted. If the initial sample has a low concentration of  
42 zooplankters, the sample may need to be further concentrated before analysis. In this instance,  
43 the sample should be concentrated using  $35 \mu\text{m}$  mesh.

1  
2

**Table 12. Density Confidence Intervals for Poisson Distributions Using the Chi-Square Statistic**

**Scaled Densities and Confidence Intervals**  
*(Assumes whole water sample volume is concentrated to 1L, with analysis of 20 one-mL subsamples from the concentrate)*

Count Data			Scaled Densities and Confidence Intervals									
Whole Water Sample Volume (V) =			<u>V = 1 m<sup>3</sup></u>		<u>V = 3 m<sup>3</sup></u>		<u>V = 10 m<sup>3</sup></u>		<u>V = 30 m<sup>3</sup></u>		<u>V = 60 m<sup>3</sup></u>	
Count	95% Upper Bound	Upper Bound / Count	Mean Density (m <sup>-3</sup> )	95% Upper Bound (m <sup>-3</sup> )	Mean Density (m <sup>-3</sup> )	95% Upper Bound (m <sup>-3</sup> )	Mean Density (m <sup>-3</sup> )	95% Upper Bound (m <sup>-3</sup> )	Mean Density (m <sup>-3</sup> )	95% Upper Bound (m <sup>-3</sup> )	Mean Density (m <sup>-3</sup> )	95% Upper Bound (m <sup>-3</sup> )
0	3.00		0	150	0	50	0	15	0	5	0	2.5
1	4.74	4.74	50	237	17	79	5	24	2	8	1	4.0
2	6.30	3.15	100	315	33	105	10	31	3	10	2	5.2
3	7.75	2.58	150	388	50	129	15	39	5	13	3	6.5
4	9.15	2.29	200	458	67	153	20	46	7	15	3	7.6
5	10.51	2.10	250	526	83	175	25	53	8	18	4	8.8
6	<b>11.84</b>	<b>1.97</b>	<b>300</b>	<b>592</b>	<b>100</b>	<b>197</b>	<b>30</b>	<b>59</b>	<b>10</b>	<b>20</b>	<b>5</b>	<b>9.9</b>
7	13.15	1.88	350	657	117	219	35	66	12	22	6	11.0
8	14.43	1.80	400	722	133	241	40	72	13	24	7	12.0
9	15.71	1.75	450	785	150	262	45	79	15	26	8	13.1
10	16.96	1.70	500	848	167	283	50	85	17	28	8	14.1
11	18.21	1.66	550	910	183	303	55	91	18	30	9	15.2
12	19.44	1.62	600	972	200	324	60	97	20	32	10	16.2
13	20.67	1.59	650	1033	217	344	65	103	22	34	11	17.2
14	21.89	1.56	700	1094	233	365	70	109	23	36	12	18.2
15	23.10	1.54	750	1155	250	385	75	115	25	38	13	19.2
16	24.30	1.52	800	1215	267	405	80	122	27	41	13	20.3
17	25.50	1.50	850	1275	283	425	85	127	28	42	14	21.2
18	26.69	1.48	900	1335	300	445	90	133	30	44	15	22.2
19	27.88	1.47	950	1394	317	465	95	139	32	46	16	23.2
20	29.06	1.45	1000	1453	333	484	100	145	33	48	17	24.2

1 **Table 13. Sample Volume Required Relative to Treatment Standards–Organisms  $\geq 50 \mu\text{m}$**

2

Concentration (individuals/m <sup>3</sup> )	N =	1	3	5
		Sample Volume Required (m <sup>3</sup> ) <sup>1</sup>		
0.1		6000	2000	1200
1		600	200	120
10		60	20	12

3 <sup>1</sup> Assumes the entire volume is concentrated to 1 L and the desired precision is the 95% Confidence Interval of the  
 4 Poisson distribution = 2 times the observed mean and not greater than the Standard Limit.

6 **Table 14. Sample Volume Required Relative to Treatment Standards Organisms  $\geq 10 \mu\text{m}$  and <  
 7  $50 \mu\text{m}$**

Concentration (individuals/mL)	N <sup>1</sup> =	2	3	4
		Sample Volume Required (L) <sup>2</sup>		
0.1		600	400	300
1		60	40	30
10		6	4	3

8 <sup>1</sup>The number of 1 mL sub-samples analyzed.

9 <sup>2</sup> Assumes the entire volume is concentrated to 1 L and the desired precision is that CV is not greater than 10%.

10  
 11  
 12 Subsamples should be analyzed immediately, and as analysis proceeds, the original sample  
 13 should be held at ambient water temperature. Previous work has shown zooplankton die-off  
 14 occurs in the sample after a hold time of 6 hours. The appropriate maximum hold time should be  
 15 validated at each test facility so that the detectable zooplankton mortality over the hold time does  
 16 not exceed 5%.

17  
 18 Subsamples should be extracted using 5-mL serological, graduated pipettes with an Eppendorf  
 19 pipette helper (or a similarly accurate instrument that can effectively capture swimming  
 20 zooplankton). Subsamples should then be examined in multi-well plates, Bogorov chambers,  
 21 Sedgewick Rafter Counting Chambers, or counting wheels. The subsample should be dispensed  
 22 into the counting chamber while still allowing for the addition of a narcotizing agent. In  
 23 addition, the counting chamber volume should be shallow enough to allow for adequate focusing  
 24 on the organisms during analysis. All direct counts should be done using counting chambers  
 25 placed under a stereo or compound microscope at magnifications ranging from 10X to 40X.

26  
 27 Lugol's iodine solution should be used as a euthanizing and preservation agent. It should be  
 28 noted that this agent works particularly well on the standard test organism *Artemia* spp. and for  
 29 ambient organisms that have chitinous exoskeletons. It has been documented, however, that  
 30 Lugol's can have distorting effects on the preservation of some marine organisms, particularly if  
 31 their bodies lack chitin or other types of hard body structure. Given the choices of preservation  
 32 or euthanizing agents available, additional validation is advised when different zooplankton are

1 present in samples, or when dealing with organisms found at specific TFs, to determine which  
2 fixative(s) work best in preserving the zooplankton concentrations for total direct counts.

3  
4 In samples from challenge water or a control tank, the zooplankton should first be examined to  
5 count the number of dead organisms, defined by a lack of visible movement during an  
6 observation time of at least ten seconds. Unmoving but intact zooplankters may be living, so  
7 they are gently touched with the point of a fine dissecting needle or probe to elicit movement.  
8 Given that each dead organism is monitored for at least 10 seconds for visible movement,  
9 viability measurements could be lengthy for samples with dense concentrations of dead  
10 organisms, thereby increasing the potential for sample bias due to sample degradation.

11  
12 Once the number of dead organisms has been tallied, the organisms within the wells should be  
13 killed and/or preserved (to eliminate motion of the live organisms) and total counts obtained.  
14 Live counts will then be calculated from the difference using the equation: Total # - # Dead = #  
15 Live. Because samples collected following treatment are expected to have few living organisms,  
16 the living organisms can be enumerated directly.

17  
18 Counts should be detailed enough to differentiate and quantify the concentrations of ambient and  
19 standard test organisms. Note that samples collected to verify challenge conditions are met may  
20 require taxonomic identification of dominant organisms.

#### 21 *5.4.6.2.3 Organisms $\geq 10 \mu\text{m}$ and $\leq 50 \mu\text{m}$ (nominally protists)*

22 Laboratory concentration of this size organisms in the whole water sample can be accomplished  
23 by gently passing the sample through a sieve with mesh  $10 \mu\text{m}$  in the diagonal. Techniques  
24 and standardized methods for the enumeration and viability analyses of protists remain an active  
25 area of investigation. This protocol recommends use a combination of two vital stains:  
26 Fluorescein Diacetate (FDA, Molecular Probes-Invitrogen Carlsbad, CA) and 5-  
27 chloromethylfluorescein diacetate (CMFDA, CellTracker™ Green; Molecular Probes-Invitrogen  
28 Carlsbad, CA). When non-specific esterases in living cells cleave the stains, the resultant  
29 molecules fluoresce green when excited with a blue light (e.g., Selvin et al., 1988;  
30 www.invitrogen.com).

31  
32 This method utilizes manual epifluorescence microscopy to evaluate samples: FDA (final  
33 concentration  $5 \mu\text{M}$ ) and CMFDA (final concentration  $2.5 \mu\text{M}$ ) are added to a 1 mL sample that  
34 is incubated in the dark for 10 minutes, the sample is loaded into a Sedgewick Rafter Counting  
35 Chamber, and it is examined under epifluorescence using a Fluorescein Isothiocyanate (FITC)  
36 narrow pass filter cube (e.g., excitation 465-495 nm, dichroic mirror wavelength 505 nm, barrier  
37 filter 515-555 nm; Drake et al., in review). Samples should be examined for a maximum of 20  
38 minutes because the signal fades as stain leaks from the cell. If a cell is labeled by either FDA or  
39 CMFDA (as exhibited by a characteristic fluorescent green color) or moves, or both, it is scored  
40 as viable. A photomicrograph should be taken of any such cells under fluorescent and brightfield  
41 (white light) illumination to create a visual record of viable cells.

42  
43 Research on the dual staining method at four locations (including marine and estuarine sites) in  
44 the U.S. has shown the method to yield variable degrees of false positives (Type I error) from as  
45 little as 3% to nearly 40% (Steinberg et al., in review). Thus, before TOs use the dual staining

1 method or any other alternative method, it is necessary that it undergo on-site validation by  
2 preparing, examining, and analyzing ambient samples that are killed (i.e., negative controls).<sup>4</sup>  
3 From the perspective of environmental protection, this type of error is conservative, as it  
4 overestimates the number of viable organisms. In contrast, Type II errors (false negatives)  
5 underestimate the number of viable organisms. Encouragingly, the Type II error rate was  
6 uniformly low across all study sites: 0% in three locations and 1% at the remaining two  
7 locations (Steinberg et al., in review). Nonetheless, the Type II error rate should also be  
8 determined during initial site validation of this method or alternative method validation on a  
9 seasonal basis, and as part of the on-going QA program.<sup>5</sup>

10  
11 The advantages of using manual microscopy with vital stains are: (1) the instrumentation  
12 required is available in most research laboratories, (2) the cost of materials is low, (3) sample  
13 incubation times are relatively short, (4) the protocol is straightforward, and (5) results can be  
14 generated fairly rapidly. The disadvantages of this method are that it takes several hours (4-5  
15 hours) to completely characterize the subsamples within a sample, and unless the microscope is  
16 equipped with a camera (which is recommended), there is no archive of the data collected.  
17 Additionally, manual counts are subject to errors from operator-specific biases as well as from  
18 fatigue effects during extended observation periods.

19  
20 Within this size class fall dormant cells or resting stages exhibited across a broad phylogenetic  
21 range of microalgae, heterotrophic protists, and metazoans (e.g., Marret and Zonneveld, 2003;  
22 Matsuoka and Fukuyo, 2000). To encompass this group, the term ‘cysts’ is used, which includes  
23 but is not limited to cysts of dinoflagellates, spores of diatoms, cysts of heterotrophic protists,  
24 and cysts of rotifers. Notably, spores of bacteria and fungi are not included; they are smaller in  
25 minimum dimension than the lower limit of the size class considered here (10 µm).

26  
27 With focus on dinoflagellates alone, many authors (e.g., Dobbs and Rogerson, 2005; Doblin and  
28 Dobbs, 2006; and references in both) have made the point that cysts in ships’ ballast water  
29 represent robust ecological hazards. Given their resistance to physiological stress, killing cysts  
30 may be the best, i.e., most stringent, test of ballast-water technologies. If cysts can be killed,  
31 then there is excellent reason to assume vegetative cells or non-resting stages will also be killed.  
32 But because of their low metabolic state and relative impermeability to stains, it may be difficult  
33 to assess the viability of cysts on an individual basis without painstaking, cultural analyses,  
34 which, if possible at all, may require weeks or months to complete. At present, no rapid, reliable  
35 method to determine cysts’ viability is in widespread use, and the FDA-CMFDA method has  
36 yielded variable results with dinoflagellates and cyst-like objects. This protocol allows for  
37 additional techniques for plankton assemblages to be developed (Section 5.4.8); should a method

---

<sup>4</sup> For example, heat-killed, negative control samples are prepared by placing ambient water samples in a 50 °C water bath. Once the sample temperature reached 50 °C, it is held in the bath for an additional 10 minutes (Drake et al., in review; Steinberg et al., in review). The sample is cooled to room temperature before being stained. Organisms should not show a green, fluorescent signal after heat killing; those that do fluoresce represent false positives and indicate the Type I error associated with the dual-stain method.

<sup>5</sup> One approach is to collect ambient protists and place them in one of four categories based on an organism’s fluorescence signal and movement: (1) fluorescent and moving, (2) fluorescent and non-moving, (3) non-fluorescent and moving, and (4) non-fluorescent and non-moving (Drake et al., in review; Steinberg et al., in review). Organisms binned as non-fluorescent and moving are obviously viable, but the combination of stains fails to indicate viability, representing the Type II (false negative) error.

1 reliably indicating cyst viability become available, it is assumed that it would allow all viable  
2 organisms within the protist size class to be enumerated.

### 3 5.4.6.2.4 Organisms <10 µm

4 Bacteria samples should not need to be concentrated from the whole water sample prior to  
5 analysis. Sample analysis will be conducted according to standard microbial techniques.  
6 Multiple bacterial growth media will be used to assess the effectiveness of a treatment for  
7 bacteria<sup>6</sup>. Use of multiple types of media enables measurement of the response of different  
8 portions of the ambient bacterial community<sup>7</sup>. The minimum number of media used will include  
9 two general-purpose (1 marine, 1 nutrient agar) media for culturable aerobic heterotrophic  
10 bacteria. Other media may be added during the development of the TQAP. The rationale and  
11 methods will be described in the TQAP.  
12

13 For culturable, aerobic, heterotrophic bacteria, 1 mL samples should be diluted in a 10-fold  
14 dilution series in sterile Phosphate Buffered Saline (PBS) or sterile ambient water. Next, 100 µl  
15 of each appropriate dilution should be spread onto the media recommended in the protocol, with  
16 triplicate plates for each dilution. Plates should be incubated at 25 °C and monitored during the  
17 incubation time to ensure overgrowth does not occur. Colonies should be counted after 5 days  
18 and recorded as colony forming units (CFUs) per 100 mL of sample water.  
19

20 For *E. coli* samples, USEPA Method 1603 should be used: 1mL, 10 mL and 100 mL water  
21 samples should be passed through 0.45 µm membranes, which should be placed on modified  
22 thermo-tolerant *E. coli* agar (mTEC) plates (Becton Dickson, Sparks, MD). Plates should be  
23 incubated at 35 ± 0.5°C for 2 hours to allow for cell wall repair. Next, plates should be incubated  
24 at 44.5°C in a waterbath for 22-24 hours. Total red and magenta colonies should be scored and  
25 data reported as *E. coli* colonies per 100 mL of sample water. Alternatively, an IDEXX Colilert  
26 kit (Westbrook, ME) can be used according to the manufacturer's protocol.  
27

28 For *Enterococci* samples, a modified version of USEPA Method 1106.1 should be used: 10 mL  
29 and 100 mL water samples should be passed through 0.45 µm membranes, the membranes  
30 transferred onto mEnterococcus agar (mEA) plates, and the plates incubated at 35 ± 2°C for 24  
31 hours. Membranes with light and dark red colonies should be transferred to bile esculin agar  
32 (BEA) plates, which should be incubated for 4 hours at 35 ± 2°C. After incubation, colonies  
33 with black halos should be scored and data reported as *Enterococci* per 100 mL. Alternatively,  
34 an IDEXX Enterolert kit (Westbrook, ME) can be used according to the manufacturer's protocol.  
35

36 Toxigenic *Vibrio cholerae* densities should be determined by a DNA colony blot hybridization  
37 method that detects *ctxA* gene (Huq A, Grim C, Colwell RR, Nair GB., 2006). Briefly, colonies  
38 are grown on TCBS agar, purified, and inoculated with 2.5% yeast extract and nalidixic acid and  
39 fixed after incubation overnight. Viable *V. cholerae* O1 and O139 cells are enumerated using a  
40 direct-fluorescent antibody kit (New Horizons Diagnostics; Columbia, MD) for serogroups O1

---

<sup>6</sup> The suggested media for marine water include 2216 Marine Agar and salt-modified R2A agar; media for fresh water species may include Plate Count Agar and Nutrient broth (plus agar (15 g/L)).

<sup>7</sup> Note it is assumed that if all culturable bacteria are killed, all non-culturable bacteria are also killed.

1 and O139 using monoclonal antibodies tagged with fluorescein isothiocyanate (FITC) under an  
2 epifluorescence microscope.

3  
4 Appropriate controls (e.g., heat to remove vegetative cells for tests using resting stages or spores)  
5 for microbial plates will be used throughout the Verification Testing. Steps will also be taken to  
6 ensure the action of any treatment (e.g., a biocide) is stopped at the time of sample collection  
7 (i.e., treatment does not continue after sample collection). Any steps and controls used to verify  
8 the effectiveness of a neutralizer will be described and justified in the TQAP.

### 9 *5.4.6.3 Auxiliary Parameters*

10 Sampling and analysis of supplemental parameters may be required depending on Vendor-  
11 specified information. For example, a Vendor may define an additional treatment effectiveness  
12 based on removal of fecal coliform bacteria or other microorganisms of public health concern.  
13 In such cases, the TO, with VO acceptance, will determine the appropriate supplemental  
14 parameters, based on Vendor-specific information, and shall determine sampling and analysis  
15 requirements for inclusion in the sample collection schedule in the TQAP.

## 16 **5.4.7 BE Validity Criteria**

17 At the conclusion of each BE verification test cycle, the TO should verify that all criteria  
18 necessary for a valid BE test were established and maintained, as appropriate. As a minimum,  
19 the test validity criteria should consist of: (1) operational parameters that demonstrate the  
20 requisite volumes were transferred and sampled and Vendor-specified flows, pressures, or other  
21 validation criteria were maintained, (2) water quality challenge conditions for uptake and  
22 discharge waters, including any toxicity sampling as required by the TQAP, were met, and (3)  
23 biological challenge conditions for ambient and STO (if used) concentration and diversity in  
24 treatment and control samples were met. Note that the Vendor-specified validation criteria  
25 should be limited to operational parameters; that is, the criteria should be employed to ensure the  
26 system was operated correctly and in accordance with the provided training and O&M manual.  
27 Parameters that document a system failure under proper usage do not invalidate a test. The types  
28 and locations for measurements in each category are summarized in Table 15, and requisite  
29 criteria are discussed by category.

30  
31 Each of the measurement criteria and its valid ranges are to be documented by the TO in the  
32 TQAP. The declared ranges should accommodate variability of ambient source water conditions  
33 as well as possible ranges for ambient and STOs (if used) present in challenge water. The  
34 declared range of valid conditions also indicates the degree to which the TO can control the test  
35 parameters. Following each individual test, the TO will produce a Test Validation Matrix that  
36 summarizes valid ranges (from the TQAP) and corresponding mean values obtained during the  
37 test. Any significant deviations from the mean noted during the testing shall be discussed in the  
38 Verification Report.

### 39 *5.4.7.1 Uptake Operations*

40 The ETV protocol provides minimum requirements for volume and flow in Section 5.2.3, with  
41 final ranges for volume, pressure and flow to be identified in the TQAP by the TO and the  
42 Vendor. For all water transfers, the minimum volume is 200 m<sup>3</sup>, and the minimum sample  
43 collection volume associated with each transfer is 3 m<sup>3</sup>. Acceptable ranges for sample collection

1 volumes, pressures, and flows are also to be identified in the TQAP. The Test Validation Matrix  
2 should provide the valid ranges and the resulting mean values to verify if valid, in-range  
3 conditions were met over the duration of the test at the locations specified in Table 16.

#### 4 *5.4.7.2 Water Quality Conditions*

5 Minimum water quality conditions for BE tests are provided in Table 2 for dissolved and  
6 particulate organics, mineral matter, total suspended solids, and temperature for the two salinity  
7 ranges. The TO, in conjunction with the Vendor, will declare valid test ranges for these and other  
8 relevant water quality parameters of interest (e.g., pH, DO, etc.) in the TQAP and provide a list  
9 of the valid ranges and mean results for each test in the Test Validation Matrix. If there is reason  
10 to measure these parameters in the discharge waters as well, these measurements should also be  
11 presented.

#### 12 *5.4.7.3 Biological Diversity and Concentrations*

13 Table 3 presented requirements for biological challenge conditions to include a minimum of 5  
14 species from 3 separate phyla across the three requisite size classes, while Table 4 provided  
15 recommendations for STOs and minimum concentrations. Ambient organisms are to make up a  
16 minimum of 75% of the total population, while STOs (if used) are required to make up a  
17 minimum of 5% of the population. Both of these specifications refer to populations at the point  
18 of treatment and entry to the control tank. Valid population densities, diversity ranges for  
19 ambient and STOs (if used) should be defined by the TO in the TQAP. These ranges are  
20 envisioned to be fairly broad to accommodate variations in ambient populations and dominant  
21 species over the duration of the ETV testing. The anticipated ranges and the measured mean  
22 values for the sampled populations are to be presented in the Test Validation Matrix.

23  
24 Organism population densities and diversity are also to be measured in the discharge samples for  
25 both treated and control waters, where minimum living concentrations are required in the control  
26 discharge as noted in Table 7. These criteria are given by totals for each size class and should be  
27 shown in the Test Validation Matrix with results broken out for the dominant 5 species present in  
28 each size class.

#### 29 *5.4.7.4 Biological Treatment Efficacy Determination*

30 Treatment efficacy will be determined by the measurement of living ambient organism  
31 concentrations in the treatment discharge for the three size classes identified in Table 3. The  
32 treatment efficacy on STOs (if used) will also be separately reported in the Verification Report  
33 for future comparison purposes or until such time as a direct correlation between test organism  
34 response and in-service performance or ambient population mortality can be made.

#### 35 *5.4.7.5 Toxicity Test for Biocide Treatments*

36 Toxicity tests conducted during the start-up for treatments involving biocides in marine and  
37 brackish waters will be selected from the following:

38  
39 Inland Silverside, *Menidia beryllina*, Larval Survival and Growth (EPA Method 1006.0):  
40 <http://www.epa.gov/OST/WET/disk1/ctm13.pdf>  
41

**Table 15. Challenge Test Validation Criteria by Location**

Parameter	Control Sample Tank	Treatment Sample Tank	Control Tank	Treatment Tank	Control Discharge Sample Tank	Treatment Discharge Sample Tank
<b>Ballasting Operations</b>						
Volume	×	×	×	×	×	×
Pressure	×	×	×	×	×	×
Flow	×	×	×	×	×	×
Vendor-specified parameters				×		
<b>Water Quality Conditions</b>						
Temperature, Salinity, pH, DO (can monitor source waters)	×	×				
Total Suspended Solids (TSS)	×	×			×	×
Dissolved Organic Carbon (DOC)	×	×			×	×
Particulate Organic Material (POM)	×	×			×	×
Mineral Matter (MM)	×	×			×	×
Environmental Contaminants						×
<b>Biological Diversity and Concentrations</b>						
Ambient organisms/m <sup>3</sup> ; ≥ 50µm (live/dead)	×	×			×	×
Ambient organisms/mL; ≥ 10 and < 50µm (live/dead)	×	×			×	×
Ambient organisms/mL; < 10 µm (live/dead)	×	×			×	×
STOs <sup>1</sup> /m <sup>3</sup> ; ≥ 50µm (live/dead)	×	×			×	×
STOs <sup>1</sup> /mL; ≥ 10 and < 50µm (live/dead)	×	×			×	×
STOs <sup>1</sup> /mL; < 10 µm (live/dead)	×	×			×	×

<sup>1</sup> If used.

Sea Urchin, *Arbacia punctulata*, Fertilization Test (EPA METHOD 1008.0: <http://www.epa.gov/OST/WET/disk1/ctm15.pdf>)

Mysid Acute Toxicity Test (EPA OPPTS Method 850.1035: [http://www.epa.gov/opptsfrs/OPPTS\\_Harmonized/850\\_Ecological\\_Effects\\_Test\\_Guidelines/Drafts/850-1035.pdf](http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-1035.pdf))

Additional guidance can be found in ASTM (1996a, 1996b) and Klemm et al. (1994). Tests and species selected for toxicity testing during Commissioning will be specified in the TQAP in accordance with the salinity ranges identified for testing.

#### 5.4.8 Alternative and Emerging Methods

New methods for analysis and enumeration of living plankton communities are being developed to meet the relatively complex and demanding needs of ballast water treatment system testing.

1 These methods include, but are not limited to, rapid analytical measures, vital stains and dyes,  
2 and molecular probes. The inclusion or substitution of these techniques to those described above  
3 is acceptable. However, at a minimum the method(s) selected for any given size class should  
4 provide a quantitative measurement of the concentration of living organisms. If non-standard  
5 methods are selected, they should be validated by the TO to the satisfaction of the VO.

#### 6 **5.4.9 Operation and Maintenance Verification Factor**

7 The operation and maintenance (O&M) of the ballast water treatment system will be verified.  
8 The verification has been designed as a minimum volume requirement, allowing sufficient time  
9 to verify operation and maintenance of the ballast water treatment system. It is anticipated that  
10 O&M testing commences in between BE test cycles to ensure some equipment run-time occurs  
11 prior to each BE verification test. In this manner, any changes in treatment efficacy due to  
12 equipment operation over time may be observed.

13  
14 The TO is responsible for monitoring and maintaining the system, in accordance with the  
15 Vendor's O&M manual, throughout the testing to ensure stable operating conditions (as mutually  
16 agreed to by the Vendor) and proper operating effectiveness. All system components will be  
17 monitored for proper operation throughout the test period. All maintenance activity completed  
18 during the verification testing shall be documented for inclusion in the Verification Report.

19  
20 All required monitoring and maintenance activities should be coordinated with the TO in  
21 advance of verification testing, and detailed in a monitoring and maintenance plan included in  
22 the TQAP. The monitoring and maintenance plan shall address the following requirements, as  
23 applicable:

- 24  
25     ▪ A monitoring and maintenance schedule for the testing period (as shipboard systems are  
26 generally designed to require minimal regular maintenance, visual inspections by the  
27 operator may be all that is required);
- 28     ▪ Equipment and component calibration methods and frequencies;
- 29     ▪ Monitoring and maintenance activities and procedures shall be described and  
30 documentation forms provided – maintenance documentation forms must identify the TF,  
31 date and time, describe the work performed, observations of the treatment system, and  
32 results of the work; and
- 33     ▪ Operating characteristics and Vendor-specified ranges required for proper operating  
34 conditions shall be described (e.g., system temperature, flows entering and exiting the  
35 system, power levels).

36  
37 Other information that must be addressed in the TQAP includes:

- 38  
39     ▪ Monitoring requirements to ensure a proper operating environment;
- 40     ▪ Continuous on-line O&M monitoring requirements, as specified by the Vendor; and
- 41     ▪ Credentials of all personnel involved in operating, monitoring and maintaining the  
42 treatment system.

43  
44 All monitoring and maintenance documentation must be maintained in a written record at the TF  
45 and will be included in the Verification Report.

1  
2 To help address predictability and reliability verification factors, qualitative and quantitative  
3 O&M performance indicators will be evaluated. The means and methods to evaluate or quantify  
4 O&M performance indicators shall be included in the TQAP and described in a schedule for  
5 collecting this information.

#### 6 *5.4.9.1 Qualitative O&M Performance Indicators*

7 Qualitative O&M performance indicators will include, but are not limited to:

##### 8 *5.4.9.1.1 Visual Observations*

9 Visual inspections of the treated ballast water quality (e.g., turbidity, color) and treatment system  
10 conditions (e.g., foaming, floating material, settled solids) will be performed at each maintenance  
11 or monitoring event. Visual observations will also include the inspection of the treatment system  
12 prior to, during and following each test cycle for equipment and process failures, corrosion,  
13 leaks, impediments of flow (entering or exiting the system) and any other system issues that  
14 could impact performance. Specific visual indicators shall be defined in the TQAP.

##### 15 *5.4.9.1.2 Operability*

16 Observations regarding the ease of start-up and operation during testing and the ease of  
17 monitoring system performance shall be noted and recorded.

##### 18 *5.4.9.1.3 O&M Manual*

19 The TO shall evaluate the usefulness and quality of the O&M manual, and a written report on the  
20 evaluation shall be prepared.

##### 21 *5.4.9.1.4 Operator Skills*

22 The level of operator expertise required to operate and maintain the treatment system shall be  
23 noted and compared with that indicated by the Vendor.

##### 24 *5.4.9.1.5 System Accessibility*

25 The ease of access and required clearances for system operation and required maintenance shall  
26 be noted.

#### 27 *5.4.9.2 Quantitative O&M Performance Indicators*

28 Quantitative O&M performance indicators shall include, but are not limited to:

##### 29 *5.4.9.2.1 Time demand*

30 Personnel time required to start-up, shutdown, operate, and maintain the treatment system shall  
31 be recorded in the monitoring and maintenance log.

##### 32 *5.4.9.2.2 Residual*

33 Volumes of residual materials, (e.g., solids removed via filtration systems, etc.), mass generation  
34 rates, and concentrations shall be determined during verification testing. Results will be  
35 recorded in m<sup>3</sup>, gallons or pounds per m<sup>3</sup>, or gallons of water treated, as appropriate. Factors

1 related to the disposal of residuals (such as storage requirements and handling hazards) shall also  
2 be addressed.

### 3 *5.4.9.2.3 Chemical Use*

4 Usage rates and concentrations of any chemicals (e.g., biocides) used as part of the treatment  
5 system and its operation during verification testing (per test cycle) will be measured and  
6 recorded. Results shall be reported for residuals and possible by-products.

### 7 *5.4.9.2.4 Power consumption*

8 The power consumed per test cycle by the treatment system will be monitored and recorded (e.g.,  
9 kWh per m<sup>3</sup> of water treated shall be calculated for use in cost factors below). The peak  
10 electrical load at system start-up will also be monitored and recorded as will fluctuations in  
11 consumption during test cycles.

### 12 *5.4.9.2.5 Other Consumables*

13 The use of any other consumables, such as filter cartridges, shall be monitored, documented, and  
14 reported.

### 15 *5.4.9.2.6 Supplemental Parameters*

16 Depending on Vendor claims, supplemental monitoring, maintenance, and O&M performance  
17 indicators may be required. These will be described, along with requirements for performance  
18 monitoring, in the TQAP.

### 19 *5.4.9.2.7 Upset Conditions*

20 Upset conditions are those events or occurrences outside the operating parameters defined in the  
21 TQAP that result in either malfunctioning of the equipment, exception from normal operating  
22 conditions, or conditions causing alarms that indicate the system is producing or discharging  
23 treated water that exceeds the stated set points or limits for effective treatment. The cause of  
24 upset conditions may be due to conditions at the TF or the technology. These events may  
25 include both events in which the system is operating within the manufacturer's specifications and  
26 those that are within specification but do not result in adequate treatment. The TO shall notify  
27 the Vendor and the VO immediately when an upset condition is identified. The TO shall correct  
28 the upset condition as soon as possible to bring the treatment system back on line. For unusual  
29 upset conditions, the TO will work with the Vendor to identify and correct the problem. The  
30 occurrence of all upset conditions, the causes, the results, and the means to correct the upset shall  
31 be documented at the time of the occurrence and shall be described in the Verification Report.

32  
33 As sampling is continuous over the course of the test, any upset conditions during the test need to  
34 be noted and a post-test review conducted to determine their cause and assess the impact on test  
35 results. (This task will be done by the TO and approved by the VO.) This review will determine  
36 where inclusion of these data is appropriate for performance assessment and the statistical  
37 analysis presented in the Verification Report. If the cause of an upset condition cannot be  
38 determined or the condition cannot be qualified as a true upset, then the sampling results shall be  
39 used in the statistical analysis for the Verification Report.

1 *5.4.9.2.8 Reliability*

2 The mechanical reliability of the technology will be determined by comparing the Vendor  
3 projected mean-time between failure (MTBF) with the maintenance events observed during  
4 testing. The comparison will be reported in the Verification Report.

5  
6 The reliability of the treatment system to achieve treatment will be determined by (1) the number  
7 of instances where the treatment system or technology does not achieve the stated performance  
8 goal per the total number of test cycles, and (2) the standard deviation of the mean for biological  
9 performance data (e.g., percent removal).

10  
11 Reliability performance measures will take into consideration any Vendor provided information  
12 that assists in the projection of the performance such as CT (concentration-time) disinfection  
13 information or power/energy curves. Any adjustments made to the system, outside of the  
14 Vendor-specified operation and maintenance claims, to achieve the performance goals will be  
15 noted in the maintenance log and specified in the Verification Report.

16  
17 Specific performance reliability indicators along with the planned methods for evaluating and  
18 reporting them will be identified in the TQAP.

19 *5.4.9.2.9 Cost Factors*

20 Verified cost factors will include the following as applicable:

21 *5.4.9.2.9.1 Power consumption*

22 Power consumption will be reported as total kWh necessary to operate all equipment to achieve  
23 desired biological treatment performance.

24 *5.4.9.2.9.2 Consumable or expendable materials*

25 Amounts of all consumables or expendables, including chemicals or other items required for  
26 treatment, shall be itemized and reported.

27 *5.4.9.2.9.3 Replacement parts used during normal maintenance*

28 The number of replacement parts will be itemized and reported. Any unanticipated replacement  
29 of parts will be specified separately.

30 *5.4.9.2.9.4 Labor time to start-up, operate, and maintain the treatment system*

31 The total number of hours for each activity will be recorded and reported.

32 *5.4.9.2.9.5 By-product or waste materials produced*

33 By-products that require treatment or disposal will be reported as an expression of total volume  
34 treated or disposed.

35 *5.4.9.2.10 Environmental Acceptability*

36 Two performance indicators will determine the Environmental Acceptability of a treatment  
37 system: Water Quality and Treatment Residuals.

38

1 The data used to evaluate the environmental acceptability of a system will be taken from the  
2 water quality data collected at the point of discharge as detailed in Section 5.3.2. These data will  
3 include but may not be limited to the following parameters:

- 4
- 5     ▪ Temperature
- 6     ▪ pH
- 7     ▪ Salinity
- 8     ▪ Total suspended solids
- 9     ▪ Particulate organic matter
- 10    ▪ Dissolved organic matter
- 11    ▪ Dissolved oxygen
- 12    ▪ Dissolved nutrients
- 13    ▪ Biochemical oxygen demand
- 14    ▪ Biological efficacy
- 15

16 The results of these tests at the point of discharge will be compared to the range of expected  
17 natural conditions and reported in the Verification Test.

18

19 Additional analytical parameters will be included as necessary for reporting on any residual  
20 material that may result from treatment; for example residual biocides and disinfection  
21 byproducts. The additional parameters, the potential impact to the environment, and the  
22 analytical methods will be detailed in the TQAP

23

24 It will be the responsibility of the TF to obtain NPDES discharge permits and to ensure that  
25 discharge is within permitted limits. Additionally, toxicity testing of any biocide treatment will  
26 be conducted, as discussed under Section 5.4.4. Verification testing will not begin unless the  
27 results of the toxicity tests are acceptable.

#### 28 5.4.9.2.11 *Safety*

29 Safety is of concern during the operation of any equipment or machinery and during the use of  
30 potential hazardous materials, but it is of particular concern while on board ship, where staff is  
31 limited and access to land based emergency infrastructure is unavailable. Therefore, the safety  
32 of the treatment system will be evaluated during verification testing.

33

34 The performance indicators for this verification factor will be technology specific, but, to the  
35 extent possible, required indicators shall include:

- 36
- 37     ▪ Listing of all dangerous or hazardous materials, including submittal of Material Safety  
38       Data Sheets (MSDS);
- 39     ▪ Potential to compromise the normal ship ballasting or deballasting cycle (i.e., impediment  
40       of flow);
- 41     ▪ Visual indicators of potential threats to shipboard operations, such as exposed or  
42       improper housing of power cables, structural stability of the system, external  
43       temperatures of the treatment system, and any other treatment-specific factors that may  
44       pose a threat to the operator or compromise the safety of ship operations; and

- 1           ▪ Review of the Vendor provided O&M manual for adequacy of cautions and guidance on  
2           ways to minimize the potential for, and directives to mitigate, a hazardous situation.  
3

4 The method for evaluating these and other items identified by the TO in reviewing the  
5 technology documentation shall be described in the TQAP.  
6  
7  
8

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## Chapter 6

### Reporting Verification Testing Results

Any deviations from this protocol shall be described in the Verification Report, which shall include supporting documentation that provided the basis for acceptance of the deviations. All testing results will be presented in the Report, including all data regarding challenge conditions, results of verification testing for all verification factors, and any Vendor supplied data or information. A summary Verification Statement, presenting the most important results of the verification testing, will also be prepared.

The outline for the report shall include:

- Verification Statement
- Executive Summary
- Introduction and Background
- Description of the Treatment System or System
- Experimental Design (including a description of all deviations from the protocol and the basis for accepting the deviations)
- Description of Challenge Conditions
- Methods and Procedures
- Results and Discussion
- Verification Testing Operation and Monitoring QA/QC

Appendices:

- TQAP
- BE Test Validation Matrix
- Vendor-supplied Operation and Maintenance Manual
- Data Generated During Testing
- QA/QC Records
- Maintenance Logs
- Any other records maintained during testing, such as chain of custody forms
- Any other information provided by the Vendor, which may be of use to the stakeholder community

Upon completion of the draft report the VO, the Vendor, and the TF QA Manager will review the document and supporting data, and provide comment. The comments will be addressed or stricken with approval of all parties and the Final Report will be submitted to NSF International (ETV Water Quality Protection Center Partner) and EPA for approval.

## Chapter 7

### Quality Assurance/Quality Control (QA/QC)

To ensure the quality and integrity of data gathered during testing activities, a Quality Assurance Project Plan (QAPP) will be prepared by the TO and included as part of the TQAP. The QAPP will describe the project scope, management, procedures for measurements and data acquisition, project assessment and oversight, and data validation and usability assessments necessary to meet the project goals. The written document will communicate all decisions related to project design and completion to the project team so work is performed according to written specifications. The generic format for a QAPP is included in Appendix A.

#### 7.1 Verification of Test Data

The QAPP will address data quality in part by the development of acceptable values for six data quality objectives: accuracy, precision, completeness, comparability, representativeness, and sensitivity. The data quality objectives will establish the locations, types and numbers of samples to be collected, the quality control samples (duplicates, blanks, spikes, etc.) required for both field and laboratory samples, and will establish the data quality criteria and measures of acceptability that are appropriate for the project. The TQAP will also detail a corrective action plan to describe actions to be taken if acceptance criteria are not met.

#### 7.2 Project Management

The QAPP will list all project participants and clearly define their roles and responsibilities. In addition, this section will describe project scheduling, data quality objectives, training and certification requirements (as applicable), and required documentation. The information included in this section will ensure that all participants understand the scope of the study and their explicit roles. Due to the complexity of testing in accordance with these protocols, it is advisable that each test cycle be preceded by a briefing or meeting in which the TF personnel critically review the plan of action, test operation, and conduct so they are familiar with the TQAP and their responsibilities. It is further recommended that this briefing or review be accompanied by a standardized test form that identifies the specific, quantitative set points and objectives that may be actively used throughout the test cycle to identify or record specific events, measurements or alarms. The consolidated and completed test form from each cycle should be included in an Appendix of the test report.

#### 7.3 Measurement and Data Acquisition

A detailed description of the experimental design and its components will be included in the QAPP. Specific requirements with regard to use, maintenance, and calibration of equipment, analytical procedures, chain-of-custody procedures, sample collection, data management and documentation, records management, project scheduling, experimental design assumptions, and disclosure of non-standard techniques or equipment will be discussed.

1 **7.4 Assessment**

2 The effectiveness of QA/QC will be monitored through assessments of general and project-  
3 specific activities. The QAPP will include detailed information on the types of assessments to be  
4 utilized (e.g., management, technical, and/or quality assurance assessments), appropriate  
5 response actions, reporting requirements, and assessment and reporting authority. To increase  
6 facility-to-facility and test-to-test comparisons, and TF internal QA/QC, standardized spiked and  
7 blank samples shall be incorporated into the sample analysis procedures. Spikes may be  
8 accomplished using inert objects such as stained, killed organisms or microbeads of appropriate  
9 size for the specific analyses. The methods to be used for spiked and blank samples shall be  
10 described in the QAPP.

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## Chapter 8

### Data Management, Analysis and Presentation

Any data collected during testing activities must be capable of withstanding challenges to its validity, accuracy, and legibility. Data will be recorded in standardized formats and in accordance with the following minimum requirements:

- Data are entered directly, promptly, and legibly;
- Hand-entered data are recorded legibly in ink; all original data records include, as appropriate, a description of the data collected, the unit, the unique sample identification, the name of the person collecting the data, and the date and time of data collection;
- Any changes to the original entry do not obscure the original entry, document the reason for the change, and are initialed and dated by the person making the change;
- All deviations from the QAPP must be documented in writing, and approved by the TO; documentation and communication include an assessment of the impact the deviation has on data quality; and
- Data in electronic format shall be included in a commercially available program for word processing, spreadsheet calculations, database processing, or commercial software developed especially for the data collection and processing on a specific hardware instrument or piece of equipment; backup of computer databases should be performed on a daily basis, if possible.

Project-specific data management requirements, including the types of data to be collected and managed and how they will subsequently be reported, shall be defined in the data handling section of the TQAP. QA/QC activities for data management will be described in the QAPP and included in the TQAP.

#### 8.2 Data Analysis and Presentation

Hand-recorded data gathered during verification testing will be entered into electronic format (a spreadsheet or other database product capable of performing graphical and simple statistical analyses). Following reduction, data will be presented in a graphical, tabular, or other logical format and accompanied by a detailed discussion to be included in the Verification Report.

Treatment effectiveness will be calculated for each size class as concentration per unit volume in the discharge and may be related to relevant standards as identified by the Vendor or the Vendor's claims. In addition, these data will be reported for both ambient and STOs (if used). Additional measures or comparisons may also be used to assess treatment efficacy, including percent organism removal by size class, or as a comparison of treated discharge to the control tank discharge. All methods will be described in the TQAP. The treatment effectiveness will be discussed in the Verification Testing Report with raw data included as an appendix.

## Chapter 9

### Environmental, Health, and Safety Plan

The TO shall develop an Environmental, Health, and Safety (EHS) Plan to be included in the TQAP. The EHS Plan shall identify all environmental concerns and potential hazards associated with the verification testing process and the TF, as well as the required measures to prevent exposure to the identified hazards. The TO shall be responsible for informing all personnel at the test site, including employees, contractors, and visitors, of the potential hazards and safety measures to be employed at the test site. The EHS Plan shall address the following issues, as applicable:

- Permitting requirements for equipment operation, effluent discharge, and waste disposal;
- Biological, chemical, mechanical, electrical, and other hazards;
- Environmental hazards will be defined in accordance with local, state and federal regulations;
- Handling, storage, and disposal of all biological material and chemicals associated with the testing;
- Safeguards and protocols to prevent the accidental release to the environment of any non-ambient organisms if used in the test process; protocols of the form supplied in Part II ANS Task Force ANS Research Evaluation Protocol are recommended ([http://www.anstaskforce.gov/Documents/Research\\_Evaluation\\_Protocol\\_ANSTF.pdf](http://www.anstaskforce.gov/Documents/Research_Evaluation_Protocol_ANSTF.pdf));
- Material Safety Data Sheets (MSDS);
- Conformance with the local electrical code;
- Conformance with the local plumbing code;
- Ventilation of equipment, trailers, or buildings housing equipment, if gases generated by the equipment could present a safety hazard;
- Confined space entry hazards;
- Fire safety; and
- Emergency contacts for 911, the nearest hospital (provide directions), local fire department, the site manager, and all other important contacts.

Any other environmental, health, or safety issues specific to the test location or ballast water treatment system to be tested must be addressed. A copy of the EHS Plan, including all MSDS, shall be maintained and readily accessible at the test site. A one-page summary of emergency contacts shall be placed inside a clear plastic cover and kept at the verification-testing unit.

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23  
24  
25  
26

## Appendix A: Quality Assurance Project Plan

A Quality Assurance Project Plan (QAPP) shall be prepared as part of the TQAP for evaluating the performance of ballast water treatment technologies. The generic format for such QAPPs includes:

### A.1 Project Descriptions, Objectives and Organization

- The purpose of the study shall be clearly stated.
- The processes to be evaluated will be described.
- The TF, apparatus and technology set-up will be fully described.
- Project objectives shall be clearly stated and identified as being primary or non-primary.
- Responsibilities of all project participants shall be identified. Key personnel and their organizations shall be identified, along with the designation of responsibilities for planning, coordination, sample collection, measurements (i.e., analytical, physical, and process), data reduction, data validation (independent of data generation), data analysis, report preparation, and quality assurance.

### A.2 Experimental Approach

- Technology installation and shakedown procedures will be identified.
- Technology startup procedures will be identified. Startup will comprise a number of tasks to implement and check operating and sampling protocols. Tasks will include establishing feed makeup and performing calibration checks on monitoring systems, identifying sampling and monitoring points and identifying the types of samples to be collected.
- Physical, analytical or chemical measurements to be taken during the study will be provided. Examples include flow rates, pH, salinity, total suspended solids, particulate organic matter, dissolved organic matter, dissolved oxygen, dissolved nutrients, biochemical oxygen demand, biological organisms, O&M performance indicators, etc.
- Sampling and monitoring points for each test unit and the type of sample to be collected (grab or composite) will be identified.
- The frequency of sampling and monitoring as well as the number of samples required will be provided. This includes the number of samples needed to meet QA/QC objectives.
- Planned approach for evaluation objectives (data analysis). This will include formulas, units, and definition of terms and statistical analyses to be performed in the analysis of the data. Example graphical relationships will be provided.
- Demobilization of the technology, including scheduling and site restoration requirements, will be described.

### A.3 Sampling Procedures

- Whenever applicable or necessary to achieve project objectives, the method used to establish steady-state conditions shall be described.
- Each sampling/monitoring procedure to be used shall be described in detail or referenced. If compositing or splitting samples is required, those procedures shall be described.
- Sampling or monitoring procedures shall be appropriate for the matrix or analyte being tested.

- 1 • If sampling/monitoring equipment is used to collect critical measurement data (e.g., used to  
2 calculate the final concentration of a critical parameter), the QAPP shall describe how and at  
3 what frequency the sampling equipment is calibrated.
- 4 • If sampling/monitoring equipment is used to collect critical measurement data, the QAPP  
5 shall describe how cross-contamination between samples is avoided.
- 6 • When representativeness is essential for meeting a primary project objective, the QAPP shall  
7 include a discussion of the procedures to be used to assure that representative samples are  
8 collected.
- 9 • A list of sample quantities to be collected, and the sample amount required for each analysis,  
10 including QC sample analysis, shall be specified in the QAPP.
- 11 • Containers used for sample collection for each sample type shall be described in the QAPP.
- 12 • Sample preservation methods (e.g., refrigeration, acidification, etc.) and holding times shall  
13 be described in the QAPP.
- 14 • A sample of the chain of custody form to be used during testing shall be provided, including  
15 records of times and other critical parameters such as storage temperatures, light condition,  
16 etc.

#### 17 **A.4 Testing and Measurement Protocols**

- 18 • Each measurement method to be used shall be described in detail or referenced in the QAPP.  
19 Modifications to EPA-approved or similarly validated methods shall be specified.
- 20 • For unproven methods, the QAPP shall provide evidence that the proposed method is capable  
21 of achieving the desired performance.
- 22 • For measurements that require a calibrated system, the QAPP shall include specific  
23 calibration procedures, and the procedures for verifying both initial and continuing  
24 calibrations (including frequency and acceptance criteria, and corrective actions to be  
25 performed if acceptance criteria are not met).

#### 26 **A.5 QA/QC Checks**

##### 27 **A.5.1 Data Quality Indicators**

- 28 • Statistical analyses shall be carried out on data obtained for all performance measurements.  
29 As part of the assessment of data quality, six data quality indicators (DQIs) can be used to  
30 interpret the degree of acceptability or utility of the data. At a minimum, the QAPP shall  
31 include a protocol for assessing the following DQIs, and acceptable limits and criteria for  
32 each of these indicators: representativeness, accuracy, precision, bias, comparability, and  
33 completeness.
- 34 • The TO shall determine acceptable values or qualitative descriptors for all DQIs in advance  
35 of verification testing as part of the experimental design. The assessment of data quality will  
36 require specific field and laboratory procedures to determine the data quality indicators. All  
37 details of DQI selection and values shall be documented in the QAPP.

##### 38 *A.5.1.1 Representativeness*

39 Representativeness refers to the degree to which the data accurately and precisely represent the  
40 conditions or characteristics of the parameter represented by the data. In this testing,  
41 representativeness will be ensured by executing consistent verification procedures.

1 Representativeness will also be ensured by using each method at its optimum capability to  
2 provide results that represent the most accurate and precise measurement it is capable of  
3 achieving. For equipment operating data, representativeness entails collecting a sufficient  
4 quantity of data during operation to be able to detect a change in operations.

#### 5 A.5.1.2 Accuracy

6 For water quality analyses, accuracy refers to the difference between a sample result and the  
7 reference or true value for the sample. Loss of accuracy can be caused by such processes as  
8 errors in standards preparation, equipment calibrations, loss of target analyte in the extraction  
9 process, interferences, and systematic or carryover contamination from one sample to the next.  
10 Loss of accuracy for microbial species can be caused by such factors as error in dilution or  
11 concentration of microbiological organisms, systematic or carryover contamination from one  
12 sample to the next, improper enumeration techniques, etc. The TO shall discuss the applicable  
13 ways of determining the accuracy of the chemical and microbiological sampling and analytical  
14 techniques in the TQAP.

15  
16 For equipment operating parameters, accuracy refers to the difference between the reported  
17 operating condition and the actual operating condition. For water flow, accuracy may be the  
18 difference between the reported flow indicated by a flow meter and the flow as actually  
19 measured on the basis of known volumes of water and carefully defined times. Meters and  
20 gauges must be checked periodically for accuracy, and when proven dependable over time, the  
21 time interval between accuracy checks can be increased. In the TQAP, the TO shall discuss the  
22 applicable ways of determining the accuracy of the operational conditions and procedures.

23  
24 From an analytical perspective, accuracy represents the deviation of the analytical value from the  
25 known value. Since true values are never known in the field, accuracy measurements are made  
26 on the analysis of QC samples analyzed with field samples. QC samples for analysis shall be  
27 prepared with laboratory control samples, matrix spikes, and spike duplicates. It is  
28 recommended for verification testing that the TQAP includes laboratory performance of one  
29 matrix spike for determination of sample recoveries. Recoveries for spiked samples are  
30 calculated in the following manner:

31  
32 
$$\% \text{ Recovery} = \frac{100(SSR - SR)}{SA} \quad (A-1)$$

33  
34 where: SSR = spiked sample result  
35 SR = sample result  
36 SA = spike amount added  
37

38 Recoveries for laboratory control samples are calculated as follows:  
39

40 
$$\% \text{ Recovery} = \frac{100(\text{foundconcentration})}{\text{trueconcentration}} \quad (A-2)$$

41

1 For acceptable analytical accuracy under the verification testing program, the recoveries reported  
2 during analysis of the verification testing samples must be within control limits, where control  
3 limits are defined as the mean recovery plus or minus three times the standard deviation.

#### 4 *A.5.1.3 Precision*

5 Precision refers to the degree of mutual agreement among individual measurements and provides  
6 an estimate of random error. Analytical precision is a measure of how far an individual  
7 measurement may be from the mean of replicate measurements. The standard deviation and the  
8 relative standard deviation recorded from sample analyses may be reported as a means to  
9 quantify sample precision. The coefficient of variation (CV) may be calculated in the following  
10 manner:

$$12 \quad \% \text{ CV} = \frac{S(100)}{X_{\text{average}}} \quad (\text{A-3})$$

13 where: S = standard deviation

14  $X_{\text{average}}$  = the arithmetic mean of the recovery values

15  
16  
17 Standard Deviation is calculated as follows:

$$19 \quad \text{Standard Deviation} = \sqrt{\frac{(X_i - X)^2}{n-1}} \quad (\text{A-4})$$

20 where:  $X_i$  = the individual recovery values

21 X = the arithmetic mean of the recovery values

22 n = the number of determinations

23  
24  
25  
26 The QAPP shall list and define all other QC checks and/or procedures (e.g., detection limits  
27 determination, blanks, spikes, surrogates, controls, etc.) used for the project.

28  
29 For each specified QC check or procedure, required frequencies, associated acceptance criteria,  
30 and corrective actions to be performed if acceptance criteria are not met shall be included in the  
31 QAPP.

### 32 **A.6 Data Reporting, Data Reduction, and Data Validation**

- 33 • The reporting requirements (e.g., units) for each measurement and matrix shall be identified  
34 in the QAPP.
- 35 • Data reduction procedures specific to the project shall be described, including calculations  
36 and equations.
- 37 • The data validation procedures used to ensure the reporting of accurate project data to  
38 internal and external clients should be described.
- 39 • The expected product document that will be prepared shall be specified.

1 **A.7 Assessments**

2 Whenever applicable, the QAPP shall identify all audits (i.e., both technical system audits  
3 [TSAs] and performance evaluations [PEs]) to be performed, who will perform these audits, and  
4 who will receive the audit reports.

5 **A.8 References**

6 References shall be provided in the QAPP in the body of the text as appropriate.

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