Protocol for the Verification of Ballast Water Compliance Monitoring Devices

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Contents

Summary

Concerns regarding the impacts of non-native species due to their transport and release in ship ballast water have resulted in agreements and regulatory requirements being implemented around the world (e.g. International Maritime Organization [IMO] Ballast Water Management Convention [BWMC], 2004; US Coast Guard [USCG], 2012; California State Lands Commission [CSLC], 2018). Consequently, effective and reliable monitoring for ship compliance with ballast water discharge standards is now critical to achieve the regulatory goal of minimizing the risk of invasive species introductions. A variety of ballast water compliance monitoring devices (CMDs) have been developed. This includes various sensors, instruments, kits, methods, and assays that have been designed to assess compliance with ballast water discharge standards and requirements. Additionally, several novel CMD approaches are also currently being explored. However, rigorous, transparent and standardized verification testing is needed for these devices to be adopted and implemented globally, by multiple administrations (i.e. countries, governments, or jurisdictions) to enforce compliance monitoring. Otherwise, CMD performance, data quality, and uncertainties will remain unknown. To address this need, this protocol has been developed by a subgroup of the ICES/Intergovernmental Oceanographic Commission (IOC)/IMO Working Group on Ballast and other Ship Vectors (WGBOSV) to serve as a standardized framework for the verification testing of CMDs.

1 Introduction

This protocol is intended to serve as a framework for the standardized performance verification of ballast water compliance monitoring devices (CMDs). These devices may be used during commissioning testing of ballast water management systems (BWMS), as data are collected during the experience-building phase associated with the BWMC (IMO 2017(a)), during regulatory ship inspections, and during routine monitoring by ships' crew members. The protocol relies on a suite of laboratory and field tests, although additional tests may be carried out when applicable. It should be noted that:

1. The protocol presented here is applicable for the two regulated size classes of viable or living organisms (organisms ≥ 50 μm, and organisms ≥ 10 μm and < 50 μm in minimum dimension), and the group of specified indicator bacteria (toxicogenic *Vibrio cholerae* [O1 and O139], *Escherichia coli*, and intestinal enterococci), prescribed by the BWMC regulation D-2 performance standard. For consistency and brevity, these three categories (i.e. the two size classes and indicator bacteria) are called "groups of organisms" in this protocol. Currently, the structure (but not the specific limits) of the D-2 standard, with the three groups of organisms, is the basis for most, if not all, other ballast water discharge standards. Different or additional groups of organisms and regulatory limits could be addressed by this protocol in the event of modifications to current regulations.
2. This protocol is based on related studies by Waldmann et al. (2010), Drake et al. (2014), and First et al. (2018). Thus, most aspects of the protocol itself have been validated.
3. CMDs include various sensors, instruments, kits, methods, and assays designed to measure ballast water discharge standards and other relevant requirements, either directly or indirectly as indicative measures. These devices can be employed in the

laboratory, with samples collected from ships' ballast discharge and transported to the laboratory for analysis; in the field, either dockside or onboard ships; as hand-held or mobile devices brought onboard; or as instruments integrated directly into a ship's ballast system, including the BWMS.

4. CMDs that are currently commercially available may not measure all three groups of organisms. Indeed, depending on the purpose for which devices will be used in the future, the vendors' claims and specifications may vary. This protocol is designed to determine a devices' data quality and reliability but not the suitability of the device for a given purpose, which should be decided by the end-user (e.g. administration, ship owner, BWMS manufacturer, etc.).
5. In the future, additional or alternative groups of organisms may be identified, and discharge limits may change. Regardless, a CMD should be verified following this protocol only for the group of organisms it is intended to quantify, as per the vendor's specifications.
6. If the vendor for a CMD indicates there are restrictions on its use (e.g. it is intended only for use in freshwater), the device should only be tested under these restricted conditions. If the vendor has not stated any restrictions, the CMD should be tested according to the full matrix of laboratory and field tests, as described in the subsequent sections and as shown in Table 1.
7. All verification of CMDs should be conducted by a third-party testing facility or organization that is independent from the device developer, manufacturer or vendor; should include appropriate quality assurance and quality control (QA/QC; e.g. International Organization for Standardization/International Electrotechnical Commission [ISO/IEC] 17025 standard), and have the specific, individual test plans and the final reports reviewed by experts.
8. For a given make/model of a CMD, one unit should be randomly selected for testing, and the same unit should be used in all verification testing (laboratory and field), which can take place over several weeks to months. If inter-device variability is of interest, three or more devices of the same make/model can also be tested for a subset of the variables shown in Table 1.

2 Verification testing parameters

At a minimum, the performance and efficacy of a given CMD make/model should be verified through measurements of the parameters listed below, under a range of conditions representative of the device's intended use. CMDs should always be tested and used according to the manufacturers' instructions for calibration, operation, and maintenance. The minimum parameters and level of replication are described in this section, and the experimental design is presented in Section 5 and in Table 1.

2.1 Accuracy

Accuracy is the closeness of a measured value to the known, or agreed-upon, reference standard value. The accuracy of an individual CMD should be determined under multiple salinities, organism communities and concentrations, and other water quality parameters that may influence device performance (e.g. optical clarity, interferences from residual chemicals used by BWMS, temperature, etc.). Accuracy should initially be determined in controlled laboratory

tests, followed by field tests. Repeated comparisons between a device's measurements and a reference standard (described in Section 3) should be completed with the appropriate level of replication to ensure statistical confidence (calculated prior to initiating the validation; e.g. n = 3, n = 5, or n = 10; see Bedson and Sargent, 1996; Hospodsky *et al.*, 2010; and Krzywinski and Altman, 2013).

2.2 Precision

Precision is the repeatability of a measurement under consistent condition(s). The precision of an individual CMD should be determined through controlled laboratory tests. The standard deviation should be calculated from multiple consecutive measurements of a single and stable reference standard solution under stable conditions. These repeated, consecutive measures should provide the appropriate level of replication to ensure statistical confidence (calculated prior to initiating the validation; e.g. n = 10, n = 15, or n = 20; see Bedson and Sargent, 1996; Hospodsky et al., 2010; and Krzywinski and Altman, 2013). This process should be repeated for other relevant stable reference solutions and environmental conditions.

2.3 Detection or quantification limits

Detection or quantification limits are the lowest and, when relevant, the highest values that can be detected with an acceptable level of confidence. Detection limits of an individual CMD should be determined in controlled laboratory tests by quantifying the signal-to-noise ratio. These repeated measurements are taken on samples with low concentrations (at and below the D-2 standard) and on blanks (known zero). From these measurements, the minimum concentration at which the known value can be quantified with a signal-to-noise ratio of 10:1 is determined. The repeated measure should be completed with the appropriate level of replication to ensure statistical confidence (calculated prior to initiating the validation; e.g. n = 3, n = 5, or n = 10; see Bedson and Sargent, 1996; Hospodsky et al., 2010; and Krzywinski and Altman, 2013).

2.4 Reliability

Reliability is the ability of an instrument to maintain its integrity or stability in operations and data collection over time. The reliability of an individual CMD should be determined in two primary ways from the data collected during all laboratory and field tests: (1) a comparison should be made of the proportion of data recovered with respect to the data that the device was intended to have collected over a set period of time (regardless of data quality); and (2) a report should be made of the total number of times, and percentage of time, where the device operated/functioned as designed, without interruption or non-scheduled maintenance, calibration, or repair. Comments on the physical condition of the device (e.g. physical damage, flooding, corrosion, battery failure, etc.) should also be recorded. When appropriate, the drift of in-use, device-reported values over time compared to blanks, or reference standards, can also be quantified (please note that these parameters are not addressed in this protocol).

3 Reference Standard

While the true, absolute concentration of viable or living organisms in discharged ballast water is often unknown, independently verified and accepted methods for quantifying organism concentrations, with known uncertainties, are currently being employed during type approval testing for BWMS. Thus, a suitable method, found within existing BWMS testing protocols (IMO, 2018; IMO, 2015; US Environmental Protection Agency [EPA], 2010) that is appropriate to the BWMS, organism group(s), and discharge limits of interest (including appropriate sample collection, handling, analysis, equipment calibration and maintenance, etc.), should be used to generate reference standard values for verifying the performance of the CMD. The selected reference standard should also be agreed to by the relevant regulatory agency, device vendor, and independent testing organization.

Please note that there is a range of available reference standards (each with its own associated uncertainties, errors and biases), and not all reference standards will be applicable to every CMD, or will be acceptable to every organization in the validation process.

4 Verification protocol

To maximize both the value and standardization of device verifications, test plans should be individual, customized, and detailed, and should:

i) be drafted separately for each specific type, make, and model of CMD;

ii) be based on existing and accepted practices for instrument and method testing (e.g. ISO, US EPA, or Alliance for Coastal Technologies [ACT]);

iii) include diverse biological communities and water quality conditions as required for BWMS certification testing;

iv) if other specific variables are known or suspected to affect the performance of a specific device, they should also be included as test parameters in verification testing (e.g. the ship's electrical noise, residual chemicals in ballast water after treatment, water temperature, etc.); and

v) include expert-review of test protocols and final reports.

To reduce the per-device testing effort, the verification protocol can be used to simultaneously evaluate multiple different devices that have a similar intended purpose (e.g. same target organisms, circumstances of use, etc.). Verification testing should not be used for direct comparisons of performance, nor for ranking among different makes/models of devices. However, when operations, specifications, and reference standards are the same or similar for multiple devices, and when appropriate, testing can occur with several CMDs using the same laboratory conditions, field conditions, and reference standards (e.g. see ACT Technology Evaluations). Each device should have its own individual test plan and final report.

Table 1. Matrix of verification tests for ballast water CMDs. Note that the measurements used to determine precision and detection limits (the last two rows of the table) may be taken from the samples prepared for accuracy testing, thereby reducing the total number of tests. Note also that the full suite of tests represented by this table would only be needed for a device that claims to quantify all groups of organisms in the D-2 performance standard, and to operate in all three salinities. Also note that the replication listed below (e.g. n ≥ 3 tests) is the minimum recommended level of replication, and additional tests could be needed depending on the statistical confidence desired. Reliability is calculated from all data as described in Section 5.7. Finally, for the tests to calculate accuracy and detection limits, the bracketing of the performance (discharge) standard (DS) is represented by < DS, ≈ DS, and > DS to indicate below, approximately equal to, and above the discharge standard, respectively.

Parameter calculated	Test type	Salinity	Replicate measurements per group of organisms								
			Bacteria			≥ 10 and < 50 μm			≥ 50 μm		
Accuracy	Laboratory - prepared challenge water	Fresh	< DS	≈ DS	> DS	< DS	≈ DS	> DS	< DS	≈ DS	> DS
			n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3
		Brackish	< DS	≈ DS	> DS	< DS	≈ DS	> DS	< DS	≈ DS	> DS
			n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3
		Marine	< DS	≈ DS	> DS	< DS	≈ DS	> DS	< DS	≈ DS	> DS
			n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3
	Laboratory - ambient challenge water	Fresh	n ≥ 3			n ≥ 3			n ≥ 3		
		Brackish	n ≥ 3			n ≥ 3			n ≥ 3		
		Marine	n ≥ 3			n ≥ 3			n ≥ 3		
	Field	Not specified	n ≥ 3			n ≥ 3			n ≥ 3		
Precision	Laboratory - prepared challenge water	1 salinity (different from detection limits test)	≈ DS			≈ DS			≈ DS		
			n ≥ 10			n ≥ 10			n ≥ 10		
Detection limits	Laboratory - prepared challenge water	1 salinity (different from precision test)	< DS	≈ DS	> DS	< DS	≈ DS	> DS	< DS	≈ DS	> DS
			n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3	n ≥ 3

5 Experimental design

Tests should be conducted in controlled laboratory, and appropriate field settings. All tests should be conducted: (1) with sample volumes consistent with those required by the CMD, and (2) with representative samples of the group(s) of organisms intended to be quantified by the device. Testing is described in the subsequent sections and in Table 1.

Laboratory testing should involve two types of challenge water, "prepared challenge water" and "ambient challenge water":

i) Prepared challenge water should meet the salinity, dissolved organic carbon (DOC), particulate organic carbon (POC), and total suspended solid (TSS) thresholds of interest. For example, if validating devices for all salinities used under the BWMC, prepared challenge water should be made to meet each of the three salinities prescribed in the BWMS Code (IMO, 2018). In these preparations, the organism concentrations should be adjusted to bracket the discharge standard (described in Section 5.2, point iii).

ii) Ambient challenge water should be collected from natural environments that meet the intended salinity range(s) of the device. It must contain diverse ambient organisms and not be manipulated.

Field testing of CMDs serves two purposes: (1) confirm the accuracy of measurements made on samples of a ships' treated, discharged ballast water, with the associated natural water quality conditions, and planktonic communities in a typically compromised physical/physiological state; and (2) assess the ability of the device to work under "real-world" environments/conditions, including dockside adjacent to a ship, onboard a ship as a handheld device, or integrated into a ship's ballast system. Field testing determines not only the reliability of the CMD, but also if its accuracy in the field is consistent with the accuracy measured in laboratory tests. Field tests are a supplement to the more comprehensive suite of laboratory tests, and should only involve the subset of parameters needed to document real-world consistency and reliability (Table 1).

5.1 Comparative analysis

For both laboratory and field testing, the CMD results should be compared to an accepted reference standard method for enumerating living or viable organisms that is relevant to the device being tested (as described in Section 3).

5.2 Laboratory tests using prepared challenge water

i) Laboratory cultures of organisms of the appropriate size can be used in these tests. For consistency in CMD verification testing by a given test organization, cultures of healthy organisms should be used, and a minimum of three relatively diverse species, should be tested together in a mixture (e.g. phytoplankton cultures in exponential growth phase, and species from three different families)

ii) It is appreciated that laboratory testing with cultures of toxicogenic strains of *Vibrio cholerae* or other pathogens of concern is challenging and requires specific safety and handling conditions and procedures. However, if the CMD is designed to quantify pathogens, then this laboratory testing, using prepared challenge water, is critical,

since toxicogenic pathogens may rarely be found in ambient waters, or during field testing.

iii) For each group of organisms, a dilution series should be created from laboratory cultures using 0.2 μm filtered water with the appropriate salinity. Each dilution series should have at least three concentrations of organisms. The concentrations should be created by diluting or concentrating the organism mix, so that the dilution series spans above and below the discharge standard. This step, and all other steps in preparing the challenge water, should be done carefully, to minimize organism mortality and loss. To ensure linearity of measurements for devices that do not have only pass/fail outputs, the highest concentration of organisms should be at least 5 x greater than the discharge standard, but no more than 50 x greater (unless the range of concentrations stipulated by the vendor exceeds this amount).

iv) DOC, POC, and TSS should be adjusted in the challenge water to meet any applicable minimum thresholds for a given salinity (e.g. device operational specifications and/or specifications in guidelines/requirements for testing of BWMS), in accordance with accepted methods for BWMS testing (IMO, 2018; IMO, 2015; US Environmental Protection Agency [EPA], 2010). Temperature should not be manipulated, but it should be measured and reported.

v) Other variables and parameters that are suspected to have a possible effect on device performance should also be incorporated and included in the suite of repeated tests, as appropriate. Examples of potential parameters/variables are optical clarity, interferences from residual chemicals used by BWMS, and temperature.

5.3 Laboratory tests using ambient challenge water

i) Ambient water should be collected from three distinct natural environments with varying salinities (see Table 1). The collected water should be used as challenge water without any dilution or concentration of organisms, or the artificial manipulation of temperature, salinity, TSS, DOC, or POC. The natural values of all these parameters should be measured and reported. As a minimum, ambient challenge water should simply reflect natural and diverse assemblages of organisms, and physical and chemical parameters, but laboratory tests with ambient water can be expanded to include a dilution series, when appropriate.

ii) The abundance and taxonomic composition of organisms in ambient challenge water should be characterized (identified) to the lowest feasible taxonomic level. Recognizing that devices may have species-specific biases, the purpose of this step is to demonstrate the diversity of organisms that are quantified by the CMD. Note that this step should be done using an accepted reference method such as microscopy, it is not intended to be performed with the CMD.

5.4 Field tests on treated discharged water

i) The nature of the field tests should be described in detail, including a description of the circumstances (if the device is used alongside or onboard a ship); documentation of sample collection, handling and analysis; type of BWMS; and history/provenance of sampled ballast water (e.g. Bradie et al., 2018).

ii) At a minimum, ballast water discharged from a ship after treatment by a functioning BWMS should be used as field testing challenge water. However, samples of ballast water upon uptake by a ship may also be tested when relevant.

iii) As in laboratory testing, the appropriate level of replication should be conducted to ensure statistical confidence in field testing. This should include a minimum of three repeated field test events, by testing one specific CMD unit either during three distinct ballasting events from a single ship, or during one ballasting event each from three distinct ships. Regardless of the approach taken, the data from the same CMD should be compared against the same reference standard as used in laboratory tests.

iv) The abundance and taxonomic composition of organisms in field test water should be characterized (identified) to the lowest feasible taxonomic level (Section 5.3, point ii), and the device accuracy (Table 1) and reliability (Section 5.7, point i) should be quantified.

5.5 Ancillary environmental data

At a minimum, water temperature, salinity (or conductance), pH, and TSS should be measured during all laboratory and field tests, using standard or approved methods or instruments. If possible, POC and DOC should be measured, as well as any other additional water quality parameters that are suspected to influence the performance of the CMD.

5.6 Test conditions and replication for accuracy, precision, and detection limits

The conditions for the accuracy, precision and detection limits laboratory tests, with prepared and ambient challenge water, and for the accuracy field tests, are shown in Table 1. In all cases, the measurement/assessment of organism concentration that is collected by the CMD should be compared to the reference standard, and the appropriate statistical analysis should be conducted (e.g. Bedson and Sargent, 1996; Hospodsky et al., 2010; and Krzywinski and Altman, 2013). Note that additional dilutions may be needed if the vendor of the CMD claims that the CMD can measure concentrations well below the discharge standard.

5.7 Reliability assessments

The reliability of the CMD should be determined as described in Section 2.4 using data collected during all testing, and specifically under the conditions of intended use (e.g. on a laboratory bench top, outdoor field conditions, the engine room of a ship, or elsewhere on board). Reliability should be calculated: (i) as the proportion of the data recovered with respect to the data the device was supposed to have collected over a given period of time; and (ii) as the percentage of time, and total number of times, that the device operated as designed, without interruption or non-scheduled maintenance, calibration, or repair. Finally, the physical condition of the device, including any physical damage, flooding, corrosion, battery failure, etc., should also be documented (e.g. with notes and photographs) and reported.

6 Data Management and Quality

i) The independent testing facility should follow standard/accepted data management and analysis procedures. For example, data logs should be recorded throughout testing, copied or duplicated, and archived daily. The datasheets should be signed by the analyst upon completion, verified by a quality officer, and stored until the data are logged into a digital file and the data themselves are verified. Data reported by the CMD should be manually transcribed on formatted data sheets and, if applicable, logged by the device itself. Additionally, data from other analyses should be recorded in standard formats, such as data collection forms, bound and paginated laboratory and field notebooks, spreadsheets, and electronic data files.

ii) Specific data analyses should be conducted as prescribed in individual device test plans. For example, accuracy should be measured relative to the reference method using a standard approach, such as percent difference, and precision should be measured as the variation among replicate readings and subsamples.

iii) All testing should occur at facilities employing a rigorous quality control/quality assurance programme that has been approved, certified, and audited by an independent accreditation body or the relevant regulatory agency. A test plan and standard operating procedures (SOPs) should be followed while conducting all tests.

iv) To ensure quality results, at a minimum, samples should be blinded, and the blinding process should be overseen and verified by a person not participating in the analysis of the samples.

v) For at least one randomly chosen subsample per test, two analysts should aliquot, distribute, process and analyse the sample using the CMD. Readings differing by ≤ 25% are considered to be within typical variation. Likewise, the variation of the reference method used should be quantified and reported in the same manner.

7 Reporting

i) The test report should include the test plan (with all laboratory and field testing details), all SOPs, all logged instrument data collected by the ballast water CMD, and all raw data (both direct verification test and ancillary environmental data).

ii) The following parameters should be described, calculated, and summarized from the test data and included in the test report: the accuracy (reported separately from laboratory and field tests), precision, detection limits, and reliability. Additionally, the probability of detecting an exceedance of the discharge standard should be calculated as in First *et al.* (2018).

iii) The CMD and reference standard should concur in their results. The level of agreement between the results of the CMD and reference standard should (1) be decided by the appropriate authority, (2) be based on the statistical confidence of the measurements, and (3) meet the requirements of the entity requiring the test.

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Please note that the guidance put forward in the publication does not represent the official views of the authors' affiliations.

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Annex 2: List of abbreviations

ACT	Alliance for Coastal Technologies
BWMC	IMO ballast water management convention
BWMS	Ballast water management system
CMD	Compliance monitoring device
CSLC	California State Lands Commission
DOC	Dissolved organic carbon
EPA	US Environmental Protection Agency
IMO	International Maritime Organization
IOC	Intergovernmental Oceanographic Commission
ISO/IEC	International Organization for Standardization and International Electrotechnical Commission
POC	Particulate organic carbon
QA/QC	Quality assurance and quality control
TSS	Total suspended solids
USCG	US Coast Guard
WGBOSV	ICES /IOC/IMO Working Group on Ballast and other Ship Vectors