



Technical Circular

No.: 054/2021

Date: 15th November 2021

Subject: Requirements for engaging Approved Firms for Commissioning Test of Ballast Water Management Systems (BWMS).

1. Amendments to Regulation E-1 of the BWM Convention were adopted at MEPC 75 vide resolution MEPC.325(75), which require a commissioning test at the time of initial/additional survey upon installation of BWMS. Although the amendments will enter into force on 1 June 2022, based on MEPC's suggestion to consider the application of the amendments as soon as possible, many Flag Administrations have already made commissioning test mandatory at the time of initial/additional survey upon installation of BWMS.
2. Commissioning test is to be conducted in accordance with 2020 Guidance for the commissioning testing of ballast water management systems (BWM.2/Circ.70/Rev.1), as may be amended. The purpose of commissioning testing is to validate the installation of a ballast water management system (BWMS) by demonstrating that its mechanical, physical, chemical and biological processes are working properly.
3. **As per IACS UR Z17, Rev.16 which will be effective from 1st January 2022, firms engaged in Commissioning Testing of Ballast Water Management Systems (BWMS), are to be approved by Classification Society.**
4. Service Suppliers are required to have a quality management system complying with the most current version of ISO 9000 series and be accredited to relevant standards such as ISO/IEC 17025 or equivalent.
5. Service Suppliers are to be independent of the BWMS manufacturer or supplier including shipyards.
6. Service suppliers are to have documented procedures for sampling collection and handling, analysis, assessment of BWMS correct operations and documenting and reporting.
7. Testing is to be conducted using indicative analysis equipment acceptable to IRS. For indicative analysis equipment planned to be used, the equipment OEM instruction manuals are to be available.
8. **Sampling & Analysis:** Sampling is to be carried out in accordance with BWM.2/Circ.42/Rev.2 (G2 Guidelines). The representative samples are to be analyzed as a minimum for the two size classes of organisms, namely $\geq 50 \mu\text{m}$ and $\geq 10 \mu\text{m}$ to <



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. While we have taken utmost care to be as factual as possible, readers/ users are advised to verify the exact text and content of the Regulation from the original source/ issuing Authority.

50 µm, specified in IMO Circular BWM.2/ Circ.70/Rev.1 - Guidance for the Commissioning Testing of Ballast Water Management Systems using indicative analysis methods. Detailed analysis of all organism type/size classes or combination of detail and indicative analysis can also be performed.

9. Operators who conduct commissioning testing are to:
 - demonstrate knowledge in the use of different ballast water testing equipment for the purpose of assessing biological efficacy;
 - have documented evidence of sufficient engineering and biological knowledge to conduct the commissioning testing
 - have knowledge of IMO BWM.2/Circ.70/Rev.1 and BWM.2/Circ.42/Rev.2 as may be amended
 - be trained in the proper use of portable indicative analysis equipment.
 - be familiar with the technologies utilized by the indicative sampling equipment and understand water quality issues.
 - be trained in the proper disposal procedures for water samples following testing
 - have knowledge of the system design limitations of the BWMS
 - have the procedures and knowledge to be able to assess the applicable self-monitoring parameters
10. Upon satisfactory verification of compliance to the requirements of IACS UR Z17, the service supplier will be issued with a Certificate of Approval.
11. In view of the above requirements, shipowners are advised to engage approved service supplier firms for Commissioning Testing of Ballast Water Management Systems.
12. Firms interested in providing the service for Commissioning Testing of Ballast Water Management Systems are advised to contact us at ho@irclass.org for further information regarding the approval process.

Enclosure:

1. IMO Resolution MEPC.325(75)
2. Relevant sections of IACS UR Z17, Rev.16
3. BWM.2/Circ.70/Rev.1
4. BWM.2/Circ.42/Rev.2

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ANNEX 2

**RESOLUTION MEPC.325(75)
(adopted on 20 November 2020)**

**AMENDMENTS TO THE INTERNATIONAL CONVENTION FOR THE CONTROL AND
MANAGEMENT OF SHIPS' BALLAST WATER AND SEDIMENTS, 2004**

Amendments to regulation E-1 and appendix I

**(Commissioning testing of ballast water management systems and
form of the International Ballast Water Management Certificate)**

THE MARINE ENVIRONMENT PROTECTION COMMITTEE,

RECALLING Article 38(a) of the Convention on the International Maritime Organization concerning the functions of the Marine Environment Protection Committee conferred upon it by international conventions for the prevention and control of marine pollution from ships,

RECALLING ALSO article 19 of the International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 (the BWM Convention), which specifies the amendment procedure and confers upon the Marine Environment Protection Committee of the Organization the function of considering amendments thereto for adoption by the Parties,

HAVING CONSIDERED, at its seventy-fifth session, proposed amendments to the BWM Convention regarding commissioning testing of ballast water management systems and the form of the International Ballast Water Management Certificate,

1 ADOPTS, in accordance with article 19(2)(c) of the BWM Convention, amendments to regulation E-1 and appendix I;

2 DETERMINES, in accordance with article 19(2)(e)(ii) of the BWM Convention, that the amendments shall be deemed to have been accepted on 1 December 2021 unless, prior to that date, more than one third of the Parties have notified the Secretary-General that they object to the amendments;

3 INVITES the Parties to note that, in accordance with article 19(2)(f)(ii) of the BWM Convention, the said amendments shall enter into force on 1 June 2022 upon their acceptance in accordance with paragraph 2 above;

4 INVITES ALSO the Parties to consider the application of the amendments to regulation E-1 with regard to commissioning testing as soon as possible to ships entitled to fly their flag, taking into account the *Guidance for the commissioning testing of ballast water management systems* (BWM.2/Circ.70/Rev.1), as may be amended;

5 RESOLVES that the analysis undertaken in the context of commissioning testing should be indicative;

6 REQUESTS the Secretary-General, for the purposes of article 19(2)(d) of the BWM Convention, to transmit certified copies of the present resolution and the text of the amendments contained in the annex to all Parties to the BWM Convention;

7 REQUESTS ALSO the Secretary-General to transmit copies of the present resolution and its annex to Members of the Organization which are not Parties to the BWM Convention;

8 REQUESTS FURTHER the Secretary-General to prepare a consolidated certified text of the BWM Convention.

ANNEX

**AMENDMENTS TO THE INTERNATIONAL CONVENTION FOR THE CONTROL AND
MANAGEMENT OF SHIPS' BALLAST WATER AND SEDIMENTS**

Regulation E-1

Surveys

1 Paragraph 1.1 is replaced by the following:

"1 An initial survey before the ship is put in service or before the Certificate required under regulation E-2 or E-3 is issued for the first time. This survey shall verify that the ballast water management plan required by regulation B-1 and any associated structure, equipment, systems, fitting, arrangements and material or processes comply fully with the requirements of this Convention. This survey shall confirm that a commissioning test has been conducted to validate the installation of any ballast water management system by demonstrating that its mechanical, physical, chemical and biological processes are working properly, taking into account the guidelines developed by the Organization.*"

2 Paragraph 1.5 is replaced by the following:

".5 An additional survey, either general or partial, according to the circumstances, shall be made after a change, replacement, or significant repair of the structure, equipment, systems, fittings, arrangements and material necessary to achieve full compliance with this Convention. The survey shall be such as to ensure that any such change, replacement or significant repair has been effectively made, so that the ship complies with the requirements of this Convention. When an additional survey is undertaken for the installation of any ballast water management system, this survey shall confirm that a commissioning test has been conducted to validate the installation of the system by demonstrating that its mechanical, physical, chemical and biological processes are working properly, taking into account the guidelines developed by the Organization.*"

* Refer to the *2020 Guidance for the commissioning testing of ballast water management systems* (BWM.2/Circ.70/Rev.1), as may be amended.

Appendix I

Form of International Ballast Water Management Certificate

3 The footnote of "IMO Number" under the item "Particulars of ship" is replaced by the following:

"IMO Ship Identification Number Scheme adopted by the Organization by resolution A.1117(30), as may be amended."

4 The text under the title "Details of ballast water management method(s) used" is replaced by the following:

"Method of ballast water management used
Date installed (if applicable) (dd/mm/yyyy)
Name of manufacturer (if applicable)"

The principal ballast water management method(s) employed on this ship is/are:

- in accordance with regulation D-1
- in accordance with regulation D-2
(describe)
- the ship is subject to regulation D-4
- other approach in accordance with regulation"

Z17 Procedural Requirements for Service Suppliers

(1997)
 (Rev.1
 June 1999)
 (Rev.2
 Nov 1999)
 (Rev.3
 July 2002)
 (Rev.4
 July 2003)
 (Rev.5
 Feb 2004)
 (Rev.6
 June 2007)
 (Rev.7
 Nov 2007)
 (Rev.8
 July 2008)
 (Rev.9
 June 2012)
 (Corr.1
 Aug 2012)
 (Rev.10
 Jan 2015)
 (Rev.11
 June 2015)
 (Rev.12
 Nov 2016)
 (Rev.13
 Jan 2018)
 (Rev.14
 Mar 2019)
 (Rev.15
 Oct 2020)
 (Corr.1
 Dec 2020)
(Rev.16
 Aug 2021)

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Annex 1 Special Requirements for Various Categories of Service Suppliers

Revision notes:

1. Rev.4 added in Annex, Section 10, 11 & 12, with reference in 3.1.2.
2. Rev.5 clarified applicability to thickness measurement companies in 3.1.1 and Annex 1, 1.1.
3. Rev.6 is to be uniformly implemented by IACS Societies and Associates from 1 January 2008.
4. Rev.7: Section 13 is added to Annex 1. This section applies to requests for recognition of test laboratories received on or after 1 January 2008.
5. Rev.8: Reference to IACS Recommendations 101 and 102 added.
6. Rev.9: Includes procedures for approval of test laboratories against res. MSC.288(87) and applies to requests for recognition of approval of testing laboratories received on or after 1 July 2013. However, deletion of the reference to PR 34 in Rev.9 applies from 1 July 2012.
7. Rev.10: Full document review carried out - Rev.10 is to be uniformly implemented by IACS Societies from 1 January 2016.
8. Rev.11: Full document review in order to verify the compliance with R.O. Code, IMO Res. MSC 349(92), carried out - Rev.11 is to be uniformly implemented by IACS Societies from 1 July 2016.
9. Rev.12: Section 15 of Annex 1 revised - Rev.12 is to be uniformly implemented by IACS Societies from 1 January 2018.
10. Rev.13: Section 3 of Annex 1 revised - Rev.13 is to be uniformly implemented by IACS Societies from 1 January 2019.
11. Rev.14: Section 13 of Annex 1 revised - Rev.14 is to be uniformly implemented by IACS Societies from 1 January 2020.
12. Rev.15: Section 5 revised, Section 17 of Annex 1 newly added - Rev.15 is to be uniformly implemented by IACS Societies from 1 July 2021.
13. Rev.16: Section 18 of Annex 1 newly added – Rev.16 is to be uniformly implemented by IACS Societies from 1 January 2022.

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(cont)**1. General**

1.1 To approve firms providing services, such as measurements, tests or maintenance of safety systems and equipment, the Society is to apply procedures in this unified requirement and relevant Annex 1.

2. Objective

2.1 The objective of this procedure is to set minimum requirements for approval and certification of service suppliers and is applicable to both initial and renewal audits.

3. Definitions

- **Manufacturer:** A company that manufactures equipment required to be periodically serviced and/or maintained.
- **Service Supplier (A Service Supplier or category of Service Supplier may be referred to here after simply as 'supplier'):** A person or company, not employed by an IACS Member, who at the request of an equipment manufacturer, shipyard, vessel's owner or other client acts in connection with inspection work and provides services for a ship or a mobile offshore unit such as measurements, tests or maintenance of safety systems and equipment, the results of which are used by surveyors in making decisions affecting classification or statutory certification and services.
- **Agent:** A Person or Company authorised to act for or to represent a Manufacturer or approved/recognized service supplier.
- **Subsidiary:** A Company partly or wholly owned by a Manufacturer or approved/recognized service supplier.
- **Subcontractor:** A Person or Company providing services to a Manufacturer or approved/recognized service supplier, with a formal contract defining the assumption of the obligations of the service supplier.

5. Procedure for Approval and Certification

5.1 Submission of documents

5.1.1 The following documents are to be submitted to the Society for review. General requirements concerning suppliers are given in 5.2, and specific requirements as relevant, in Annex 1.

- Outline of company, e.g. organisation and management structure, including subsidiaries to be included in the approval/certification
- List of nominated agents, subsidiaries and subcontractors
- Experience of the company in the specific service area
- For categories of Service Suppliers that require ~~authorization~~ certification from manufacturers, manufacturer's documentary evidence that the Service Supplier has been ~~authorized~~ certified or licensed to service the particular makes and models of equipment for which approval is sought shall be provided
- List of operators/technicians/inspectors documenting training and experience within the relevant service area, and qualifications according to recognised national, international or industry standards, as relevant
- Description of equipment used for the particular service for which approval is sought
- A guide for operators of such equipment
- Training programmes for operators/technicians/inspectors
- Check lists and record formats for recording results of the services referred to in Annex 1
- Quality Manual and/or documented procedures covering requirements in 5.5
- Documented procedures for communication with the crew prior to commencing work, so that it is safe to decommission the equipment being maintained, and to provide a safe system of work in place
- Evidence of approval/acceptance by other bodies, if any
- Information on the other activities which may present a conflict of interest
- Record of customer claims and of corrective actions requested by certification bodies
- Operators/technicians/inspectors documentation they have acknowledged the code of conduct

5.2 General requirements:

5.2.1 Extent of Approval – The supplier shall demonstrate, as required by 5.2.2 – 5.2.11, that it has the competence and control needed to perform the services for which approval is sought.

5.2.2 Training of personnel – The supplier is responsible for the qualification and training of its personnel to a recognised national, international or industry standard as applicable. Where such standards do not exist, the supplier is to define standards for the training and qualification of its personnel relevant to the functions each is authorised to perform. The personnel shall

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also have adequate experience and be familiar with the operation of any necessary equipment. Operators/technicians/inspectors shall have had a minimum of one year tutored on-the-job training. Where it is not possible to perform internal training, a program of external training may be considered as acceptable.

5.2.3 Supervision – The supplier shall provide supervision for all services provided. The responsible supervisor shall have had a minimum of two years of experience as an operator/technician/inspector within the activity for which the supplier is approved. For a supplier consisting of one person, that person shall meet the requirements of a supervisor.

5.2.4 Personnel records – The supplier shall keep records of the approved operators/technicians/inspectors. The record shall contain information on age, formal education, training and experience for the services for which they are approved.

5.2.5 Equipment and facilities – The supplier shall have the necessary equipment and facilities for the service to be supplied. A record of the equipment used shall be kept and available. The record shall contain information on maintenance and results of calibration and verifications. The Society shall assess and record the validity of previous measuring results when the equipment is found not to conform to requirements. The Society shall take appropriate action on the equipment affected.

5.2.6 Control of data:

When computers are used for the acquisition, processing, recording, reporting, storage, measurement assessment and monitoring of data, the ability of computer software to satisfy the intended application shall be documented and confirmed by the service supplier. This shall be undertaken prior to initial use and reconfirmed as necessary.

Note: Commercial off-the-shelf software (e.g. wordprocessing, database and statistical programmes) in general use within their designed application range may be considered to be sufficiently validated and do not require any subsequent confirmation.

5.2.7 Where several servicing stations are owned by a given company, each station is to be assessed and approved except as specified in 5.5.3

5.2.8 Procedures – The supplier shall have documented work procedures covering all services supplied.

5.2.9 Subcontractors – The supplier shall give information of agreements and arrangements if any parts of the services provided are subcontracted. Particular emphasis shall be given to quality management by the supplier in following-up such subcontracts. Subcontractors providing the services of the approved service supplier shall also meet the requirements of section 5.

5.2.10 Verification – The supplier shall verify that the services provided are carried out in accordance with approved procedures.

5.2.11 Reporting – The report shall be prepared in a form acceptable to the Society. The report should detail the results of inspections, measurements, tests, maintenance and/or repairs carried out. Special guidelines may be given in Annex 1. The report shall include a copy of the Certificate of Approval.

5.2.12 Documented procedures and instructions should be available for the recording of damages and defects found during inspection, servicing and repair work. This documentation is to be made available upon request.

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5.3 Auditing of the Supplier – Upon reviewing the submitted documents with satisfactory result, the supplier is audited in order to ascertain that the supplier is duly organised and managed in accordance with the submitted documents, and that it is considered capable of conducting the services for which approval/certification is sought.

5.4 Certification is conditional on a practical demonstration of the performance of the specific service as well as satisfactory reporting being carried out. At renewal audits, evidence of performance, verified by class surveyor, since the previous audit is sufficient to satisfy this requirement.

5.5 Quality System

5.5.1 The supplier shall have a documented system covering at least the following:

- code of conduct for the relevant activity
- maintenance and calibration of equipment
- training programmes for operators/technicians/inspectors
- supervision and verification to ensure compliance with operational procedures
- recording and reporting of information
- quality management of subsidiaries, agents and subcontractors
- job preparation
- periodic review of work process procedures, complaints, corrective actions, and issuance, maintenance and control of documents

5.5.2 A documented Quality system complying with the most current version of ISO 9000 series and including the above items, would be considered acceptable.

5.5.3 If a manufacturer of equipment (and/or its service supplier) applies to a Society for inclusion of its nominated agents and/or subsidiaries (excluding any subcontractor), in the approval, then it must have implemented a quality system certified in accordance with the most current version of ISO 9000 series. The quality system must contain effective controls of the manufacturer's (and/ or service supplier's) agents and/or subsidiaries. The nominated agents/subsidiaries must also have in place an equally effective quality system complying with the most current version of ISO 9000 series. Such approvals shall be based upon an evaluation of the quality system implemented by the parent company against the most current version of ISO 9000 series. The Society may require follow-up audits on such agents or subsidiaries against the most current version of ISO 9000 series to confirm adherence to this quality system.

5.6 Service Suppliers Relations with the Equipment Manufacturer

5.6.1 A company which works as a service station for manufacturer(s) of equipment (and as a service supplier in this field), shall be assessed by the manufacturer(s) and nominated as their agent. The manufacturer shall ensure that appropriate instruction manuals, material etc. are available for the agent as well as proper training of the agent's technicians. Such suppliers shall be approved either on a case by case basis, or in accordance with 5.5.3.

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(cont)**6. Certification**

6.1 Upon satisfactory completion of both the audit of the supplier and the demonstration test, as applicable, the Society may issue a Certificate of Approval stating that the supplier's service operation system has been found to be satisfactory and that the results of services performed in accordance with that system may be accepted and utilised by the Society's Surveyors in making decisions affecting classification or statutory certification, as relevant. The Certificate shall clearly state the type and scope of services and any limitations or restrictions imposed including type of equipment and/or names of Manufacturers of equipment where this is a limiting restraint. The supplier may also be included in the Society's record of approved service suppliers.

6.2 Renewal or endorsement of the Certificate is to be made at intervals not exceeding five (5) years by verification through audits that approved conditions are maintained or, where applicable, on expiry of the supplier's approval received from an equipment Manufacturer, whichever comes first. In the latter case, the Society is to be informed in due course by the Service Supplier. Individual Societies may require renewal or endorsement of the Certificate at intervals shorter than five (5) years and may require intermediate audits. For firms engaged in thickness measurements, renewal/endorsement of the Certificate is to be made at intervals not exceeding 3 years by verification that original conditions are maintained.

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(cont)**7. Information Regarding Alterations to the Certified Service Operating System**

7.1 When any alteration to the certified service operating system of the supplier is made, such alteration is to be immediately informed to the Society. Re-audit may be required when deemed necessary by the Society.

8. Cancellation of Approval

8.1 The Society reserves the right to cancel the approval and to inform the IACS Members accordingly (For Firms engaged in thickness measurements refer to PR23).

8.2 Approval may be cancelled in the following cases:

8.2.1 Where the service was improperly carried out or the results were improperly reported.

8.2.2 Where a Surveyor finds deficiencies in the approval service operating system of the supplier and appropriate corrective action is not taken.

8.2.3 Where alterations have been made to the Company's Quality System relevant to the service supplier certificates, without written notification to the Society.

8.2.4 Where the intermediate audit, if requested as per 6.2, has not been carried out.

8.2.5 Where wilful acts or omissions are ascertained.

8.2.6 Where any deliberate misrepresentation has been made by the Service Supplier.

8.3 A supplier whose approval was cancelled, may apply for re-approval provided it has corrected the non-conformities which resulted in cancellation, and the Society is able to confirm it has effectively implemented the corrective action.

8.4 Expiration or cancellation of the Supplier's parent company approval automatically invalidates approval of all agents and subsidiaries if these are certified according to 5.5.3.

9. Existing Approvals

Approvals for the categories of service suppliers granted before the date of implementation of UR Z17 by a society may remain valid as stated in the respective certificates for a period up to but not exceeding 3 years. Renewals of such certificates must be carried out in accordance with UR Z17.

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18. Firms engaged in Commissioning Testing of Ballast Water Management Systems (BWMS)

18.1 Extent of engagement - Sampling and Analysis of ballast water and Verification of the self-monitoring equipment during Commissioning Testing of Ballast Water Management Systems (BWMS), for Statutory purposes.

18.2 Procedure

18.2.1 Service suppliers are to have documented procedures including:

- Procedures for sampling collection and handling, analysis, assessment of BWMS correct operations and documenting and reporting. The procedures are to outline how the ballast water sampling and analysis is conducted with respect to each size class of organisms;
- Operating procedures for the ballast water test equipment specified including calibration, adjustment and maintenance

18.2.2 Service Suppliers are to be familiar with the BWMS operation including features and limits of each treatment technology, and self-monitoring parameters.

18.2.3 Service Suppliers are to be accredited to relevant standards such as ISO/IEC 17025 or equivalent, as applicable.

18.2.4 Service Suppliers are to be independent of the BWMS manufacturer or supplier including shipyards.

18.3 Operators – Service Suppliers are expected to be able to perform both the biological sampling and assessment of self-monitoring parameters and has responsibility for document that the requirements to the operator are satisfied. Therefore, operators who conduct commissioning testing are to:

- demonstrate knowledge in the use of different ballast water testing equipment for the purpose of assessing biological efficacy;
- have documented evidence of sufficient engineering and biological knowledge to conduct the commissioning testing;
- have knowledge of IMO BWM.2/Circ.70/Rev.1, as may be amended - 'Guidance for the Commissioning Testing of Ballast Water Management Systems' and IMO BWM.2/Circ.42/Rev.2 - 'Guidance on Ballast Water Sampling and Analysis for Trial Use in accordance with the BWM Convention and Guidelines (G2)', as may be amended;
- (* be trained in the proper use of portable indicative analysis equipment. Review of training records and/or interviews should be conducted to confirm the equipment will be properly used during testing;
- (* be familiar with and understand the design concepts of the Guidelines G2 sampling devices installed on the vessel's water ballast system. Personnel shall understand the need to maintain the G2 sampling devices clean and free of contaminants and the importance of controlling the ballast water sample flow rates from the G2 device (to avoid organism mortality in the sample);
- (* be familiar with the technologies utilized by the indicative sampling equipment and understand water quality issues that are both conducive to successful use of the equipment and circumstances that could challenge the use of the equipment;

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- : (*) be trained in the proper disposal procedures for water samples following testing.
- : (Δ) have knowledge of the system design limitations of the BWMS (as stated in the BWMS type approval certificate) and knowledge of the BWMS self-monitoring parameters, such as flow rate, pressure, TRO concentration, UV transmittance/intensity, etc. and how the BWMS notifies the operator in case he operates BWMS outside its system design limitations. This knowledge is relevant for evaluating whether the self-monitoring equipment of the BWMS indicates correct operation of the BWMS. In case Service Supplier are not present during ballasting operations, the Service Supplier shall have knowledge of how to access the BWMS log to evaluate that the BWMS operated correctly during ballasting operations;
- : (Δ) have the procedures and knowledge to be able to assess the applicable self-monitoring parameters (e.g., flow rate, pressure, TRO, UV intensity, etc.) of the BWMS, taking into account the System Design Limitations of the BWMS;

Notes: (1) the points marked with (*) are qualifications for operators performing sampling and analysis of ballast water; (2) the points marked with (Δ) are the qualifications for operators performing verification of the self-monitoring equipment ;(3) the points above without symbol are the common qualifications for service supplier.

18.4 Equipment and facilities

Equipment, procedures and methods for detailed analysis, where applicable, are to be in accordance with relevant International standard and/or accepted Industry standards. Laboratories conducting sample enumeration are to be accredited to ISO/IEC 17025 standard, or equivalent.

Testing should be conducted using indicative analysis equipment accepted by Society. information and reference to the acceptance documents for the equipment used should be submitted to the Society in the report which includes the results from the commissioning test as per IMO BWM.2/Circ.70/Rev.1, as may be amended. In case the indicative analysis equipment used has not been previously accepted by the Society, the following information is to be submitted to the Society;

- : Equipment information - type, model, technology used, evidence of calibration, detection range, Organism type/size classes that can be analyzed.
- : Test results conduct for the verification of accuracy, detection range and repeatability.
- : Certificate of standards, if available.

For indicative analysis equipment planned to be used, the equipment OEM instruction manuals shall be available. The manuals shall include, at least, clear guidance for the proper storage, handling, operation, maintenance, repair, and calibration.

Note: Each Service Supplier applicant will present the Surveyor their confidential internal procedures for conducting the indicative testing. Not all the equipment listed in the references will be used. For all equipment planned to be used, the instruction manuals shall be available.

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The Service Supplier will need to use specialty devices (e.g., sieves, screens, etc.) to separate the different organism sizes classes (i.e., $\geq 10 \mu\text{m}$ to $< 50 \mu\text{m}$, and $\geq 50 \mu\text{m}$, and indicator microbes) to support analysis of each size class.

Equipment used for the analysis of other physical-chemical water parameters is to be suitable for the intended use.

Indicative analysis equipment should be properly stored or transported to avoid damage and disturbance to calibrations, etc. when transporting from the Service Suppliers facilities to the vessels.

18.5 Sampling and Analysis

Service Suppliers are to follow relevant guidelines on sampling of ballast water. A standard operating procedure is to be defined for sampling of uptake water. Discharge sampling shall follow the IMO's 'Guidelines for Ballast Water Sampling (G2)'.

The representative samples shall be analyzed as a minimum for the two size classes of organisms, namely $\geq 50 \mu\text{m}$ and $\geq 10 \mu\text{m}$ to $< 50 \mu\text{m}$, specified in IMO Circular BWM.2/Circ.70/Rev.1 - Guidance for the Commissioning Testing of Ballast Water Management Systems using indicative analysis methods. Detailed analysis of all organism type/size classes or combination of detail and indicative analysis can also be performed.

Service Suppliers shall maintain a record of:

- : Operation of the BWMS during test period, including any recorded data or operator observations associated with the performance deviations, alarms or abnormal/unexpected operations.
- : Applicable self-monitoring parameters.

In case the commissioning testing requires the Service Supplier's personnel to work in hazardous areas (e.g., pump room for tankers, etc.), the Service Supplier shall either have equipment certified for the spaces or provide the Surveyor with a list of vessels for which they would not be able to conduct testing.

18.6 Reporting

Service Suppliers are to provide reports detailing the results of sampling and analysis of ballast water and assessment of self-monitoring parameters during commissioning testing. The format is to be acceptable to Society. The report, as a minimum, will contain the following:

- : Manufacturer's name
- : Model name
- : BWMS Technology limiting operating conditions and system design limitations
- : Operation required, e.g., ballasting, de-ballast, circulation, one pass, in tank, etc

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- Treatment rated capacity (TRC) in m³/h
- Relevant performance parameters (e.g. TRO, UV dose, UVI, flow rate or other relevant performance parameter).
- Alarms developed during operation.
- Installation location.
- Type Approval issued by and Certificate No
- Date installed
- Results of Sample analysis
- Pump flow rate, ballast tanks and volume
- Comments/Options: Filter and other major components, Process measurements.

18.7 Reference Documents

The Service Supplier is to have access to the following documents, as may be amended:

- IMO Resolution MEPC.300(72) – Code for Approval of Ballast Water Management Systems (BWMS Code)
- IMO Resolution MEPC.173(58) – Guidelines for Ballast Water Sampling (G2)
- IMO Circular BWM.2/Circ.42/Rev. 2 – Guidance on Ballast Water Sampling and Analysis for Trial Use in accordance with the BWM Convention and Guidelines (G2)
- IMO Circular BWM.2/Circ.70/Rev.1 - Guidance for the Commissioning Testing of Ballast Water Management Systems
- IMO Circular BWM.2/Circ.61 - Guidance on Methodologies that may be used for Enumerating Viable Organisms for Type Approval of Ballast Water Management Systems
- IMO Circular BWM.2/Circ.69 - Guidance on System Design Limitations of Ballast Water Management Systems and their Monitoring
- IMO Resolution MEPC.279(70) - 2016 Guidelines for Approval of Ballast Water Management Systems (G8)
- IMO Resolution A.1120(30) – Survey Guidelines under the Harmonized System of Survey and Certifications (HSSC), 2017 (for BWMS that were Type Approved to the 2016 G8)

End of Document

4 ALBERT EMBANKMENT
LONDON SE1 7SR
Telephone: +44 (0)20 7735 7611 Fax: +44 (0)20 7587 3210

BWM.2/Circ.70/Rev.1
9 December 2020

**INTERNATIONAL CONVENTION FOR THE CONTROL AND MANAGEMENT
OF SHIPS' BALLAST WATER AND SEDIMENTS, 2004**

2020 Guidance for the commissioning testing of ballast water management systems

- 1 The Marine Environment Protection Committee (MEPC), at its seventy-third session (22 to 26 October 2018), approved the *Guidance for the commissioning testing of ballast water management systems*.
- 2 MEPC 74 (13 to 17 May 2019) invited submissions to the Sub-Committee on Pollution Prevention and Response (PPR) concerning proposals on any necessary changes to the Guidance in light of the draft amendments to regulation E-1 of the BWM Convention.
- 3 MEPC 75 (16 to 20 November 2020) approved the *2020 Guidance for the commissioning testing of ballast water management systems*, prepared by PPR 7 (17 to 21 February 2020), as set out in the annex.
- 4 Member Governments and international organizations are invited to bring the annexed Guidance to the attention of all parties concerned.
- 5 This circular revokes BWM.2/Circ.70.

ANNEX

2020 GUIDANCE FOR THE COMMISSIONING TESTING OF BALLAST WATER MANAGEMENT SYSTEMS

Context

1 The purpose of commissioning testing is to validate the installation of a ballast water management system (BWMS) by demonstrating that its mechanical, physical, chemical and biological processes are working properly. Commissioning testing is not intended to validate the design of type-approved BWMS that are approved by the Administration.

2 The following Guidance for the commissioning testing of BWMS has been developed for use by persons fitting and verifying the installation of BWMS in accordance with:

- .1 regulation E-1 of the Convention;
- .2 paragraph 8.2.5 of the BWMS Code, which requires that the Administration issuing the international ballast water management certificate verify that installation commissioning procedures are on board the ship in a suitable format;
- .3 paragraph 8.3.6 of the BWMS Code, which requires that the installation commissioning procedures have been completed prior to the issuance of the IBWMC following the installation of a BWMS; and
- .4 paragraph 1.18 of resolution MEPC.174(58), which provides that, when a type-approved ballast water management system is installed on board, an installation survey according to section 8 should be carried out.

Commissioning testing

3 Local ambient water should be used for testing regardless of the level of challenge it poses to the BWMS.

4 The following steps should be undertaken following installation of the BWMS on board the ship, and after all ballasting equipment (e.g. pumps and piping) has been fully installed and tested, as appropriate:

- .1 a sample may be collected during ballast water uptake to characterize the ambient water, by any means practical (e.g. in-line sample port or direct harbour sample). Characterization of the ambient water does not require detailed analysis of the uptake water, however an indicative analysis may be undertaken;
- .2 a representative sample should be collected during the corresponding ballast water discharge after the full treatment has been applied. Samples should be collected from the sampling point as described in the *Guidelines on ballast water sampling* (G2). The total sample volume should be at least 1 m³. If a smaller volume is validated to ensure representative sampling of organisms, it may be used;

- .3 the representative samples should be analysed for the two size classes of organisms, namely $\geq 50 \mu\text{m}$ and $\geq 10 \mu\text{m}$ to $< 50 \mu\text{m}$, as specified in the D-2 standard, using indicative analysis methods listed in BWM.2/Circ.42/Rev.2, as may be amended; and
- .4 the applicable self-monitoring parameters (e.g. flow rate, pressure, TRO concentration, UV transmittance/intensity, etc.) of the BWMS should also be assessed, taking into account the system design limitations of the BWMS, and the correct operation of all sensors and related equipment should be confirmed.

5 The commissioning test is successful if the indicative analysis indicates that the discharge samples do not exceed the D-2 standard for the size classes analysed (see paragraph 4.3) and the self-monitoring equipment indicates correct operation. Indicative analysis equipment used should be to the satisfaction of the Administration. Indicative analysis is defined in BWM.2/Circ.42/Rev.2, as may be amended.

6 In the case that the ambient water is not appropriate for the commissioning testing (e.g. salinity of ambient water is outside the system design limitations of the BWMS), testing should be evaluated to the satisfaction of the Administration.

7 The collection and analysis of the representative samples should be independent of the BWMS manufacturer or supplier and to the satisfaction of the Administration.

Documentation

8 A written report, including methods, results (including raw data) and information on the self-monitoring parameters, should be provided to the Administration.

4 ALBERT EMBANKMENT
LONDON SE1 7SR
Telephone: +44 (0)20 7735 7611 Fax: +44 (0)20 7587 3210

BWM.2/Circ.42/Rev.2
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**INTERNATIONAL CONVENTION FOR THE CONTROL AND MANAGEMENT
OF SHIPS' BALLAST WATER AND SEDIMENTS, 2004**

**2020 Guidance on ballast water sampling and analysis for trial use in accordance with
the BWM Convention and Guidelines (G2)**

- 1 The Marine Environment Protection Committee, at its fifty-eighth session (October 2008), following the adoption of the *Guidelines for ballast water sampling (G2)* (resolution MEPC.173(58)), instructed the Sub-Committee on Bulk Liquids and Gases (BLG) to develop, as a matter of high priority, a circular to provide sampling and analysis guidance.
- 2 MEPC 65 (13 to 17 May 2013) approved BWM.2/Circ.42 on *Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)*, as agreed by BLG 17 (4 to 8 February 2013).
- 3 MEPC 66 (31 March to 4 April 2014) invited Member Governments and international organizations to submit further information and proposals related to ballast water sampling, analysis and contingency measures to the Sub-Committee on Pollution Prevention and Response (PPR), with a view to further developing and improving the relevant guidance documents and guidelines.
- 4 MEPC 68 (11 to 15 May 2015) approved the revised *Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)*, as agreed by PPR 2 (19 to 23 January 2015).
- 5 MEPC 75 (16 to 20 November 2020) approved the *2020 Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)*, as agreed by PPR 7 (17 to 21 February 2020), set out at annex.
- 6 Member Governments are invited to bring the annexed Guidance to the attention of all parties concerned.
- 7 This circular revokes BWM.2/Circ.42/Rev.1.

ANNEX 1

2020 GUIDANCE ON BALLAST WATER SAMPLING AND ANALYSIS FOR TRIAL USE IN ACCORDANCE WITH THE BWM CONVENTION AND GUIDELINES (G2)

1 INTRODUCTION

1.1 The purpose of this Guidance is to provide general recommendations on methodologies and approaches to sampling and analysis to test for compliance with the standards described in regulations D-1 and D-2 of the International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 (BWM Convention). This Guidance is an updated version of the guidance contained in document BLG 16/WP.4, taking into account advances in research since the document was first drafted, and should be read in conjunction with the BWM Convention, the *Guidelines for port State control under the BWM Convention* (resolution MEPC.252(67)) and the *Guidelines for ballast water sampling (G2)* (resolution MEPC.173(58)). Furthermore, and as instructed by MEPC 64, the sampling and analysis procedures to be used for enforcement of the BWM Convention should result in no more stringent requirements than what is required for Type Approval of ballast water management systems (BWMS).

1.2 This Guidance consists of two parts,

- .1 a discussion of the principles of sampling, accompanied by a list of recommended methods and approaches for analysis and sampling protocols available for compliance testing to the D-1 and D-2 standards in section 5; and
- .2 background information on sampling and analysis methodologies and approaches, set out in the annex.

1.3 Sampling and analysis for compliance testing is a complex issue. According to the *Guidelines for ballast water sampling (G2)*, testing for compliance can be performed in two steps. As a first step, prior to a detailed analysis for compliance, an indicative analysis of ballast water discharge may be undertaken to establish whether a ship is potentially in compliance with the Convention.

1.4 When testing for compliance, the sampling protocol used should result in a representative sample of the whole discharge of the ballast water from any single tank or any combination of tanks being discharged.

2 DEFINITIONS

For the purpose of this Guidance, the definitions in the BWM Convention apply and:

- .1 A *sample* means a relatively small quantity intended to show what the larger volume of interest is like.
- .2 *Representative sampling* reflects the relative concentrations and composition of the populations (organisms and/or chemicals) in the volume of interest. Samples should be taken in accordance with the annex, part 1 and/or part 2 of the *Guidelines on ballast water sampling (G2)*.
- .3 *Analysis* means the process of measuring and determining the concentrations and composition of the populations of interest (organisms and/or chemicals) within the sample.

- .4 An *indicative analysis* means a compliance test that is a relatively quick indirect or direct measurement of a representative sample of the ballast water volume of interest:
- .1 an indirect, indicative analysis may include measurements whose parameters do not provide a value directly comparable to the D-2 standard, including biological, chemical or physical parameters (e.g. dissolved oxygen levels, residual chlorine levels, Adenosine triphosphate (ATP), nucleic acid, *chlorophyll a*, and that by variable fluorescence, etc.). The practicalities, applicability and limitations of these methods should be understood before they are used in compliance testing;
 - .2 a direct measurement, which is directly comparable to the D-2 standard (i.e. the determination of the number of viable organisms per volume) may also be indicative if it has:
 - .1 a large confidence interval; or
 - .2 high-detection limits; and
 - .3 an indicative analysis is an analysis performed in accordance with sections 4.1 and 4.2.
- .5 A *detailed analysis* means a compliance test that is likely to be more complex than indicative analysis and is a direct measurement of a representative sample used to determine the viable organism concentration of a ballast water volume of interest. The result of such measurement:
- .1 should provide a direct measurement of viable organism concentration in the ballast water discharge which is directly comparable to the D-2 standard (number of viable organisms per volume);
 - .2 should be of sufficient quality and quantity to provide a precise measurement of organism concentration (+/- [X] organisms per volume) for the size category(ies) in the D-2 standard being tested for; and
 - .3 should use a measurement method with an adequate detection limit for the purpose for which it is being applied.
- A detailed analysis is an analysis performed in accordance with the methods and approaches in sections 4.3 and 4.4. Detailed analysis should usually be undertaken on a sample taken in accordance with the procedures in section 4.4.
- .6 *Testing for compliance* using indicative analysis and detailed analysis can employ a range of general approaches or standard methods. These approaches or methods are divided into those that sample a small proportion of the volume of interest to indicate or confirm compliance or a larger proportion of the volume of interest that can be utilized to indicate and confirm compliance. Those that provide a wide confidence interval should not be used to confirm compliance unless the result and confidence limit are demonstrably over the D-2 standard as measured directly or indirectly. Approaches/Standards are highlighted in sections 4.1, 4.2 and 4.4 for indicative analysis and sections 4.3 and 4.4 for detailed analysis.

- .7 *Method* means a detailed step-by-step analysis procedure (for indicative or detailed analysis) or sampling methodology, which the laboratory or organization undertaking the work can follow, be audited against and be accredited to.
- .8 *Approach* means a detailed step-by-step analysis procedure (for indicative or detailed analysis) or sampling methodology, which the laboratory or organization undertaking the work can follow. These procedures will not have been validated by an international or national standards organization.
- .9 *General approach* means a conceptual description or broad methodology of sample collection or analysis.
- .10 *The precision* of a measurement system is the degree to which repeated measurements under unchanged conditions show the same results.
- .11 *The detection limit* is the lowest concentration level that can be determined to be statistically different from a blank sample within a stated confidence interval. Limits of detection are method and analysis specific.
- .12 *Plankton* means *phytoplankton* (e.g. diatoms or dinoflagellates) and *zooplankton* (e.g. bivalve larvae or copepods) that live in the water column and are incapable of swimming against a current.
- .13 *Confidence interval* means a statistical measure of the number of times out of 100 that test results can be expected to be within a specified range. For example, a confidence level of 95% means that the result of an action will probably meet expectations 95% of the time.
- .14 *Operational indicator* means a parameter used to monitor and control the operation of the BWMS as defined during testing for Type Approval, e.g. limit values of physical or chemical parameters such as flow rates, dose, etc.
- .15 *Performance indicator* means a biological parameter (e.g. ATP, *chlorophyll a*, direct counts) used to estimate or measure the performance of the BWMS in achieving the D-2 standard.

3 PRINCIPLES FOR SAMPLING AND ANALYSIS FOR BALLAST WATER DISCHARGES

3.1 All samples and analysis carried out to determine whether a ship is in compliance with the BWM Convention should be performed under reliable and verified QA/QC procedures (note that any method, approach or sampling procedure should be rigorously validated and practicability should be assessed).

3.2 The first premise of any sampling and/or any analysis protocol is to identify the purpose of the protocol, i.e. to prove whether the discharge of a ship is meeting the D-1 standard or meeting the D-2 standard. There are many ways in which this can be done; however, they are limited by:

- .1 the requirements of the methodologies available for sampling the ballast water discharge;
- .2 the methods of analysis of samples being collected;

- .3 the methods involved in statistically processing the results of these analyses;
- .4 the specific operation of the ballast water management system (including when the treatment is applied during the ballast cycle and the type of treatment used); and
- .5 the practicalities of sampling a very large volume of water and analysing it for very low concentrations of organisms.

3.3 Successful sampling and analysis is also based on identifying the viable biological population being sampled and its variability. If this population is homogenous, it is much easier to sample than one that is known to be heterogeneous. In the case of ballast water, the sample is drawn from a discharge with a population that can vary significantly. Consequently, the samples collected for indicative or detailed analysis should be representative samples.

3.4 Sampling a ballast water discharge is restricted even further when parts of the ballast water may have already been discharged. Very few inferences can be made on the quality of that ballast water already discharged based on sampling the remaining discharge as it happens. The challenge is to determine the volume of interest and how to sample it.

3.5 The qualitative difference between indicative analysis and detailed analysis often relies on the level of statistical confidence, which, in detailed analysis may be superior.

3.6 Indicative analysis (using operational or performance indicators) can be undertaken at any time throughout the discharge. In cases where indicative analysis identifies that a system is grossly exceeding the D-2 standard, it may be sufficient to establish non-compliance, however, the practicalities, application and limitations of the methodology being used for indicative analysis need to be understood fully.

3.7 Based on the discussion in paragraph 3.3, two different potential detailed sampling approaches can therefore be considered:

- .1 sampling the entire discharge from a vessel during a port visit. During this approach:
 - .1 it will be impossible, by definition, for vessels to discharge prior to sampling;
 - .2 large numbers of samples are likely to be required over a long period of time;
 - .3 large sample volumes may be required over a long period of time; and
 - .4 sampling personnel would be required on the vessel over a significant period of time; and
- .2 collecting a representative sample of the ballast water being discharged during some chosen period of time, e.g. one sample or a sequence of samples. During this approach:
 - .1 the sampling can be developed to fit the situation on board the vessel; and

- .2 a representative sample of the discharge can be taken, and that volume can be selected in many ways, providing the opportunity for identifying and sampling specific volumes of the discharge if appropriate, e.g. choosing a percentage of the discharge or sampling duration.

3.8 The D-2 standard expresses a low concentration of organisms to identify in the analysis. The confidence in the result of any sampling and analysis depends on the error inherent in the sampling method and on the error inherent in the method used for analysing the sample. The cumulative error of both must be taken into account when evaluating the result.

3.9 The tables in sections 4.1, 4.2 and 4.3 set out the range of methodologies and approaches, currently identified for use to analyse ballast water discharges and how they relate to the specific sampling protocols in section 4.4. These methodologies and approaches are stand-alone techniques that need to be combined with specific sampling protocols. These protocols should recognize the limitations of each methodology, its inherent sampling requirements, and how it can fit into a comprehensive sampling protocol for compliance testing.

3.10 Although some methodologies and approaches used in type approval testing may also be applicable in compliance testing, the latter, especially indicative sampling, may also require other approaches.

Table 1

Definition and differences between indicative and detailed analysis for the D-2 standard

	Indicative analysis	Detailed analysis
Purpose	To provide a quick, rough estimate of the number of viable organisms	To provide a robust, direct measurement of the number of viable organisms
Sampling		
Volume	Small or large depending on specific analysis	Small or large depending on specific analysis
Representative sampling	Yes, representative of volume of interest	Yes, representative of volume of interest
Analysis method		
Analysis parameters	Operational (chemical, physical) and/or performance indicators (biological)	Direct counts (biological)
Time-consuming	Lower	Higher
Required skill	Lower	Higher
Accuracy of numeric organism counts	Poorer	Better
Confidence with respect to D-2	Lower	Higher

4 METHODOLOGIES FOR COMPLIANCE TESTING UNDER THE BWM CONVENTION

4.1 Table 2: Analysis methods that may provide an indication of compliance with the D-1 standard¹

Indicator	General approach	Standard method	Notes	Level of confidence or detection limit and citation for validation studies
Salinity	Conductivity meter to monitor salinity.	No international standard for ballast water analysis at this time although standard methods for measuring salinity do exist.	External elements can affect the salinity.	To be determined.
Salinity	Refractometer to monitor salinity.	No international standard for ballast water analysis at this time although standard methods for measuring salinity do exist.	Temperature can affect the readings.	To be determined.
Types of organisms in discharge – oceanic, coastal, estuarine or fresh water	Visual identification.	No international standard for ballast water analysis at this time.	Expensive, time-consuming, needs extensively trained personnel; may produce false results if encysted organisms from previous ballasting operations hatch.	To be determined.
Turbidity	Portable turbidity sensors.	No international standard for ballast water analysis at this time.	Requires understanding of turbidity characteristics in relation to the distance from shore.	To be determined.
Dissolved inorganic and organic constituents (nutrients, metals coloured dissolved organic matter (CDOM))	Portable nutrient sensors.	No international standard for ballast water analysis at this time.	Requires understanding of inorganic or organic constituent characteristics in relation to the distance from shore.	To be determined.

¹ Additional information can be found in document BLG 16/4.

4.2 **Table 3: Indicative analysis methods for use when testing for potential compliance with the D-2 standard²**

Indicator	General approach	Standard method	Notes	Level of confidence or detection limit and citation for validation studies
Viable organisms $\geq 50 \mu\text{m}$	Visual counts or stereo-microscopy.	No international standard for ballast water analysis at this time.	Can be expensive and time-consuming, needs moderately trained personnel. (Note that OECD Test Guideline for Testing of Chemicals 202, " <i>Daphnia</i> sp. Acute immobilization test and reproduction test" could be used as basis for standard methodology.)	To be determined.
Viable organisms $\geq 50 \mu\text{m}$	Visual inspection.	No international standard for ballast water analysis at this time.	Visual inspection is likely to only register organisms bigger than 1,000 micro-metres in minimum dimension.	To be determined.
Viable organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Variable fluorometry.	No international standard for ballast water analysis at this time.	Only monitors photosynthetic phytoplankton and thus may significantly underestimate other planktonic organisms in this size fraction.	To be determined.
Viable organisms $\geq 50 \mu\text{m}$ and $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Photometry, nucleic acid, ATP, bulk fluorescein diacetate (FDA), <i>chlorophyll a.</i> , ChemChrome V6.	No international standard for ballast water analysis at this time.	Semi-quantitative results can be obtained. However, some of these organic compounds can survive for various lengths of time in aqueous solution outside the cell, potentially leading to false positives. Welschmeyer and Maurer (2012). The reference to organic compound survival does not refer to CV6; further information on CV6 can be found	To be determined.

² Additional information can be found in document BLG 15/5/4.

Indicator	General approach	Standard method	Notes	Level of confidence or detection limit and citation for validation studies
			in documents MEPC 74/INF.17 and PPR 7/INF.5.	
Viable organisms ≥ 50 µm and ≥ 10 µm and < 50 µm	Flow cytometry.	No international standard for ballast water analysis at this time.	Very expensive.	To be determined.
Enterococci	Fluorometric diagnostic kit.	No international standard for ballast water analysis at this time.	Minimum incubation time 6 h. Semi-quantitative results from portable methods (see paragraph 2.2.2 of annex 1).	To be determined.
<i>Escherichia coli</i>	Fluorometric diagnostic kit.	No international standard for ballast water analysis at this time.	Minimum incubation time 6 h. Semi-quantitative results from portable methods (see paragraph 2.2.2 of annex 1).	To be determined.
<i>Vibrio cholerae</i> (O1 and O139)	Test kits.	No international standard for ballast water analysis at this time.	Relatively rapid indicative test methods are available.	To be determined.
Viable organisms ≥ 50 µm and ≥ 10 µm and < 50 µm	Pulse counting fluorescein diacetate (FDA).	No international standard for ballast water analysis at this time.	Sampling kit can be larger than that for bulk fluorescein diacetate (FDA).	To be determined.
Total living bacteria including Enterococci, <i>Escherichia coli</i> , <i>Vibrio cholerae</i>	Second-generation ATP	No international standard for ballast water analysis at this time.	Semi-quantitative results can be obtained	PPR 7/INF.4

4.3 **Table 4: Detailed analysis methods for use when testing for compliance with the D-2 standard**

Indicator	General approach	Standard method	IMO citation	Notes	Level of confidence or detection limit and citation for validation studies
Viable organisms $\geq 50 \mu\text{m}$ and $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Visual counts or stereo-microscopy examination. May be used with vital stains in conjunction with fluorescence + movement.	No international standard for ballast water analysis at this time, but see US EPA ETV Protocol, v. 5.1	BLG 15/5/5 and BLG 15/5/6 BLG 15/INF.6	Can be expensive and time-consuming, needs trained personnel. (Note that OECD Test Guideline for Testing of Chemicals 202, "Daphnia sp. Acute immobilization test and reproduction test" could be used as basis for standard methodology.)	To be determined.
Viable organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Visual counts with use of vital stains.	No international standard for ballast water analysis at this time, but see US EPA ETV Protocol, v. 5.1	BLG 15/5/10 (method) BLG 15/5/5 and BLG 15/5/6 (approach) MEPC 58/INF.10	Requires specific knowledge to operate them. It should be noted that there may be limitations using vital stains with certain technologies.	To be determined. Steinberg et al., 2011
Viable organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Flow cytometers (based on <i>chlorophyll a</i> and vital stains).	No international standard for ballast water analysis at this time.	BLG 15/5/5 and BLG 15/5/6	Expensive and require specific knowledge to operate them. It should be noted that there may be limitation using vital stains with certain technologies.	To be determined.
Viable organisms $\geq 50 \mu\text{m}$ and Viable organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Flow cameras (based on <i>chlorophyll a</i> and vital stains).	No international standard for ballast water analysis at this time.	BLG 15/5/5 and BLG 15/5/6	Expensive and require specific knowledge to operate them. It should be noted that there may be limitations using vital stains with certain ballast water management systems.	To be determined.

Indicator	General approach	Standard method	IMO citation	Notes	Level of confidence or detection limit and citation for validation studies
Viable organisms $\geq 50 \mu\text{m}$ and Viable organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Culture methods for recovery, regrowth and maturation.	No international standard for ballast water analysis at this time.	BLG 15/5/5, BLG 15/5/6 and PPR 7/INF.10	Densities are expressed as the sum of cultivable autotrophs after a 2-week incubation time and motile heterotrophs as determined by epifluorescence microscopy.	Validation available in Cullen (2019).
Enterococci	Culture methods.	ISO 7899-1 or ISO 7899-2	BLG 15/5/5 and BLG 15/5/6	Requires specific knowledge to conduct them. At least 44-h incubation time. EPA Standard Method 9230	To be determined.
<i>Escherichia coli</i>	Culture methods.	ISO 9308-3 or ISO 9308-1	BLG 15/5/5 and BLG 15/5/6	Requires specific knowledge to conduct them. At least 24-h incubation time. EPA Standard Method 9213D	To be determined.
<i>Vibrio cholerae</i> (O1 and O139)	Culture and molecular biological or fluorescence methods.	ISO/TS 21872-1/13/	BLG 15/5/5 and BLG 15/5/6	Requires specific knowledge to conduct them. 24-48 h incubation time. US EPA ETV Fykse et al., 2012 (semi-quantitative pass/fail-test) Samples should only be cultured in a specialized laboratory.	To be determined.

Indicator	General approach	Standard method	IMO citation	Notes	Level of confidence or detection limit and citation for validation studies
Enterococci, <i>Escherichia coli</i> , <i>Vibrio cholerae</i> (O1 and O139)	Culture with fluorescence-in-situ hybridization (FISH)	No international standard for ballast water analysis at this time.		Requires specific knowledge to conduct them. Quantitative and qualitative results after 8 h. Samples should only be cultured in a specialized laboratory.	To be determined.
Viable organisms $\geq 50 \mu\text{m}$ and viable organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Visual counts using stereo-microscopy examination and flow cytometry.	No international Standard for ballast water analysis at this time.	BLG 17/INF.15	A Sampling Protocol that identifies whether a system is broken or not working and producing a discharge that is significantly above the D-2 standard. Designed to detect gross non-compliance with 99.9% confidence. Needs to be Validated.	To be determined.

4.4 **Table 5: General approaches for sampling use when testing for compliance with the BWM Convention**

General approaches for sampling	Discharge line or BW tank	Citation for validation study or use	Sample error and detection limit	Relative sample error among approaches
Filter skid + isokinetic sampling	Discharge line	Drake et al., 2014; First et al., 2012 (land-based testing); shipboard validation underway, Prototype 01, SGS	To be determined.	Lower
Cylinder containing plankton net + isokinetic sampling	Discharge line	MEPC 57/INF.17	To be determined.	Lower
Sampling tub containing plankton net + isokinetic sampling	Discharge line	Gollasch, 2006 and Gollasch et al., 2007 Cangelosi et al., 2011	To be determined.	Lower
Continuous drip sampler + isokinetic sampling	Discharge line	Gollasch and David, 2010, 2013	To be determined.	Lower
Grab sample	BW tank	David and Perkovic, 2004; David et al. 2007, BLG14/INF.6	To be determined.	Higher

4.5 **Table 6: Sampling and analysis methods/approaches for use when testing compliance with the BWM Convention. A checkmark indicates an appropriate combination of sampling and analysis.**

Analysis type size class or indicator microbe analysis method/approach	Filter skid + isokinetic sampling ³	Plankton net + isokinetic sampling	Continuous drip sampler + isokinetic sampling	Grab sample
<u>Indicative Analysis</u> ≥ 50 µm Visual inspection Stereomicroscopy counts Flow cytometry Nucleic acid ATP <i>Chlorophyll a</i> , Bulk FDA	✓	✓		
<u>Indicative Analysis</u> < 50 µm and ≥ 10 µm variable fluorometry Flow cytometry Nucleic acid ATP <i>Chlorophyll a</i> , Bulk FDA			✓	✓

³ Methods other than using an isokinetic approach as defined in the Guidelines (G2) for acquiring a representative sample may be used in certain circumstances. Such methods should be validated prior to use.

Analysis type size class or indicator microbe analysis method/approach	Filter skid + isokinetic sampling ³	Plankton net + isokinetic sampling	Continuous drip sampler + isokinetic sampling	Grab sample
<u>Indicative Analysis</u> Enterococci, <i>E. coli</i> Fluorometric diagnostics			✓	✓
<u>Indicative Analysis</u> <i>Vibrio cholerae</i> Test kits Culture methods + microscopy			✓	✓
<u>Detailed Analysis</u> ≥ 50 µm Stereomicroscopy counts Flow cytometry/Flow camera	✓	✓		
<u>Detailed Analysis</u> < 50 µm and ≥ 10 µm Visual counts + vital stain(s) Flow cytometry/Flow camera Culture methods			✓	
<u>Detailed Analysis</u> Enterococci, <i>E. coli</i> Culture methods FISH with pre-cultivation			✓	
<u>Detailed Analysis</u> <i>Vibrio cholerae</i> Culture methods FISH with pre-cultivation			✓	

4.6 References

- Cullen JJ (2019). The best available science describing type-approval testing methods and protocols for ballast water management systems that render nonviable organisms in ballast water. <http://doi.org/10.5281/zenodo.2656597>
- David M & Perkovic M (2004). Ballast Water Sampling as a Critical Component of Biological Invasions Risk Management, *Marine Pollution Bulletin*, Vol. 49, 313-318.
- David M, Gollasch S, Cabrini M, Perkovič M, Bošnjak D & Virgilio D (2007). Results from the First Ballast Water Sampling Study in the Mediterranean Sea – the Port of Koper Study. *Marine Pollution Bulletin* 54(1), 53-65.
- Drake LA, Moser CS, Robbins-Wamsley SH, Riley SC, Wier TP, Grant JF, Herring PR, First MR (2014). Validation trials of a shipboard filter skid (p3SFS) demonstrate its utility for collecting living zooplankton. *Marine Pollution Bulletin* 79, 77–86.
- First MR, Lemieux EJ, Hyland WB, Grant JF, Moser CS, Riley SC, Robbins-Wamsley SH, Steinberg MK, Wier TP, Drake LA (2012). Validation of a closed-housing filter skid for in-line sampling of aquatic organisms. *Journal of Plankton Research* 34:321-331.
- Fykse EM, Nilsen T, Nielsen AG, Tryland I, Delacroix S, Blatny JM (2012). Real-time PCR and NASBA for rapid and sensitive detection of *Vibrio cholerae* in ballast water. *Marine Pollution Bulletin* 64:200-206.
- Gollasch S (2006). A new ballast water sampling device for sampling organisms above 50 micron. *Aquatic Invasions*, Volume 1, Issue 1: 46-50.
- Gollasch S, David M, Voigt M, Dragsund E, Hewitt C & Fukuyo Y (2007). Critical review of the IMO International Convention on the Management of Ships' Ballast Water and Sediments. In Hallegraeff, G.M. (ed.): *Harmful Algae* 6, 585-600.
- Gollasch S & David M (2013). Recommendations for Representative Ballast Water Sampling. Final report of research study of the Bundesamt für Seeschifffahrt und Hydrographie (BSH), Hamburg, Germany. Order Number 4500025702. 28 pp.
- Gollasch S & David M (2010). Testing Sample Representativeness of a Ballast Water Discharge and developing methods for Indicative Analysis. Final report of research study undertaken for the European Maritime Safety Agency (EMSA), Lisbon, Portugal, 124 pp.
- Steinberg MK, Lemieux EJ, Drake LA (2011). Determining the viability of marine protists using a combination of vital, fluorescent stains. *Marine Biology* 158:1431-1437.
- Thronsen J (1978). Chapter 7.6: The dilution-culture method. In *Phytoplankton manual*, Ed: Sourina, A., UNESCO, France, p. 218-224.
- U.S. Environmental Protection Agency (2010). Environmental Technology Verification Program (ETV) Generic protocol for the verification of ballast water treatment technology, Version 5.1. Report number EPA/600/R-10/146, United States Environmental Protection Agency, Washington, D.C.
- Welschmeyer N & Maurer B (2012). A portable, sensitive plankton viability assay for IMO shipboard ballast water compliance testing. In: *Proceeding of the Global R and D forum on Compliance Monitoring and Enforcement*, Eds. A. Olgun, F.T. Karokoc and F. Haa.

ANNEX 2

TECHNICAL DISCUSSION FOR THE 2020 GUIDANCE TO BALLAST WATER SAMPLING AND ANALYSIS IN ACCORDANCE WITH THE BWM CONVENTION AND GUIDELINES (G2)

1 INTRODUCTION

1.1 The purpose of this annex is to provide background information on:

- .1 the development and use of methodologies for both indicative and detailed analysis and appropriate sampling; and
- .2 analysis of the sample at an accredited laboratory.

1.2 This annex highlights the advantages, disadvantages and limitations of many different measures. Although recommendations are given in this document on what methodologies may be used, there are distinct benefits in using certain technologies at certain times. This should not stop the use of any of the methodologies, as long as the limitations are taken into account.

1.3 Any methods for analysis used for assessing compliance with the BWM Convention should be carefully validated under a range of operating conditions.

2 INDICATIVE ANALYSIS: METHODOLOGY AND APPROACHES

2.1 The D-1 standard

2.1.1 The D-1 standard requires the vessel to exchange its ballast water 200 NM from the coastline in waters 200 m deep, or if this cannot be achieved for safety reasons, 50 NM from the coastline in waters of the same depth. Therefore, the water in exchanged ballast water should have a similar salinity to that of mid-ocean water.

2.1.2 Indicative analysis for the D-1 standard of the BWM Convention could rely on the chemical parameters (e.g. salinity) of the water in the ballast water discharge, or on an estimate of species present. However, the latter might need trained personnel. If the ballast water discharge being tested has a salinity significantly less than that of 30 PSU, then it is likely that the ballast water has not been exchanged en route under the conditions required in the D-1 standard, or that the exchange has not been completed successfully.

2.1.3 Two exceptions to this are:

- .1 when ballast water is taken up in port areas that are located in high-salinity environments, above 30 PSU. In such a case ballast water with a PSU of 30 may not originate from mid-ocean waters and therefore the ship may not be compliant with the D-1 standard; or
- .2 when ballast water has been exchanged in designated ballast water exchange areas within 50 NM from the coastline in waters that may be of less salinity than the mid-ocean water. In this case the ballast water exchange would be compliant.

Therefore, the origin of the last ballast water exchange should be known before interpreting the results of salinity analysis.

2.1.4 Checking salinity could be backed up by further analysis of the organisms in the ballast water discharge to determine the origin of the ballast water; however, this would take time and need experienced staff. This can be done in line with the visual analysis methodologies outlined in paragraph 2.4.3 below. However, it should be noted that there are many external factors that could affect the salinity and the organisms in the ballast water, such as wet sediments in the ballast tanks, the state of the tide in the port concerned during its uptake and the fact that exchange may not remove all coastal organisms.

2.1.5 There are many ways to quickly and easily monitor the salinity of water on the market, and generic salinity measures should be used for indicative analysis.

2.2 Bacteria levels in the D-2 standard

2.2.1 Bacterial levels could be tested by a wealth of available portable methods. However, as the D-2 standard for bacteria is measured in colony forming units (CFU), the systems utilized may have to include a specific incubation time of the samples, which for commercially available systems is never shorter than 4 hours. Therefore, the time it takes for incubation limits the use of such systems for indicative analysis.

2.2.2 Advances in fluorometric diagnostics have resulted in a methodology that identifies the presence or absence of bacteria in a sample of the ballast water discharge. This methodology is based upon the detection of enzymes produced by the target bacteria in unconcentrated fresh water or marine samples and presently easily portable test kits for *E. coli* and Enterococci are available. This method can identify low levels of bacteria in water samples in less than 10 minutes, but the results are only semi-quantitative, i.e. a low level reading equates to a low level of bacteria. However, although the presence of bacteria can be shown, whether or not these organisms are living (i.e. form colonies) cannot be proven with this method at the present time. These diagnostic methods could be used in indicative analysis if very large numbers of organisms are identified.

2.3 Organisms of less than 50 micrometres and greater than or equal to 10 micrometres in minimum dimension¹ in the D-2 standard

2.3.1 Methods to measure the organisms in this category of the D-2 standard can be divided into two categories as follows:

- .1 the use of biological indicators for organisms:
 - .1 nucleic acid;
 - .2 adenosine triphosphate (ATP), a coenzyme used as the main energy storage and transfer molecule in the cells of all known organisms; and
 - .3 indicators for the presence of organisms, such as *chlorophyll a*;

¹ The "Minimum Dimension" means the minimum dimension of an organism based upon the dimensions of that organism's body, ignoring e.g. the size of spines, flagellae or antenna. The minimum dimension should therefore be the smallest part of the "body", i.e. the smallest dimension between main body surfaces of an individual when looked at from all perspectives. For spherical-shaped organisms, the minimum dimension should be the spherical diameter. For colony-forming species, the individual should be measured as it is the smallest unit able to reproduce that needs to be tested in viability tests. This should be considered whenever size is discussed in this document.

- .2 the use of direct counts of living organisms (coupling a means to determine viability and manual or automatic counting of individual organisms).

2.3.2 The presence of nucleic acid or ATP in a sample may be taken as an indication of life, but it should be noted that this nucleic acid or ATP could come from any living organism of any size within the sample. There are no definitive methods available to correlate the amount of nucleic acid or ATP with the amount or viability of organisms in the sample and, therefore, the presence of these chemicals is limited as an indicative analysis methodology. However, zero measurements of these chemicals may indicate that no organisms are in the sample, i.e. the treatment process was successful and the D-2 standard is being met. Additionally, if nested filters are used to isolate specific size groups, then ATP, which degrades relatively quickly, can provide an indication of the potential presence of a large concentration of organisms in one size class. If linked to thresholds of ATP concentrations, this can be used to indicate samples which are highly likely to be above the standard.

2.3.3 The same problems occur when using other bio-chemical indicators to monitor the number of organisms in this category. As many of the organisms in this size range are likely to be phytoplankton, an obvious step would be to measure the level of *chlorophyll a*, a photosynthetic pigment which is essential for photosynthesis in the sample. Zero concentrations may indicate that there is no phytoplankton in the sample and *chlorophyll a* may also be a good indicator as to whether a BWMS using an oxidizing process was working to design dosages, as it might be expected to bleach such pigments. However, caution has to be exercised as:

- .1 *chlorophyll a* can persist in seawater outside of a cell, therefore, sampling should only be limited to the particulate phase. However, nucleic acid and ATP can exist in dead organisms, detrital material, senescent or dead cells, decomposing macroalgae, plant detritus from terrestrial ecosystems and other non-living particles, etc.;
- .2 there may be zooplankton in the sample being analysed;
- .3 no cell count can be directly measured from a *chlorophyll a* measurement, as many small cells may provide a similar signal strength to that of fewer bigger cells; and
- .4 no size distinction can be made and the *chlorophyll a* could derive from phytoplankton in the larger size category of the D-2 standard.

As a consequence, direct concentration measurements of this chemical would be difficult to use in indicative analysis. A wealth of portable tools exists to document the *chlorophyll a* content in seawater.

2.3.4 One potential exception is the pulse-amplitude modulated fluorometer (PAM) which measures the *chlorophyll a* fluorescence in living cells by exciting *chlorophyll a* molecules and registering the subsequent fluorescent signal. Such a response is only available in living cells and it should be noted that this method only provides an indirect measurement of those phytoplankton that use *chlorophyll a* in the sample, in both size categories of the D-2 standard. Testing this methodology on ballast water discharges suggests that there is a correlation between the ratio of variable and maximum fluorescence and the number of phytoplankton in this size category. However, the relationship between fluorescence signals and mixed assemblages of phytoplankton from different locations needs to be validated.

2.3.5 For analysis of organisms above 10 microns in minimum dimension, a flow cytometer may also be used. A common element of these systems is that they automatically count objects, including organisms, per size class in a fluid. The more simplified systems cannot separate organisms from sediment and detritus, or living from dead organisms. More sophisticated systems can also assess organism viability for phytoplankton by using organism stains together with flow cytometry. The separation of living phytoplankton from detrital material and zooplankton is based on the presence of auto chlorophyll fluorescence of phytoplankton cells. It should be noted, however, that using *chlorophyll a* fluorescence as an indicator of living organisms may result in overcounting, as the molecule can remain intact for a significant amount of time as has been proved in preparing fixed (dead) samples. The practicability to use such devices on board a ship should be carefully assessed before use. To make a stable stream to produce adequate size of water particles, the device should be set in perfectly horizontal. Also, any vibration should be isolated for accurate measurement.

2.3.6 Systems using flow cytometry deliver automated results promptly and may be used to assess the number of living phytoplankton in a sample after treatment with a viability stain. However, readings provided by the flow cytometer should also be examined manually to verify the automated readings. Concerns have been raised by users that the viability of smaller algae may not always be categorized correctly in these systems, as the viability signal may be too low for detection. Other concerns include the efficiency of portable versions and the limited ability of some of them to monitor organisms greater than or equal to 50 micrometres in minimum dimension. Although these systems may become a major tool in the future, there are elements, such as the reliability of portable versions of the systems that limit their use at the present time, which is especially the case for organisms greater than or equal to 50 micrometres in minimum dimension. Also, it is not clear if the time to analyse a sample is greater than can be allotted in compliance testing. These can be overcome by taking the sample off the ship and using a fixed or mobile system near to the ship or the port.

2.3.7 Visual inspection could be another method of indicative analysis that is a quick and simple way to justify the need for detailed analysis. Taking an appropriate sample, concentrating it if necessary, and visually inspecting it against the light may show living organisms in the sample, but it should be noted that without magnification a visual inspection is likely to result in only organisms greater than or equal to 1,000 micrometres in minimum dimension being detected, unless chains or clumps are formed by colony-forming organisms or the density of organisms is sufficiently large to colour the water. An assessment of the viability in such an inspection is limited to complete body movements of the organisms as organ activity and antennae or flagella movements may not be seen. As samples from BWMS that are not compliant are likely to contain organism levels that are orders of magnitude above the D-2 performance standard, visual inspections could be used in indicative analysis. However, it is assumed that only organisms bigger than 1,000 micrometres in minimum dimension may be determined in such way, therefore, its use for this size category is limited.

2.3.8 Visual inspection can also be undertaken using a field stereomicroscope with a low magnification (e.g. x 10). However, this methodology may require concentration of the sample and may need analysis by a trained operator to detect viable organisms. It should also be noted that this methodology would be more efficient and practicable for organisms greater than or equal to 50 micrometres in minimum dimension.

2.4 Organisms greater than or equal to 50 micrometres in minimum dimension in the D-2 standard

2.4.1 Many of the methodologies for monitoring organisms less than 50 micrometres and greater than or equal to 10 micrometres in minimum dimension may also be valid for monitoring organism levels in this category. However, nucleic acid and ATP methodologies encounter the

same problems as outlined in paragraphs 2.3.2 and 2.3.3; and monitoring *chlorophyll a* levels, through fluorometers or the PAM methodology described above, has limited value for this size category of the D-2 standard, as the majority of organisms in this category are likely to be zooplankton.

2.4.2 Visual inspections may significantly underestimate the number of organisms in this size category due to the issues described in paragraph 2.3.8. However, the method may be robust enough to determine whether the BWMS is working at orders of magnitude above the D-2 standard based on a simple extrapolation from the sample to the D-2 standard. Detailed analysis may be needed to confirm this, especially when levels near the D-2 standard are encountered.

2.4.3 Additionally, stereomicroscopy can also be used to identify viable organisms greater than or equal to 50 micrometres in minimum dimension. The sample should be concentrated appropriately. Viability assessment should be based on movements of intact organisms. This movement may be stimulated. In addition, organ activity should be observed and fully intact non-moving organisms which show organ activity should be counted as living. Stains might also be used to help in viability determination – though methods are still under development. The viable organism numbers should be recorded and the numbers extrapolated up to the total volume of water filtered.

2.4.4 If the results in paragraphs 2.4.2 and 2.4.3 show elevated levels of organisms, then this result will indicate that the D-2 standard is not being met.

2.4.5 Further research must be encouraged; innovative methods for assessing for D-2 compliance, preferably based on in situ, automatic sampling and analytical procedures, should facilitate the most uniform implementation of the BWM Convention.

2.5 Operational indicators

Other indirect parameters and indicators could be used to indicate whether a BWMS is meeting the D-2 standard. These include, but are not limited to, indicators from the electronic self-monitoring of the BWMS and residual chemicals (or lack of) from the BWMS, such as dissolved oxygen levels, residual chlorine, etc.

3 DETAILED ANALYSIS METHODOLOGIES AND APPROACHES

3.1 Once detailed analysis has been instigated by the port State, they should be prepared to undertake full analysis of the sample at an appropriate laboratory.

3.2 Bacteria

3.2.1 There are already international standards in place to analyse for the bacteriological indicators contained within the D-2 standard.

3.2.2 For Enterococci, ISO 7899-1 or 7899-2; or Standard Method 9230 (in the United States) should be used, and ISO 9308-3, ISO 9308-1 or Standard Method 9213D (in the United States) are appropriate for *Escherichia coli*. The methods used should be quantitative and based on a 95-percentile statistical evaluation. The number of laboratory samples should be sufficient to define the mean and standard deviation of Log 10 bacterial enumerations.

3.2.3 For *Vibrio cholerae* ISO/TS 21872-1/13 is appropriate. 100 ml of ballast water should be filtered and incubated according to ISO/TS 21872-1. Analysis needs to be undertaken in a specialist laboratory.

3.3 Organisms of less than 50 micrometres and greater than or equal to 10 micrometres in minimum dimension

3.3.1 Many of the analysis methods used to ascertain the numbers of organisms within this category have already been discussed in section 2. However, section 2 focuses on indicative analysis, rather than the more detailed analysis. Therefore, the following sections examine these methodologies in more detail. Some of these methodologies discussed here also relate to organisms greater than or equal to 50 micrometres in minimum dimension.

3.3.2 Simple upright and inverted microscopes are very useful for the enumeration of morphologically healthy organisms and motile organisms, as well as for measuring the size of organisms. Using this technology needs some skill and experience to evaluate the health of the individual organisms in the sample. However, this technology and experience should be available globally.

3.3.3 Fluorescence generated from photosynthetic pigments can be used for more detailed analysis of the morphological health of organisms and for the evaluation of stained organisms and a microscope with fluorescence capabilities is needed. However, this methodology only identifies phytoplankton (both living and dead) in the sample and makes no size differentiation. Zooplankton should be analysed through the methods highlighted in section 3.4.

3.3.4 Fluorescein di-acetate (FDA), chloromethylfluorescein diacetate (CMFDA) and Calcein-AM vital stains have both been used to determine viability. When non-specific esterases (enzymes found in live cells) are present, they cleave the acetate groups from the stains, and the resultant fluorescein molecules fluoresce green when illuminated with a blue light from an epi-fluorescence microscope. This method works best with live samples. Microscopes with a fluorescence capability and operators with skills and experience of analysis should be available at universities and research laboratories worldwide. However, it should be noted that these stains do not always work on all species or at all salinities and further research to validate this approach may be needed to support the use of these stains for this type of analysis.

3.3.5 Flow cytometers are advanced technologies which can be used in a laboratory to determine size, and viability of organisms in ballast water when a reliable vital stain(s) is (are) used to indicate organism viability. Cytometer detected particles, including organisms, can be processed visually or by a computer to quantify viable organisms in that sample. These systems reduce manual labour but require specific knowledge to operate them. High particle loads in ballast water may reduce the detection limits of these methodologies and the volume of samples analysed. At present, portable versions of these technologies have not fully been proven for use on ballast water discharges, however, samples could be taken off the ship and analysed using a fixed or mobile system near to the ship or the port.

3.3.6 Regrowth experiments, in which the visual appearance of photosynthetic organisms in a sample is followed by a specific period in order to quantify the most probable number (MPN), are methods to evaluate the number of organisms in a sample. However, these are slow and are work intensive. In addition, a major drawback of this methodology may be that specific growth factors during the incubation may not be fulfilled, giving a risk of bias. Regrowth and reproduction may be seasonably variable, giving different results at different times. Further, a viable organism may be in good health and reproducing rapidly, or in poor health, not reproducing until health has improved. Finally, this is likely to be time-consuming.

3.3.7 Bulk parameter measurements, such as photosynthetic activity, are also not suitable for detailed analysis (please see paragraphs 2.3.2 and 2.3.3), but can be used as supporting data for other methods used to determine the number of viable organisms in the ballast water samples.

3.3.8 Planktonic organisms may be fragile and samples may need to be concentrated further to aid the accurate quantification of organisms. There are many methods to achieve this, however, care has to be taken to reduce physical stress as this may result in reduced viability levels. A simple, rapid, flexible and cautious method for concentrating plankton cells is the use of transparent membrane filters. If the sample analysis is performed on board the sample can be filtered directly on to this membrane, which can subsequently be placed directly under a microscope for examination. The sample volume to be analysed would need to be adjusted depending on the cell density, however, live, vital stained and fixed organisms within this size category can be evaluated on these filters. If the representative analysis is performed at a laboratory, this process for concentration should be performed at the laboratory just before starting the staining process to avoid under-estimate of viable organisms. Importantly, the loss (if any) of organisms (i.e. those cells passing through the filter and recovered in the filtrate) would need to be determined. Alternatively, a filter mesh may be used to concentrate the sample and the concentrated organisms may, after filtration, be transferred into an observation chamber. Again, the loss of organisms through damage must be quantified.

3.4 Organisms greater than or equal to 50 micrometres in minimum dimension in the D-2 standard

3.4.1 Paragraphs 3.3.2 to 3.3.8 are also applicable to the analysis of organisms in this size category.

3.4.2 In addition, the following issues need to be considered when developing a methodology for analysing organism numbers in this size category:

- .1 testing the sample for movement and response to different stimuli are simple techniques for the examination of viable/dead zooplankton under a stereomicroscope. The observation for organ activity, such as heartbeats, may also contribute to the viability assessment. The use of a filtering mesh (e.g. 50 microns in diagonal dimension) under the Petri dish of the stereomicroscope, or the addition of 50 micron micro beads to the sample, may help with size calculations and vital stains may also add value to these methodologies. Separate guidelines on this issue are being developed through the land-based facilities and the ETV protocol in the United States;
- .2 methods using a combination of flow cytometry and microscopy have the disadvantage of high complexity, high price and small sample sizes, which means the ballast water samples would have to be concentrated further; and
- .3 the storage condition and time before analysis is likely to be critical to reduce mortality in the sample.

3.4.3 It is therefore recommended that simple microscopic examination of organisms in this size category is used for compliance monitoring. The microscopic examination of organisms is a robust, simple and cheap methodology which can be completed in laboratories worldwide.

4 SOURCES OF ERROR

4.1 The ideal method for compliance monitoring is a procedure that:

- .1 detects organisms in the ballast water discharge;
- .2 has an appropriate limit of detection;

- .3 is precise;
- .4 is accurate;
- .5 is economical;
- .6 is quick;
- .7 can be carried out with minimal technical expertise; and
- .8 can be obtained in all parts of the world.

However, any result obtained would have to include confidence limits based on both the sampling error and analytical error.

4.2 Sources of error include, but are not limited to, errors arising within:

- .1 sampling, including:
 - .1 sample loss (e.g. during filtration);
 - .2 incorrect use of equipment;
 - .3 day-to-day variations in the conditions in which the sampling is taking place; and
 - .4 the experience of the technicians;
- .2 processing the sample, including:
 - .1 incorrect use of equipment;
 - .2 day-to-day variations in the conditions in which the sampling is taking place; and
 - .3 the experience [and fatigue] of the technicians;
- .3 analysis of the sample:
 - .1 incorrect use of equipment;
 - .2 the experience [and fatigue] of the technicians;
 - .3 day-to-day variations in the conditions in which the sampling is taking place;
 - .4 the number of organisms counted. The distribution of organisms in a range of samples usually follows the Poisson distribution and higher numbers of samples give a lower relative variation and sample error;
 - .5 the inherent variation and errors arising from the methods used for analysis. This is especially so when the evaluation of organism numbers in a sample is based on manual counting methods due to human error. For example, although the definition of the minimum

dimension of an organism in the Guidelines (G2) is quite detailed, analytical results may be influenced by practical issues. These include situations when the size of an organism is determined on a two-dimensional microscope, which cannot view the organism "from all perspectives"; and

- .6 poor harmonization between laboratories and quality control within the laboratory. In the field of chemical analysis, inter-laboratory calibration occurs and is tested. Inter-laboratory calibration of biological samples is also common practice, but the difficulty in the compliance monitoring context is that the viability of the organisms needs to be documented and the viability may be impaired by the mode and duration of sample shipments to different laboratories. Therefore, laboratories should be well managed, and uncertainty limits (the analysis variation) should be calculated for each laboratory. This should be achieved in conjunction with ISO 17025, which provides a standard for the general requirements needed by laboratories to prove they are competent to carry out tests and/or calibrations, including sampling.

4.3 The variation arising from sampling should be added to that from analysis to determine the confidence limits within which the true value of the organism number lies. This has an important bearing on how the result can be used for enforcement of the BWM Convention.

4.4 The sampling uncertainty can be obtained by setting up a null-hypothesis, that is a general or default position that is expected in the results, e.g. the average concentration of organisms is equal to the D-2 standard at a selected level of significance and then the data would be analysed using one of the following tests:

Table 1: Statistical handling of the results

Distribution of the results	Test	Notes
Normal distribution	t-test	It is unlikely this test will be used, as it is not used with "rare" populations, i.e. the expected population of organisms in treated ballast water
A distribution that is not normal	Non-parametric Wilcoxon rank test	Not normal due to the small number of samples
Poisson distribution	Chi-square test	Used when the analytical results are treated as one sample (i.e. the numbers of organisms over the entire volume are very rare [low] and combined).

Ideally, an analysis of the distribution should be performed before the data are statistically evaluated.

4.5 There has been much discussion within IMO on whether the results of the analysis should be averaged to assess compliance or that every result should have to meet

the D-2 standard. This is a unique debate at IMO due to the biological nature of the subject matter being analysed, and different States have significantly different views on this issue. Therefore, it will be very difficult to arrive at a conclusion as in the case of non-compliance the results of the analysis are likely to be used in the legal jurisdictions of each IMO Member State, and each of those States may require different evidence to support any enforcement action.

4.6 If the results of detailed analysis are to be averaged, then both the sample variation and the analysis variation need to be calculated and applied to the result. However, some analysis of the sample variation may be needed, as it may be unacceptably high. For example, for five treated ballast water samples, viable organism number results of 9,9,9,9 and 9 will provide the same average as 0,0,0,0 and 45. Both systems would pass the D-2 standard, if averaged; however, the variation is considerably bigger for the second set of results and may prove to be unacceptable because of the one large value.

4.7 If each of the results is treated as an individual value that has to meet the D-2 standard, then again the confidence limits would have to be calculated from the sampling and analytical errors. Here if all results are less than the D-2 standard, then the sampling has proved that the BWMS is meeting the standard.

4.8 The basic difference between instantaneous and average approaches is that the results of the average approach describe the variations of the concentration of organisms during the deballasting event, whereas the results of the instantaneous approach describe the variation based on the assumptions of the Poisson distribution. However, the average approach, based on the results of a few samples, has the disadvantage that the variation may be too high, is unacceptable and needs to be improved, which could invalidate the evaluation and lead to inconclusive results.

4.9 The instantaneous approach has the disadvantage that variations in the organism levels at different times of the discharge are not taken into account, which should not be a problem if all the samples meet the D-2 standard. If the discharge is not always under the D-2 standard, the problem can be mitigated by using a flow-integrated sample over set periods of time, which, if taken properly, represents an average of the organisms in the treated ballast water over that time when presented with variance estimates and confidence intervals. This constitutes a better representation of the ballast water quality than separate samples. In addition, a lower variation should be obtained because a larger sample is being analysed. The average approach is likely to have the same disadvantages unless the samples are very large and collected over most of the discharge.

4.10 The differences between applying an instantaneous sampling regime or an average sampling regime to the result are less extreme when taking numerous flow-integrated samples. This is because for each discharge there will be a number of results arising from samples that have been averaged over a specific time.

5 DETAILED ANALYSIS: THE SAMPLE PROTOCOL

5.1 Sample protocols for discharges of treated ballast water through a distinct discharge point fall into two categories, the first based on specified and replicated volumes and the second based on flow integration over a specified time. The first entails taking a specific number of set volumes of the ballast water discharge, whilst the second takes a continuous sample over a set time period. The flow integration sampling protocol can be achieved by either continuously sub-sampling a small amount throughout the entire duration of the discharge, therefore, collecting one sample over time, or taking multiple sub-samples over a specific time scale (i.e. 5 minutes, 10 minutes or 15 minutes) repeatedly throughout the discharge, providing a result for each sub-sample.

5.2 However, for sampling protocols based on specified and replicated volumes, defining both the number of samples and their volume to ensure representativeness, takes time. As a representative sampling procedure is needed to ensure compliance with the BWM Convention, then the flow integration protocols based on set times should be implemented.

5.3 Using a sampling protocol that continuously sub-samples small amounts throughout the entire duration of the discharge, may significantly underestimate the amount of larger organisms (i.e. organisms greater than or equal to 50 micrometres in minimum dimension) in the sample due to damage to the organisms held in the cod-end of the filter. If such a system is used then a protocol for replacing the cod end needs to be developed.

5.4 The arrangements for detailed analysis should take into account the requirements of the methods and/or approaches they intend to use for detailed and/or indicative analysis. Special consideration should be given and contingencies arranged for sampling in remote ports, where it is likely to take time to mobilize samplers and sampling resources.

6 DETAILED METHODOLOGY

6.1 As described in paragraph 5.1, there are two distinct ballast water sampling protocols, one based on flow integration and one based on the use of specified and replicated volumes. As they both use filtration and concentration of the sample the following section can apply to both methods.

6.2 For in-line sampling, a sampling system should be set up which:

- .1 collects organisms greater or equal to 50 µm;
 - .2 allows samples of the ballast water to be taken and filtered;
 - .3 enables the amount of ballast water sampled to be measured to allow for extrapolation of the results; and
 - .4 allows the filtered ballast water to be discharged safely without affecting the stability and safety of the ship, its crew and the samplers or other discharges from the vessel such as bilge water.
-