BWM - Guidance for best practices on sampling

INTRODUCTION

1.1. GOALS AND PURPOSE

The Ballast Water Management Convention or BWM Convention (full name International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004) is a treaty adopted by the International Maritime Organization (IMO) in order to help prevent the spread of potentially harmful aquatic organisms and pathogens in ships' ballast water. The Convention was adopted by consensus at a Diplomatic Conference held at IMO Headquarters in London on 13 February 2004 and it entered into force on the 8th of September 2017.

This document is intended to provide guidance for a harmonised approach to ballast water sampling procedures, identifying best practice according to the different standards, D-1 and D-2 for ascertaining the compliance with the Ballast Water Convention (hereafter referred to as 'the Convention').

1.2. BALLAST WATER CONVENTION, SCOPE OF APPLICATION & RELEVANT DOCUMENTATION

Ballast Water Convention

As a general obligation the Convention says that "Parties shall give complete effect to the provisions of the Convention and the Annex in order to prevent, minimize and ultimately eliminate the transfer of harmful aquatic organisms and pathogens through the control and management of ship's ballast water and sediments".

Scope of Application

The Convention shall apply to:

a) Ships entitled to fly the flag of a Party (States that have been ratified the Convention),
b) Ships not entitled to fly the flag of a Party but which operate under the authority of a Party,

which take up and use ballast water during international voyages. However some countries have applied the regulations to domestic shipping.

This means that each Party shall require ships flying its flag or operating under its authority to comply with the requirements set in forth in the Convention, including the Annex, and shall take effective measures to ensure that those ships comply with those requirements.

The Convention shall not apply to ships not carrying ballast water or ships with permanent ballast water in sealed tanks on ships that is not subject to discharge, warships, naval auxiliary and governmental ships.

Under some exceptional circumstances, discharge of ballast water can be allowed[1].

Relevant documentation

In order to establish whether a ship is in compliance with the requirements of the Convention, ships have to carry:

• A ballast water management plan - specific to each ship, the ballast water management plan includes a detailed description of the actions to be taken to implement the ballast water management requirements;

• A ballast water record book - to record when ballast water is taken on board; circulated or treated for ballast water management purposes; and discharged into the sea. It should also record when ballast water is discharged to a reception facility and accidental or other exceptional discharges of ballast water; and

[1] E.g. in case of damage to the ship or its equipment, and in case of securing the safety of a ship or saving life at sea (Convention Annex, Reg.A-3).

• An International Ballast Water Management Certificate (required on ships of 400 GT and above under a flag which is a Party to the Convention)[2] – this is issued by or on behalf of the Administration (flag State) and certifies that the ship carries out ballast water management in accordance with the BWM Convention and specifies which standard the ship is complying with, as well as the date of expiry of the Certificate.

1.3. STANDARDS, REGULATIONS D-1 & D-2

The Convention establishes that ships are required to manage their ballast water and sediments according to a certain standard (Regulation D-1 or Regulation D-2). Regulation D-1 establishes the standards for the water exchange and Regulation D-2 sets the standard for the performance of the ballast water system.

Regulation D-1

The D-1 standard requires ships to exchange water in open seas. By doing this, fewer organisms will survive and so ships will be less likely to introduce potentially harmful species when they release the ballast water. Convention proposes to different options for the exchange of water:

1. do it with an efficiency of at least 95 percent volumetric exchange of Ballast Water or,

2. if the ships exchange Ballast Water by the pumping-through method, pumping through three times the volume of each Ballast Water tank shall be considered to meet the standard described in paragraph 1. But if the ship can demonstrate that at least 95 percent volumetric exchange is met pumping through less than three times the volume it may be accepted.

Systems approved by IMO to comply with D-1 standard are:

• Sequential method – a process by which a ballast tank intended for the carriage of ballast water is first emptied and then refilled with replacement ballast water to achieve at least a 95 per cent volumetric exchange.

• Flow-through method – a process by which replacement ballast water is pumped into a ballast tank intended for the carriage of ballast water, allowing water to flow through overflow or other arrangements.

• Dilution method – a process by which replacement ballast water is filled through the top of the ballast tank intended for the carriage of ballast water with simultaneous discharge from the bottom at the same flow rate and maintaining a constant level in the tank throughout the ballast exchange operation.

Regulation D-2

The second standard proposed in the Convention, the standards for the Ballast Water Performance, Regulation D-2, establishes the maximum quantity of organism that can be discharged:

1. Ships conducting Ballast Water Management in accordance with this regulation shall discharge less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension and less than 10 viable organisms per millilitre less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension; and discharge of the indicator microbes shall not exceed the specified concentrations described in paragraph 2 below.

2. Indicator microbes as a human health standard, shall include but not be limited to:

- toxicogenic Vibrio cholerae (O1 and O139): <1 colony forming unit (cfu) per 100 ml or < 1 cfu per 1 g (wet weight) zooplankton samples,
- escherichia coli: < 250 cfu per 100 ml,
- intestinal Enterococci: < 100 cfu per 100 ml.

[2] Flag States which are not a Party to the Convention, or Recognized Organizations acting on their behalf, may issue a Statement of Compliance instead of the Certificate.

1.4. IMPLEMENTATION SCHEDULE

Once the Convention has entered into force, the schedule for implementation has been agreed by the MEPC and compliance with the D-2 standard will be phased in over time for individual ships, up to 8 September 2024, from this date all ships falling under the Convention have to comply with D-2 standard.

From 8 September 2017:

- New ships must meet the D-2 standard.
- All ships must have:
  - A ballast water management plan;
  - A ballast water record book; and
  - An International Ballast Water Management Certificate (only on ships falling under the Convention).
- Existing ships must meet at least the D-1 (ballast water exchange) standard; they may also choose to install a ballast water management system or otherwise meet the D-2 (discharge) standard but this is not mandatory until the corresponding compliance date.

The compliance date is coupled to the renewal data of the IOPP certificate:

- If the IOPP Certificate Renewal survey is after 8 September 2019, the ship will need to meet the D-2 standard by the date of this IOPP renewal survey.
- If the IOPP Certificate Renewal survey is between 8 September 2017 and 8 September 2019:
  - If the previous IOPP Certificate renewal survey was between 8 September 2014 and 8 September 2017, then the ship must comply with D-2 standard by this IOPP renewal survey.
  - If the previous IOPP Certificate renewal survey was before 8 September 2014, then the ship can wait until the first upcoming IOPP renewal survey after the 8th September 2019 to comply with D-2 Standard.

1.5. DEFINITIONS

For the purpose of this best practice's guidance, the definitions in the BWM Convention apply and:

1. **A sample** means a relatively small quantity of ballast water intended to show what the larger volume of interest is like.

2. **Representative sampling** represents a series of samples that reflect the relative concentrations and composition of the populations (organisms and/or chemicals) in the volume of interest.

3. **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.

4. **Sampling Point** means that place in the ballast water piping where the sample is taken.

5. **Sampling Facilities** means the equipment installed to take the sample.

6. **Sampling protocol** is a procedure used to select samples to be measured. The goal of the sampling protocol is to select samples that are representative of the population with respect to the attribute of interest. The sampling protocol should stablish how and when the samples are selected and how many samples are selected.

7. **Analysis** means the process of measuring and determining the concentrations and composition of the populations of interest (organisms and/or chemicals) within the sample.

8. **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:

- 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;
- 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 a large confidence interval, or high-detection limits.

9. **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:

- 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);
- 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
- should use a measurement method with an adequate detection limit for the purpose for which it is being applied.

10. **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.

11. **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.

12. **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.

13. **General approach** means a conceptual description or broad methodology of sample collection or analysis.

14. **The precision** of a measurement system is the degree to which repeated measurements under unchanged conditions show the same results.

15. **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.

16. **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.

17. **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.

18. **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.

19. **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.

20. **Mixotrophic species** means organism that combine autotrophic and heterotrophic modes of nutrition. It is widely spread in various microorganisms, particularly in such important plankton groups as dinoflagellates and cyanobacteria.

21. **PSU (Practical Salinity Unit)** is a unit based on the properties of sea water conductivity. The averaged salinity in the global ocean is 35.5 PSU, varying from less than 15 PSU at the mouth of the rivers to more than 40 PSU in the Dead Sea.

BALLAST WATER SAMPLING

In accordance with Article 9 of the Convention, a Party may sample the ship's ballast water for the purpose of determining whether the ship is in compliance with the Convention, however, sampling for enforcement should be no more stringent that what is required for type approval of ballast water management systems. Sampling for compliance with the BWM Convention should to be done in accordance with IMO Guidelines for ballast water sampling G2, and IMO Circular BWM.2/Circ.42/Rev.1 - Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention.

It is important to bear in mind that different approaches in the sampling process influence the results regarding organism concentrations. The organisms in the discharge are affected by the sampling method; therefore the selection of the "wrong" sampling approach may influence the compliance control result. The organism concentrations in the ballast water discharge may therefore be underestimated, and a "faulty" ballast water treatment system (BWTS) could be recognised as compliant. Conversely organism concentrations may be overestimated, and a BWTS complying with the D-2 Standard may fail in compliance tests.

It is important to note that there is currently no standard sampling method recognized by IMO. Although there exists ISO standard ISO11711-1, it is however under review. Discussions at IMO are focused in establishing a standard in other to harmonize the sampling procedure all over the word.

Inspectors or personal involved in sampling should be duly trained and authorised by the Member States to perform sampling in relation to the IMO Ballast Water Convention.

In addition, organisations/laboratories duly accredited or applying quality management system practices in accordance with EN ISO/IEC-17025 or equivalent standards accepted at international level should be available in case a sample of ballast water needs to be analysed to ascertain its compliance with the Convention. Although at this time there is no agreed or validated direct or indirect analyses for compliance monitoring of ballast water.

IMO Circular BWM.2/Circ.42/Rev.1 lists some analysis methods that can provide an indication of compliance. There are some ISO standards for testing Enterococci, Escherichia coli and Vibrio cholera however there is no accepted international standard method for ballast water analysis. Moreover the level of confidence or detection limit and citation for validation studies has to be determined.

IMO Circular BWM.2/Circ.61[3] provides also Guidance on methodology to be used for enumerating viable organisms.

[3] Guidance on methodologies that may be used for enumerating viable organism for type approval of ballast water

2.1. PRINCIPLES FOR SAMPLING & ANALYSIS

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. Any method, approach or sampling procedure should be rigorously validated and practicability should be assessed.

With the aim to ensure consistency of approach between Convention's Parties, IMO has established some principles:

1.- the sampling protocol should be in line with IMO G2 Guidelines;

2.- the sampling protocol should result in samples that are representative of the whole discharge of ballast water from any single tank or any combination of tanks being discharged;

3.- the sampling protocol should take account of the potential for a suspended sediment load in the discharge to affect sample results;

4.- the sampling protocol should provide for samples to be taken at appropriate discharge points, if possible;

5.- the quantity and quality of samples taken should be sufficient to demonstrate whether the ballast water being discharged meets with the relevant standard;

6.- sampling should be undertaken in a safe and practical manner;

7.- samples should be concentrated to a manageable size;

8.- samples should be taken, sealed and stored to ensure that they can be used to test for compliance with the Convention;

9.- samples should be fully analysed within test method holding time limit using an accredited laboratory which apply quality management system practices in accordance with EN ISO/IEC-17025 or other equivalent standards accepted at international level; and

10.- samples should be transported, handled and stored with the consideration of the chain of custody.

Identify the purpose of the sampling.

The first premise of any sampling is to identify the purpose of the sampling, specifically whether the ballast water management system of the ship is meeting the D-1 standard or the D-2 standard. It should be noted that different treatments and the site of discharge of ballast water may require different sampling methods.

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 tank or a discharge with a population that can vary significantly. Consequently, the samples collected for indicative or detailed analysis should be representative samples.

The second challenge regarding sampling is to determine the volume of interest and how to sample it.

Where to take the sample.

Samples may be taken from the discharge line or directly from ballast water tanks.

As a first option, samples should be taken from the discharge line through designated sampling points but in there are some cases where this is no possible and samples have to be taken from the tanks.

These cases are:

a. when tanks are emptied through direct overboard discharge valves, as in upper side wing tanks, rather than through the ballast pumps. In such cases, tank sampling may be an appropriate approach;
b. cases where the ballast system design does not enable sampling from the discharge line;
c. if any part of the treatment process occurs during the ballast water discharge, then in- tank sampling will be inappropriate;
d. ballast coming from pre-notified contaminated or problematic areas; or,

e. or when the ballast water management system is found to be not working properly and the discharge is forbidden.

In-tank samples may be taken via sounding or air pipes and manholes by using pumps, sampling bottles or other water containers.

Indicative Analysis versus Detailed Analysis

The qualitative difference between indicative analysis and detailed analysis relies on the level of statistical confidence, which, in detailed analysis will be superior. So that, the sampling procedure to be use is independent of this parameter. Indicative sampling of a small amount of the discharge may lead to detailed analysis when a representative sample should be taken.

2.2. SAMPLING FOR COMPLIANCE WITH D-1 STANDARD

Sampling for analysis for compliance with D-1 Standard can be undertaken from the discharge line or from the tanks.

Analysis for compliance with the D-1 standard of the BWM Convention should rely on either the chemical/biochemical parameters of the ballast water discharged, such as salinity, or on an estimation of species present in the water discharge to check whether the plankton types are oceanic or coastal in nature. Although the latter might need trained personnel and would take time. These parameters should be use together to prove that ballast water exchange has been done, or not, according with regulation D-1.

As a general principle, 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 in route under the conditions required in the D-1 standard, or that the exchange has not been completed successfully.

However, the origin of the ballast water exchange should be known before interpreting the results of the salinity analysis because there are some exceptions to this general rule. The exceptions are: ballast water taken up in ports areas located in high-salinity environments of above 30 PSU where water with a PSU of 30 may not originate from the mid-ocean waters, or when the exchanged has been carried out in areas within 50 nm from the coastline with less salinity than the middle ocean, in that case salinity less than 30 PSU could be compliant. Therefore, the origin of the last ballast water exchange should be known before interpreting the results of salinity analysis, this information can be obtained from the Ballast Water Management record book.

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.

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.

Checking salinity should be backed up by further analysis of the organisms in the ballast water discharge to determine the origin of the ballast water; however, as it was said this would take time and need experienced staff.

2.3. SAMPLING FOR COMPLIANCE WITH D-2 STANDARD

In 2010, EMSA financed a project with the objective to find methods to overcome the issue of how to take a sample for being representative of the whole ballast water discharge and also to answer the question of how to conduct sampling for an indicative analysis of ballast water for compliance with D-2 standard, two problems that Maritime Administrations face in sampling for enforcement of BWM Convention. The project conclusions have been used to prepare this guidance for best practices on sampling.

http://emsa.europa.eu/implementation-tasks/environment/ballast-water/download/1019/605/23.html

As explained in point 2.1 as a general rule samples will be taken for the discharge line, two different potential sampling approaches can be considered:

1. sampling the entire discharge from a vessel during a port visit, or,

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, two, three or more during the discharge.

Sampling the entire discharge has some disadvantages as large numbers of samples and large volumes are required over a long period of time and sampling personnel would be required on the vessel over a significant period of time.

In the case of a sequence of samples, the sampling can be developed to fit the situation on board the vessel. Different types, sizes and cargo profiles of vessels trigger very different ballast water discharge profiles and times. Ballast water discharge may be conducted “at once” or “in sequence”, lasting from some hours up to several days depending on the length of the cargo operation. It is important to take this factor into account as it is difficult to imagine that a PSC officer and/or sampling team would stay on-board the vessel for several days.

Considering the above and taking into account the EMSA project results, it is recommended for undertaking a representative sampling to perform a sequential sampling during the discharge operation which is feasible and relatively easy.

Volume of water to sampling

The EMSA study showed that to obtain the most representative results it is recommended that:

- for the bigger organism standard (greater or equal to 50 micrometres in minimum dimension): 300 to 450 litres should be filtered and concentrated;
- for the smaller organism standard (less than 50 micrometres and greater or equal to 10 micrometres in minimum dimension): a "continuous drip" sample totalling approximately 5 litres (i.e., collecting approximately 0,5 litres of sample water every minute during the entire sampling time duration, or collecting about 0.5 litres of sample water every 30 to 45 litres sampled, depending on the flow rate) should be taken. The resulting 5 litres of sample water should be mixed and sub-sampled to create two sets of samples, one alive and another preserved. It is recommended sub-sample volumes of 60 to 100 ml;
- for the bacterial standard: a sample of approximately 1 litre should be taken as a sub-sample from the 5 litre "continuous drip" sample after it has been mixed.

Sampling timing

The EMSA study found that organism concentrations may vary considerably if the sampling is conducted at the very beginning or at the very end of the discharge process because of the patchy distribution of organisms inside ballast water tanks.

Therefore, it is not recommended to take a sample at the very beginning (i.e., the first 5 min) or at the very end of the discharge (i.e., the last 5 min), as an underestimation as well as an overestimation of organism concentrations may be expected.

Based on this it is recommended that the sampling is conducted randomly anytime in the middle of the discharge, starting after 5 minutes from the start of discharge and ending 5 minutes before the end of the discharge.

Sampling duration

Sampling duration, timing, number of samples and sampled water quantity are the main factors that influence the results regarding organism concentrations.

The EMSA study showed that longer sampling times result in an underestimation of the viable organism concentration in the discharge, especially for bigger organisms. Bigger organisms are negatively affected by longer sampling times due to mortality caused by the concentration of the sample through a net.

Considering that the EMSA study shows that a shorter sampling time is still representative, the recommended sampling time should be approximately 10 minutes.

Number of samples

Organism concentrations in all organism groups vary due to the patchy distribution of organisms inside the ballast water tanks; hence a single 10 minutes sequential sample may underestimate or overestimate the concentration of organisms being discharged.

The EMSA study showed that an average of organism concentrations from 2 random samples from sequential sampling provides very similar results to the average of the 3 random samples. Based on this it is recommended that sampling is conducted by undertaking at least 2 random samples, which are analysed immediately after each sampling event has ended, and that the organism concentration results are averaged.

The challenge may become to obtain a representative sample of the whole discharge, when the vessel will be discharging ballast water from more than one ballast water tank. In such cases it is recommended that at least one sequential sampling protocol per tank is taken. If a tank was filled from multiple sources this does not trigger the necessity for more sampling.

Other recommendations

It is also assumed that the sampling flow rates may influence the results. Lower flow rates obtained by partially closed valves of the sampling line may damage organisms in the discharge, and a similar negative effect may be caused by to strong flow rates affecting mainly the filtering process of the bigger organisms. Hence, the flow rate, or "valve" effect, may cause an underestimation of the organism concentration as organisms may die during the sampling process.

To avoid this negative influence, it is recommended that the valve at the sampling point is opened as much as possible, however it should not exceed the flow rate of 50 litres/min, so that the water pressure is not too high during concentration of the sample, as this may impair organism survival. This will be further explained in point 3.1.

Conclusions

Considering all the points above and taking into account the EMSA project the best practice for a representative sampling protocol for D-2 compliance is to complete an in-line sequential sampling during the discharge operation.

Two samples should be taking during 10 minutes maximum with at least 5 minutes between the two samples. The samples should not be taking during the first and last 5 minutes of the discharge. The result should be the average of the values of these samples.

The valves should be fully open and a flow rate less or equal to 50 litres/min should be maintained.

Sampling from the tanks should be only considered when the discharge the water is forbidden or not recommended.

It should be noted that as the sample is taken from near the discharge point, once the sample is taken the ballast water will be discharged so the sample cannot be replicated for this point in the discharge.

SAMPLING COLLECTION

In this chapter, how to collect samples for detailed analysis to check compliance with D-2 Standard will be explained for the two possible sampling options, sampling from the ballast water discharge line and sampling from the ballast water tanks.

3.1. SAMPLING FROM THE BALLAST WATER DISCHARGE LINE

Sampling from the ballast water discharge has some advantages, as the biota present in the ballast water discharge line is most likely to accurately represent the concentration of organisms in the actual discharge, which is of primary concern in assessing compliance with the discharge regulations.

But this method also has some disadvantages. On most ships in- line sampling should be carried out in the engine room, where space may be limited, and the handling of water once the samples are concentrated may be impracticable.

Sampling can be done using traditional net sampling kits (plankton net and buckets) or using ballast water sampling kits that some companies have developed to simplify the sampling process. However, these systems are still under development and their performance has to be confirmed by further studies. We hope that in the future these kits can allow sampling to be completed in a quicker and easier way than the existing equipment.

In order to undertake an accurate measurement on the organism concentration in the ballast water, ships should have an "isokinetic" sampling point to ensure that a sample contains the same proportions of the various flow constituents as the flow stream being sampled and to ensure that the velocities of both the sample and the main flow are equal at the point at which the sample is separated from the main flow.

Sample port diameter

Ships should have sampling ports installed and computational fluid dynamics modelling should have been used to calculate the isokinetic diameter of the sample pipe. Sample port diameters rates between 1.5 and 2.0 times showed that flow transitions from the main stream were best as they had smooth transitions and pressure profiles that allowed for direct sampling without the need of a pump to induce sample collection. This should be checked before sampling.

See IMO Resolution MEPC173(58) for more information about flow calculation.

However as was explained in point 2.3.2, and as per the EMSA study, it is recommended that the valve at the sampling point is opened as much as possible and the flow rate doesn't exceed of 50 litres/min.

Sampling process

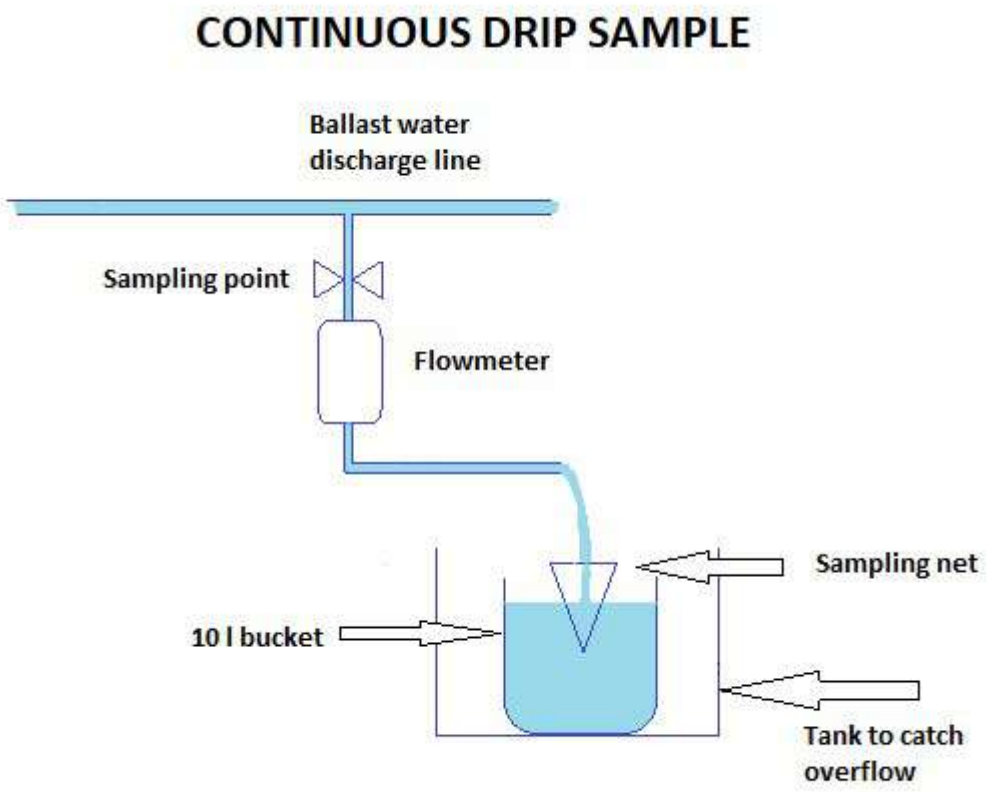
In EMSA's study the sampling was achieved by sampling the discharge line using traditional plankton net kits and the process explained here is based on the use of this equipment which has been used extensively during the type approval testing of Ballast Water Management Systems.

As a general approach samples should be taken by using the in-line sampling points of the ships ballast water pipework. The sampling should be from a straight part of the discharge line as near to the ballast water discharge overboard as practicable.

Water from a sampling point should be directed via flowmeters, to exactly measure the amount of water sampled, into plankton nets with a meshsize of 50 micrometres in diagonal dimension fitted with a cod end inserted in large buckets to collect the water.

The sampler should ensure that the plankton net sits in water at all times to avoid organism damage and mortality. Through the entire sampling process, a continuous drip sample of at least 5 litres should be collected into a 10 litres bucket. At the end of the sampling process the net has to be rinsed down with the filtered water to the cod-end at the bottom end of the plankton net, which should be unscrewed from the net and covered with a lid and stored in a 10 litres bucket filled with water until on-board processing or analysis.

This "continuous drip sample" could be used as water source for the sampler to undertake a detailed sampling of phytoplankton and bacteria. This filtered water in the cod end of the plankton net should be used to analyse organism above 50 microns and the filtered water for organisms between 10 and 50 microns and for bacterial analysis.



Sampling kit

The sampling kit for discharge line sampling should in minimum consist of:

• a flow meter,

• Suitable piping to direct water from the sampling point to the plankton net or sieve;

• 50-micron plankton net or sieve with a cod end to concentrate the sample (with replacement material of identical mesh size);

• containers to collect sieved water for rinsing sieve or net when sampling is completed;

• a funnel to ease filling of sample container;

• sample containers including sterile containers for microbial analysis;

• all necessary forms including sample data reporting/chain of custody forms;

• toolkit to enable net or sieve replacement, etc.;

• tape to seal the sample jar lid to the jar; and

• a first aid kit.

3.2. SAMPLING FROM THE BALLAST WATER TANKS

D-2 compliance should be assessed at ballast water discharge, whenever this is possible. However, there may be circumstances when in-tank sampling to provide an indication of compliance or non-compliance with the ballast water performance standard D-2 may be found appropriate as it was explained in point 2.1. Also some older vessels may have ballast water tanks that discharge water by emptying the entire tank at one time, so this may be the only option for some ships.

In-tank sampling may be also appropriate for assessing D-1 compliance.

Manholes

Sampling of ballast water via manholes allows direct access to ballast tanks and ballast holds. The disadvantages of this type of sampling access include the need for opening and closing manholes and hatches which may increase light in the tank and cause stratification of the organisms in the tank. Further, overlaying cargo may prevent access for sampling. Also, hatches and horizontal openings inside tanks are not aligned one below the other, which means that although the tank may have three or more decks, only the top deck may be accessible for sampling. Further, in some ships, access hatches and vertical openings are on the side of the tank and thus are not accessible unless the tank is empty. Another disadvantage is ladders and platforms may inhibit access to the full depth of the tank. Sampling from some certain parts of the ballast water tank may result in a lack of representation of the whole ballast water discharge.

Samples should be collected using scientific sampling equipment including plankton nets and pumps, as appropriate, for the sampling and analytical method intended for use.

Whenever possible samples should be taken from multiple water depths inside the ballast tank.

When employing plankton nets:

1.- sample should be taken in a vertical net haul from the deepest sampling point accessible in the tank;

2.- all plankton nets should be lowered to the maximum accessible depth inside the ballast tank and retrieved at a speed of approximately 0.5 m/s; and

3.- multiple vertical net hauls may be needed to meet the required sample volume. The water volume sampled may be measured by flow meters in the opening of the net or by noting the sampling depth and net opening diameter and making the necessary calculations.

When employing pumps:

1.- pump intake pipes should be lowered to multiple depths (if possible) for different samples to obtain a vertical sample; and

2.- the water volume sampled may be measured by flow meters in the hose or by using larger containers to measure the pumped water volume.

The sampling kit for manhole sampling should in minimum consist of:

• a plankton net with an associated flow meter - scientific trials have shown that plankton nets equipped with a cone shaped opening and filtering cod-end provide the most accurate samples. Nets to be lowered down into the tank should further not exceed 1 m in length and 30 cm in diameter to reduce the risk to become entangled inside the tank. Aspare net including an extra cod end should be added to the sampling kit in case damages occur. A weight (minimum 1 kg) should be used to keep the wire vertical during the net haul;

• a rope to lower down net (the rope should be metered to document net haul depth);

• a net or sieve to concentrate sample (with replacement material of identical mesh size) spare sieves with identical mesh size should be added to the sampling kit in case damages occur;

• a method for collecting sieved water for rinsing sieve and plankton net when sampling is completed;

• a water bottle to rinse net or sieve;

• a funnel to ease filling of sample container;

• sample containers including sterile containers for microbial analysis;

• all necessary forms including sample data reporting/chain of custody forms;

• a toolkit to enable net or sieve replacement, etc.;

• tape to seal the sample jar lid to the jar; and

• a first aid kit.

Sounding pipes or air pipes

Sampling by sounding pipes, when available, could be appropriate if they are accessible. However, there are some limitations when using this point to test for compliance. The use of sounding pipes will be more effective when the ship's sounding pipes are perforated along their length, ensuring better mixing of ballast water and that within the sounding tube. However, care must be taken if initial water samples from a sounding pipe indicate no or insufficient exchange even though the ship's records document otherwise. Experience has shown that in some cases water within unperforated sounding pipes is not affected during an exchange. This may occur during flow-through because the water in pipes is not exposed to the mixing within the tank. This may also occur during the process of emptying and refilling the tanks when water in the sounding pipes is held within the pipe by vacuum pressure while the tanks are drained and then filled.

Samples should be collected using scientific sampling equipment as appropriate.

The sampling kit for sounding or air pipe sampling should in minimum consist of:

• a pump (e.g., suction, power or air driven);

• a hose (optional with weight to ease lowering down the hose);

• a net or sieve to concentrate sample (with replacement material of identical mesh size);

• at least two containers to measure water volume pumped on deck. The container is further needed to collect sieved water for rinsing sieve when sampling is completed and to rinse hose;

• a water bottle to rinse net or sieve;

• a funnel to ease filling of sample container;

• sample containers including sterile containers for microbial analysis;

• all necessary forms including sample data reporting/chain of custody forms;

• a toolkit to enable net or sieve replacement, opening of sounding or air pipes, etc.;

• tape to seal the sample jar lid to the jar; and

• a first aid kit.

Use of pumps

Pumps of various types may be used to sample via sounding or air pipes. However the use of pumps may be limited by inability to overcome the pumping head, i.e. when the vertical distance from the pump to the water level in tank exceeds 10 metres, suction pumps cannot be used. Pumps can also contribute to the mortality of organism, so we have to bear that in mind when using pumps and use the less damaging types.

Pump intake pipes should be lowered to multiple depths (if possible) for different samples to obtain a vertical sample. The water volume sampled may be measured by flow meters in the hose or by using larger containers to measure the pumped water volume. In principle, intrinsically safe pumps should be used in all circumstances.

3.3. PRESERVATION OF SAMPLES

Sample processing for organisms greater than or equal to 50 micrometres in minimum dimension.

For these organisms, studies show that samples should be concentrated, the plankton net cod-end containing the concentrated sample should be emptied into a 20-micrometre filter. Once filtered the sample concentrate has to be transferred into a 100 ml container and mixed well before subsampling it for analysis. Subsamples of 1 or 2 ml volume have to be extracted from the 100 ml container by using a pipette and transferred into counting chambers for counting the organisms.

There are different counting chambers on the market, the most common ones are "Petri dishes". However, with these chambers organism counting may not be accurate as any movement, such as ship movement or movement on the microscope, induces water movements in the Petri dish, so some organisms may be counted twice and some may be excluded.

To avoid this, there are other kinds of chambers that could be used such as the “Bogorov” counting chamber, these chambers work very well to minimise any sample movement, and their efficienty has been proved during sea trials. However, with increasing ship movements the Bogorov chamber loses its advantage.

To solve the problem with the ship movements companies have designed several counting chambers which may be used with increasing ship movements. These chambers give greater accuracy reducing the risk to overlook organisms.

For the size measurements a piece of the filtering mesh (50 micrometres in diagonal dimension) should be placed underneath the stereomicroscope dish. This transparent mesh can then be used as a scale for counting all living organisms greater than or equal to 50 micrometres in minimum dimension.

The organisms have to be counted as soon as possible after sample processing as longer waiting times may negatively impact organism survival.

Alternatively, electronic means of count and measuring these organisms can be used and there are some portable analysis devices in the market that have been proved to be useful for ballast water compliance monitoring.

Sample processing for organisms less than 50 and greater than or equal to 10 micrometres in minimum dimension.

Several studies have shown that the organisms in this size class should not be concentrated as the concentration process damages the organisms, please see the EMSA Study for details. Samples taken for analysed on-board the vessel can be taken directly from the buckets.

For analysis at laboratories small bottles (e.g. 80ml.) have to be filled with sample water taken from the sample bucket after mixing. Samples have to be properly labelled.

Living samples for preliminary analysis should be kept in the dark at a temperature of between 4 and 10 °C. They can be kept unpreserved for up to 24 h in polypropylene or polyethylene bottles. However to preserve the samples for later analyses, several procedures must be applied directly after the collection to prevent the adverse effects of light, temperature and microorganisms which might cause rapid decay of organisms, samples can be kept alive by stored in a fridge or preserved into some solution.

The more common preservation solutions currently employed are:

1. Formaldehyde-based solutions
2. Lugol’s solution and its adaptations
3. Glutaraldehyde and its adaptations

Glutaraldehyde is quite toxic with hazardous fumes and formaldehyde-based solutions are irritating at very low concentrations in the air and may lead to allergic reactions. Based on that the more suitable solution for being using on board ship will be Lugol’s solution. This solution was also used for preservation of the samples during the EMSA study.

Lugol’s solution has some advantages and also disadvantages. As advantages we can consider that it stains cells a dark brown colour, making counting easier and the resulting solution is relatively harmless (not very toxic) compared to the others.

Disadvantages include that the Lugol’s solution breaks down in sunlight, so samples and stock solution need to be kept in a dark and cool environment. Samples preserved in Lugol's fixative do not have a long shelf life, so samples stored for more than one year are of little use as the solution loses preservative power over time. Re-adding the Lugol’s solution every 6 months may overcome this issue.

Another disadvantage is that Lugol’s can mask chlorophyll fluorescence, which may be needed to recognize mixotrophic species. Lugol’s also dissolves hard structures such as coccoliths and diatom frustules and, therefore, is not ideal for long-term storage of many plankton taxa.

Sample processing for Bacteria

Analysis of bacteria should be done in a proper laboratory as the samples need an incubation time. This should be between 24 and 48 hours. Although samples could be prepared on board it will be better if they are processed by trained people in laboratory premises.

For bacteria analysis, the water in the 10 l bucket should be mixed well and filtered through pore filters of 0.45 micrometre and placed in 10 and 100 ml bottles.

3.4. SAMPLE DATA FORM

The following minimum information is recommended for sample documentation in the IMO Guidelines for Ballast Water Sampling (G2):

Sampling date	
Ship particulars	Name of ship: Distinctive number or letters Port of registry: Gross tonnage: IMO Number: Date of construction: Ballast water capacity:
Identification of sampled tank*	
Type and position of sampled tank*	
Capacity of sampled tank*	(m^3)
Type of ballast water management undertaken	(type of exchange or treatment)
Make of ballast water management system	
Date of ballast water management undertaken	
Sample identification code	(including number of replicate)
Sample type	(larger, smaller plankton, microbes)
Sampling techniques used	net (including depth of vertical net haul, net opening size, mesh size) pumps (including sampling depth, pumping capacity in l/min.) bottle (incl. sampling depth, bottle capacity in l.) specify other sampling technique if used
Sampling time/start	
Sampling end time	
Origin of water sampled*	(latitude/longitude/port)

* If appropriate.

Other information :

Type of sampling access point	
Location of sampling access point	
Water volume sampled	(by volume)
In case sample is concentrated on board specify filter or net sizes (if applicable)	(µm)
Preservative (if used)	
Transport to laboratory	cooling container, dark storage, etc.
Sample results	

* If appropriate.

More information than necessary should be included in the table.

SAMPLING UNDER THE PSC REGIME

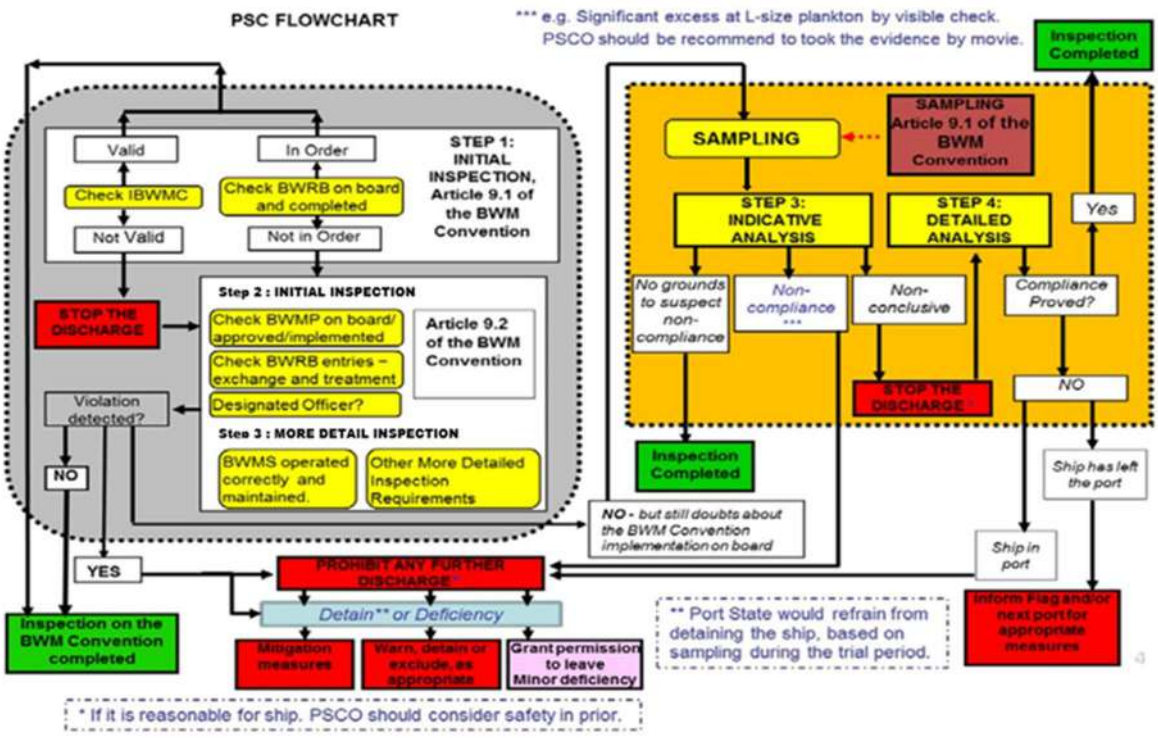
During Port State Control (PSC), inspectors can also check the compliance with the Ballast Water Convention, as per Convention regulation 9.2. The IMO developed Guidelines for Port State Control[4] and the Paris MOU also developed the corresponding "PSC Guidelines on Ballast Water Management Convention 2004".

Sampling may be undertaken during the 3rd step of the PSC process as follows:

1. "Initial inspection", inspection focuses on documentation review, Ballast Water Management (BWM) Plan, Record book, system's document and ensuring that an officer has been nominated for ballast water management on board the ship and the officer is familiar with the system;
2. "More detailed inspection", where the operation of the BWMS is checked and the PSCO clarifies whether the BWM System has been operated adequately according to the Ballast Water Management Plan and the self-monitored operational indicators verified;
3. "Sampling" which relies on indicative analysis is the 3rd step and envisaged to identify whether the ship is meeting the ballast water management performance standard described in regulation D-1 and D-2; and
4. If necessary, detailed analysis is carried out to verify compliance with the D 2 standard.

In the IMO Guidelines for ballast water sampling Part 6, some recommendations are included for undertaking ballast water sampling during PSC, including an example of a minimum sapling kit.

Also, following the Paris MOU Guidelines, inspections should be done according to the following flow chart:



However, sampling during a Port State Control inspection for compliance with D-1 and D-2 standards should only be carried out after an initial inspection, when clear grounds of non-compliance have been identified. Sampling is a very complex issue as we have seen, and it will be responsibility of the appropriate authority in the port State and every effort should be made to avoid any unduly delays to the ship.

[4] IMO Resolution MEPC.252(67)

If the indicative analysis indicates that the ship poses a threat to the environment, human health, property or resources and a detailed analysis may be required, in accordance with BWM.2/Circ.62 Guidance on contingency measures under the BWM Convention, the PSCO should stop the discharge and decide on one or more of the following actions:
• Actions predetermined in the Ballast Water Management Plan of the ship;
• Retention of all ballast water on board;
• Require the vessel to undertake any repairs required to the BWMS;
• Permit the vessel to proceed to exchange ballast water in a location acceptable to the Administration, provided this action does not pose a threat;
• Allow the vessel to discharge ballast to an appropriate shore reception facility; or
• Allow the vessel to treat the ballast water or a portion of it on board in accordance with a method approved by the Port Administration.

SAMPLING ANALYSIS

This chapter follows the recommendations given by IMO in its Circular, BWM.2/Circ.42/Rev.1, Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2).

5.1. ANALYSIS FOR COMPLIANCE WITH D-1 STANDARD

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	Portable nutrient sensors.	No international standard for ballast water analysis at this	Requires understanding of inorganic or organic	To be determined.

(Nutrients, metals coloured dissolved organic matter (CDOM))		time.	constituent characteristics in relation to the distance from shore.	

5.2. ANALYSIS FOR COMPLIANCE WITH D-2 STANDARD

The table below shows different indicative analysis methods for use when testing for potential compliance with the D-2 standard.

Indicator	General approach	Standard method	Notes	Level of confidence or detection limit and citation for validation studies
Viable organisms ≥ 50 µm	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, "Daphnia sp. Acute immobilization test and reproduction test" could be used as basis for standard methodology.)	To be determined.
Viable organisms ≥ 50 µm	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 ≥ 10 µm and < 50 µm	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 ≥ 50 µm and ≥ 10 µm and < 50 µm	Photometry, nucleic acid, ATP, bulk fluorescein diacetate (FDA), chlorophyll a.	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).	To be determined.
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.	To be determined.

Escherichia coli	Fluorometric diagnostic kit.	No international standard for ballast water analysis at this time.	Minimum incubation time 6 h. Semi-quantitative results from portable methods.	To be determined.
Vibrio cholera (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.

The next table shows different detailed analysis methods for use when testing for potential compliance with the D-2 standard.

Indicator	General approach	Standard method	Notes	Level of confidence or detection limit and citation for validation studies
Viable organisms ≥ 50 µm and ≥ 10 µm and < 50 µm	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	Can be expensive and time-consuming, needs moderately 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 ≥ 10 µm and < 50 µm	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	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 ≥ 10 µm and < 50 µm	Flow cytometers (based on chlorophyll a and vital stains).	No international standard for ballast water analysis at this time.	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 ≥ 50 µm and Viable organisms ≥ 10 µm and < 50 µm	Flow cameras (based on chlorophyll a and vital stains).	No international standard for ballast water analysis at this time.	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.
Viable organisms ≥ 50 µm and Viable organisms ≥ 10 µm	Culture methods for recovery, regrowth	No international standard for ballast water analysis at this	Require specific knowledge to conduct	To be determined

and < 50 µm	and maturation.	time.	them. Densities are expressed as Most Probable Numbers (the MPN method). Most species do not manage to grow using this method therefore cannot be used alone. 2-3 weeks incubation time needed.	
Enterococci	Culture methods.	ISO 7899-1 or ISO 7899-2	Requires specific knowledge to conduct them. At least 44-h incubation time. EPA Standard Method 9230	To be determined.
Escherichia coli	Culture methods.	ISO 9308-3 or ISO 9308-1	Requires specific knowledge to conduct them. At least 24-h incubation time. EPA Standard Method 9213D	To be determined.
Vibrio cholerae (O1 and O139)	Culture and molecular biological or fluorescence methods.	ISO/TS 21872-1/13/	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.
Enterococci, Escherichia coli, Vibrio cholerae (O1 and O139)	Culture with 11holera11ence-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 ≥ 50 µm and viable organisms ≥ 10 µm and < 50 µm	Visual counts using stereo-microscopy examination and flow cytometry.	No international standard for ballast water analysis at this time.	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.

5.3. METHODOLOGIES THAT MAY BE USED FOR ENUMERATING VIABLE ORGANISM

According to the IMO Circular, BWM.2/Circ.61, Guidance on methodologies that may be used for enumerating viable organism for type approval of ballast water, the following table shows the different Methodologies that may be used for enumerating viable organisms for type approval of BWMS.

Methodologies for enumerating viable organisms	Organism size class or indicator	Assessed criteria of viability	Examples of how the methodologies are applied	Applicability to ballast water treatment technologies
FDA/CMFDA + Motility	Viable organisms ≥ 10 µm to < 50 µm	Membrane integrity Enzyme activity Motility	PPR 4/7, appendix 1; PPR 4/INF.10	Suitable for assessing treatment technologies intended to kill or remove organisms
MPN Dilution Culture + Motility	Viable organisms ≥ 10 µm to < 50 µm	Reproduction capacity, motility	PPR 4/7, appendix 2	Suitable for assessing all treatment technologies

REFERENCES

[1] EMSA Study: Gollasch S., David M. Testing Sample Representativeness of a Ballast Water Discharge and Developing Methods for Indicative Analysis, Final Report, 2010

[2] IMO Resolution MEPC.173(58) Guidelines for Ballast Water Sampling (G2)

[3] IMO Resolution MEPC.252(67), Guidelines for Port State Control under the BWM Convention

[4] IMO Circular, BWM.2/Circ.42/Rev.1, Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)

[5] IMO Circular, BWM.2/Circ.61, Guidance on methodologies that may be used for enumerating viable organism for type approval of ballast water

[6] IMO Circular, BWM.2/Circ.62, Guidelines on Contingence measures under the BWM Convention

[7] Paris MoU, PSC Guidelines on Ballast Water Management Convention 2004.