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**Ships and marine technology —  
Aquatic nuisance species —**

**Part 2:  
Ballast water sample collection and  
handling**

*Navires et technologie maritime — Espèces aquatiques nuisibles —*

*Partie 2: Prélèvement et manipulation des échantillons d'eau de  
ballast*

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Published in Switzerland

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

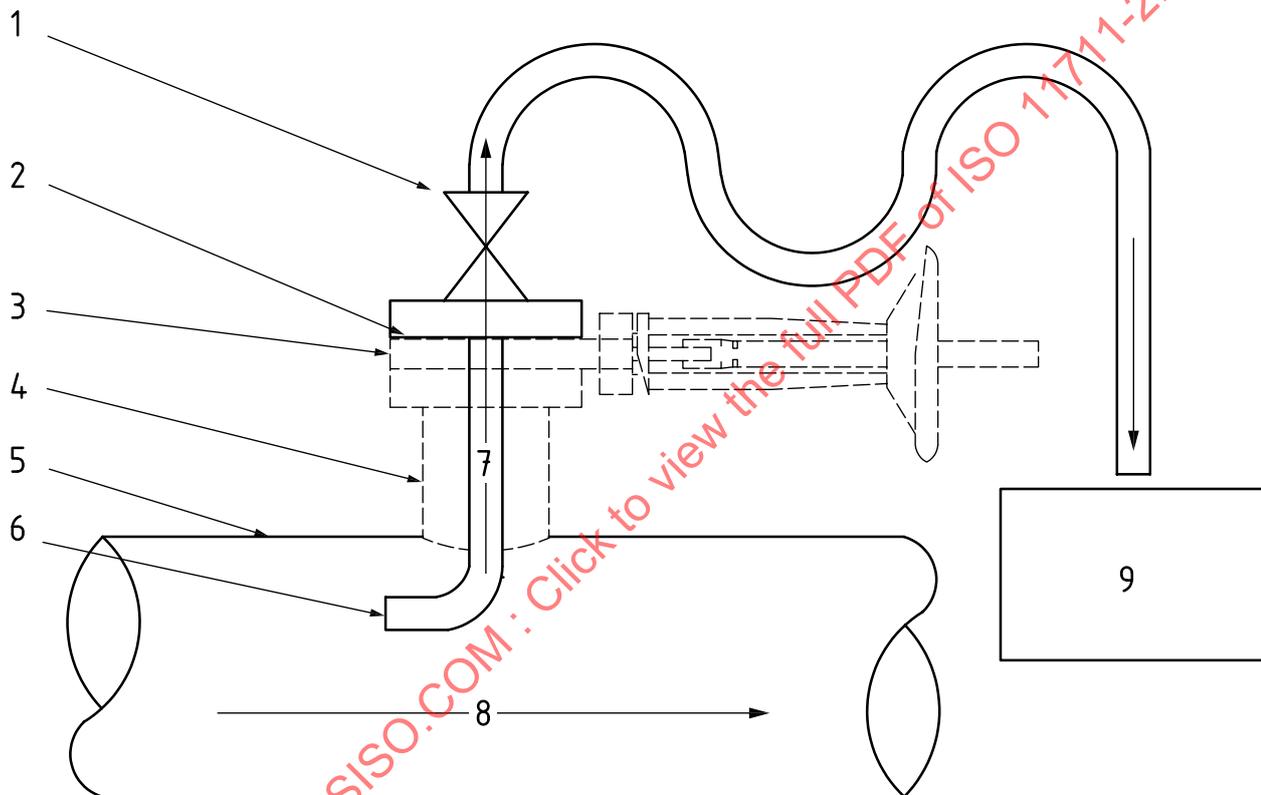
This document was prepared by Technical Committee ISO/TC 8, *Ships and marine technology*.

A list of all parts in the ISO 11711 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## Introduction

The sampling guidance provided by the ISO 11711 series is intended to standardize the measurement of organism concentrations through sampling of a ship's ballast discharge consistent with the requirements of the International Maritime Organization (IMO) Regulation D-2<sup>[1]</sup>. The ISO 11711 series currently includes two parts, where ISO 11711-1 provides guidance on the shipboard arrangements for piping and fittings necessary for sampling and return ports, and standardizes the presentation of the sampling port to accommodate various sampling probe configurations. This document addresses the process of collecting and processing ballast water samples for subsequent analysis such as required for type approval, according to IMO Resolution MEPC.300(72) (BWM Code)<sup>[2]</sup>. It provides guidance to ballast water sampling teams and other concerned parties on the apparatus, installation, and procedures required to obtain representative samples of ballast water discharges from sample ports on a ship. These concepts are illustrated in [Figure 1](#).



### Key

- |   |   |   |                          |
|---|---|---|--------------------------|
| 1 | sample collection device isolation valve                          | 6 | sample probe             |
| 2 | sample port access flange   | 7 | sample water flow        |
| 3 | sample port valve   | 8 | ballast water flow       |
| 4 | sample port   | 9 | sample collection device |
| 5 | ballast discharge pipe  |   |                          |
|   | ISO 11711-1 Ballast water sample port - fitting arrangements      |   |                          |
|   | ISO 11711-2 On-board ballast water sampling and sample processing |   |                          |

NOTE 1 Figure not to scale.

NOTE 2 The figure shows a sample port arranged perpendicular to the main ballast flow.

NOTE 3 See [Annex A](#) for examples of configurations of sample collection devices and their connection to ballast piping.

**Figure 1 — Illustration of the scopes of ISO 11711-1 and 11711-2**

Specifically, this document defines appropriate sample probe and sample flow control to achieve representative sampling and minimize measurement uncertainty consistent with measurement requirements. Appropriate sample volumes and collection times provide statistical confidence for viable organism counts at the discharge limit. Regulation D-2<sup>[1]</sup> requires the measurement of two organism size classes:  $\geq 10 \mu\text{m}$  and  $< 50 \mu\text{m}$  ( $< 10$  organisms  $\text{ml}^{-1}$ ) and  $\geq 50 \mu\text{m}$  ( $< 10$  organisms  $\text{m}^{-3}$ ), and three indicator microbes: toxigenic *Vibrio cholerae* (serotypes O1 and O139,  $< 1$  cfu  $100 \text{ ml}^{-1}$  or  $< 1$  cfu  $\text{g}^{-1}$  wet weight zoopl.), *Escherichia coli* ( $< 250$  cfu  $100 \text{ ml}^{-1}$ ), and intestinal enterococci ( $< 100$  cfu  $100 \text{ ml}^{-1}$ ). Sampling approaches for each are provided, where both indicative and detailed analyses of viable organisms are supported, as defined by BWM.2/Circ.42/Rev.2 <sup>[3]</sup>, as may be amended, and considering the criteria in ISO 17025 for quality management, measurement uncertainty, and standardized procedures. The ISO 11711 series does not intend to add any requirements to the BWM Convention or related documents of IMO but provides supplemental guidance for sampling of ballast water.

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# Ships and marine technology — Aquatic nuisance species —

## Part 2: Ballast water sample collection and handling

### 1 Scope

This document provides requirements and recommendations to ballast water sampling teams or other concerned parties on the selection and use of sampling apparatus to collect and process ballast water discharge samples aboard a ship from sample ports installed in accordance with ISO 11711-1. It includes an overview of the sampling process, and a discussion on the design and maintenance of sample probes, the necessary sample flow rates, the sample collection devices that incorporate sample flow control to maintain representative sampling conditions, and the handling of samples for subsequent analyses.

This document primarily addresses the collection of ballast water discharge samples. However, it can also be applied to uptake samples with consideration of appropriate sample volumes given anticipated organism concentrations in ambient (as opposed to treated) waters.

NOTE While this document is focused on installations aboard a ship, it can be used for land-based facilities.

### 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

MEPC.173(58), *Guidelines for Ballast Water Sampling* (G2)

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 11711-1:2019, *Ships and marine technology — Aquatic nuisance species — Part 1: Ballast water discharge sample port*

ISO 17602, *Ships and marine technology — Metal valves for use in flanged pipe — Face-to-face and centre-to-face dimensions*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

### 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

#### 3.1

##### **absolute pore size**

pore size based on empirical measurements of the pores in a *filter* (3.9)

**3.2**

**capture efficiency**

measurement of organism retention in a sample collection apparatus, typically expressed as a percentage

**3.3**

**closed loop configuration**

sampling arrangement that returns the filtered water to the ballast discharge pipe

**3.4**

**closed system**

*sample collection device* (3.23) that houses a *filter* (3.9) within a sealable container having inlet and outlet connections

Note 1 to entry: The filter used to concentrate organisms is typically made of metal or nylon mesh (see 6.1.3).

Note 2 to entry: A closed system can be operated in either *open loop configuration* (3.19) or *closed loop configuration* (3.3).

**3.5**

**collection container**

container used to obtain, hold, and transport water samples

**3.6**

**concentration factor**

ratio of *filtrate* (3.11) volume to *filtrand* (3.10) volume

**3.7**

**depth filtration**

filtration method where particles are captured within the filter media rather than on the surface of the *filter* (3.9)

**3.8**

**effective surface area**

*filter* (3.9) area available for filtration

**3.9**

**filter**

barrier that is introduced to retain organisms and particles of a given size while smaller particles are allowed to pass through

**3.10**

**filtrand**

concentrated sample, used in whole or in part for analysis, that is collected during the concentration process

**3.11**

**filtrate**

water that passes through a *filter* (3.9)

**3.12**

**filtration velocity**

*flow velocity* (3.13) through *filter* (3.9) pores

Note 1 to entry: In SI units, this parameter is expressed in metres per second.

**3.13**

**flow velocity**

distance travelled by a fluid per unit time (independent of any pipe dimensions)

Note 1 to entry: In SI units, this parameter is expressed in metres per second.

**3.14****hot-tap sample probe assembly**

sample probe assembly that can be installed into and removed from a water-filled, pressurized ballast pipe

**3.15****maximum allowable working pressure****MAWP**

maximum pressure that the weakest component in a pressurized system is designed to withstand

**3.16****measurement requirement**

specific requirement needed to support the purpose of the measurement, including sample timing, volume, duration, a specific ballast tank or sampling location, and acceptable measurement uncertainty

Note 1 to entry: See [4.3.2](#).

**3.17****nominal pore size**

pore size specified by the *filter* ([3.9](#)) manufacturer to identify the size of particles typically retained by the filter

**3.18****open loop configuration**

sampling arrangement that returns the filtered water to an unpressurized container (e.g. a bilge)

**3.19****open system**

*sample collection device* ([3.24](#)) that houses a *filter* ([3.9](#)) within an open container, e.g. an open tank with a *plankton net* ([3.21](#))

**3.20****operation, maintenance, and safety manual****OMSM**

reference manual supplied by the manufacturer for a ballast water management systems (BWMS) product that identifies factors that affect the operation of the BWMS, including any warm-up or other requirements that must be completed to achieve operational stability

Note 1 to entry: The OMSM specifies what constitutes stable operating conditions for the BWMS, factors that can affect operating conditions, and any adjustments required to reach or to maintain a stable operating condition.

**3.21****plankton net**

conical *filter* ([3.9](#)) device that collects organisms in a removable cod endNote 1 to entry: the filter material is a fabric net with a specific pore size, and the device can be towed in open waters or used as a filter in an *open system* ([3.19](#)) for organism concentration

**3.22****representative sampling**

sampling methodology that obtains concentrations and compositions of constituent materials and organisms that are in the proportions and physical state of the source volume of interest

Note 1 to entry: In the case of ballast water sampling, representative conditions are considered under fully turbulent flow, where an appropriate sited sample probe obtains a sample at a *flow velocity* ([3.13](#)) of 0,25 to 1 times the flow velocity of the water in the ballast discharge pipe, thus sample flow is isokinetic or sub-isokinetic [\[4\]](#), [\[5\]](#).

**3.23**

**sample collection device**

device that can concentrate and collect the larger class of organisms [via a *filter* (3.9) or *plankton net* (3.21)], collect a whole water sample, or both

Note 1 to entry: A sample collection device can consist of multiple individual systems, e.g. multiple *closed systems* (3.4), each housing a filter.

[SOURCE: ISO 11711-1:2019, 3.13, modified — Note 1 to entry has been added]

**3.24**

**sample collection team**

personnel responsible for setting up the *sample collection device* (3.23), collecting and retrieving the ballast water samples

**3.25**

**sample collection device isolation valve**

full port valve used to isolate the *sample collection device* (3.23) from the ballast discharge pipe

**3.26**

**sample flow control valve**

valve used to regulate the sample flow rate

**3.27**

**sample hold time**

duration between end of sample collection and start of analysis

**3.28**

**sample probe bend radius**

radius of the curvature in the sample probe, as measured at the centreline

**3.29**

**sample probe insertion length**

distance from the diametric centre of the sample probe entrance to the sample port access flange when installed into the ballast discharge pipe

**3.30**

**sample probe opening**

entrance through which the water from the ballast discharge pipe enters the sample probe

**3.31**

**test cycle**

testing iteration (including uptake, treatment, holding and discharge, as appropriate) under a given set of requirements used to establish the ability of a ballast water management system (BWMS) to meet the set discharge standards

[SOURCE: IMO MEPC.300(72), BWMS Code, 3.15]

**3.32**

**testing organization**

company, corporation, firm, enterprise, authority or institution, or part or combination thereof, whether incorporated or not, public or private, that has its own functions and administration, that conducts the testing of ballast water

[SOURCE: IEC 62507-1:2010, 3.17]

**3.33**

**volumetric flow rate**

volume of fluid passing per unit time, calculated from *flow velocity* (3.13) and cross section area

Note 1 to entry: In ballast water operations, this parameter is typically expressed in cubic metres per hour.

Note 2 to entry: In SI units, this parameter is expressed in cubic metres per second.

## 4 Sample collection process

### 4.1 General

The sampling team shall conduct shipboard sampling operations appropriate to the ship, its ballast system, and discharge procedures in accordance with IMO sampling guidance MEPC.173(58), Guidelines for Ballast Water Sampling (G2). Prior to sampling, essential information shall be collected to choose sampling equipment appropriate for the anticipated ballast discharge; ideally, this is done prior to boarding the ship, but this may not always be possible. Consultation and coordination with the ship's crew are necessary to understand ballast water management system (BWMS) operations, determine ballasting parameters, identify sampling locations (typically in a machinery space), install or remove the sampling equipment, and coordinate the start and stop of sampling during the ballast operations. Sampling parameters are dictated by the measurement requirements. For example, the timing for a type approval sampling event may be conducted over an entire discharge of a specific ballast tank, while collection of an indicative compliance sample may occur over a specified number of minutes from a tank of convenience.

In practice, activities of the sampling team shall be coordinated with the actions of the ship and crew. This is necessary both for safety and to schedule the collection and processing of ballast water samples. In preparation for sampling, the sample probe shall be installed in the shipboard sample port and connected to the sample collection apparatus. Similarly, the return port when present, is connected as needed. Selection of the sample probe and other sample collection apparatus appropriate for the ship discharge is determined by the sample collection team according to the guidance in this document, and the timing of sample collection is determined by the measurement requirements, as described in [4.3.2](#), for the ballast discharge.

Certain information regarding the ship's ballasting and ballast water management system is required by the sampling team in advance of the sampling event in order to determine the appropriate safety procedures, materials, equipment, and sample collection parameters. A sampling requirements worksheet is provided in [Annex B](#) to facilitate documenting this information. However, sampling teams requires additional information not identified in this document, so they shall also address logistics for ship access that are outside the scope of this document.

In general, the sampling process includes the following.

- a) Installation of an appropriately sized sample probe into the ship's sample port.
- b) Connection of the sample collection device, typically an open or closed sample system which can collect large volumes ( $\geq 1 \text{ m}^3$ ) of filtered water to analyse organisms  $\geq 50 \text{ }\mu\text{m}$ , and/or smaller volumes of whole water ( $\geq 10 \text{ l}$ ) to analyse smaller organisms.
- c) Preparation of rinse water (microfiltered, free of organisms in the size class(es) of interest) from the ship's ballast water.
- d) Collection of ballast water samples over a duration appropriate to the measurement requirements and ship operations.
- e) Processing of collected samples.
- f) Transport of samples for off-ship analyses, if necessary.
- g) Disassembling, removing, and cleaning of sampling apparatus from the ship.

A minimum volume of  $3 \text{ m}^3$  of filtered water to analyse organisms  $\geq 50 \text{ }\mu\text{m}$  is recommended; this volume is consistent with the requirements of BWMS type approval testing<sup>[2]</sup>.

## 4.2 Fundamental principles

### 4.2.1 General

At all times, the sampling team shall consider safety of personnel and equipment when conducting shipboard operations. Any actions that require access or interaction with the ship's ballast system shall be performed in consultation with the ship's crew and in accordance with ship policy. Only the ship's crew or their delegate shall operate the ballast system or ship's equipment (e.g. electrical supply, bilges, pneumatic supplies).

The purpose and required statistical confidence of measurements dictate the sample collection timing (e.g. beginning, middle, end of the discharge), duration (i.e. collection time), volume collected, and volume analysed. Thus, these measurement requirements shall be defined by the sampling team for each sampling event; this document defines the necessary parameters but does not specify their values.

Sample collection apparatus and handling procedures shall be designed to collect representative samples under fully turbulent flow conditions and shall minimize effects on organisms and the potential for contamination. Sample probes shall be cleaned according to standard operating procedures (SOPs) and verified free of visual foreign matter prior to installation. The probes may be installed for the duration of the test cycle but should be removed for cleaning after no more than one week. Semi-permanent or permanent sample probes shall not be used for sample collection.

Flow measurement is required for both the ballast discharge flow and the sample collection flow. As internal dimensions of the ballast discharge pipe are not readily observable, measuring the flow velocity near the sample probe is preferred. Measurement using the ship's flow meter, the BWMS flow meter, or other direct measurement in a section of ballast discharge pipe with the same diameter and flow stream (e.g. at the return port) is also acceptable. Electronic logging of flow data are preferred to logging measurements by hand. Flow measurement device(s) shall be calibrated following the manufacturer's requirements and in accordance with the sampling team's protocols.

### 4.2.2 Sample collection flow, duration, and volume

The requirements for organism density resolution and measurement uncertainty shall dictate the sample volumes to be collected. The sample collection duration shall also be based on the end use requirements of the organism density measurement (e.g. port state control, type approval, general compliance). The uncertainty of a single sample event should be considered, as multiple sample events can reduce measurement uncertainty. Best practices shall be employed to avoid sampling bias in the selection of sampling times and sample handling protocols. Note that flow velocity is independent of the size of the pipe (and thus facilitates comparisons between different pipe sizes), while volumetric flow rate incorporates the cross-sectional area of the pipe; either term may be used.

Ship ballast discharge operations can dictate the time available for sample collection and the ballast discharge flow rates. The ballast discharge flow rate, ballast discharge pipe inner diameter, sample volume, and sample collection duration are parameters necessary to determine the appropriate sample collection flow velocity. To ensure isokinetic to sub-isokinetic conditions, the sample flow velocity shall be between the range of 0,25 to 1 times the ballast discharge flow velocity. The details for expansion from the G2 guidelines of 0,5 to 1 times flow velocity are given in Reference [5]. Sample collection flow velocity shall not exceed  $3,0 \text{ m s}^{-1}$  and subsequent sample handling shall minimize any effect on organism viability.

The volume of the sample that is analysed may be less than the volume of sample collected; the analysis volume shall be based on the methodology employed and the required statistical confidence of the measurement (see 4.3.2).

## 4.3 Preparation

### 4.3.1 General

The sample collection team shall determine the measurement-specific information discussed in this subclause prior to boarding the ship. Information describing the installed BWMS and its treatment process shall also be identified prior to boarding the ship. Any requirements for personal protective equipment shall be in accordance with the ship and the sampling organization. The sample collection worksheet in [Annex B](#) can be helpful as a template for documenting the measurement-specific information discussed below.

### 4.3.2 Measurement requirements and purpose of the sample

Measurement requirements shall specify measurement uncertainty and thus the minimum volume of sample collected for a given analysis method. The duration of the sample collection shall also be specified. In general, detailed measurements require suitable volumes in order to resolve concentrations of sparsely distributed organisms; this depends on the discharge limits for the organism size class. The required volumes, therefore, depend upon the reporting limit and uncertainty and therefore vary with analysis objectives. Special requirements (e.g. sampling of discharges from a specific tank) shall be identified during preparation for the sample collection and analysis activities. The purpose of the sample (i.e. regulatory, compliance, type approval, self-monitoring) generally drives the requirements for measurement uncertainty and identifies the waters to be sampled.

### 4.3.3 Ship access and sampling facilities in machinery spaces

Access shall be arranged in accordance with the ship's requirements (and local maritime authorities if necessary). The sample collection team shall communicate the requirements for the transport of the equipment to the ship, and request information on the ship's ballast system. As sampling locations are in machinery spaces, for example, the engine room of the ship, sampling apparatus shall be designed to be carried by hand or assembled in place unless other arrangements are made in advance. The information requested from the ship should identify the following.

- Special safety requirements in the space where the sample port is located (e.g. explosion proof).
- Compliance of the sample port with ISO 11711-1 (thus is also compliant with the G2 guidelines of BWM Convention).
- Type of port configuration (in-line, 45°, or 90°).
- Availability of a nearby return port, or arrangements for disposal of sampled water (e.g. volume of 1 m<sup>3</sup>-3 m<sup>3</sup>).
- Make, model, treatment processes, holding time (as stipulated on the type approval certificate), and treatment rated capacity of ballast water management system(s).
- Locations and types of any chemical injection in the ballast system.
- Availability of a piping diagram (e.g. photograph of the piping diagram from the ship's operation, maintenance, and safety manual).
- Ship power specifications:
  - distance of available outlets from sampling location,
  - plug configuration,
  - voltage, frequency, and current ratings for available outlets.
- Bench/table space availability for sample processing and analysis.

Any information which cannot be obtained prior to boarding the ship shall be obtained from the crew as soon as possible after boarding.

#### 4.3.4 Coordination with the ship's crew

To facilitate ship access as well as installation and proper use of sampling equipment, the primary contact on the ship and the number and position of crew members needed shall be identified prior to the sampling event. Where no return port is available, the ship's crew should be informed of the volume of filtered sample water that needs removal to confirm the method of disposal. A coordination meeting between the sample collection team and designated ship crew contacts should occur immediately after boarding to review communication and safety protocols, accessible and non-accessible areas, explosion proof or hazardous spaces, and emergency procedures. Any ship crew concerns regarding sampling operations should be noted and addressed. Discussions with ship crew shall concern expected maximum and minimum flow rates, times for ballast discharge and, if needed, the nominal diameter or size of the ballast discharge pipe. Discussions may also include the BWMS on board; its control location; how it operates; standard/recent maintenance; discussion of logged data that can be useful to the sampling team; and identity of crew trained to operate the BWMS. Also, the sampling team should be aware of active substances (e.g. oxidants) and any hazards associated with the BWMS, including any neutralizer, so that they can use the appropriate personal protective equipment.

#### 4.4 Maintenance of sampling apparatus

The sampling apparatus consists of sample probes, sample collection devices, interconnecting hoses or piping, equipment used to prepare filtered water for rinsing and dilution, and any support equipment that are used for measurements made on the acquired sample. All such apparatus shall be maintained in proper working order and properly cleaned to prevent contamination of the collected samples. The sample collection team shall have and follow SOPs for the operation, maintenance, cleaning, and inspection of the sampling apparatus specific to their organization and equipment.

#### 4.5 Quality management

The testing organization, including the sample collection team, shall conduct sampling and any subsequent analysis in accordance with a rigorous quality management programme. The testing organization shall operate in accordance with ISO/IEC 17025 or another standard acceptable to the Administration or contracting body. The quality management programme documentation shall consist of:

- a) A quality management plan (QMP) or equivalent, which addresses the quality control management structure and policies of the testing organization (including subcontractors and outside laboratories).
- b) A quality assurance project plan (QAPP) or equivalent, which defines methods, procedures, and quality assurance/quality control (QA/QC) protocols used by the sample collection and analysis team. It identifies the team members, and it includes all relevant SOPs, typically as appendices.

The inclusion of a test quality assurance plan (TQAP) or equivalent that is specific to the ship is optional but can be mandatory in some cases (e.g. type approval testing).

### 5 Sample probe

#### 5.1 General

The design of the sample probe, which includes the operational flow rates and geometrical shape, can affect sampling efficiency. A sample probe having an opening facing into the flow with isokinetic to sub-isokinetic flow velocities (i.e. sample probe flow velocities in the range of 0,25 to 1 times the flow velocity of the ballast discharge pipe) minimizes turbulence and shear forces near the probe opening and optimizes the capture efficiency of living organisms. The isokinetic diameter,  $D_{ISO}$ , for a sample

probe is specified as that diameter where, for a given volumetric sample flow rate, the flow velocity in the sample probe is equal to the flow velocity in the ballast discharge pipe.

## 5.2 Design of the sample probe

### 5.2.1 Sample probe sizing and flow rates

#### 5.2.1.1 General

The sample probe shall be sized to extract the volume of ballast water needed to achieve measurement requirements, within a specific timeframe, and at a flow velocity that is within the guidance range. For example, if 3 m<sup>3</sup> of ballast water shall be collected over a period of one hour, then the sample probe should be sized to support that demand while maintaining a sample flow velocity between 0,25 and 1 times the flow velocity of the ballast discharge pipe. There are three options for determining the appropriate flow rate for a particular sample probe size: using lookup tables; using the sample flow rate formula; and, measuring the flow velocity in the ballast discharge pipe.

#### 5.2.1.2 Option A: Lookup tables

Use the information in the lookup tables in [Annex C](#) to determine the appropriate range of sample flow rates for a given nominal sample probe diameter, main ballast diameter, and main ballast flow rate. The tables may also be used to select an appropriate sample probe size (based on corresponding flow rate) for a desired sample collection time. For parameters outside the scope of the table, proceed to option B ([5.2.1.3](#)).

For non-circular sample probes, the hydraulic diameter,  $D_h$ , should be calculated using [Formula \(1\)](#):

$$D_h = \frac{4A_c}{p} \quad (1)$$

where

$A_c$  is the cross sectional area of the pipe;

$p$  is the perimeter of the sample probe opening.

With the hydraulic diameter, the sample flow rate should be indexed to the nearest larger sample probe diameter.

#### 5.2.1.3 Option B: Sample flow rate formula

The appropriate maximum sample flow rate,  $Q_{ISO}$ , is dependent on the inner diameter of the ballast discharge pipe,  $D_m$ , the inner diameter of the sample probe,  $D_{ISO}$ , and the volumetric flow rate through the ballast discharge pipe,  $Q_m$ . It can be calculated using [Formula \(2\)](#):

$$Q_{ISO} = Q_m * \left( \frac{D_{ISO}}{D_m} \right)^2 \quad (2)$$

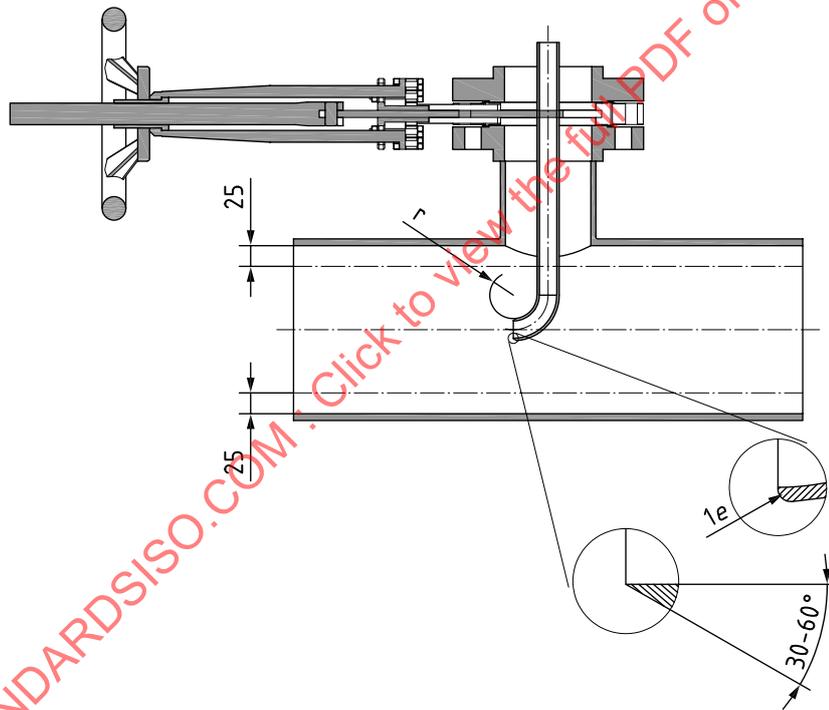
#### 5.2.1.4 Option C: Velocity based flow control

The sample flow velocity is controlled from direct measurement of the ballast discharge pipe flow velocity (e.g. m s<sup>-1</sup>). Here, the sample flow shall be maintained to the range of 0,25 to 1,0 times the main ballast discharge flow velocity.

5.2.2 Sample probe geometry

The sample probe shall be designed such that its dimensions allow it to physically fit within the sample port dimensions specified in ISO 11711-1 and meet the design requirements to optimize capture efficiency. For all three sample port configurations of ISO 11711-1, the installed sample probe opening shall be normal to the ballast flow (Figures 2 to 4). Any transition in the direction of flow should be curved (i.e. a smooth sample probe bend radius and not an abrupt angle), and the cross-sectional area of the probe interior shall never be less than that of the probe opening; it may increase in area. For 90° and 45° probe configurations, the sample probe insertion length shall be such that the sample probe opening shall be as close as possible to the centre of the ballast discharge pipe in the region of fully turbulent flow (i.e. not located in the boundary layer). Thus, the entirety of the probe entrance shall be no closer than 25 mm from the inside wall of the ballast discharge pipe, based on a maximum flow rate of 3 m s<sup>-1</sup>. For in-line sample probe configurations, the position of the sample probe opening shall be greater than two times the ballast discharge pipe diameter from the ballast discharge pipe centreline (Figure 4). The sample probe shall have a leading-edge chamfer between 30°-60° or have a leading-edge radius of the probe wall thickness. Ideally, the sample probe includes an entrance length of one pipe diameter to provide a smooth flow transition. However, a full pipe diameter may not be easily achieved in larger probe diameters and still allow entrance through the sample port configuration of ISO 11711-1. Note that these illustrations are only one example of a probe design, other configurations are possible.

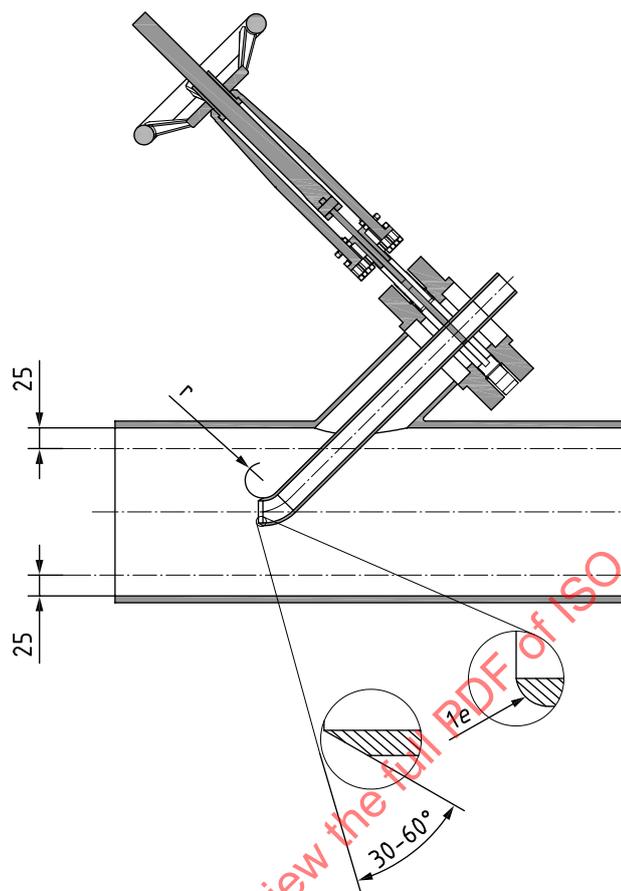
Dimensions in millimetres



Key

- r sample probe inner bend radius
- e sample probe wall thickness

Figure 2 — Sample probe inserted into ballast discharge pipe with a perpendicular (90°) port configuration

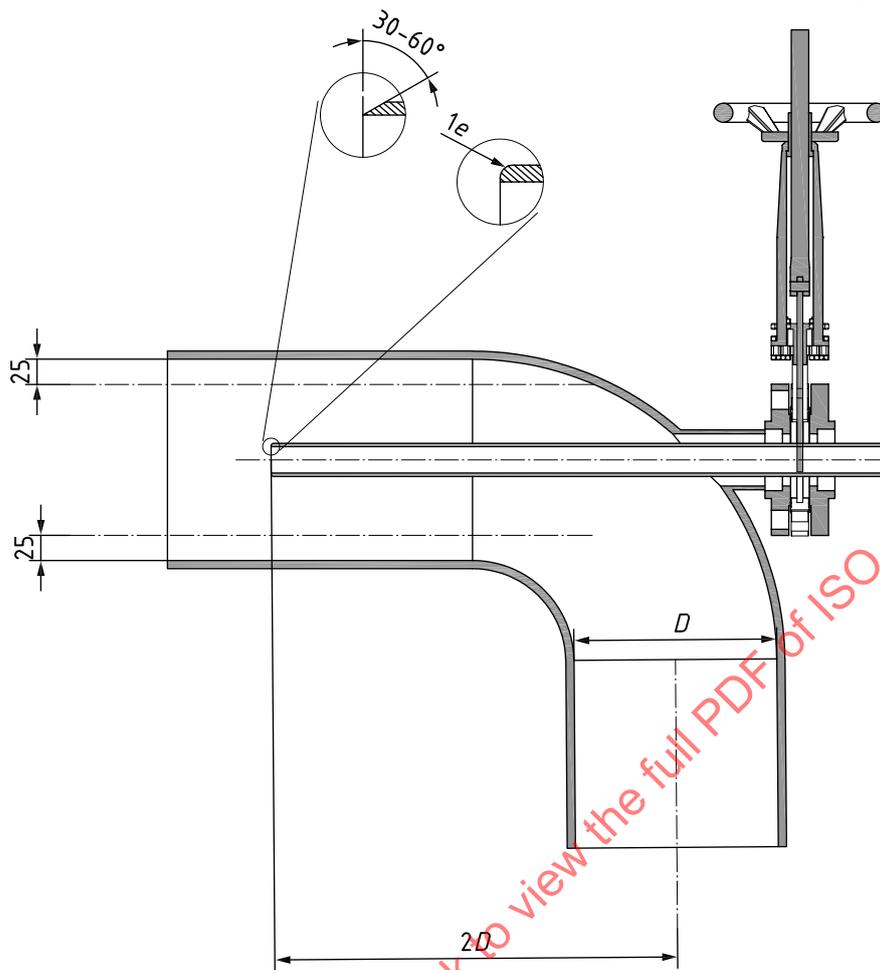


**Key**

- r sample probe inner bend radius
- e sample probe wall thickness

**Figure 3 — Sample probe inserted into ballast discharge pipe with a 45° probe configuration**

Dimensions in millimetres



**Key**

- $D$  ballast discharge pipe diameter
- $e$  sample probe wall thickness

**Figure 4 — Sample probe inserted into ballast discharge pipe with an in-line probe configuration**

**5.2.3 Sample probe structural design and materials**

The sample probe shall be structurally designed to withstand hydrodynamic forces from turbulent flows. The portion of the probe that extends outside the ballast discharge pipe and upstream from the sample probe isolation valve shall be designed to the maximum allowable working pressure (MAWP) of the ballast system, but no less than 1 MPa. Pipe strength requirements shall be compatible with the pipes to which they are attached, suitable for effective operation at MAWP, and in compliance with applicable classification society rules.

Materials shall be chosen that are non-toxic, resistant to seawater corrosion, and in compliance with applicable classification society rules and Administration regulations.

**5.2.4 Installation and removal considerations**

When installed for use, the sample probe should be inserted following the insertion length specified in 5.2.2 and oriented with the probe opening facing upstream, i.e. against the direction of the ballast discharge flow. The seal between working parts shall be at least 1,5 of the MAWP of the ballast system. The probe shall be removed after each test cycle and inspected for fouling, condition, and corrosion.

The sample probe should be cleaned and disinfected (referencing QAPP standard procedure) after the test cycle and again prior to the next test cycle.

### 5.3 Hot-tap sample probe assembly

#### 5.3.1 General

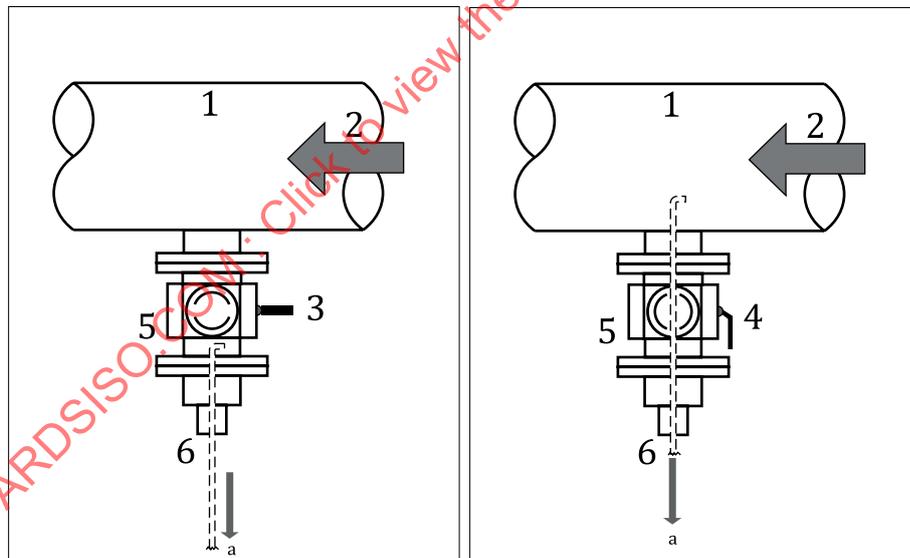
Design guidance and typical operation procedures for the hot-tap sample probe assembly are provided in 5.3.2 to 5.3.4.

#### 5.3.2 Configuration

A hot-tap sample probe assembly allows insertion of a sample probe into the ballast discharge pipe when the pipe is under pressure or filled with water. Such an assembly enables insertion of a sample probe into the main ballast water pipe without having to stop the main ballast water flow or to empty the pipe. For a hot-tap sample probe to be used, it needs to be able to:

- attach to the sample port valve while the sample port valve is closed;
- slide through the sample port valve when it is in fully opened position;
- prevent leakage and withstand pressure during insertion and sampling;
- withstand the anticipated stresses within the ballast discharge pipe.

Figure 5 shows the general principles of a hot-tap probe (not to scale).



#### Key

- |   |                           |   |                              |
|---|---------------------------|---|------------------------------|
| 1 | ballast discharge pipe    | 5 | sample port                  |
| 2 | ballast discharge flow    | 6 | sample probe                 |
| 3 | sample port valve, closed | a | To sample collection device. |
| 4 | sample port valve, open   |   |                              |

**Figure 5 — Hot-tap probe configuration before insertion with sample port valve closed (left) and after insertion with sample port valve open (right)**

### 5.3.3 Design criteria

- Materials shall meet the same requirements as all sample probes, i.e. they shall be corrosion resistant and non-toxic, see [5.2.3](#).
- Probe insertion depth and geometry shall meet the same requirements as all sample probes, i.e. obtain minimum distance from ballast discharge pipe wall in accordance with [5.2.2](#).
- The device shall be designed to a specific MAWP including the attachment to the sample port and the sample probe seals.
- The device shall undergo hydrostatic qualification to not less than 1,5 times its MAWP.
- The device shall be designed to fit through a sample port valve type and size as described in ISO 11711-1, where standard valve dimensions shall be in accordance with ISO 17602.
- Sample probe designed in accordance with [5.2.2](#) is able to fit through a fully opened ball valve or gate valve.
- The sample outlet of the device shall provide an isolation valve for the connection to the sample collection device.

### 5.3.4 Typical operations

Follow the manufacturer instructions for hot-tap sample probe operations. Typical insertion procedures include the following steps.

- a) Confirm that the ballast discharge pipe pressure is compatible with the hot-tap sample probe design.
- b) Ensure the sample port valve is fully closed and no sample probe is currently in place. (If a sample probe is currently installed, it shall be removed prior to installing the hot-tap probe. A previously installed probe that is not of a hot-tap design requires that the ballast pipe be isolated, depressurized, and potentially drained for removal; this shall be performed by the ships' crew).
- c) Remove the blind flange from the sample port valve, if one is in place.
- d) Install the hot-tap sample probe assembly to the sample port valve per the orientation in [Figure 5](#). Inspect and confirm that all sealing devices and associated fasteners are in place and secured. Inspect and confirm that the sample collection device isolation valve(s) are closed.
- e) Open the sample port valve and inspect for any water leaks.
- f) Slide the sample probe into the sampling port to its design length. Inspect and confirm the sample probe direction, i.e. with the opening facing into the ballast discharge flow.
- g) Make any additional adjustments as per the sample probe manufacturer instructions, such as further securing of sealing or locking devices.
- h) The hot-tap sample probe can then be connected to a sample collection device just like any other sample probe.
- i) Proceed with normal sampling. At the end of sampling, close any sample collection device isolation valves.
- j) Make any adjustments as per the sample probe manufacturer instructions, such as loosening seals or locking devices as needed to assist device removal.
- k) Withdraw the hot-tap sample probe and close the sample port valve.
- l) Disconnect and disassemble the sample probe as per the manufacturer's instructions.
- m) Reconnect the blind flange (if any).

## 6 Sample collection device

### 6.1 Initial considerations

#### 6.1.1 General

The sample collection device is used to extract ballast water samples from the sample probe through the sample transfer piping, for processing and subsequent analysis. The sample collection device shall be able to collect samples for the required size classes of organisms (e.g.  $\geq 50 \mu\text{m}$ ,  $\geq 10 \mu\text{m}$  and  $< 50 \mu\text{m}$ , and bacteria). Samples may be filter-concentrated, filtrate, or unprocessed whole-water. Sample water concentration can occur in open systems, which operate at ambient pressures, or closed systems, which can operate at the ballast discharge pipe pressure. The filtered water may be returned to the ballast discharge pipe (closed loop configuration) or to an unpressurized container such as the bilge (open loop configuration). [Annex A](#) provides illustrations for combinations of systems and loop configurations.

The sample collection device shall be set up with a method for controlling (e.g. sample flow control valve) and measuring (e.g. flow meter) the sample flow rate and volumes (e.g. flow totalizer) of samples collected. The device shall include a valve for isolating the sample (and return) flow from the main ballast flow. Some authorities can require multiple samples to increase confidence intervals. All wetted components of the sample collection device should be corrosion resistant and non-toxic (including to the sampled organisms).

#### 6.1.2 Open system

A sample collection device in an open system uses piping and equipment (e.g. collection apparatus) that are open to the ambient atmospheric pressure once the sample water leaves the sample transfer piping. One possible open system uses a plankton net with a cod end that is submerged in a collection container. The cod end should be configured such that the retained materials can be easily and completely rinsed into the collection container. An open system may be operated in either open loop or closed loop configuration.

#### 6.1.3 Closed system

A sample collection device in a closed system uses piping and equipment that are fully sealed such that they can operate at the ballast discharge piping pressure and are not open to ambient atmospheric pressure. Such apparatus commonly uses a filter installed inside of a sealed housing to concentrate organisms in ballast water. A closed system can be operated in either open loop or closed loop configuration.

#### 6.1.4 Open loop configuration

A sample collection device in an open loop configuration directs the filtrate water to the ship's bilge, other holding tanks, or another suitable location, e.g. overboard. In this case, crew should be consulted about the disposal of the filtered water in the planning stages for the sampling event.

#### 6.1.5 Closed loop configuration

A sample collection device in a closed loop configuration returns the filtrate water to the ballast discharge pipe without exposing it to ambient conditions. A closed loop configuration can require a booster pump or ballast discharge pipe restriction to create the differential pressure required to return water via the return port.

### 6.1.6 Concentration methods $\geq 50 \mu\text{m}$ (filtered samples)

#### 6.1.6.1 Plankton net concentration of organisms in the $\geq 50 \mu\text{m}$ size class

Plankton nets are used as means for concentrating a large-volume water sample ( $\geq 1 \text{ m}^3$ ). The plankton net (and cod end) should have no larger than  $35 \mu\text{m}$  nominal pore size. Organisms or other particles greater than the rated pore size of the plankton net are retained on the surface of the net. The sampling team SOPs should address qualification, maintenance, and wear limits of plankton nets. Sampling SOPs shall include a process to monitor flow through plankton nets to prevent clogging or damage to the net.

NOTE Mesh or pore sizes  $< 35 \mu\text{m}$  retain organisms that fall outside the  $\geq 50 \mu\text{m}$  size class, and they quickly clog.

#### 6.1.6.2 Filters used for concentration of organisms in the $\geq 50 \mu\text{m}$ size class

Filters are commonly used in the closed system defined above. The principal function of the filter is to provide a barrier for organisms  $\geq 50 \mu\text{m}$  to be collected on the surface of the filter so that they can be retrieved for analysis; thus, depth filtration is not recommended.

NOTE A nylon monofilament filter bag is an example of a filter type for installation within a filter housing to collect organisms on the interior surface of the bag. Another type is a stainless-steel mesh filter basket installed inside of a housing with pore openings to capture organisms of a given size. A third type incorporates pores made using a laser punch.

#### 6.1.6.3 Filter attributes

The conditions during filtration such as the shape of the organism or particle, the operating pressure, the concentration of particles, and other factors can have an effect on the retention of particles (including organisms) on the surface of the filter. There shall be an SOP for inspecting the filter(s) and housing(s) prior to use. Consider the following for the design of filters used to concentrate organisms.

- Proper fit and sealing of the filter to the filter housing to ensure there is no fluid bypass of the filter pores.
- Filter openings and pores are rated to retain objects  $\geq 50 \mu\text{m}$ . For woven mesh filters, nominal pore size should be  $35 \mu\text{m}$ . Absolute pore size of a filter can be quite difficult to measure accurately, thus the filter rating should be based on nominal pore size.
- In general, to reduce organism mortality from sheer stresses, the filter should be designed with an effective surface area to maintain a maximum filtration velocity of  $0,5 \text{ m s}^{-1}$ . The maximum differential pressure across the filter shall be defined during validation testing with organisms, and the sample operation shall remain below this limit. The mortality that can be caused by the filter and sample collection device should be estimated (e.g. through a validation study) and provided by the manufacturer of the filter and sample collection device, as appropriate.

### 6.1.7 Collection methods for whole water samples

The sample collection device shall be fitted with a means for obtaining a whole water sample (not concentrated). Because the sample from the sample probe is “representative” of the ballast discharge pipe, a separate sample probe that is built into the sample collection device upstream of the filter may be used but is not required. Alternatively, a side stream tap of the sample collection hose upstream of the filter can be used to direct whole water samples to a collection container. The sample water entering the collection container shall flow through a hose extending to the bottom of the container to minimize harm to organisms, and the method for collection shall follow a SOP validated by the sample collection team. There shall be a means for controlling the whole water flow rate (e.g. with a flow control valve, see [6.2.2.3](#)) and for measuring the volume of sample obtained. There shall be a SOP for sample container preparation and inspection prior to use. The following should be considered for the selection of sample collection containers:

- they are non-toxic;

- they are of autoclavable plastic construction (to avoid breakage), e.g. high density polyethylene or polypropylene;
- they provide instructions on filling the bottle (e.g. do not rinse, fill only to fill-line);
- they are pre-labelled to indicate e.g. sterility, to not discard neutralizer liquid, the target organisms ( $\geq 50 \mu\text{m}$ ,  $\geq 10 \mu\text{m}$  and  $< 50 \mu\text{m}$ , indicator microbes).

The sample collection team shall use their judgement and best practices to ensure the appropriate containers and proper neutralizer chemicals are used, consistent with their SOPs.

NOTE Some geometry considerations for the collection containers include:

- wide-mouth with leak-prevention features (ring seal or liner, continuous thread);
- clear or semi-transparent material with a clearly marked fill line;
- fill line marking near top to minimize headspace;
- sized to hold the entire sample volume and leave headspace for homogenization;
- rectangular for ease in packing multiple samples in an insulated box for storage and transport.

### 6.1.8 Sample retrieval and rinsing

Samples concentrated by filters use either an open system (e.g. through use of a plankton net) or closed system (the filter surface). Filtrate samples shall be retrieved prior to sample analysis, and this requires a rinsing operation. As such, the rinse water shall not introduce additional organisms to the sample and shall also not cause any adverse effects to the captured organisms in the filtrate (i.e. osmotic shock, physical trauma, toxicity). Temperature of rinse water shall be within 5 °C of the sample water. The following list provides options for making the rinse water.

#### a) Filtered ballast water used to rinse nets or filters

Rinse water prepared from ballast water shall be filtered to remove organisms, and when residual disinfectants remain from chemical treatment, the treatment shall be neutralized (see 7.3). Requirements to filter rinse water are defined as follows.

- For the  $\geq 50 \mu\text{m}$  size class: filter having nominal pore size of  $\leq 10 \mu\text{m}$ , or filtrate from the sample concentration process for this size class (e.g. water filtered by the plankton net with mesh  $\leq 35 \mu\text{m}$ ).
- For the  $\geq 10 \mu\text{m}$  and  $< 50 \mu\text{m}$  size class: filter having a nominal pore size of  $\leq 1 \mu\text{m}$ .
- Neutralizer, if used, shall be defined and not create any harmful effects to organisms.
- Water source shall be from tanks that are being sampled (may be filtrate from sample processing).
- Non-sterile containers shall be rinsed three times prior to filling when not using neutralizer; sterile bottles may be used without rinsing.

#### b) Synthetic water used to rinse nets or filters

Synthetic water is equipollent to rinse water prepared from ballast water. The following can be used:

- neutralized tap, potable, or deionized water;
- salinity adjusted with artificial sea salt to within 1 part per thousand (ppt) of the sample water;
- reference SOP for application of rinse water.

### 6.1.9 Sample volumes by size class

Three size classes are considered for collection; measurement requirements define the volumes sampled to achieve target uncertainties. These volumes may be processed during sample collection to reduce the volume handled during analysis as described in the list below and as summarized in [Table 1](#).

a)  $\geq 50 \mu\text{m}$  size class sample volumes

- The target sample volumes and duration shall be determined prior to starting a sampling operation, with a maximum collection time per sample to consider sampling bias according to sampling team SOPs; the minimum volume of water sent to the filter (or plankton net) shall be  $1 \text{ m}^3$ ; the recommended volume is at least  $3 \text{ m}^3$ .
- The sample volumes shall be sufficient for the type of analysis being conducted.
- The final concentrated sample volume to be transported to lab shall be 1 000 ml or as defined by the SOP (can be further concentrated during analysis).

b)  $\geq 10 \mu\text{m}$  and  $< 50 \mu\text{m}$  size class sample volumes

- The target sample volumes and duration shall be determined prior to starting a sampling operation, the volume of collected water shall be at least 10 l.
- Unfiltered whole water is recommended; a filtrate passing through a plankton net or filter may be used with validation.
- The sample volumes shall be sufficient for the type of analysis being conducted; the final sample volume to be transported to the lab shall be 1 000 ml.

c)  $< 10 \mu\text{m}$  (indicator microbes) sample volumes

- Unfiltered whole water is recommended; a filtrate passing through a plankton net or filter may be used with validation.
- It is not necessary to take an isokinetic sample.
- Minimum volumes:
  - Total volume sampled: 10 l; may be shared with volume collected in [6.1.9 b\)](#).
  - Final sample volume to be transported to the lab: at least 1 000 ml or as defined by SOP.

**Table 1 — Sample and deliverable volumes for each organism size class**

Size class	Sample volume (l)	Final volume (ml)
$\geq 50 \mu\text{m}$	$\geq 1\ 000$ , $\geq 3\ 000$ recommended	1 000
$\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	$\geq 10$	1 000
$< 10 \mu\text{m}$	$\geq 10$ (may be shared with $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ sample)	1 000

## 6.2 Sample collection device control system

### 6.2.1 General

Sampling shall be controlled to ensure that representative sampling is achieved, organism mortality is minimized, and critical parameters are recorded. Controls shall ensure:

- monitoring of differential pressure across filter (per above validation, [6.1.6.3](#));
- the required sample volume was collected (as can be determined mathematically from flow and time);

- the ballast discharge and sample flow rates remain within established ranges needed for representative sampling.

## 6.2.2 Parameters monitored

### 6.2.2.1 Open system parameters

The operation should be continuously monitored and managed to prevent clogging of the plankton net or filter. If a plankton net is used, ensure that the net does not overflow and that the majority of the net and cod end remain submerged during sample concentration. If the sensors are capable of logging data automatically, the log interval should be  $\leq 5$  s. Manually recorded data values should be logged at  $\leq 5$  min intervals. Parameters logged shall include:

- sample flow (volumetric or flow velocity);
- time of sample collection (start, end);
- sample volume (flow totalizer or by recording the end volume within the sample bin);
- ballast discharge flow (units consistent with sample flow).

### 6.2.2.2 Closed system parameters

The operation should be continuously monitored and managed to ensure differential pressure does not exceed and the sample flow rate remains within operational requirements. If the sensors are capable of logging data electronically, the log interval should be  $\leq 5$  s. Manually recorded data values should be logged at  $\leq 5$  min intervals. Parameters logged shall include:

- sample flow (volumetric or flow velocity);
- differential pressure across the filter;
- time of sample collection (start, end);
- sample volume (flow totalizer);
- ballast discharge flow (units consistent with sample flow).

### 6.2.2.3 Flow control

The sample collection device shall provide both an isolation valve and a sample flow control valve. The isolation valve shall allow the sample collection device to be isolated from the main ballast piping and shall be operated in either the fully open or fully closed position; the valve position shall be visibly indicated. The sample flow control valve is used to ensure representative sampling is conducted at the isokinetic to sub-isokinetic flow velocities as defined by the measurement requirements. A sample flow control valve located upstream of a concentrating filter or plankton net shall be a diaphragm type valve to minimize organism mortality.

Monitoring of sample flow shall utilize an in-line flow meter. Flow meters shall have an averaging of no more than 5 s. Those installed upstream of a concentrating filter or plankton net shall be of a non-contact type (e.g. magmeter). Open systems shall locate the flow meter upstream of the plankton net or filter, while closed systems may monitor flow either upstream or downstream of the concentrating filter.

The motive force for the sample flow may be provided by pressure differential or by a sample pump. Only a diaphragm, positive displacement pump, or other type validated to minimize organism mortality, may be installed upstream of a concentrating filter or plankton net. Suitable piping or hoses shall be used to return flow to the ballast piping return port or other suitable location.

#### 6.2.2.4 Main ballast flow rate

The following should be considered for obtaining the main ballast flow rate:

- the ballast water management system flow meter can be observed to obtain the main ballast flow rate;
- a sample probe mounted paddle wheel may be used;
- the sampling team can supply an external flow meter (e.g. ultrasonic) and use it to obtain the ballast flow rate (follow manufacturer specifications on where/how it is installed);
- the ship's ballast control system may be used to supply ballast flow data.

The sampling team should understand and describe the limitations (e.g. min/max flow capacity) to account for uncertainties of the main ballast flow measurement technique and any impact on representativeness of the sampling event.

### 6.3 Sample transfer piping

Sample transfer piping connects the sample probe to the sample collection device, and, in some cases, returns the processed water to the ballast discharge pipe. Sample transfer piping may include both hard piping and hoses. A flow meter installed in the transfer piping shall be installed according to the manufacturer's instructions. The following shall be considered.

- All connections, plumbing and hoses shall be compatible with ballast water and should have a pressure rating that follows applicable ship classification society rules.
- The connection between the sample probe and the sample collection device isolation valve shall be hard piped (rather than connected via hose). Hose may be used downstream of the isolation valve.
- All wetted parts for sample collection (e.g. sample probe, filter, sample container) shall be made from materials that are not toxic to the organisms of interest (e.g. copper alloys or polyurethanes should be avoided).
- The diameter of transfer piping (or hoses) in areas upstream of the sample collection device should be equal to or greater than the nominal diameter of the sample probe to prevent increases in flow velocity ahead of the sample collection device.
- The transfer piping shall be fitted with a means for cleaning, inspecting, and flushing with process water before use, and this process should be well practiced and verifiable by the sampling team prior to sampling. The transfer piping shall be checked for leaks during this operation, and all leaks stopped prior to the sampling operation in accordance with SOPs.
- Transfer piping shall have an air bleed located at the high point(s) in the system (closed system).
- The sample probe shall be isolated from the sample collection device (and transfer piping) by means of a valve that indicates its position (open/closed).
- Connections to the return port shall be isolated from the sample collection device and transfer piping by means of a valve that indicates its position (open/closed).
- The sample transfer piping shall be as short as possible and obstruct pathways or walk ways to the smallest degree possible. Anyone in the area of sampling should be alerted to the ongoing operations and to possible trip hazards.
- Appropriately sized gaskets and gasket materials should be used in instances where gaskets are needed (i.e. flange connections). The hardware for assembling flanges to the sampling device and sample transfer hoses (bolts, washers and nuts) should be brought on board by the sampling team and should be the appropriate size to match the flanges used in the sample transfer piping (i.e. the diameter and length of the bolt should be correct for the flange pressure rating).

- The use of threaded fittings should be avoided because of their susceptibility to leaking, but, if used, they shall have the proper thread sealants applied.
- If hoses are used in the sample transfer piping, they should attach to the pipe sections through the use of proper hose fittings.

## 7 Handling and identification of the sample

### 7.1 Sample handling

General sample handling procedures shall be conducted as specified in ISO 5667-3, which provides details on sample containers, identification, preservation of samples for biological evaluation, sample transport and storage.

The following material are required:

- pre-labelled sample containers;
- pen or marker;
- chain-of-custody (CoC) forms;
- disposable gloves;
- waste bucket;
- lint-free, disposable wipes (e.g. Kimwipes®<sup>1)</sup>);
- alcohol wipes [70 % isopropyl alcohol (IPA), or equivalent];
- insulated container;
- frozen icepacks;
- packaging material.

### 7.2 Sample identification

Sample containers shall be pre-labelled with laboratory tape, label gun tape, or waterproof stickers. To facilitate handling and processing:

- a) tape and labels shall be adhesive-backed and not easily or accidentally removed;
- b) tape and label surfaces shall be markable with a pen or marker and resist ink smearing and bleeding;
- c) the sample record shall include the following data to be recorded at the time of sampling:
  - 1) sample identification code and date,
  - 2) sampling start and end times,
  - 3) source (ship name and IMO number),
  - 4) sample type ( $\geq 10 \mu\text{m}$  and  $< 50 \mu\text{m}$ ,  $\geq 50 \mu\text{m}$ , or indicator microbes),
  - 5) volume,
  - 6) concentration factor,

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1) Kimwipes® are an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

- 7) identification of sampling personnel,
  - 8) residual chemical signature [i.e. if residual total residual oxidant (TRO) was measured],
- d) the sample label shall have all prompts for all critical data to be recorded at the time of sampling including:
- 1) sample identification code and date,
  - 2) source (ship name and IMO number),
  - 3) sample type ( $\geq 10 \mu\text{m}$  and  $< 50 \mu\text{m}$ ,  $\geq 50 \mu\text{m}$ , or indicator microbes),
  - 4) concentration factor;
- e) sterilized sample containers should be marked (e.g. with autoclave tape) to indicate sterility;
- f) if a neutralization agent is pre-added (see 7.3), its identity, concentrations, volume, and expiration date should be indicated on the sample label.

### 7.3 Neutralization agents

A neutralization agent, such as sodium thiosulfate, may be pre-added to the sample containers as a concentrated solution. This step is relevant for samples where TRO is measured. The neutralization agent stops the biocidal or biostatic activity of the residual oxidants.

Neutralization agents may be pre-added to an empty, cleaned sample container, but should not exceed 10 % of the sample volume. The neutralization agent should be filter- or heat-sterilized for bacterial samples. The neutralization agent should be appropriate for the chemical being neutralized.

The following shall be considered related to the neutralizer pre-dosed in collection containers:

- a neutralizer may be included;
- the residual chemical dose shall be measured and recorded (i.e. measure the residual TRO of a sodium hypochlorite system);
- the neutralizer shall only be used in sampling of systems where a residual chemical may be present that can affect organism mortality within the sample (i.e. oxidant type BWMS);
- the neutralizer chemical shall be appropriate to the type of chemical reaction being applied to the ballast water (e.g. sodium thiosulphate in a sodium hypochlorite BWMS treatment operation);
- the concentration of neutralizer shall be set up such that it does not cause adverse treatment effects to the organisms within the sample (shall determine concentration and cite references for how neutralizer chemicals do not hurt organisms).

### 7.4 Filling the container

Whole water samples from the sample collection device are likely be collected into a container at a valve spigot or a connected hose. The exterior section of the spigot or hose that can come into contact with the sample container shall be wiped with an alcohol wipe (70 % IPA or equivalent) to minimize bacterial contamination, should the spigot or surface contact the inside of the container.

Prior to sample collection, the sample flow rate shall be adjusted to the target value per the measurement requirement (4.3.2). The sample stream should be diverted to a waste bucket, with a minimum of 1 l volume and duration of 2-min sample flow. Sample containers may be filled once the flow rate is set and at least 1 l is discharged.

To reduce the risk of sample contamination, the containers shall be:

- kept closed until the time of sampling;

- opened only during the duration of filling.

Personnel handling the sample shall wear disposable gloves. For bacteria samples, personnel shall wipe the gloves with an alcohol wipe (70 % IPA or equivalent), allowing the liquid to evaporate prior to opening the container. The sample container shall be opened and placed into the flow stream, such that:

- sample water flows down the sides of the container, held at a slight angle;
- fluid agitation (caused by splashing and bubbling) is minimized.

The filling procedure can affect the gas exchange with the atmosphere and keeping the fluid stream intact and directed along the inside surfaces of the container reduces gas exchange and aerosol formation.

Once the sample level reaches the fill line, the sample flow shall be stopped, and the container removed from the flow stream, capped and dried with a lint-free wipe. The analyst shall note the sample start and end times on the container and record other information as needed in sample logs. Multiple bottles may be filled sequentially from the flowing water, but only one sample container shall be opened at a time.

Directly sampling the flow stream is recommended, but it may be necessary to collect the sample in a secondary vessel, such as a beaker or graduated cylinder, prior to filling the sample container. To maintain sample integrity, all materials in contact with the sample water shall be well-cleaned, sterilized (for bacterial samples), and rinsed three times with sample water. Rinsing can be performed for a partially filled vessel by tilting and rotating the vessel. Transfer of fluids shall minimize contamination and gas exchange (as above). If only a portion of entire volume of the vessel is transferred into the sample container, the vessel water shall be homogenized prior to filling the sample containers. This can be performed by covering, then inverting the vessel; swirling the vessel; or mixing the water with a clean and sterile bar.

## 7.5 Sample storage

Filled sample containers shall be checked to ensure that the labelling and identification is complete and correct. If sample containers may be inverted during storage or transport, the neck and cap of the vessel shall be sealed with Parafilm®<sup>2)</sup> wax or equivalent, pulled tightly around the circumference of the gap between the neck and the cap of the vessel.

Sample containers shall be stored in an insulated box to minimize temperature fluctuations during storage and transport. Icepacks are used to keep the insulated container temperature fluctuations less than ambient temperatures, as insulated container temperatures generally slow biological activities. Internal temperatures shall be maintained in accordance with the sampling team's SOPs. Samples shall be placed upright and secured from movement within the insulated container by placing icepacks or packaging material in the open areas. The time to transport and hold samples prior to analysis shall ensure analysis occurs within the sample hold time specified by the SOP.

## 7.6 Chain-of-custody

A chain-of-custody (CoC) form shall be included with each set of samples and for each insulated container containing samples. The CoC form identifies the samples within the containers and provides basic information about the samples (number of containers, volume, analysis, sample location, date and time). The sampling personnel shall verify the information is complete and correct, then sign the CoC forms (one is included in the sample container; a duplicate is for records). The personnel accepting the CoC form, if different than the initiator of the form, shall verify its information then sign the document.

---

2) Parafilm® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

## 7.7 Transport

Insulated boxes for sample containers shall be closed and sealed during transport, and labelled with information including: the contact information for the project personnel, the nature of the samples (“shipboard water samples”), and any notifications required by shipboard or port authorities. The insulated container shall also indicate that it is “not for food” and “not harmful chemicals”. The person in custody of the samples shall keep them secured in the insulated container, which shall be kept out of direct sunlight and temperature extremes. Transport to the receiving facility for analysis shall be direct, if possible. The receiving facility or analyst shall be alerted to the estimated time of arrival and the contents of the insulated container. Upon receipt, the receiving personnel shall review the CoC form, verify its information, then sign the form.

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## Annex A (informative)

### Example configurations of sample collection devices and ballast piping connections

This annex illustrates possible configurations of the sample collection devices discussed in [6.1](#):

a) Open system

The sample water in an open system ([Figure A.1](#) and [Figure A.3](#)) is exposed to the ambient (atmospheric) pressure conditions after leaving the sample transfer piping, such as when concentrating organisms in a plankton net.

b) Closed system

The sample water in a closed system ([Figure A.2](#) and [Figure A.4](#)) is fully contained within the sample collection device and interconnecting piping and typically operates at higher than atmospheric pressure. Such apparatus commonly uses a filter installed inside of a sealed housing to concentrate organisms in ballast water.

c) Open loop configuration

An open loop configuration ([Figure A.1](#) and [Figure A.2](#)) directs the water leaving the sample collection device (filtrate) to the ship's bilge, other holding tanks, or another suitable location, e.g. overboard. In this case, crew should be consulted about the disposal of the filtrate in the planning stages for the sampling event.

d) Closed loop configuration

A closed loop configuration ([Figure A.3](#) and [Figure A.4](#)) returns the filtrate water to the ballast discharge pipe via closed piping. A closed loop configuration may require a booster pump or ballast discharge pipe restriction to create the differential pressure required to return water via the return port.

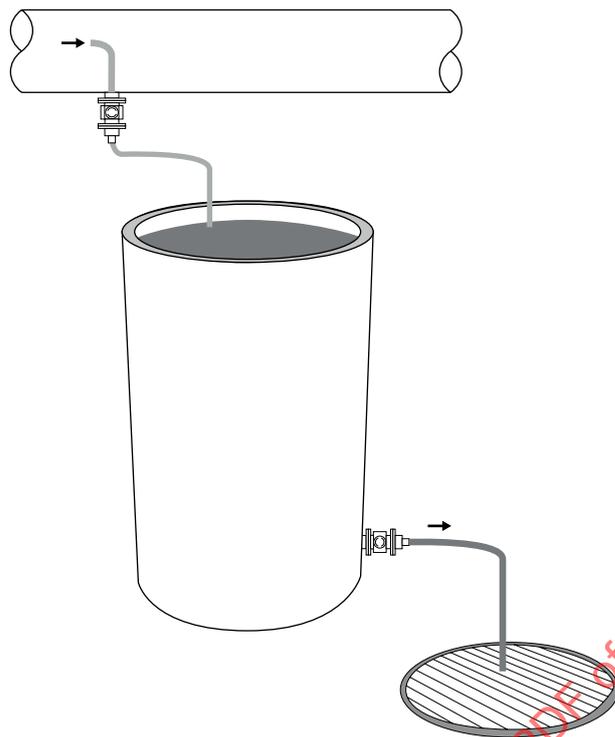


Figure A.1 — Open system with disposal of filtrate to ship drain via open loop configuration

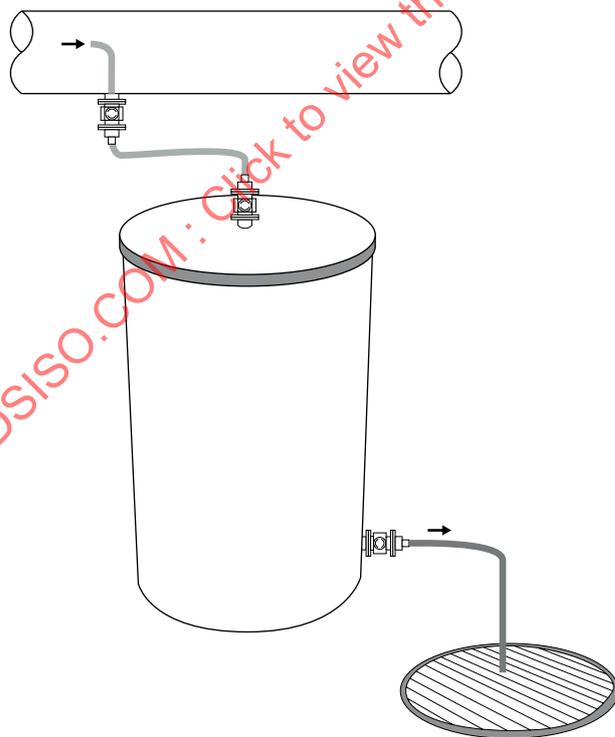


Figure A.2 — Closed system with disposal of filtrate to ship drain via open loop configuration

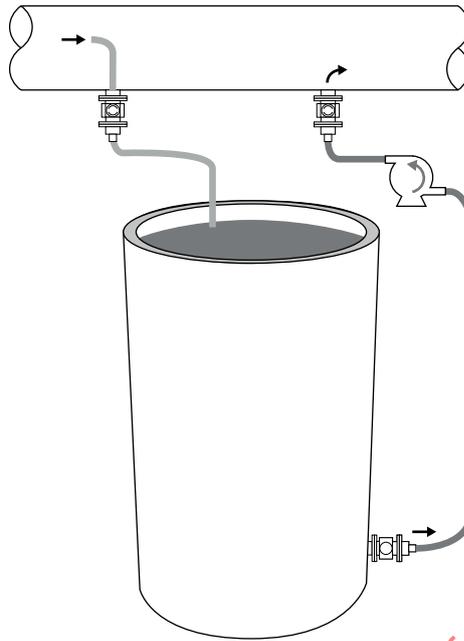


Figure A.3 — Open system with filtrate pumped back to ballast discharge line via closed loop configuration

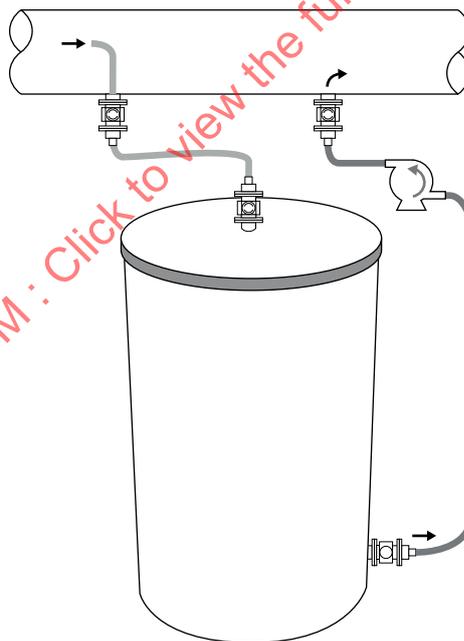


Figure A.4 — Closed system with filtrate pumped back to ballast discharge line via closed loop configuration

## Annex B (informative)

### Shipboard sample collection worksheet

This annex provides a template for recording the information necessary in shipboard sample collection.

#### Shipboard sample collection worksheet

#### ----- SHIP AND PERSONNEL -----

Name and IMO number of ship:	Sample label code for ship:
End use of data (e.g. PSC, ship/owner, Research, type approval, other (specify):	Location of ship:
Sampling organization:	Sample collection dates:
Sampling team lead:	Email:
Office phone:	Mobile phone:
Sampling team members:	

#### Ship crew

Ballast engineer/POC:	
Chief mate:	
Other (ID role):	

POC = Point of contact; PSC = Port State Control.

**SHIP BALLAST WATER MANAGEMENT SYSTEM**

BWMS manufacturer:	Type approval Administration, certificate no.
BWMS model:	<input type="checkbox"/> Filter <input type="checkbox"/> EC <input type="checkbox"/> UV <input type="checkbox"/> Other treatment:
Ballast pipe nominal diameter:	<input type="checkbox"/> mm <input type="checkbox"/> inch
Sample port location:	<input type="checkbox"/> in-line <input type="checkbox"/> 45° <input type="checkbox"/> 90° <input type="checkbox"/> ISO 11711-1 compliant <input type="checkbox"/> Entry required to explosion proof area
Return port location:	
Distance to nearest power outlet:	
voltage / current / frequency (Hz or DC):	<input type="checkbox"/> 3 phase

EC = Electrochlorination, UV = Ultraviolet

**Required personal protective equipment (PPE)**

hard hat    steel toe shoes    hearing protection    safety glasses

\_\_\_\_\_    \_\_\_\_\_

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-----MEASUREMENT REQUIREMENTS-----

**ORGANISM (org) ANALYSES BY SIZE CLASS**

≥50 µm	<input type="checkbox"/> N/R <input type="checkbox"/> <10 org m <sup>-3</sup> <input type="checkbox"/> ≥10 org m <sup>-3</sup> <input type="checkbox"/> Taxonomy <input type="checkbox"/> (Specify):
≥10 µm and <50 µm	<input type="checkbox"/> N/R <input type="checkbox"/> <10 org ml <sup>-1</sup> <input type="checkbox"/> ≥10 org ml <sup>-1</sup> <input type="checkbox"/> Taxonomy <input type="checkbox"/> MPN <input type="checkbox"/> PAM <input type="checkbox"/> Chlorophyll a <input type="checkbox"/> mortal stain: <input type="checkbox"/> vital stain: <input type="checkbox"/> (Specify):
Indicator microbes	<input type="checkbox"/> N/R <input type="checkbox"/> HPC <input type="checkbox"/> (Specify): <input type="checkbox"/> vibrio method: <input type="checkbox"/> E. coli method: <input type="checkbox"/> Enterococci method: <input type="checkbox"/> (Specify):
Neutralization	<input type="checkbox"/> N/R <input type="checkbox"/> Chemical and dose:
Other tests or specific analytical requirements	(Specify):

N/R = Not required; MPN = Most probable number; PAM = Pulse amplitude modulation; HPC = Heterotrophic plate count.

**WATER CHEMISTRY ANALYSES**

- DOC  POC  TOC  TSS
- Temperature  pH  Salinity
- \_\_\_\_\_
- \_\_\_\_\_

**Other tests or specific analytical requirements (specify)**

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----- **SAMPLING REQUIREMENTS** -----

Duration of sample collection: Minimum \_\_\_\_\_ (min) Maximum \_\_\_\_\_ (max)

Expected discharge flow rate (min/max): \_\_\_\_\_ / \_\_\_\_\_ (m<sup>3</sup> h<sup>-1</sup>)

Total volume to be discharged: \_\_\_\_\_ (m<sup>3</sup>)

Minimum volume to be sampled: \_\_\_\_\_ (m<sup>3</sup>)

Sample discharge from specific ballast tank. Tank ID(s): \_\_\_\_\_  
 --

Uptake location of ballast water (specify or N/A): \_\_\_\_\_  
 ----

Sample type (X out box if not required)	Minimum collection volume	Number of replicate samples	Minimum number subsamples to be analysed per replicate
<input type="checkbox"/> ≥50 µm	(m <sup>3</sup> )		
<input type="checkbox"/> ≥10 µm and <50 µm	(l)		
<input type="checkbox"/> indicator microbes <sup>a</sup>	(l)		
<input type="checkbox"/> water chemistry <sup>a</sup>	(l)		
<sup>a</sup> Indicate "WWSS" (for whole water sub-sample) if indicator microbe and water chemistry samples are to be subsampled from a ≥10 µm and <50 µm whole water sample volume.			

**Other sampling requirements or information (specify)**

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**SAMPLE COLLECTION EQUIPMENT**

- Sample probe inner diameter: \_\_\_\_\_  mm
- Sample probe serial number: \_\_\_\_\_  clean for use
- Sample hose serial number: \_\_\_\_\_  clean for use
- Sample collection device serial number: \_\_\_\_\_  clean for use
- Sample collection filter bag/net serial number: \_\_\_\_\_  clean for use
- Isokinetic sample probe flow at maximum ballast rate: \_\_\_\_\_  m·s<sup>-1</sup>  l·min<sup>-1</sup>
- Isokinetic sample probe flow at minimum ballast rate: \_\_\_\_\_  m·s<sup>-1</sup>  l·min<sup>-1</sup>
- 0,25 × isokinetic sample probe flow at minimum ballast rate: \_\_\_\_\_  m·s<sup>-1</sup>  l·min<sup>-1</sup>
- Target minimum sample probe flow: \_\_\_\_\_  m·s<sup>-1</sup>  l·min<sup>-1</sup>
- Target maximum sample probe flow: \_\_\_\_\_  m·s<sup>-1</sup>  l·min<sup>-1</sup>
- Target collection time: \_\_\_\_\_ min
- Other equipment identifiers or information (specify)**

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