Global TestNet Member‘s Methodology Comparison Charts
June 2025

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## 4. Abbreviations

| | |
|---|---|
| **BWTS/BWMS**: | Ballast water management System / Ballast water treatment system |
| **Conc**: | Concentrated |
| **DOC:** | Dissolved Organic Carbon |
| **NC / C:** | No Concentration / Concentration |
| **NR:** | Neutral Red |
| **OET:** | Entire Experimental Time (=entire discharge time / pumping time) |
| **POC:** | Particulate Organic Carbon |
| **Temp:** | Temperature |
| **TSS**: | Total Suspended Solids |

# 5. Background

## 5.1. Introduction

With our aim to promote comparable and accurate test results on the performance evaluation of technologies and methodologies to control the risk of bio-invasion and harmful species introductions by shipping, Global TestNet members have been sharing information on their testing approach and made these available to public. The present set of information was first compiled after the Annual meeting in Istanbul in 2012 (“the Istanbul paper”) and has been updated over the course of the Global TestNet successive annual meetings and internal surveys. Improvement of methodologies applied by members as well as the development of new regulations has promoted an increased transparency in the testing protocols and the organisation is confident that this may be considered as “best practices” in ballast water - sampling and testing.

## 5.2. Documentation regulating testing of ballast water management systems

The testing carried out by the members is done according to the following documentation:

- Guidelines for Approval of Ballast Water Management Systems (G8). Res. MEPC.125(53); Res. MEPC.174(58), MEPC.279(70)
- Code for Approval of Ballast Water Management Systems (BWMS CODE) Res. MEPC.300(72)
- Procedure for Approval of Ballast Water Management Systems that Make Use of Active Substances (G9). Res. MEPC. 169(57)
- U.S. Coast Guard. Standards for Living Organisms in Ships’ Ballast Water Discharged in U.S. Waters. 33 CFR Part 151 and 46 CFR Part 162.
- U.S. Environmental Protection Agency, Environmental Technology Verification Program. Generic Protocol for the Verification of Ballast Water Treatment Technology. EPA/600/R-10/146.
- BWM2/Circ.70. (as revised) Guidance for the commissioning testing of ballast water management systems (IMO)
- Guidelines for ballast water sampling (G2) Res. MEPC.173(58)

## 5.3. Test Organisations

Test facilities active in the Global TestNet have evolved and changed overtime and for this reason only the information from active facilities is included here (e.g. NIOZ & GCDC integrated into Control Union, closing of DHI Singapore). Active testing organizations are listed on Global TestNet website. Organization may be active worldwide but only reference to the country of their headquarter in noted (CN=China, DE=Germany, DK = Denmark, JP=Japan; NL=The Netherlands, NO=Norway, RoK=Republic of Korea, CH = Switzerland, TUR=Türkiye, USA=United States of Americas, UK=United Kingdom)

# 6. Sampling for Commissioning and Compliance Testing

**Table 1: Sampling details, commissioning tests for the discharge of treated water, organisms > 50 micron, 10 – 50 micron, and <10 micron**

| Testing organisation | Sampling Approach (OET or Sequences) | Sampling Equipment (open nets or closed sampler) | Flow meter position (before or after sampling) | Duration of Sample Collection | Total Volume Sampled (>50um) |
|---|---|---|---|---|---|
| Ankron Water Services (DE) | OET (If possible) | Open Net | Before sampling | < 1 hour | ≥ 3 $m^3$ |
| Control Union (NL) | Continuous, in line sampling / isokinetic | Open net | Before sampling | 15 - 60 min. | >1 $m^3$ |
| DHI (DK) | Sequence | Open net | Before sampling | < 1 hour | >2 x 500 L |
| | Sequence | Sample container | | | |
| | Sequence | Sample container | | | |
| GBRC (USA) | OET | Open net | Before sampling | < 1 hour | ≥ 3 $m^3$ |
| | Sequence | Sample container | | | |
| | Sequence | Sample container | | | |
| KARLABS (TUR) | OET | Sample container | Before sampling | >20 min. | 1 $m^3$ |
| KOMERI (RoK) | OET | Open net | Before sampling | < 1 hour | ≥ 1 $m^3$ |
| MBRIJ (JP) | Sequence | Open net | Before sampling | < 1 hour | ≥ 1 $m^3$ |
| | | Sample container | | | |
| | | Sample container | | | |
| NIVA (NO) | OET (if possible) | Open Net | None, flow calculated (time and volume) | <1 hour | ≥ 1 $m^3$ |
| OEMA (UK) | OET (sample @ beginning, middle & end of one tank discharge | Open Nets | Before sampling | Uptake Sample: 10 min. During uptake; Discharge Sample: 3 x 10 min. | 1.5 $m^3$ |
| SGS (CH) | Continuous, in line sampling / isokinetic | Closed sampler SGS BWS 1 or BWM2 | Flow meter installed after the sampler | < 1 hour | 1-3 $m^3$ |

# 7. Analyses of samples

## 7.1. Methods for the analysis used during commissioning testing

**Table 2: Analyses details for organisms > 50 micron, 10 – 50 micron, and <10 micron**

| Facility | Indicative method used | Detailed method used | Time used between sampling and analyses |
|---|---|---|---|
| Ankron Water Services (DE) | ATP | > 50 µm: Microscopy | Immediate |
| | PAM fluorometry | 10-50 µm: FDA/CM FDA | |
| | | < 10: E.*coli, Enterococcus, Cholera* | |
| Control Union (NL) | BallastWISE or ATP | N/A | <6 hr |
| DHI (DK) | | >50 µm: Microscopy | Immediate |
| | Combination of PSD+ fluorescence | 10-50 µm: FDA/CMFDA | Immediate |
| | | < 10: E.coli, Enterococcus, Cholera | <24 hr |
| GBRC (USA) | | > 50 µm: Microscopy | < 2 hr |
| | | 10-50 µm: FDA/CMFDA | < 2 hr |
| | | < 10: E.coli, *Enterococcus, Cholera* | < 24 hr |
| KARLABS (TUR) | ATP | >50 µm: Microscopy | Immediate |
| | | 10-50 µm: Na | |
| | | <10: *E.coli, Enterococcus, Cholera* | |
| KOMERI (RoK) | ATP | > 50 µm: Microscopy | < 6 hr |
| | ATP | 10-50 µm: FDA/CMFDA | |
| | ATP | < 10: *E.coli, Enterococcus, Cholera* | |
| MBRIJ (JP) | Pulse counting FDA | > 50 µm: Microscopy (NR, FDA/CMFDA) | < 6 hr |
| | Pulse counting FDA | 10-50 µm: Microscopy (NR,FDA/CMFDA) | |
| | | < 10: *E.coli, Enterococcus, Cholera* | |
| NIVA (NO) | Not in use yet | >50 µm: Microscopy | Immediate (< 6 hr) |
| | | 10-50 µm: FDA/CMFDA | |
| | | | |
| OEMA (UK) | | >50 µm: Microscopy | |
| | | 10-50 µm: microscopy (FDA/CMFDA) | |
| | | < 10: *E.coli, Enterococcus, Cholera*< *10: E.coli, Enterococcus, Cholera* | |
| SGS (CH) | ATP | > 50 µm: Microscopy | Immediate for ATP / < 6 hr for microscopy |
| | ATP and / or PAM fluorometry | 10-50 µm: microscopy (FDA/CMFDA) | Immediate for ATP / < 6 hr for microscopy |
| | ATP | < 10: *E.coli, Enterococcus, Cholera*, Total heterotrophic counts | < 24 hr |

## 7.2. Methods for detailed analysis and viability assessments (Type approval testing)

**Table 3: Methods for counting organisms and viability assessment.**

| Test facility | Greater than 50 µm | 10 – 50 µm | Counts | Resting stages |
|---|---|---|---|---|
| Control Union (NL) | Movement, poking (ETV protocol) | (NC[)]) FDA/CMFDA, MPN | Microscope, Fluorescent microscope, MPN | Not assessed |
| DHI (DK) | Movement, poking | (NC) FDA/CMFDA, MPN and Lugol's samples | Microscope Fluorescence for MPN | Counted, viability assessment not always possible |
| GBRC (USA) | Movement, poking | FDA/CMFDA, MPN and Lugol's samples, other corroborative assays (e.g. flow cytometry, PAM, BWI) | Microscope Fluorescence for MPN | Rarely encountered Not counted |
| KIOST (RoK) | Organism integrity, stain (Neutral Red), poking | (C and NC) Growth experiments, FDA stain, FDA + CMFDA-stain, organism movement | Microscope | Not assessed |
| KOMERI (RoK) | Organism integrity, poking | (C and NC) Growth experiments, FDA/CMFDA-stain, organism movement, MPN. | Microscope, Fluorescent microscope | Not assessed |
| MBRIJ (JP) | Movement, stain (Neutral Red or FDA/CMFDA) | (C and NC) stain (Neutral Red or FDA/CMFDA), Movement, Growth experiments (MPN) | Nomal-microscope and Fluorescence-microscope | Incubation(Zooplankton eggs and Phytoplankton cyst) |
| NIVA (NO) | Movement, poking | ((NC) Dual staining FDA/CMFDA, MPN | Microscope, Fluorescence for MPN | Rarely seen, if found noted |
| OEMA (UK) | Organism movement, organism integrity, poking | (NC) FDA/CMFDA stain, fixed (Lugol's) samples for archiving samples | Std Microscopy & Epi-fluorescence microscopy | Rarely encountered, if found numbers noted |
| SHOU (CN) | Movement, poking | (NC) Dual staining FDA/CMFDA | Fluorescence-microscope | (Not seen any) |

## 7.3. Sizing of organisms (also used during type approval)

There are two fundamentally different principles to identify the minimum dimension. One way to do this is to measure the maximum width of the smallest visible axis of the organism excluding cilia, spikes and appendages. In the other approach the smallest dimension of the smallest visible axis is measured.

For organisms forming chains and colonies, single cells are measured and counted.

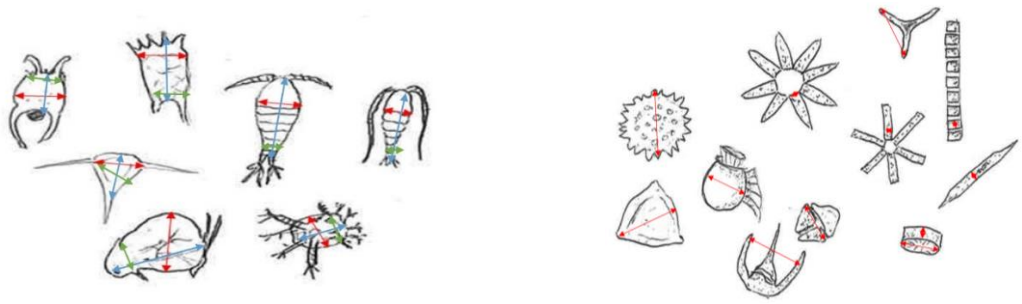

**Figure 1: Examples how to measure the organism size. Red line maximum "body" dimension on smallest axis, green line minimum "body" dimension on the smallest axis and blue line maximum dimension in length of the organism.**

**Table 4: Method used to measure minimum dimension.**

| Test facility | Minimum dimension measurement | Test facility | Minimum dimension measurement |
|---|---|---|---|
| Control Union (NL) | Minimum size on the smallest visible axis | MBRIJ (JP) | Smallest part of the length, width and thickness of individual |
| DHI (DK) | | NIVA (NO) | Maximum size on the smallest visible axis |
| GBRC (USA) | Maximum size on the smallest visible axis | OEMA (UK) | Minimum size on the smallest visible axis |
| KIOST (RoK) | Minimum size on the smallest visible axis | SHOU (CN) | Minimum size on the smallest visible axis |
| KOMERI (RoK) | Minimum size on the smallest visible axis | SGS (CH) | Maximum size on the smallest visible axis |

# 8. Type approval Testing

## 8.1. Water quality and preparation of challenge water during land-based testing

### 8.1.1. Water quality background at testing sites

Different facilities face different test water conditions (Table 5: Summary of ambient water parameters at different land-based test sites.). The differences are affected by, e.g., climate, river runoffs, urban influence and impact of resource users. These differences ensure that testing is done under “world-wide conditions” but at the same time this is also a challenge regarding test result comparability.

**Table 5: Summary of ambient water parameters at different land-based test sites.**

| Test Facility | Temp (°C) | Salinity (PSU) | TSS (mg $l^{-1}$) | POC (mg $l^{-1}$) | DOC (mg $l^{-1}$) | Organisms ≥ 50 µm $m^{-3}$ | Organisms < 50 µm and ≥ 10 µm $ml^{-1}$ | Bacteria $ml^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| Control Union (NL) | Variable | 0.3 - 34 | 5 - 400 | 5 - 20 | 1 - 5 | 10,000 - 1,000,000 | 100 - 100,000 | 10,000 - 1,000,000 |
| DHI (DK) | Variable | 0 - 25 | 1.7 - 20 | 0 - 0.5 | 3 - 5 | 30,000 - 1,000,000+ | 50 - 5,000 | Variable |
| GBRC (USA) | Variable | 0 - 28 | 20 - 100+ | 0.5 - 2 | 2 - 5 | 25,000 - 1,000,000+ | 100 -1,300 | > 1,000 |
| KIOST (RoK) | 12.0 – 21.5 | 0.6 - 30 | 63.3 - 72.3 | 8.42 - 15.6 | 5.84 - 6.96 | 171,667 - 376,667 | 1,230 - 5,247 | 3.8 - 7.0 x $10^5$ |
| KOMERI[1] (RoK) | 4 - 30 | 0 - 33 | Variable | Variable | Variable | 1,000 - 500,000 | 100 - 3,000 | 1,000 - 800,0001,000 - 800,000 |
| MBRIJ (JP) | 4 - 30 | 0 - 34 | 1 - 50 | <0.1 - 5 | 1 - 5 | 10,000 - 300,000 | <1 - 200 | 10,000 - 500,000 |
| NIVA (NO) | 4 - 25 | 0 – 34 | 1 - 10 | 1 - 2 | 1 - 4 | 20,000 – 1,000,000 | 100 - 8,000 | >$10^3$ |
| SHOU (CN) | 16 - 22 | 32 – 33 | 1 - 5 | ca. 5 | ca. 2 | Standard met | 50 % of standard | Standard met |

[1] For the test, water quality used by KOMERI have a wide range. The challenge water is used the natural seawater and fresh waters. The seawater is directly supplied in a nearby sea using the pump, and natural fresh water is indirectly supplied in a nearby river using a tank lorry. For the challenge water, natural viable or living organisms are collected by mechanical concentration method.

### 8.1.2. Adjustment of water quality parameters during testing

IMO and USCG require certain water conditions to challenge ballast water management systems. Some conditions need to be manipulated to meet these requirements. Table 6: Additives used and methodologies used for challenge waterTable 6: Additives used and methodologies used for challenge water shows what test facilities do to meet the challenge water conditions.

**Table 6: Additives used and methodologies used for challenge water**

| Test facility | Manipulation of water parameters | Use of Standard Test Organisms (STO) | % of STO | Test tank mixing during hold time | Challenge water application |
|---|---|---|---|---|---|
| Control Union (NL) | Ligno Sulfonate, Sodium Citrate, Corn starch, Bentonite clay | *Tetraselmis sp*<br>Concentrate of local organisms<br>Wild cultures of local organisms | Up to 50% (variable) | No | 500 L, TSS/DOC/POC injection prior to BWTS / 30 $m^3$ organism concentrate / culture injection prior to BWTS |
| DHI ( DK) | Ligno Sulfonate, Sodium Citrate, Corn starch, Kaolin clay, Brine | *Tetraselmis sp* | Up to 500 cells/mL | Mixing with propeller | Mixed separately and injected into test tank priot testing |
| GBRC (USA) | Ligno Sulfonate, Sodium Citrate, Corn starch, Kaolin clay | Ambient phytoplankton grow-out.<br>Ambient zooplankton concentration.<br>Various species. | Up to 90% phytoplankton (ambient organisms). Typically 0% zooplankton. | Air lift in source tank | Organisms mixed into source tank. Water quality 1,000 L injection into uptake water. |
| KIOST (RoK) | Glucose, Starch, Silica, A2 Fine dust(ISO 12103-1) | *Artemia sp, Tetraselmis sp.* | None | + | 230 $m^3$ and/or 430 $m^3$, i.e. used directly for test (runs tests sequentially) |
| KOMERI (RoK) | Glucose, Starch, Lignin | Only natural organism | 0 % | Agitation and/or bubbling | Depends on natural condition (normally 3 - 30 $m^3$) |
| MBRIJ (JP) | Carbon, TSS | *Brachionus rotundiformis, Tetraselmis sp. Synchaeta sp. Rotaria sp. and Scenedesmus sp.* | Up to 90 % | Bubbling | 1,000 L injected |
| NIVA (NO) | Lignine Sulfonate, Sodium Citrate, Corn starch, Kaolin clay | *Tetraselmis sp Chlamydomonas sp.* | Up to 50% | No | Additives, STOs and 100-150$m^3$ local organism inoculum mixed separately in test tank prior to testing. |

## 8.2. Sampling procedures (Land based tests)

**Table 7: Sampling details, land-based tests for the discharge of treated water, organisms above 50 micron in minimum dimension.**

| Facility | Sampling point location | OET or sequences | Sample port | Volume | Duration sample collection | Method | Conc. sample volume | Second Conc. sample volume | % of sample volume analysed | Max time Conc. sample storage | Time end of collection to end of analysis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control Union (NL) | In-line | 3 sequences | G2-isokinetic, Pitot-tube, 1 sampling point | > 3,000 L (>1m³/ sequence) | Ca. 1 hour | Plankton nets 50 µm (diagonal) in 1m³ sampling tanks | 250-750 ml | - | 100 | 2 - 4 hours | < 6 hour |
| DHI (DK) | In-line | OET or 3 sequences for operation exceeding 2 hours | G2-isokinetic, Pitot-tube, 1 sampling point, use manifold to split the flow into 3 sampling net | > 3,000 L (3 samples) | Througout the operation, Depending on flow rate. For operation exceeding 2 hours, OET is swapped to 3 sequences | Plankton nets with minimum mesh size of 20 µm (conical net bag with an upper diameter of 40 cm and a length of 100 cm) in 1m³ sampling tanks | 400 - 1,000 mL (3 samples) | **Very turbid samples:** min. volume per lab replicate 10 mL (no additional concentration). **Not turbid samples:** min. 3 lab replicates with a maximum volume per lab replicate of 30 mL. | Until >30 organisms are found or: **Very turbid samples:** as many as possible sub-samples are counted within 6 hours Not turbid samples: 100%. | >0.75 <1 hour | <6 hour |
| GBRC (USA) | In-line | OET | G2-isokinetic, Pitot-tube, 1 sampling point, use flow splitter for 3 parallel samples | > 3,000 L (3 samples) | 1 to 2 hours depending on flow rate | Plankton nets 50 µm (diagonal) in 1m³ sampling tanks | 400 mL | 60 mL | 100 | < 1.5 hour | < 1.5 hour |
| KIOST (RoK) | In-line | OET | G2-isokinetic, Pitot-tube, 1 sampling point | > 3,000 L | Ca. 1 hour | Nets 50 µm (diagonal) | 1,000 mL | 20-100 mL | 100 | < 2 hours | < 6 hours |
| KOMERI (RoK) | In-line | OET | G2-isokinetic, Pitot-tube, 1 sampling point | > 3,000 L | Ca. 1 hour | Nets 50 µm (diagonal) | 1,000 mL | 20-100 mL | 100 | < 2 hours | < 6 hours |
| MBRIJ (JP) | In-line | 3 sequences | G2-isokinetic, Pitot-tube, 3 sampling point | > 1,000 L (for each sequence) | > 1 hour | Plankton nets 35 µm <50 µm (diagonal) | 500 mL | 10-50 mL | 100 | < 2 hours | < 6 hours |

| Facility | Sampling point location | OET or sequences | Sample port | Volume | Duration sample collection | Method | Conc. sample volume | Second Conc. sample volume | % of sample volume analysed | Max time Conc. sample storage | Time end of collection to end of analysis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NIVA (NO) | In-line | OET or 3 sequences | G2-isokinetic, Pitot-tube, 2 sampling ports | > 1,000 L (3consecutive samples) | >3x 6-15 mins per sample | Plankton nets 50 µm(diagonal) in 1m³ sampling tanks | 100 mL | For very turbid samples: Concentrated volume may be diluted with filtered water of relevant quality. | 100 | < 2 hours | 2-6 hours |
| SHOU (CN) | In-line | OET | G2-isokinetic, Pitot-tube, 1 sampling point | > 3,000 L (3 samples) | Ca. 1 hour depending on flow rate | Plankton nets 50µm(diagonal) in 1m³ sampling tank | 1,000 mL | | 100 | < 2 hours | < 6 hours |

## 8.3. Sampling procedures (Type Approval Ship-board tests)

**Table 8: Sampling details, ship-board tests for the discharge of treated water, organisms above 50 micron in minimum dimension.**

| Facility | Sampling point location | OET or sequences | Volume | Duration sample collection | Conc. sample volume | Second Conc. sample volume | % of sample volume analysed | Max time Conc. sample storage | Time end of collection to end of analysis | Flowmeter | Method details |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control Union (NL) | In-line | OET or 3 sequences | > 1000 L (3 OET samples in parallel or 1 sequence in each beginning, middle and end) | Dependent on vessel specifics typically 30 mins to 1 hour | 250 mL | 100 mL | 20 - 100 | 15 - 60 min. | < 6 hours | Flowmeter capacity 20-200 L/min. | Plankton nets 50 µm (diagonal) in a sampling bin of ca. 200 L capacity |
| DHI (DK) | In-line | OET | > 1,000 L (3 samples) | Dependent on vessel specifics | 1,000 ml | Minimum 3 lab replicates with a maximum volume per lab replicate of 30 mL. | 100 | 2 hours | < 6 hours | Yes | Plankton nets with minimum mesh size of 20 µm (conical net bag with an upper diameter of 40 cm and a length of 100 cm) in 1m³ sampling tanks |

| Facility | Sampling point location | OET or sequences | Volume | Duration sample collection | Conc. sample volume | Second Conc. sample volume | % of sample volume analysed | Max time Conc. sample storage | Time end of collection to end of analysis | Flowmeter | Method details |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GBRC (USA) | In-line | OET | > 1,000 L (3 samples) | Ca 2 hour | 400 mL | 60 mL | 100 | < 1.5 hour | < 1.5 hour | | Plankton nets 50 µm (diagonal) |
| KIOST (RoK) | In-line | OET | > 3,000 L | Dependent on vessel specifics, typically ca. 1 hour | 1,000 mL | 100 mL | 100 | < 2 hours | < 6 hours | YES | Plankton nets 50 µm (diagonal) net in a sampling plastic buckets (about 70 L capacity). |
| KOMERI (RoK) | In-line | OET | > 3,000 L | Dependent on vessel specifics, typically ca. 1 hour | 1,000 mL | 100 mL | 100 | < 2 hours | < 6 hours | Flowmeter capacity 20- 200 L/min | Plankton nets 50 µm (diagonal) in a sampling bin of ca. 100 L capacity |
| MBRIJ (JP) | In-line | 3 sequences | > 1,000 L (9 samples) | Dependent on vessel specifics , typically 4 to 10 min. | 500 mL | 50 mL | 100 | < 2 hours | < 6 hours | Flowmeter capacity 20-250 L/min | Plankton nets 50 µm (diagonal) net in a sampling plastic buckets (70 L capacity). |
| SHOU (CN) | In-line | OET | > 1,000 L (3 samples) | Dependent on vessel specifics, typically ca. 1 hour | 1,000 mL | | 100 | < 2 hours | < 6 hours | Yes | Plankton nets 50 µm (diagonal) |
| NIVA (NO) | In-line | OET | > 1,000 L (>3-9 samples) | Dependent on vessel specifics typically 10 mins per sequence | 100 mL | For very turbid samples: Concentrated volume may be diluted with filtered water of relevant quality. | 100 | < 2 hours | < 6 hours | Flow measurement with or without flowmeter | Plankton nets 50 µm (diagonal) |

*** END ***