ETV
Verification statement

Technology: BallastWISE®

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The verification process, whose results are summarized in this Statement, complies with the ISO Standard 14034 on Environmental Management: Environmental Technology Verification.

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1. Technology description

BallastWISE developed by MicroWISE ApS is a Compliance and Monitoring Device (CMD) for accurate measuring of living organisms in water samples including samples of ballast water.

The technology combines light-emitting diodes and high-resolution cameras with image analysis algorithms to detect and count living heterotrophic and autotrophic microorganisms within two size-ranges: 1: ≥10µm and <50µm and 2: ≥50µm. (BallastWISE does not detect non-motile and non-pigmented organisms and spores). The variable fluorescence analysis is based on Pulse-Amplitude-Modulation (PAM) fluorometry to ensure a selective identification of phototrophic organisms containing chlorophyll to avoid false positive detections. Fluorescence analysis is combined with image analysis that allows for the identification and measurement of individual organisms.

The technology and video analysis enables tracking and quantification of movement. The movement tracking is especially important for analysis of zooplankton, as movement is a criterion for a living organism. False positives are avoided by controlling convection flow within chambers to a degree where particles moving with the flow have a velocity that is less than the swimming speed threshold for live swimming organisms.

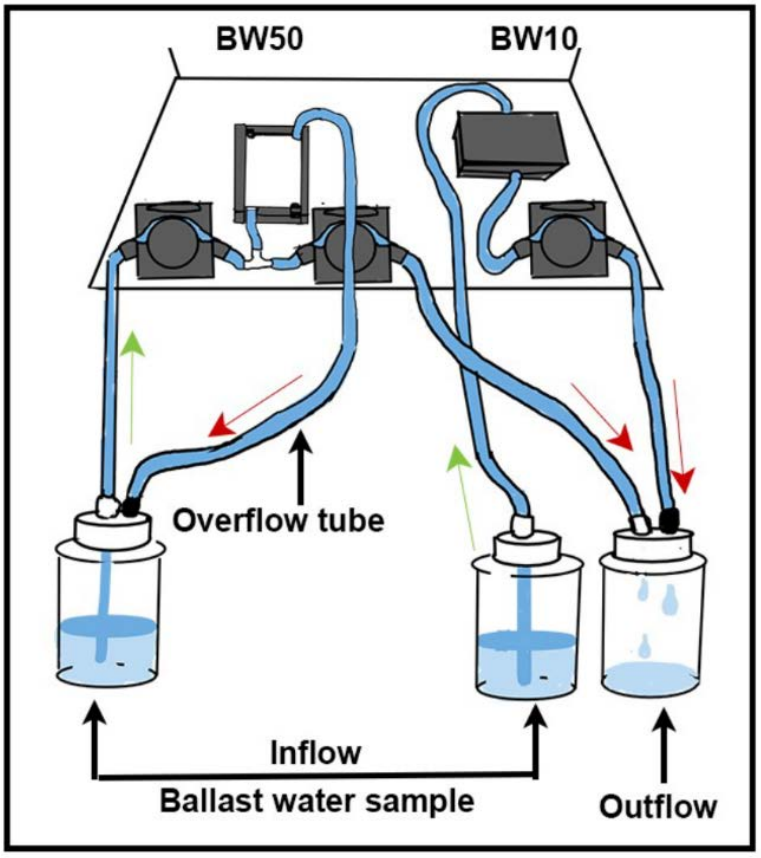

Figure 1 Illustration of BallastWISE operation for the two size fractions.

2. Application

2.1 Matrix

Ballast water from the shipping industry.

2.2 Purpose

Automatic counting of viable organisms in ballast water samples to confirm that a ballast water management system (BWMS) installed on a ship meets the D-2 discharge standard (DS) of the IMO Ballast water convention (number of viable organisms per volume: <10/m^3 for organisms ≥50µm and <10/ml for organisms ≥10µm to 50µm)[1]. The bacterial fraction described in the D-2 standard is not tested. There are only very few practical solutions for fast and reliable testing for compliance. Currently, the widely accepted solution is to use manual microscopic counting also known as detailed analysis. The collected samples need to be analyzed within 6 hours of sampling, and it is therefore most practical to analyze them on site using a compliance monitoring device (CMD). Using detailed analysis can be challenging in terms of logistics and cost, and there is thus a high demand for a portable instrument that can test for compliance and is easy to use.

2.3 Conditions of operation and use

The BallastWISE system must be operated in temperatures between 4-40°C and with water sample temperatures above freezing point. BallastWISE is not affected by vibration within reasonable levels. Vibration levels on board vessels with machinery running at idle are acceptable. BallastWISE analysis does not require the addition of chemicals, laboratory facilities or extended training or education to use. Samples analyzed for organism density in the size category ≥50µm must be concentrated by a factor 1.000 by filtration prior to testing on the BallastWISE system.

Each BallastWISE system is calibrated at the factory. Annual checks are recommended and calibration of focus and lighting if necessary. MicroWISE offers regular (annual) service for customers. The same chamber and tubing can be used for several measurements, however, if the chambers are re-used, they should be cleaned thoroughly with demineralized water under pressure. It is recommended to only use the same chamber and tubing for a maximum of 20 measurements after which the parts should be replaced with new ones.

The intended use in commissioning and monitoring of ballast water is well within the limitations of the BallastWISE system as the discharge standards for compliance are 10 organisms per mL and 10 organisms per m^3 for the size fraction ≥10-50µm and ≥50µm respectively.

Table 1 The upper limit for the number of particles that are detectable by BallastWISE. The upper limit includes particles and organisms with the given minimum size. For motion analysis particles or organisms must have a minimum speed to be registered. For fluorescence analysis in the size fraction ≥10-50µm the size limit given below applies.

Organism sizes	Minimum particle size (µm)	Particles per measuring chamber	Motioning analysis minimum speed (µm/s)	Particles per volume
≥50µm	40	500	500	20.000 particles/L
≥10-50µm	4	500	100	20.000 particles/mL

[1] Centre for International Law. (2004). 2004 INTERNATIONAL CONVENTION FOR THE CONTROL AND MANAGEMENT OF SHIPS' BALLAST WATER AND SEDIMENTS. London: Centre for International Law.

2.4 Verification parameters definition summary

BallastWISE is capable of measuring and calculating:

- Density of organisms ≥ 50 µm: number/m^3
- Density of organisms ≥10 to >50 µm: number/ml
- Size distribution (%)
- Movement pattern of organisms
- Variability in fluorescence

The focus of this verification process has been to verify that the BallastWISE system can deliver comparable results to the current industry standard for measuring organism density and identifying non-compliance in ballast water samples.

3. Test and analysis design

3.1 Existing and new test data

Danish Hydraulic Institute, DK (DHI) and Control Union Vessel Performance Center, NL (VPC) were test bodies and provided new test data. The test design was based on protocols from the International Maritime Organization (IMO)[2] and the International Organization for Standardization (ISO)[3]. Both protocols were under development when the test design was in the planning phase. No existing data was included in the verification process.

3.2 Laboratory and field conditions

All testing was performed under accreditation (VPC: ISO 17025, DHI: ISO 17025 and ISO 9001) at DHI and VPC. Several field tests were performed onboard a ship to complete the onboard usability assessment.

3.3 Matrix compositions

Tests were performed on matrices with varying salinities, treatments and organism densities. See Table 2 for the tested matrices.

[2] BWM.2/Circ.78 - INTERNATIONAL CONVENTION FOR THE CONTROL AND MANAGEMENT OF SHIPS' BALLAST WATER AND SEDIMENTS, 2004 (Protocol for the verification of ballast water compliance monitoring devices). London: IMO (International Maritime Organization).

[3] ISO 3725 - International Standard - Ships and marine technology - Aquatic nuisance species - Methods for evaluating the performance of compliance monitoring devices for ballast water discharges. Geneva: ISO (the International Organization for Standardization).

Table 2 Test setup. Matrix composition presented as tests were performed by DHI and VPC.

Matrix composition: Test setup - location, source, treatment, organism density, salinity, and number of replicates			Concentration	Salinity category	Samples n
DHI	Trueness (Laboratory)	Untreated prepared challenge water	Blank*	Fresh	1
			<DS (DS x 0,5)		3
			≈DS		3
			>DS (DS x 1,5)		3
			Blank	Brackish	1
			<DS (DS x 0,5)		3
			≈DS		3
			>DS (DS x 1,5)		3
			Blank	Marine	1
			<DS (DS x 0,5)		3
			≈DS		3
			>DS (DS x 1,5)		3
		Prepared challenge water treated with an oxidative technology	-	Fresh	3
			-	Brackish	3
			-	Marine	3
		Prepared challenge water treated with an UV technology	-	Brackish	3
			-	Marine	3
		Laboratory – non augmented water containing minimum 3 species	-	Fresh	3
			-	Brackish	3
			-	Marine	3
	Precision	Laboratory – Prepared challenge water	(≈)DS	Fresh	10
			(≈)DS	Brackish	10
			(≈)DS	Marine	10
VPC		Laboratory – Prepared challenge water	>DS (approx. DS * 2,5)	Marine	10
	Trueness (Field test)	Seawater Field test #1 - 22FEB2024; Dina Supplier; Optimarin UV	-	Marine	3
		Seawater Field test #2 - 20MAR2024; Dina Supplier; Optimarin UV	-	Brackish	3
		FW field test #3- 09JUL2024; Eems exe; Alfa Laval UV	-	Fresh	3

3.4 Test and analysis parameters

Performance parameters were tested in accordance with the IMO and ISO protocols.

3.5 Tests and analysis methods summary

See Table 3.

3.6 Parameters measured

See Table 3.

Table 3 Parameters measured and calculated in the verification process. Parameters are split into two groups depending on if the data is related to the performance of BallastWISE or if it is related to the condition of the water sample. The three columns contain (from left to right) Parameter category, specific parameter and the applied analyses methods.

Category	Parameter	Analysis method
Performance parameters:	Density of organisms ≥ 50 µm: number/m^3	**BallastWISE:** Movement tracking (motility) **Reference (Standard method):** Standard microscopy counting of organisms (standard movement and response to stimuli)
	Density of organisms ≥10 to >50 µm: number/ml	**BallastWISE:** Imaging Pulse-Amplitude-Modulation fluorometry (iPAM) and Movement tracking (motility) **Reference (Standard method):** Epifluorescence microscopy (CMFDA+FDA staining)
	Calculated parameters: - Numerical precision and trueness - Categorical outcome precision and trueness - Lower Limit of Detection (LLoD) and Limit of Blank (LoB) - Rate of false positives	Regular statistical calculations and inter-rater agreement calculations (Cohen's kappa) based on specifications in the ISO protocol.
	Reliability and usability: - Time in operation with no issues - Down time - Count for non-scheduled occurences	Regular statistical calculations and experience from use.
	Background data for organism density from BallastWISE (not reported): - Size distribution (%) - Movement pattern of organisms - Variability in fluorescence	BallastWISE automated image and video analysis and iPAM
Water quality parameters:	Chemical parameters: - DOC, POC, TSS, pH, temperature, salinity	Regular chemical analysis methods and online sensors
	Biological parameters: - Taxonomy	Microscopy

4. Verification results

4.1 Performance

Available data for precision and detection limits are found to be comparable between BallastWISE and the reference method (see Table 4). Regarding detection limits BallastWISE is found able to measure 0 organism per volume and values much higher than the value of the discharge standard of 10 organisms per volume.

BallastWISE consistently measures lower numerical values than the reference method as seen in Figure 2. This is expected as the two methods evaluate viability or living/dead criteria in different ways. BallastWISE requires organisms to be detectable via motility with a lower threshold value for movement speed or via iPAM.

Taking the differences in analyses methods into account the optimal value of the internal BallastWISE limit for categorizing a sample as in compliance (meets the discharge standard) was investigated. The optimal value was found to be 3 organisms per volume as seen in Figure 3.

Table 4 Results for calculated parameters of the combined testing performed at DHI and VPC. Cells with no data is either marked "-" or "N/A" where the first indicates that the reference data is used as the "true" value and the latter indicates insufficient data points for calculating the parameter.

		Reference	BallastWISE	Reference	BallastWISE
	Unit	≥10-50 µm		≥50 um	
Precision:					
Numerical precision (CV%)		21%	24%	N/A	27%
Categorical precision		93%	88-98%	N/A	100%
LLoD:					
Limit of Blank (LoB)	org./vol.	-	0,28	-	2,60
Calculated according to protocol		12,5	10,6	N/A	N/A
Lowest value measured		0	0	0	0
Highest value measured		2.582	1.277	273.333	235.600
Trueness:					
Numerical error (%)		N/A	60%	N/A	61%
Categorical agreement (κ) (IL_3)		-	0,85	-	0,71
False positives		N/A	0% - 4%	N/A	0% - 4%

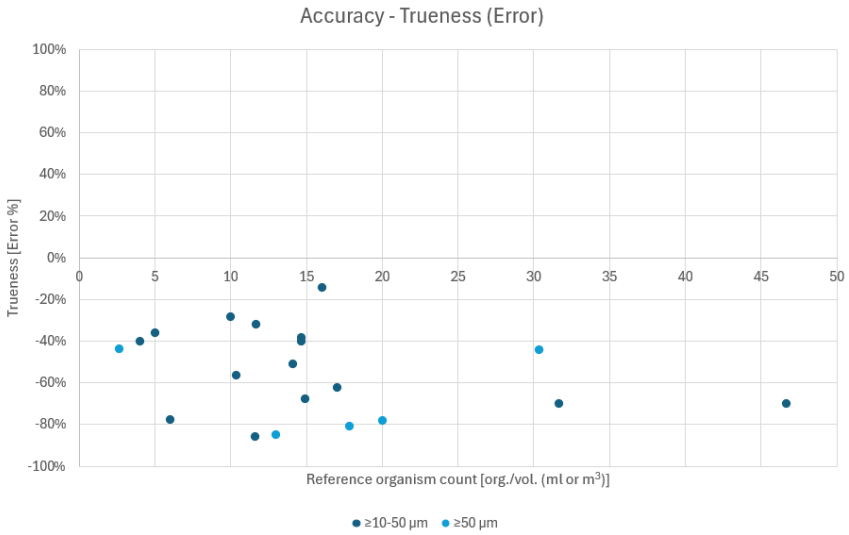

Figure 2 Data shown for numerical accuracy. BallastWISE data points are consistently lower than the values of the reference method. The average numerical error is found to be approximately 60% for both size categories.

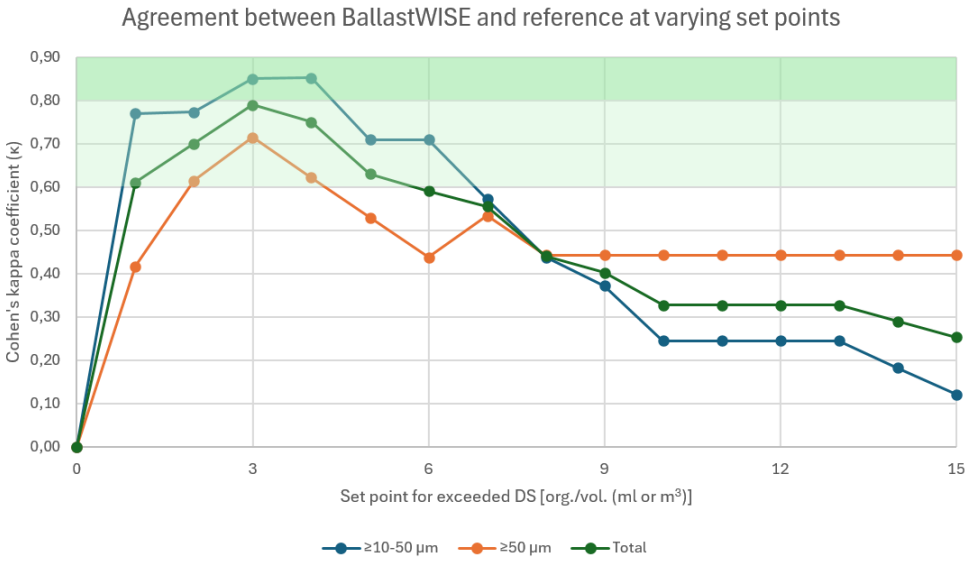

Figure 3 Data for agreement in terms of the Cohen's kappa value at varying set points for the internal BallastWISE limit for categorizing a test series as in compliance or non-compliance with the international discharge standard set for the discharge of ballast water. The optimal set point is found to be when the reference method limit is equal to the discharge standard of 10 organisms per volume (mL or m³) and the internal BallastWISE limit is equal to 3 organisms per volume.

Cohen's kappa is a measurement of interrater agreement that accounts for expected agreement due to chance. This parameter for evaluating the agreement between two test methods with categorical outcomes (compliance/non-compliance) is specified in the ISO protocol which states that a Cohen's kappa value of 0,8 is registered as strong agreement and that values down to 0,6 can be accepted in certain circumstances. Cohen's kappa values for the two size fractions ≥10-50µm and ≥50µm were 0,85 (agreement on 25 of 27 test series (93%)) and 0,71 (agreement on 18 of 21 test series (86%)) respectively which is well within the acceptable limits set by the ISO protocol. Registered categorical outcomes for BallastWISE, and the reference method can be found in Table 5 Categorical outcome tables for BallastWISE and the reference method at the optimal set point (3 organisms per volume). Values in cells placed on the diagonal (top left to bottom right in each table) represent test series where the two methods agree on the outcome.Table 5.

BallastWISE was found to be comparable to the reference method in terms of categorical outcomes based on the analysis of Cohen's kappa values.

Table 5 Categorical outcome tables for BallastWISE and the reference method at the optimal set point (3 organisms per volume). Values in cells placed on the diagonal (top left to bottom right in each table) represent test series where the two methods agree on the outcome.

Categorical outcome tables:

All values (≥10-50 µm)

Reference method \ BallastWise	Exceeds DS	Meets DS	Indeter-minate	
Exceeds DS	14	1	0	$\sum R_1$ 15 0,56
Meets DS	1	11	0	$\sum R_2$ 12 0,44
Indeter-minate	0	0	0	$\sum R_3$ 0 0,00
	$\sum BW_1$ 15 0,56	$\sum BW_2$ 12 0,44	$\sum BW_3$ 0 0,00	Total (n) 27 1,00

Cohen's kappa: 0,85

≥50 um

Reference method \ BallastWise	Exceeds DS	Meets DS	Indeter-minate	
Exceeds DS	9	2	0	$\sum R_1$ 11 0,52
Meets DS	1	9	0	$\sum R_2$ 10 0,48
Indeter-minate	0	0	0	$\sum R_3$ 0 0,00
	$\sum BW_1$ 10 0,48	$\sum BW_2$ 11 0,52	$\sum BW_3$ 0 0,00	Total (n) 21 1,00

Cohen's kappa: 0,71

Combined

Reference method \ BallastWise	Exceeds DS	Meets DS	Indeter-minate	
Exceeds DS	23	3	0	$\sum R_1$ 26 0,54
Meets DS	2	20	0	$\sum R_2$ 22 0,46
Indeter-minate	0	0	0	$\sum R_3$ 0 0,00
	$\sum BW_1$ 25 0,52	$\sum BW_2$ 23 0,48	$\sum BW_3$ 0 0,00	Total (n) 48 1,00

Cohen's kappa: 0,79

BallastWISE was found to have a rate of false positives of 0% at the standard limit for compliance and a rate of 4% when the internal limit for compliance was optimized to increase agreement between the categorical outcomes between BallastWISE and the reference method.

4.2 Reliability and usability

VPC recorded operational time, down time and occurrences of non-scheduled interruptions of operations across laboratory and field tests performed by their personnel and observed 100% reliability. No issues with usability or vulnerabilities were observed.

4.3 Operational

Water quality parameters from Table 3 were monitored to ensure compliance with the IMO and ISO protocols.

5. Additional information

BallastWISE records and saves all relevant images used for analysis and results of size distribution, fluorescence and movement patterns. This ensures that results can be qualified and validated by investigating recorded data. The saved data can also be made available for external control and verification.

6. Quality assurance and deviations

The personnel and experts responsible for quality assurance as well as for the different quality assurance tasks can be seen in Table 6. All relevant reviews were prepared using the DANETV review report template. An audit of the test system was performed at the test site of DHI and VPC.

Table 6 QA-plan for verification of BallastWISE

	Verification body	Proposer	External expert
Task	Danish Technological Institute	MicroWISE ApS	EnviDan
Specific Verification Protocol		Review and approve	Review
Test plan	Review and approve	Review and approve	
Test system at test site			
Test report	Review and approve	Review	
Verification Report		Review	Review
Statement of Verification		Acceptance	Review

6.1 Deviations

The test protocol was designed to fulfil the requirements given in the IMO protocol (BWM.2/Circ.78) for the verification of a CMD. The corresponding test protocol described in ISO 3725:2023 is basically a sub-set of the IMO protocol but contains some valuable recommendations on data analysis.

Minor deviations from the specific verification protocol were registered regarding number of replicates and applied preparation method for certain samples. No critical deviations were registered.