ANNEX D

Indicator	Methodology	standard method	Comments	Confidence level/threshold detection
Viable Organisms ≤ 50 µm	Score look or Stereomicrosc Opia	currently without international standardization for ballast water analysis.	It can be costly and time consuming. Requires moderately trained personnel. OECD Test Guideline for Testing of Chemicals 202 (Daphnia sp. Acute Immobilization Test) can be used as a basis for standard methodology.	To be determined.
Viable Organisms ≤ 50 µm	Visual inspection.	Currently without international standardization for ballast water analysis.	Visual inspections usually only detect organisms > 1 mm in minimal dimensions.	To be determined.
Viable Organisms ≤ 10 µm - < 50 µm	Fluorescence variable.	currently without international standardization for ballast water analysis.	Only detects photosynthetic phytoplankton and thus underestimates other organisms planktonic in this size class.	To be determined.
Organisms viable ≤ 50 µm and ≤ 10 µm - < 50 µm	photometry, acid nucleic, ATP, diacetate of fluorescein (FDA), chlorophyll *The.*	currently without international standardization for ballast water analysis.	Semi-quantitative results can be obtained. However, some of these organic compounds can survive for long periods of time in aqueous solutions outside of the cell, potentially generating false positives (Welschmeyer & Maurer, 2012).	To be determined.
Organisms viable ≤ 50 µm and ≤ 10 µm - < 50 µm	Flow cytometry.	currently without international standardization for ballast water analysis.	Costly.	To be determined.
Enterococci	kit of Detection Fluorimetry here.	currently without international standardization for ballast water analysis.	Minimum incubation time of 6 hours. Semi-quantitative results detected with portable methods.	To be determined.

Indicator	Methodology	standard method	Comments	Confidence level / limit of detection
Escherichia coli	Detection Kit Fluorimetric.	currently not standardized international standards for ballast water analysis.	Minimum incubation time of 6 hours. Semi-quantitative results detected with portable methods.	To be determined.
Vibrio cholerae(O1 and O139)	Test Kits.	Currently without international standardization for ballast water analysis.	Relatively quick tests are available.	To be determined.
viable organisms ≤ 50 μm and ≤ 10 μm - < 50 μm	pulse count FDA.	Currently without international standardization for ballast water analysis.	The sampling kit may be larger than the one used for FDA.	To be determined.

Indicator	Approach	standard method	Comments	Level in confidence/limit detection
viable organisms ≤ 50 µm and ≤ 10 µm - < 50 µm	visual count or stereomicroscopy Vital dyes can be used in conjunction with fluorescence and movement.	At the moment without Standardization international standards for ballast water analysis. See US EPA ETV v.5.1 Protocol	It can be costly and time consuming. Requires moderately trained personnel. OECD Test Guideline for Testing of Chemicals 202 (Daphnia sp. Acute Immobilization Test) can be used as a basis for standard methodology.	To be determined.
Viable organisms ≤ 10 µm - < 50 µm	Visual counting using visual dyes.	At the moment without Standardization international standards for ballast water analysis. See US EPA ETV v.5.1 Protocol	Requires specific knowledge for operation. It should be noted that the use of vital dyes has limitations.	As to be determined. Steinberg et al., 2011.
Viable organisms ≤ 10 µm - < 50 µm	Flow cytometers (based on chlorophyll *The* and vital dyes)	At the moment without Standardization international standards for ballast water analysis.	Costly and requires specific knowledge to operate. It should be noted that the use of vital dyes has limitations.	To be determined.
Viable organisms ≤ 50 µm and viable organisms ≤ 10 µm - < 50 µm	Flow chambers (based on chlorophyll *A* and vital dyes)	currently without Standardization international standards for ballast water analysis.	Costly and requires specific knowledge to operate. It should be noted that the use of vital dyes has limitations.	To be determined.
viable organisms ≤ 50 µm and viable organisms ≤ 10 µm - < 50 µm	methods of culture (recovery, regrowth and maturation).	At the moment without Standardization international standards for ballast water analysis.	Requires specific knowledge to conduct the methods. Densities are expressed as Most Probable Numbers (Dilution Method – MPN). Many species do not grow using the method and therefore cannot be used alone. Incubation time 2-3 weeks.	To be determined.

Indicator	Approach	standard method	Comments	Confidence level / detection limit
Enterococci	Culture methods.	ISO 7899-1 or ISO 7899-2	Requires specific knowledge to conduct the methods. At least 44 hours of incubation time. EPA Standard Method 9230	To be determined.
Escherichia coli	Culture methods.	ISO 9308-3 or ISO 9308-1	Requires specific knowledge to conduct the methods. At least 24 hours incubation time. EPA Standard Method 9213D	To be determined.
Vibrio cholerae (O1 and O139)	Culture and methods as molecular, biological or fluorescence.	ISO/TS 21872-1/13/	Requires specific knowledge to conduct the methods. Incubation time: 24-48 hours. US EPA ETV Fykse et al., 2012 (semi-quantitative / pass/ fail test). Samples should only be cultured in a specialized laboratory.	To be determined.
Enterococci, *Escherichia coli, Vibrio cholerae*(O1 and O139)	Culture with 11holera11ence- in-situ hybridization (FISH)	At the moment without standardization International for ballast water analysis.	Requires specific knowledge to conduct the methods. Quantitative and qualitative results after 8 hours. Samples should only be cultured in a specialized laboratory.	To be determined.
Viable organisms ≤ 50 μm and viable organisms ≤ 10 μm - < 50 μm	Score visual using stereomicroscopy and flow cytometry.	At the moment without standardization International for ballast water analysis.	A sampling protocol that identifies whether the system is broken or not functioning and producing a discharge significantly above the D-2 standard. Designed for non-compliance detection with 99% confidence. It needs to be validated.	To be determined.