REPORT ON PERFORMANCE OF BALLAST WATER COLLECTION AND ANALYSIS DEVICES

RV METEOR CRUISE M116/2

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Executive Summary

Promising approaches for ballast water sample collection and analysis have been developed, but require further study in the field to examine their utility for compliance monitoring. To address this gap, a voyage was undertaken by 20 international researchers on board the RV Meteor from June 4-15, 2015. During this time 28 trials were conducted to evaluate three ballast sampling devices (plankton net and 2 sampling skids) and a number of analytic devices (>200µm: 1 technique, ≥50µm: 5 techniques, >10µm and <50µm: 10 techniques, bacteria: 6 techniques). Water samples were collected using paired sampling devices and analyzed in parallel by all analytic methods to determine whether results were similar between devices and whether quick, indicative methods offer comparable results to standard, time-intensive testing methods (e.g. microscopy) and high-end scientific approaches.

For sample collection, some differences were observed in the number of viable organisms collected by the various sampling devices (net, sampling skids in open and closed configurations), but these differences were not consistent across size classes and, in many cases, there was no significant difference between samples collected with a net or with a skid. For sample analysis, several promising indicative methods were identified that showed high correlation with microscopy results but allow much quicker processing.

This study is the first to concurrently test a large number of analytic tools and multiple sampling devices under operational conditions. Results are useful to identify the merits of each method and can serve as a basis for further improvement and development of these tools and methodologies for compliance monitoring.

1.0 Introduction

The Convention for the Control and Management of Ships' Ballast Water and Sediments, (2004; BWMC) aims to "prevent, minimize and ultimately eliminate the transfer of harmful aquatic organisms and pathogens". Ballast water is used to trim merchant vessels in order to enhance stability and to keep the propeller under the water line, but since it can contain organisms that are present in the water during ballasting, it can transfer organisms between regions. In fact, ballast water is considered one of the most prominent vectors for the transfer of marine non-indigenous species (Verling *et al.*, 2005). In this way, organisms reach foreign environments and may cause serious commercial, health and/or environmental damage (NRC 1996, Ruiz 2000, Williams 2013). Being aware of these hazards, the International Maritime Organization (IMO) began to address this problem at the beginning of the 1990s which resulted in the 2004 adoption of the BWMC. The BWMC requires compliance with certain standards limiting the number of living organisms of specified size classes discharged in ballast water (IMO 2008). These standards include restricting the concentration of organisms at discharge to (i) < 10 viable organisms per cubic meter ≥ 50 micrometers in minimum dimension, (ii) < 10 viable organisms per millilitre < 50 micrometers in minimum dimension and ≥10 micrometers in minimum dimension (hereafter, 10-50 μm), and the following concentrations of indicator microbes, as a human health standard: (iii) < 1 Colony Forming Unit (cfu) of Toxigenic *Vibrio cholerae* (serotypes O1 and O139) per 100 millilitres or per 1 gram (wet weight) of zooplankton samples, (iv) < 250 cfu of *Escherichia coli* per 100 millilitres, and (v) < 100 cfu Intestinal Enterococci per 100 millilitres. At present, most ships are expected to meet these standards through shipboard Ballast Water Management Systems (BWMS).

Much effort has been undertaken worldwide to develop BWMS, which usually consist of a filtration unit and a subsequent disinfection procedure that destroys or deactivates the remaining organisms. However, the BWMC is not yet in force. One reason non-subscribing States have been reluctant to ratify the BWMC is because there is some doubt about the reliability of the existing compliance monitoring methods. Ballast water compliance monitoring can be divided into two main parts: sample collection and sample analysis. Several promising approaches have been developed that could be applied during a "trial period", but these sampling and analysis methods need to be further studied in the field to understand their methodological differences, including benefits and drawbacks, and also assess their comparability, accuracy, and precision.

1.1 Sample collection devices

Traditionally, shipboard type approval testing has been conducted using an open collection system with plankton nets. Samples are collected using in-line, "L" shaped sampling probes (i.e. pitot tubes) installed in the vessel's ballast water piping. Ballast water sampled by each sampling probe is pressure-fed by the vessel's ballast system through connected piping and a flowmeter and into a conical plankton net with 35 μm mesh (49.497 μm in diagonal) within a wetted sample tub. Flowrates are adjusted to maintain approximately isokinetic flow (isokinetic according to IMO G-2 Guidelines). The sample retained inside the plankton net is

collected for analysis of organisms in the ≥50 µm size class, recognizing that the flexible nature of the mesh and many organisms may lead to some error in the capture rate. For organisms in the 10-50 µm size class, and water quality analyses, a composite sample totalling ~5 L is taken by collecting ~400 ml of water from the sample tubing (before or after water passes through the plankton net) every two to five minutes during the entire sampling duration. In general, 350-3000 L of water is collected for assessment of ≥ 50 µm size organisms at low density (US Coast Guard, 2010; Briski et al. 2014), so it is necessary to dispose of 'waste' water. 'Waste' water can be, for example, returned to the ship's ballast system downstream via a second purpose-built return port or an existing connection such as a drain valve (Briski et al. 2014), sent overboard using a submersible pump and hose, or deposited into the bilge to be processed and discharged by the ship, which is seldom the preferred option on board vessels.

More recently, collection systems (i.e. sampling skids) have been developed that enable filtration and sample collection of large volumes of water in a small space and, optionally, the ability to return filtered 'waste' water into the ship's ballast water pipe (closed systems). Again, the sampling skid connects to the ship's ballast system using in-line, "L" shaped sampling probes. The sampled ballast water flows through a filter housing with a filter basket having filter material sized according to the BWMC regulation (mesh size 50µm in diagonal/diameter). Large size organisms (≥50 µm) are collected inside the filter assembly, and a bypass after the filter housing equipped with a valve allows for taking 10-50 µm plankton samples and bacterial samples as single samples with small volumes (e.g. 300ml) or drip samples across a longer period of time.

1.2 Analytic Methods

Standard procedures used for testing during type approval of BWMS include "detailed" analysis, such as microscopic examinations, that require extensive scientific expertise and equipment, and a timeframe generally too long for a compliance and enforcement scenario. However, as an alternative, a variety of rapid, easy, but mostly "indicative" analysis methods have been developed that do not directly count live organisms, but provide an indication of potential [gross] non-compliance with the BWMC discharge standards. Indicative methods rely on various indicators to assess the biomass and or viability of individuals in samples, including adenosine triphosphate (ATP) methods which detect the amount of cellular energy, pulse amplitude-modulation (PAM) methods which rely on the natural autofluorescence properties of the chlorophyll-protein complex present in algal cells, and fluorescein diacetate (FDA) methods which variously use FDA stain to measure enzymatic activity (group of esterase enzymes). Some indicative methods provide a calibration curve that allows an estimation of the number of individuals in samples based on a biomass estimate.

1.3 Project description and objectives

The RV Meteor is a 97.5 m research vessel operated by the German Research Foundation. The RV Meteor Cruise M116/2, which took place from June 4-15, 2015, was used to run a series of investigations to compare methods for ballast water sample collection and analysis by conducting replicated, comparative testing of water samples. During this voyage, 28

trials were conducted to evaluate three ballast sampling devices (plankton net, SGS skid, Triton skid NP 6007 TG 18) and a number of analytic tools (>200µm: 1 technique, ≥50µm: 7 techniques, 10-50µm: 9 techniques, <10 µm: 5 techniques; bacteria: 7 techniques). A full list of the analytic devices used on board, the targeted size class(es) for each device, and the participants responsible for each analysis are detailed in Table 1.

The METEOR experiments collected data to examine 4 main objectives: 1) Comparison of ballast water sample collection methods (plankton net, SGS sampling skid, TRITON sampling skid), 2) Comparison of ballast water analysis methods for measuring abundance of organisms ≥50 µm in the maximum dimension on the smallest axis, and 3) Comparison of ballast water analysis methods for measuring abundance of 10-50 µm organisms, and 4) Comparison of analysis methods for organisms <10 µm in the maximum dimension on the smallest axis (bacteria and phytoplankton). In addition, we obtained data to address 2 minor objectives: 1) Test potable water used as ballast for compliance with D-2 standard as per the request of the ship's management/operator, and 2) Examine if analysis methods are equally effective on treated and untreated ballast water. This study thus supports ongoing efforts to establish uniform methods for ballast water sampling and analysis under the Convention for the Control and Management of Ships' Ballast Water and Sediments adopted by the International Maritime Organization.

2.0 Methods

2.1 Test vessel and experimental design

All trials were conducted onboard the RV Meteor while in transit from Mindelo, Cape Verde to Hamburg, Germany. The RV Meteor is equipped with an Optimarin BWMS consisting of a filter and UV reactor, and multiple sampling ports to facilitate sample collection for compliance monitoring. The ballast water pipe system onboard the RV Meteor comprises DN10 galvanized steel pipes (diameter 100mm) and an uncompensated piston pump with a maximum capacity of 65m³/h. During the sampling experiments the system pressure ranged from 0.2 to 1.6 bar and the mean pump capacity was 56 m³/h resulting in a mean flow velocity of 1.98m/s. The variable pressure and periodic entrance of air into the ballast system presented a challenge for maintaining sub-isokinetic flow according to G-2.

Figure 1 gives a schematic overview of the BWMS with sampling points and valves that can be used to produce different flow paths through the ship's ballast system. Sample point A is located before the BWMS, sample port B is located after the BWMS, and two additional sampling points (C and D) are installed after the BWMS; port C is used for water collection and Port D is used for water return with a closed sampling system. The sampling ports A, B, and C installed in the ballast water pipes were equipped with an "L" shaped sampling probe with a leg facing upstream as required by the IMO Guidelines for Ballast Water Sampling (cf. IMO document MEPC.173(58), October 10th, 2008). At port D a return probe was installed with the opening facing downstream. The sampling probe had a diameter of 25.4mm.

A total of 28 trials were run with sampling devices used in pairs. The BWMS was turned off for 24 trials that focused on examining differences in organism density due to (i) sampling device and sampling port and (ii) analytic method used. During these tests, water was taken directly from the sea with no holding period (sea to sea). The BWMS was turned on for four trials which were used to (i) explore how analytic devices performed at high and low organism concentrations and (ii) examine the ability of analytic tools to rapidly detect the effects of UV treatment. One of these trials used water that had been treated and held in the ballast tank for two days before a second treatment upon discharge. The first UV trial was run with the UV system regulated and the remaining trials had unregulated UV treatment (see Table 2). When the system is run with regulated UV, a sensor evaluates the dose being achieved by the system and adjusts bulb output to achieve the required dose (i.e. if water has high DOC content, system increases bulb output, and in very clear water, system reduces bulb output). In trials with unregulated UV, the system operates at 100% UV power regardless of the water conditions. Unregulated UV was used in the final three trials since air bubbles were observed in the ballast sample collection systems (see Discussion) and it was unknown how these air bubbles might affect the UV regulation. Since our objectives were to examine the ability of the analytic tools to detect the effect of treatment, rather than examine the performance of the BWMS, this setting should not be important for our results. All trials using the BWMS were sampled using a net as the collection device both before and after treatment. Table 2 details sampling devices and water sources for each trial. Table A1 in Appendix A includes more detailed trial information including salinity, temperature, sampling time and locations, ballast water flow rate, and total volume of water that passed through the ballast system during the trial.

2.2 Sample collection

For each trial, the normal ballasting procedures onboard the RV Meteor were used to pump seawater through the ship's ballast water pipe system. The sampling ports already installed on the ballast water pipe system (described above) allowed for the simultaneous collection of paired samples of the same water at two different sampling ports with two different sampling techniques. Each of the three sampling devices have a 'cod' end (plankton net) or inbuilt filter (sampling skids), where the sample of organisms ≥ 50 µm is collected and concentrated using 50 µm mesh. For collection of 10-50 µm organisms, the devices have differing methods. For trials 1-5, the plankton net 'frequent grab' sample was taken by collecting 500 mL of unfiltered ballast water from the sample hose every two minutes for the duration of the collection. For trials 6-28, this water was collected directly from the containment drum after passing through the plankton net; this change was enacted following discussion in the on-board scientific meeting to eliminate a redundant filtration step during sample processing to separate ≥50 µm particles. For the SGS filter skid, a ball valve was installed through which a 'frequent grab' sample was collected from the water which has already passed through the 50 µm inbuilt filter. The Triton filter skid offers two options: (i) to collect a 'frequent grab' sample from the water that has already passed through the 50µm inbuilt filter and (ii) to collect 'continuous drip' samples without the 50µm filtration step; during the course of the voyage, option (i) was used. When possible, sampling devices were rotated

between sampling ports during testing. Since the closed filter skid systems can only be positioned at sample port (C) in order to return backflow water at (D), they were paired with an open method at port position (A). Based on the very short sections of straight pipe and number of bends in the ballast system, theoretically, the flow of ballast water at each sample port location was considered to be turbulent, such that upstream sampling would not affect downstream sampling (Richard et al. 2008). However, for all tests, the sampling port position was recorded to enable the statistical evaluation of any potential influence of sample port position. Devices used and their positions for each trial are detailed in Table 2.

For most trials, open ocean water was pumped through the ballast water pipe system while in transit, with the exception of two trials that tested organism densities in the ship's potable water, one trial that used Mindelo port water that had been held in a ballast tank for three days, and one trial that used treated ocean water that had been stored in a ballast tank for two days (see Table 2). The 'potable water testing' examined water that had been treated using the ship's potable water system and stored in ballast tanks for a period of time prior to testing to evaluate the use of potable water as ballast. During each trial, each collection device filtered ~1000 L of water for analysis of ≥50 µm size class that was condensed to 1 L (some exceptions; see Table A2); the actual volume of water filtered by each device was quantified and recorded so that appropriate densities could be back-calculated for device comparisons. The volume of water filtered was quantified using a Seametrics WMP104-100 magnetic flow meter for net samples, and built-in flow meters for the sampling skids. Additionally, each device collected between 10 and 16 L of water for analysis of 10-50 µm organisms and microbes.

2.3 Sample preparation

For each trial, a common batch of filtered seawater was prepared for use as rinse water by all participants during sample collection and sample analysis. Rinse water was prepared by filtering local seawater water obtained using the vessel's seawater taps through 1000 µm and 500 µm mesh. This filtrate was further sieved through 35 µm and 8 µm plankton mesh filters, and finally, passed through a 0.2 µm passive (gravity-fed) filter cartridge (Whatman Polycap TC).

For each trial, each participant received one sample bottle for each analysis method corresponding to the appropriate size fraction of water required. All sample collection, sample splitting, sample fractionation/sieving was completed in a uniform way, so that observed variability is more likely explained by analysis method rather than sample handling.

For water samples containing taxa ≥ 50 µm, concentration was performed during sample collection, so post-collection processing was not required. To prepare individual sample bottles for each analysis method, each sample was mixed by inverting five times, and then sample bottles were half-filled (7 bottles, total volume 35-300 ml depending on analysis requirements). This procedure was repeated to fill the sample bottles. This splitting procedure (5x inversion, half fill, 5x inversion, fill remainder) was used to fill all sample bottles as detailed below (see also Sample Splitting SOP, Appendix B).

Water samples containing particles < 50 µm were processed to generate the fractions required for the remaining analyses (<50 µm, 10-50 µm, and <10 µm). First, the sample bottles for the < 50 µm fraction were filled (7 bottles, 50-600 ml). The remaining water was filtered on a 10 µm (diagonal) Sterlitech polyester track etch (PETE) membrane filter. The filtrate containing organisms < 10 µm was split into 8 sample bottles (125-500 ml), and the retained particles were resuspended in filter-sterile seawater with a final concentration up to 16x times the original volume (see Table A2). The concentrated sample was split into sample bottles for analysis of the 10-50 µm fraction (9 samples, 25-350 ml; see Sample Splitting SOP, Appendix B).

After each trial, all sampling gear and sample bottles were cleaned in a dilute (100-200 ppm) or concentrated (2500 ppm) commercial bleach bath (depending on equipment hardiness) to prevent cross-contamination of living organisms between tests. After bleaching, all equipment was rinsed with MilliQ water 3 times and nets were soaked in rinse water and hung to dry. Prior to re-use, all sample bottles were rinsed with filtered seawater 3 times to remove any residues.

2.4 Protocol for spiking ballast water samples

Since the numbers of IMO-relevant human pathogen bacteria levels are low in the open ocean, samples were spiked with bacteria in 4 trials in order to better compare analytic methods for bacteria. A stock culture of each *Escherichia coli* and *Enterococcus faecalis* were prepared from type cultures obtained from Public Health England, culture collections UK, by aseptically adding one Lenticule disc to a sterile bijou tube containing 5 ml of sterile TSB medium. Cultures used were *E. coli,* NCTC 9001, Cat # CRM09001L, Batch 2037-15 and *E. faecalis,* NCTC 775, Cat # CRM00775L, Batch 2297-14. The culture was incubated for four hours to resuscitate the organisms under ideal conditions. After four hours, 1 ml of each organism suspension was inoculated into 20 ml of sterile Tryptic Soy Broth (TSB) medium prepared in a diluent of 75% reverse osmosis (RO) water and 25% filter-sterilised marine water and cultured overnight to acclimatise the organisms to high salt environments and reduce osmotic shock when spiking samples of marine water. The resulting cultures were used as "working stocks" and stored at 4 ^{0}C until required for spiking experiments. In parallel, these were assessed visually (experience was used to predict the number of organisms present based on turbidity) and serially diluted in sterile RO:marine water (75%:25%). 0.1 ml of the serial dilutions expected to contain between 10 and 100 cfu were plated onto Tryptose Soya Agar plates in triplicate and incubated overnight at 37 ^{0}C. These were counted and the mean result used to confirm the actual cfu value of the working stocks.

For ballast water trials, working stocks were serially diluted in sterile RO:marine water (25%:75%) to give target values in the bulk sample containers as indicated in the notes column of Table 2. Note that target values are affected by storage and handling of the working stocks, so the cfu values in the ballast water trials should be considered target values rather than exact numbers. Further, there is the potential that bacteria were killed by osmotic shock when mixed into the sample water; this is especially pertinent for trial 6 where bacteria were not acclimatized to the salinity before spiking.

2.5 Sample Analysis

Samples were analyzed in parallel using the analytic methods listed in Table 1. A short description of each method is provided in Table 3 and detailed methodology for each analytic method is provided in Appendix C. In order to minimize potential bias, all samples were provided to analysts in a 'blind' form (i.e. no information given about status as treated/untreated or sample collection method). From each sample bottle, participants were asked to draw three replicate subsamples for measurement. Samples were inverted five times before withdrawing each subsample to ensure samples were well mixed, and all steps of sample analysis were performed independently on each replicate sample. Due to time constraints and operational considerations (e.g. limited materials), not all analytic methods could be performed for all trials. Detailed information about the number of replicates analyzed for each trial by method is provided in Tables 4 through 7 for size classes ≥50 µm, 10-50 µm, <10 µm, and bacteria, respectively.

2.6 Statistical Analysis

All analyses were performed in the R statistical programming environment (R Core Team 2015). To account for any concentration steps performed by individual researchers (see Appendix C), values were standardized to equivalent concentrations in the raw sample where necessary. Further, since sample volumes collected by each sampling device varied, results were also standardized based on the total volume of water filtered by each sampling device (Table A1) for analyses involving sampling skids, ballast water treatment, and potable water testing.

2.6.1 Analytic Device Comparison

To compare results for each analytic device versus traditional microscopy, scatterplots were made that compared all measurements taken for each sample (1 sample per sampling port per trial (i.e. 2 samples per trial); 56 samples total) with replicates randomly paired. A line of best fit was generated using Deming's regression which accounts for error in both variables (Ripley and Thompson, 1987). Since Deming's regression does not provide a method to characterize strength of fit, the Pearson correlation coefficient was used to quantify the strength of the relationship between the two methods.

A model for methods comparisons was used to simultaneously compare results between analytic methods (Carstensen, 2010):

$$y_{mir} = \alpha_m + \beta_m(\mu_i + c_{mi}) + e_{mir}$$

where y_{mir} is the measured value for a given method (m), item (i) and replicate (r), α_m and β_m describe the linear relationship between the true value (μ_i) and the measurement made on item i by method m. The c_{mi} term is a random method by item effect ($c_{mi} \sim N(0,\tau_m^2)$) and e_{mir} represents the residual variation ($e_{mir} \sim N(0, \sigma^2)$). Note that the true value (μ_i) is estimated during the fitting process and is essentially a weighted mean across estimates by different

methods. Deming regression was used to verify model assumptions (constant variance and constant bias), and data were transformed when necessary to meet assumptions. Where logarithmic transformations are indicated, these refer to the natural log. Where bias was not constant (all analyses herein), Markov Chain Monte Carlo methods were used to fit the model which can account for linear bias between methods. Model validation was performed graphically by inspecting the traces and posterior densities for convergence. Note that not all methods could be analyzed using these methods due to inability to meet the assumptions of constant variance using transformation. These analyses were performed in R using the *MethComp* package (Carstensen et al. 2015).

2.6.2 Sampling Device Comparison

Sampling devices were compared using linear mixed effect models with fixed effects for sampling device and sampling port, and random effects for analytic device and trial. Measurement data from each analytic tool was z-transformed to standardize measurements from each device to mean = 0 and standard deviation =1 so that all devices influenced results equally (i.e. devices that report results with a high magnitude do not influence results more than those that report in units with a low magnitude). Models were run separately for each size class using *lme4* (Bates *et al*., 2015) in R (R Core Team, 2015). Variance structures were specified to account for different variances for both trials and analytic tools. Model selection was done using likelihood ratio tests and model assumptions were validated by visual inspection of residuals and using Levene's test. P-values for the effect of sampling port were obtained using likelihood ratio tests comparing models with and without sampling port included. P-values for differences between sampling devices were obtained by running pairwise Tukey contrasts using the general linear hypothesis testing function in the *Multcomp* package (Hothorn *et al.,* 2008).

For all analyses, trials 1 and 2 were excluded due to errors in sample handling (see Table 2), and trials that compared water before and after treatment were excluded since treatment negates a paired comparison between collection devices. For the ≥ 50 µm size class, Welschmeyer ATP, bulk FDA, and Hach BW680 results were excluded from the model since they were used infrequently and caused the assumption of residual homogeneity to be violated. For the 10-50 µm size class, trials 1 through 5 were excluded since net samples for trials 1-5 did not exclude taxa ≥50 µm.

2.6.3 Analysis of Samples before and after Treatment

In four trials, water was collected before and after treatment with a BWMS. One-way t-tests with Welch's correction for unequal variance were used to determine if each analytic method was able to detect a decrease in organism density immediately following treatment.

2.6.4 Potable Water Testing

One-way t-tests were used to determine whether the organism densities found in the tanks containing 'potable water' exceeded the IMO D-2 standards. For these tests, data from

all analysis methods and sampling devices were pooled, with the exception of data for which conversions to individuals by volume were unavailable (i.e. ≥50µm: ATP, PAM, bulk FDA; 10-50 µm: ATP, PAM, bulk FDA).

3.0 Results and Discussion

All trials were conducted during transit from Mindelo, Cape Verde to Hamburg, Germany (Figure 2). Natural variation in the plankton communities in the water led to a great difference in the densities of individuals of each size class found throughout the voyage. The beginning of the cruise was mostly spent in oligotrophic water where biological variation was low and organism concentrations were at or below ballast discharge standards. Near the end of the cruise, very high densities were reached which were more suitable for testing water before/after treatment. Organism densities in the ≥50 µm size class ranged between 1 individual/m^3 and ~800,000 individuals/m^3 (as estimated by microscopy) and organism densities in the 10-50 µm size class ranged between 0.3 and 60.3 individuals/mL (as estimated by microscopy after FDA staining). While the phytoplankton biomass varied largely, the phytoplankton viability, based on photosynthetic efficiency, remained fairly constant (based on flow cytometry results) and, with one exception (trial 7), the Fv/Fm ranged from a value of 0.3 to 0.65, indicative of healthy phytoplankton (Figure 3). The relative contribution of the 10-50 µm fraction was on average ~10% of the total phytoplankton counts (based on flow cytometry results).

For each trial, water samples containing organisms of varying size classes were collected and split for analysis. Due to random chance, it is not expected that every subsample measured would contain the exact same number of organisms. Instead, the true number of individuals in a sample is expected to be represented by a distribution of possibilities where the mean is equal to the true number of individuals and the variance equals the mean (i.e. Poisson distribution; Haigh, 1967). Variation in measurements between subsamples is therefore expected, and the amount of variation is expected to increase as the true population density increases. Importantly, as a result, observed differences between measurements may represent a combination of true differences between subsamples and measurement error.

3.1 Comparison of Analytic Devices

Information regarding the time requirements, costs, training requirements and portability of each analytic method tested is provided in Table 7.

3.1.1 Comparison of analytic devices for organisms ≥ 50 µm in dimension

Eight methods were used to analyze water samples containing particles ≥ 50 µm:

- microscopy (visual inspection for motile organisms; staining for phytoplankton), Satake Pulse Counter, bulk FDA,
- two methods for PAM, measuring only phytoplankton,
    - Hach BW680

    - Walz-Water PAM)
- two methods for ATP
    - Welschmeyer ATP method
    - SGS ATP (aqua-tools) method.

In general, most analytic methods were sensitive enough to detect organisms in the water samples at a range of densities (i.e. most analytic devices produced non-zero values where the microscope method detected live individuals; Figure 4; Deming regression coefficients are shown in Table 9). Since most measurement tools were unable to estimate the number of individuals in samples, it is not possible to determine whether the devices are equivalent to microscope measures. The Satake Pulse Counter is the only method which was able to provide density estimates in individuals/volume. This method tended to underestimate the density of individuals as compared to microscope counts (see Figure 4; most data points fall below 1:1 line). Notably, some other analytic methods used here (e.g. SGS ATP (aqua-tools)) may be able to estimate organism density in the future, but this is still under development.

A method comparison model was run to compare log+1 transformed data from SGS ATP (aqua-tools), microscopy, Walz-Water PAM, and the Satake Pulse Counter (Figure 5; Full model details in Appendix E.1); Hach BW 680, Welschmeyer ATP, and Bulk FDA methods were excluded due to limited data (see Table 4). The model allows measurement conversion between devices, but the confidence interval is wide due to uncertainty in the conversion. Note that while residual variation is reported by the model (Appendix E.1), it is not appropriate to draw conclusions on the precision of instruments based on these values since measurements are reported in different units.

An additional analysis was done to examine the results of the Optical Zooplankton Analyser (OZA) which counts zooplankton that are > 200 µm in size. Results were compared both to the microscope counts for zooplankton only and the total microscope counts (motile taxa and stained taxa). In both cases, the OZA underestimated organism densities (Figure 6), but correlation was much higher with zooplankton only microscope counts (r=0.574 vs 0.107).

3.1.2 Discussion of results for organisms ≥50 µm in dimension

During the voyage, it was observed that phytoplankton were present, often at high abundance, in the ≥ 50 µm size class. In response, the microscopy methods were adjusted to count phytoplankton ≥ 50 µm using FDA staining since movement is not a reliable indicator of viability for non-motile taxa. Essentially, a second count was added so that results included two sets of counts: (i) motile taxa counted visually, and (ii) non-motile taxa counted with FDA stain. Examinations of the efficacy of FDA stain for marine phytoplankton have focused on the 10-50 µm size class (e.g. Steinberg *et al.* 2011) and error rates for larger phytoplankton are unknown. Currently, most ballast water counting protocols do not add this extra step and instead count ≥50 µm phytoplankton at the same time as motile counts, with the assumption that intact cells are viable (e.g. First *et al.*, 2015). Based on results from this voyage, these two methodologies may yield very different results, suggesting that this issue should be considered further in future work.

The composition of the plankton communities is likely to affect the level of concordance between analytic methods. Since some methods used herein (e.g. PAM methods) are only able to detect phytoplankton, it is possible that results for total organism counts would be very different between phytoplankton-only methods and total organism methods if the samples had not been dominated by phytoplankton during this voyage. However, note that phytoplankton-only methods can indirectly detect zooplankton if phytoplankton have been consumed by the zooplankton.

3.1.3 Comparison of analytic devices for organisms in the 10-50 µm size class

Nine methods were used to analyze water samples containing 10-50 µm particles:

- microscopy (FDA staining); direct counts,
- Satake Pulse Counter; direct counts
- bulk FDA; indicative method,
- four methods based on different properties of the autofluorescence of the chlorophyll in phytoplankton
    - Hach BW680; indicative method converted into numbers
    - bbe 10cells; indicative method converted into numbers
    - BallastCheck2; indicative method converted into numbers
    - Walz Water PAM, indicative method
- flow cytometry; direct counts (counts both live and dead organisms), and
- Welschmeyer ATP; indicative method.

Five methods were able to estimate the number of individuals in samples: Hach BW680, 10cells, BallastCheck2, flow cytometry, and the Satake Pulse Counter. 10cells and the BallastCheck2 had the strongest correlation with microscopy results (Figure 7; Pearson correlation coefficient= 0.92 and 0.90 respectively). The regression line for the BallastCheck2 matched the 1:1 line most closely indicating that the device was neither systematically under- or over-estimating organism densities (Table 10). The Satake Pulse Counter underestimated organism densities as compared to microscope measurements (see Figure 7; most data points fall below 1:1 line) and flow cytometry overestimated organism densities as compared to microscopy (see Figure 7; most data points fall above the 1:1 line). The flow cytometer counts both live and dead organisms, so this is not unexpected. Many samples analysed by the Hach BW680 were below its detection limit; in 17 trials it did not detect a signal in any replicate where the microscope method detected viable organisms. In contrast, 10cells and BallastCheck2 methods reported zeros where the microscope method reported viable organisms at most once (i.e. in one replicate). For methods that did not report results in individuals/volume, the Walz-Water PAM had the strongest correlation with microscopy results (Pearson's correlation coefficient= 0.879).

Concentrations estimated by PAM devices are based on conversion factors which are particular to each device. To eliminate this source of variation, PAM devices were each compared to each other and to microscopy counts using their raw output, Fv (Figure 8). The Fv

values for these devices had high levels of correlation (range 0.75-0.96) with BallastCheck2 and 10cells showing the highest similarity. Fv values were also highly correlated with microscope densities (r=0.82-0.87; Figure 8).

A method comparison model was run to compare methods. The model compared log+1 transformed data from the 10cells, Hach BW680, BallastCheck2, Walz-Water PAM and microscopy (Figure 9; full model details in Appendix E.2). The slope of each conversion curve varies around 1 and the 95% confidence intervals show a much tighter fit than was observed for the ≥50 µm fraction indicating greater certainty in the conversion lines.

3.1.4 Discussion of results for organisms in the 10-50 µm size range

Results from the PAM fluorometers (i.e. 10cells, BallastCheck2, Walz-Water PAM and Hach BW680) were highly correlated with microscope results, but the fluorometers differed in their sensitivity (Figures 7, 8). PAM fluorometers measure F_o, total chlorophyll biomass, and F_m, total fluorescence after excitation and use these values to estimate F_v, which relates to live chlorophyll fluorescence ($F_v=F_m-F_o$). Chlorophyll biomass estimates based on F_v reduce the sensitivity of the instrument response, because the noise in each measure is combined making it easy to generate 'negative' values. Fluorometers in this category, such as the Hach BW680, are thus less sensitive. The 10cells also bases its estimates on F_v, but minimizes sensitivity loss by increasing signal strength through filtration.

PAM results (F_v,F_m, or F_o) can be converted to densities in cells/mL based on an internal calibration coefficient that is determined at the factory or by the end-user. The calibration constant is specific to each device, and consequently, different PAM devices can generate different density estimates even if they have the same fluorescence reading. This variation in calibration factors affects the slope between devices (Figure 7), and for this reason, results were analyzed both by looking at the raw fluorescence measurements (specifically, F_v; Figure 8) and by looking at the calculated cell densities which can be compared to microscopy counts (Figure 7).

In reality, the true relationship between fluorescence and cell density varies between algal communities depending on chlorophyll cellular content (can vary by a magnitude of 5x due to photoadaption and an additional 5x due to nutrient limitation/saturation) and the size of cells in the community (Veldhuis et al, 1997). Cell size in the 10-50 µm group represents a range of cell volumes (biomass) of potentially 125x (volume = $4/3\ \pi\ r^3$); as such there is no 'standard' cell or community to which these devices are calibrated. During the METEOR voyage, the average cellular chlorophyll fluorescence of the entire phytoplankton community, and the 10-50 µm fraction in particular, varied by a factor of 2 to 3, with fluorescence increasing, in general, during the course of the voyage, with the exception of trials 10 through 14 when the average chlorophyll fluorescence was at its lowest (Figure 10). Variation in chlorophyll content per cell is a major source of error for estimating cell numbers on the basis of bulk chlorophyll. Simply, if cells have a higher chlorophyll concentration, fewer cells are needed for the same amount of chlorophyll fluorescence.

To examine this further, the number and size of phytoplankton cells and their chlorophyll fluorescence were determined by applying flow cytometry (Veldhuis & Kraay, 2000) to formalin-preserved samples. This data allows the determination of an independent chlorophyll fluorescence biomass (FCMchlor) which is distinct from that determined using standard fluorometry (e.g. WALZ Water PAM), and can be compared with the chlorophyll fluorescence properties (F_0) of the PAM-type of fluorometers. For the METEOR voyage, the total number of phytoplankton cells (all sizes and < 10 µm) show a reasonable correlation with the bulk florescence as measured with the WALZ-PAM. In contrast, the relationship between cell number and chlorophyll biomass for 10-50 µm phytoplankton is very poor (Figure 11). If the average cellular chlorophyll fluorescence of each cell is considered (recall that it varied throughout the voyage), we see a substantial increase in the relationship with total biomass, especially for the 10-50 µm fraction (i.e. the size class of interest; Figure 12). This implies that variation in size and therefore cellular chlorophyll content can have a significant effect on the relationship between fluorescence signal and cell number, and a simple conversion value for chlorophyll fluorescence to cell numbers will therefore not be completely reliable.

3.1.5 Comparison of analytic devices for taxa <10 µm (excl. bacteria)

Five methods were used to analyze water samples containing < 10 µm particles: Walz Water PAM, BallastCheck2, bulk FDA, Hach BW680, and Welschmeyer ATP. Since microscopy data was not available, each method was compared to both Water PAM and BallastCheck2 since they were analyzed in all trials (Figure 13; Table 11). All methods showed some level of positive correlation with both Walz-Water PAM and BallastCheck2, but many samples registered below the detection limit of the Hach BW 680. A method comparison model was run to compare data for BallastCheck2, Bulk FDA and Welschmeyer ATP (Figure 14; full model details in Appendix E.3).

3.1.6 Discussion of results for taxa < 10 µm (excl. bacteria)

While many methods were used to analyze the < 10 µm fraction during the voyage, only PAM and BallastCheck2 were used consistently. There is a strong relationship between results for PAM and BallastCheck2, but there are a considerable number of outliers. More complete data from other methods would be useful to make inferences about these non-matching data points. Since all measurements are reported in different units, it is not possible to make inferences about the relative precision of each method.

3.1.7 Comparison of analytic devices for bacteria

Methods for measuring bacteria focused on either total bacteria (IDEXX Simplate and SGS ATP (aqua-tools), *Enterococcus* (IDEXX Enterolert, Speedy Breedy Enterococcus), or *E. coli* (Triton BW BacTest EC, IDEXX Colilert, Speedy Breedy *E. coli*). Bacteria levels were low throughout the voyage, with nearly all samples indicating levels of *E. coli* and *Enterococci* that were below the detection threshold for all measurement instruments except when samples were spiked (see methods). Analytic tools to detect total bacteria (IDEXX Simplate and SGS ATP (aqua-tools) were able to detect bacteria throughout the voyage, but there was low correlation

between measurements by each method (Figure 15; r=0.239). For many trials, SGS ATP (aqua-tools) detected bacteria when IDEXX Simplate readings were below the detection limit. Note that the detection limit of IDEXX Simplate varies depending on dilution used and that the SGS ATP (aquatools) methodology is designed to detect all 'living' ATP, whereas IDEXX Simplate is specific to bacteria that grow at 35°C.

Similarly, there was no clear relationship between the amount of *E. coli* measured by Speedy Breedy *E. coli* and Triton BW BacTest EC (Figure 16), though both devices detected *E. coli* in 3 of 4 trials where E. coli was spiked (trials 12, 21, and 22; note that bacteria may have been killed by osmotic shock in trial 6 because acclimation was not performed). In contrast, IDEXX Colilert measured all samples as below the detection limit with the exception of 1 sample in trial 4 where neither Speedy Breedy nor Triton BW BacTest EC detected *E. coli*.

Measurements of *Enterococcus* by IDEXX Enterolert and Speedy Breedy Enterococcus had a much stronger agreement (Figure 17; r=0.765) within their detection limits. Both devices detected *Enterococci* in trials 12, 21, and 22 (where it had been spiked) and IDEXX Enterolert additionally detected *Enterococci* in trial 6 (where it had been spiked) and trial 15 (at a low level).

3.1.7 Discussion of results for bacteria

Since bacteria levels were low throughout the voyage, we had limited data to use for comparison between these analytic methods. While bacteria spiking was used to ensure bacteria were present in some samples, there are at least two potential issues with this approach. First, it is expected that salinity shock killed bacteria in at least the first spiking experiment. While steps were taken to acclimate bacteria in subsequent trials (see methods), it is possible that salinity shock continued to be an issue and could influence differences between results depending on the length of exposure prior to analysis. Second, after the voyage, it was noticed that the strain of *E. coli* used for spiking may be lacking the glucuronidase enzyme needed for detection by IDEXX Colilert.

3.2 Comparison of Sampling Devices

Since analytic tools reported measurements in various units (e.g. individuals/mL, pgATP/mL, fluorescein production rate) and all of this data was pooled and transformed to analyze the effect of sampling device (see methods), the outcome variable which relates to organism density is not readily interpretable; instead it represents a mean difference in measurements which can be attributed to sampling device. Thus, while it is possible to detect whether there are differences between the sampling devices, the amount of difference between the methods is unintelligible and we are unable to comment on whether a significant difference is operationally relevant.

3.2.1 Results for sampling devices

For the ≥ 50 µm size class, there was a significant effect of both port ($L = 9.8$(df=1, $p<0.05$)) and sampling device ($L=23.4$ (df=4, $p<0.001$)). Densities of samples collected using the SGS skid (open or closed) and the Triton skid (closed) were not significantly different from samples collected using the net (Tukey contrasts, all $p>0.05$; Figure 18), however samples collected using the Triton skid in the open configuration were significantly lower than those collected using the net (Tukey contrast; $p<0.001$; Figure 18).

For the 10-50 µm size class, there was a highly significant effect of sampling device ($L=28.3$ (df=4, $p<0.001$)), but no significant effect of sampling port ($L=0.2$ (df=1, $p>0.05$)). Densities of samples collected using the SGS skid (closed configuration) and Triton skid (open and closed configuration) were not different from net samples (Tukey contrasts; all $p>0.05$). Organism densities were slightly higher in samples collected using the SGS sampling skid (open configuration) than samples collected using the net (Tukey contrast; $p<0.01$; Figure 19).

For the < 10 µm size class, there was no significant effect of sampling device ($L=4.6$ (df=4, $p>0.05$)), but there was a significant effect of sampling port ($L=3.9$ (df=1, $p<0.05$).

3.2.2 Discussion for sampling devices

Our experiments were designed to replicate the operational use of sampling skids as much as possible. While IMO rules state that isokinetic sampling is not strictly necessary, it is important to ensure representative sampling and to minimize stress on organisms. The ISO standards for ballast water sampling (2013) and a US Naval Research Lab report on this ISO standard (Drake *et al.*, 2015) require sampling probe diameters between 1.5 to 2.0 times the isokinetic diameter to facilitate sampling at sub-isokinetic speeds. As such, we attempted to sample accordingly throughout the voyage and we were largely successful. However, in three trials isokinetic sampling was not achieved. In trials 1 and 2, the ballast pump was pumping very slowly (17 and 29 m^3/hr; compared to overall average of 57 m^3/hr during voyage). This was not recognized during sampling, so we were unable to alter our sampling rate to compensate. Resultantly both SGS and Triton sampled at super-isokinetic speeds (in trial 1 and trials 1 and 2, respectively), since pressure differences between the ballast system and the atmosphere vented through the sampling system as the path of least resistance (this is only possible when sampling skids are in the open configuration). Additionally, in trial 26 (net/net sampling), flow rates were super-isokinetic for both nets. At super isokinetic speeds, samples are expected to contain lower densities, but there should not be a difference between devices at similar velocities. These trials had already been excluded from the sampling device analysis due to methodology (trials 1 and 2, see table 2) or because they sampled using the same device (trial 26), so these violations of isokinetic sampling will not have any influence on our results.

In a related matter, the actual flow velocity in relation to the isokinetic velocity may be an important factor to consider. During the METEOR voyage, most sampling devices sampled at roughly the same rates (mean sampling velocities ranged between 43.53 m^3/h and 56.48 m^3/h for different sampling devices), but in some cases sampling rates were much lower (e.g. Triton closed system, mean 24.43 m^3/h, about 1/3 speed of the paired device). We were not able to

explicitly analyze the effect of sampling speed herein, but additional research may be warranted to determine whether it influences the density of organisms captured.

Operational challenges experienced during the course of the voyage may have affected the performance of the sampling devices. For example, during several trials, the filter cup of the Triton skid and the net sampling system were observed to contain a massive intrusion of micro-air bubbles. While the filter housing of the SGS skid is not transparent, it may be assumed that the SGS sampling system experienced the same issue. This would presumably have a considerable impact on the pressure regime in the BW pipe system, and could have exposed plankton organisms to hydrodynamic stress and impacted their viability (L. Schillak, pers. comm). This impact should have been comparable across sampling methods, so it should not influence our conclusions with respect to differences between devices. The air bubbles may have also had an effect on flow meter readings and since each device used a different type of flow meter (i.e. paddle wheel, magnetic, and positive displacement), these impacts may not be consistent across sampling devices. It is expected that any differences between flow meters would not have a large effect on the results (if correctly installed, max 2% error; C. Gianoli, pers. comm.).

Moreover, during several trials there was a high amount of sediment in the water. The IMO G2 regulations, Annex 3, para 6.2.3., page 5 state that "*the sampling protocol should take account of the potential for a suspended sediment load in the discharge to affect sample results*". Each skid developer has a different perspective on how high organism densities or high sediment load should affect sampling. From the SGS perspective, it is expected that samples with a high concentration of particles could be compliant and thus the SGS skid is designed to maintain operations even when particle loads are high. In contrast, Triton assumes that a blocked filter is a symptom of non-compliance and sampling should be stopped, since either a high sediment load or a high organism density during sampling would generate irreversible negative impacts on the vitality of the live organisms targeted for analysis. During the METEOR voyage, the filter of the Triton skid was clogged by very high organism densities and the filter cartridge jumped off its seat and became inoperational; this would be interpreted as a compliance failure.

3.3 Results and Discussion for Analytic Methods Before and After Treatment

A significant decrease in organism densities after treatment was detected by each analytic method in both size classes (≥50 µm and 10-50 µm; Figures 20, 21; one-way paired t tests on log+1 transformed data with Welch's correction for unequal variance; all $p<0.05$), with the exception of bulk FDA (≥ 50 µm and 10-50 µm), Welschmeyer ATP (≥50 µm), and flow cytometry (10-50 µm). Note that these methods did detect a decrease in organism densities, but due to limited usage may not have had enough statistical power to detect a difference. In particular, the PAM methods and the Satake Pulse Counter showed the greatest decrease in detection post-treatment.

For trials where treated water was held in the ballast tank for two days before being re-treated, all methods showed a decrease in organism density after both treatments (Figures 22,

23 for ≥ 50 µm and 10-50 µm, respectively), but some devices registered an increase in organism concentrations during the two-day hold period. This may be the result of a true increase in organism densities in tanks or it may be the result of leaky valves (see potable water testing results). Alternatively, since tanks were not cleaned before the trials, it is also possible that the increase is simply due to organisms already being present in the tank's residual ballast before the treated samples were added.

It is important to note the minimum detection limits (see Table 8) when comparing results, since instruments that are unable to detect low organism densities may appear to show a greater effect of treatment simply because they are registering zero values when only a few organisms remain.

3.4.1 Results and Discussion for Potable Water Testing

The 'potable water testing' trials examined water that had been treated using the potable water system and stored in ballast tanks for a period of time prior to testing. These trials were undertaken to evaluate the potential to use potable water as ballast (IMO, 2013). For organisms ≥ 50µm, the D2-Standard requires a discharge of < 10 individuals per cubic metre of ballast water. Pooled data indicate that the organism concentration exceeded the D-2 standard (Figure 24; $t(23)=4.73$, $p<0.001$). Since density estimates were quite different between analytic devices (Table 12), we re-ran the analysis to determine if results would be the same if we were only analyzing the samples using traditional microscopy. Microscopy results also indicate that the organism concentration exceeded the D-2 standard ($t(11)=2.63$, $p=0.011$).

For 10-50 µm organisms, the D-2 standard requires a discharge of <10 individuals per milliliter of ballast water. Pooled data across all devices indicate that the organism concentration complies with the D-2 standard for this size fraction (Figure 24; $t(82)=-5.50$, $p>0.05$). Density estimates for each analytic device are shown in Table 13.

Importantly, salinity readings for these trials indicated that the water tested was brackish (trial 1= 13.8 ppt, trial 2=23.5 ppt), which is not consistent with potable water. As such, there is speculation that the water tested may have been a mix of potable water and ballast water, potentially due to leaking between tanks or because tanks were not completely empty when the potable water was introduced. Furthermore, in the second trial, the rinse water did not match the salinity of the test water (see Table 2). This may have caused stress to organisms in the ≥ 50 µm fraction, which may have resulted in death of some organisms, but this would not change the outcome of these results since taxa concentrations exceeded the threshold nonetheless.

Tables and Figures

Table 1. List of analysis methods conducted on the METEOR voyage, the size class targeted, and the participant(s) responsible for conducting the analysis.

Analysis Method/Type	Size Class (µm)	Participant
Optical zooplankton analysis	>200	Wouter van der Beek
Microscopy (movement + FDA staining)	≥50	Sarah Bailey
Satake Pulse Counter	≥50	Satake
MLML (steamlined) bulk FDA method	≥50	Nick Welschmeyer
ATP assay with improved extraction protocol	≥50	Nick Welschmeyer
SGS ATP (aqua-tools)	≥50	SGS
WALZ-Water PAM	<50	Marcel Veldhuis
Microscopy (FDA)	10-50	Julie Vanden Byllaardt
Ballast Check™ 2 (Turner fluorometer)	10-50	Lawrence Younan
10cells (PAM fluorometer)	10-50	André Zaake
Hach BW680 (PAM)	10-50	Nick Welschmeyer
Satake Pulse Counter	10-50	Satake
ATP assay with improved extraction protocol	10-50	Nick Welschmeyer
MLML (steamlined) bulk FDA method	10-50	Nick Welschmeyer
WALZ-Water PAM	10-50	Marcel Veldhuis
ATP assay with improved extraction protocol	<10	Nick Welschmeyer
MLML (steamlined) bulk FDA method	<10	Nick Welschmeyer
Hach BW680 (PAM)	<10	Nick Welschmeyer
WALZ-Water PAM	<10	Marcel Veldhuis
Flow cytometry after staining nuclear DNA	Bacteria	Marcel Veldhuis
IDEXX Simplate	Bacteria	SGS
SGS ATP (aqua-tools)	Bacteria	SGS
Speedy Breedy	*Enterococcus*	Derek Price
IDEXX Enterolert	*Enterococcus*	SGS
TRITON BW BacTest EC	*E. coli*	Lothar Schillak
IDEXX Colilert	*E. coli*	SGS
Speedy Breedy	*E. coli*	Derek Price

Table 2. Details for trials run on board the METEOR including the trial number (#), the sampling devices and ports used, the source for the water pumped through the ballast system and the destination for the water after being pumped through the ballast system, whether the OptiMarin BWTS was turned on (+) or off (-) and whether it was regulated/unregulated (see methods), and notes regarding spiking and any issues experienced during the trial.

#	Date	Sampling Device combination				Source	Destination	Treatment	Notes
		Port A	Port B	Port C	Port D				
1	4.6.2015	Triton		SGS		potable water tank 9	Ocean	-	Data only useful for comparison between analytic methods (not sampling methods), since size fractions for S2 were mixed up in splitting.
2	5.6.2015	Triton		SGS		potable water tank 9	Ocean	-	Data only useful for comparison between analytic methods, since different rinse water was used in engine room for ≥50 µm samples (SGS used 13 ppt rinse water, TRITON used 38 ppt).
3		Triton		Net		Ocean	Ocean	-	Net sample for the 10-50 fraction included all organisms >10. This may introduce variation between results for this fraction.
4	6.6.2015	Net		Net		Ocean	Ocean	-	Net sample for the 10-50 fraction included all organisms >10.
5		SGS		Net		Ocean	Ocean	-	Net sample for the 10-50 fraction included all organisms >10. This may introduce variation between results for this fraction.
6	7.6.2015	Triton		Net		Ocean	Ocean	-	Bacteria samples were spiked with target densities of 500 cfu/100 ml E. coli & 100cfu/100ml E. faecalis in sample 1 and 100 cfu/100 ml E. coli & 500 cfu/100ml E. faecalis in sample 2.
7		Net		SGS		Mindelo water tank 9	Ocean	-	

8		SGS		Net		Ocean	Ocean	-	
9	8.6.2015	Triton		SGS - closed		Ocean	Ocean	-	
10		Net		SGS - closed		Ocean	Ocean	-	
11		Net		Net		Ocean	Ocean	-	
12	9.6.2015	SGS		Triton - closed		Ocean	Ocean	-	Bacteria samples were spiked with target densities of 500 cfu/100 ml E. coli & 50cfu/100ml E. faecalis in sample 1 and 50 cfu/100 ml E. coli & 500 cfu/100ml E. faecalis in sample 2.
13		Net		Triton - closed		Ocean	Ocean	-	
14		Net		Triton		Ocean	Ocean	-	
15	10.6.2015	Net	Net			Ocean	Tank 9	+ Regulated	
16		Net	Net			Ocean	Tank 10	+ Unregulated	
17		Net		SGS - closed		Ocean	Ocean		
18	11.6.2015	Net	Net			Ocean	Forepeak	+ Unregulated	
19		Triton		SGS		Ocean	Ocean	-	
20		Net		SGS - closed		Ocean	Ocean	-	
21	12.6.2015	SGS		Triton - closed		Ocean	Ocean	-	Bacteria samples were spiked with target densities of 100 cfu/100 ml *E. coli* & 500cfu/100ml *E. faecalis* in sample 1 and 500 cfu/100 ml *E. coli* & 100 cfu/100ml *E. faecalis* in sample 2. Triton skid malfunctioned due to high densities causing clogging. Obtained <50µm samples from both sampling ports, but ≥50µm for SGS only.
22		Net		SGS - closed		Ocean	Ocean	-	Bacteria samples were spiked with target

									densities of 50 cfu/100 ml *E. coli* & 50cfu/100ml *E. faecalis* in sample 1 and 250 cfu/100 ml *E. coli* & 250 cfu/100ml *E. faecalis* in sample 2.
23		Net		Net		Ocean	Ocean	-	
24	13.6.2015	Net		SGS - closed		Ocean	Ocean	-	
25		Net		Net		Ocean	Ocean	-	
26		Net	Net			Forepeak	Ocean	+ Unregulated	
27	14.6.2015	SGS		Net		Ocean	Ocean	-	
28		Net		SGS - closed		Ocean	Ocean	-	

Table 3. Brief description of analytic methods used on the METEOR voyage. Full description and SOP is available in Appendix C.

Analysis Method	Description
Optical zooplankton analysis (>200 µm)	The OZA method distinguishes viable zooplankton larger than 200µm from dead organisms and debris based on their swimming capability by analyzing successive images. Samples are placed in the OZA device and analyzed by capturing images and processing them using MATLAB.
Microscopy (movement; ≥50 µm)	Water samples are placed in a Bogorov chamber and live zooplankton (≥50) are enumerated by observing movement.
Microscopy (staining; ≥50 µm and 10-50 µm)	Samples are stained using FDA to identify live phytoplankton and counted using an epifluorescence microscope.
Satake Pulse Counter (≥50 µm and 10-50 µm)	The Satake pulse counter employs pulse counting FDA method to estimate the number of viable organisms in ballast water samples by counting fluorescence pulses over specific threshold from FDA stained organisms. Samples are placed in the portable pulse counter system and an estimated number of viable organisms is displayed on the screen after one minute.
MLML (streamlined) bulk FDA method	The technique is a 'live' incubation. The organisms are captured on a GF/F filter (representing the 'volume filtered'). The filter is placed in an incubation fluid (tagged with FDA). the activity of the organisms converts the FDA to fluorescein and it is mixed and homogenized within the full volume of the incubation buffer (2 mL). We remove a small aliquot of the endpoint incubation buffer to determine the Fluorescein concentration within that 2 mL volume. From the final [fluorescein] concentration, taking into account the seawater volume filtered and the incubation volume we calculate the fluorescein production rate, ng fluorescein/(L h), and the concentration of live cells can be calculated.
Welschmeyer ATP	The technique parallels bulk FDA, but it is NOT an incubation; it is an extraction. Quantitative volumes of seawater are captured on the GF/F filter, the filter is immersed in a small volume (1.2 mL) of strong extraction fluid and after a 1 hour 'soak time' the extraction tubes were frozen (on the METEOR) and analyzed in batches after thawing. The extraction fluid is mixed thoroughly and measured for luminescence in the presence of luciferase enzyme; the luminescence produced is linearly proportional to ATP concentration, as standardized by ATP standards.
SGS ATP (aqua-tools)	Aqua-tools Ballast Water kit enable measuring in few minutes the whole living flora in collected samples (≥50µm; ≥10 and <50µm; and bacteria indicators fractions) for a rapid assessment of your ballast water quality. This test kit allows a rapid estimation of all biological contaminant through the quantification of bioluminescent signal coming from the reaction of the Luminase™ with intracellular Adenosine TriPhosphate (cATP) – the energy carrier of any living cell.
WALZ-Water PAM	The WALZ-Water PAM is a desktop PAM device that is used to estimate phytoplankton biomass and photosynthetic activity. Measurements can be performed using whole water samples or size fractionated or otherwise collected water samples containing phytoplankton.

Ballast Check™ 2 (Turner fluorometer)	Turner Designs' Ballast Check-2 is a quick indicative compliance tool used for estimating the abundance and assessing the viability of phytoplankton from the 10-50 micron size class. This instrument uses the organism's fluorescence characteristics to provide a quick analysis of whether ballast water is in compliance with current discharge standards.
bbe 10cells (PAM fluorometer)	10cells is an indicative instrument for analyzing living cells based on variable fluorescence (Fv) of chlorophyll of living algae. Phytoplankton is filtered onto a filter paper which is placed inside the 10cells for measurement. The 10cells displays the estimated number of living cells per ml.
Hach BW680 (PAM)	Hach BW680 is a pocket-sized, battery operated fluorometer which uses PAM to determine the average variable fluorescence response. Water samples are deposited into a cuvette and the device displays BWI values and estimated risk. Live cell concentrations can be estimated based on BWI.
Bacteria- Flow cytometry after staining nuclear DNA	Bacterial DNA is stained using PicoGreen and samples are run through the flow cytometer. Flow cytometer counts for bacteria are calculated by using the size and presence/absence of chlorophyll to distinguish bacteria from phytoplankton.
IDEXX Simplate	SimPlate* for HPC method is used for the quantification of heterotrophic plate counts (HPC) in water. It detects viable bacteria in water by testing for the presence of key enzymes. The sample and media are added to a SimPlate, incubated, and then examined for fluorescing wells. The number of fluorescing wells corresponds to a Most Probable Number (MPN) of total bacteria in the original sample.
Speedy Breedy (Enterococcus and E. coli)	Speedy Breedy is a portable, sensitive, precision respirometer which detects and monitors microbial activity. Samples are added to a culture vessel containing a suitable nutrient medium and where present, contaminating microorganisms begin to grow. Speedy Breedy detects pressure transients due to metabolic processes where microbes are present.
IDEXX Enterolert	Enterolert* is based on IDEXX's patented Defined Substrate Technology* (DST*). When enterococci utilize their ß-glucosidase enzyme to metabolize Enterolert's nutrient-indicator, 4-methyl-umbelliferyl ß-D-glucoside, the sample fluoresces. Enterolert detects enterococci at 1 cfu per 100 mL sample within 24 hours
TRITON BW BacTest EC	The Triton BW Bac Test EC method was derived from PCR. Similar to PCR, primers designed for this test detect a group of genes within E. coli and induce the multiplication of an RNA with integrated fluorescein which gives a signal in a fluorometer.
IDEXX Colilert	Colilert-18 either simultaneously detects total coliforms and E. coli or fecal coliforms in water. It is based on IDEXX's patented Defined Substrate Technology*(DST*). When total or fecal coliforms metabolize Colilert-18's nutrient-indicator, ONPG, the sample turns yellow. When E. coli metabolize Colilert-18's nutrient-indicator, MUG, the sample fluoresces. Colilert-18 can simultaneously detect these bacteria at 1 cfu/100 ml within 18 hours even with as many as 2 million heterotrophic bacteria per 100 ml present.

Table 4. Total number of replicates run for each trial for each analytic device for measurement of organisms ≥50 µm in minimum dimension. Note that this number includes the sum total of replicates taken for both samples collected in the trial.

Trial #	Wels. ATP	SGS ATP (aqua-tools)	Bulk FDA	Hach BW680	Microscopy visual	Microscopy stain	Walz-Water PAM	Satake Pulse Counter
1	6	6	6	0	6	0	0	6
2	4	6	4	0	6	0	0	6
3	4	6	4	5	6	0	0	6
4	4	6	4	6	6	0	0	6
5	6	6	6	6	6	3	0	6
6	6	6	6	5	6	6	0	6
7	6	6	0	6	6	6	0	6
8	0	0	0	0	0	0	0	6
9	0	6	0	0	6	6	6	6
10	0	6	0	6	6	6	6	6
11	0	0	0	6	0	0	6	6
12	0	6	0	6	6	6	6	6
13	0	6	0	0	6	6	6	6
14	0	0	0	0	0	0	6	6
15	0	6	2	5	0	0	6	6
16	2	6	2	6	0	0	6	6
17	0	0	0	0	0	0	0	6
18	0	0	0	0	6	6	2	6
19	0	6	0	0	6	6	6	6
20	0	0	0	0	0	0	0	6
21b	0	3	0	0	3	3	3	3
22	0	6	0	0	6	6	6	6
23	0	6	0	0	0	0	0	6
24	0	6	0	0	6	6	6	6
25	0	6	0	0	6	6	6	6
26	0	6	0	0	0	0	6	6
27	0	6	0	0	6	6	6	6
28	0	6	0	0	6	6	6	6

Table 5. Number of replicates run for each trial for each analytic device for measurement of organisms >10 µm and <50 µm in minimum dimension. Note that this number includes the sum total of replicates taken for both samples collected in the trial.

Trial #	Wels. ATP	Ballast Check2	10cells	Bulk FDA	Flow cytometry	Hach	Microscopy	Walz-Water PAM	Satake Pulse Counter
1	6	6	6	6	2	6	6	6	6
2	4	6	6	4	2	6	6	6	6
3	3	2	6	4	2	6	6	6	5
4	4	3	6	4	2	6	6	6	4
5	6	4	6	6	2	6	6	6	6
6	6	5	6	6	2	3	6	6	6
7	6	4	6	0	2	2	6	6	6
8	0	2	6	0	2	0	0	6	5
9	6	6	6	6	2	6	6	6	6
10	6	4	6	6	2	6	6	6	6
11	6	5	6	6	2	4	0	6	6
12	4	6	6	4	2	6	6	6	6
13	0	6	6	0	2	0	6	6	6
14	0	6	6	0	2	0	0	6	6
15	6	6	6	6	2	8	0	6	6
16	6	6	6	6	2	6	0	6	6
17	0	6	6	0	2	0	0	6	6
18	6	6	6	6	2	6	6	6	6
19	0	6	6	0	2	0	6	6	6
20	0	6	6	0	2	0	0	6	6
21a	0	6	6	0	2	0	6	6	6
22	0	6	6	0	2	0	6	6	6
23	0	6	6	0	2	0	0	6	6
24	0	6	6	0	2	0	6	6	6
25	0	6	6	0	2	0	6	6	6
26	6	6	6	5	2	6	0	6	6
27	3	6	6	2	2	8	6	6	6
28	0	6	6	0	2	3	6	6	5

Table 6. Number of replicates run for each trial for each analytic device for measurement of organisms <10 µm in minimum dimension. Note that this number includes the sum total of replicates taken for both samples collected in the trial.

Trial #	Welschmeyer ATP	BallastCheck2	Bulk FDA	Hach	Walz-Water PAM	Flow cytometry
1	6	6	6	6	6	2
2	4	6	4	6	6	2
3	4	6	4	6	6	2
4	4	6	4	6	6	2
5	6	6	6	6	6	2
6	6	6	6	2	6	2
7	6	6	0	2	6	2
8	0	3	0	0	6	2
9	0	6	0	0	6	2
10	0	6	0	0	6	2
11	0	6	0	0	6	2
12	0	6	0	0	6	2
13	0	6	0	0	6	2
14	0	6	0	0	6	2
15	6	6	6	7	6	2
16	6	6	6	6	6	2
17	0	6	0	0	6	2
18	0	6	0	0	6	2
19	0	6	0	0	6	2
20	0	6	0	0	6	2
21a	0	6	0	0	6	2
22	0	6	0	0	6	2
23	0	6	0	0	6	2
24	0	6	0	0	6	2
25	0	6	0	0	6	2
26	0	6	0	0	6	2
27	0	6	0	5	6	2
28	0	6	0	0	6	2

Table 7. Number of replicates run for each trial for each analytic device for measurement of bacteria. Trials indicated with an asterisk were spiked for bacteria. Note that this number includes the sum total of replicates taken for both samples collected in the trial.

	Total bacteria		*Enterococcus*		*E coli*		
Trial #	IDEXX Simplate	SGS ATP (aqua-tools)	IDEXX Enterolert	Speedy Breedy	Triton BW BacTest EC	IDEXX Colilert	Speedy Breedy
1	6	6	6	6	6	6	6
2	6	6	6	6	6	6	6
3	6	6	6	6	6	6	6
4	6	6	6	6	6	6	6
5	6	6	6	6	6	6	6
6*	6	6	6	6	6	6	6
7	6	6	6	6	6	6	6
8	6	0	6	0	6	6	0
9	6	6	6	6	6	6	6
10	6	6	6	6	6	6	6
11	6	4	6	0	6	6	0
12*	6	6	6	6	5	6	6
13	6	6	6	6	6	6	6
14	6	6	6	0	6	6	0
15	6	6	6	6	6	6	6
16	6	6	6	0	6	6	0
17	6	0	6	0	6	6	0
18	6	6	6	0	6	6	0
19	6	6	6	0	6	6	0
20	6	6	6	0	6	6	0
21*	6	6	6	10	6	6	12
22*	6	6	6	6	5	6	5
23	6	6	6	0	6	6	0
24	0	6	6	0	6	6	0
25	0	6	6	0	6	6	0
26	0	6	6	0	4	6	0
27	0	6	0	0	5	0	12
28	0	6	0	0	6	0	0

Table 8. Comparison of analysis methods including cost, time, and training requirements.

Analysis Method	Size Class (µm)	Time Required		Cost		Detection limits (min/max)	Required training/ expert knowledge	Portable
		Active processing	Time to result	Acquisition	Running			
Optical zooplankton analysis	>200	10-30 min	10-30 min	Tool in early development stages				Yes
Microscopy (movement)	≥50	20-30 min	20-30 min	$7000 CDN	NA	Lower limit: 0	Microscopy training and knowledge of zooplankton taxa required	Limited
Microscopy (FDA staining)	≥50 10-50	20 min	30 min	$15000 CDN	$0.02 CDN	Lower limit: 0	Microscopy training and some knowledge of phytoplankton taxa required	
Satake Pulse Counter	≥50	<5 min	10 min	Undecided		Lower limit: 0	Minimal	Yes
	10-50	<5 min	30 min			Lower limit: 0		Yes
MLML (streamlined) bulk FDA method	≥50 10-50 <10		1-2 hours					
Welschmeyer ATP	≥50 10-50 <10							
SGS ATP (aqua-tools)	≥50	50 min	50 min	7700 €	31.83 €	Lower limit: <0.01 pg ATP Upper limit: Not applicable	Minimal	Yes
	10-50	50 min	50 min		31.83 €			
	bacteria	15 min	15 min		13.33 €			
Walz-Water PAM	≥50 <50 10-50 <10	2 min	2 min	15,000 €	5 €		Minimal training	Yes
Ballast Check™ 2 (PAM)	10-50	2 min	2 min				Minimal	Yes
bbe 10cells	10-50	2 min	2 min	4300 € (bulk	1€		Minimal	Yes

(PAM)				ware version) 13800 € (advanced scientific version)				
Hach BW680 (PAM)	10-50	2 min	2 min	$4907 CDN		7	Minimal	Yes
Speedy Breedy	bacteria	<5 min	12 hours	3500£			Minimal	Yes
IDEXX Simplate	bacteria	1 hour	48 hours	1000 €	3.36 €	Lower: 2 Upper: 738 (MPN/ml) *Assume 10x dilution	Minimal	No
IDEXX colilert	E. coli	1 hour	18 hours	5500 €	5.02 €	Lower: 10 Upper: 2005 (MPN/100ml) *Assume 10x dilution	Minimal	No
IDEXX enterolert	Enterococcus	1 hour	24 hours	5500 €	6 €	Lower: 10 Upper: 2005 (MPN/100ml) *Assume 10x dilution	Minimal	No
TRITON BW BacTest EC	E. coli	25 min	25 min	9008€	0.8-1€		Minimal	Yes

Table 9. Intercept, slope, and confidence intervals (CI) based on Deming regression of each method versus microscopy for organisms ≥50 µm.

Method	Intercept	Lower 95% CI	Upper 95% CI	Slope	Lower 95% CI	Upper 95% CI
Satake Pulse Counter	-0.364	-0.694	-0.034	0.740	0.617	0.862
SGS ATP (aqua-tools)	7.623	6.533	8.713	0.722	0.474	0.971
Welschmeyer ATP	7.803	6.850	8.756	0.534	-0.112	1.181
Bulk FDA	6.902	5.193	8.611	0.486	-0.322	1.294
Hach BW680	2.599	1.428	3.769	1.453	0.974	1.932
Walz-Water PAM	0.052	-2.951	3.056	1.946	1.257	2.635

Table 10. Intercept, slope, and confidence intervals (CI) based on Deming regression of each method versus microscopy for 10-50 µm size class of organisms.

Method	Intercept	Lower 95% CI	Upper 95% CI	Slope	Lower 95% CI	Upper 95% CI
Hach BW680	-0.023	-0.440	0.395	0.862	0.599	1.125
bbe 10cells	-0.107	-0.289	0.075	1.187	1.097	1.276
BallastCheck2	0.098	-0.137	0.332	1.103	1.009	1.197
Flow cytometry	2.392	-0.278	5.062	0.588	-1.449	2.626
Satake Pulse Counter	0.086	-0.082	0.255	0.261	0.136	0.386
Walz-Water PAM	1.127	0.808	1.446	1.424	1.191	1.657
Welschmeyer ATP	-20.617	-129.141	87.906	34.886	-125.388	195.159
Bulk FDA	-8.540	-53.189	36.109	16.979	-53.376	87.334

Table 11. Intercept, slope, and confidence intervals (CI) based on Deming regression of each method versus BallastCheck2 and Walz-Water PAM for <10 µm size class of organisms**.**

Method 1	Method 2	Intercept	Lower 95% CI	Upper 95% CI	Slope	Lower 95% CI	Upper 95% CI
BallastCheck2	Walz-Water PAM	-5.626	-9.099	-2.154	2.772	1.852	3.691
BallastCheck2	Bulk FDA	0.435	-1.099	1.970	1.173	0.679	1.667
BallastCheck2	Hach BW680	-12.591	-53.023	27.840	4.548	-6.022	15.118
BallastCheck2	ATP	-22.555	-96.469	51.359	9.188	-15.915	34.292
Walz-Water PAM	BallastCheck2	2.049	1.448	2.649	0.361	0.240	0.481
Walz-Water PAM	Bulk FDA	3.582	3.025	4.139	0.112	-0.008	0.233
Walz-Water PAM	Hach	11.331	1.882	20.780	-1.210	-2.952	0.532
Walz-Water PAM	Welschmeyer ATP	3.422	2.844	4.000	0.334	0.198	0.470

Table 12. Density of ≥50 µm organisms in potable water across both trials.

Analytic Device	Density (individuals/ m^3)
	Mean +/ -SD
Microscopy	89.6 +/ - 104.9
Satake Pulse Counter	344.4 +/ - 222.9

Table 13. Density of 10-50 µm organisms in potable water across both trials. (*) indicates that the individuals were non-viable. (**) indicates that all samples were below the detection limit for the device.

Analytic Device	Density (individuals/mL)
	Mean +/ -SD
BallastCheck2	2.9 +/- 2.5
10cells	0.2 +/- 0.1
Bulk FDA	6.5 +/- 6.0
Flow cytometry*	39.8* +/- 29.4
Hach fluorometer**	0.5+/- 0.0
Microscopy	0.3 +/- 0.3
Satake Pulse Counter	1.3 +/- 2.2

Figure 1. Schematic view of the BWMS with sampling points at position (A), (B), (C) and (D).

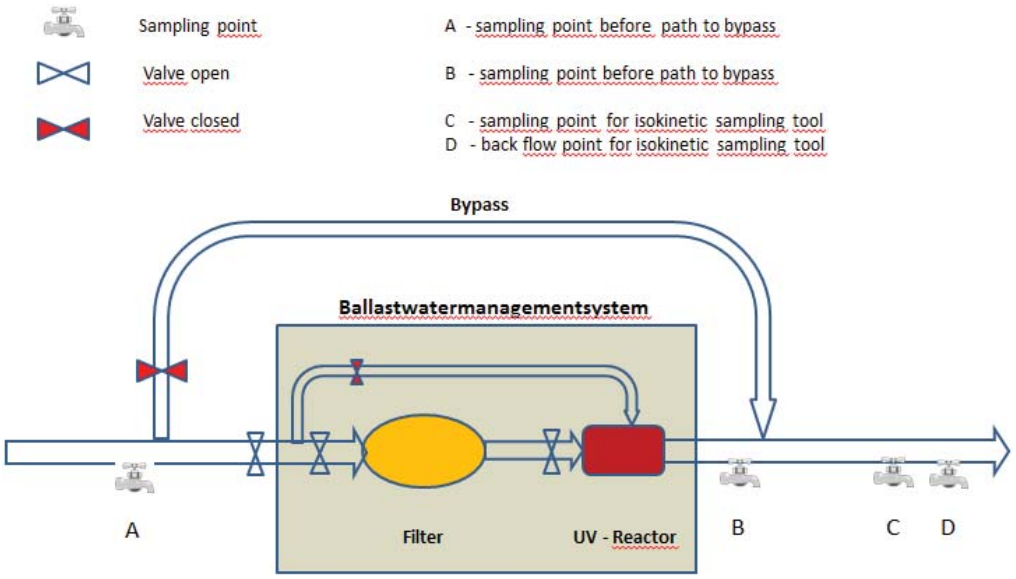


Figure 2. Map showing ship path and sampling locations for each trial.

Figure 3. Phytoplankton viability from Cape Verde Islands to Hamburg based on results from the Walz-Water PAM. Values between 0.3 and 0.6 are indicative of healthy phytoplankton.

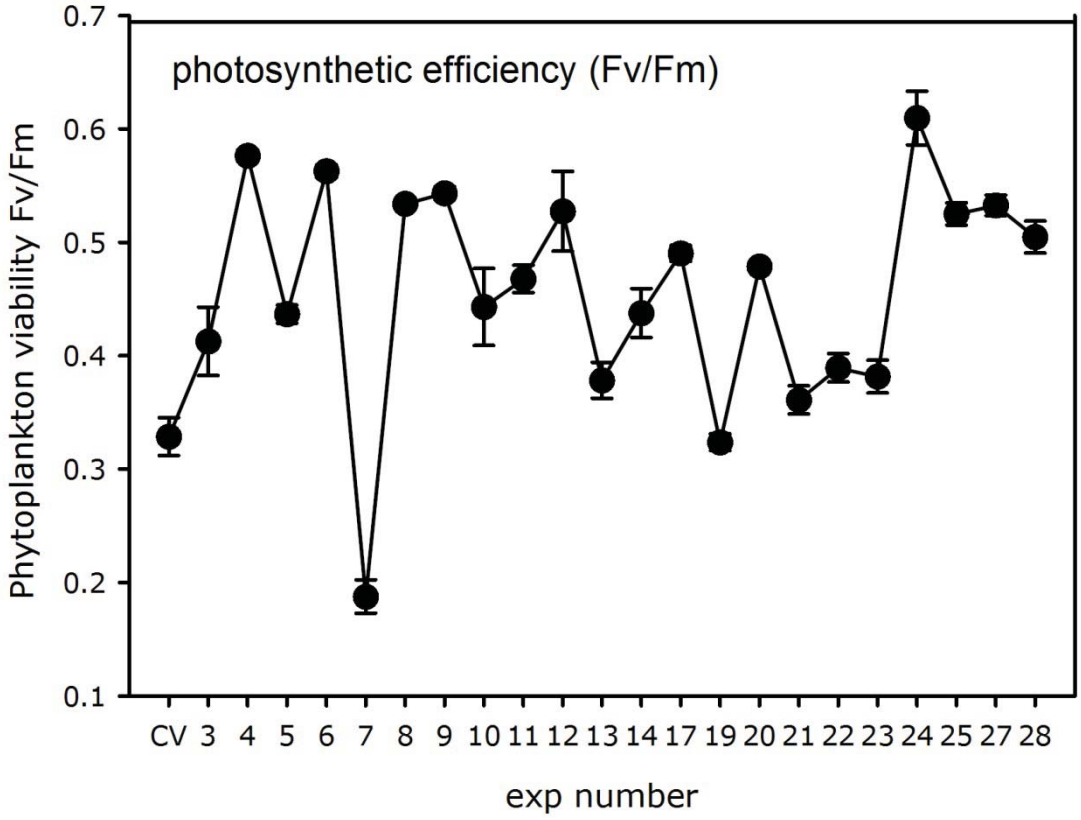


Figure 4. Scatterplots of each analytic tool for the ≥50 µm size class versus total microscopy counts. All values are standardized to raw sample concentration to account for any concentrations performed by individual researchers. Red dots are used to distinguish samples that were treated with the ballast water management system. The solid line indicates the line of best fit found using Deming regression (full details in Table 8) and the dashed line indicates the 1:1 line for devices reporting values in the same units. The Pearson correlation coefficient for each plot is indicated above the figure. Note that coefficients should not be directly compared since plots differ in the number of data points. The shaded rectangles indicate a region where no data was available for a given technique (see Table 4).

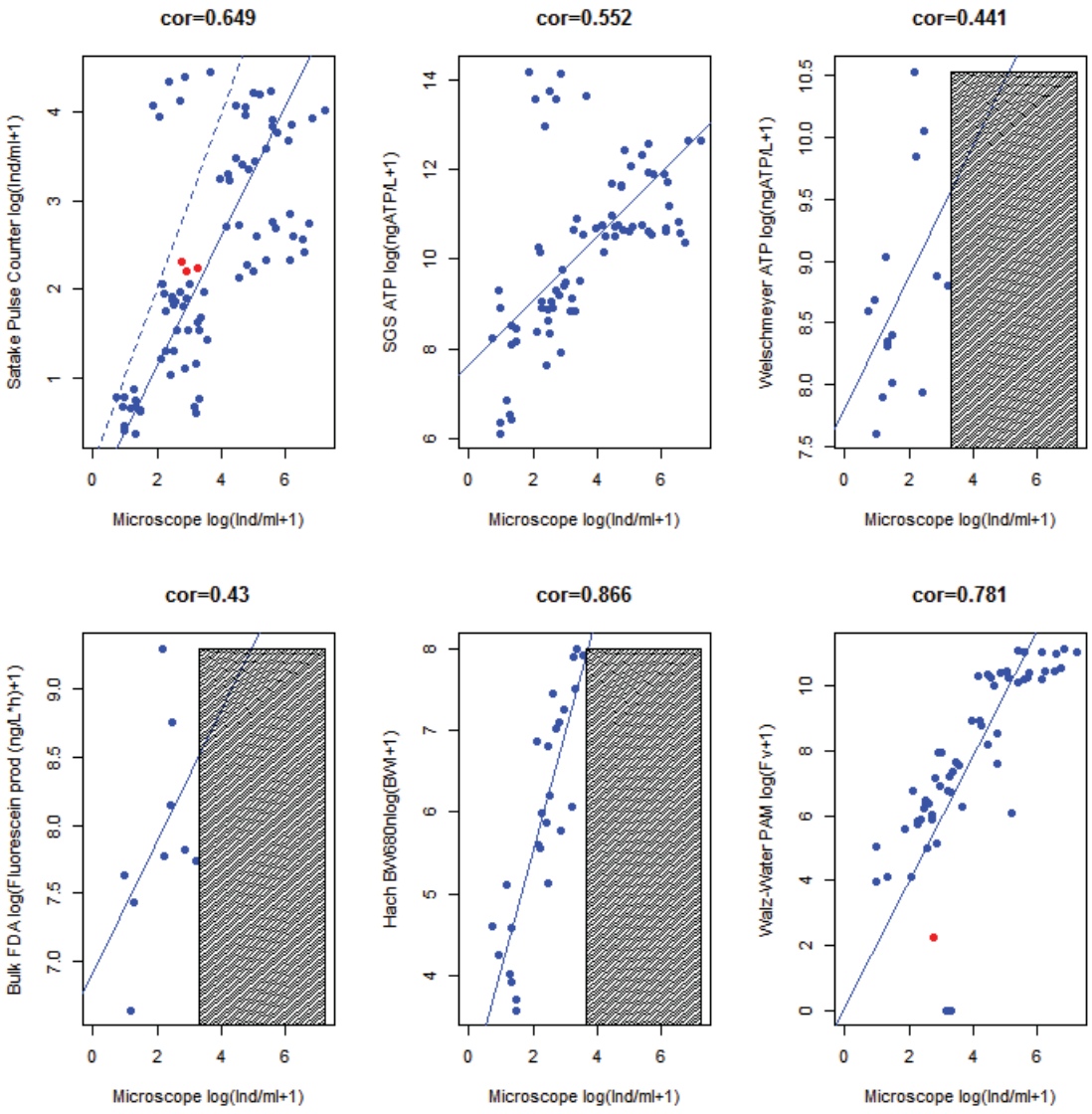


Figure 5. Estimated conversion lines (thick black line) and 95% confidence intervals (thin black lines) to convert between measurements made using different analytic devices for ≥50 µm organisms. Data points are indicated as black dots; all values are standardized to raw sample concentration to account for any concentrations performed by individual researchers and log+1 transformed to satisfy model requirements. Full model data is available in Appendix E.1.

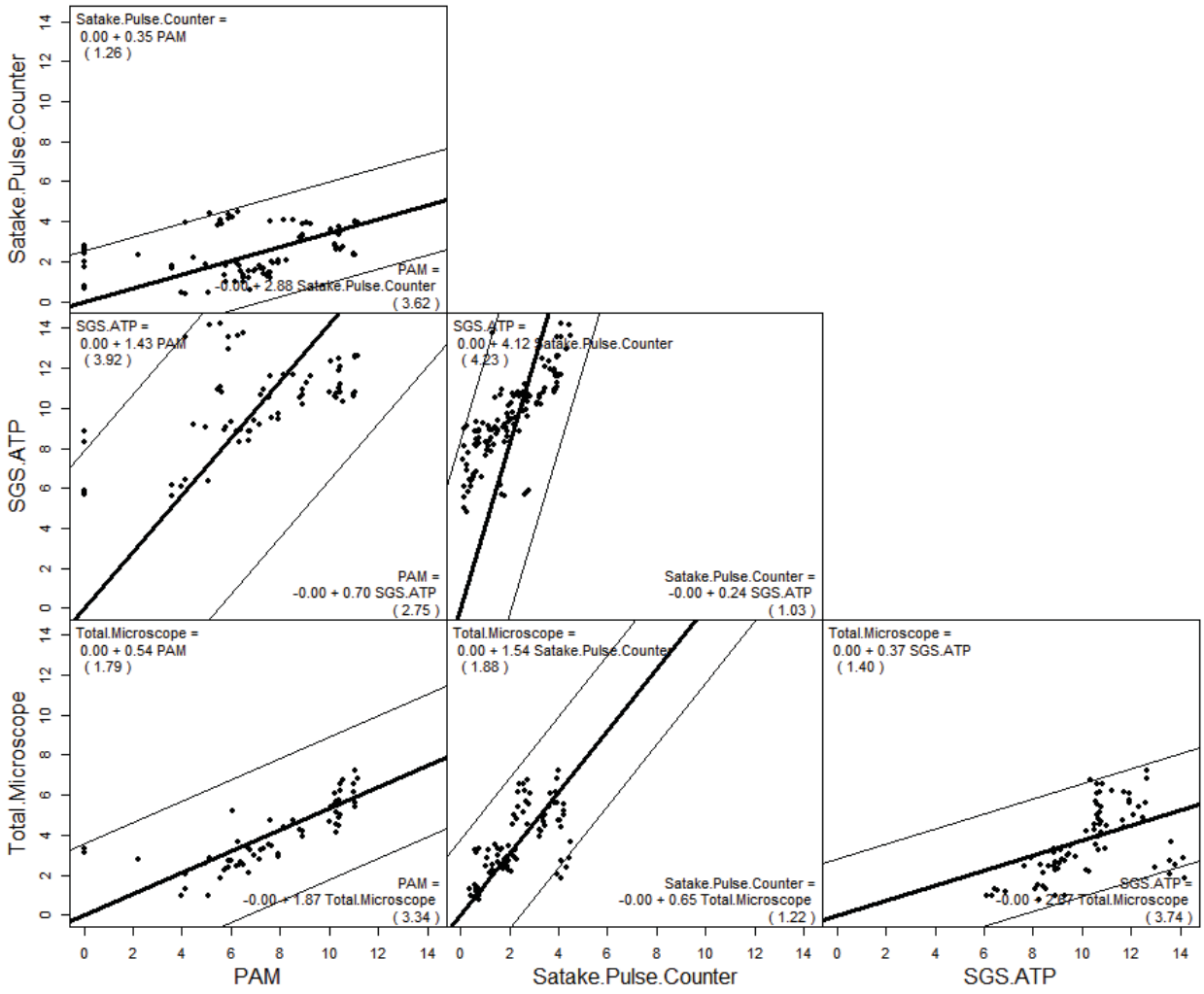


Figure 6. Scatterplots of each results for the Optical Zooplankton Analyzer (>200 µm) versus motile and total microscopy counts. All values are standardized to raw sample concentration to account for any concentrations performed by individual researchers. The solid black line indicates the line of best fit found using Deming regression and the dashed line indicates the 1:1 line. The Pearson correlation coefficient for each plot is indicated above the figure.

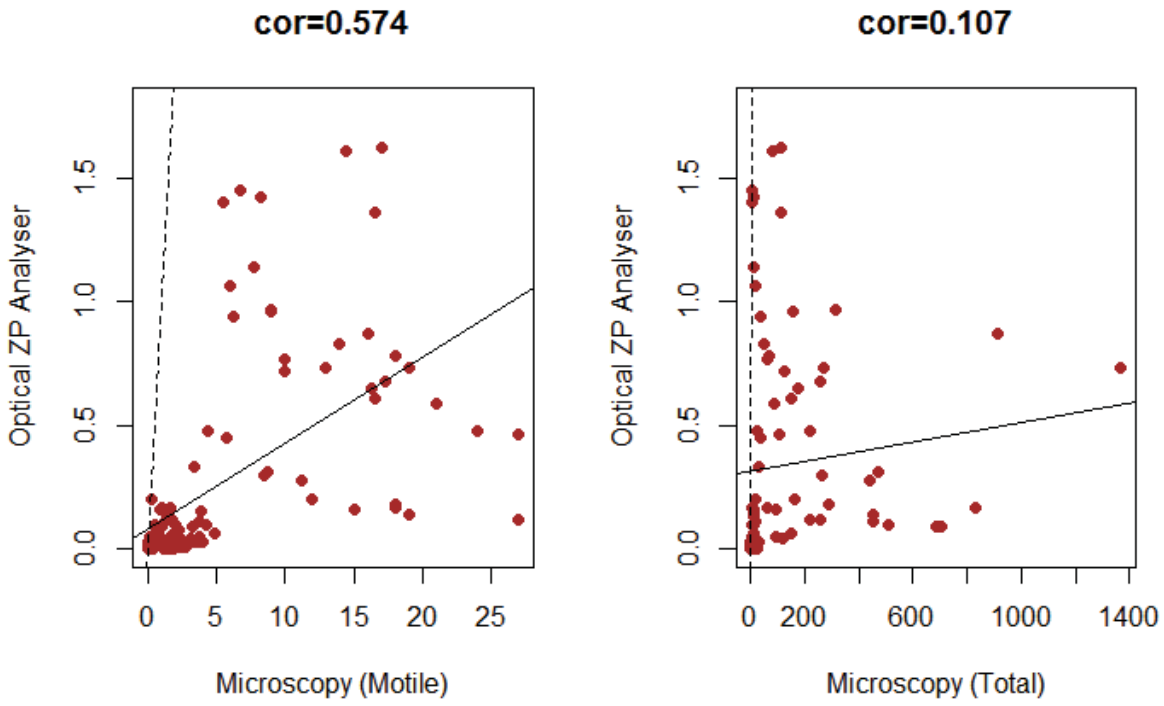


Figure 7. Scatterplots of each analytic tool for the 10-50 µm size class versus total microscopy counts. Red points are used to distinguish samples that were treated with the ballast water management system. Grey points indicate values that were below the detection range of the Hach BW680 and were plotted assuming the minimum detection limit. All values are standardized to raw sample concentration to account for any concentrations performed by individual researchers. The solid line indicates the line of best fit found using Deming regression and the dashed line indicates the 1:1 line for devices reporting values in the same units. The Pearson correlation coefficient for each plot is indicated above the figure. Note that coefficients should not be directly compared since plots differ in the number of data points. The shaded rectangles indicate a region where no data was available for a given technique (see Table 5).

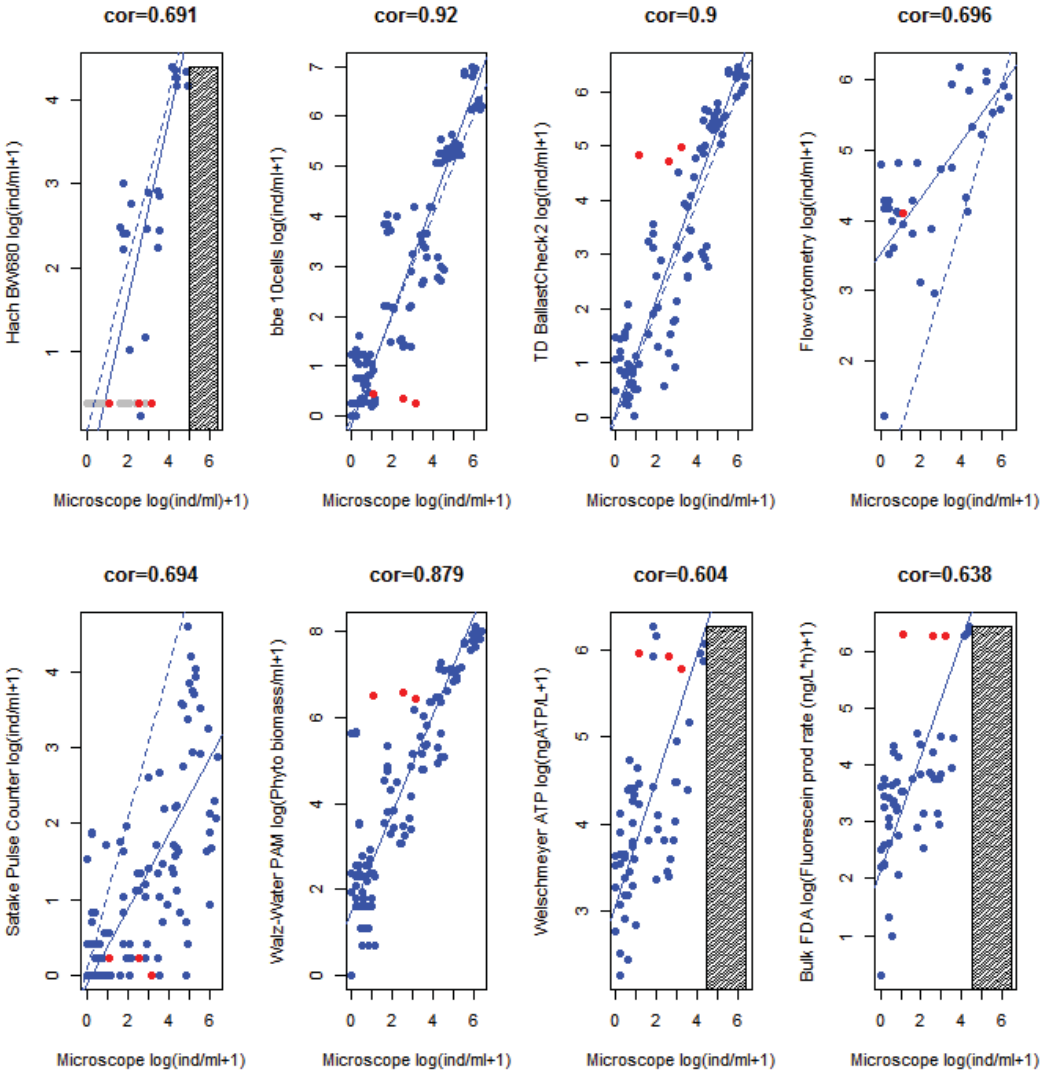


Figure 8. Pairwise scatterplots of Fv values for each PAM tool and microscopy counts (ind/mL) for the 10-50 µm size class. The boxes above the diagonal show the Pearson correlation coefficient (r) for each pair.

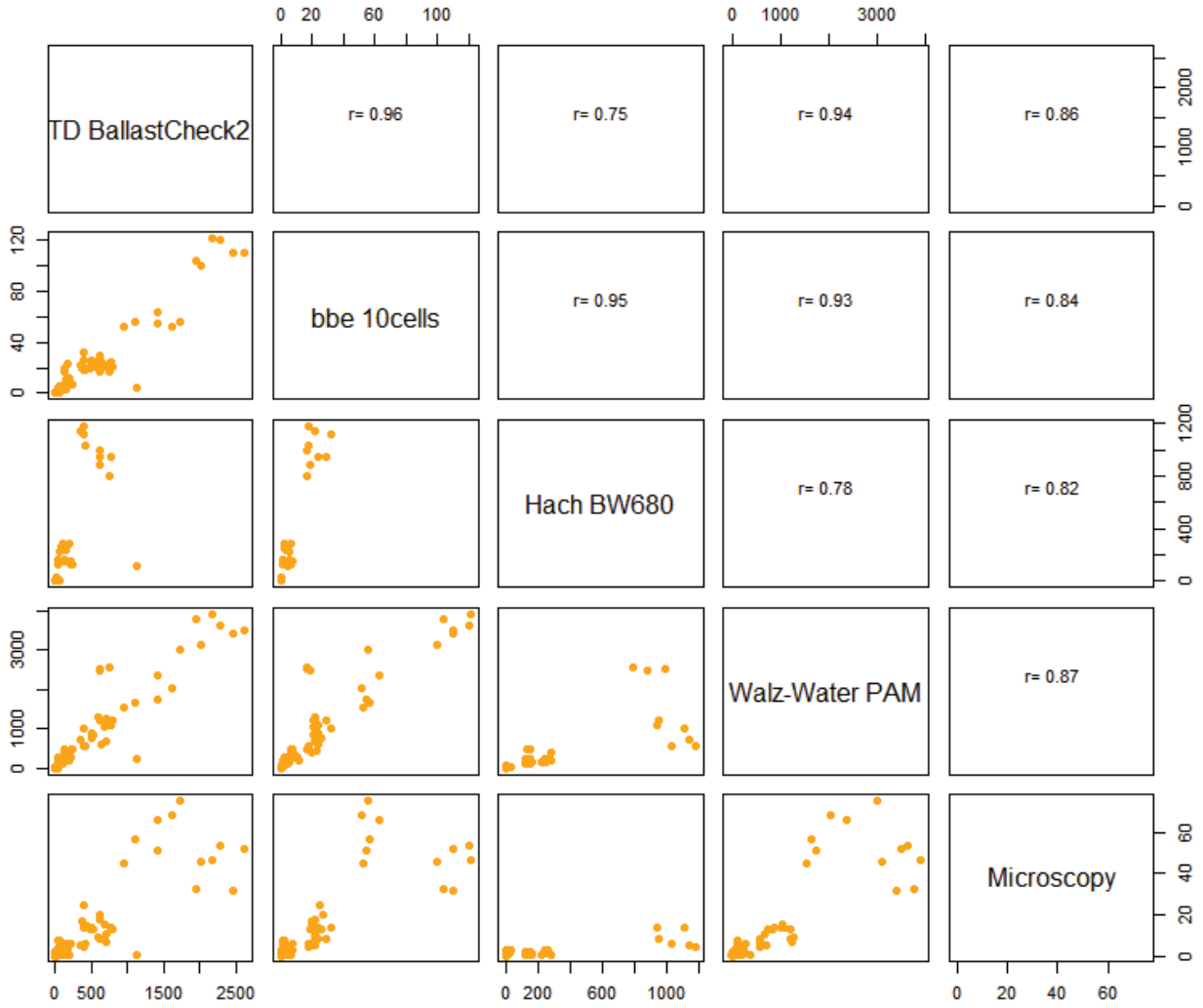


Figure 9. Estimated conversion lines (thick line) and 95% confidence intervals (thin lines convert between measurements made using different analytic devices for 10-50 µm organisms (bbe 10cells, Hach BW680, BallastCheck2, Walz-Water PAM (labelled PAM) and Microscopy). Data points are indicated as black dots. Full model data is available in Appendix E.2.

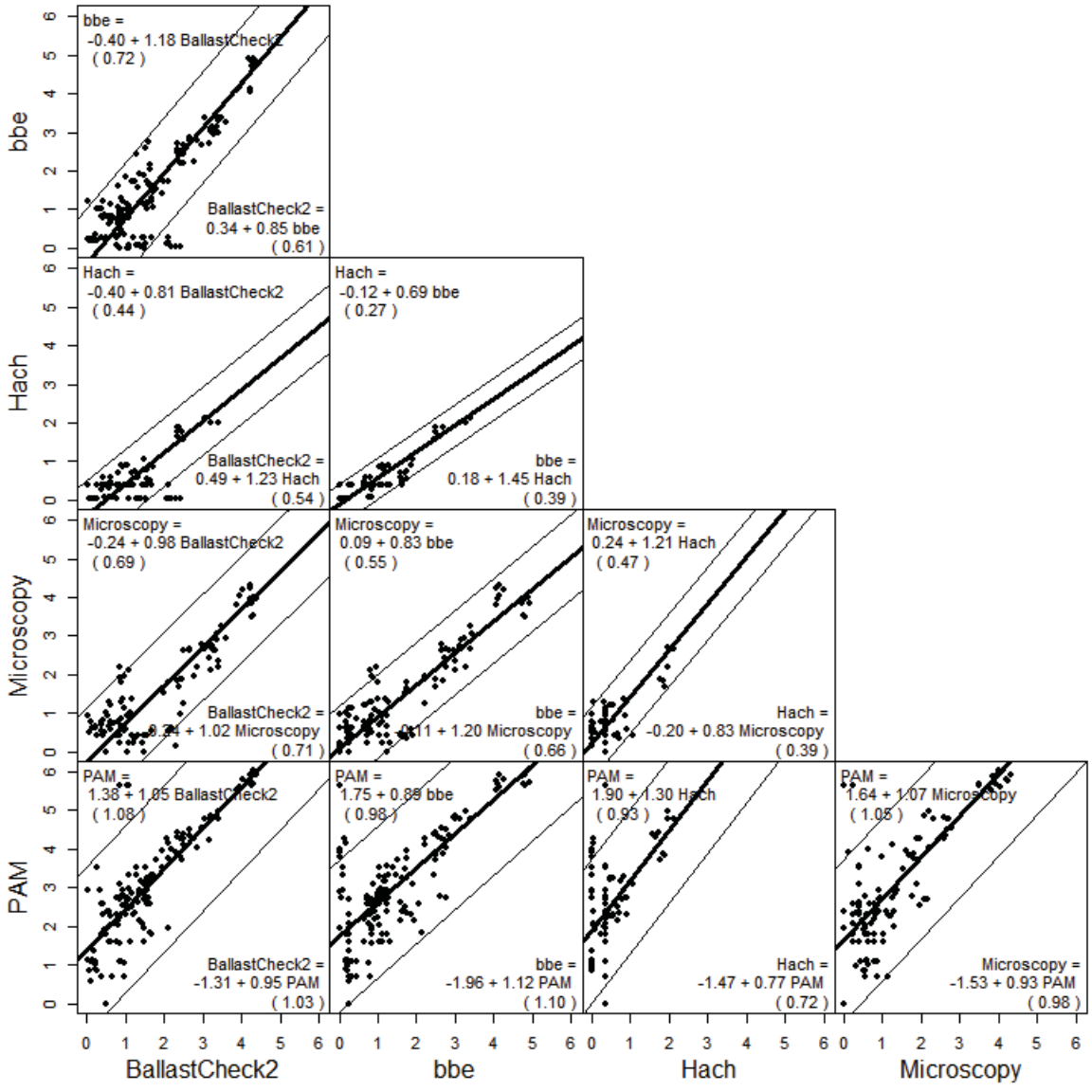


Figure 10. Average chlorophyll fluorescence per cell for total phytoplankton (2 – 50 µm; black dots) and 10-50 µm cells (blue dots) based on the mean of 2 replicates measured using the Walz-Water PAM. Note different scales on the y-axes.

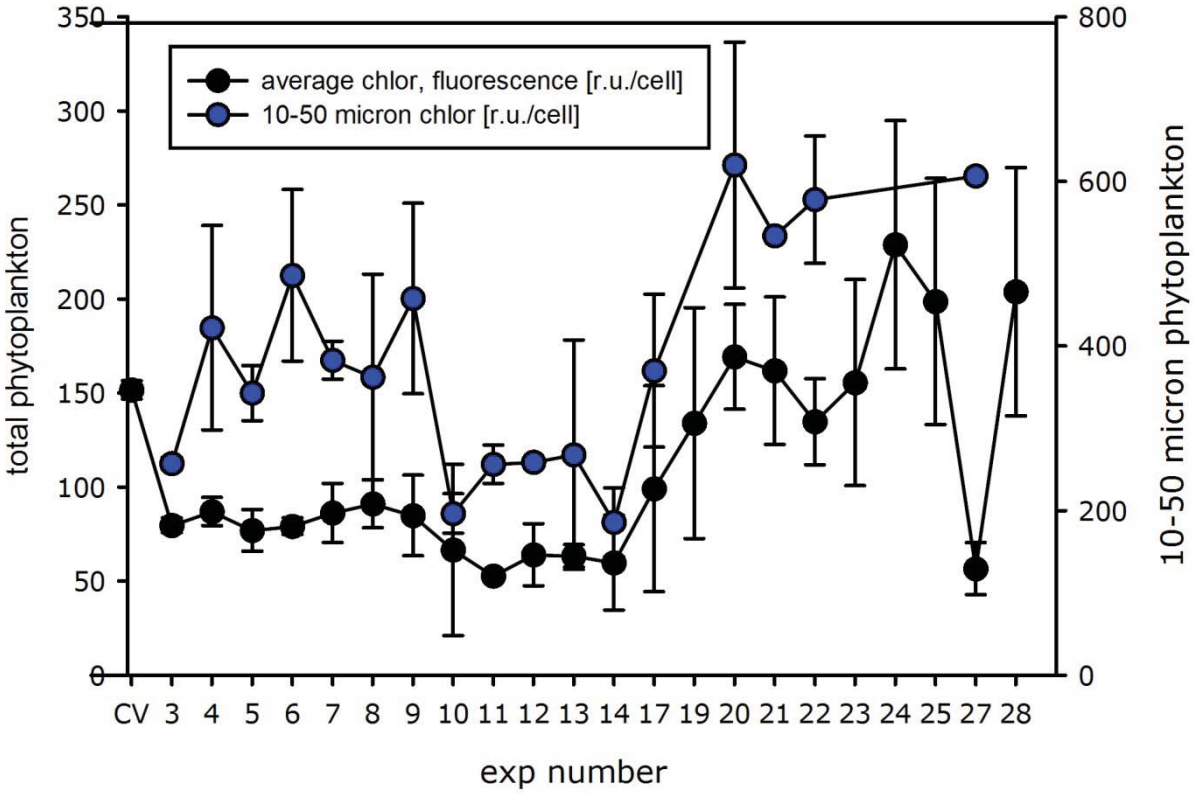


Figure 11. Cell number as estimated by flow cytometry versus WALZ-Pam chlorophyll fluorescence for total phytoplankton (blue diamonds), 10-50 µm phytoplankton (red squares) and < 10 µm phytoplankton (green triangles). Linear regression equations and coefficient of determination (R^2) are shown for each group.

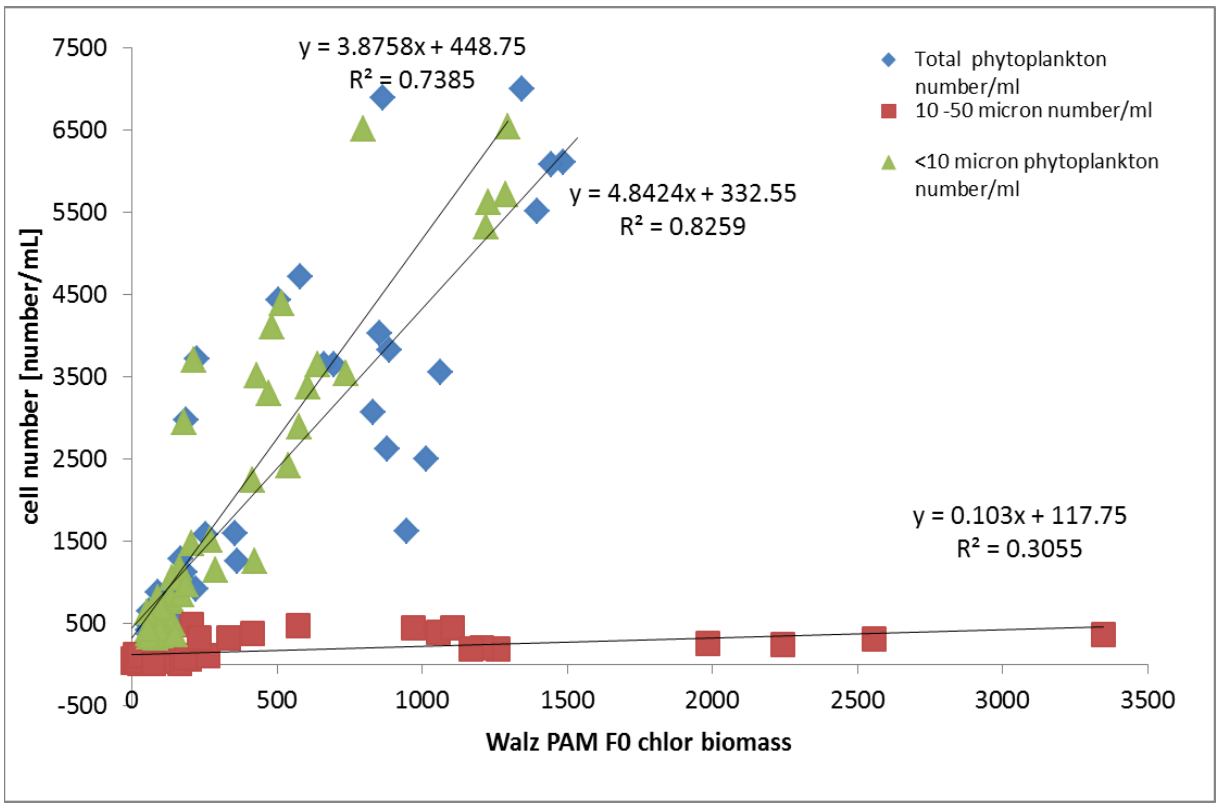


Figure 12. Integrated chlorophyll biomass of the phytoplankton (FCMchlor = flow cytometer counts * cellular chlorophyll fluorescence) versus WALZ-Pam chlorophyll fluorescence for total phytoplankton (blue diamonds), 10-50 µm phytoplankton (red squares) and < 10 µm phytoplankton (green triangles). Linear regression equations and coefficient of determination (R^2) are shown for each group.

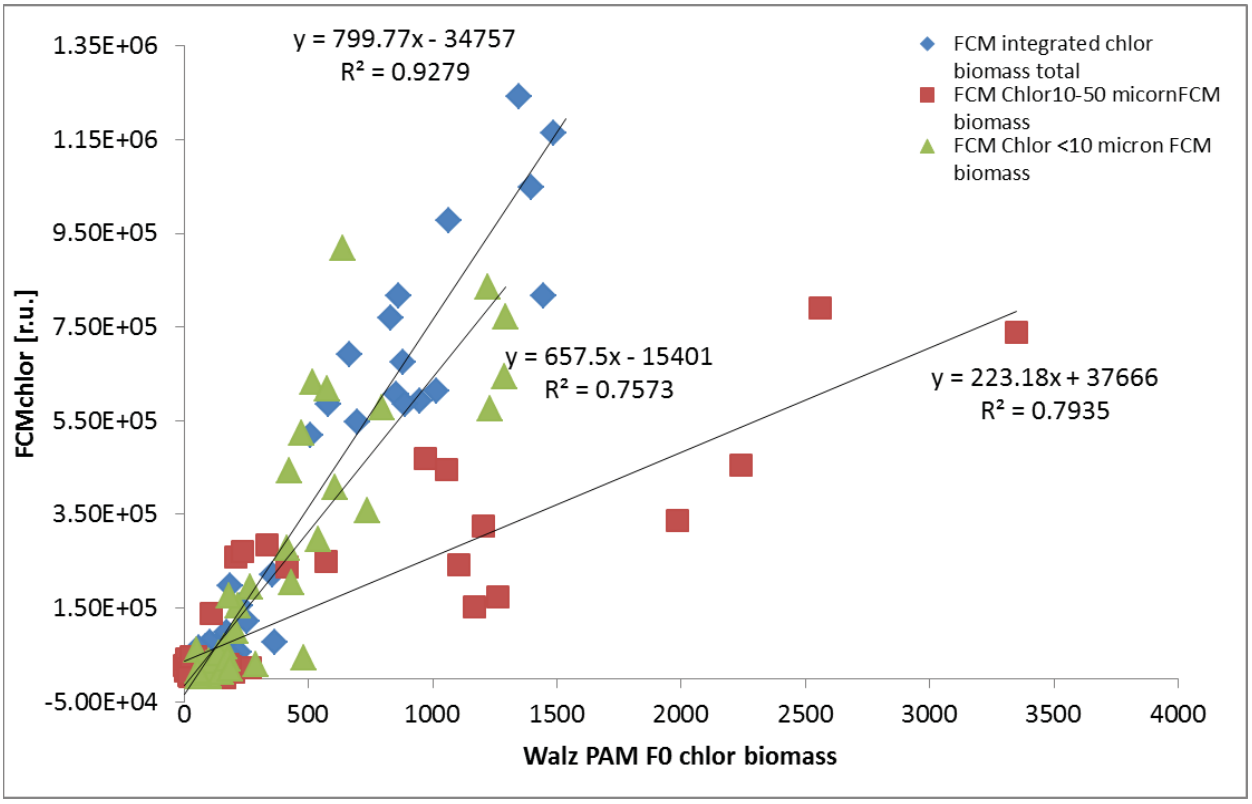


Figure 13. Scatterplots of each analytic tool for the < 10 µm size class versus BallastCheck2 (top row) and Walz-Water PAM (bottom row). Red points are used to distinguish samples that were treated with the ballast water management system. Grey points indicate values that were below the detection range of the Hach BW680, and are plotted assuming the minimum detection limit for points below the detection limit. All values are standardized to raw sample concentration to account for any concentrations performed by individual researchers. The blue line indicates the line of best fit found using Deming regression. The Pearson correlation coefficient for each plot is indicated above the figure. Note that coefficients should not be directly compared since plots differ in the number of data points (see Table 6).

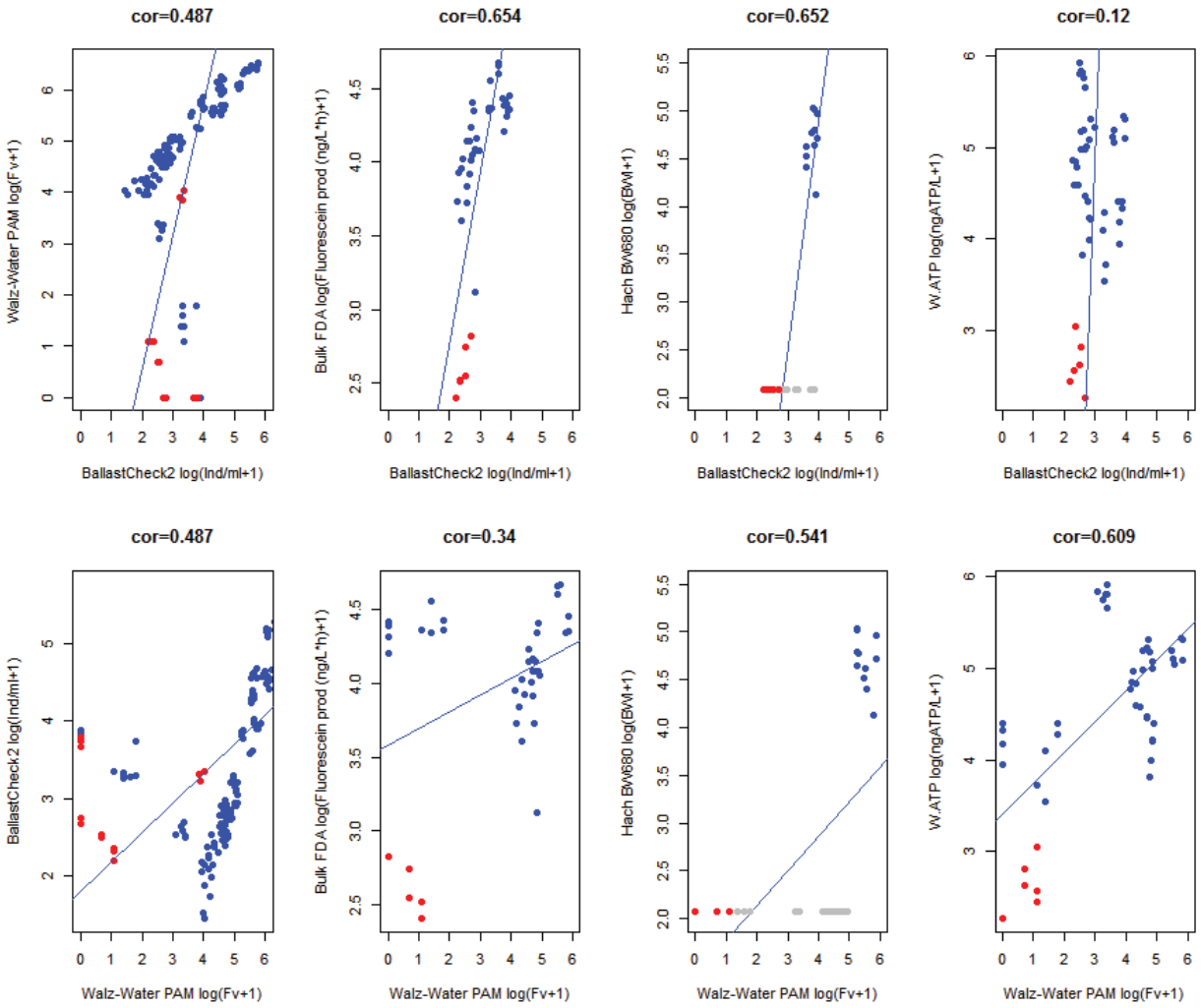


Figure 14. Estimated conversion lines (thick line) and 95% confidence intervals (thin lines) to convert between measurements made using different analytic devices for < 10 µm size class (BallastCheck2, Welschmeyer ATP, and bulk FDA). Data points are indicated as black dots. Full model data is available in Appendix E.3.

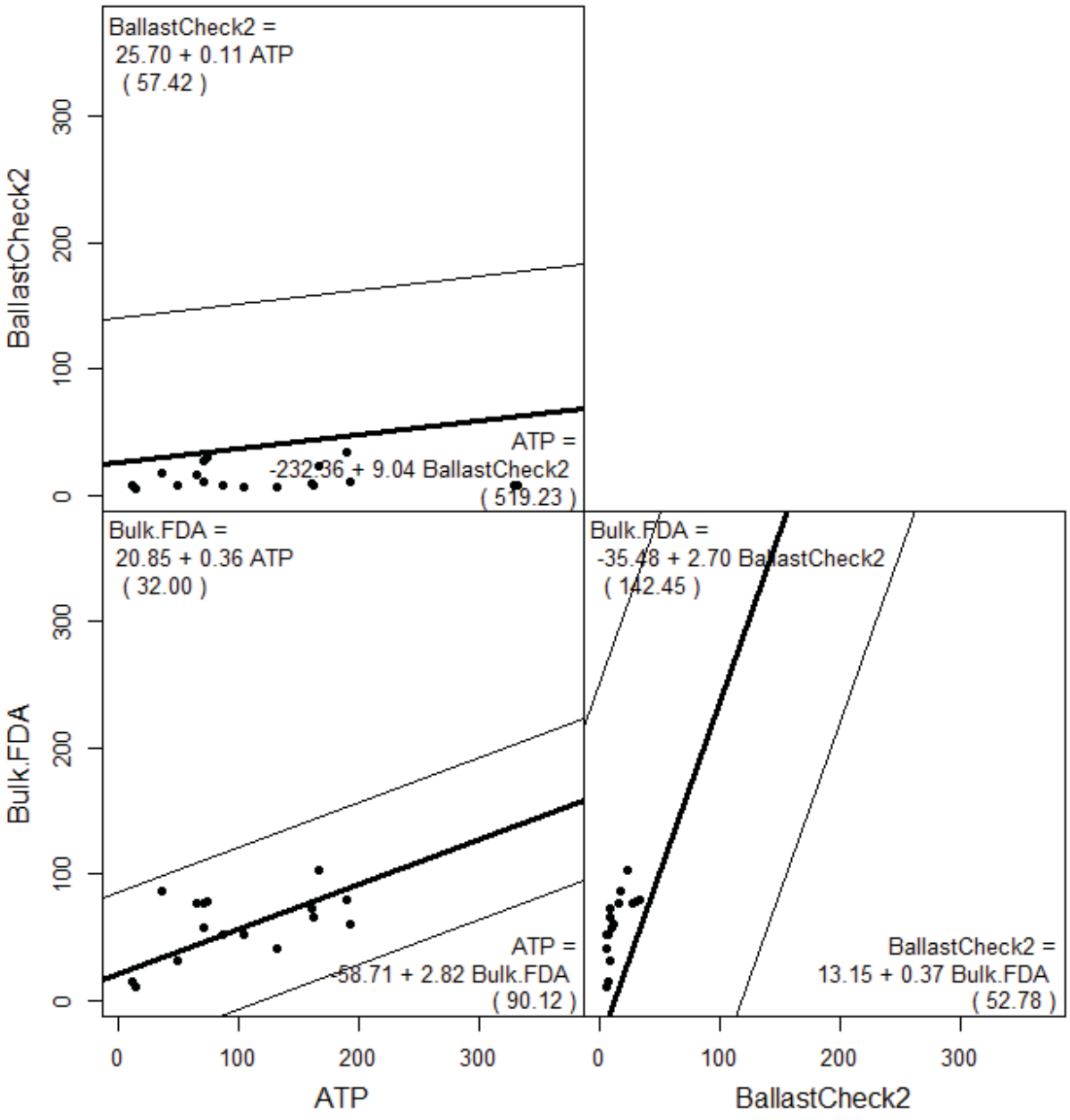


Figure 15. Scatterplot of estimated bacterial densities by IDEXX Simplate and SGS ATP (aqua-tools). Green points represent points where a measurement was given for each device and blue points represent values that were below the detection limit of IDEXX Simplate. Blue points were plotted assuming zero values for measurements below detection limit. All values are standardized to raw sample concentration to account for any concentrations performed by individual researchers. The green line indicate the line of best fit found using Deming regression and the Pearson correlation coefficient is indicated above the figure; both are based only on points where true measures were obtained from both devices.

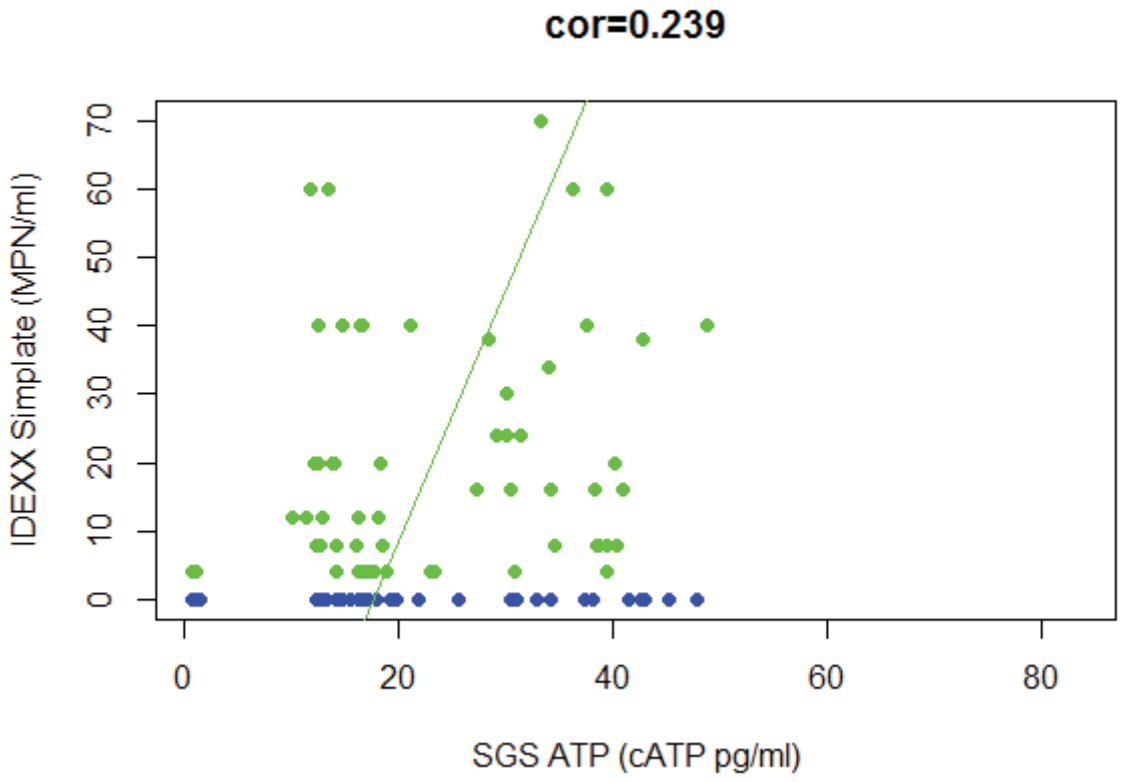


Figure 16. Scatterplot of estimated *E. coli* densities by Triton BW BacTest EC and Speedy Breedy. Green points represent points where a measurement was given for each device and blue points represent values that were under the detection range of Triton BW BacTest EC. Blue points were plotted assuming the zero values for samples below the detection limit. All values are standardized to raw sample concentration to account for any concentrations performed by individual researchers. The green line indicate the line of best fit found using Deming regression and the Pearson correlation coefficient is indicated above the figure; both are based only on points where true measures were obtained from both devices.

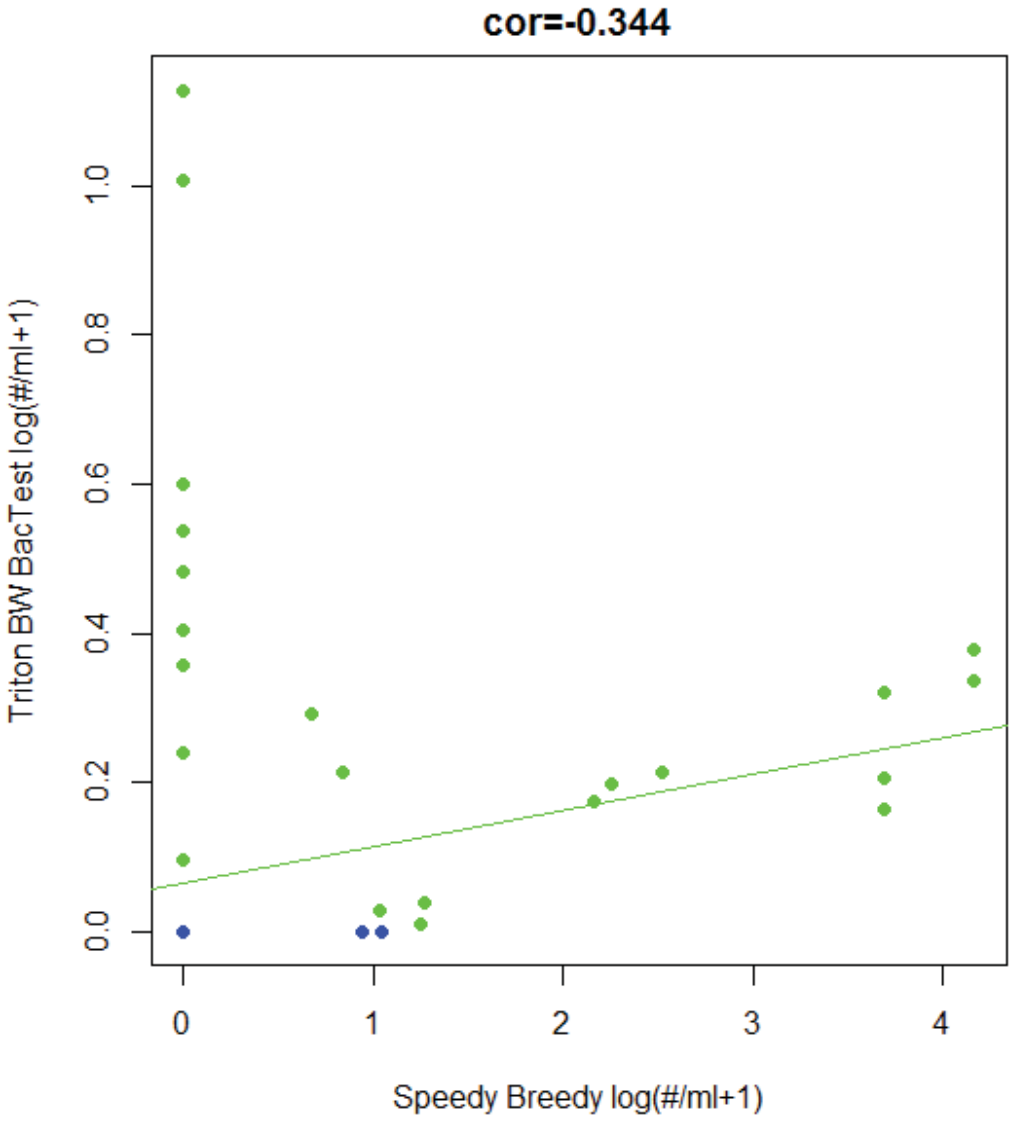


Figure 17. Scatterplot of estimated *Enterococcus* densities by IDEXX Enterolert and Speedy Breedy. Green points represent points where a measurement was given for each device and blue points represent values that were over the detection range of IDEXX Enterolert. Blue points were plotted assuming the maximum detection limit for points above the detection limit. All values are standardized to raw sample concentration to account for any concentrations performed by individual researchers. The green line indicate the line of best fit found using Deming regression and the Pearson correlation coefficient is indicated above the figure; both are based only on points where true measures were obtained from both devices.

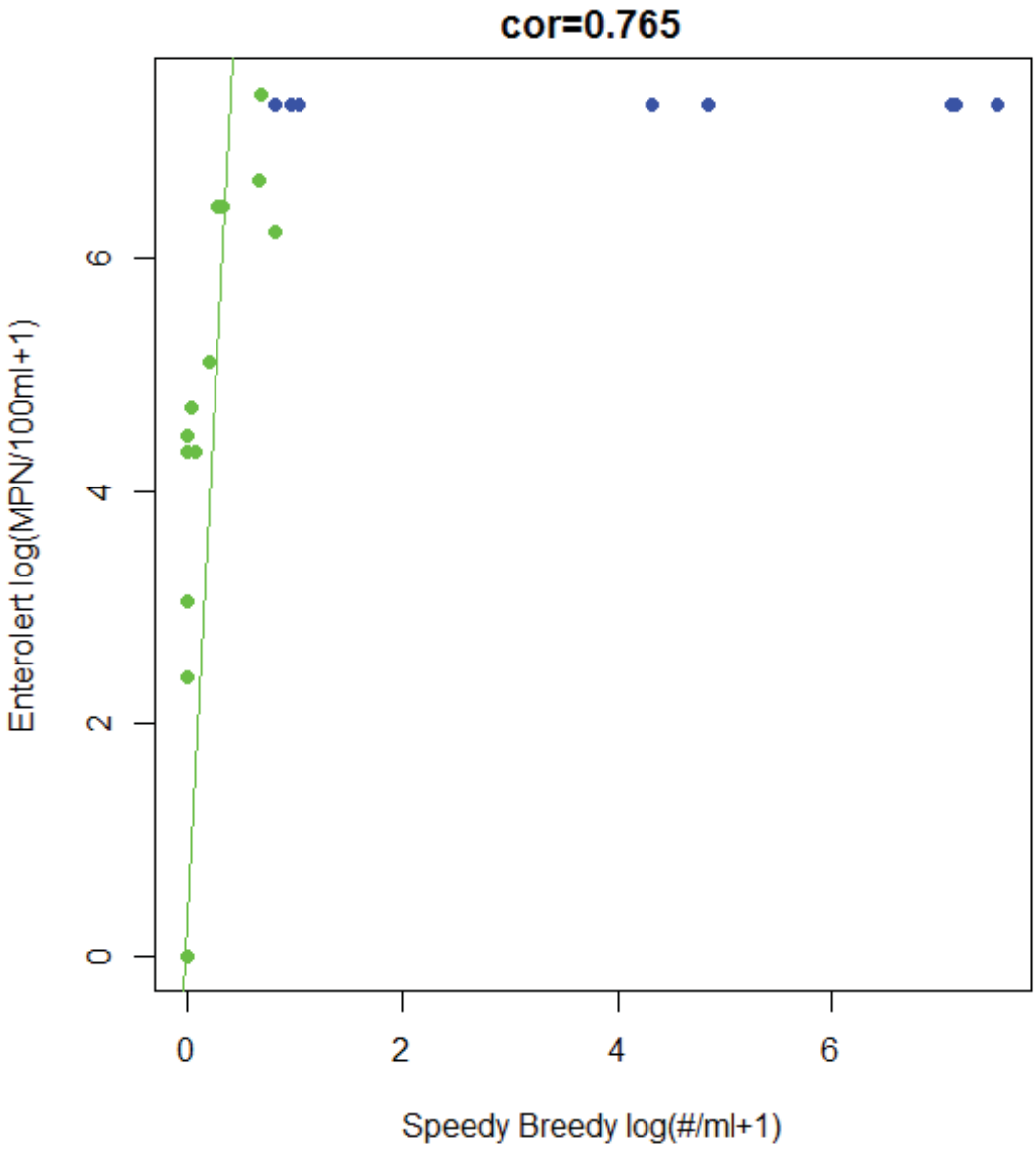


Figure 18. Results of Tukey pairwise contrasts for measurements made on samples collected using different collection devices for organisms ≥ 50 µm. The dot indicates the expected difference between samples collected using the devices indicated based on a linear mixed model (see methods and results) and the lines show the 95% confidence interval. There is no significant difference between collection devices where the confidence interval overlaps the vertical dashed line.

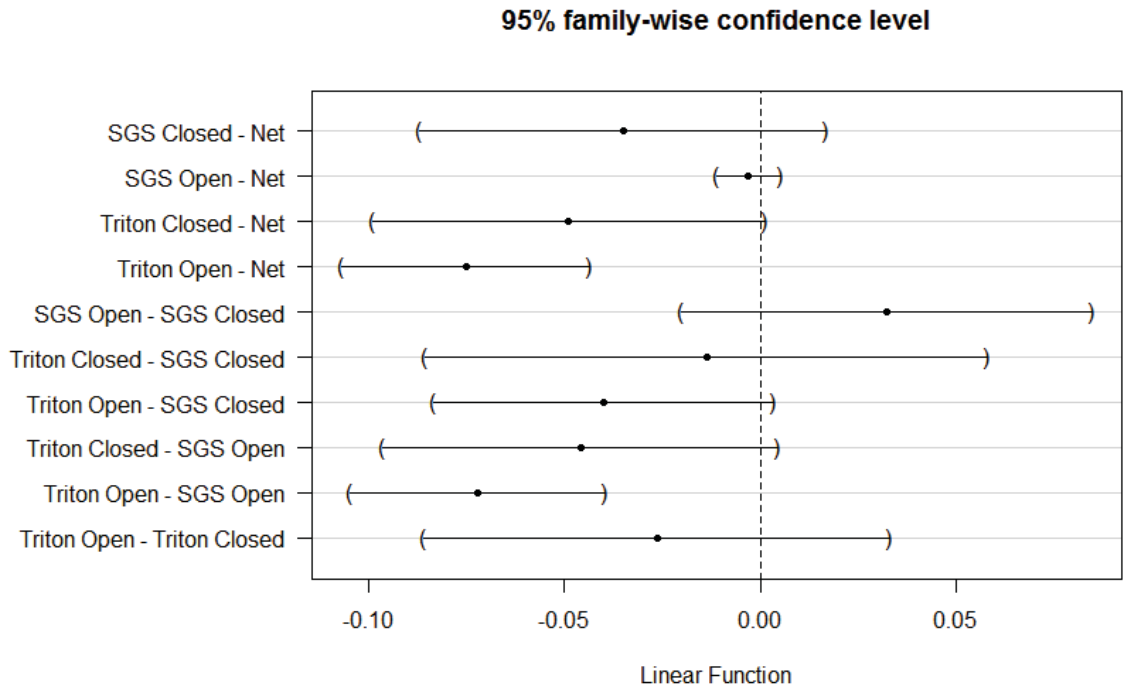


Figure 19. Results of Tukey pairwise contrasts for measurements made on samples collected using different collection devices for 10-50 µm organisms. The dot indicates the expected difference between samples collected using the devices indicated based on a linear mixed model (see methods and results) and the lines show the 95% confidence interval. There is no significant difference between collection devices where the confidence interval overlaps the vertical dashed line.

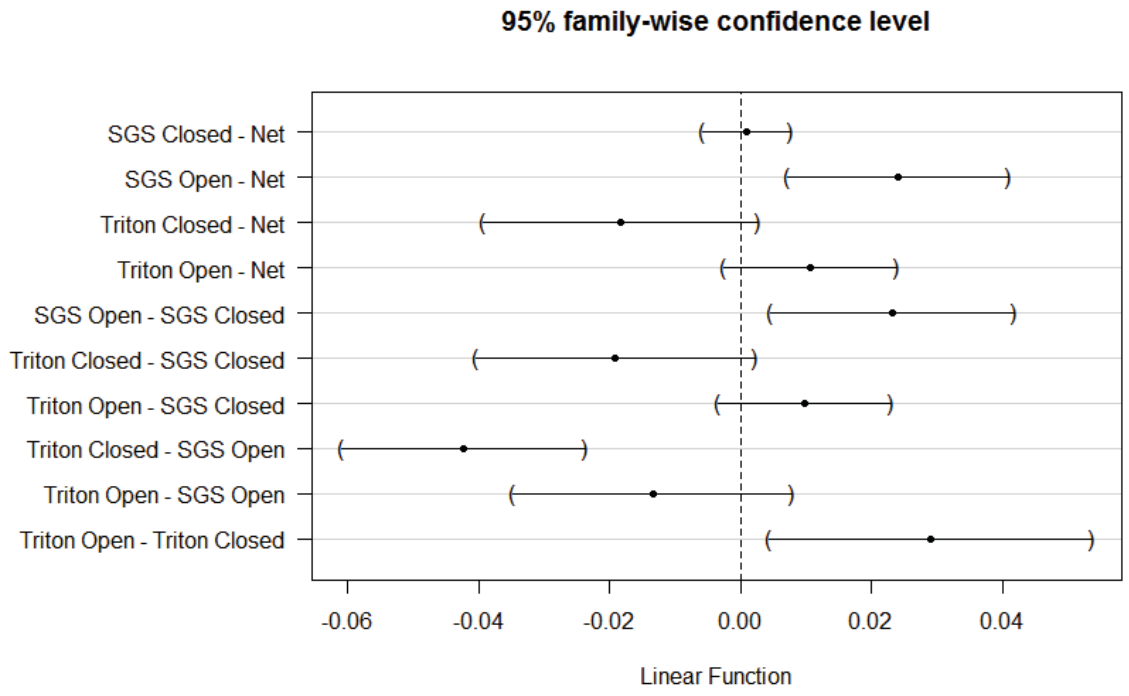


Figure 20. Bar graphs of estimated mean densities (+/- SD) before and after treatment with the OptiMarin BWMS for organisms ≥ 50 µm. Red bars show estimated densities before treatment, and blue bar shows densities after treatment. All values are standardized to raw ballast concentration. (*) indicates that only 1 replicate was run during trials. A significant decrease in densities were seen for all analytic devices except bulk FDA and Welschmeyer ATP (One-way paired t tests on log+1 transformed data with Welch's correction for unequal variance; all $p<0.05$).

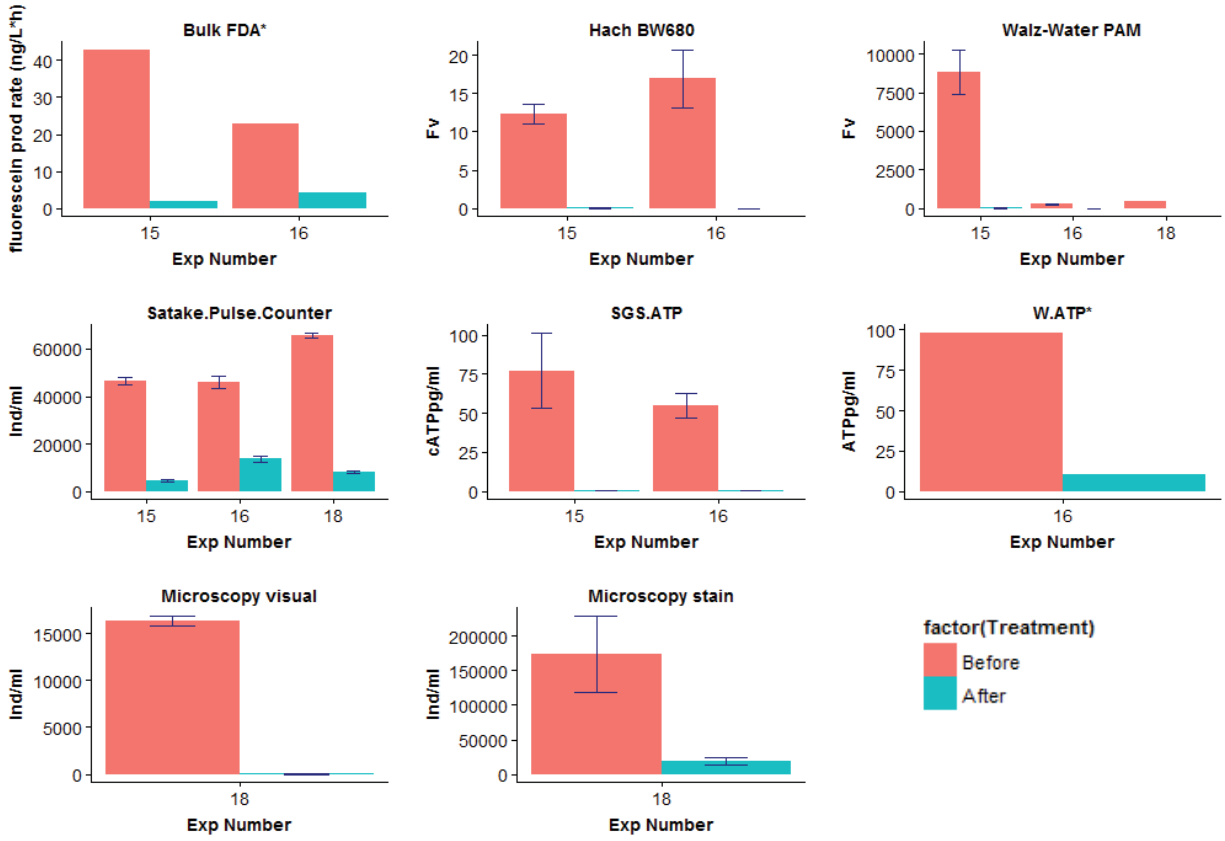


Figure 21. Bar graphs of estimated mean densities (+/- SD) before and after treatment with the OptiMarin BWMS for 10-50 µm organisms. Red bars show estimated densities before treatment, and blue bars show densities after treatment. All values are standardized to raw ballast concentration. Only 1 replicate was run per trial for flow cytometry. A significant decrease in densities were seen for all analytic devices except flow cytometery and bulk FDA (One-way paired t tests on log+1 transformed data with Welch's correction for unequal variance; all $p<0.05$).

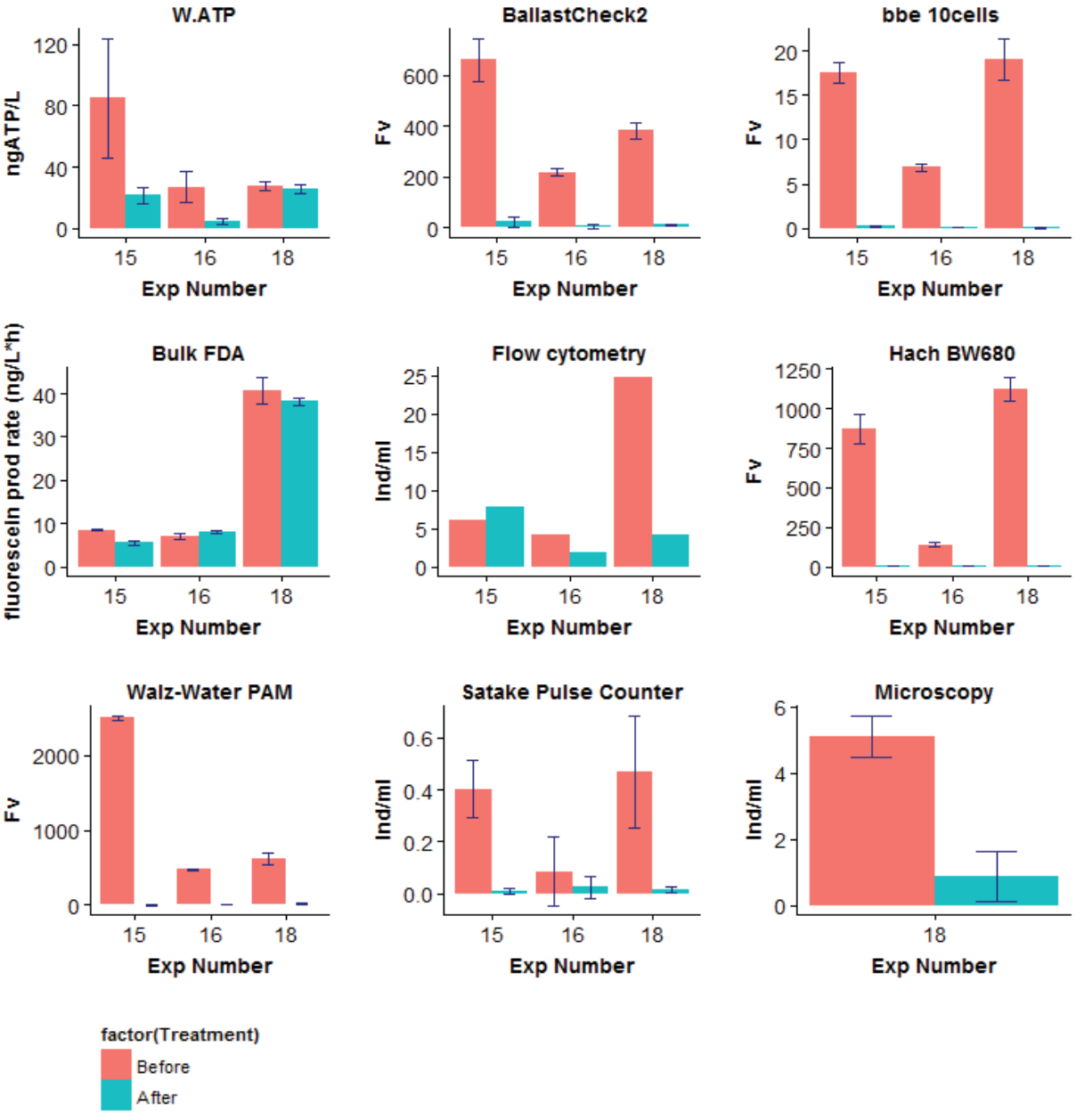


Figure 22. Bar graph of estimated mean densities (+/- SD) for ≥ 50 µm organisms in water that was treated using the OptiMarin BWMS and retreated after being held in a ballast tank for 2 days. All values are standardized to raw ballast concentration. (18, Before) shows estimated densities before treatment, (18, After) shows densities after the first treatment, (26, Before) shows densities before the second treatment, and (26, After) shows densities after the second treatment.

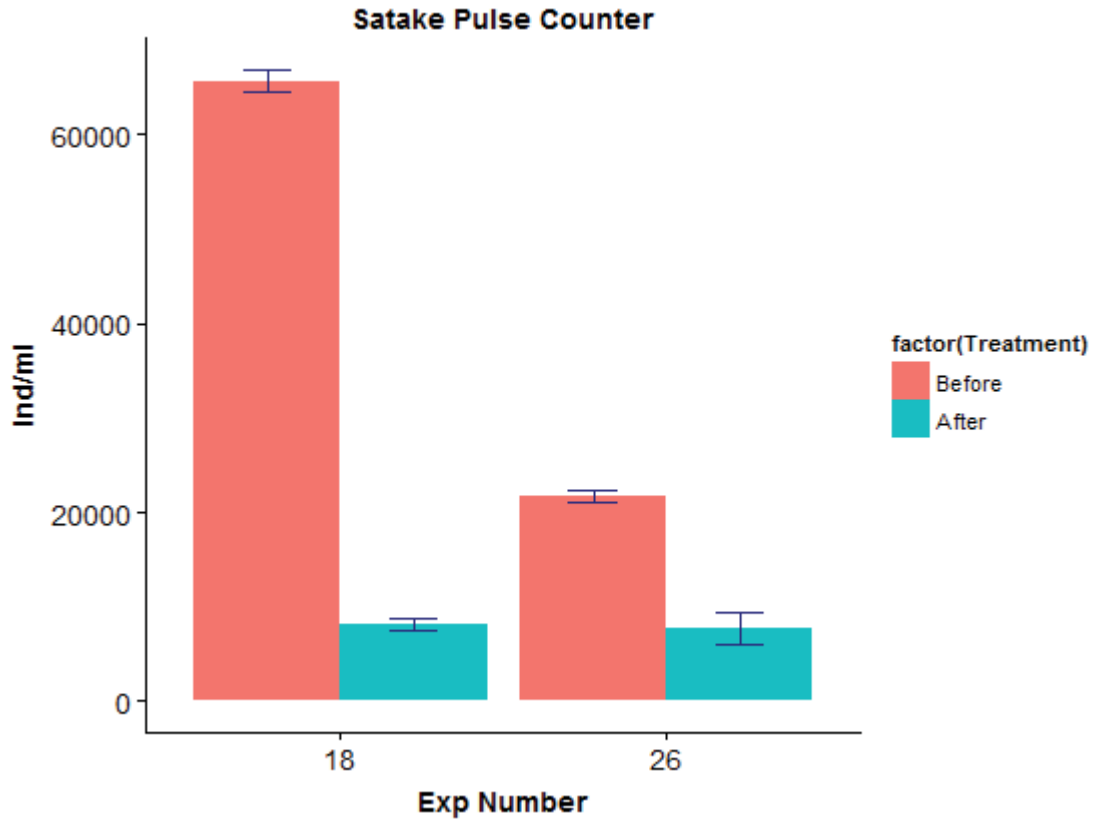


Figure 23. Bar graphs of estimated mean densities (+/- SD) for 10-50 µm organisms in water that was treated using the OptiMarin BWMS and retreated after being held in a ballast tank for 2 days. All values are standardized to raw ballast concentration. (18, Before) shows estimated densities before treatment, (18, After) shows densities after the first treatment, (26, Before) shows densities before the second treatment, and (26, After) shows densities after the second treatment.

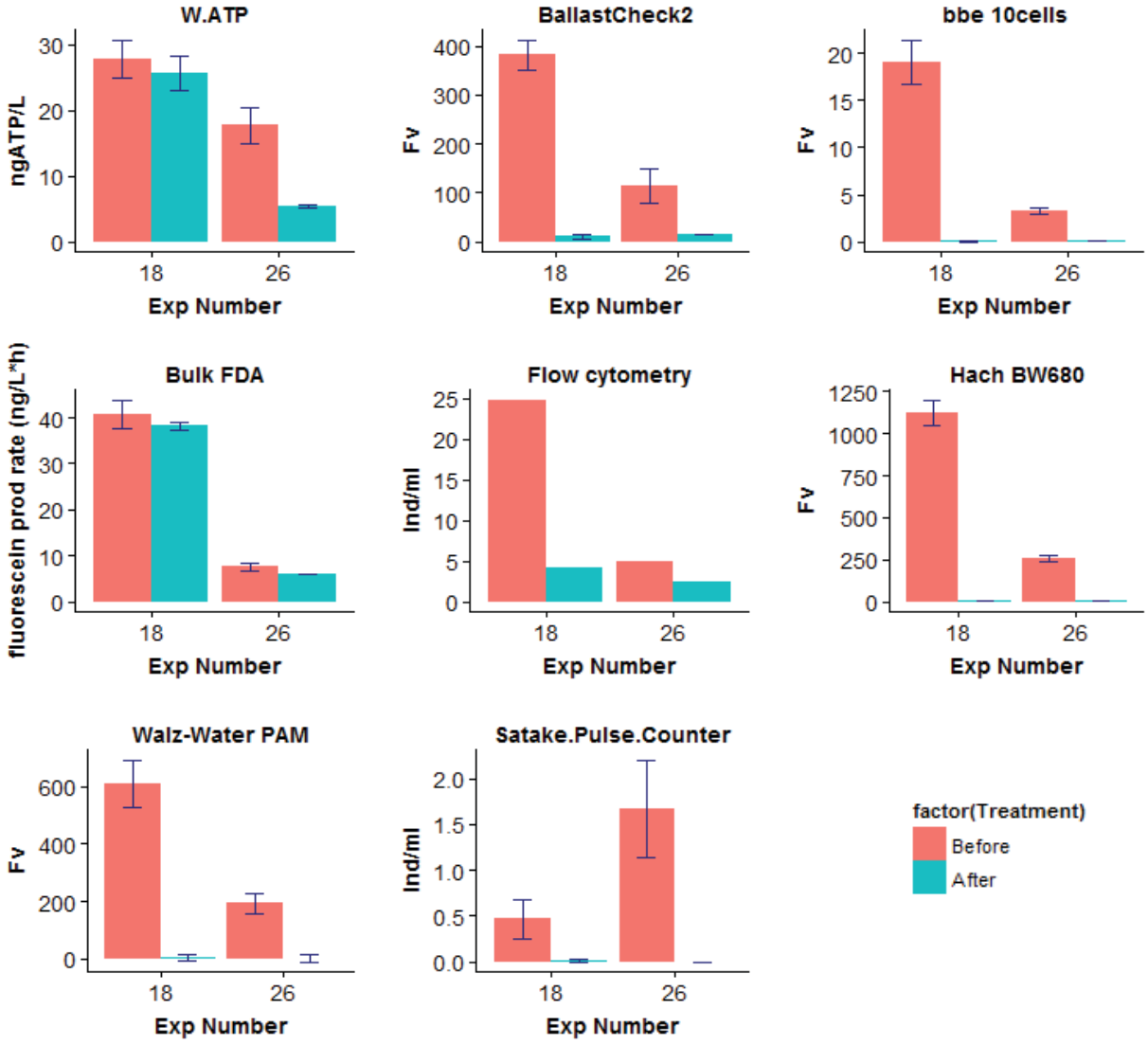
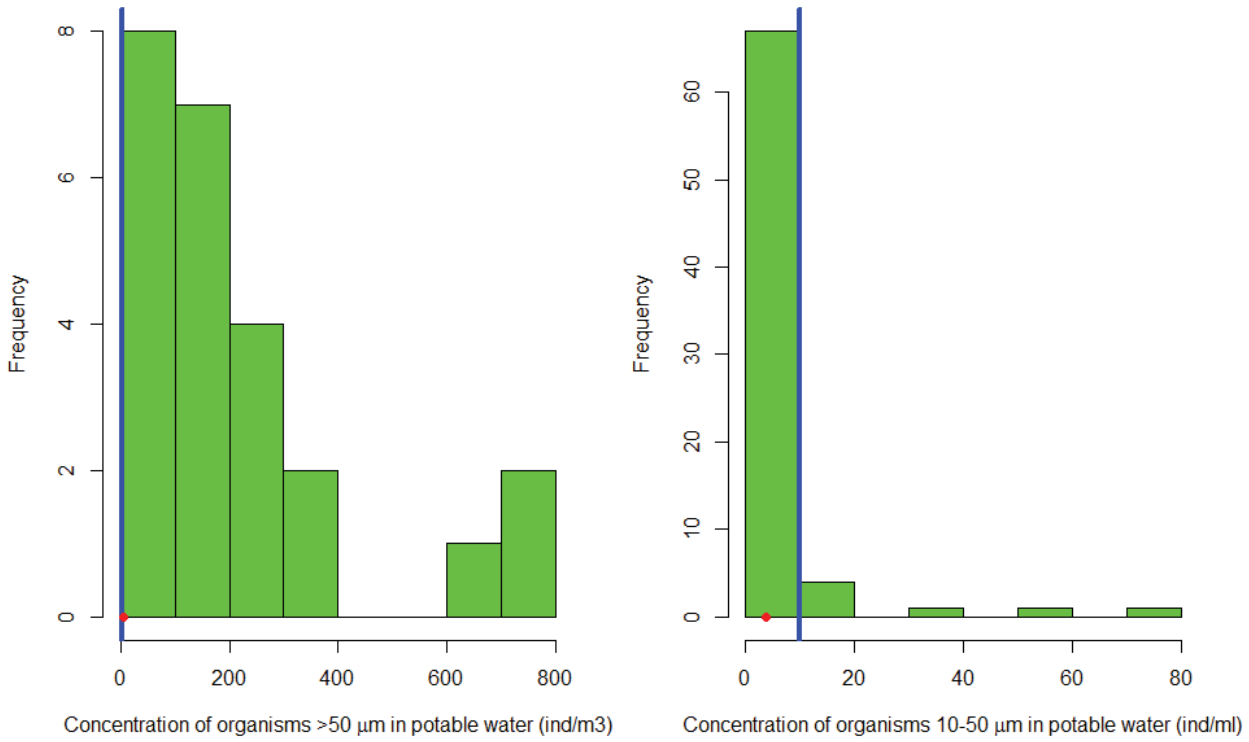


Figure 24. Histogram of the concentration of organisms ≥ 50µm in individuals/m^3 (left panel) and organisms 10-50 µm in individuals/mL (right panel) in potable water samples. The vertical blue line indicates the D2-standard and the red dot on the x-axis marks the mean.



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APPENDICES

Appendix A: Detailed sampling information.

Table A1: Detailed sample collection information. Columns detail the total ballast volume that passed through the ship's ballast pipes during sampling, the pumping rate of the ballast water, the start and end time of each trial, the salinity and temperature of sampled water, the ship starting and ending position, and the source of water used for the trial.

Exp Num	Total ballast volume (m^3)	Flow Rate (m^3/hr)	Start Time	End Time	Sample Salinity (psu)	Sample Temp (C)	Start Position Longitude	Start Position Latitude	End Position Longitude	End Position Latitude	Water Source
1	6.57	17	17:22:00	17:45:00	13.84	23.12	18.08	-24.47	18.14	-24.44	Tank 0.7
2	14.02	29	09:42:00	10:11:00	23.5	22.3	20.97	-23.19	21.04	-23.13	Tank 0.7
3	17.7	58	13:20:00	13:38:00	35.75	22.2	21.61	-22.92	21.67	-22.89	Ocean
4	16.15	57	09:15:00	09:32:00	34	21.7	24.86	-21.46	24.90	-21.43	Ocean
5	17.73	56	13:01:00	13:20:00	34.86	22.8	25.43	-21.19	25.49	-21.17	Ocean
6	16.43	58	08:59:00	09:16:00	35.57	20.81	28.73	-19.66	28.77	-19.64	Ocean
7	14.4	48	12:47:00	13:12:00	35.63	20.97	29.35	-19.36	29.42	-19.33	Tank 0.9
8	17	60	15:40:00	15:57:00	36.07	20.74	29.83	-19.13	29.88	-19.11	Ocean
9	22.23	58	08:14:00	08:37:00	36.15	20.22	32.66	-17.76	32.72	-17.73	Ocean
10	21.85	57	12:23:00	12:46:00	35.09	19.32	33.34	-17.36	33.40	-17.32	Ocean
11	18.37	58	15:13:00	15:32:00	35.36	19.27	33.81	-17.07	33.86	-17.03	Ocean
12	16.5	55	08:24:00	08:44:00	35.59	19.2	36.69	-15.21	36.75	-15.17	Ocean
13	18.15	55	12:15:00	12:35:00	34.95	18.47	37.31	-14.80	37.36	-14.77	Ocean
14	24.75	55	15:28:00	15:55:00	34.78	18.77	37.83	-14.46	37.91	-14.41	Ocean
15	16.8	56	08:30:00	08:48:00	35.06	17.13	40.40	-12.71	40.43	-12.69	Ocean
16	16.15	57	12:25:00	12:42:00	34.79	17.07	40.75	-12.47	40.78	-12.45	Ocean
17	22.8	57	15:45:00	16:09:00	34.94	16.7	41.14	-12.20	41.18	-12.17	Ocean
18	16.8	56	08:15:00	08:33:00	34.93	15.75	43.23	-10.71	43.28	-10.68	Ocean
19	17.7	59	12:18:00	12:38:00	35.08	16	43.82	-10.24	43.87	-10.20	Ocean
20	21.47	56	15:35:00	15:58:00	34.51	15.81	44.32	-9.80	44.38	-9.74	Ocean
21a	16.8	56	08:44:00	09:02:00	35.2	15.52	47.22	-7.08	47.27	-7.03	Ocean
21b	15.87	56	09:27:00	09:44:00	34.95	15.1	47.35	-6.97	47.40	-6.92	Ocean
22	16.5	55	12:25:00	12:43:00	34.65	14.11	47.90	-6.46	47.96	-6.41	Ocean

23	15.87	56	15:48:00	16:05:00	34.42	14.9	48.49	-5.90	48.53	-5.86	Ocean
24	21.46	56	08:25:00	08:48:00	34.07	13.19	50.12	-1.15	50.14	-1.04	Ocean
25	17.3	56	12:19:00	12:38:00	33.96	13.12	50.29	-0.12	50.30	-0.04	Ocean
26	15	50	15:46:00	15:59:00	34.31	13.77	50.45	0.81	50.46	0.87	Tank 0.1
27	15.87	56	08:35:00	08:52:00	34.02	13.7	52.80	4.18	52.84	4.22	Ocean
28	16.5	55	12:20:00	12:40:00	33.29	13.79	53.29	4.64	53.34	4.68	Ocean

Table A2: Sample volumes and concentrations for ≥50 µm and < 50 µm samples.

Exp Num	Port	Collection Device	Total volume filtered (≥50 µm)	Condensed Volume (≥50 µm)	Total Volume Collected (<50 µm)	Concentration Factor (<50 µm) (Total / Condensed volume)
1	A	Triton Open	1001	1	10	1
1	C	SGS Open	1020	1	10	1
2	A	Triton Open	1417	1	10	1
2	C	SGS Open	729	1	10	1
3	A	Triton Open	1001	1	10	1
3	C	Net	1036	1	10	1
4	A	Net	1048	1	10	1
4	C	Net	1014	1	10	1
5	A	SGS Open	1003	1	10	1
5	C	Net	1135	1	10	1
6	A	Triton Open	1001	1	10	1
6	C	Net	1032	1	10	1
7	A	Net	1003	1	11	1
7	C	SGS Open	1073	1	10	1
8	A	SGS Open	1000	1	10	1
8	C	Net	1059	1	10	1
9	A	Triton Open	1157	1	15	6.5
9	C	SGS Closed	1005	1	15	6
10	A	Net	1264	1	15	14.5
10	C	SGS Closed	1001	1	15.5	15.5
11	A	Net	1020	1	16	16
11	C	Net	1060	1	15	14
12	A	SGS Open	1072	1	15	12
12	C	Triton Closed	307	1	15	14
13	A	Net	1114	1	15	11.5
13	C	Triton Closed	231	1	15	13.5
14	A	Net	1568	1	15	14
14	C	Triton Open	1002	1	15	14
15	A	Net	1051	1	15	14
15	B	Net	1017	1	15	13.6
16	A	Net	1025	1	15	14
16	B	Net	1005	1	15	14
17	A	Net	1172	1	15	15
17	C	SGS Closed	1002	1	15	14
18	A	Net	1019	1	15	14
18	B	Net	1040	1	15	14
19	A	Triton Open	1000	1	15	14

19	C	SGS Open	1129	1	15	14
20	A	Net	1203	1	15	14
20	C	SGS Closed	1001	1	15	14
21a	A	SGS Open	1069	1	15	8
21a	C	Triton Closed	835	1	10	8
21b	A	SGS Open	854	1	NA	NA
21b	C	Triton Closed	NA	1	NA	NA
22	A	Net	1102	1	15	8
22	C	SGS Closed	1001	1	15	8
23	A	Net	1009	1	15	10
23	C	Net	1013	1	15	10
24	A	Net	1162	1	15	8
24	C	SGS Closed	1001	1	15	8
25	A	Net	1038	1	15	8
25	C	Net	1017	1	15	8
26	A	Net	1012	1	15	14
26	B	Net	1052	1	15	14
27	A	SGS Open	1001	1	15	10
27	C	Net	1021	1	15	10
28	A	Net	1058	1	15	10
28	C	SGS Closed	662	1	15	10

Appendix B: Sample Splitting Procedure

The following procedure was used to split samples collected into the engine room into sample bottles for each individual method:

≥50 µm (1 L)

1. Gently invert the 1-L bottle 5 times.
2. Half-fill 1 bottle of each AA, AB, AC, AD, AE, AF and "extra."
3. Gently invert the 1-L bottle 5 times and top up the volumes.

<50 µm (15 L)

1. Gently invert the 10-L carboy 5 times.
2. Half-fill 1 bottle of each DD, DE, DG, DJ, BK, BC-1 and "extra."
3. Gently invert the 10-L carboy 5 times and top up the volumes.
4. Filter remaining water on a 10 µm (diagonal) Sterlitech polyester track etch (PETE) membrane filter.
5. Resuspend retained particles in 1.5L sterile seawater for 10-50 µm fraction.
6. Retain ~3L filtrate containing organisms in the smaller than 10 µm size faction.

> 10 < 50 µm (1.5 L)

1. Gently invert the carboy containing the resuspended particles 5 times.
2. Half-fill 1 bottle of each BB, BC-2, BD, BE, BF, BG, BH, and BJ.
3. Gently invert the carboy 5 times and top up the volumes.

< 10 µm (~3 L)

1. Gently invert the carboy 5 times.
2. Half-fill 1 bottle of each DH, DI, BC-3, CC, CD, CE, CF and CG.
3. Gently invert the carboy 5 times and top up the volumes.
4. Rinse the carboy with filtered seawater 5 times and allow to drip-dry.

Appendix C: Protocols for Analytic Methods

Optical Zooplankton Analysis (OZA)
Size Class: >200µm
Researcher: Wouter van de Beek

Description: The OZA method distinguishes viable zooplankton larger than 200µm from dead organisms and debris based on their swimming capability. An LED in the OZA device projects shadows of the organisms in the sample onto a partially transparent projection screen. To ensure very sharp projections of the organisms, the light coming from the LED must approach perfect light collimation. To do so, the divergent light coming from the LED is collimated by a lens and the collimation is further optimized using mirrors to make the distance from lens to sample as long as possible. Two successive images of the projection are captured by camera and loaded in Matlab for analysis. Matlab subtracts one image from the other (analyzed image from reference image), so that every object that has the same location in both images disappears. Resultantly, the only objects that remain are those that have moved: swimming, viable zooplankton. The final image is made binary and the number of objects are automatically counted.

Methods:

1. A 200 mL sample is poured into a sample bottle and filled to the top with the same salinity water (diluting); this reduces movement in the sample caused by the ship's movement.
2. The sample is placed in the OZA device and left for 15 minutes to allow debris to settle and ensure debris that is still settling does so at a lower speed than detectable.
3. The camera is focused manually and 2 pictures are captured.
4. The camera is removed from the device and connected to a computer. Images are downloaded and converted from RAW to TIFF for Matlab analyses.
5. Images are placed in chronicle order and filenames are input to MATLAB code for processing. The MATLAB script takes approximately 10-15 seconds to display the results which include the analyzed image with the motile organisms identified as purple dots, and organism counts, which are displayed above the image. This process is repeated after reversing the order of the images (i.e. analyzed image becomes reference image), and the average is taken of these two results to get the final result.
6. Steps 3-5 are repeated in triplicate for each sample. Note that the waiting time is only necessary for the first run, since the same 200mL is used for all three tests.

Microscopy
Size class: ≥50 µm; 10 µm > x > 50 µm
Researchers: Julie Vanden Byllaardt, Johanna Bradie, Sarah Bailey
Description: Microscopy is the historical standard for the analysis of ballast water samples.

Methods (≥50 µm):

1. Initially, a 50 ml sample [AA] of the ~1000L concentrated water was received for enumeration of live organisms greater than 50 µm. Due to very low densities of large motile invertebrates at the beginning of the voyage, this was increased to 200ml beginning with test run #4. The density of large motile invertebrates varied widely as the vessel sailed – when required, the sample received was further concentrated up to 20x using 50 µm (in diagonal) plankton mesh to achieve a target range of 20-50 motile invertebrates per ml. The concentration factor utilized was recorded for each sample such that an estimate of densities in the unprocessed raw water could be calculated.
2. Each [concentrated] sample was well mixed by gentle inversion five times.
3. Three one ml subsamples were drawn using a 1000 µl Eppendorf Research® pipette. The end of the pipette tip was cut shorter by approximately 5 mm to enlarge the diameter of the tip opening to facilitate capture of larger organisms.
4. To facilitate counting at sea, we modified standard Bogorov chambers by adding plexiglass blocks in each curve, dividing the chamber into five channels, each having one ml capacity. Each subsample was deposited into a channel for analysis under a Nikon SMZ800N dissecting microscope at 30x magnification.
5. All organisms retained in the sample were assumed to be larger than 50um in minimum dimension since the sample had been processed 2 or 3 times on 50 µm mesh – however, protists obviously smaller than 50um were occasionally observed and omitted from counts. Motile organisms were counted if they exhibited any movement autonomously or in response to gentle nudging using an insect pin. Non-motile organisms, such as diatoms, were counted using FDA stain as described for organisms in the 10-50 µm size range.
6. Three additional one ml subsamples were taken from the [concentrated] sample for staining, with 0.25 or 0.5 ml examined under an epifluorescence microscope, depending on density.

   All counts were completed within 6 hours of the start of sample collection.

Methods (10 µm > x > 50 µm):

1. Each [concentrated] sample was well mixed by slow inversion of the sample bottle 5 times.
2. In triplicate, 5 ml of the water was drawn using a 5000 µl Eppendorf Research® pipette, placed in a 20 ml glass scintillation vial and stained with 10 µl of 2.5 mM fluorescein diacetate (FDA; in solution with dimethyl sulfoxide) to create a final concentration of 5 µM FDA.
3. Stained subsamples were dark incubated for 10 minutes, after which 1 ml was loaded into a gridded 1 mm^2 Sedgewick-Rafter counting chamber.

4. Fluorescing cells were enumerated at 200x magnification using a Zeiss Axio Vert.A1 inverted epifluorescence microscope fitted with a blue light excitation-green bandpass emission filter cube (FITC; excitation 465 - 495 nm, dichoric 505 nm, barrier 515 - 555 nm) and light-emitting diode (LED) illumination.
5. Due to the low concentration of algae in the sample water, the entire chamber was viewed and fluorescing cells were counted if <u>any</u> dimension was greater than 10 µm, using the gridded line thickness of 18 µm as a size reference. Individuals that were only partly fluorescing were counted as viable. Cells within colonies were only included in counts if the individual cell had a dimension greater than 10 µm. Stained aliquots were examined within 20 minutes of dark incubation and all samples were processed within 4 hours of the start of sample collection. FDA stain was kept frozen and in the dark when not in use.

Satake Pulse Counter
Size Class: ≥50µm and 10µm<x<50µm
Researchers: Akiko Nakata and Jianjun He, Satake Corporation
Description: The Satake pulse counter, which employs pulse counting FDA method, estimates the number of viable organisms in ballast water samples by counting fluorescence pulse over specific threshold from FDA stained organisms in short time. This system consists of main body (detection unit, touch panel display and CPU), sample cell, portable casing and AC adapter. This system is small enough to be carried on board by one person, and therefore suitable for PSC inspection. Figure A1-1 shows a schematic diagram of the detection unit of the Satake pulse counter. The detection unit consists of LEDs, a stirrer, a detector, and optical filters. The LED has an excitation light source that provides blue light with a specific wavelength which makes stained organisms fluorescent. A photomultiplier tube is used as a detector in order to detect weak fluorescence. The optical filters are employed to cut stray light in order to enhance sensitivity. Figure A1-2 shows an image of detected pulse signals. When fluorescence intensity from one organism exceeds a pre-fixed threshold, the number of organisms which pass on the monitoring area will be counted.

Methods:

1. L size plankton (x ≥50 µm) measurement

- Unfreeze "Reagent L" (FDA) at room temperature.
- Put the stirring bar into sample cell.
- Add 100 mL of sampling water into sample cell
- Add "Reagent L" (FDA) to sample cell
- Wait for ten minutes.
- Switch on, then press "L size" button on screen.
- Set sample cell in the body, and press start button.
- Estimated number of viable organisms will be displayed on the screen.

2. S size plankton (10~50 µm) measurement

- Unfreeze "Reagent S" (FDA) at room temperature.
- Add sampling water into "Reagent S" bottle up to 5 mL scale line using a dropper.
- Wait for thirty minutes.
- Put the stirring bar into sample cell.
- Transfer 5 mL stained sampling water to sample cell.
- Add more sampling water without FDA staining up to 100 mL.
- Switch on, then press "S size" button on screen.
- Set sample cell in the body, and press start button.
- Estimated number of viable organisms will be displayed on the screen.

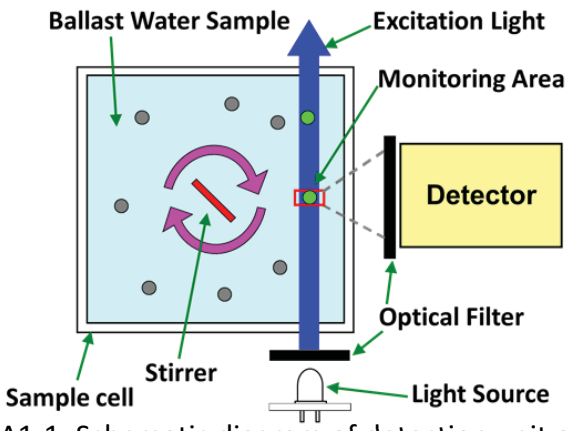

Figure A1-1: Schematic diagram of detection unit of Satake pulse counter

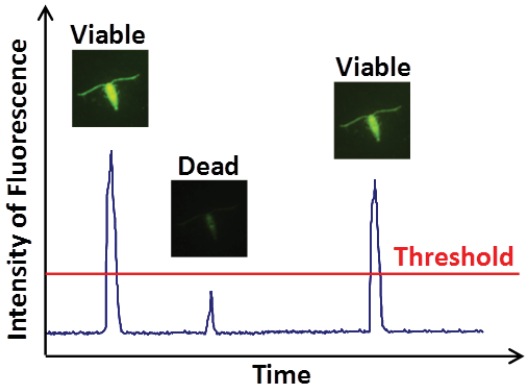

Figure A2-2: Image of detected pulse signals

bbe 10cells (PAM)
Size Class: 10µm>x>50µm
Researcher: André Zaake, bbe
Description: bbe 10cells is an indicative Instrument for analyzing living cells in ballast water. It is based on variable fluorescence (Fv) of chlorophyll of living algae. During a filtration step, the algae are placed on a filter inside a bbe filter kit. This filter will be placed in the instrument and a measurement can be started. By changing the volume of filtration or filter size it can be adapted to different environments and requirements. It is easy to use, takes approx. 1 min and no chemicals are needed.
Methods:

1. Switch the instrument on by pressing "Power".
2. Optionally, filter sample using a 50µm pre filter.
3. Fill a 10ml syringe to 10ml with sample.
4. Pull the syringe to fill 2ml additional air in it.
5. Take a bbe 8µm filter kit and push/screw the syringe on.
6. Press the syringe gently while holding the syringe with filter kit vertically. Press until the syringe is completely empty (include air).
7. Pull the filter kit to release from syringe.
8. Open filter kit by open latch.
9. Remove filter strip while holding it horizontally.
10. Dry filter by pressing a tissue on bottom of filter.
11. Insert filter into 10cells and close cap.
12. Press start.
13. Wait approximately 1min --> result will be displayed on screen.
14. Remove filter strip.

Turner Designs' Ballast-Check 2 (PAM)
Size Class: 10µm>x>50µm
Researcher: Lawrence Younan, Turner Designs

Description: Turner Designs' Ballast Check-2 is a quick indicative compliance tool used for estimating the abundance and assessing the viability of phytoplankton from the 10-50 micron size class. This high precision instrument uses the organism's fluorescence characteristics to provide a quick analysis of whether ballast water is in compliance with current discharge standards. The Ballast Check-2 is a small, lightweight, durable instrument operated by four AAA batteries. It can store up to 1000 data points, which can be viewed using the instrument's display or downloaded directly to your computer. Factory set, it is ready for use right out of the box, no calibration necessary.

Methods:

All Standard Operating Procedures pertaining to calibration check, sample processing, measurement, and maintenance/cleaning can be found in the Ballast-Check 2 User's Manual; sections referenced for each procedure.

Sample Analysis:

For each trial, analysis was completed on 3 size fractionated samples (< 50 µm, 10-50 µm, and < 10 µm) in triplicate from each collection device (S1 and S2). All measurements were corrected for blank using rinse water that was available for each sampling event. All subsamples (fractionated samples) were analyzed independently, using rinse water as a blank value, to calculate individual cells per milliliter and associated activity of those cells. The final analysis used both the < 50 micron and < 10 micron samples, using the < 10 micron filtrate sample as a blank, to calculate individual cells per milliliter, activity of those cells, and the risk associated with the water collected for that sampling event, for that sampling method (S1 or S2).

Sample Measurement:

The following procedure was used to analyze the three subsamples independently:

1) The sample bottle was gently inverted 5x
2) A 1-cm square glass cuvette was rinsed 3x using the mixed sample
3) 3.5 milliliters of sample was transferred into the cuvette using a 5 ml pipet
4) Cuvette walls were wiped using KIMTECH Kimwipes
5) Cuvette was inserted into the Ballast Check-2
6) Sample was measured
    a. If a 10 micron filtered sample was not required, sample analysis was done and results were logged
    b. If a 10 micron filtered sample was requested, a secondary 1-cm glass cuvette containing rinse water was inserted into the Ballast Check-2 and read in place of the 10 micron sample, then sample analysis was done and results were logged
7) Steps 1-6b were repeated 3x for each subsample provided
8) Logged results were downloaded and transcribed into provided data sheets

The following procedure was used to run a final analysis using 2 of the three subsamples provided (< 50 micron and < 10 micron samples):

1) Both sample bottles were gently inverted 5x
2) Two 1-cm square glass cuvette were rinsed 3x using the mixed sample (one cuvette for the < 50 micron sample and one cuvette for the < 10 micron sample)
3) 3.5 milliliters of each sample was transferred into its respective cuvette using a 5 ml pipet
4) Cuvette walls were wiped using KIMTECH Kimwipes
5) The < 50 micron sample cuvette was inserted into the Ballast Check-2
6) Sample was measured
    a. If a 10 micron filtered sample was not required, sample analysis was done and results were logged
    b. If a 10 micron filtered sample was requested, the < 10 micron sample cuvette was inserted into the Ballast Check-2 and measured, then sample analysis was done and results were logged
7) Steps 1-6b were repeated 3x for each sampling method (S1 and S2)
8) Logged results were downloaded and transcribed into provided data sheets

Calibration Check Procedure (See section 2.3 in the Ballast-Check 2 Manual)
Sampling Procedure (See section 2.5 in the Ballast-Check 2 Manual)
Data Download Procedure (See section 2.7 in the Ballast-Check 2 Manual)

Calculations

There are two gain states that may be used to read a sample depending on the sample's fluorescence intensity. If the sample read has a high amount of fluorescence, the 1x gain state is used for the measurement. For low fluorescing samples, the 10x gain state is used. The Ballast-Check 2 will log the gain state used for each sample measured so the appropriate blank and calibration values can be applied when manually calculating Abundance.

Abundance is calculated as:

Abundance = [(Unfiltered Fo–Blank) – (10micron filtered Fo–Blank)] * [(Cal Val)/(Cal RFU)]

where "Blank" and "Cal RFU" are gain specific

The Activity calculation requires a blank to be subtracted from the Unfiltered Fo and Fm values to accurately estimate algal viability. To manually calculate Activity, the gain state the sample was read in needs to be known so the appropriate blank can be applied.

If the sample did not require filtration, then Activity is calculated as:

Activity = (Unfiltered Fm–Blank 10x) – (Unfiltered Fo–Blank 10x) (Unfiltered Fm–Blank 10x)

If the sample required filtration, then Activity is calculated as:

Activity = (Unfiltered Fm–10micron filtered Fm) – (Unfiltered Fo–10micron filtered Fo) (Unfiltered Fm–10micron filtered Fm)

Walz-Water Pulse-Amplitude-Modulation (PAM)
Size Class: 10 µm > x >50 µm; ≥50 µm; <10 µm
Researcher: M.Veldhuis, MEA-nl, RUG

Description: The aim of PAM is to estimate the phytoplankton biomass as chlorophyll (in terms of autofluorescence) and the photosynthetic activity (as estimate of viability) of phytoplankton. Measurements can be performed using whole water samples or size fractionated or otherwise collected water samples containing phytoplankton.

Principle: Chlorophyll a fluorescence, particulary Fv/Fm, which has shown to be a sensitive indicator of photosynthetic performance (Krause & Weis, 1991). Modulated fluorometry (Schreiber et al,, 1986) allows the recording of photosynthetic performance parameters that are obtained using saturation pulses applied during fluorescence induction by an actinic light source (Heraud & Berdall, 2000). The PAM fluorometer measures different stages of the fluorescence of the chlorophyll-protein complex of phytoplankton. After dark adaptation the measured fluorescence is the auto, minimum or ground fluorescence signal (F0).

During the activation the sample is illuminated with light saturation pulses by means of pulse amplitude modulation (PAM). During process the maximum yield of photochemical energy conversions will be measured of the chlorophyll-protein complex (Fm). The difference between both fluorescence signals is the variable fluorescence (Fv). This variable fluorescence is an indicator of the efficiency of the photosynthetic activity of the chlorophyll-protein complex of the algal cell. In healthy and fast growing phytoplankton cells the difference is high but in stressed or dead phytoplankton cells the both values of fluorescence signals are nearly identical, hence difference is only minimal.

Definitions
Actinic light: light that will facilitate photosynthesis or stimulate light sensitive species
PAM: Pulse Amplitude Modulation
r.u.: relative unit
F_0: total chlorophyll biomass, estimated in relative units (r.u.)
F_m: chlorophyll fluorescence after excitation (r.u.)
F_v: variable chlorophyll fluorescence: $F_v = F_m - F_0$
F_v/F_m: photosynthetic efficiency of the phytoplankton community. Typical values range between 0 and ca 0.8

Figure 1: PAM fluorometry; a fast method to determine (bulk) phytoplankton biomass and the photosynthetic activity of the phytoplankton. Left Universal Control Unit, right Water ED/B

Reagents and consumables

- Sample water (GF/F or 0.2 µm filtered)
- Mili Q or equivalent water

Equipment and tools

- PAM-Universal-Control unit, WALZ
- WATER-ED/B Emitter-Detector-Unit, WALZ
- Water-K, Quartz glass cuvette
- PC or laptop with WALZ measurement software (PAM WALZ Win-Control v3.23)

Sampling and sample handling

Obtain different water samples during the sampling and fractionation procedure and store them in the dark prior to analysis. Avoid bright light are high temperatures. Analyse samples as soon as possible after receiving the bottles.

Procedure

5.1. Processing

- Analysis of the samples can be done when samples were stored in the dark for at least 30 min.

5.2. Analysis

- Start the computer and the software program for the PAM (=Wincontrol version 3.23).
- Prior to this measurement the operator should be familiar with the procedures as described in the PAM control manual (PAM control unit and Water PAM manual).
- Program selects automatically last save protocol for given set of measurements.
- First a Blank sample needs to be measured; fill (at least 2-3 mL) the PAM cuvette with filtered sample water (0.2 micron) of challenge water and cover the cuvette with the lid.
- The Ft signal needs to stabilize for at least 30 seconds.
- To increase the stability highlight ML-F-high, this will increase the number of saturation pulses
- Mix water sample gently and fill a cuvette with a sample (2-3 mL)
- The Ft signal needs to stabilize for at least 30 seconds.
- Note: Ft measurement is not reliable when the value is below 100 r.u. or above 1000 r.u. If this is the case, increase or decreases the PM-Gain, respectively. Note the PM-Gain down (10, 15 or 20). Make sure to calibrate settings again for zero with filtered water sample
- Press the F0, Fm button to activate saturation light pulse for reading of F0, Fm and yield (Fv/Fm) (Saturation pulse intensity reaches up to 3000 µmol quanta m-2s-1).
- Note the values of F0, Fm and Fv/Fm ratio as well as the run number
- Data should be saved (floppy icon) using a date and unique identification code
- Repeat the whole procedure three times, a higher gain may be needed when measuring treated water samples with low phytoplankton biomass and photosynthetic efficiency
- When analysis with the PAM is finished, rinse the PAM cuvette with mili Q water.
- Save data, close the program on the computer and turn off the computer and the PAM control.

Calculation and Report

F0 fluorescence of dark adapted phytoplankton
Fm maximum fluorescence yield after saturated light induction
Fv variable amount of fluorescence = Fm=F0

Phytoplankton photosynthetic efficiency Fv/Fm = (Fm – F0) / Fm (no unit, ratio)
With a dark-adapted sample: ΔF/Fm = (Fm-Fo)/F = Fv/Fm, corresponding to the maximal yield of photochemical energy conversion.

Raw data are presented as chlorophyll biomass (F0), not corrected for instrument settings. Viability is presented as a quantitative measurement ranging from 0 to approximately 0.8 as the sample measured and can be compared for the different fractions.

To convert the raw data into a number (viable) cells a set of specific calibration curves need to be made for each size class of phytoplankton and is because of its complexity outside the scope of this exercise.

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WATER-PAM Chlorophyll Fluorometer Instrument Description and Information for Users 2.137 / 11.00 2. Edition: May 2013 waterp3e.doc

Hach BW680 (PAM)
Researcher: Nick Welschmeyer
Size classes: ≥50 µm; 10µm>x>50µm; <10µm

Summary: The determination of variable chlorophyll *a* (chl) fluorescence from natural, whole-cell phytoplankton serves as a rapid method to assess relative ballast water compliance. The Standard Operating Procedure (SOP) here provides details on the application of variable chl *a* fluorometry to ballast water compliance testing (see Protocol Overview). The method is specifically configured to yield easily measured data that scales proportionally with the total living phytoplankton biomass within the 10 - 50 µm size class. A calibrated conversion factor allows simple calculation of the equivalent numerical live count for purposes of compliance evaluation against published ballast water discharge standards (BWDSs).

Scope and Application: The protocol described here utilizes modulated fluorescence instrumentation to measure variable chl *a* fluorescence for the rapid evaluation of ballast water compliance. The method is based on simple, whole-cell evaluation of small sample volumes (2.5 mL) without the requirement for reagents nor biological incubations. The method is rapid (<2 min) and requires no special training. The method does have its limitations for direct comparison to ballast water discharge standards because the detection response is restricted to autotrophic, chl-containing organisms only, thus ignoring heterotrophs. The trade-off, however, lies in the 1) *speed* (1-2 min), 2) *simplicity* (no reagents) and *convenience* (portable, pocket-sized instrumentation) of the procedure; these are considered ideal traits for rapid ballast water compliance monitoring.

To our knowledge, the method, as described here, could be executed by any of the currently marketed variable chl fluorometers capable of delivering stable measurements of Fv (Schrieber et al. 2000) at levels of chlorophyll approximating those concentrations expected for successfully treated ballast water (see Protocol Overview). The list of vendors include BBE Moldaenke, Schwentinental, Germany; Chelsea Instruments, Ltd., West Molesey, United Kingdom; Hach Corp, Loveland, CO USA; Hansatech, Ltd., Pentney, United Kindgom; Heinz Walz GmbH, Effeltrich, Germany; Opti-Sciences, Hudson, NH USA; Photon Systems, Inc., Brno, Czech Republic; Qubit Systems, Inc., Kingston Ontario, Canada; Satlantic Instruments, Dalhousie, Canada; Turner Designs, Inc., Sunnyvale CA USA.

In the SOP here, we describe the use of the pocket-sized, battery operated BW680 fluorometer, originally developed by PSI Inc. (Czech Republic) and now modified and marketed for ballast water work by Hach Corporation (USA). The instrument is preprogrammed with repetitive flash sequences, utilizing both blue and red excitation energy (to optimally cover fluorescence excitation of all algal taxa, including cyanophytes) yielding a single averaged variable fluorescence response, termed the Ballast Water Index (BWI). The BWI response shares all the characteristics of Fv, the standardized term for 'variable fluorescence' generated by all variable chl fluorescence instruments (Schreiber et al. 2000). The convenient features of the BW680 instrument for rapid ballast water testing relate to its simplicity: 1) no instrument blanking is required, 2) the wide dynamic range of the detector requires no gain adjustments from its

minimum detection level of 0.05 µg Chl *a*/L to over 40 µg Chl a/L, and 3) no user-based programming manipulations are required (nor offered); it operates on the push of a single button.

Methods:

- Hold filter-fractionated samples in the dark for a minimum of 3 minutes before making measurements. The correct determination of BWI (also Fv) must be made on dark-adapted samples. A room temperature darkened cabinet/drawer is sufficient; conversely, samples can be held in darkened, amber sample bottles, held out of direct sunlight.
- Pipet or decant 2.5 mL dark-adapted sample water into a clean, plastic, fluorometer cuvette. Keep volumes consistent, since changes in volume level will affect optical environment within the cuvette chamber.
- Place sample cuvette in the instrument sample chamber, close the lid and press 'MEASURE'; the BWI response will be displayed in ca. one minute.
- Measure the unprocessed sample. Record measurement; data are also stored in internal memory, accessed by USB data downloads.
- The programmed flash sequence will expose sample water to high saturating levels of light that may require some recovery time; the same cuvette fluid should not be measured twice. Therefore, replicate measurements, if desired, should be made on fresh sample additions.
- Calculate estimated numeric concentration of cells using Eq. 1 below; compare to ballast water discharge standards of 10 live cells/mL for the 10-50 µm size class.

Calculation of equivalent numeric cell concentration

Equations 1 and 2, below, provides the means to estimate the equivalent numeric concentration of live cells corresponding to the measured BWI signal (or Fv) of any unknown ballast water sample, processed as described above. The exponentially-weighted mean cell size of the 10-50 µm plankton size class is calculated to be 15 µm ESD (Welschmeyer 2013) based on organism size distribution models for coastal systems, characterized by a Log biomass vs. Log cell volume slope of -0.1 (Vidondo et al. 1997). A cell specific BWI response factor (RF) of 14.98 BWI/(cell mL-1) for the 15 µm ESD cell size was determined empirically, and specifically, for the BW680 fluorometer from sized-based analysis of several freshwater and marine phytoplankton species; data shown in Fig. 1 (from Welschmeyer 2013). The BWI signal for the 10-50 µm size category is calculated by subtracting Fraction 2 (<10 µm) from Fraction 1 (<50 µm):

Eq. 1: BWI(10-50µm) = BWI(<50µm) – BWI(<10µm)

As noted above, the calculated 15 µm mean cell diameter refers to the 10-50 µm size category. The estimate of live autotrophic cells within the 10-50 µm size class is then calculated as:

Eq. 2: Live cells/mL(10-50µm) = Sample BWI(10-50µm) / (14.98 BWI/(cell mL-1)

NOTE: The Response Factor (RF) for 'Fv' determined on modulated fluorometers from other manufacturers must be determined empirically, as in Fig. 1, due to unique characteristics for each manufacturer's optical design and detector responses (e.g., raw fluorescence signals). Eq. 2 is specific to the BW680 (Hach Corrp.)

Method detection limits
0.05 µg Chl/L, equivalent to ca. 5 live cells/mL for 15 µm ESD cells.

List of Abbreviations
BWI Ballast Water Index (fluorescence parameter)
Chl Chlorophyll *a*
CV Coefficient of Variation
ESD Equivalent Spherical Diameter
RF Response Factor

Health and Safety
No health and safety issues.

Interferences
Turbidity greater than 20 NTU will cause decreases in BWI

Personnel Qualifications and Responsibilities
Non-technical personnel can execute all aspects of the method; no special training or practice is required.

Special Equipment and Materials
- Hach BW680 handheld modulated fluorometer (or equivalent).
- Disposable plastic 1 cm cuvettes; 4 sides clear, standard VIS transparency.
- Pipet capable of dispensing 2.5 mL sample volume.
- Extra AAA batteries (4) or USB data cord for drawing computer power. New batteries will last several days under full time use.
- 10 µm syringe filters (Pall Life Sciences, Acrodisc Versapor Membrane, 10 µm, PN AP-4001T).
- 50 µm nylon sieve filter (50 µm on diagonal); this coarse filter can be reused after rinsing.

Equipment and Supplies
- Instrument carry case
- Squeeze bottle for rinsing cuvettes (distilled water)
- Plastic waste receptacle for fluids.
- Test tube rack to hold samples (50 mL each, nominal)
- Sample tubes.
- 10 µm syringe filters

Quality Control

The instrument sensitivity is calibrated at the factory. Once calibrated, the solid state, fixed-optics fluorometer is not expected to drift, unless physically mishandled (e.g., dropped on steel deck). Warm up time (<5 sec) is factory programmed into the startup procedure when the instrument is turned on. Instrument will be supplied with a permanent solid standard to check for sensitivity drift.

Flow Cytometry
Size Class: 2 µm > x >50 µm
Researcher: M.Veldhuis, MEA-nl, RUG;
Author of methods: L. Douwes

Summary: This SOP describes the procedure which should be followed when measurements of the total number of phytoplankton in the size range from 2 ≤ cell diameter < 50 µm are needed. Phytoplankton numbers are determined based on the presence of their red fluorescence caused by the presence of chlorophyll. SOP-317.

Aim: The aim of this SOP is to describe sampling, sample storage and analysis of phytoplankton in the size range 2 ≤ cell diameter <50 micron and using flow cytometry (FCM). Of each individual particle, size, optical density and three different fluorescent properties are analyses and stored for detailed analysis. Size class differentiation can be made on basis of filter fractionation or using an internal standard.

Definitions:
Total phytoplankton cells: total number of free phytoplankton cells with cell size varying from 2≤ d <50 µm present in the water per mL based on counting cells possessing chlorophyll auto-fluorescence.
Calibration of flow cytometer: see 'Coulter XL basic training manual'. This calibration procedure provides estimate of sample flow rate (µL/s)

Conversion of actual counts to absolute counts :

Run numbers are unique and linked to a control, treatment activity, and date of intake or discharge.

Absolute Counts = (counted cells – blank)* 1000/counted volume (mL)
Phytoplankton numbers/mL counted cells: number of chlorophyll containing phytoplankton cells
Blank: number of chlorophyll containing phytoplankton cells counted in 0.2 micron filtered sample (average over whole sample set)
Absolute number of cells is averaged of the 3 samples bottles.

Information of Green, Orange auto fluorescence is additional data not immediately necessary. In addition, when present same data of subpopulations can be added to the data sheet.

Principle

To determine the number of phytoplankton in the size range 2 ≤ cell diameter <50 micron and using flow cytometry (FCM).

Reagents and consumables

- Sampling water
- Prefiltered sampling water (0.2 µm prefiltered)
- Sample tubes

Equipment and tools

- Dark sample bottles (500 – 1000 mL)
- Pipettes and pipette tips (1-10 mL)
- Beckman Coulter EPICS XL-MCL flow cytometer
- Refrigerator or cool-box

Sampling and handling

Obtain water samples, this can be freshly collected samples or samples preserved and stored in the freezer. Fresh samples should be stored in a cool box or equivalent container in the dark and cool until analysis. Measurements should be conducted within 4 hours after samples. In the case of preserved samples, samples should be thawed (avoid exposure to light) and measured within 2 hours.

Procedure

1. Mix sample gently prior to taking subsample.
2. A volume of 2.5 – 3.0 mL subsample is pipetted in a clean flow cytometer sample tube of 5 mL (1- 3 replicates). No additions are made to the sample.

Analysis

1. Sample tubes are transferred to manual or carousel of the flow cytometer for analysis.
2. Start up, maintenance and preparatory phase to calibrate the flow cytometer is described in 'Coulter XL basic training manual'.
3. Blank sample is taken prior to the analysis of actual samples (0.2 micron filtered sample).
4. In case of carousel 6 – 12 samples are in the carousel to avoid heating of the sample. This number may vary depending on ambient water temperature of the water samples.
5. Add ND 10 filter in front of FS detector to reduce sensitivity.
6. Analyse sample in the flow cytometer, by manual or through the carousel using test protocol (usually Phyto-10NDX). X may vary for water type or type of treatment.
7. Raw analysis is stored as 3.0 FCM list mode data file and includes setting of flow cytometer parameters for scatter and different fluorescence wavelengths, each sample has unique number (xxxxxxx.lmd).
8. Stored RAW data file is analysed with commercially available software package FCS Express V3 (DeNovo version 3 or higher) using predefined protocol (MEA-Phyto-10ND-vX.fey). Phytoplankton cells are separated from other particles based on scatter and the red fluorescence of the chlorophyll present in the phytoplankton cell (PMT4, > 610 nm emission). The higher limit of the size range of phytoplankton is determined using 10 micron size spherical beads as an internal standard. Phytoplankton numbers of collective phytoplankton community and each subpopulation are collected in spread sheet and converted into absolute counts are correcting for flow rate and sample dilution factors (when appropriate).

Calculation and Report

Absolute number (unit mL) = (counted number of phytoplankton)* f1 = phytoplankton > 10 micron number/mL

f1: (Conversion factor * counted number of phytoplankton) / counting time (seconds)

Conversion factor = number of seconds to analyse 1 mL of sample

Absolute number of phytoplankton in the size range from 2 – 10 micron is average of 1-3 replicates. In addition to the number of phytoplankton cells, the size of cells (in relative units) and auto-fluorescence of the chlorophyll (FL4) and, when present, phycoerythrin (PE) fluorescence (FL2) are measured. PE is a photopigment typically present in cyanobacteria.

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Bulk FDA Viability Assay
Researcher: Nick Welschmeyer
Size classes: ≥50 µm; 10µm>x>50µm; <10µm

Summary The bulk FDA viability assay is a cuvette based fluorometric technique that provides quantitative measurements of total living biomass contained in ballast water samples (freshwater, brackish or marine). The method relies on the active conversion of fluorescein diacetate (FDA) to its fluorescent by-product fluorescein, by natural enzyme activity in live cells. The bulk FDA assay responds to all living organisms, whether autotrophic or heterotrophic, and produces low, nearly undetectable signals when cells are dead (Welschmeyer and Maurer, 2011).

Scope and Application Here we describe a technique to measure total living biomass based on enzyme-activated fluorescein production derived from the substrate, FDA (Welschmeyer and Maurer 2011). The method relies on the capture of particulate matter on selective size filters followed by incubation of the particles in a sterile buffer solution that is quantitatively tagged with FDA. The intracellular, live-cell production of FDA-derived fluorescein is diffusively passed to the extracellular fluid in the buffer suspension, and the concentration of fluorescein is determined fluorometrically in standard 1 cm fluorometer cuvettes. The procedure described here is applied to the 10-50 µm size class of ballast water organisms using simple size-fractionation filter capture. The method utilizes syringe-based manipulations for 1) filtration, 2) reagent addition and 3) incubation, thus reducing potential shipboard contamination and providing pipet-free protocol execution. The expected production rate of fluorescein for cell sizes characteristic of the 10-50 µm range is known (see below). Therefore, the nominal concentration of live cells responsible for the measured fluorescein production rate can be calculated. Measurement of ballast water with unknown live cell concentrations can thus be tested for compliance relative to defined, numeric Ballast Water Discharge Standards (BWDS).

Methods

1. Collect sample water by passing the desired volume through a 50 µm sieve, thus removing organisms >50 µm. Nominally, 500 mL is adequate for up to three replicate assays.
2. Filter 100 mL sample water through a 10 µm pore size, 2.5 cm diameter, sealed syringe filter (Pall Life Sciences, Acrodisc Versapor Membrane, 10 µm pore, PN AP-4001T), using a clean 140 mL plastic syringe. Discard filtrate.
3. Remove syringe filter from large sample syringe. Replace with empty, but fully depressed, 5 ml plastic syringe/plunger on top of sealed syringe filter, fasten luer lock connection. Place empty 5 mL FDA syringe barrel (w/ female connector) on bottom of sealed syringe filter, fasten luer lock connection.
4. Invert syringe pair and decant pre-measured volume (2.5 mL) of Reagent A Buffer into empty FDA injection syringe barrel; add one drop FDA Reagent. Cap barrel with syringe plunger gently to seal, preventing pressurized loss of fluid through filter. Mix solutions.

5. Invert syringe pair so that top of sealed filter faces upwards and slowly inject Reagent A+B mixture upward through the bottom of the syringe filter, fully depressing the plunger. Keep this vertical orientation (see Note 2 below).
6. Store syringe pair vertically in a darkened, room temperature environment (cabinet), with fluid on top. Incubate for 1 hour; record ambient temperature. Sealed filter should be facing up.
7. Plunge fluid downward through filter, depressing plunger fully. Invert syringe pair and again plunge fluid downward through filter, depressing plunger fully. This step ensures well-mixed incubation fluid. Syringe-pair will now be upside-down, with FDA syringe on top, receiver syringe on the bottom.
8. Draw the plunger of the bottom receiver syringe down slightly (ca. 0.5 cm) to relieve pressure within syringe. Remove empty top syringe (female connector, FDA syringe) from the sealed syringe filter. Invert remaining syringe and syringe filter and dispense reaction fluid through syringe filter into a clean 1 cm fluorometer cuvet.
9. Add two drops finishing solution to cuvette contents, cap and mix gently (invert 3 times).
10. Measure fluorescence on hand-held calibrated fluorescein fluorometer.

Calculation of equivalent numeric cell concentration

Equation 1 below provides the means to estimate the equivalent numeric concentration of live cells corresponding to the measured fluorescein production rate of any unknown ballast water sample, processed as described above. This assumes the sample was captured by filter size fractionation corresponding to the 10-50 µm size class and was incubated at 20 C. The exponentially-weighted mean cell size of the 10-50 µm plankton size class is calculated to be 15 µm ESD (Welschmeyer and Maurer 2013) based on organism size distribution models for coastal systems, characterized by a Log biomass vs. Log cell volume slope of -0.1 (Vidondo et al. 1997). A cell-specific response factor of 2.86 (pg fluorescein cell-1 h-1) for the 15 µm ESD cell size (at 20 C) was determined empirically from sized-based analysis of several freshwater and marine phytoplankton species; data shown in Fig. 1 (from Welschmeyer and Maurer 2013).

$$\text{Eq. 1. } \frac{live\ cells}{ml} = \frac{sample\ fluorescein\ \frac{\mu g}{L} \cdot cuvette\ volume\ (mL)}{\frac{2.86 pg\ fluorescein}{cell \cdot hr} \cdot\ volume\ filtered\ (L) \cdot incubation\ time\ (h)}$$

Method Detection Limits

0.5 µg fluorescein/L (equivalent to ca. 5 live cells per mL, nominal 15 µm equivalent spherical diameter, assuming 100 mL filtration, 2.5 mL incubation reagent, 1 hour incubation)

List of Abbreviations

BWDS Ballast Water Discharge Standard
ESD Equivalent Spherical Diameter
FDA Fluorescein diacetate
RF Response Factor

Health and Safety

FDA solubilized in DMSO (flammable; 5 mL per stock vial; refrigerated, thus frozen). MSDS posted at http://biooce.mlml.calstate.edu/home/resources/

Cautions
Avoid skin contact with DMSO solution.

Interferences
If ballast treatment includes UV inactivation of cells, there may be residual enzyme activity in the treated cells for several days after the last UV exposure, thus yielding residual fluorescein production. Bulk FDA response will be reduced relative to uptake water (control) but may indicate numeric equivalent live cell concentrations higher than actual.

Personnel Qualifications and Responsibilities
Non-technical personnel can execute all aspects of the method, but practice with tap water is recommended for syringe manipulations to avoid leaks and spills and, thus, sample loss.

Special Equipment and Materials

- Turner AquaFluor handheld fluorometer (or equivalent); 480 nm/520 nm, excitation/emission.
- Disposable plastic 1 cm cuvettes; 4 sides clear, standard VIS transparency.
- Sealed syringe filter, 10 µm pore (Pall Life Sciences, Acrodisc Versapor Membrane, 10 µm, PN AP-4001T).
- Reagent A (Incubation Buffer), Reagent B (FDA Solution), Reagents are available in trial kits from Moss Landing Marine Laboratories until a distribution network is established.
- 10 µg/L fluorescein standard in capped 1 cm fluorometer cuvette.

Equipment and Supplies

- Clean sampling bottles, preferably polycarbonate (nominal 500 mL).
- Squeeze bottle of distilled water for rinsing, or equivalent
- 2 – 5 mL clean plastic syringes (male/female) per sample
- 1 – 140 mL (or equivalent) plastic syringe for initial sample filtration.
- 1 – 10 cm x 10 cm 35 µm (50 µm on the diagonal) prefilter nylon mesh to remove >50 µm organisms.
- Plastic waste receptacle for fluids.
- Test tube rack or open container to hold sample syringe-pair vertical during incubation.
- Thermometer (± 1 C) for determination of mean incubation temperature.

Quality Control
Calibrate the Turner AquaFluor fluorometer, using the sealed 10 ug/L fluorescein standard provided (see instrument instructions). Once calibrated, the solid state, fixed-optics fluorometer is not expected to drift, unless physically mishandled (e.g., dropped on steel deck). Warm up time (<5 sec) is factory programmed into the startup procedure when the instrument is turned on. Reagents may be stored at refrigerated temperature for 6 months; up to one year if frozen.

References

Welschmeyer, N. and B. Maurer. 2011. A portable, sensitive plankton viability assay for IMO shipboard ballast water compliance testing. In: Proceedings of the Global R&D Forum on Compliance Monitoring and Enforcement – Ballast Water Management Systems, Istanbul, Turkey, p117-130, Eds. A. Olgun, F.T. Karokoc and F. Haag.

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Welschmeyer, N. 2013. Rapid, indirect ballast water compliance methods and their conversion to regulated numeric ballast water discharge standards: Exploiting plankton size distribution models in aquatic ecology. (in preparation).

Vidondo, B., Y.T. Prairie, J.M. Blanco and C.M. Duarte. 1997. Some aspects of the analysis of size spectra in aquatic ecology. Limnol. Oceanogr. 42:184-192.

SGS ATP (aqua-tools)
Size Class: ≥50 µm; 10µm>x>50µm; bacteria
Researcher: Sebastien Peuchet

Description: ATP (adenosine triphosphate) have a long history on determination of bacteria concentration; development of new generation of ATP analysis answer to the necessity to have an on-board system on the ship that is robust, reliable and non-expensive. AQUA-TOOLS, in collaboration with SGS Group (Switzerland) and LuminUltra (Canada), has developed a full, easy and rapid method to ensure that ballast water treatment system are compliant. Ballast Water Treatment Monitoring Kit by ATP 2G can give fast and accurate results in few hours compare to the traditional methods for the different fractions of organisms (≥50µm, 10-50µm and total bacteria). Aqua-tools Ballast Water kit enable measuring in few minutes the whole living flora in collected samples (≥50µm; ≥10 and <50µm; and bacteria indicators fractions) for a rapid assessment of your ballast water quality. This test kit allows a rapid estimation of all biological contaminant through the quantification of bioluminescent signal coming from the reaction of the Luminase™ with intracellular Adenosine TriPhosphate (cATP) – the energy carrier of any living cell. This kit dedicated to ballast water analysis is based on second generation of ATP test kit, providing quantitative results without interferences thanks to adapted protocol and calibration solution.

Methods:

Calibration (All size classes)

1. Rehydrate Luminase with the Luminase buffer. Gently mix from time to time.
2. Put 100µL of Luminase in a luminometer tube.
3. Measure the background noise. If >15RLU, discard the tube and prepare a new one.
4. After the measure add 2 drop of UltraCheck1 and measure the signal.
5. Subtract the noise value from the signal and record it (RLUuc1).

Protocol (>50 µm):

1. After mixing the AE bottle (200mL), pour 60mL into a graduated 100mL Becher
2. Filter that volume through the 36µm mesh handfilter
3. Rinse the 100mL Becher with the “Rinse” water
4. Rinse the handfilter with the “Rinse” water
5. Regroup the organisms in define area and transfer them into a small 10mL Becher using the “Rinse” water
6. Measure the volume of concentrated sample (noted as RV50)
7. Homogenize and take 1mL and transfer it into a grinding tube
8. Add 5mL of Modified UltraLyse30 solution into the grinding tube
9. Switch on the grinder and set it in mode 2, speed 9, for 50 sec (6000rpm; 50sec)
10. Attach the tube and push start
11. When the rotation stops, remove the tube, shake it, re-attached the tube, and push the start button again.

12. When the rotation stops, remove the tube, shake it, re-attached the tube, and push the start button again. (it's not a copy and paste mistake I really do that 2 times)
13. When the rotation stops, remove the tube and let it on the table still for 5min
14. (During this time, clean Becher and filtration equipment, and start filtrating replicate 2)
15. After the incubation time, take 100µL and transfer it into a 5mL UltraLute tube
16. Mix the UltraLute tube and finish the replicate2 and 3 filtration / extraction
17. Take a luminometer tube, put 100µL of Luminase inside and measure the background noise tube (RLUempty ; if >15RLU discard the tube and prepare a new one)
18. After the measure, add 100µL of the UltraLute tube into the same luminometer and measure the signal (RLUcATP50)
19. Ask for the volume of ballast water that has been filtrated through the filtration device and put the value in the table (ZV)

Protocol (10µm>x>50µm):

1. After mixing the BI bottle (350mL), pour 100mL into a graduated 100mL Becher
2. Filter that volume through the 7µm mesh handfilter.
3. Rinse the 100mL Becher with the "Rinse" water
4. Rinse the handfilter with the "Rinse" water
5. Regroup the organisms in define area and transfer them into a small 10mL Becher using the "Rinse" water
6. Measure the volume of concentrated sample (noted as RV10-50)
7. Homogenize and take 1mL and transfer it into a grinding tube
8. Add 5mL of Modified UltraLyse30 solution into the grinding tube
9. Switch on the grinder and set it in mode 2, speed 9, for 50 sec (6000rpm; 50sec)
10. Attach the tube and push start
11. When the rotation stops, remove the tube, shake it, re-attached the tube, and push the start button again.
12. When the rotation stops, remove the tube, shake it, re-attached the tube, and push the start button again. (it's not a copy and paste mistake I really do that 2 times)
13. When the rotation stops, remove the tube and let it on the table still for 5min
14. (During this time, clean Becher and filtration equipment, and start filtrating replicate 2)
15. After the incubation time, take 100µL and transfer it into a 5mL UltraLute tube
16. Mix the UltraLute tube and finish the replicate2 and 3 filtration / extraction
17. Take a luminometer tube, put 100µL of Luminase inside and measure the background noise tube (RLUempty ; if >15RLU discard the tube and prepare a new one)
18. After the measure, add 100µL of the UltraLute tube into the same luminometer and measure the signal (RLUcATP10-50)
19. Ask for the volume of ballast water that has been filtrated through the filtration device and put the value in the table (PV)

<u>Blank measurement (>50 and 10-50µm)</u>

1. Homogenize the "Rinse" water take 1mL and transfer it into a grinding tube
2. Add 5mL of Modified UltraLyse30 solution into the grinding tube
3. Switch on the grinder and set it in mode 2, speed 9, for 50 sec (6000rpm; 50sec)

4. Attach the tube and push start
5. When the rotation stops, remove the tube, shake it, re-attached the tube, and push the start button again.
6. When the rotation stops, remove the tube, shake it, re-attached the tube, and push the start button again. (it's not a copy and paste mistake I really do that 2 times)
7. When the rotation stops, remove the tube and let it on the table still for 5min
8. (During this time, clean Becher and filtration equipment, and start filtrating replicate 2)
9. After the incubation time, take 100µL and transfer it into a 5mL UltraLute tube
10. Mix the UltraLute tube
11. Take a luminometer tube, put 100µL of Luminase inside and measure the background noise tube (RLUempty ; if >15RLU discard the tube and prepare a new one)
12. After the measure, add 100µL of the UltraLute tube into the same luminometer and measure the signal (RLUartesaw)

Protocol (Bacteria)

1. Take a 60mL syringe, remove it from the packaging, discard the paper part and use the plastic part to lay the disassembled syringe (plunger and barrel)
2. Attach the 2.7µm disc filter to the barrel, attach the 0,7µm disc filter to the other disc
3. Pour 50mL of DH sample into the barrel, add the plunger and filter ONLY the liquid, not the air
4. Remove the two filters, remove the plunger, reattach the filters, pour 50mL of DH sample add the plunger and filter ONLY the liquid, not the air.
5. Remove the filters, remove the plunger, discard the 2.7µm disc filter, reattach the 0,7µm filter directly to the barrel.
6. Open a 9mL UltraLute tube and put the syringe on the top
7. Take1mL of UltraLyse7 solution and transfer it into the barrel, add the plunger and filter the entire content of the barrel (liquid and air)
8. Mix the UltraLute tube to dilute the ATP extract correctly
9. Take a luminometer tube, put 100µL of Luminase inside and measure the background noise tube (RLUempty ; if >15RLU discard the tube and prepare a new one)
10. After the measure, add 100µL of the UltraLute tube into the same luminometer and measure the signal (RLUcATPbact)

Welschmeyer ATP
Size Class: ≥50 µm; 10µm>x>50µm; bacteria
Researcher: Nick Welschmeyer

No methods have been provided.

Triton BW BacTest EC
Size Class: Bacteria
Researcher: Lothar Schillak, Triton Marine Science&Consult, Germany

Description: The Triton BW Bac Test EC was especially designed for the analysis of the concentration of Escherichia coli in ballast water. The basic method is derived from the Polymerase Chain Reaction-PCR method. Similar to the PCR, primers with their codons, especially developed for this test, detect a predefined group of genes within the genome of Escherichia coli. When the primer binds to this group of genes it induces the multiplication of the RNA and with integrated fluorescein the multiplication gives a signal in a fluorometer. However, different to the PCR the reaction is held on an isothermal level.

The development of the multiplication follows the enzymatic kinetics of a sigmoid curve, in which the inflection point, for the Triton BW Bac Test EC set to 5.000 fluorescence units (F), is defined as "positive". Since the multiplication develops exponentially, the inflection point 5 kF is trespassed the earlier the higher the concentration of Escherichia coli is in the sample.

The fluorometer software is adapted to the Triton BW Bac Test EC and data can be transferred and stored in a laptop using an respective laptop Triton BW Bac Test EC software.

Materials: (to perform analysis of 1 ballast water sample in triplicate)

- Water, sterile filtered 0.2 µm, approximately 200mL
- Buffer tube (available from *Triton Marine Science and Consult*)
- Lyophyllisate teststrips (8 wells; available from *Triton Marine Science and Consult*))
- Triton fluorometer with Triton Data Processing Software (available from *Triton Marine Science and Consult*)
- Heating block for tubes
- Laptop
- 2 plankton filters (35µm mesh and 7µm mesh)
- 3 beakers (600ml, 500ml, 250ml)
- 2 syringes, luer-lock (140ml and 60ml)
- Fibre glass syringe luer-lock filter, 1-2µm nominal mesh, Ø 23mm (*winlab* no. 101265251)
- Syringe filter holder, Ø 13mm (*Sartorius stedim biotech* no. 80238-004-50)
- Cellulose nitrate membrane filter, 0,45 pore size Ø 13mm (*Sartorius stedim biotech* no. 11306—13-----N)
- Vario pipette (Eppendorf „research plus", 10-100µl)
- Pipette tips, 100µl (*StarLab* no. S1120-1840)
- Tube rack for tubes
- Tweezers

Methods:

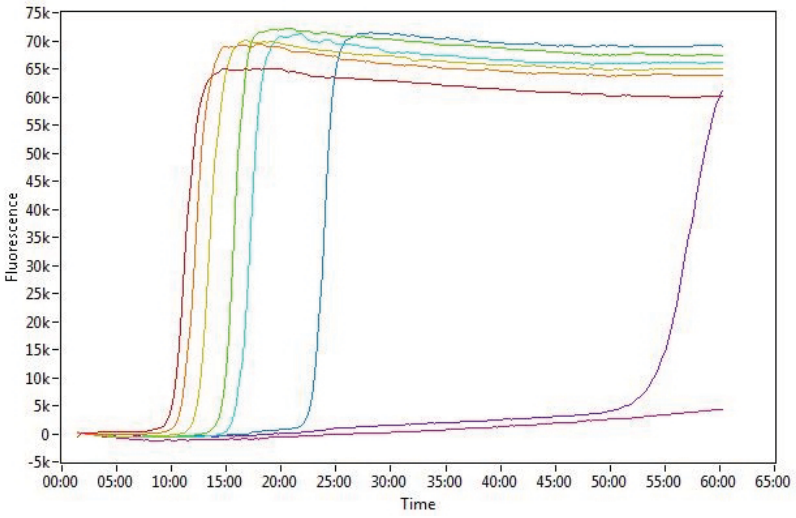
1. Place cellulose nitrate filters into syringe filter holders
2. Filter 600ml of ballast water sample through a 35µm mesh filter
3. Filter filtrate from step 2 through a 7µm mesh filter

4. Take 140ml of filtrate from step 3 into syringe 140ml, attach fibre glas filter to syringe and filter into a beaker 250ml, discard filter
5. Take 100ml of filtrate from step 4 into syringe 140ml, attach syringe filter holder and filter 100ml through the cellulose nitrate membrane filter inside the filter holder, detach filter holder
6. Take 20ml water sterile filtered 0,2µm into syringe 60ml, attach filterholder from step 5 to syringe 60ml, push the 20ml water through the cellulose nitrate membrane filter inside the filter holder, detach filter holder
7. Open filter holder and carefully take out the cellulose nitrate membrane filter from inside the filter holder with tweezers, place it into a buffer tube, gently destroy the filter inside the buffer tube
8. Repeat step 4 to step 7 for triplicates 2 and 3. The triplicate buffer tubes 1,2 and 3 are stable for several days (store at 4°C)
9. Set-up analysis programme in the fluorometer as instructed on the fluorometer screen
10. Place triplicate buffer tubes 1,2 and 3 into heating block, incubate 2 minutes at 99°C. Let tubes return to ambient room temperature
11. Take 25µl from each buffer tube 1,2 and 3 into wells nos. 1, 2 and 3 of a test strip
12. Take 25µl from buffer tube no. 3 into well number 4 (control) of the test strip, make sure all wells are closed
13. Place test strip into the pre-set slot of the fluorometer and start analysis
14. The sigmoid curves of wells 1, 2, 3 and 4 are shown on the screen of the fluorometer until completion of the analysis
15. The analysis is followed by an annealing and multiplication rate control
16. After termination of the analysis, transfer the respective analysis file to the laptop, start data programme and transfer readings of sigmoid curves into cfu/100ml as instructed by the programme.

Figure 1: BW Bactest EC: fluorometer with two slots for test strips

Figure 2: BW Bactest EC: test strip

Figure 3: BW Bactest EC: typical graphs for determination of final value



Bacteria quantification by flow cytometry after staining nuclear DNA
Size Class: Bacteria
Researcher: Marcel Veldhuis

The cellular DNA content of the phytoplankton community was analyzed with the green nuclear stain PicoGreen (MP, P-7581) according to the method described by Veldhuis et al. (1997, J. Phyml. 33, 527-541) on a flow cytometer (COULTER-Elite-ESP, 75mW laser power, 488 nm excitation wavelength). The method used differed in that RNAse and Triton X100 were not added prior to analysis. Although it increased the peak width of the DNA signal slightly the chlorophyll signal could be better separated from the DNA signal. Flow cytometer counts for bacteria were calculated by using the size and presence/absence of chlorophyll to distinguish bacteria from phytoplankton.

Speedy Breedy – By Bactest
Size Class: Bacteria
Researcher: Derek Price

Description: Speedy Breedy is a portable, sensitive, precision respirometer which detects and monitors microbial activity. Detection of microbial activity is determined as a consequence of pressure transients relating to gaseous exchanges within a closed culture vessel of 50 ml working volume, as a result of microbial respiration. Samples are added to a culture vessel containing a suitable nutrient medium and where present, contaminating microorganisms begin to grow. Speedy Breedy has advanced mixing technology that homogenises the culture conditions for rapid growth of microbes and efficiently converts gaseous changes in the culture medium into pressure variances in the headspace, therefore allowing detection of pressure transients due to metabolic processes where microbes are present. Time to detection is indicative of the degree of contamination in the sample.

Methods: Testing for E. coli or Enterococcus in potable or marine ballast water.

It is recommended to wear latex gloves while processing samples, cleaning them before and after the procedure with medical wipes or 70% ethanol in water.

1. Filter sterilise 45 ml of milli Q water (using a 0.2 um syringe filter) directly into a new sterile Speedy Breedy culture vessel.
   For E. coli tests use vessels pre-filled with MacConkey Broth capsules.
   For Enterococcus tests use vessels pre-filled with M-Enterococcus Broth capsules.
2. Using a sterile 5 ml syringe, remove 5 ml of sample from its container.
3. Using aseptic technique and touching only the outer rim, remove the filling cap from the culture vessel by rotating it anticlockwise and fill the vessel with the 5 ml sample.
4. Replace the cap and make sure it is firmly located by turning it clockwise to engage it. A small arrow on the top of the vessel indicates the correct closed position.
5. Note: an alternative method is to dilute the sample 1 in 10 in a large sterile container and add 50 ml of this diluted sample to the Speed Breedy vessel.
6. Ensure that the Speedy Breedy instrument is plugged in and press the “on” button (first button on the central display).
7. When you first switch on your Speedy Breedy in test mode, you will see the message “Speedy Breedy Booting” followed by “Speedy Breedy by Bactest” and finally “MAIN MENU: Setup Test” appear on the display.

To start a test:

1. To begin a test using Speedy Breedy, select “Set up test” from the Main Menu using the arrow buttons, and confirm by pressing the Select (□) button.
2. You will then be prompted to SELECT PROTOCOL. Scroll through Protocols present on the device’s SD card by pressing the arrow keys and then confirm the Protocol you would like to use for your test by pressing the Select (□) button.
3. Once a test Protocol is selected, Speedy Breedy prompts the user to select which chamber(s) you wish to conduct a test in. Test Protocols typically allow the user to

select one or both of the test chambers. To select which chamber you wish to use, cycle through options using the arrow buttons, and confirm using the Select ( ) button.

4. Once you have chosen the chamber(s) you wish to run a test in, Speedy Breedy prompts you to BEGIN TEST. It is at this point that Speedy Breedy Culture Vessels should be introduced into the test chamber(s). Speedy Breedy will not allow a test to begin without a vessel present in the chamber(s) and without the lid closed.
5. Once you have introduced the Culture Vessel and closed the lid, press the Select ( ) button to begin your test.
6. Speedy Breedy will indicate contamination by a change of colour of the flashing LED on the central display from green to red. If no red LED is seen and the protocol completes its time course, the sample is not contaminated.
7. If a PC or lap top is connected via USB to Speedy Breedy, results can be visualised as pressure curves. The time to detection is found by placing the cursor over the (red) detection line on the graph. If the protocol is linked to a calibration curve (some supplied) then the degree of contamination in the original sample is also indicated.

SimPlate* for HPC

Unit Dose

English

Introduction

SimPlate* for HPC method is used for the quantification of heterotrophic plate counts (HPC) in water. It is based on IDEXX's patented Multiple Enzyme Technology*, which detects viable bacteria in water by testing for the presence of key enzymes known to be present in these organisms. It uses multiple enzyme substrates that produce a blue fluorescence when metabolized by waterborne bacteria. The sample and media are added to a SimPlate, incubated, and then examined for fluorescing wells. The number of fluorescing wells corresponds to a Most Probable Number (MPN) of total bacteria in the original sample. The MPN values generated by the SimPlate for HPC method correlate with the Pour Plate method using Total Plate Count Agar incubated at 35°C for 48 hours as described in *Standard Methods for the Examination of Water and Wastewater*[1].

Contents

- 25 sterile media tubes for 10 mL samples
- 25 sterile SimPlate plates with lids
- MPN table
- This insert

Storage

Store at 2–30°C and away from light. Expiration date is printed on the box of media tubes.

Test Procedure

1. Add 10±0.2 mL of sample to a media tube, re-cap and shake to dissolve. See image #1.
2. Pour the contents of the tube onto the center of the plate base. See image #2.
3. Cover the plate with the lid and gently swirl to distribute the sample into all the wells. See image #3.
   NOTE: Air bubbles in the wells do not interfere with the test.
4. Tip the plate 90–120° to drain excess sample into the absorbent pad. See image #4.
5. Invert the plate and incubate for 48 hours at 35±0.5°C. See image #5.
6. Count the number of wells showing any fluorescence by holding a 6-watt, 365-nm UV light 5 inches above the plate. Direct light away from your eyes and towards the sample. Alternatively, you may read fluorescent wells through the back of the inverted SimPlate base.
7. Refer to the MPN table provided to determine the Most Probable Number of heterotrophic plate count bacteria in the original sample. The table takes into account the sample/media poured off in step #4 above.

Procedural Notes

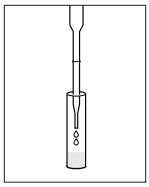
1. Follow aseptic technique.
2. Chlorinated samples should be treated with sodium thiosulfate prior to testing.
3. Results can be read from 45 to 72 hours after start of incubation.
4. Dispose of sample and media in accordance with Good Laboratory Practices.
5. Samples may be diluted before adding to the media as long as the final volume (sample plus sterile diluent) is 10±0.2 mL. Adjust the MPN result to reflect dilutions. For example, if 1 mL of sample and 9 mL of sterile diluent are tested (a 10-fold dilution), multiply the MPN table number by 10 to find the correct MPN/mL.

Quality Control Procedure

The following procedure is recommended for each lot of SimPlate for HPC product:

1. A. Positive Control: IDEXX-QC HPC/TVC[2]: *Enterococcus faecalis*.
   B. Negative Control/Blank: Use 10 mL of rehydrated HPC media.
2. Follow steps 1–7.
3. Negative Control/Blank wells should not fluoresce after incubation.

1



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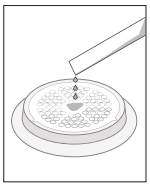

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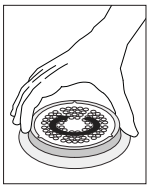

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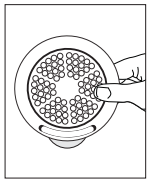

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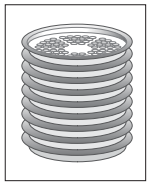

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idexx.com/water

1. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 2005. Washington, DC.
2. IDEXX-QC HPC/TVC, IDEXX Catalog #UN3373-WQC-HPC.

One IDEXX Drive
Westbrook, Maine 04092 USA

Unit Dose
SimPlate For HPC
Most Probable Number (MPN) Table

# Positive Wells	MPN	95% confidence limits	
		lower	upper
0	<0.2	<0.03	<1.4
1	0.2	0.0	1.4
2	0.4	0.1	1.6
3	0.6	0.2	1.9
4	0.8	0.3	2.2
5	1.0	0.4	2.5
6	1.2	0.6	2.7
7	1.5	0.7	3.0
8	1.7	0.8	3.3
9	1.9	1.0	3.6
10	2.1	1.1	3.9
11	2.3	1.3	4.2
12	2.6	1.5	4.5
13	2.8	1.6	4.8
14	3.0	1.8	5.1
15	3.3	2.0	5.4
16	3.5	2.2	5.8
17	3.8	2.3	6.1
18	4.0	2.5	6.4
19	4.3	2.7	6.7
20	4.5	2.9	7.0
21	4.8	3.1	7.4
22	5.1	3.3	7.7
23	5.3	3.5	8.0
24	5.6	3.8	8.4
25	5.9	4.0	8.7
26	6.2	4.2	9.1
27	6.5	4.4	9.4
28	6.8	4.7	9.8
29	7.1	4.9	10.2
30	7.4	5.1	10.6
31	7.7	5.4	10.9
32	8.0	5.6	11.3
33	8.3	5.9	11.7
34	8.6	6.2	12.1
35	9.0	6.4	12.6
36	9.3	6.7	13.0
37	9.7	7.0	13.4
38	10.0	7.3	13.9
39	10.4	7.6	14.3
40	10.8	7.9	14.8
41	11.2	8.2	15.2
42	11.6	8.5	15.7

# Positive Wells	MPN	95% confidence limits	
		lower	upper
43	12.0	8.8	16.2
44	12.4	9.1	16.7
45	12.8	9.5	17.3
46	13.2	9.8	17.8
47	13.7	10.2	18.3
48	14.1	10.6	18.9
49	14.6	10.9	19.5
50	15.1	11.3	20.1
51	15.6	11.7	20.7
52	16.1	12.1	21.3
53	16.6	12.5	22.0
54	17.1	13.0	22.7
55	17.7	13.4	23.4
56	18.3	13.9	24.1
57	18.9	14.4	24.9
58	19.5	14.9	25.7
59	20.2	15.4	26.5
60	20.9	15.9	27.3
61	21.6	16.5	28.2
62	22.3	17.1	29.2
63	23.1	17.7	30.2
64	23.9	18.3	31.2
65	24.8	19.0	32.3
66	25.7	19.7	33.5
67	26.6	20.4	34.7
68	27.6	21.2	36.1
69	28.7	22.0	37.5
70	29.9	22.9	39.0
71	31.1	23.8	40.7
72	32.4	24.8	42.5
73	33.9	25.8	44.4
74	35.5	27.0	46.6
75	37.2	28.2	49.1
76	39.2	29.6	51.9
77	41.4	31.1	55.1
78	44.0	32.8	58.9
79	47.0	34.8	63.6
80	50.7	37.1	69.5
81	55.5	39.8	77.5
82	62.3	43.2	89.9
83	73.8	47.6	114.6
84	>73.8	>47.6	>114.6

MPN is per mL of the 10 mL sample added to the media tube (pour-off is accounted for).

SimPlate* for HPC

Méthode SimPlate* pour HPC • Dose Unitaire

Française

Introduction

La méthode SimPlate* pour HPC est utilisée pour la quantification de la flore totale revivifiable dans l'eau ("heterotrophic plate counts" ou HPC). Ce test repose sur la technologie multienzymatique (Multiple Enzyme Technology*) d'IDEXX breveté qui détecte les bactéries viables dans l'eau en recherchant les enzymes clés, connues pour être présentes dans ces microorganismes. Cette méthode utilise plusieurs substrats enzymatiques produisant une fluorescence bleue lorsqu'ils sont métabolisés par les bactéries présentes dans l'eau. L'échantillon et le milieu nutritif sont déposés sur la plaque SimPlate. L'ensemble est incubé puis examiné sous UV pour compter les puits fluorescents. Le nombre de puits fluorescents correspond au nombre le plus probable ("Most Probable Number" ou MPN) de bactéries totales présentes dans l'échantillon original. Les valeurs NPP générées par la méthode SimPlate pour HPC sont conformes à la méthode en boîte de Pétri utilisant une numération totale sur plaque en milieu gélosé à une incubation de 35°C pendant 48 heures, tel que décrit dans *Standard Methods for the Examination of Water and Wastewater*[1] (*Méthodes traditionnelles pour l'analyse de l'eau et des eaux usées*).

Contenu

- 25 tubes de milieu stériles pour échantillons de 10 ml
- 25 plaques SimPlate stériles avec couvercles
- Tableau des valeurs NPP
- Cette notice

Conservation

Conserver entre 2–30°C, à l'abri de la lumière. La date de péremption est imprimée sur la boite.

Procédure de test

1. Ajouter 10±0,2 ml d'échantillon dans un tube de milieu nutritif, remettre le bouchon puis agiter pour mélanger. Voici l'illustration n° 1.
2. Verser le contenu du tube au centre de la plaque. Voici l'illustration n° 2.
3. Recouvrir la plaque avec le couvercle puis répartir l'échantillon dans tous les puits en effectuant un mouvement circulaire à la plaque. Voici l'illustration n° 3.
   REMARQUE: Les bulles d'air présentes dans les puits n'interfèrent pas avec le test.
4. Incliner la plaque à 90–120° pour verser l'excès de liquide dans le tampon absorbant. Voici l'illustration n° 4.
5. Retourner la plaque et laisser incuber pendant 48 heures à 35±0,5°C. Voici l'illustration n° 5.
6. Compter le nombre de puits présentant une fluorescence en tenant une lampe à UV (6 W, 365 nm) à 12,5 cm au-dessus de la plaque. Diriger la lampe vers l'échantillon et non vers les yeux. Egalement, vous pouvez lire des puits fluorescents quand vous inversez le SimPlate.
7. Consulter le tableau des valeurs NPP fourni pour déterminer le nombre le plus probable de bactéries présentes dans l'échantillon original. Le tableau tient compte de l'échantillon/du milieu éliminé à l'étape 4 ci-dessus.

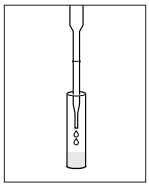
Notes concernant la procédure de test

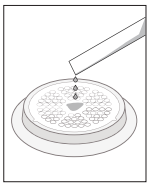
1. Respecter une technique aseptique.
2. Les échantillons chlorés doivent être traités au thiosulfate de sodium avant le test.
3. Les résultats doivent être lus entre 45 et 72 heures après le début de l'incubation.
4. Eliminer l'échantillon et le milieu conformément aux Bonnes Pratiques de Laboratoire.
5. Les échantillons peuvent être dilués avant leur addition au milieu tant que le volume final (échantillon plus diluant stérile) est de 10±0,2 ml. Ajuster le résultat NPP pour tenir compte des dilutions. Par exemple, si 1 ml d'échantillon et 9 ml de diluant stérile sont testés (dilution de 1:10), il faut multiplier par 10 la valeur donnée dans le tableau des valeurs NPP pour obtenir le NPP/ml correct.

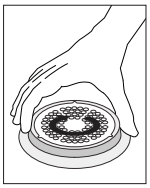
Contrôle de qualité

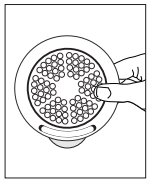
La procédure suivante est recommandée pour chaque lot de produit Simplate pour HPC:

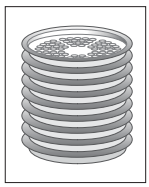
1. A. Contrôle positif: IDEXX-QC HPC[2]: *Enterococcus faecalis*.
   B. Contrôle négatif/à blanc: utiliser 10 ml de milieu HPC réhydraté.
2. Suivre les étapes 1 à 7.
3. Les puits de contrôle négatif/blanc ne doivent pas être fluorescents après l'incubation.











Pour obtenir l'assistance technique, veuillez appeler :

Europe: +00800 4339 9111

idexx.fr/eau

1. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater* (*Méthodes traditionnelles d'analyses de l'eau et des eaux usées*), American Public Health Association, 2005. Washington, DC.
2. HPC/TVC d'IDEXX-QC, IDEXX Catalogue n° UN3373-WQC-HPC.

One IDEXX Drive
Westbrook, Maine 04092 USA

Dose Unitaire
SimPlate pour HPC
Tableau des valeurs MPN

# Puits Positifs	MPN	Limites de confiance à 95%	
		minimum	maximum
0	<0,2	<0,03	<1,4
1	0,2	0,0	1,4
2	0,4	0,1	1,6
3	0,6	0,2	1,9
4	0,8	0,3	2,2
5	1,0	0,4	2,5
6	1,2	0,6	2,7
7	1,5	0,7	3,0
8	1,7	0,8	3,3
9	1,9	1,0	3,6
10	2,1	1,1	3,9
11	2,3	1,3	4,2
12	2,6	1,5	4,5
13	2,8	1,6	4,8
14	3,0	1,8	5,1
15	3,3	2,0	5,4
16	3,5	2,2	5,8
17	3,8	2,3	6,1
18	4,0	2,5	6,4
19	4,3	2,7	6,7
20	4,5	2,9	7,0
21	4,8	3,1	7,4
22	5,1	3,3	7,7
23	5,3	3,5	8,0
24	5,6	3,8	8,4
25	5,9	4,0	8,7
26	6,2	4,2	9,1
27	6,5	4,4	9,4
28	6,8	4,7	9,8
29	7,1	4,9	10,2
30	7,4	5,1	10,6
31	7,7	5,4	10,9
32	8,0	5,6	11,3
33	8,3	5,9	11,7
34	8,6	6,2	12,1
35	9,0	6,4	12,6
36	9,3	6,7	13,0
37	9,7	7,0	13,4
38	10,0	7,3	13,9
39	10,4	7,6	14,3
40	10,8	7,9	14,8
41	11,2	8,2	15,2
42	11,6	8,5	15,7

# Puits Positifs	MPN	Limites de confiance à 95%	
		minimum	maximum
43	12,0	8,8	16,2
44	12,4	9,1	16,7
45	12,8	9,5	17,3
46	13,2	9,8	17,8
47	13,7	10,2	18,3
48	14,1	10,6	18,9
49	14,6	10,9	19,5
50	15,1	11,3	20,1
51	15,6	11,7	20,7
52	16,1	12,1	21,3
53	16,6	12,5	22,0
54	17,1	13,0	22,7
55	17,7	13,4	23,4
56	18,3	13,9	24,1
57	18,9	14,4	24,9
58	19,5	14,9	25,7
59	20,2	15,4	26,5
60	20,9	15,9	27,3
61	21,6	16,5	28,2
62	22,3	17,1	29,2
63	23,1	17,7	30,2
64	23,9	18,3	31,2
65	24,8	19,0	32,3
66	25,7	19,7	33,5
67	26,6	20,4	34,7
68	27,6	21,2	36,1
69	28,7	22,0	37,5
70	29,9	22,9	39,0
71	31,1	23,8	40,7
72	32,4	24,8	42,5
73	33,9	25,8	44,4
74	35,5	27,0	46,6
75	37,2	28,2	49,1
76	39,2	29,6	51,9
77	41,4	31,1	55,1
78	44,0	32,8	58,9
79	47,0	34,8	63,6
80	50,7	37,1	69,5
81	55,5	39,8	77,5
82	62,3	43,2	89,9
83	73,8	47,6	114,6
84	>73,8	>47,6	>114,6

Le résultat final est exprimé en NPP/ ml (résultat final qui tient compte des 10ml analysés et du volume d'échantillon absorbé).

SimPlate* for HPC

Metodo SimPlate* per HPC • Dosaggio Unitario

Italiano

Introduzione

Metodo SimPlate* per HPC è utilizzato per la quantificazione HPC (conte in piastra di batteri eterotrofi) nell'acqua. Si basa su una tecnologia IDEXX (Multiple Enzyme Technology*) brevettato, che rileva i batteri vitali nell'acqua testando la presenza di enzimi chiave la cui presenza in questi organismi è certa. Utilizza substrati enzimatici multipli che sviluppano una fluorescenza blu allorché metabolizzati dai batteri contenuti nell'acqua. Il campione e il terreno vengono posti in una piastra SimPlate, incubati e quindi esaminati alla ricerca di pozzetti fluorescenti. Il numero di pozzetti SimPlate fluorescenti corrisponde all'MPN (Most Probable Number, numero più probabile) di batteri totali nel campione originario. I valori MPN generati mediante il metodo SimPlate per HPC sono correlati con il metodo Pour Plate utilizzando Total Plate Count Agar incubato a 35°C per 48 ore, come illustrato in *Standard Methods for the Examination of Water and Wastewater [Metodi standard per l'esame dell'acqua e delle acque di scarico]*[1].

Contenuto

- 25 provette di terreno sterile per campioni di 10 ml
- 25 piastre SimPlate con relativo coperchio
- Tabella MPN
- Il presente foglietto illustrativo

Conservazione

Conservare a 2–30°C, al riparo dalla luce. La data di scadenza è stampata sulla scatola.

Procedura del test

1. Versare 10±0,2 ml di campione in una provetta di terreno, ritappare e agitare per sciogliere. Vedere la Figura 1.
2. Versare il contenuto della provetta al centro della base della piastra. Vedere la Figura 2.
3. Chiudere la piastra con il coperchio e agitarla delicatamente per distribuire il campione in tutti i pozzetti. Vedere la Figura 3.
   NOTA: le bolle d'aria eventualmente presenti nei pozzetti non interferiscono con il test.
4. Eliminare il eccesso dei campioni inclinando la piastra con un angolo di 90–120° sulla spugnetta. Vedere la Figura 4.
5. Capovolgere la piastra e incubare per 48 ore a 35±0,5°C. Vedere la Figura 5.
6. Contare il numero di pozzetti che presentano fluorescenza tenendo una lampada UV da 6-watt, 365 nm, 12,5 cm al di sopra della piastra. Rivolgere la lampada verso il campione, non verso gli occhi. Diversamente, si puo' effettuare la lettura della fluorescenza attraverso il retro della base del SimPlate.
7. Consultare la tabella MPN allegata alla confezione per determinare l'MNP delle conte in piastra di batteri eterotrofi nel campione originario. La tabella tiene conto del campione/terreno versato al punto 4 della presente procedura.

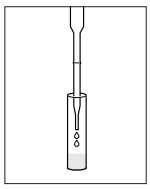
Note procedurali

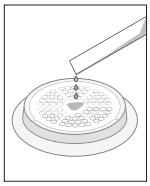
1. Adottare una tecnica asettica.
2. Prima del test, trattare con tiosolfato di sodio i campioni clorati.
3. E' possibile leggere i risultati nell'arco di tempo da 45 a 72 ore dall'inizio dell'incubazione.
4. Smaltire il campione e il terreno in conformità alle procedure standard di laboratorio.
5. E' possibile diluire i campioni prima di aggiungerli al terreno fino ad ottenere un volume finale (campione più diluente sterile) di 10±0,2 ml. Regolare l'MNP in modo da riflettere le diluizioni. Per esempio, in caso di test condotto su 1 ml di campione e 9 ml di diluente sterile (una diluizione di dieci volte), moltiplicare il numero riportato nella tabella MPN per 10 per trovare l'MPN/ml corretto.

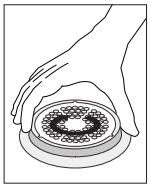
Procedura di controllo di qualità

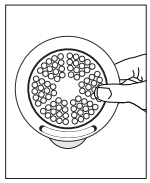
Per ciascun lotto di SimPlate per il prodotto HCP è raccomandata una delle seguenti procedure:

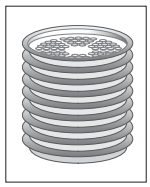
1. A. Controllo positivo: HPC IDEXX-QC[2]: *Enterococcus faecalis*.
   B. Controllo negativo/in bianco: usare 10 ml di terreno colturale HPC reidratato.
2. Seguire i passaggi da 1 a 7.
3. I pozzetti per il controllo negativo/in bianco non dovrebbero diventare fluorescenti dopo l'incubazione.











Per ricevere assistenza tecnica contattare:

Europa: +3902 31920351

idexx.it/acqua

1. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 2005. Washington, DC.
2. HPC/TVC IDEXX-QC, Catalogo IDEXX N. UN3373-WQC-HPC.

One IDEXX Drive
Westbrook, Maine 04092 USA

Dosaggio Unitario
SimPlate per HPC
Tabella MPN

# Pozzetti positivi	MPN	Limiti fiduciali del 95% inferiore	superiore
0	<0,2	<0,03	<1,4
1	0,2	0,0	1,4
2	0,4	0,1	1,6
3	0,6	0,2	1,9
4	0,8	0,3	2,2
5	1,0	0,4	2,5
6	1,2	0,6	2,7
7	1,5	0,7	3,0
8	1,7	0,8	3,3
9	1,9	1,0	3,6
10	2,1	1,1	3,9
11	2,3	1,3	4,2
12	2,6	1,5	4,5
13	2,8	1,6	4,8
14	3,0	1,8	5,1
15	3,3	2,0	5,4
16	3,5	2,2	5,8
17	3,8	2,3	6,1
18	4,0	2,5	6,4
19	4,3	2,7	6,7
20	4,5	2,9	7,0
21	4,8	3,1	7,4
22	5,1	3,3	7,7
23	5,3	3,5	8,0
24	5,6	3,8	8,4
25	5,9	4,0	8,7
26	6,2	4,2	9,1
27	6,5	4,4	9,4
28	6,8	4,7	9,8
29	7,1	4,9	10,2
30	7,4	5,1	10,6
31	7,7	5,4	10,9
32	8,0	5,6	11,3
33	8,3	5,9	11,7
34	8,6	6,2	12,1
35	9,0	6,4	12,6
36	9,3	6,7	13,0
37	9,7	7,0	13,4
38	10,0	7,3	13,9
39	10,4	7,6	14,3
40	10,8	7,9	14,8
41	11,2	8,2	15,2
42	11,6	8,5	15,7

# Pozzetti positivi	MPN	Limiti fiduciali del 95% inferiore	superiore
43	12,0	8,8	16,2
44	12,4	9,1	16,7
45	12,8	9,5	17,3
46	13,2	9,8	17,8
47	13,7	10,2	18,3
48	14,1	10,6	18,9
49	14,6	10,9	19,5
50	15,1	11,3	20,1
51	15,6	11,7	20,7
52	16,1	12,1	21,3
53	16,6	12,5	22,0
54	17,1	13,0	22,7
55	17,7	13,4	23,4
56	18,3	13,9	24,1
57	18,9	14,4	24,9
58	19,5	14,9	25,7
59	20,2	15,4	26,5
60	20,9	15,9	27,3
61	21,6	16,5	28,2
62	22,3	17,1	29,2
63	23,1	17,7	30,2
64	23,9	18,3	31,2
65	24,8	19,0	32,3
66	25,7	19,7	33,5
67	26,6	20,4	34,7
68	27,6	21,2	36,1
69	28,7	22,0	37,5
70	29,9	22,9	39,0
71	31,1	23,8	40,7
72	32,4	24,8	42,5
73	33,9	25,8	44,4
74	35,5	27,0	46,6
75	37,2	28,2	49,1
76	39,2	29,6	51,9
77	41,4	31,1	55,1
78	44,0	32,8	58,9
79	47,0	34,8	63,6
80	50,7	37,1	69,5
81	55,5	39,8	77,5
82	62,3	43,2	89,9
83	73,8	47,6	114,6
84	>73,8	>47,6	>114,6

Il Numero Più Probabile é riferito ad 1 ml dei 10 ml di campione aggiunto alla fiala di reagente (tenendo in acconto l'eccesso).

SimPlate* for HPC

Método SimPlate* para RPH • Dosis Unitaria

Español

Introducción

El método SimPlate* para RPH se usa para la cuantificación de recuentos de plaquetas heterotróficas (RPH) en agua. Se basa en la tecnología de enzimas múltiples de IDEXX patentado (Multiple Enzyme Technology*), que detecta bacterias viables en el agua comprobando la presencia de enzimas clave que se sabe existen en esos organismos. Se vale de sustratos de enzimas múltiples que producen una fluorescencia azul al ser metabolizados por la bacteria que se encuentra en el agua. La muestra y el medio se añaden a una placa SimPlate, se incuban y luego se examinan para determinar la presencia de pocillos fluorescentes. El número de pocillos fluorescentes corresponde al Número Más Probable (NMP) de bacteria total en la muestra original. Los valores del MPN generados por la SimPlate para el método de recuento de heterótrofos en placa (Heterotrophic Plate Count, HPC) coinciden con el método de vertido en placa utilizando agar para el recuento total en placa incubado a 35 °C durante 48 horas según se describe en la publicación *Standard Methods for the Examination of Water and Wastewater*[1].

Contenido

- 25 probetas de medio, esterilizadas, para muestras de 10 ml
- 25 placas SimPlate, esterilizados y con tapa
- Tabla de NMP
- Este folleto

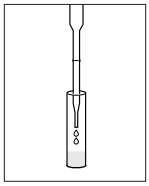
Conservación

Conservar entre 2–30°C y fuera del alcance de la luz. La fecha de caducidad es en la caja.

Procedimiento de prueba

1. Agregue 10±0,2 ml de muestra a una probeta de medio, ponga la tapa y agite para disolver. Vea la imagen N° 1.
2. Vierta el contenido de la probeta en el centro de la base de la placa. Vea la imagen N° 2.
3. Cubra la placa con la tapa y agite suavemente para distribuir la muestra en todos los pocillos. Vea la imagen N° 3.
   NOTA: las burbujas de aire en los pocillos no interfieren con la muestra
4. Coloque la placa en un ángulo de 90° a 120° para verter el exceso en el paño absorvente. Vea la imagen N° 4.
5. Invierta la placa e incube durante 48 horas a 35±0,5°C. Vea la imagen N° 5.
6. Cuente el número de pocillos que tienen fluorescencia: sujete una bombilla de 6 wats, 365 nm de luz UV a una distancia de 12,5 cm por encima de la placa. Apunte la bombilla hacia la muestra. Alternativamente, usted puede leer los pozos fluorescentes a través de la parte posterior de la base invertida del SimPlate.
7. Consulte la tabla de NMP (MPN) proporcionada para determinar el número más probable de bacteria de recuento de plaqueta heterotrófica en la muestra original. La tabla tiene en cuenta la muestra/medio eliminado en el paso 4.

1



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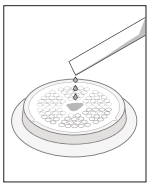

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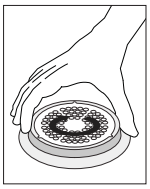
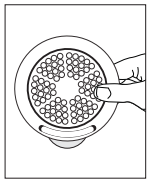


4



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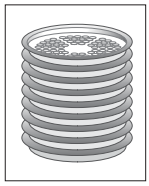


Notas del procedimiento

1. Proceda con técnica aséptica.
2. Las muestras clorinadas se deben tratar con tiosulfato de sodio antes de hacer la prueba.
3. Los resultados se pueden leer desde 45 hasta 72 horas después de iniciada la incubación.
4. Elimine la muestra y el medio siguiendo buenas prácticas de laboratorio.
5. Las muestras se pueden diluir antes de agregar al medio siempre que el volumen final (muestra más diluyente esterilizado) sea 10±0,2 ml. Ajuste el NMP para reflejar las diluciones. Por ejemplo, si se ponen a prueba 1 ml de muestra y 9 ml de diluyente estéril (dilución de 1:10), multiplique el número de la tabla de NMP por 10 para encontrar el NMP/ml correcto.

Procedimiento de control de calidad

El siguiente procedimiento se recomienda para cada lote de SimPlate para productos sometidos al HPC:

1. A. Control positivo: IDEXX-QC HPC[2]: *Enterococcus faecalis*.
   B. Control negativo/blanco: use 10 ml de medios de HPC rehidratados.
2. Siga los pasos del 1 al 7.
3. Los pocillos de control negativo/blanco no deben quedar fluorescentes después de la incubación.

Contacte con el servicio técnico en los siguientes teléfonos:

Europa: +00800 4339 9111

idexx.es/agua

1. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater* (*Métodos estándares para el análisis del agua y las aguas residuales*). American Public Health Association (Asociación Americana de Salud Pública), 2005. Washington, DC.
2. IDEXX-QC HPC/TVC, IDEXX Catalog #UN3373-WQC-HPC.

One IDEXX Drive
Westbrook, Maine 04092 USA

Dosis Unitaria
SimPlate de RPH
Tabla de NMP

# Pocillos positivos	NMP	Límites de confianza del 95%	
		inferior	superior
0	<0,2	<0,03	<1,4
1	0,2	0,0	1,4
2	0,4	0,1	1,6
3	0,6	0,2	1,9
4	0,8	0,3	2,2
5	1,0	0,4	2,5
6	1,2	0,6	2,7
7	1,5	0,7	3,0
8	1,7	0,8	3,3
9	1,9	1,0	3,6
10	2,1	1,1	3,9
11	2,3	1,3	4,2
12	2,6	1,5	4,5
13	2,8	1,6	4,8
14	3,0	1,8	5,1
15	3,3	2,0	5,4
16	3,5	2,2	5,8
17	3,8	2,3	6,1
18	4,0	2,5	6,4
19	4,3	2,7	6,7
20	4,5	2,9	7,0
21	4,8	3,1	7,4
22	5,1	3,3	7,7
23	5,3	3,5	8,0
24	5,6	3,8	8,4
25	5,9	4,0	8,7
26	6,2	4,2	9,1
27	6,5	4,4	9,4
28	6,8	4,7	9,8
29	7,1	4,9	10,2
30	7,4	5,1	10,6
31	7,7	5,4	10,9
32	8,0	5,6	11,3
33	8,3	5,9	11,7
34	8,6	6,2	12,1
35	9,0	6,4	12,6
36	9,3	6,7	13,0
37	9,7	7,0	13,4
38	10,0	7,3	13,9
39	10,4	7,6	14,3
40	10,8	7,9	14,8
41	11,2	8,2	15,2
42	11,6	8,5	15,7

# Pocillos positivos	NMP	Límites de confianza del 95%	
		inferior	superior
43	12,0	8,8	16,2
44	12,4	9,1	16,7
45	12,8	9,5	17,3
46	13,2	9,8	17,8
47	13,7	10,2	18,3
48	14,1	10,6	18,9
49	14,6	10,9	19,5
50	15,1	11,3	20,1
51	15,6	11,7	20,7
52	16,1	12,1	21,3
53	16,6	12,5	22,0
54	17,1	13,0	22,7
55	17,7	13,4	23,4
56	18,3	13,9	24,1
57	18,9	14,4	24,9
58	19,5	14,9	25,7
59	20,2	15,4	26,5
60	20,9	15,9	27,3
61	21,6	16,5	28,2
62	22,3	17,1	29,2
63	23,1	17,7	30,2
64	23,9	18,3	31,2
65	24,8	19,0	32,3
66	25,7	19,7	33,5
67	26,6	20,4	34,7
68	27,6	21,2	36,1
69	28,7	22,0	37,5
70	29,9	22,9	39,0
71	31,1	23,8	40,7
72	32,4	24,8	42,5
73	33,9	25,8	44,4
74	35,5	27,0	46,6
75	37,2	28,2	49,1
76	39,2	29,6	51,9
77	41,4	31,1	55,1
78	44,0	32,8	58,9
79	47,0	34,8	63,6
80	50,7	37,1	69,5
81	55,5	39,8	77,5
82	62,3	43,2	89,9
83	73,8	47,6	114,6
84	>73,8	>47,6	>114,6

El NMP es por ml de los 10 ml de la muestra añadida al reactivo en tubo (teniendo en cuenta el producto que se pueda derramar).

SimPlate* for HPC

SimPlate* Methode für HPC • Einheitsdosis

Deutsch

Einführung

Die SimPlate* Methode für HPC wird zur quantitativen Bestimmung der Zahl heterotropher Bakterien (HPC, heterotrophic plate counts) in Wasser verwendet. Der Test beruht auf der zum Patent angemeldeten Mehrfach-Enzymtechnologie von IDEXX (Multiple Enzyme Technology*). Mit dieser Methode können lebensfähige Bakterien in Wasser nachgewiesen werden, indem sie auf die Anwesenheit wichtiger Enzyme, die in diesen Organismen bekanntermaßen vorhanden sind, getestet werden. Dabei werden mehrere Enzymsubstrate verwendet, die blau fluoreszieren, wenn sie durch im Wasser vorhandene Bakterien metabolisiert werden. Probe und Medien werden auf eine SimPlate Platte gegeben, inkubiert und anschließend auf fluoreszierende Vertiefungen überprüft. Die Zahl der fluoreszierenden Vertiefungen entspricht einer Wahrscheinlichsten Gesamtbakterienzahl (MPN, most probable number) in der ursprünglichen Probe. Die von der SimPlate for HPC-Methode erzeugten MPN-Werte korrelieren mit der Pour-Plate-Methode, bei der Total Plate Count Agar verwendet wird, der 48 Stunden bei 35°C wie in *Standard Methods for the Examination of Water and Wastewater*[1] beschrieben inkubiert wurde.

Inhalt

- 25 sterile Medienröhrchen für 10-ml-Proben
- 25 sterile SimPlate Platten mit Deckel
- MPN-Tabelle
- Die vorliegende Packungsbeilage

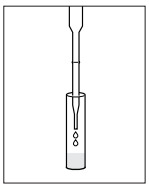
Lagerung

Bei 2–30°C vor Licht geschützt aufbewahren. Das Verfallsdatum ist auf dem Kasten aufgedruckt.

Testverfahren

1. 10±0,2 ml Probe in ein Medienröhrchen geben, Verschluss wieder aufsetzen, und zur Auflösung schütteln. Siehe Abb. 1.
2. Inhalt des Röhrchens in die Mitte einer Platte gießen. Siehe Abb. 2.
3. Platte mit Deckel verschließen und vorsichtig kreisförmig bewegen, um die Probe gleichmäßig auf alle Vertiefungen zu verteilen. Siehe Abb. 3. *HINWEIS:* Luftblasen in den Vertiefungen beeinflussen den Test nicht.
4. Platte 90–120° kippen, damit die überschüssige Flüssigkeit von dem Schwamm absorbiert wird. Siehe Abb. 4.
5. Platte umdrehen und 48 Stunden bei 35±0,5°C inkubieren. Siehe Abb. 5.
6. Die Zahl der fluoreszierenden Vertiefungen bestimmen, indem eine UV-Lampe mit einer Leistung von 6-Watt bei einer Wellenlänge von 365 nm etwa 12,5 cm über die Platte gehalten wird. Dabei das Licht von den Augen weg und zur Platte hinrichten. Sie können alternativ die fluoreszierenden Vertiefungen auch durch die Rückwand der SimPlate ablesen.
7. Die Wahrscheinlichste Zahl (MPN) der heterotrophen Bakterien in der ursprünglichen Probe wird mit Hilfe der mitgelieferten MPN-Tabelle bestimmt. Die Tabelle berücksichtigt die in Schritt 4 abgegossene Probe inkl. Medium.

1



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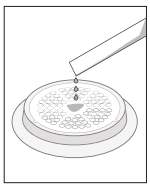

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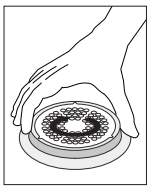

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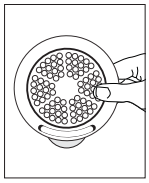

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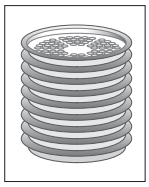


Verfahrenshinweise

1. Aseptisch arbeiten.
2. Chlorierte Proben müssen vor dem Testen mit Natriumthiosulfat behandelt werden.
3. Die Ergebnisse können 45 bis 72 Stunden nach Beginn der Inkubation abgelesen werden.
4. Proben und Medien müssen in Übereinstimmung mit anerkannten Laborverfahren entsorgt werden.
5. Proben können vor Zugabe zum Medium verdünnt werden, solange das endgültige Volumen (Probe plus steriles Verdünnungsmittel) 10±0,2 ml beträgt. Der MPN-Wert muss dann entsprechend korrigiert werden, um die Verdünnung zu berücksichtigen. Werden z.B. 1 ml Probe und 9 ml steriles Verdünnungsmittel getestet (10 fache Verdünnung), muss der aus der MPN-Tabelle bestimmte Wert mit 10 multipliziert werden, um den korrekten MPN-Wert pro ml zu erhalten.

Qualitätskontrollverfahren

Das folgende Verfahren wird für jede SimPlate for HPC-Produktcharge empfohlen:

1. A. Positivkontrolle: IDEXX-QC HPC[2]: *Enterococcus faecalis*.
   B. Negativkontrolle/Blindprobe: 10 ml rehydriertes HPC-Medium verwenden.
2. Schritte 1 bis 7 durchführen.
3. Die Wells der Negativkontrolle/Blindprobe dürfen nach der Inkubation keine Fluoreszenz aufweisen.

Telefonnummern des technischen Supports:

Europa: +00800 4339 9111

idexx.de/wasser

1. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater* (*Standardverfahren für die Wasser- und Abwasseruntersuchung*). American Public Health Association, 2005. Washington, DC, USA.
2. IDEXX-QC HPC/TVC, IDEXX BestellNr. UN3373-WQC-HPC.

One IDEXX Drive
Westbrook, Maine 04092 USA

Einheitsdosis
SimPlate für HPC
MPN - Tabelle

# Positiven Vertiefungen	MPN	95% Vertrauensgrenze Untere	Obere
0	<0,2	<0,03	<1,4
1	0,2	0,0	1,4
2	0,4	0,1	1,6
3	0,6	0,2	1,9
4	0,8	0,3	2,2
5	1,0	0,4	2,5
6	1,2	0,6	2,7
7	1,5	0,7	3,0
8	1,7	0,8	3,3
9	1,9	1,0	3,6
10	2,1	1,1	3,9
11	2,3	1,3	4,2
12	2,6	1,5	4,5
13	2,8	1,6	4,8
14	3,0	1,8	5,1
15	3,3	2,0	5,4
16	3,5	2,2	5,8
17	3,8	2,3	6,1
18	4,0	2,5	6,4
19	4,3	2,7	6,7
20	4,5	2,9	7,0
21	4,8	3,1	7,4
22	5,1	3,3	7,7
23	5,3	3,5	8,0
24	5,6	3,8	8,4
25	5,9	4,0	8,7
26	6,2	4,2	9,1
27	6,5	4,4	9,4
28	6,8	4,7	9,8
29	7,1	4,9	10,2
30	7,4	5,1	10,6
31	7,7	5,4	10,9
32	8,0	5,6	11,3
33	8,3	5,9	11,7
34	8,6	6,2	12,1
35	9,0	6,4	12,6
36	9,3	6,7	13,0
37	9,7	7,0	13,4
38	10,0	7,3	13,9
39	10,4	7,6	14,3
40	10,8	7,9	14,8
41	11,2	8,2	15,2
42	11,6	8,5	15,7

# Positiven Vertiefungen	MPN	95% Vertrauensgrenze Untere	Obere
43	12,0	8,8	16,2
44	12,4	9,1	16,7
45	12,8	9,5	17,3
46	13,2	9,8	17,8
47	13,7	10,2	18,3
48	14,1	10,6	18,9
49	14,6	10,9	19,5
50	15,1	11,3	20,1
51	15,6	11,7	20,7
52	16,1	12,1	21,3
53	16,6	12,5	22,0
54	17,1	13,0	22,7
55	17,7	13,4	23,4
56	18,3	13,9	24,1
57	18,9	14,4	24,9
58	19,5	14,9	25,7
59	20,2	15,4	26,5
60	20,9	15,9	27,3
61	21,6	16,5	28,2
62	22,3	17,1	29,2
63	23,1	17,7	30,2
64	23,9	18,3	31,2
65	24,8	19,0	32,3
66	25,7	19,7	33,5
67	26,6	20,4	34,7
68	27,6	21,2	36,1
69	28,7	22,0	37,5
70	29,9	22,9	39,0
71	31,1	23,8	40,7
72	32,4	24,8	42,5
73	33,9	25,8	44,4
74	35,5	27,0	46,6
75	37,2	28,2	49,1
76	39,2	29,6	51,9
77	41,4	31,1	55,1
78	44,0	32,8	58,9
79	47,0	34,8	63,6
80	50,7	37,1	69,5
81	55,5	39,8	77,5
82	62,3	43,2	89,9
83	73,8	47,6	114,6
84	>73,8	>47,6	>114,6

Der MPN Wert ist pro 1 ml der 10 ml Probe, welche zum Reagenzröhrchen dazugegeben wird.

SimPlate* for HPC

HPC用SimPlate* • Unit Dose

日本語版

商品の紹介

HPC用SimPlate*は、水中の従属栄養細菌数（HPC）の定量検査用キットです。これは、IDEXXが特許を取得した「Multiple Enzyme Technology*」を基にしています。この技術は、細菌の存在の指標である主な酵素の有無を調べることによって、水中の生菌を検出します。水中の細菌による代謝によって青い蛍光を発する複数の酵素基質を利用しています。検体と培地はSimPlate平板で培養し、蛍光を発するウェルの数を数えます。蛍光を発するウェルの数から、検体中の全細菌の最確数（MPN値）を求めます。HPC用SimPlateで求められた最確数（MPN値）は、「*Standard Methods for the Examination of Water and Wastewater*[1]（水および排水標準検査法）」に掲載されている、35°Cで48時間培養された標準寒天培地を使用した混釈平板法と相関があります。

内容

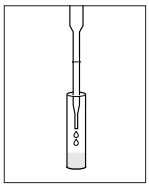
- 10ml用滅菌培地チューブ25本
- 滅菌SimPlate25枚（蓋付き）
- MPN表
- 使用説明書

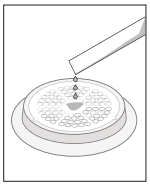
保存

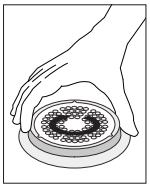
直射光を避け、2～30°Cで保管してください。使用期限は培地チューブの箱に印字されています。

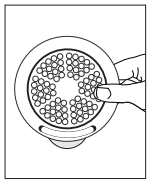
操作手順

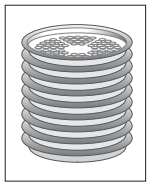
1. 培地チューブに10 ± 0.2 mlの検体を加え、蓋を閉めて振り、ゆっくり混ぜてください。図1を参照。
2. SimPlateの中央に培地チューブ内の検体を全て注入してください。図2を参照。
3. SimPlateに蓋をし、全ウェルに検体が分注されるよう、水平に動かしてください。図3を参照。
   注：ウェル内に気泡があっても、結果には影響はありません。
4. SimPlateを90°～120°に傾け、余分な検体を吸収パッドに吸引させてください。 図4を参照。
5. SimPlateを逆さまにし、35 ± 0.5°Cで48時間培養してください。図5を参照。
6. 蓋を外し、SimPlateの上5インチ（13 cm）に6W・365nm UVランプを置き、蛍光を発するウェルの個数を数えてください。光が目に向けないように注意し、検体に向けてください。また、シンプレートを逆さまにして、裏側から蛍光発色をカウントすることもできます。
7. 専用MPN表を参照して、検体中の従属栄養細菌の最確数（MPN値）を求めてください。この表は、上記ステップ4で吸引した検体や培地を考慮しています。











操作上の注意

1. 無菌操作を行ってください。
2. 塩素処理した検体は、検査前に、チオ硫酸ナトリウムで処理してください。
3. 培養開始後、45～72時間で結果判定できます。
4. 検体と培地はGLPに従って廃棄してください。
5. 最終的な量（検体と培地の合計）が10 ± 0.2 mlであれば、ごく少量の検体を検査することができます。その際は、使用した検体の量に応じて、MPN値を調整します。例えば、1 mlの検体と9 mlの培地を使用した場合、MPN表の数値は、0.1 ml当たりの数値です。1 ml当たりのMPNに換算するには、その数値を10倍します。

品質管理手順

HPC用SimPlateの各ロット毎に次の操作手順を行うことをお薦めします。

1. A. 陽性対照：IDEXX-QC HPC/TVC[2]：Enterococcus faecalis（フェカリス菌）。
   B. 陰性対照／ブランク：再水和されたHPC培地10 mlを使用。
2. 手順1～7を実施してください。
3. 陰性対照／ブランクウェルは、培養後に蛍光を発しないはずです。

アイデックス ラボラトリーズ株式会社

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idexx.co.jp/water

アイデックス ラボラトリーズ株式会社東京都三鷹市北野３－３－７

1. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 2005. Washington, DC.
2. IDEXX-QC HPC/TVC, IDEXX カタログ番号 UN3373-WQC-HPC。

One IDEXX Drive
Westbrook, Maine 04092 USA

Unit Dose

HPC用SimPlate

最確数（**MPN**）表

陽性ウェルの数	**MPN**	**95%**信頼限界	
		下限	上限
0	<0.2	<0.03	<1.4
1	0.2	0.0	1.4
2	0.4	0.1	1.6
3	0.6	0.2	1.9
4	0.8	0.3	2.2
5	1.0	0.4	2.5
6	1.2	0.6	2.7
7	1.5	0.7	3.0
8	1.7	0.8	3.3
9	1.9	1.0	3.6
10	2.1	1.1	3.9
11	2.3	1.3	4.2
12	2.6	1.5	4.5
13	2.8	1.6	4.8
14	3.0	1.8	5.1
15	3.3	2.0	5.4
16	3.5	2.2	5.8
17	3.8	2.3	6.1
18	4.0	2.5	6.4
19	4.3	2.7	6.7
20	4.5	2.9	7.0
21	4.8	3.1	7.4
22	5.1	3.3	7.7
23	5.3	3.5	8.0
24	5.6	3.8	8.4
25	5.9	4.0	8.7
26	6.2	4.2	9.1
27	6.5	4.4	9.4
28	6.8	4.7	9.8
29	7.1	4.9	10.2
30	7.4	5.1	10.6
31	7.7	5.4	10.9
32	8.0	5.6	11.3
33	8.3	5.9	11.7
34	8.6	6.2	12.1
35	9.0	6.4	12.6
36	9.3	6.7	13.0
37	9.7	7.0	13.4
38	10.0	7.3	13.9
39	10.4	7.6	14.3
40	10.8	7.9	14.8
41	11.2	8.2	15.2
42	11.6	8.5	15.7

陽性ウェルの数	**MPN**	**95%**信頼限界	
		下限	上限
43	12.0	8.8	16.2
44	12.4	9.1	16.7
45	12.8	9.5	17.3
46	13.2	9.8	17.8
47	13.7	10.2	18.3
48	14.1	10.6	18.9
49	14.6	10.9	19.5
50	15.1	11.3	20.1
51	15.6	11.7	20.7
52	16.1	12.1	21.3
53	16.6	12.5	22.0
54	17.1	13.0	22.7
55	17.7	13.4	23.4
56	18.3	13.9	24.1
57	18.9	14.4	24.9
58	19.5	14.9	25.7
59	20.2	15.4	26.5
60	20.9	15.9	27.3
61	21.6	16.5	28.2
62	22.3	17.1	29.2
63	23.1	17.7	30.2
64	23.9	18.3	31.2
65	24.8	19.0	32.3
66	25.7	19.7	33.5
67	26.6	20.4	34.7
68	27.6	21.2	36.1
69	28.7	22.0	37.5
70	29.9	22.9	39.0
71	31.1	23.8	40.7
72	32.4	24.8	42.5
73	33.9	25.8	44.4
74	35.5	27.0	46.6
75	37.2	28.2	49.1
76	39.2	29.6	51.9
77	41.4	31.1	55.1
78	44.0	32.8	58.9
79	47.0	34.8	63.6
80	50.7	37.1	69.5
81	55.5	39.8	77.5
82	62.3	43.2	89.9
83	73.8	47.6	114.6
84	>73.8	>47.6	>114.6

MPNは、培地チューブに添加した検体10 ml中のml単位の値です（吸収した検体や培地を考慮しています）。

Enterolert* Test Kit

Introduction
Enterolert* detects enterococci, such as *E. faecium* and *E. faecalis*, in fresh and marine water. It is based on IDEXX's patented Defined Substrate Technology* (DST*). When enterococci utilize their ß-glucosidase enzyme to metabolize Enterolert's nutrient-indicator, 4-methyl-umbelliferyl ß-D-glucoside, the sample fluoresces. Enterolert detects enterococci at 1 cfu per 100 mL sample within 24 hours.

Storage
Store at 2–30°C away from light.

Presence/Absence (P/A) Procedure
1. Add contents of one pack to a 100 mL sample in a sterile, transparent, nonfluorescing vessel.
2. Cap vessel and shake.
3. Incubate at 41 ± 0.5°C for 24 hours.
4. Read results according to Result Interpretation table below.

Quanti-Tray* Enumeration Procedure
1. Add contents of one pack to a 100 mL water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray* or Quanti-Tray*/2000 and seal in an IDEXX Quanti-Tray* Sealer.
4. Place the sealed tray in a 41 ± 0.5°C incubator for 24 hours.
5. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

Result Interpretation

Appearance	Result
Lack of fluorescence	Negative for enterococci
Blue fluorescence	Positive for enterococci

- Look for fluorescence with a 6–watt, 365 nm, UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.
- Enterolert results are definitive at 24–28 hours. In addition, positives for enterococci observed before 24 hours and negatives observed after 28 hours are also valid.

Procedural Notes
- Marine water samples must be diluted at least tenfold with sterile fresh water. Multiply the MPN value by the dilution factor to obtain the proper quantitative result.
- Use only sterile, nonbuffered, oxidant-free water for dilutions.
- For comparison, a water blank can be used when interpreting results.
- This insert may not reflect your local regulations. For compliance testing, be sure to follow appropriate regulatory procedures.
- Enterolert can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater*[1] MPN tables should be used to find Most Probable Numbers.
- Enterolert is a primary water test. Enterolert performance characteristics do not apply to samples altered by any pre-enrichment or concentration.
- Aseptic technique should always be followed when using Enterolert. Dispose of in accordance with Good Laboratory Practices.

Quality Control Procedures
1. One of the following quality control procedures is recommended for each lot of Enterolert:
   A. IDEXX-QC Enterococci[2]: *Enterococcus faecalis*, *Escherichia coli*, and *Streptococcus bovis*.
   B. i. For each of the American Type Culture Collection (ATCC)[3] bacterial strains (*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862, and *Aerococcus viridans* ATCC 10400), streak the culture onto labeled TSA or Blood Agar plates and incubate at 35 ± 2°C for 18–24 hours.
   ii. For each bacterial strain, touch a sterile 1 µL inoculating loop to a colony and use it to inoculate a labeled test tube containing 5 mL of sterile deionized water. Close cap and shake thoroughly.
   iii. For each bacterial strain, take a 1 µL loop from the test tube and use it to inoculate a labeled vessel containing 100 mL of sterile deionized water. These are your controls.
2. Follow the P/A Procedure or Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

1. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. Washington, DC: American Public Health Association; 2005.
2. IDEXX-QC Enterococci – IDEXX Catalog #UN373-WQC-ENT
3. American Type Culture Collection 1-800-638-6597 atcc.org

Kit d'analyse Enterolert*

Introduction
Enterolert* permet la détection d'entérocoques tels que *E. faecium* et *E. faecalis* dans l'eau douce et l'eau de mer. Ce test est basé sur la technologie brevetée Defined Substrate Technology* (DST*) d'IDEXX. Lorsque les entérocoques utilisent leur enzyme ß-glucosidase pour métaboliser l'indicateur de nutriants Enterolert, le 4-méthyl-umbelliféryl ß-D-glucoside, l'échantillon devient fluorescent. Enterolert détecte les entérocoques à 1 cfu par prélèvement de 100 mL en 24 heures.

Conditions de conservation
Conserver entre 2–30°C à l'abri de la lumière.

Procédure de présence/absence (P/A)
1. Ajouter le contenu d'un sachet dans un prélèvement de 100 ml placé dans un récipient stérile, transparent et non fluorescent.
2. Fermer le récipient et agiter.
3. Incuber à 41 ± 0,5°C pendant 24 heures.
4. Interpréter les résultats en se référant au tableau d'interprétation des résultats ci-dessous.

Procédure de numération Quanti-Tray*
1. Ajouter le contenu d'un sachet dans un prélèvement de 100 ml d'eau placé un récipient stérile.
2. Fermer le récipient et agiter jusqu'à dissolution.
3. Verser le mélange prélèvement/réactif dans un Quanti-Tray* ou Quanti-Tray*/2000 et fermer hermétiquement dans un IDEXX Quanti-Tray* Sealer.
4. Placer le plateau hermétiquement fermé dans un incubateur à 41 ± 0,5°C pendant 24 heures.
5. Interpréter les résultats en se référant au tableau d'interprétation des résultats ci-dessous. Compter le nombre de puits positifs et se référer au tableau MPN fourni avec les plateaux pour obtenir le Chiffre le plus probable (MPN).

Interprétation des résultats

Aspect	Résultat
Absence de fluorescence	Négatif pour entérocoques
Fluorescence bleue	Positif pour entérocoques

- Évaluer la fluorescence dans l'obscurité, avec une ampoule UV de 6 watts et 365 nm placée à 13 cm du prélèvement. Orienter la lumière vers le prélèvement, dans la direction opposée à celle des yeux de l'opérateur.
- Les résultats de Enterolert doivent être lus entre 24–28 heures. En outre, les résultats positifs pour entérocoques notés avant 24 heures, de même que les résultats négatifs notés après 28 heures, sont également valides.

Remarques concernant la procédure
- Le prélèvement d'eau salée doit être dilué au moins au dixième avec de l'eau douce stérile. Multiplier la valeur MPN par le facteur de dilution pour obtenir le résultat quantitatif correspondant.
- Utiliser uniquement de l'eau stérile, non tamponnée et sans oxydant pour les dilutions.
- Pour comparer, il est possible d'utiliser une eau pure stérile (water blank) pour interpréter les résultats.
- Cette notice peut différer des réglementations en vigueur dans votre pays. Pour tout test de conformité, suivre les procédures réglementaires appropriées.
- Enterolert peut être effectué en format de tubes multiples. Utiliser des méthodes standard et les tableaux MPN pour le contrôle des eaux et des eaux usées[1] afin de déterminer les Chiffres les plus probables (MPN).
- Enterolert est avant tout un test pour eau. Les caractéristiques de performance d'Enterolert ne s'appliquent pas aux prélèvements altérés par tout enrichissement préalable ou toute concentration.
- Utiliser systématiquement des techniques aseptiques dans l'emploi d'Enterolert. Mettre au rebut conformément aux Bonnes pratiques de laboratoire.

Le contrôle de la qualité
1. Une des procédures de contrôle qualité suivantes est recommandée pour chaque lot d'Enterolert:
   A. IDEXX-QC Enterococci[2]: *Enterococcus faecalis*, *Escherichia coli* et *Streptococcus bovis*.
   B. i. Pour chacune des souches bactériennes American Type Culture Collection (ATCC)[3] (*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862 et *Aerococcus viridans* ATCC 10400), placer la culture sur des plaques TSA ou de gélose au sang dûment étiquetées et incuber à 35 ± 2°C pendant 18 à 24 heures.
   ii. Pour chaque souche bactérienne, prélever 1 µl d'une colonie à l'aide d'une anse et inoculer un tube à essai dûment étiqueté contenant 5 ml d'eau désionisée stérile. Fermer le bouchon, puis secouer vigoureusement.
   iii. Pour chaque souche bactérienne, prendre une anse de 1 µl du tube à essai et l'utiliser pour inoculer un récipient dûment étiqueté contenant 100 ml d'eau désionisée stérile. Il s'agit de vos contrôles.
2. Suivre la procédure P/A ou la procédure de numération Quanti-Tray ci-dessus pour tester ces contrôles.
3. Les résultats doivent correspondre avec le tableau d'interprétation des résultats ci-dessus.

1. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. (Méthodes traditionnelles pour l'analyse de l'eau et des eaux usées). Washington, DC: American Public Health Association; 2005.
2. Entérocoques d'IDEXX-QC - Catalogue IDEXX n° UN3373-WQC-ENT
3. American Type Culture Collection 1-800-638-6597 atcc.org

Kit di analisi Enterolert*

Introduzione
Il kit Enterolert* rileva gli enterococci come l'*E. faecium* e l'*E. faecalis* in acque dolci e marine. Il kit si basa sulla tecnologia brevettata IDEXX chiamata Defined Substrate Technology* (DST*). Quando gli enterococci utilizzano il loro enzima ß-glucosidasi per metabolizzare il nutriente-indicatore di Enterolert, il 4-metil-umbellliferil ß-D-glucoside, il campione diventa fluorescente. Enterolert rileva gli enterococci in concentrazioni di 1 cfu (unità formante colonia) per campione da 100 mL entro 24 ore.

Conservazione
Conservare a 2–30°C lontano dalla luce.

Procedura di presenza/assenza (P/A)
1. Aggiungere il contenuto di una bustina (pack) ad un campione di 100 ml, in un recipiente sterile, trasparente e non fluorescente.
2. Chiudere il recipiente ed agitarlo.
3. Incubare a 41 ±0,5°C per 24 ore.
4. Leggere i risultati in base alla Tabella di interpretazione dei risultati qui sotto.

Procedura di enumerazione Quanti-Tray*
1. Aggiungere il contenuto di una bustina (pack) ad un campione di acqua di 100 ml, in un recipiente sterile.
2. Chiudere il recipiente ed agitarlo fino alla dissoluzione.
3. Versare la miscela campione/reagente in un Quanti-Tray* o un Quanti-Tray*/2000 e sigillarlo con un IDEXX Quanti-Tray* Sealer.
4. Mettere il vassoietto sigillato in un incubatore a 41 ±0,5°C per 24 ore.
5. Leggere i risultati in base alla Tabella di interpretazione dei risultati qui sotto. Contare il numero dei pozzetti positivi e consultare la tabella MPN in dotazione con i vassoietti per ottenere il Numero più probabile (MPN).

Interpretazione del risultato

Aspetto	Risultato
Mancanza di fluorescenza	Negativo per gli enterococci
Fluorescenza azzurra	Positivo per gli enterococci

- Individuare la presenza di fluorescenza con una luce a raggi ultravioletti da 6 watt, 365 nm a distanza di 13 cm, in ambiente oscuro. Rivolgere la luce in direzione opposta ai propri occhi e verso il campione.
- I risultati di Enterolert sono definitivi a distanza di 24–28 ore. Inoltre, sono validi anche i risultati positivi per gli enterococci osservati prima delle 24 ore ed i risultati negativi osservati dopo 28 ore.

Note procedurali
- Il campione di acqua marina deve essere diluito almeno dieci volte con acqua dolce sterile. Moltiplicare il valore MPN per il fattore di diluizione per ottenere il risultato quantitativo giusto.
- Per le diluizioni usare solo acqua sterile, non tamponata e senza ossidanti.
- A scopi comparativi, quando si interpretano i risultati si può utilizzare un campione di acqua come modello.
- Questo inserto informativo potrebbe non riflettere i regolamenti locali dell'utente. Per i test di conformità, assicurarsi di seguire le appropriate procedure normative.
- Il test Enterolert può essere eseguito in qualsiasi formato con provette multiple. Per trovare i Numeri più probabili (MPN) vanno impiegate le tabelle MPN dei Metodi standard per l'analisi dell'acqua e delle acque di scarico (*Standard Methods for the Examination of Water and Wastewater*[1]).
- L'analisi Enterolert è un'importante analisi dell'acqua. Le caratteristiche di prestazione Enterolert non sono applicabili a campioni alterati da qualsiasi pre-arricchimento o concentrazione.
- Quando si usa Enterolert vanno sempre seguite tecniche asettiche. Eliminare seguendo le Buone pratiche di laboratorio.

Procedure per il controllo di qualità
1. Per ciascun lotto di Enterolert si consiglia una delle seguenti procedure di controllo della qualità:
   A. Enterococchi[2] IDEXX-QC: *Enterococcus faecalis*, *Escherichia coli* e *Streptococcus bovis*.
   B. i. Per ciascun ceppo batterico della American Type Culture Collection (ATCC)[3] (*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862 e *Aerococcus viridans* ATCC 10400), strisciare la coltura su piastre TSA o su piastre Agar sangue e incubare a 35 ± 2°C per 18–24 ore.
   ii. Per ciascun ceppo batterico, toccare una colonia con un'ansa sterile per inoculazione da 1 µl e utilizzarla per inoculare una provetta etichettata contenente 5 ml di acqua deionizzata sterile. Chiudere il tappo e agitare bene.
   iii. Per ciascun ceppo batterico, prelevare un'ansa da 1 µl dalla provetta e utilizzarla per inoculare un contenitore etichettatocontenente 100 ml di acqua deionizzata sterile. Questi sono i controlli.
2. Seguire la procedura P/A o la procedura di enumerazione Quanti-Tray di cui sopra.
3. I risultati dovrebbero corrispondere alla tabella di interpretazione dei risultati di cui sopra.

1. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. Washington, DC: American Public Health Association; 2005.
2. Enterococchi IDEXX-QC - Catalogo IDEXX N. UN3373-WQC-ENT
3. American Type Culture Collection 1-800-638-6597 atcc.org

Enterolert* Testkit

Einführung
Enterolert ist zum Nachweis von Enterokokken, wie z. B. *E. faecium* und *E. faecalis*, in Süß- und Meereswasser bestimmt. Der Test basiert auf der patentierten Defined Substrate Technology* (DST*) von IDEXX. Wenn die Enterokokken mit ihrem ß-Glucosidase-Enzym den Nährstoff-Indikator 4-Methylumbelliferyl-ß-D-Glucosid von Enterolert metabolisieren, fluoresziert die Probe. Enterolert weist Enterokokken im Bereich von 1 CFU (KbE; koloniebildende Einheit) pro 100 mL Probe innerhalb von 24 Stunden nach.

Lagerung
Bei 2–30 °C und vor Licht geschützt lagern.

Presence/Absence (P/A)-Test
1. Den Inhalt einer Packung zu einer 100 ml Probe in einem sterilen, transparenten, nicht fluoreszierenden Gefäß hinzufügen.
2. Gefäß verschließen und schütteln.
3. 24 Stunden bei 41 ± 0,5 °C inkubieren.
4. Das Ergebnis gemäß der nachstehenden Ergebnisauswertetabelle ablesen.

Quanti-Tray* Auszählmethode
1. Den Inhalt einer Packung zu einer 100 ml Probe in einem sterilen Gefäß hinzugeben.
2. Gefäß verschließen und schütteln, bis sich das Präparat völlig aufgelöst hat.
3. Die aus Probe und Reagenz bestehende Mischung in ein Quanti-Tray* oder Quanti-Tray*/2000 gießen und in einem IDEXX Quanti-Tray* Sealer verschließen.
4. Das verschlossene Tray 24 Stunden bei 41 ± 0,5 °C inkubieren.
5. Die Ergebnisse anhand der nachstehenden Ergebnisauswertetabelle ablesen. Die Anzahl der positiven Probenvertiefungen zählen und die wahrscheinlichste Zahl (MPN; Most Probable Number) anhand der MPN-Tabelle, die den Trays beiliegt, ermitteln.

Ergebnisauswertung

Erscheinung	Ergebnis
Keine Fluoreszenz	Negativ für Enterokokken
Blaue Fluoreszenz	Positiv für Enterokokken

- Prüfung auf Fluoreszenz mit einer 6-Watt-, 365-nm-UV-Lampe aus einem Abstand von 13 cm in einer dunklen Umgebung. Dabei die Lampe nur auf die Probe, nicht auf die Augen, richten.
- Die Enterolert-Ergebnisse sind nach 24–28 Stunden definitiv. Außerdem sind vor Ablauf von 24 Stunden beobachtete positive und nach Ablauf von 28 Stunden beobachtete negative Reaktionen auf Enterokokken ebenfalls gültig.

Verfahrenshinweise
- Meereswasser muss mindestens zehnfach mit sterilem Süßwasser verdünnt werden. Dann ist der MPN-Wert mit dem Verdünnungsfaktor zu multiplizieren, um das richtige quantitative Ergebnis zu erhalten.
- Nur steriles, nicht gepuffertes, keine Oxidantien enthaltendes Wasser zur Verdünnung verwenden.
- Für Vergleichszwecke kann bei der Ergebnisauswertung eine Blindprobe (Wasser) herangezogen werden.
- Diese Packungsbeilage gibt möglicherweise nicht Ihre örtlichen Vorschriften wieder. Bei Qualitätstests sind die anwendbaren aufsichtsbehördlichen Verfahren zu befolgen.
- Das Enterolert-Verfahren kann in jedem Multiple-Tube-Format durchgeführt werden. Zur Ermittlung der MPNs (wahrscheinlichste Zahlen) sollten MPN-Tabellen für Standardverfahren zur Untersuchung von Wasser und Abwasser* herangezogen werden.
- Enterolert ist primär ein Wassertest. Die Leistungsmerkmale von Enterolert gelten nicht für Proben, die durch Voranreicherung oder Konzentration modifiziert wurden.
- Bei der Verwendung von Enterolert ist ein aseptisches Vorgehen vorgeschrieben. Entsorgung gemäß Standard-Laborpraktiken.

Qualitätskontrollverfahren
1. Eines der folgenden Qualitätskontrollverfahren wird für jede Enterolert-Charge empfohlen:
   A. IDEXX-QC *Enterococci*[2]: *Enterococcus faecalis*, *Escherichia coli* und *Streptococcus bovis*.
   B. i. Für jeden American Type Culture Collection (ATCC)[3]-Bakterienstamm (*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862 und *Aerococcus viridans* ATCC 10400) die Kultur auf etikettierte TSA- oder Blutagarplatten streichen und 18–24 Stunden bei 35 ± 2 °C inkubieren.
   ii. Für jeden Bakterienstamm eine Kolonie mit einer sterilen 1-µl-Impföse berühren und ein etikettiertes Probenröhrchen, das 5 ml steriles deionisiertes Wasser enthält, inokulieren. Kappe verschließen und gründlich schütteln.
   iii. Für jeden Bakterienstamm mit einer 1-µl-Öse eine Probe aus dem Teströhrchen entnehmen und ein etikettiertes Gefäß, das 100 ml steriles deionisiertes Wasser enthält, damit inokulieren. Dies sind Ihre Kontrollen.
2. Das oben beschriebene P/A-Verfahren oder das Quanti-Tray-Auszählverfahren anwenden.
3. Die Ergebnisse sollten mit der Tabelle zur Ergebnisauswertung (siehe oben) übereinstimmen.

1. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. (*Standardverfahren für die Wasser- und Abwasseruntersuchung*). Washington, DC: American Public Health Association; 2005.
2. IDEXX-QC Enterococci – IDEXX Bestellnr. UN373-WQC-ENT
3. American Type Culture Collection 1-800-638-6597 atcc.org

Kit de análisis Enterolert*

Introducción

Enterolert* detecta enterococos tales como enterococos *E. faecium*, y enterococos *E. faecalis* en agua superficial de bano y agua de mar. Se basa en Defined Substrate Technology® (Tecnología de substrato definido [DST®]), patentada por IDEXX. Cuando los enterococos utilizan su enzima ß-glucosidasa para metabolizar el indicador de nutriente de Enterolert, 4-metil-umbeliferil ß-D-glucosida, la muestra fluoresce. Enterolert detecta enterococos en una muestra de 1 ufc por 100 mL dentro de 24 horas.

Almacenamiento

Almacenar a temperatura de 2–30°C, alejado de la luz.

Procedimiento de presencia/ausencia (P/A)

1. Añadir el contenido de un paquete a una muestra de 100 ml en un recipiente estéril transparente, no fluorescente.
2. Tapar y agitar el recipiente.
3. Incubar a 41 ±0,5°C durante 24 horas.
4. Leer los resultados de acuerdo con el cuadro de interpretación de resultados, más abajo.

Procedimiento de enumeración Quanti-Tray*

1. Añadir el contenido de un paquete a una muestra de 100 ml de agua, en un recipiente estéril.
2. Tapar y agitar el recipiente hasta disolver.
3. Verter la mezcla de muestra/reactivo en una Quanti-Tray* o una Quanti-Tray*/2000 y sellar en un IDEXX Quanti-Tray* Sealer.
4. Colocar la bandeja sellada en una incubadora a 41 ±0,5°C durante 24 horas.
5. Leer los resultados de acuerdo con el cuadro de interpretación de resultados, más abajo. Contar el número de pocillos positivos y referirse a la tabla NMP proporcionadas con las bandejas para obtener el número más probable.

Interpretación de resultados

Apariencia	Resultado
Ausencia de fluorescencia	Negativo para enterococos
Fluorescencia azul	Positivo para enterococos

- Buscar fluorescencia usando una luz ultravioleta de 6 vatios, 365 nm a distancia de unas 5 pulgadas (13 cm) de la muestra, en un entorno oscuro. Apuntar el haz de luz en dirección contraria a los ojos y hacia la muestra.
- Los resultados Enterolert son definitivos a las 24–28 horas. Además, los positivos para enterococos observados antes de las 24 horas y los negativos observados después de las 28 horas también son válidos.

Notas sobre el procedimiento

- La muestra de agua de mar deberá diluirse por lo menos diez veces con agua superficial de bano estéril. Multiplicar el valor NMP por el factor de dilución para obtener el resultado cuantitativo apropiado.
- Utilizar solamente agua estéril, no tamponada, libre de oxidantes, para efectuar las diluciones.
- Para comparación, se puede utilizar blanco testigo de agua al interpretar resultados.
- Este prospecto tal vez no refleje sus reglamentaciones locales. Para realizar pruebas en cumplimiento, asegúrese de seguir los procedimientos reglamentarios apropiados.
- Enterolert puede procesarse en cualquier formato de múltiples tubos. Deben utilizarse métodos estándares para examen del agua y las tablas NMP de aguas residuales (*Standard Methods for the Examination of Water and Wastewater*[1]) para encontrar el resultado de NMP.
- Enterolert es un a prueba primaria de agua. Las características de desempeño de Enterolert no se aplican a muestras alteradas por cualquier enriquecimiento o concentración previos.
- Siempre debe aplicarse una técnica asséptica cuando se utilice Enterolert. Se debe desechar en cumplimiento con las buenas prácticas de laboratorio.

Procedimientos de control de calidad

1. Se recomienda uno de los siguientes procedimientos de control de calidad para cada lote de Enterolert:
   A. IDEXX-QC Enterococci[2]: *Enterococcus faecalis*, *Escherichia coli* y *Streptococcus bovis*.
   B. i. Para cada una de las cepas bacterianas de Colección Americana de Cultivos Tipo (American Type Culture Collection, ATCC)[3](*Enterococcus faecium* ATCC 35667, *Serratia marcescens* ATCC 43862, y *Aerococcus viridans* ATCC 10400), siembre en estrías el cultivo sobre placas etiquetadas de agar sangre o agar triptícasa de soja (Tripticase Soy Agar, TSA) e incube a 35 ±2°C durante 18 a 24 horas.
   ii. Para cada cepa bacteriana, agregue 1 µl de asa de inoculación a una colonia y úsela para inocular una probeta etiquetada que contenga 5 ml de agua estéril desionizada. Cierre la tapa y agite enérgicamente.
   iii. Para cada cepa bacteriana, tome 1 µl de asa de la probeta y úsela para inocular un recipiente etiquetado que contenga 100 ml de agua estéril desionizada. Estos son los controles que debe realizar.
2. Siga el procedimiento P/A o el procedimiento de enumeración de Quanti-Tray que se indica más arriba.
3. Los resultados deben coincidir con la tabla de interpretación de resultados que se indica más arriba.

1. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. (Métodos estándares para el análisis del agua y las aguas residuales). Washington, DC: American Public Health Association (Asociación Americana de Salud Pública); 2005.
2. IDEXX-QC Enterococci—IDEXX Catalog #UN3373-WQC-ENT
3. American Type Culture Collection 1-800-638-6597 atcc.org

*Enterolert, Defined Substrate Technology, DST y Quanti-Tray son marcas o marcas registradas de IDEXX Laboratories, Inc. o sus filiales en los Estados Unidos de América y/o en otros países.
Información sobre la patente: idexx.com/patents.

Enterolert*（エンテロラート）テストキット

はじめに

Enterolert* は、水および海水中の*E. faecium*, *E. faecalis*などの腸球菌を検出します。検出方法は、IDEXXが特許を所有する特定酵素基質法 Defined Substrate Technology® (DST®) に基づいています。腸球菌がß-グルコシダーゼ酵素によってEnterolert中の栄養指標である4-メチルーウンベリフェリルß-D-グルコシドを代謝すると、検体は蛍光を発します。Enterolertは、24時間以内に1 cfu/100mLの感度で腸球菌を検出することができます。

保管

直射日光を避け、2～30° Cで保管してください。

定性検査・操作手順

1. 透明な滅菌容器に検体100 mlを入れ、スナップパック1個を加えてください。
2. 容器の蓋を閉め、混ぜてください。
3. 41±0.5°Cで24時間培養してください。
4. 以下の「結果判定表」に従って結果判定してください。

Quanti-Tray* 定量法

1. 滅菌容器に検体100 mlを入れ、スナップパック1個を加えてください。
2. 容器の蓋を閉め、ゆっくり混ぜてください。
3. Quanti-Tray*/2000に注ぎ、IDEXX Quanti-Tray*シーラーで密封してください。
4. 密封したトレーを41 ±0.5°Cで24時間培養してください。
5. 下記の「結果判定表」に従って結果を判定してください。陽性ウェルの数を数え、専用MPN表を参照して最確数 (MPN) を求めてください。

結果判定

培養液の状態	結果
蛍光なし	腸球菌陰性
青く蛍光発色	腸球菌陽性

- 暗所で検体から5インチ (13 cm) 以内の距離から6W・365nmのUVランプを使用して判定してください。光は目に向けないようにし、検体に向けてください。
- Enterolertの結果は24～28時間で判定してください。24時間以内で腸球菌が陽性となった場合、陽性判定が有効となり、28時間以上培養して陰性となった場合は、陰性判定が有効です。

操作上の注意

- 海水検体は滅菌水で10倍以上に希釈してください。MPN値に希釈係数を掛けて適切な定量結果を求めてください。
- 希釈には、緩衝剤や酸化物質の入っていない、滅菌水のみを使用してください。
- 比較のため、結果判定時にみのみのブランクを使用してください。
- 本使用説明書の内容は、各国の法律・条例に適合していないこともあります。法律・条例に準拠したテストをする為に、適切な規定の手順に必ず従ってください。
- Enterolertは5本法などの最確数法でも検査可能です。MPN表は、最確数 (MPN) を求めるためにご使用ください。
- Enterolertは水の一次検査です。Enterolertの性能特性上で、増菌培地で培養または濃縮によって変質した検体に適用できません。
- Enterolertを使用しているときは、常に無菌操作を行ってください。GLPに従って廃棄してください。

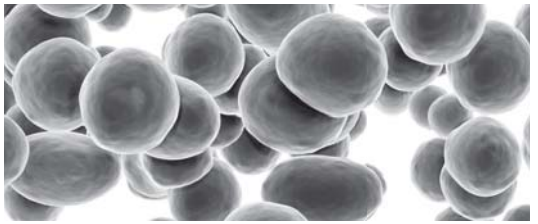
品質管理手順

1. Enterolertを使用する場合、ロット毎に次の品質管理手順のいずれかを行うことをお薦めします。:
   A. IDEXX-QC腸球菌*: *Enterococcus faecalis*、大腸菌、*Streptococcus bovis*。
   B. i. 米国培養細胞系統保存機関 (ATCC) の各菌種 (*Enterococcus faecium* ATCC 35667、*Serratia marcescens* ATCC 43862および*Aerococcus viridans* ATCC 10400) については、ラベルを付けたTSA培地もしくは血液寒天培地上に菌を画線し、35 ±2°Cで18～24時間培養します。
   ii. 各菌種につき、1 µlの滅菌接種ループで、コロニーを滅菌脱イオン水 5 mLが入った試験管に接種します。蓋を閉め、十分に振ってください。
   iii. 各菌種につき、試験管から1 µlをループで取り、滅菌脱イオン水100 mLを入れた容器に接種します。これが対照群 (コントロール) となります。
2. 上記の定性法 (P/A) 手順、またはQTトレイ定量法操作手順に従ってください。
3. 結果は上記の結果解釈表と一致するはずです。

1. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH, eds. *Standard Methods for the Examination of Water and Wastewater*. 21 st ed. Washington, DC: American Public Health Association; 2005.
2. IDEXX-QC 腸球菌—IDEXX カタログ番号 UN3373-WQC-ENT
3. American Type Culture Collection 1-800-638-6597 atcc.org

*Enterolert Defined Substrate Technology, DST, 及びQuanti-Trayは、米国およびその他国のIDEXX Laboratories,Inc.またはその関連会社の、商標または登録商標です。
特許情報：idexx.com/patents

Enterolert*



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IDEXX

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Colilert® -18 Test Kit

Introduction
Colilert-18 either simultaneously detects total coliforms and *E. coli* or fecal coliforms in water. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When total or fecal coliforms metabolize Colilert-18's nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert-18's nutrient-indicator, MUG, the sample fluoresces. Colilert-18 can simultaneously detect these bacteria at 1 cfu/100 ml within 18 hours even with as many as 2 million heterotrophic bacteria per 100 ml present.

Contents
- WP200i-18 contains 20 Snap Packs for 100 mL samples
- WP200i-18 contains 200 Snap Packs for 100 mL samples

Storage
Store at 2-25°C away from light.

Presence/Absence (P/A) Procedure
1. Add contents of one pack to a 100 mL sample in a sterile, transparent, nonfluorescing vessel.
2. Cap vessel and shake.
3. If sample is not already at 33–38°C, then place vessel in a 35°C waterbath for 20 minutes or, alternatively a 44.5°C waterbath for 7–10 minutes.
4. Incubate at 35°C ±0.5°C for the remainder of the 18 hours.
5. Read results according to Result Interpretation table below.

Quanti-Tray® Enumeration Procedure
1. Add contents of one pack to a 100 mL room temperature water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer.
4. Place the sealed tray in a 35°C ± 0.5°C (or 44.5°C ±0.2°C for fecal coliforms) incubator for 18 hours (prewarming to 35°C is not required). For incubation in a water bath, submerge the Quanti-Tray, as is, below the water level using a weighted ring.
5. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

Result Interpretation

Appearance	Result
Less yellow than the comparator[1] when incubated at 35°C ±0.5°C or 44.5°C ±0.2°C	Negative for total coliforms and *E. coli*, Negative for fecal coliforms
Yellow equal to or greater than the comparator when incubated at 35°C ±0.5°C	Positive for total coliforms
Yellow equal to or greater than the comparator when incubated at 44.5°C ±0.2°C	Positive for fecal coliforms
Yellow and fluorescence equal to or greater than the comparator when incubated at 35°C ±0.5°C	Positive for *E. coli*

- Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and toward the sample.
- Colilert-18 results are definitive at 18–22 hours. In addition, positives for both total coliforms and *E. coli* or fecal coliforms observed before 18 hours and negatives observed after 22 hours are also valid.

Procedural Notes
- A slight tinge may be observed when Colilert-18 is added to the sample.
- If excess foam causes problems while using Quanti-Tray, you may choose to use IDEXX Antifoam Solution (Catalog# WAFDB) or IDEXX 120 ml vessels with Antifoam (Catalog# WV120SBAF-200).
- This insert may not reflect your local regulations. For compliance testing, be sure to follow appropriate regulatory procedures. For example, samples run in Europe should be incubated at 36 ±2°C for 18 – 22 hours.
- When following AFNOR validated method for drinking water testing, place the sealed tray in a 36°C ±2°C incubator for 18 hours (prewarming to 36°C is not required).
- Colilert-18 can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater*[2] MPN tables should be used to find Most Probable Numbers (MPNs).
- If a water sample has some background color, compare inoculated Colilert-18 sample to a control blank of the same water sample.
- Colilert-18 can be used for *E. coli* (but not coliforms) in marine water. Samples must be diluted at least tenfold. Multiply the MPN value by the dilution factor to obtain the proper quantitative result.
- Use only sterile, nonbuffered, oxidant-free water for dilutions.
- Colilert-18 is a primary water test. Colilert-18 performance characteristics do not apply to samples altered by any pre-enrichment or concentration.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert-18. If this is seen, consider sample invalid and discontinue testing.
- Aseptic technique should always be followed when using Colilert-18. Dispose of in accordance with Good Laboratory Practices.

Quality Control Procedures
The following quality control procedure is recommended for each lot of Colilert-18 when used for total coliform and *E. coli* testing at 35°C ±0.5°C:
1. Fill three sterile vessels with 100 mL sterile water and inoculate one with an *E. coli*, one with a total coliform and one with a non-coliform using one of the following products :
   A. Quanti-Cult™[3] *E coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*
   B. A sterile loop of ATCC® strains, *E. coli* ATCC 25922 or 11775, *Klebsiella pneumoniae* ATCC 31488 and *Pseudomonas aeruginosa* ATCC 10145 or 27853
2. Follow the P/A Procedure or Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

The following quality control procedure is recommended for each lot of Colilert-18 when used for fecal coliform testing at 44.5°C ±0.2°C:
1. Fill two sterile vessels with 100 mL sterile water and inoculate one with a fecal coliform (*E. coli*) and one with a non-fecal coliform (*Pseudomonas aeruginosa*) using the following strains :
   - A sterile loop of ATCC strains, *E. coli* ATCC 25922 or 11775 and *Pseudomonas aeruginosa* ATCC 10145 or 27853
2. Follow the Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

[1] IDEXX P/A Comparator, catalog # WP104; Quanti-Tray Comparator # WQTC, or Quanti-Tray/2000 Comparator #WQT2KC
[2] Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 1998. Washington, DC.
[3] Quanti-Cult™ cultures - IDEXX catalog # WKIT-1001
[4] American Type Culture Collection 1-800-638-6597

*Colilert, Defined Substrate Technology, DST and Quanti-Tray are trademarks or registered trademarks of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries. Quanti-Cult is a trademark of Remel Inc.

Manufactured under one or more of the following U.S. patents: 5,610,029; 5,518,892; 5,620,895; and 5,753,456. Other U.S. and/or foreign patents issued or pending.

Kit d'analyse Colilert®-18

Introduction
Colilert-18 permet de détecter dans l'eau soit la présence de coliformes totaux et de bactéries *E. coli*, soit la présence de coliformes fécaux. Ce test est basé sur la technologie brevetée Defined Substrate Technology® (DST®) d'IDEXX. Lorsque les coliformes totaux ou fécaux métabolisent ONPG, le substrat chromogène-indicateur de Colilert-18, l'échantillon vire au jaune. Lorsque *E. coli* métabolise MUG, le substrat chromogène-indicateur de Colilert-18, le prélèvement devient fluorescent. Colilert-18 peut détecter simultanément ces bactéries à 1 cfu/100 ml en 18 heures, même en présence de bactéries hétérotrophes à une concentration de 2 millions par 100 ml.

Contenu
- WP200i-18 contient 20 Sachets hermétiques pour prélèvements de 100 ml
- WP200i-18 contient 200 Sachets hermétiques pour prélèvements de 100 ml

Conditions de conservation
Conserver entre 2–25°C à l'abri de la lumière.

Procédure de Présence/Absence (P/A)
1. Ajouter le contenu d'un sachet dans un prélèvement de 100 ml placé dans un récipient stérile, transparent et non fluorescent.
2. Fermer le récipient et agiter.
3. Si le prélèvement n'est pas déjà à 33–38°C, placer le récipient dans un bain-marie à 35°C pendant 20 minutes ou dans un bain-marie à 44,5°C pendant 7 à 10 minutes.
4. Incuber à 35°C ±0,5°C pendant les 18 heures qui suivent.
5. Interpréter les résultats en se référant au tableau d'interprétation des résultats ci-dessous.

Procédure de numération Quanti-Tray®
1. Ajouter le contenu d'un sachet dans un prélèvement de 100 ml d'eau à température ambiante placé dans un récipient stérile.
2. Fermer le récipient et agiter jusqu'à dissolution.
3. Verser le mélange prélèvement/réactif dans un Quanti-Tray ou un Quanti-Tray/2000 et fermer hermétiquement dans un dispositif hermétique Quanti-Tray IDEXX.
4. Placer le plateau hermétiquement fermé dans un incubateur à 35°C ±0,5°C (ou à 44,5°C ±0,2°C pour les coliformes fécaux) pendant 18heures (aucun réchauffage préalable à 35°C n'est requis). Si le plateau est incubé dans un bain-marie, immerger le Quanti-Tray à l'aide d'un anneau lesté.
5. Interpréter les résultats en se référant au tableau d'interprétation des résultats ci-dessous. Compter le nombre de puits positifs et se référer au tableau NPP fourni avec les plateaux Quanti-Tray pour obtenir le Nombre le plus probable (NPP).

Interprétation des résultats

Aspect	Résultat
Moins jaune que le comparateur[1] après une incubation à 35°C ± 0,5°C ou à 44,5°C ± 0,2°C	Négatif pour les coliformes totaux et *E. coli*, négatif pour les coliformes fécaux
Aussi jaune ou plus jaune que le comparateur après une incubation à 35°C ± 0,5°C	Positif pour les coliformes totaux
Aussi jaune ou plus jaune que le comparateur après une incubation à 44,5°C ± 0,2°C	*Positif pour les coliformes fécaux*
Couleur jaune et fluorescence égales ou supérieures au comparateur après une incubation à 35°C ± 0,5°C	Positif pour *E. coli*

- Évaluer la fluorescence avec une lampe UV de 6 watts et 365 nm placée à 13 cm de l'échantillon, dans un endroit obscur. Orienter la lumière vers l'échantillon en l'éloignant des yeux de l'opérateur.
- Les résultats de Colilert-18 doivent être lus entre 18 et 22 heures. Les échantillons positifs pour les coliformes totaux et *E. coli*, ou pour les coliformes fécaux observés avant 18 heures, de même que les résultats négatifs observés après 22 heures, sont également valides.

Remarques concernant la procédure
- Il est possible d'observer une légère coloration lorsque Colilert-18 est ajouté au prélèvement.
- Si l'excès de mousse pose des problèmes avec le Quanti-Tray, il est possible d'utiliser la solution antimousse d'IDEXX (réf n° WAFDB) ou les récipients IDEXX de 120 ml (réf. n° WV120SBAF-200).
- Cette notice peut ne pas refléter votre réglementation locale. Pour le test de conformité mis en œuvre dans le cadre du contrôle sanitaire, assurez-vous de suivre les procédures réglementaires appropriées. Par exemple, les tests réalisés en Europe doivent être incubés à 36 ±2°C pendant 18 à 22 heures.
- Lors de l'utilisation de la méthode testée que validée par AFNOR certification pour l'analyse de l'eau potable, placer le plateau hermétiquement fermé dans un incubateur à 36°C ± 2°C pendant 18 heures (aucun réchauffage préalable à 36°C n'est requis).
- Colilert-18 peut être effectué en tubes multiples. Utiliser les tableaux NPP des *Méthodes de référence pour l'analyse de l'eau et des eaux usées*[2] afin de déterminer les Nombres les plus probables (NPP).
- Si un prélèvement d'eau présente une couleur de fond, comparer le prélèvement inoculé avec Colilert-18 à un échantillon non inoculé du même prélèvement d'eau.
- Colilert-18 peut être utilisé pour détecter *E. coli* (mais non les coliformes) dans l'eau de mer. Les prélèvements doivent être dilués au moins au dixième. Multiplier la valeur NPP par le facteur de dilution pour obtenir le résultat quantitatif correspondant.
- Utiliser uniquement de l'eau stérile, non tamponnée et sans oxydant pour les dilutions.
- Colilert-18 est avant tout un test pour eau. Les caractéristiques de performance de Colilert-18 ne s'appliquent pas aux prélèvements modifiés par tout enrichissement préalable ou toute concentration.
- Avec les prélèvements présentant un excédent de chlore, il peut se produire une rapide lueur bleuâtre lors de l'ajout de Colilert-18. Si tel est le cas, le prélèvement n'est pas valide et il faut cesser le test.
- Il est recommandé de toujours utiliser des techniques aseptiques avec Colilert-18. À éliminer conformément aux Bonnes pratiques de laboratoire.

Procédures de contrôle de qualité
Il est recommandé d'utiliser la procédure de contrôle qualité suivante pour chaque lot de Colilert-18 utilisé pour tester la présence de coliformes totaux et *E. coli* à 35°C ± 0,5°C:
1. Remplir trois récipients stériles avec 100 ml d'eau stérile et inoculer l'un des récipients avec *E. coli*, un autre avec des coliformes totaux et le dernier avec une bactérie non coliforme en utilisant l'un des produits suivants:
   A. Quanti-Cult™[3] *E. coli, Klebsiella pneumoniae* et *Pseudomonas aeruginosa*
   B. Une anse stérile de souches ATCC® *E. coli* ATCC 25922 ou 11775, *Klebsiella pneumoniae* ATCC 31488 et *Pseudomonas aeruginosa* ATCC 10145 ou 27853
2. Suivre la procédure P/A ou la procédure de numération Quanti-Tray.
3. Les résultats doivent correspondre aux résultats du tableau d'interprétation ci-dessus.

Il est recommandé d'utiliser la procédure de contrôle qualité suivante pour chaque lot de Colilert-18 utilisé pour tester la présence de coliformes fécaux à 44,5°C ± 0,2°C :
1. Remplir deux récipients stériles avec 100 ml d'eau stérile et inoculer l'un des récipients avec *E. coli* et le second avec la bactérie non coliforme *Pseudomonas aeruginosa* en utilisant les produits suivants :
   •Une anse stérile de souches ATCC, *E. coli* ATCC 25922 ou 11775, et *Pseudomonas aeruginosa* ATCC 10145 ou 27853
2. Suivre la procédure de numération Quanti-Tray.
3. Les résultats doivent correspondre aux résultats du tableau d'interprétation ci-dessus.

[1] Comparateur P/A IDEXX, réf. n° WP104; Comparateur Quanti-Tray n° WQTC ou Quanti-Tray/2000 Comparateur n° WQT2KC
[2] Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 1998. Washington, DC.
[3] Cultures Quanti-Cult™ IDEXX réf. n° WKIT-1001
[4] American Type Culture Collection 1-800-638-6597

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Manufacturé sous un ou plusieurs du suivant brevets U.S. nos 5,610,029; 5,518,892; 5,620,895; et 5,753,456. Autres brevets américains et/ou étrangers enregistrés ou en cours d'enregistrement.

Kit di analisi Colilert®-18

Introduzione
Colilert-18 rileva simultaneamente i coliformi totali e *E. coli* o i coliformi fecali in acqua. Si basa su una tecnologia di substrato definito (Defined Substrate Technology) brevettata IDEXX® (DST®). Quando i coliformi totali o fecali metabolizzano il nutriente indicatore ONPG di Colilert-18, il campione diventa giallo. Quando *E. coli* metabolizza l'indicatore di nutrienti del Colilert-18, MUG, il campione diventa fluorescente. Il Colilert-18 è in grado di rilevare simultaneamente questi batteri in concentrazioni di 1 UFC/100 ml entro 18 ore anche se sono presenti addirittura 2 milioni di batteri eterotrofici per 100 ml.

Contenuto
- WP200i-18 contiene 20 pacchetti Snap per campioni da 100 ml
- WP200i-18 contiene 200 pacchetti Snap per campioni da 100 ml

Conservazione
Conservare a 2–25°C lontano dalla luce.

Procedura relativa a Presenza/Assenza (P/A)
1. Unire il contenuto di un pacchetto ad un campione da 100 ml in un a provetta sterile, trasparente e non fluorescente.
2. Incappucciare la provetta ed agitarla.
3. Se il campione non è già a 33–38°C, mettere la provetta a bagno maria a 35°C per 20 minuti oppure, alternativamente, a bagno maria a 44,5°C per 7–10 minuti.
4. Incubare a 35°C ±0,5°C per il resto delle 18 ore.
5. Leggere i risultati secondo la tabella di interpretazione dei risultati qui sotto.

Procedura di enumerazione Quanti-Tray®
1. Unire il contenuto di un pacchetto ad un campione di acqua da 100 ml a temperatura ambiente in una provetta sterile.
2. Chiudere la provetta e agitarla fino a dissoluzione.
3. Versare la miscela campione/reagente in un vassoietto Quanti-Tray o Quanti-Tray/2000 e sigillarlo in un Sigillatore Quanti-Tray IDEXX.
4. Posizionare il vassoietto sigillato in un'incubatrice a 35°C ± 0,5°C (o 44,5°C ±0,2°C per i coliformi fecali) per 18 ore (non si richiede il pre-riscaldamento a 35°C). Per l'incubazione in un bagno termostatato, immergere completamente il Quanti-Tray nell'acqua utilizzando un anello appesantito.
5. Leggere i risultati secondo la tabella di interpretazione dei risultati qui sotto. Contare il numero di gialli positivi e consultare la tabella MPN fornita insieme ai vassoietti per ottenere il numero più probabile.

Interpretazione dei risultati

Aspetto	Risultato
Colore giallo meno intenso del comparatore[1] quando viene incubato a 35°C ±0,5°C o a 44,5°C ±0,2°C	Negativo per coliformi totali ed *E. coli*; negativo per coliformi fecali
Colore giallo uguale o più intenso del comparatore quando viene incubato a 35°C ±0,5°C	Positivo per coliformi totali
Colore giallo uguale o più intenso del comparatore quando viene incubato a 44,5°C ±0,2°C	Positivo per coliformi fecali
Colore giallo e fluorescenza uguali o più intensi del comparatore quando viene incubato a 35°C ±0,5°C	Positivo per *E. coli*

- Individuare la fluorescenza con una luce a raggi ultravioletti da 6 watt, 365 nm, entro circa 13 cm dal campione in un ambiente non illuminato. Dirigere la luce verso il campione, in direzione opposta ai propri occhi.
- I risultati di Colilert-18 sono considerati definitivi dopo 18–22 ore. Inoltre, sono validi anche i risultati positivi sia per i coliformi totali che per *E. coli* o i coliformi fecali osservati prima di 18 ore e i risultati negativi osservati dopo 22 ore.

Note sulla procedura
- Una leggera colorazione si può osservare quando il Colilert-18 viene aggiunto al campione.
- Se la schiuma in eccesso causa problemi mentre si usa il Quanti-Tray, si può scegliere di usare la Soluzione antischiuma IDEXX (Codice catalogo WAFDB) o provetta IDEXX da 120 ml con antischiuma (Codice catalogo WV120SBAF-200).
- Questo inserto informativo potrebbe non riflettere le normative locali. Per i test sulla conformità, assicurarsi di seguire le procedure normative corrispondenti. Ad esempio, campioni effettuati in Europa dovrebbero essere incubati a 36 ±2°C per 18–22 ore.
- Quando viene eseguito il metodo validato da AFNOR per l'analisi dell'acqua potabile, mettere il vassoietto sigillato in un'incubatrice a 36°C ±2°C per 18 ore (non si richiede il pre-riscaldamento a 36°C).
- Il Colilert-18 si può eseguire in qualsiasi formato a provetta multipla. I metodi standard[2] per l'esame delle tabelle MPN dell'acqua e delle acque di scarico vanno usati per ottenere i numeri più probabili (MPN).
- Se un campione di acqua dovesse presentare della colorazione di sfondo, confrontare il campione Colilert-18 inoculato con controllo vuoto dello stesso campione di acqua.
- Il Colilert-18 può essere usato per *E. coli* (ma non per i coliformi) in acqua di mare. I campioni devono essere diluiti almeno dieci volte. Moltiplicare il valore MPN per il fattore di diluizione per ottenere il risultato quantitativo adeguato.
- Per le diluizioni usare solo acqua sterile, non tamponata, priva di ossidanti.
- Il Colilert-18 è un test primario per l'acqua. Le caratteristiche di prestazione del Colilert-18 non sono applicabili a campioni alterati da qualsiasi pre-arricchimento o da concentrazione.
- In campioni con cloro eccessivo, quando si aggiunge il Colilert-18 si potrebbe vedere un lampo azzurro. In questo caso, considerare il campione non valido e interrompere l'analisi.
- Quando si usa il Colilert-18 va sempre seguita la tecnica asettica. Eliminare secondo le buone pratiche di laboratorio.

Procedure di controllo della qualità
La seguente procedura di controllo di qualità deve essere eseguita su ogni lotto di Colilert-18 quando viene utilizzata per la misurazione dei coliformi totali e di *E. Coli* a 35°C ± 0,5°C:
1. Riempire tre provette sterili con 100 ml di acqua sterile e inoculame una con *E. coli*, una con i coliformi totali e una con un microrganismo diverso dai coliformi utilizzando uno dei seguenti prodotti:
   A. Quanti-Cult™[3] *E. coli, Klebsiella pneumoniae* e *Pseudomonas aeruginosa*
   B. Un'ansa sterile con ceppi ATCC® *E. coli* ATCC 25922 o 11775, *Klebsiella pneumoniae* ATCC 31488 e *Pseudomonas aeruginosa* ATCC 10145 o 27853
2. Seguire la procedura P/A o la procedura di enumerazione Quanti-Tray sopra riportata.
3. I risultati devono corrispondere a quelli della tabella di interpretazione dei risultati indicata sopra.

La seguente procedura di controllo di qualità deve essere eseguita su ogni lotto di Colilert-18 quando viene utilizzata per la misurazione dei coliformi fecali a 44,5°C ±0,2°C:
1. Riempire due provette sterili con 100 ml di acqua sterile e inoculame una con un coliforme fecale (*E. coli*) e una con un coliforme non fecale (*Pseudomonas aeruginosa*) utilizzando i seguenti ceppi:
   •Un'ansa sterile con ceppi ATCC, *E. coli* ATCC 25922 o 11775 e *Pseudomonas aeruginosa* ATCC 10145 o 27853
2. Seguire la procedura di enumerazione Quanti-Tray sopra riportata.
3. I risultati devono corrispondere alla tabella di interpretazione dei risultati indicata sopra.

[1] Comparatore P/A IDEXX, codice catalogo WP104; Comparatore Quanti-Tray N. WQTC o Quanti-Tray/2000 Comparatore N. WQT2KC
[2] Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 1998. Washington, DC.
[3] Ceppi Quanti-Cult™ N. di catalogo IDEXX WKIT-1001
[4] American Type Culture Collection 1-800-638-6597

*Colilert, Defined Substrate Technology, DST e Quanti-Tray sono marchi commerciali o marchi depositati della IDEXX Laboratories, Inc. negli Stati Uniti e/o in altri Paesi. Quanti-Cult è un marchio commerciale della Remel Inc.

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Colilert®-18 Testkit

Einführung
Colilert-18 ist zum gleichzeitigen Nachweis von Gesamtcoliformen und *E. coli* oder Fäkalcoliformen in Wasser bestimmt. Es basiert auf der patentierten Defined Substrate Technology® (DST®) von IDEXX. Bei der Metabolisierung von ONPG, des Nährstoffindikators von Colilert-18, durch die Gesamtcoliformen oder Fäkalcoliformen verfärbt sich die Probe gelb. Bei der Metabolisierung des Nährstoffindikators MUG durch *E.coli* fluoresziert die Probe. Colilert-18 kann diese Bakterien gleichzeitig im Bereich von 1 CRU/100 ml innerhalb von 18 Stunden nachweisen, selbst wenn 2 Mio. heterotrophe Bakterien pro 100 ml vorhanden sind.

Inhalt
- WP200i-18 enthält 20 Snap Packs für 100 ml Proben
- WP200i-18 enthält 200 Snap Packs für 100 ml Proben

Lagerung
Bei 2–25°C und nicht im Licht lagern.

Presence/Absence (P/A)-Test
1. Den Inhalt einer Packung zu einer 100 ml Probe in einem sterilen, transparenten, nicht fluoreszierenden Gefäß hinzugeben.
2. Das Gefäß verschließen und schütteln.
3. Wenn die Probe noch nicht im Temperaturbereich von 33–38°C ist, das Gefäß 20 Minuten in ein Wasserbad von 35°C oder alternativ 7–10 Minuten lang in ein Wasserbad von 44,5°C stellen.
4. Für den verbleibenden 18-Stunden-Zeitraum bei 35°C ±0,5°C inkubieren.
5. Die Ergebnisse gemäß der nachstehenden Ergebnisauswerte-Tabelle ablesen.

Quanti-Tray® Auszähl-Methode
1. Den Inhalt einer Packung zu einer 100 ml Wasserprobe mit Zimmertemperatur in einem sterilen Gefäß hinzugeben.
2. Das Gefäß verschließen und so lange schütteln, bis der Inhalt aufgelöst ist.
3. Die aus Probe und Reagenz bestehende Mischung in ein Quanti-Tray oder Quanti-Tray/2000 gießen und in einem IDEXX Quanti-Tray Sealer fest verschließen.
4. Den versiegelten Quanti-Tray bei 35°C ±0,5°C (oder bei -Fäkalcoliformen bei 44,5°C ±0,2°C) 18 Stunden inkubieren (ein Vorwärmen auf 35°C ist nicht erforderlich). Sollte die Inkubation des Quanti-Tray im Wasserbad erfolgen, den Tray so beschweren, dass er vollständig von Wasser bedeckt ist.
5. Die Ergebnisse anhand der nachstehenden Ergebnisauswerte-Tabelle ablesen. Die Anzahl der positiven Vertiefungen zählen und die wahrscheinlichste Zahl (MPN; Most Probable Number) anhand der MPN-Tabelle, die den Trays beiliegt, ermitteln.

Ergebnisauswertung

Aussehen der Probe	Mögliche Ergebnisse
Geringere Gelbtärbung als der Comparator[1] bei Inkubation bei 35°C ±0,5°C oder bei 44,5°C ±0,2°C	Negativ für Gesamtcoliforme und *E. coli*; negativ für fäkale Coliforme
Gleiche oder stärkere Gelbtärbung als der Comparator[1] bei Inkubation bei 35°C ±0,5°C	Positiv für Gesamtcoliforme
Gleiche oder stärkere Gelbtärbung als der Comparator[1] bei Inkubation bei 44,5°C ±0,2°C	Positiv für Fäkalcoliforme
Gleiche oder stärkere Gelbtärbung und Fluoreszenz als der Comparator[1] bei Inkubation bei 35°C ±0,5°C	Positiv für *E. coli*

- Die Anwesenheit von Fluoreszenz wird mit einer UV-Lampe von 6 Watt bei einer Wellenlänge von 365 nm im Dunkeln in einem Höchstabstand von 12 cm von der probe geprüft. Dabei muß das UV-Licht von den Augen des Anwenders weg- und zur Probe gerichtet sein.
- Colilert-18 zeigt nach 18–22 Stunden ein definitives Ergebnis. Außerdem sind positive Ergebnisse für Gesamtcoliforme und *E. coli* oder Fäkalcoliforme, die vor dem Ablauf von 18 Stunden beobachtet werden und negative Ergebnisse, die nach 22 Stunden beobachtet werden, ebenfalls gültig.

Verfahrenshinweise
- Beim Hinzugeben von Colilert-18 zur Probe kann eine leichte Färbung beobachtet werden.
- Wenn die Verwendung des Quanti-Tray durch übermäßige Schaumentwicklung erschwert wird, kann die IDEXX Antifoam Solution (Antischaum-Lösung; Best.-Nr. WAFDB) oder IDEXX 120 ml Gefäße mit Antifoam (Best.-Nr. WV120SBAF-200) verwendet werden.
- Diese Packungsbeilage gibt möglicherweise nicht Ihre örtlichen Vorschriften wieder. Bei den Qualitätstests sind die anwendbaren aufsichtsbehördlichen Verfahren zu befolgen. Proben aus Europa bspw. Sollten bei 36°C ±2°C für 18-22 hours inkubiert werden.
- Bei Durchführung eines vom AFNOR validierten Verfahrens zur Untersuchung von Trinkwasser das verschlossene Tray 18 Stunden in den Inkubator stellen und im Temperaturbereich von 36°C ±2°C inkubieren (Vorwärmen auf 36°C ist nicht erforderlich).
- Das Colilert-18 Verfahren kann in jedem Multiple-Tube-Format durchgeführt werden. Zur Ermittlung der MPNs (wahrscheinlichste Zahlen) sollten MPN-Tabellen für Standardverfahren[2] zur Untersuchung von Wasser und Abwasser verwendet werden.
- Wenn eine Wasserprobe etwas Hintergrundfarbe aufweist, ist die inokulierte Colilert-18 Probe mit einer Kontrollprobe derselben Wasserprobe zu vergleichen.
- Colilert-18 kann für *E. coli* (jedoch nicht Coliforme) in Meereswasser verwendet werden. Die Proben müssen mindestens zehnfach verdünnt werden. Der MPN-Wert ist mit dem Verdünnungsfaktor zu multiplizieren, um das korrekte quantitative Ergebnis zu erhalten.
- Nur steriles, nicht gepuffertes, keine Oxidantien enthaltendes Wasser zur Verdünnung verwenden.
- Colilert-18 ist ein primärer Wassertest. Die Leistungsmerkmale von Colilert-18 gelten nicht für Proben, die durch Voranreicherung oder Konzentration modifiziert wurden.
- In Proben mit übermäßigem Chlorgehalt wird bei der Zugabe von Colilert-18 u.U. ein blaues Aufleuchten beobachtet. In diesem Fall ist die Probe als ungültig zu betrachten und der Test abzubrechen.
- Bei der Verwendung von Colilert-18 ist ein aseptisches Vorgehen vorgeschrieben. Entsorgung gemäß Standard-Laborpraktiken.

Qualitätskontrollverfahren
Das folgende Qualitätskontrollverfahren wird für jede Colilert-18-Charge empfohlen, wenn Colilert-18 zum Test auf Gesamtcoliforme und *E. coli* bei 35°C ±0,5°C verwendet werden soll:
1. Drei sterile Gefäße mit 100 ml sterilem Wasser füllen und eines mit *E. coli*, eines mit einem Gesamtcoliformund eines mit einem Nichtcoliform inokulieren; dabei eines der folgenden Produkte verwenden:
   A. Quanti-Cult™[3] *E. coli, Klebsiella pneumoniae* und *Pseudomonas aeruginosa*
   B. Eine sterile Öse mit ATCC®-Stämmen, *E. coli* ATCC 25922 oder 11775, *Klebsiella pneumoniae*, ATCC 31488, und *Pseudomonas aeruginosa*, ATCC 10145 oder 27853
2. Das oben beschriebene P/A-Verfahren oder das Quanti-Tray Auszählverfahren befolgen.
3. Die Ergebnisse sollten mit der Ergebnisauswerte-Tabelle oben übereinstimmen.

Das folgende Qualitätskontrollverfahren wird für jede Colilert-18-Charge empfohlen, wenn Colilert-18 zum Test auf Fäkalcoliforme bei 44,5°C ±0,2°C verwendet werden soll:
1. Zwei sterile Gefäße mit 100 ml sterilem Wasser füllen und ein Gefäß mit einem fäkalen coliformen Bakterium (*E. coli*) und das andere mit einem nicht-fäkalen coliformen Bakterium (*Pseudomonas aeruginosa*) unter Verwendung folgender Stämme inokulieren:
   •Eine sterile Öse mit ATCC Stämmen, *E. coli* ATCC 25922 oder 11775 und *Pseudomonas aeruginosa* ATCC 10145 oder 27853
2. Das oben geschilderte Quanti-Tray Auszählverfahren befolgen.
3. Die Ergebnisse müssen mit der Auswertungstabelle oben übereinstimmen.

[1] IDEXX P/A Comparator, Best.-Nr. WP104; Quanti-Tray Comparator WQTC oder Quanti-Tray/2000 Comparator WQT2KC
[2] Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 1998. Washington, DC
[3] Quanti-Cult™ Kulturen - IDEXX Best.-Nr. WKIT-1001
[4] American Type Culture Collection 1-800-638-6597

*Colilert, Defined Substrate Technology, DST und Quanti-Tray sind Warenzeichen oder eingetragene Warenzeichen von IDEXX Laboratories, Inc. in den Vereinigten Staaten und/oder anderen Ländern. Quanti-Cult ist ein Warenzeichen von Remel Inc.

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Colilert®-18 Kit para Prueba

Introducción

Colilert-18 detecta de manera simultánea los coliformes totales y *E. coli* o coliformes fecales en el agua. Se basa en la Defined Substrate Technology® (Tecnología de sustrato definido [DST®]), patentada por IDEXX. Cuando los coliformes totales o los coliformes fecales metabolizan el indicador de nutrientes de Colilert-18, la muestra se vuelve de color amarillo. Cuando *E. coli* metaboliza el indicador MUG de nutrientes de Colilert-18, la muestra fluoresce. Colilert-18 puede detectar simultáneamente estas bacterias a una concentración de 1 ufc/100 ml dentro de las 18 horas, hasta en presencia de 2 millones de bacterias heterotróficas por cada 100 ml

Índice

- WP020I-18 contiene 20 paquetes Snap para muestras de 100 ml
- WP200I-18 contiene 200 paquetes Snap para muestras de 100 ml

Almacenamiento

Almacenar a temperatura de 2–25°C, alejado de la luz.

Procedimiento de presencia/ausencia (P/A)

1. Añadir el contenido de un paquete a una muestra de 100 ml, en un recipiente estéril transparente, no fluorescente.
2. Tapar y agitar el recipiente.
3. Si la muestra no está ya a temperatura de 33–38°C, colocar el recipiente en un baño de agua a 35°C durante 20 minutos o, como alternativa, en un baño de agua a 44,5°C durante 7 a 10 minutos.
4. Incubar a 35°C ±0,5°C durante el resto de las 18 horas.
5. Leer los resultados de acuerdo con el cuadro de interpretación de resultados, más abajo.

Procedimiento de enumeración Quanti-Tray®

1. Añadir el contenido de un paquete a una muestra de 100 ml de agua a temperatura ambiente, en un recipiente estéril.
2. Tapar y agitar el recipiente hasta disolver.
3. Verter la reacción de muestra/reactivo en una Quanti-Tray o una Quanti-Tray/2000 y sellar en un sellador de Quanti-Tray de IDEXX.
4. Colocar la bandeja sellada en una estufa de incubación a 35°C ±0,5°C (o a 44,5°C ±0,2°C para los coliformes totales) durante 18 horas (no se necesita precalentar a 35°C). Si se incuba en un baño termostático, sumergir la Quanti-Tray con la ayuda de un anillo pesado, hasta situarla por debajo del nivel del agua.
5. Leer los resultados de acuerdo con el cuadro de interpretación de resultados, más abajo. Contar el número de pocillos positivos y referirse al cuadro NPP proporcionado con las bandejas para obtener el número más probable.

Interpretación de resultados

Aspecto	Resultado
Menos amarillo que el comparador[1] cuando se incuba a 35°C ±0,5°C o a 44,5°C ±0,2°C	Negativo para coliformes totales y *E. coli*; negativo para coliformes fecales
Amarillo igual o mayor que el del comparador[1] cuando se incuba a 35°C ±0,5°C	Positivo para coliformes totales
Amarillo igual o mayor que el del comparador[1] cuando se incuba a 44,5°C ±0,2°C	Positivo para coliformes fecales
Amarillo y fluorescencia iguales o mayores que los del comparador[2] cuando se incuba a 35°C ±0,5°C	Positivo para *E. coli*

- Buscar fluorescencia usando una luz UV de 6 vatios, 365 nm a distancia de unos 13 cm de la muestra en un ambiente oscuro. Apuntar el haz de luz en dirección contraria a los ojos y hacia la muestra.
- Los resultados Colilert-18 son definitivos después de 18-22 horas. Además, los positivos para coliformes totales y para *E. coli* o coliformes fecales observados antes de las 18 horas y los negativos observados después de las 22 horas también son válidos.

Notas sobre el procedimiento

- Cuando se agrega Colilert-18 a la muestra es posible que se observe una tinción leve.
- Si el exceso de espuma causa problemas mientras se usa Quanti-Tray, se puede decidir utilizar solución antiespumante IDEXX (Nº de catálogo WAFDB) o recipientes de 120 ml IDEXX con antiespumante (Nº de catálogo WV120SBAF-200).
- Este prospecto tal vez no refleje sus reglamentaciones locales. Para probar el cumplimiento, asegurarse de seguir los procedimientos reglamentarios apropiados. Por ejemplo, muestras procesadas en Europa deben ser incubadas a 36 ±2°C por 18-22 horas.
- En caso de utilizar el método validado por la AFNOR para el análisis de agua potable, colocar la bandeja sellada en una estufa de incubación a 36°C ±2°C durante 18 horas (no se necesita precalentar a 36°C)
- Colilert-18 puede procesarse en cualquier formato de múltiples tubos. Deben usarse los Métodos estándares[3] para examen del agua y los cuadros NPP de aguas residuales para encontrar los números más probables (NPP).
- Si la muestra de agua tiene un cierto color de fondo, comparar la muestra inoculada de Colilert-18 con un blanco testigo de la misma muestra de agua.
- Colilert-18 puede usarse para *E. coli* (pero no para coliformes) en aguas marinas. Las muestras deben diluirse al menos diez veces. Multiplicar el valor NPP por el factor de dilución para obtener el resultado cuantitativo apropiado.
- Usar solamente agua estéril, no tamponada, libre de oxidantes, para efectuar las diluciones.
- Colilert-18 es una prueba primaria del agua. Las características de rendimiento de Colilert-18 no se aplican a muestras alteradas por enriquecimiento o concentración previos.
- En el caso de muestras con un exceso de cloro, tal vez se observe un destello azul al añadir Colilert-18. Si se observa, considerar que la muestra no es válida y suspender la prueba.
- Siempre debe utilizarse una técnica aséptica cuando se use Colilert-18. Desechar en cumplimiento con las Buenas Prácticas de Laboratorio.

Procedimientos de control de calidad

Se recomienda el siguiente procedimiento de control de calidad para cada lote de Colilert-18 cuando se use para analizar coliformes totales y *E. coli* a 35°C ±0,5°C:

1. Limpar tres recipientes estériles con 100 ml de agua estéril e inocular en uno de ellos *E. coli*, en otro coliformes totales y en otro no coliformes usando uno de los siguientes productos:
   A. Quanti-Cult®[4] *E. coli*, *Klebsiella pneumoniae* y *Pseudomonas aeruginosa*
   B. Una asa estéril con cepas ATCC, *E. coli* ATCC 25922 o 11775, *Klebsiella pneumoniae* ATCC 31488 y *Pseudomonas aeruginosa* ATCC 10145 o 27853
2. Seguir el procedimiento P/A o el procedimiento de enumeración Quanti-Tray mencionado anteriormente.
3. Los resultados deben corresponder a los del Cuadro de Interpretación de resultados, más arriba.

Se recomienda el siguiente procedimiento de control de calidad para cada lote de Colilert-18 cuando se use para analizar coliformes fecales a 44,5°C ±0,2°C:

1. Limpar dos recipientes estériles con 100 ml de agua estéril e inocular en uno de ellos un coliforme fecal (*E. coli*) y en el otro un coliforme no fecal (*Pseudomonas aeruginosa*) usando los siguientes productos:
   •Un asa estéril con cepas ATCC, *E. coli* ATCC 25922 o 11775 y *Pseudomonas aeruginosa* ATCC 10145 o 27853
2. Seguir el procedimiento de enumeración Quanti-Tray mencionado anteriormente.
3. Los resultados deben corresponder a los del cuadro de interpretación de resultados, más arriba.

[1] IDEXX, Comparador P/A Nº de catálogo WP104; Comparador Quanti-Tray Nº WQTC o Quanti-Tray/2000 Comparador Nº WQT2KC
[2] Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 1998, Washington, DC.
[3] Cultivos Quanti-Cult®, Nº de catálogo IDEXX WM11-001
[4] American Type Culture Collection 1-800-638-6597

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Fabricado bajo una o más de las patentes siguientes de los E.U.: 5,610,029; 5,518,892; 5,620,895; y 5,753,456. Otras patentes norteamericanas y/o extranjeras concedidas o pendientes de concesión.

Colilert®-18 コリラート18 テストキット

はじめに

コリラート18は水中の大腸菌群と大腸菌または糞便性大腸菌群を同時に検出します。検出方法はIDEXXが特許を取得したDefined Substrate Technology® (DST®)（特定酵素基質法）に基づいています。大腸菌群や糞便性大腸菌群がコリラート18の発色酵素基質ONPGを代謝すると、検体は黄変します。大腸菌がコリラート18の発色酵素基質MUGを代謝すると、検体は蛍光を発します。コリラート18は100ml当たり最大200万の従属栄養菌が存在したとしても、18時間以内に、1cfu/100mlの感度で大腸菌群および大腸菌を同時に検出することができます。

内容

- WP020I-18 は、検体100ml用のスナップパック20個入りです
- WP200I-18 は、検体100ml用のスナップパック200個入りです

保管

直射日光を避け、2～25°Cで保管してください。

定性法(P/A)の手順

1. 1パックの中身を、滅菌済みの透明な蛍光を発しない容器の中に入った100mlの検体に加えてください。
2. 容器の蓋を締め、ゆっくり振ってください。
3. 検体がこの時点で 33～38°Cでない場合、35°Cの恒温槽に20分、または、44.5°Cの恒温槽に7～10分間置いてください。
4. 35°Cで、18時間培養してください。
5. 以下の結果判定表に従って、結果判定してください。

Quanti-Tray®／QTトレイの計算手順

1. 1パックの中身を、滅菌容器の中に入った室温の検水100mlに加えてください。
2. 容器の蓋を締め溶けるまで静かに振ってください。
3. QTトレイまたはQTトレイ/2000に検水/コリラート18混合を注ぎ、シーラーで密封してください。
4. 密封されたトレイを 36°C±0.5°C（糞便性大腸菌群の場合は44.5°C±0.2°C）の培養器の中に18時間置いてください（前もって35°Cにする必要はありません）。ウォーターバスで培養する場合、QTトレイを重りリングを使って沈めてください。
5. 以下の結果判定表に従って、結果を判定してください。陽性ウェルの数を数え、専用MPN表を参照して、最確数を求めてください。

結果判定

培養後の状態	結果
36°C±0.5°Cまたは44.5°C±0.2°Cで培養した場合、比色管より薄い黄色	大腸菌群と大腸菌共に陰性
36°C±0.5°Cで培養した場合、比色管と同等かそれより濃い黄色	大腸菌群陽性
44.5°C±0.2°Cで培養した場合、比色管と同等かそれより濃い黄色	糞便性大腸菌群陽性
36°C±0.5°Cで培養した場合、比色管と同等かそれより濃い黄色および蛍光	大腸菌陽性

- 暗所で、検体の5インチ（12.7cm）以内で6W-365 nmのUVランプを使用して、判定してください。光は、目に向けないようにし、検体に向けてください。
- コリラート18の結果は、18～22時間で判定してください。また、培養18時間以内で大腸菌群および大腸菌が共に陽性となった場合、陽性判定が有効となり、培養を22時間以上行った場合に共に陽性となった場合は、陽性判定が有効です。

操作上の注意

- コリラート18を検体に加えた時、かすかな色が見られる場合があります。
- QTトレイを使用中に、泡が問題になる場合は、IDEXX 消泡液(カタログ# WAFDB)、または消泡剤入りのIDEXX 120 ml容器(カタログ# WV120SBAF-200)の使用を選択できます。
- 全ての国や地方の法律・条例に適合していないこともあります。法律・条例に準拠したテストをするために、適切な規定の手順に必ず従ってください。例）ヨーロッパでは、培養は36 ±2°Cで18～22時間と決められています。
- AFNORが確認した飲料水検査方法に従う場合は、密封されたトレイを 36°C±2°C の培養器の中に18時間置いてください（前もって36°Cにする必要はありません）。
- コリラート18は、5本法などの最確数法でも実施できます。*MPN*表は、最確数 *MPN*を求めるために使用してください。
- 検水に何らかの着色がある場合、同じ検水を用いたブランクと比較してください。[1]
- コリラート18は、海水中の大腸菌に使用可能です(大腸菌群を除く)。検体を10倍以上に希釈してください。 MPN値に希釈倍数を掛けて、適切な定量結果を求めてください。
- 希釈には、緩衝液や微生物質の入っていない、滅菌された水だけを使用してください。
- コリラート18は、水の一次検査です。コリラート18の性能特性として、増菌増殖で培養または遺伝によって変異した検体に適用できません。
- コリラート18を加えるとき、過剰の塩素がある検体で、青色を呈する場合があります。これが見られる場合、検体はテストに適さないので、テストを中止してください。
- コリラート18を使用する際は、常に無菌操作を行ってください。GLPに従って、廃棄してください。

品質管理手順

36±0.5°Cでの大腸菌群および大腸菌の検査に用いる場合、以下の品質管理は、コリラート18の各ロットについて行うことをお薦めします：

1. 滅菌容器3本にそれぞれ滅菌水100mLを入れ、下記の製品のどれか一つを使用して1本目は大腸菌、2本目は大腸菌群、3本目は非大腸菌群で接種してください。
   A. Quanti-Cult®[4]大腸菌、*Klebsiella pneumoniae*、*Pseudomonas aeruginosa*
   B. ATCC[4]菌株の滅菌ループ、大腸菌ATCC 25922または11775、*Klebsiella pneumoniae* ATCC 31488、*Pseudomonas aeruginosa* ATCC 10145 または 27853
2. 上記の定性法(P/A)手順またはQTトレイ定量法手順に従ってください。
3. 結果は上の結果解釈表と一致するはずです。

44.5°C±0.2°Cでの糞便性大腸菌の検査に用いる場合、以下の品質管理は、コリラート18の各ロットについて行うことをお薦めします：

1. 滅菌容器2本にそれぞれ滅菌水100mLを入れ、下記の菌株を使用して1本目は糞便性大腸菌群（大腸菌）、2本目は非糞便性大腸菌群（*Pseudomonas aeruginosa*）で接種してください。
   •ATCC菌株の滅菌ループ、大腸菌ATCC25922または11775、*Pseudomonas aeruginosa* ATCC 10145または27853
2. 上記のQTトレイ定量法手順に従ってください。
3. 結果は上の結果解釈表と一致するはずです。

[1] IDEXX P/A 比色管, カタログ # WP104 ; QTトレイ比色カレイ #WQTC, または QTトレイ/2000比色トレイ#WQT2KC
[2] Eaton AD, Clesceri LS, Greenberg AE. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 1998, Washington, DC
[3] Quanti-Cult® - 培地カタログ # WM11-001
[4] American Type Culture Collection 1-800-638-6597

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以下の米国特許の1つまたはそれ以上のもとに製造されています: 5,610,029; 5,518,892; 5,620,895および5,753,456。他の米国及び/またはは外国で発行された特許または、出願中の特許。

Colilert®-18

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ALTERNATIVE METHOD OF ANALYSIS
Certified by AFNOR Certification

AFNOR Validation

The AFNOR VALIDATION mark is granted to IDEXX for the following method: Colilert®-18/Quanti-Tray® for the quantification of *E. coli* and coliforms in drinking water.

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Appendix D: Description of Sampling Skids

Triton NP 6007 TG 18 ballast water sampling system

The Triton NP 6007 TG 18 ballast water sampling system has a modular design and consists in minimum of five major elements: a diaphragm valve, a filter housing equipped with filter cartouche and a transparent filter cup, an isokinetic bypass (elbow), a volume count and a ball valve. The material of all elements fulfil international quality requirements for pipe installations in the food industry and in the clinical sector. With the installation of a diaphragm valve at the intake and a ball valve at the outlet the Triton NP 6007 TG 18 sampling system fulfils the IMO Guidelines for Ballast Water Sampling (cf. IMO document MEPC.173(58), October 10th, 2008)

In its minimal composition the footprint of the Triton NP 6007 TG 18 sampling system is 40x40x45cm.

The Triton NP 6007 TG 18 can be operated as an open sampling system (i.e. the waste water is collected in a container or is directed into the bilge of the ship) or as a closed sampling system (i.e. the waste water is re-directed into the ballast water main pipe). With the NP 6007 TG 18 two different types of ballast water samples can be generated: (i) frequent grap samples and (ii) drip samples.

Connected to a sampling port the diaphragm valve of the NP 6007 TG 18 allows for the regulation of the flow volume through the NP 6007 TG 18 and with shutting both valves, the diaphragm valve and the ball valve, the NP 6007 TG 18 is completely cut off from the ballast water main pipe.

The ballast water diverted from the main pipe enters the transparent filter housing and passes through a filter cartouche with a filter material that has been developed especially for the sampling of plankton organisms ≥ 50µm from ballast water onboard ships. Having passed the filter housing the water is directed through an isokinetic elbow, which, equipped with a small ball valve, allows for the sampling of ballast water for the analysis of plankton organisms <50µm and of bacteria, too. After the isokinetic elbow the waste water finally passes a digital volume count, which allows for the monitoring of flow volume, total volume and flow velocity.

Depending on the flow velocity in the ballast water main pipe and the flow volume through the NP 6007 TG 18 the time for sampling of 1,0m³ of ballast water can be easily calculated.

At the start of the sampling procedure both valves, the diaphragm valve and the ball valve, are fully opened and the flow volume through the NP 6007 TG 18 is adjusted with the diaphragm valve to a value which ensures that the sensitive plankton organisms ≥50µm are not impacted by the filtration process itself. While the ballast water passes through the NP 6007 TG 18 additional samples can be taken through the small ball valve of the isokinetic elbow for the analysis of plankton organisms <50µm and bacteria.

After the termination of the sampling process both valves are closed. To retrieve the plankton organisms retained in the filter cartouche the transparent cup of the filter housing is screwed off. The filter cartouche can easily be removed from the filter head by a half turn of the cartouche. Since the filter cartouche is closed with a funnel and a small ball valve at its lower

end, the plankton organisms trapped inside the filter cartouche can easily be rinsed into a beaker for further sample processing and analysis.

Figure D.1: Triton NP 6007 TG 18 skid: basic set of elements

Figure D.2: Triton NP 6007 TG 18 skid: installations during Meteor cruise

Figure D.3: Triton NP 6007 TG 18 skid: installation during hydraulic tests

SGS BWS 1

1 THE SGS BWS1

The SGS Ballast Water Sampler (SGS BWS1) has a footprint of 45x25 cm and weighs 6.5 kg. Figure 1 shows the SGS BWS1 with the sampling pipe, filter housing containing the steel filter, two pressure gauges to monitor differential pressure across the filter, bypass sampling port and the volume count at the discharge connection. In certain configurations the discharge connection will also be equipped with an L-shaped pipe.

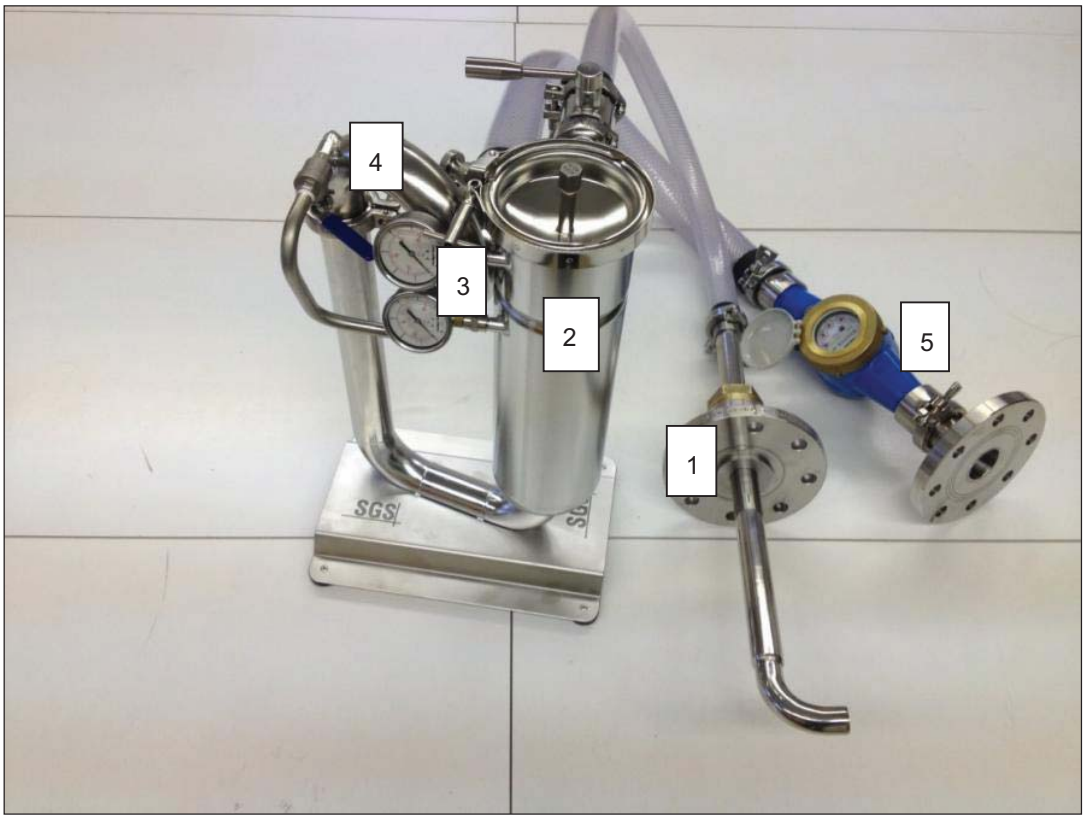

Figure 1: The SGS Ballast Water Sampler v02. The parts are numbered in the order that the water passes through the sampler. 1: Sampling pipe. 2: Filter housing. 3: Pressure gauges. 4: Bypass sampling port. 5: Volume count.

The ballast water enters the sampling system via the sampling pipe and is directed into the large filterhousing, in which the plankton organisms ≥50µm are retained. The water then leaves the filterhousing through a straight pipe section, in which a small isokinetic bypass sampling port (figure 2) allows for the sampling of ballast water for analysis of plankton organisms ≥10µm<50µm and bacteria.

Figure 2: The isokinetic bypass sampling port, dismantled (left) and installed (right)

The different parts, like hoses, volume count and sampling pipes are connected by a system of clamps (figure 3), which allows for easy dismantling and cleaning.

Figure 3: The connecting system and additional ball valves

The flange which fixes the sampling pipe into the ship's main ballast water pipe can is of DN100 size, in line with the ISO 11711-1:2013 standard for ballast water sampling connections and the recommendations by the USCG for modifying this standard (USNRL Report 3900 6130/1550). Figure 4 shows the parts of the sampling pipe assembly; figure 5 shows the completed assembly.

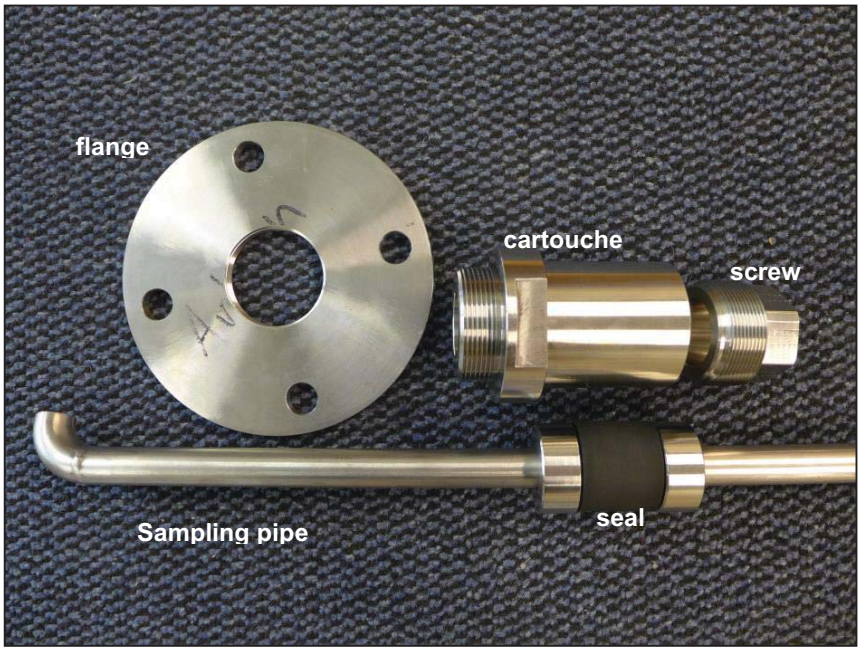

Figure 4: The components of the sampling pipe. The sampling pipe is mounted inside the cartouche and fastened in place with the screw. The seal prevents leaking. This assembly is then screwed into the flange so it can be attached to the piping on-board.

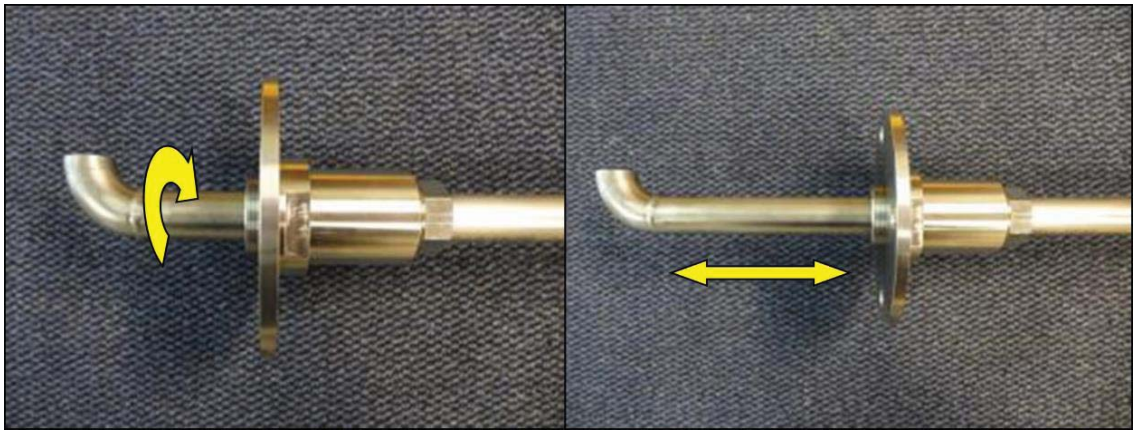

Figure 5: The complete sampling pipe assembly, arrows indicate the possibilities to adjust the position of the sampling pipe

Once the flange with the cartouche and the sampling pipe are fixed to the main ballast water pipe, the position of the sampling pipe inside the main ballast water pipe can easily be adjusted. Figure 5 presents the mounted cartouche with yellow arrows indicating the possibilities to adjust the sampling pipe inside the main ballast water pipe, i.e. the opening of the sampling pipe inside the main ballast water pipe is positioned concentrically and faces upstream.

Attaching the SGS BWS1 directly to a flange on the ballast water discharge pipe is only possible if the pipe is empty. Since this is often not the case on ships, the sampling pipes are designed to be able to fit through a ball valve (figure 6). The flange with the sampling pipe can be attached while the ball valve is closed. When the ball valve is opened the sampling pipe can then be inserted into the ballast water pipe. Using this configuration the sampling pipe can be inserted without emptying the ballast water discharge line.

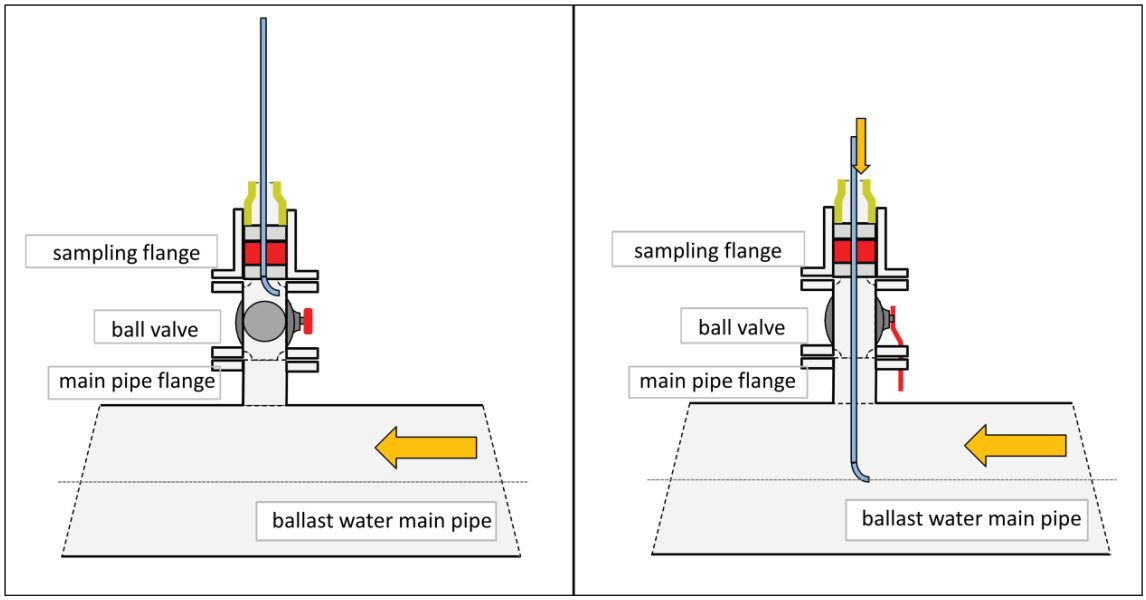

Figure 6: Installation of the isokinetic pipe into main ballast water pipes on-board ships without emptying the main ballast water pipe

The SGS BWS1 is equipped with a steel filter screen with laser-perforations of 50 µm (figure 7 and 8). In addition to being extremely sturdy, it also has a very smooth surface which is very suitable for washing particles and organisms down to the collection tap. Cleaning of the filter is also a fast and easy process.

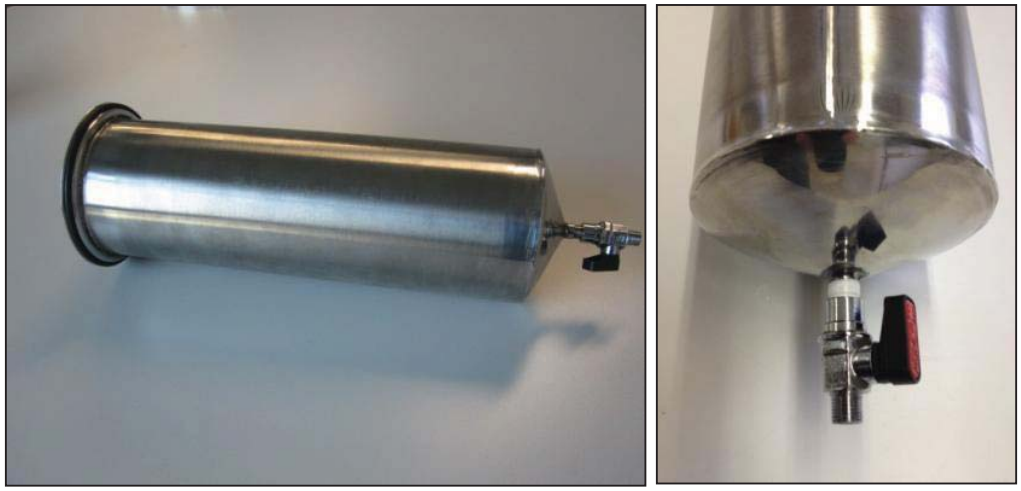

Figure 7: The stainless steel screen filter inlet of the SGS BWS1, the rubber ring at the top prevents water and organisms from bypassing the filter. The valve at the bottom allows easy collection of the sample.

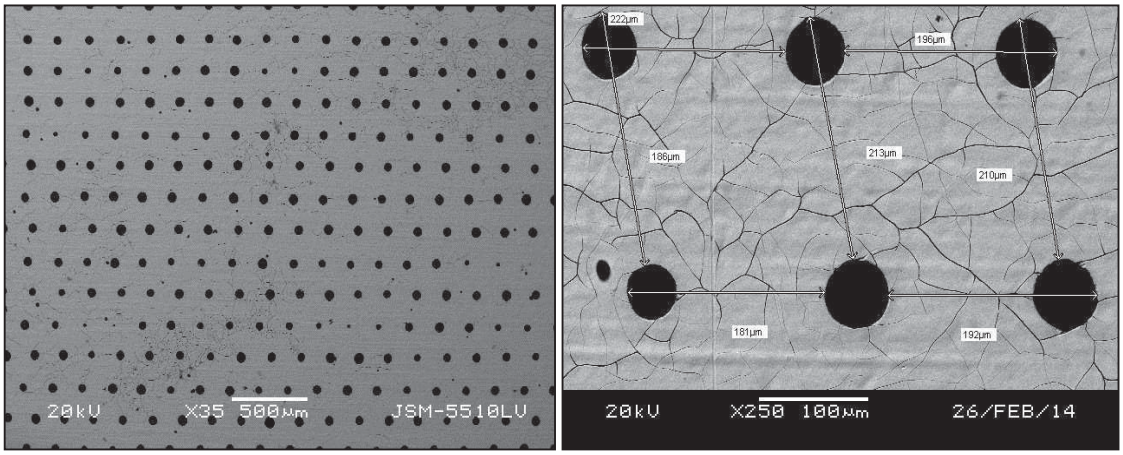

Figure 8: Scanning Electron Microscopy pictures of the steel filter screen.

The SGS BWS1 can be used in either closed loop (figure 9) or open loop configuration (figure 10). The configurations are very similar, in open loop no return port is used.

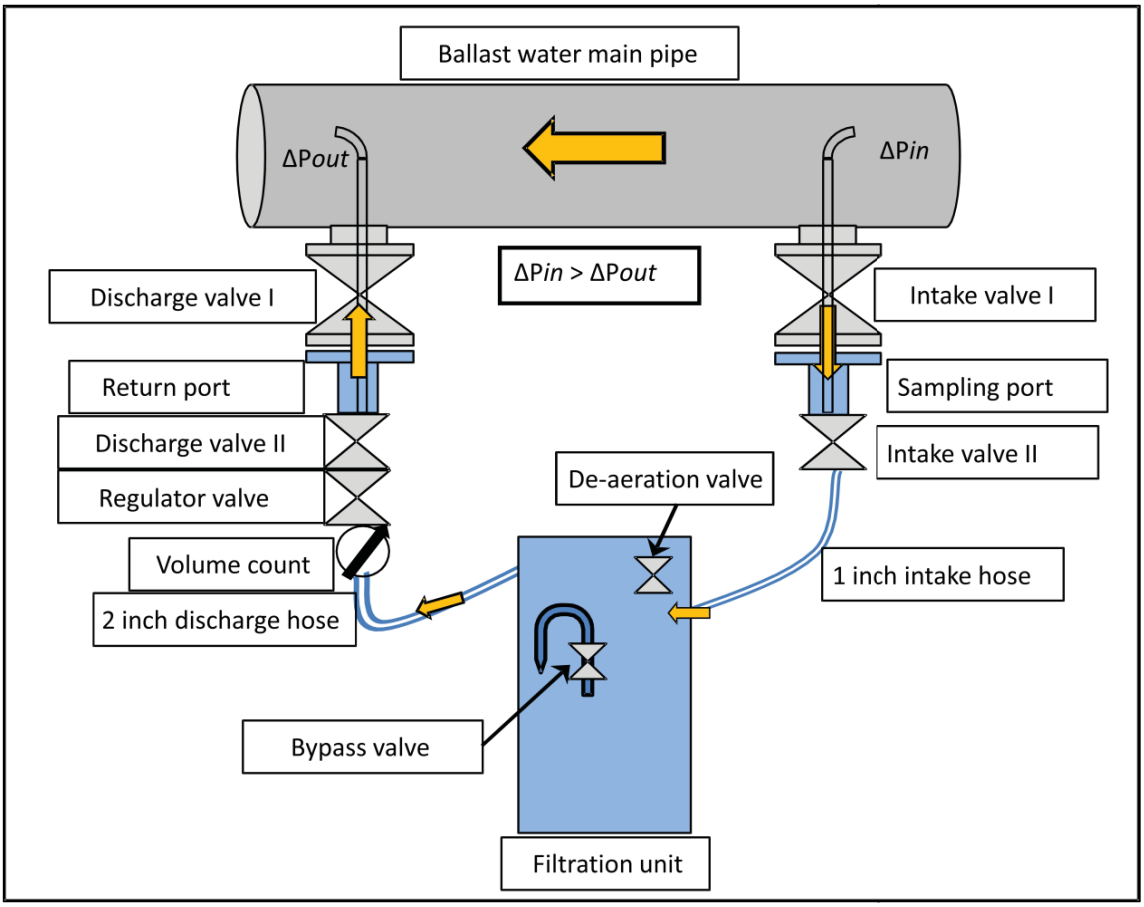

Figure 9. Schematic of the sampling system configuration in closed loop.

Since the main connecting valves (intake valve I and discharge valve I) cannot be closed when the sampling probes are inserted, closing the sampler off from the main system is done by the additional valves that come with the system (intake valve II and discharge valve II).

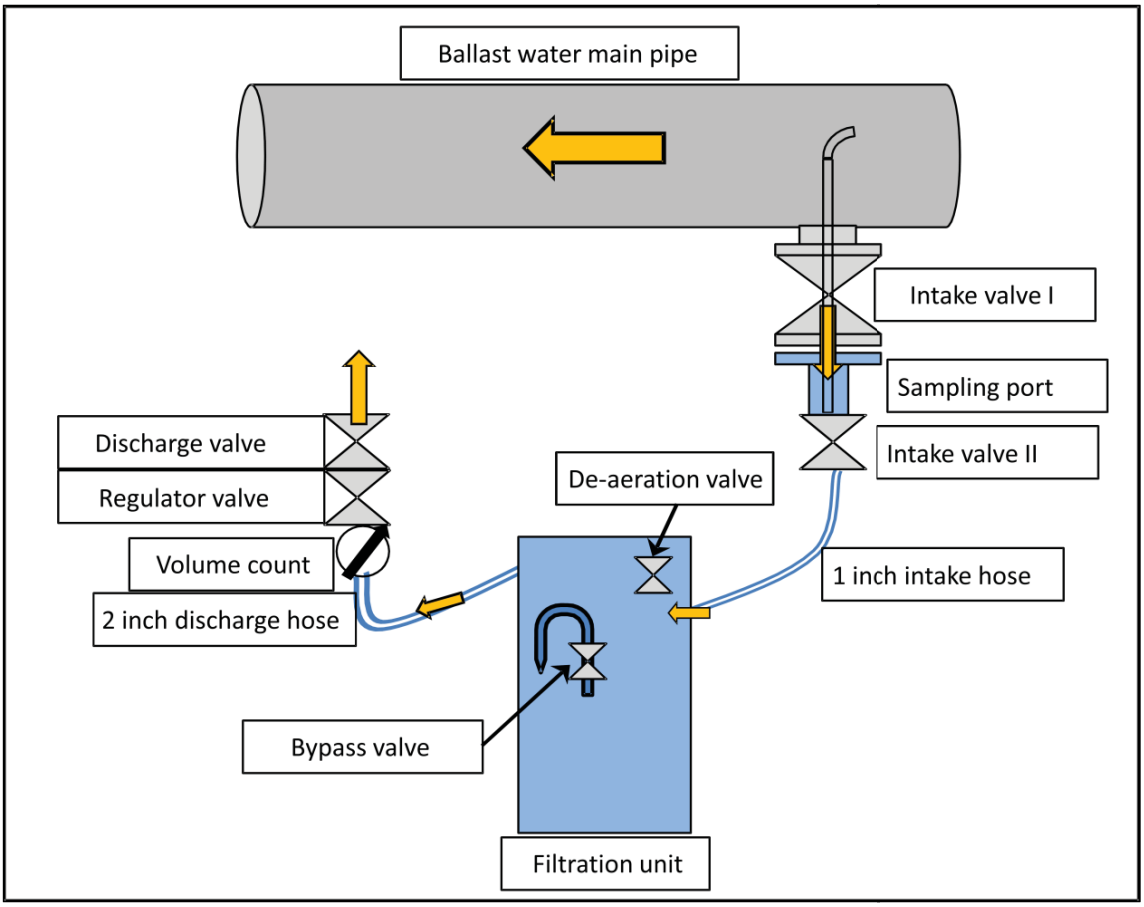

Figure 10. Schematic of the sampling system configuration in open loop.

In both configurations the correct flow for safe sampling of plankton is maintained using the regulator valve.

All recommendations and terminology in this document is in line with ISO 11711-1:2013 and the US Naval Research Institute report: 'Ballast Water Sample Ports, Sample Probes, and Sample Collection Devices: Recommendations for Standardization' (Report 3900 6130/1550).

Appendix E: Method Comparison Statistics results

Appendix E.1: G50 Method Comparison Results: SGS ATP, Microscopy, PAM, and Satake Pulse Counter: log+1 transformed data

```
Conversion between methods:
                                          alpha   beta sd.pred int(t-f) slope(t-f) sd(t-f)
To:                  From:
PAM                  PAM                  0.000  1.000   4.151    0.000       0.000   4.151
                     Satake.Pulse.Counter  4.595  0.885   2.994    4.875      -0.122   3.176
                     SGS.ATP               0.352  0.645   3.088    0.428      -0.431   3.754
                     Total.Microscope      3.131  0.974   3.284    3.173      -0.027   3.328
Satake.Pulse.Counter PAM                  -5.191  1.130   3.365   -4.875       0.122   3.160
                     Satake.Pulse.Counter  0.000  1.000   0.360    0.000       0.000   0.360
                     SGS.ATP              -4.710  0.717   0.991   -5.487      -0.330   1.154
                     Total.Microscope     -1.482  1.063   1.447   -1.437       0.061   1.403
SGS.ATP              PAM                  -0.545  1.550   4.766   -0.428       0.431   3.738
                     Satake.Pulse.Counter  6.570  1.395   1.388    5.487       0.330   1.159
                     SGS.ATP               0.000  1.000   1.805    0.000       0.000   1.805
                     Total.Microscope      4.505  1.485   2.340    3.625       0.390   1.883
Total.Microscope     PAM                  -3.216  1.027   3.323   -3.173       0.027   3.278
                     Satake.Pulse.Counter  1.394  0.941   1.384    1.437      -0.061   1.426
                     SGS.ATP              -3.033  0.673   1.596   -3.625      -0.390   1.907
                     Total.Microscope      0.000  1.000   1.861    0.000       0.000   1.861

 Variance components (sd):
                      s.d.
Method                 IxR   MxI   res
  PAM                    0 2.734 1.056
  Satake.Pulse.Counter   0 0.239 0.088
  SGS.ATP                0 1.079 0.678
  Total.Microscope       0 1.263 0.361

Variance components with 95 % cred.int.:
    method   PAM                      Satake.Pulse.Counter   SGS.ATP              Total.Microscope
    qnt      50%  2.5% 97.5%          50%  2.5% 97.5%        50%  2.5% 97.5%     50%  2.5% 97.5%
SD
IxR        0.000 0.000 0.000          0.000 0.000 0.000      0.000 0.000 0.000   0.000 0.000 0.000
MxI        2.734 1.904 3.784          0.239 0.012 0.758      1.079 0.653 1.457   1.263 0.893 1.780
res        1.056 0.888 1.287          0.088 0.077 0.101      0.678 0.585 0.795   0.361 0.304 0.443
tot        2.935 2.173 3.930          0.255 0.087 0.763      1.276 0.938 1.608   1.316 0.964 1.811

Mean parameters with 95 % cred.int.:
                                             50%    2.5%  97.5% P(>0/1)
alpha[Satake.Pulse.Counter.PAM]           -5.192 -61.703 -0.367   0.008
```

```
alpha[SGS.ATP.PAM]                             -0.543 -79.066  5.674   0.472
alpha[Total.Microscope.PAM]                    -3.208 -55.646  1.226   0.126
alpha[PAM.Satake.Pulse.Counter]                 4.594   0.893  6.984   0.992
alpha[SGS.ATP.Satake.Pulse.Counter]             6.561   5.354  7.421   1.000
alpha[Total.Microscope.Satake.Pulse.Counter]    1.396  -0.241  2.689   0.958
alpha[PAM.SGS.ATP]                              0.353  -8.542  6.229   0.528
alpha[Satake.Pulse.Counter.SGS.ATP]            -4.716  -7.073 -2.799   0.000
alpha[Total.Microscope.SGS.ATP]                -3.033  -6.944  0.549   0.049
alpha[PAM.Total.Microscope]                     3.139  -3.261  6.682   0.874
alpha[Satake.Pulse.Counter.Total.Microscope] -1.480  -6.217  0.158   0.042
alpha[SGS.ATP.Total.Microscope]                 4.505  -1.729  6.612   0.951
beta[Satake.Pulse.Counter.PAM]                  1.130   0.420  9.395   0.565
beta[SGS.ATP.PAM]                               1.550   0.642 12.876   0.778
beta[Total.Microscope.PAM]                      1.027   0.364  8.595   0.513
beta[PAM.Satake.Pulse.Counter]                  0.885   0.106  2.383   0.435
beta[SGS.ATP.Satake.Pulse.Counter]              1.395   1.037  1.933   0.985
beta[Total.Microscope.Satake.Pulse.Counter]     0.941   0.435  1.544   0.414
beta[PAM.SGS.ATP]                               0.645   0.078  1.558   0.222
beta[Satake.Pulse.Counter.SGS.ATP]              0.717   0.517  0.964   0.015
beta[Total.Microscope.SGS.ATP]                  0.673   0.317  1.066   0.051
beta[PAM.Total.Microscope]                      0.974   0.116  2.746   0.487
beta[Satake.Pulse.Counter.Total.Microscope]     1.063   0.648  2.296   0.586
beta[SGS.ATP.Total.Microscope]                  1.485   0.938  3.152   0.949
```

```
 Note that intercepts in conversion formulae are adjusted to get conversion formulae that represent the
same line both ways, and hence the median intercepts in the posterior do not agree  exactly with those
given in the conversion formulae.
```

Appendix E.2: 10-50 Method Comparison Results: BallastCheck2, bbe, Hach BW680, Microscopy

```
Note: Response transformed by:  function (y) {    log(y + 1)}
```

Conversion between methods:

To:	From:	alpha	beta	sd.pred	int(t-f)	slope(t-f)	sd(t-f)
BallastCheck2	BallastCheck2	0.000	1.000	0.750	0.000	0.000	0.750
	bbe	0.338	0.849	0.616	0.366	-0.163	0.666
	Hach	0.488	1.232	0.546	0.437	0.208	0.489
	Microscopy	0.242	1.020	0.709	0.239	0.020	0.702
	PAM	-1.313	0.950	1.026	-1.347	-0.052	1.053
bbe	BallastCheck2	-0.398	1.178	0.723	-0.366	0.163	0.664
	bbe	0.000	1.000	0.511	0.000	0.000	0.511
	Hach	0.178	1.453	0.390	0.145	0.369	0.318
	Microscopy	-0.109	1.203	0.659	-0.099	0.184	0.598
	PAM	-1.955	1.120	1.094	-1.844	0.113	1.032
Hach	BallastCheck2	-0.396	0.812	0.441	-0.437	-0.208	0.487
	bbe	-0.122	0.688	0.269	-0.145	-0.369	0.318
	Hach	0.000	1.000	0.117	0.000	0.000	0.117
	Microscopy	-0.198	0.829	0.389	-0.217	-0.187	0.426
	PAM	-1.468	0.772	0.720	-1.657	-0.257	0.812
Microscopy	BallastCheck2	-0.237	0.980	0.693	-0.239	-0.020	0.700
	bbe	0.091	0.832	0.549	0.099	-0.184	0.600
	Hach	0.239	1.207	0.471	0.217	0.187	0.427
	Microscopy	0.000	1.000	0.641	0.000	0.000	0.641
	PAM	-1.532	0.932	0.974	-1.586	-0.070	1.008
PAM	BallastCheck2	1.383	1.053	1.084	1.347	0.052	1.056
	bbe	1.745	0.893	0.981	1.844	-0.113	1.037
	Hach	1.902	1.295	0.934	1.657	0.257	0.813
	Microscopy	1.644	1.073	1.051	1.586	0.070	1.014
	PAM	0.000	1.000	1.306	0.000	0.000	1.306

Variance components (sd):

Method	s.d. IxR	MxI	res
BallastCheck2	0	0.489	0.204
bbe	0	0.341	0.119
Hach	0	0.064	0.052
Microscopy	0	0.385	0.238
PAM	0	0.875	0.294

Appendix E.3: <10 Method Comparison Results

Conversion between methods:

To:	From:	alpha	beta	sd.pred	int(t-f)	slope(t-f)	sd(t-f)
ATP	ATP	0.000	1.000	95.792	0.000	0.000	95.792
	BallastCheck2	-232.360	9.043	514.574	-46.275	1.602	102.479
	Bulk.FDA	-58.713	2.816	106.476	-30.773	0.952	55.807
BallastCheck2	ATP	25.696	0.111	57.421	46.275	-1.602	103.407
	BallastCheck2	0.000	1.000	74.953	0.000	0.000	74.953
	Bulk.FDA	13.146	0.371	55.044	19.183	-0.919	80.324
Bulk.FDA	ATP	20.851	0.355	32.005	30.773	-0.952	47.235
	BallastCheck2	-35.477	2.699	142.448	-19.183	0.919	77.025
	Bulk.FDA	0.000	1.000	28.261	0.000	0.000	28.261

Variance components (sd):

	s.d.		
Method	IxR	MxI	res
ATP	0	65.320	17.343
BallastCheck2	0	52.912	2.915
Bulk.FDA	0	19.023	6.051

Variance components with 95 % cred.int.:

method	ATP			BallastCheck2			Bulk.FDA		
qnt	50%	2.5%	97.5%	50%	2.5%	97.5%	50%	2.5%	97.5%
SD									
IxR	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MxI	65.320	1.577	162.364	52.912	2.250	67.894	19.023	1.502	35.962
res	17.343	13.556	22.873	2.915	2.585	3.352	6.051	4.659	8.204
tot	67.735	16.256	163.363	53.000	3.768	67.951	19.984	6.103	36.445