

STANDARD OPERATING PROCEDURE Procedure For Quantifying Heterotrophic Plate Counts (HPCs) Using IDEXX's SimPlate® for HPC Method

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STANDARD OPERATING PROCEDURE
Procedure For Quantifying Heterotrophic Plate Counts (HPCs) Using
IDEXX's SimPlate® for HPC Method

BACKGROUND

The Great Ships Initiative (GSI) is a regional effort devoted to ending the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System and globally. In support of that goal, the GSI has established superlative freshwater ballast treatment evaluation capabilities at three scales—bench, land-based, and on board ship. Each scale is dedicated to addressing specific evaluation objectives. These include:

GSI Bench-Scale Tests

- Range finding for effective doses under a range of ambient conditions;
- Chemical degradation over time under a range of ambient conditions;
- Detection of any residual toxicity under a range of ambient conditions; and
- Confirmation of treatment process.

GSI Land-Based Tests

- Detection of scale-up, mechanical operation issues;
- Effectiveness of a dose with respect to the full range of ambient organisms; and
- Detection of any whole water effluent toxicity.

GSI Shipboard Tests

- Confirmation of biological and operational performance as expected in the ship environment; and
- Confirmation of performance as expected under a broad range of ambient conditions.

The GSI awards its independent status-testing services to developers of ballast treatment systems and processes determined to be promising. GSI status-testing is performed at the scale appropriate to the state of development of the target treatment system, with the goal of facilitating the rapid progression of meritorious ballast treatment systems through the research and development and approval processes to a market-ready condition.

GSI has no involvement, intellectual or financial, in the mechanics, design or market success of the actual treatment systems it tests. To ensure that GSI tests are uncompromised by any real or perceived individual or team bias relative to test outcomes, GSI test activities are subject to rigorous quality assurance/quality control (QAQC) procedures and documentation. This attention to QAQC assures high quality and credible evaluation of GSI and its findings.

INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes the procedure used to quantify Heterotrophic Plate Counts (HPCs) Using IDEXX's SimPlate® for HPC Method¹. The HPC method is used to determine the number of viable heterotrophic bacteria in a water sample. Although there are other media and methods that may be relevant, this SOP describes the use of IDEXX's SimPlate® for HPC method, which 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® plate, 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, 19th Edition.

EQUIPMENT LIST

- Gloves.
- Lab coat.
- Goggles which provide protection from UV light.
- 1% bleach solution or 70 % ethanol for disinfecting bench tops.
- SimPlates® with lids.
- Pipettor and sterile tips.
- Dilution tubes.
- Peptone saline diluent.
 - Incubator at 35 °C ± 2 °C.
 - UV light-6 watt, 365 nm.
- Bunsen burner.
- Vortex mixer.
- Autoclave.
- MPN table (see appendix 1).
- Foil-packed sterile SimPlate® media in 100 mL vessels.
- Sterile deionized (DI) water.

PROCEDURE

Preparation

1. On the day prior to sampling, prepare one SimPlate® for each batch of sterile DI water

¹ SimPlate® is a trademark or a registered trademark of BioControl Systems, Inc. and is used by IDEXX under license from BioControl Systems, Inc. Covered by U.S. Patent Nos. 5,700,655; 5,985,594; 6,287,797; 6,387,650; 6,472,167. Other patents pending.

used to prepare SimPlate® media, and one tube of diluent. Incubate overnight to check for media contamination.

2. Disinfect bench top with a germicide solution and light a Bunsen burner in the center of the work area in order to provide an aseptic environment. Follow aseptic technique throughout procedure.
3. Label each SimPlate® with sample ID, replicate number, dilution, and date.
4. Check accuracy of pipettors according to manufacturer's instructions.
5. Check incubator temperature for accuracy.

Sample Collection

1. For samples from the GSI Land-Based RDTE Facility collect and transport samples in 1-L sterile sample containers as outlined in *GSI/SOP/LB/RA/SC/4 - Procedure for Microbial Sample Collection*; for bench-scale samples follow *GSI/SOP/BS/RA/MA/2 - Procedure for Assessing Antimicrobial Activity Using Time-Kill Method*.
2. Inactivate or neutralize the active substance at the time of sample collection or when a defined exposure period has been reached (i.e., sodium thiosulfate to neutralize chlorine).
3. Store samples in a refrigerator at 2°C – 8°C until analysis.
4. Analyze samples within 4-6 hours of collection. In exceptional circumstances, i.e., if there is a delay, store samples under the above conditions for a maximum of 24 hours before beginning analysis.

Media

1. Prepare Peptone Saline diluent (PSD) according to the following recipe:
 - a. Peptone saline diluent: 1 g peptone, 8.5 g NaCl, 1 L water (filtered, sterilized harbor water, or reagent grade deionized water). Add all dry ingredients to water; adjust pH to 7.0, and autoclave at 121°C (15 lb pressure) for 15 minutes
2. Prepare SimPlate® media:
 - a. Add sterile DI water to 100 mL mark on the media vessel to hydrate the Simplate® media. Recap and shake to dissolve. Label with media name, date prepared, and initials. Use within five days.

Sample Analysis

1. Using a sterile tip, aseptically transfer 1 mL of sample to the center of a Simplate®. Note: A smaller sample volume may be used as long as the final volume (sample plus hydrated media) is 10 ± 0.2 mL. If bacteria counts are expected to be high, and less than 0.1 mL is needed to get countable numbers, prepare dilutions as described in “Preparing a Dilution Series” section in *GSI/SOP/BS/RA/MP/1 - General Microbiology Preparation Procedures*. Note: The goal of the dilution(s) is to produce countable plate(s) that contain between 1 and 83 fluorescing wells or an MPN of less than 738.
2. Slowly pipette 9 mL of rehydrated media directly onto the sample in the center of the plate. If less than 1 mL of sample is used, adjust media volume so that total volume is 10 ± 0.2 mL.
3. Cover plate with lid and swirl gently to distribute sample into each well. It is acceptable to have air bubbles but double check to be sure all wells contain some sample.
4. Tilt the plate 90° - 120° to drain excess liquid into absorbent pad.
5. Invert the plate and incubate for 48 hours at $35 \pm 2^\circ\text{C}$. Note that results can be read from 45 to 72 hours after the start of incubation but it is important to be consistent in the incubation time. All samples within one test should have equal incubation time.
6. Repeat steps 3-5 using 10 mL of rehydrated media and no sample to act as a media blank.
7. Refrigerate any unused SimPlate® media and discard if not used within five days
8. Dispose of sample and media in accordance with Good Laboratory Practice.

Counting and Reporting

1. After Incubation time, remove cover and count the number of wells showing any fluorescence by holding a 6 watt, 365 nm, UV light five inches above the plate. Face light away from your eyes and towards the sample. Fluorescing wells may be counted on the bottom of the plate instead. No wells should fluoresce in the controls.
2. Refer to the Most Probable Number (MPN) table (see appendix 1) to determine the MPN of heterotrophic plate count bacteria in the original sample. Note: The table takes into account the sample/media poured off. Adjust the MPN to reflect the sample volume used. For example, if 0.1 mL of sample and 9.9 mL of hydrated media were used, then the MPN table number is MPN per 0.1 mL. To convert this to MPN per mL, multiply by 10.

$$\text{MPN/mL} = \frac{\text{MPN for number of fluorescing wells counted}}{\text{Actual volume of sample in dish, mL}}$$

3. Report the average CFU/mL per plate in addition to method used, incubation temperature and time, and media used.

Technical Assistance

1. For IDEXX Technical Assistance in the U.S. and Canada, call 1-800-321-0207 or 1-207-856-0496. In other countries, visit www.idexx.com/water.

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

1. Conduct all QAQC procedures according to *GSI/QAQC/QAPP/BS/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale Tests (2010)* or *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests*.
2. Follow all procedures outlined in this SOP. Any deviations known ahead of time must be approved by the GSI Principal Investigator and communicated to a GSI QAQC Officer.
3. Ensure that a minimum of 10 % of samples are analyzed in duplicate.
4. Method blanks are prepared daily, or at each analysis period in order to monitor any contamination which may take place throughout the procedure.

DATA STORAGE AND ARCHIVING

1. Store and archive data according to *GSI/QAQC/QAPP/BS/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale Tests (2010)* or *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests*.
2. Archive all hard- and electronic-copies of data and records generated for a period of five years.

REFERENCES AND RELATED DOCUMENTS

Eaton AD, Clesceri LS, Rice EW & Greenberg AE, Eds. (2005). Standard Methods for the Examination of Water & Wastewater.

GSI/QAQC/QMP/1 – Great Ships Initiative Quality Management Plan (2010).

GSI/QAQC/QAPP/BS/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale Tests (2010).

GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2010).

GSI/SOP/BS/RA/MA/2 - Procedure for Assessing Antimicrobial Activity Using Time-Kill Method.

GSI/SOP/G/RA/SC/2 - Procedure for Custody of GSI Land Based Samples RDTE Facility Samples.

GSI/SOP/G/RA/SC/3 – Procedure for Labeling Samples Collected at the GSI Land-Based RDTE Facility.

GSI/SOP/G/RA/SC/4 – Procedure for Labeling GSI Bench Scale Samples.

GSI/SOP/LB/RA/SC/1 – Procedure for Collecting Biological Samples via In-Line Sample Ports.

GSI/SOP/LB/RA/SC/4 - Procedure for Microbial Sample Collection.

GSI/SOP/BS/RA/MP/1 - General Microbiology Preparation Procedures

Great Ships Initiative website: <http://www.greatshipsinitiative.org>.

Great Ships Initiative Standard Operating Protocols: <http://www.nemw.org/GSI/protocols.htm>.

Instructions for Simplate® for HPC MultiDose Test Kit.

Appendix 1. Multi-Dose IDEXX SimPlate® for HOC Most Probable Number (MPN)

Multi-Dose IDEXX SimPlate for HPC Most Probable Number (MPN)			
#PositiveWells	MPN	95% confidence limits	
		lower	upper
0	<2	<0.3	<14
1	2	0.3	14
2	4	1	16
3	6	2	19
4	8	3	22
5	10	4	25
6	12	6	27
7	15	7	30
8	17	8	33
9	19	10	36
10	21	11	39
11	23	13	42
12	26	15	45
13	28	16	48
14	30	18	51
15	33	20	54
16	35	22	58
17	38	23	61
18	40	25	64
19	43	27	67
20	45	29	70
21	48	31	74
22	51	33	77
23	53	35	80
24	56	38	84
25	59	40	87
26	62	42	91
27	65	44	94
28	68	47	98
29	71	49	102
30	74	51	106
31	77	54	109
32	80	56	113
33	83	59	117
34	86	62	121
35	90	64	126
36	93	67	130
37	97	70	134
38	100	73	139
39	104	76	143
40	108	79	148
41	112	82	152
42	116	85	157
43	120	88	162
44	124	91	167
45	128	95	173
46	132	98	178
47	137	102	183
48	141	106	189
49	146	109	195
50	151	113	201
51	156	117	207
52	161	121	213
53	166	125	220
54	171	130	227
55	177	134	234
56	183	139	241
57	189	144	249
58	195	149	257
59	202	154	265

Multi-Dose IDEXX SimPlate for HPC Most Probable Number (MPN)

#PositiveWells	MPN	95% confidence limits	
		lower	upper
60	209	159	273
61	216	165	282
62	223	171	292
63	231	177	302
64	239	183	312
65	248	190	323
66	257	197	335
67	266	204	347
68	276	212	361
69	287	220	375
70	299	229	390
71	311	238	407
72	324	248	425
73	339	258	444
74	355	270	466
75	372	282	491
76	392	296	519
77	414	311	551
78	440	328	589
79	470	348	636
80	507	371	695
81	555	398	775
82	623	432	899
83	738	476	1146
84	>738	>476	>1146

MPN is per ml of the one ml added to 9 ml rehydrated media (pour-