From:	McCambridge, Michael
To:	Miller, Lindsey
Cc:	Brown, Don; Fox, Tim; McGill, Richard
Subject:	Federal Register 10/29/2002; HPC for SimPlate EPA Approval with summary
Date:	Thursday, May 2, 2019 1:45:00 PM
Attachments:	HPC SimPlate EPA method description 001128.pdf image001.png image003.png

Exactly what I need. Thank you.

Don Brown: Add this e-mail exchange to the record of the R19-16 SDWA update to comport with the Ethics Act prohibition against *ex parte* contacts.

From: Miller, Lindsey <Lindsey-Miller@idexx.com>
Sent: Thursday, May 2, 2019 1:37 PM
To: McCambridge, Michael <Michael.McCambridge@illinois.gov>
Subject: [External] RE: Federal Register 10/29/2002; HPC for SimPlate EPA Approval with summary

Hello Michael,

I apologize, I thought you asked for the document from 2002. Here is out method description from November 2000, attached.

Best,

Lindsey Miller
Technical Support
IDEXX Water
Customer Support
IDEXX Livestock, Poultry, and Dairy
1-800-321-0207 (Water Support)
1-800-548-9997 (LPD Support)
(207) 556-8651 (direct line)

One IDEXX Drive
Westbrook, ME 04092 USA
idexx.com/water

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From: McCambridge, Michael [mailto:Michael.McCambridge@illinois.gov]
Sent: Thursday, May 02, 2019 2:36 PM
To: Miller, Lindsey <Lindsey-Miller@idexx.com>
Subject: RE: Federal Register 10/29/2002; HPC for SimPlate EPA Approval with summary

Thank you for your prompt attention, but you did not send the document that I need. I have the *Federal Register* notice from 2002 approving the method.

What I need is a copy of the November 2000 version of the SimPlate method as approved in that notice. I cannot find one in our records. Those available on your website are copyrighted 2012.

Illinois law prohibits incorporation by reference of any version later than that approved by USEPA. Illinois law further requires that we maintain a copy of every document incorporated by reference.

If I cannot find a copy, the Board will be required to remove the incorporation by reference from the Illinois rules.

Michael J. McCambridge, Attorney
Illinois Pollution Control Board
312-814-6924 (9:00 a.m. to 7:00 p.m. Mon. through Thurs.)
219-614-5082 (personal cell at all other times)

From: Miller, Lindsey <Lindsey-Miller@idexx.com>
Sent: Thursday, May 2, 2019 1:19 PM
To: McCambridge, Michael <Michael.McCambridge@illinois.gov>
Subject: [External] Federal Register 10/29/2002; HPC for SimPlate EPA Approval with summary

Good afternoon Michael,

Please see pages 65891-65901 of the attached Federal Register for mention of SimPlate approval. Please let me know if you need anything further.

Best,

Lindsey Miller
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Westbrook, ME 04092 USA

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IDEXX SimPlate™ HPC Test Method for Heterotrophs in Water

IDEXX SimPlate™ Test Method for Heterotrophic Bacteria in Water

1.0 Scope and Application

1.1 This method is intended for use in the detection and quantification of heterotrophic bacteria in water. High levels (>500/ml) of heterotrophic bacteria in drinking water is an indication of no chlorine or chlorine residuals less than 0.2 mg/l. (1)

1.2 The minimum, non-zero number of bacterial counts detectable with this method is a function of the dilution scheme used when processing the sample (see package insert).

1.3 The SimPlate HPC method can be applied to fresh waters and drinking waters.

1.4 Since there can be a wide range of levels in waters, dilutions can be used with this method for detecting and enumerating the actual level.

1.5 The SimPlate for HPC has not been validated for use with marine water samples.

2.0 Summary of Method

2.1 This method is based on a multiple enzyme technology, which detects viable bacteria in water by testing for the presence of key enzymes known to be present in these organisms. The bacteria are detected as fluorescent wells on a SimPlate. The bacterial MPN value associated with a water sample is determined by counting the positive wells, looking up the corresponding MPN value using a provided table and applying any dilution factor which may have been introduced during sample preparation.

3.0 Definitions

3.1 In this method, heterotrophic bacteria are those bacteria which produce a fluorescent signal in the SimPlate wells under a 6 watt, 365nm UV light after incubation at 35°C for 48 hours.

4.0 Interferences

4.1 There are no chemical or microbiological substances known to interfere with the SimPlate HPC method.

5.0 Safety

5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory preparing using and disposing of samples, reagents and materials, and while operating sterilizing equipment.

5.2 Mouth-pipetting is prohibited.

6.0 Equipment and Supplies

6.1 Pipettes, sterile, T.D. bacteriological or Mohr, glass or plastic of appropriate volume.

6.2 Vessels, sterile, plastic or glass, 100 to 200 ml volume.

6.3 Incubator maintained at 35 ± 0.5° C.

6.4 6 Watt, 365 nm, UV Light.

7.0 Reagents

7.1 Purity of reagents: Regent grade chemicals shall be used. Unless otherwise indicated, chemicals shall conform to specifications of the American Chemical Society (2)

7.2 Sterile deionized or distilled water: Water conforming to specification D1193 (*check this out),* Reagent water conforming Type II, Annual Book of ASTM Standards (3). Autoclave at 121° C (15 lb presure) for 15 min.

7.3 Buffered Dilution Water (May be purchased or see Standard methods, Section 9050 C (5)

7.3.1 Composition:
Potassiun Dihydrogen Phosphate 3.4 g
Magnesium Chloride -6 H_2O
1 N Sodium Hydroxide

7.3.2 Preparation: Dissolve 3.4 grams of Potassium Dihyrogen Phosphate in 75 ml of reagent water and adjust pH to 7.2 ± 0.5 with 1 N Sodium Hydroxide. Dilute to 100 ml with reagent water (Stock Phosphate). Weigh 8.1 g of Magnesium Chloride in 80 ml of reagent water. Mix to dissolve and dilute to 100 ml with reagent water (stock Magnesium Chloride). To a 1 L flask add 1.25 ml of Phosphate stock and 5 ml of Magnesium Chloride stock and dilute to volume With reagent water. Autoclave after preparation at 121°C (15 lb presure) for 15 min.

7.4 0.1% Peptone Water

7.4.1 Preparation: Dissolve 1 g in 1 liter of reagent water in a flask. Autoclave after preparation at 121°C (15 lb presure) for 15 min.

7.5 Sodium Thiosulfate used to dechlorinate drinking water samples.

7.6 SimPlate for HPC Reagent (Store at 4-30 °C away from light. Expiration date is printed on each package).

7.6.1 Multi-Dose package:
10 foil packed sterile media vessels for 100 ml of media when hydrated.
100 ml sterile IDEXX SimPlates with lids.
MPN Table and package insert.

7.6.2 Unit-Dose package:
25 sterile media tubes for 10 ml samples.
25 ml sterile IDEXX SimPlates with lids.
MPN Table and package insert.

8.0 Sample Collection, Preservation and Storage

8.1 Sampling procedures as described in detail in the USEPA microbiology methods manual, Section II, A (4) and in Standard Methods for the examination of Water and Wastewater (5).

8.1.1 Storage Temperature and Handling Conditions

Ice or refrigerate bacteriological samples at a temperature or 1-4°C during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that sample vessels are not totally immersed in water during transit.

8.1.2 Holding Time Limitations

Examine samples as soon as possible after collection. Do not hold samples longer than 8 h between collection and initiation of analyses for compliance samples and not longer than 24 h for non-compliance samples.

9.0 Quality Control

9.1 See recommendations on quality control for microbiological analyses in the USEPA microbiology manual, Part IV, C (4).
9.2 See 11.0 Procedure for sterility testing of media. Use 10 ml of reagent. No wells should fluoresce after incubation.

10.0 Calibration and Standardization

10.1 Check temperatures in incubators daily to insure operation within stated limits.

10.2 Check thermometers at least annual against NIST certified thermometer or one that meets the requirements of NIST Monograph SP 250-23.

11.0 Procedure

11.1HPC Multi Dose

11.11 Hydrate media by filling vessel to the 100 ml mark with sterile diluent (see 7.0 reagents). Recap and shake to dissolve.

11.12 Pipette 1.0 ml sample and then 9 ml of the rehydrated media onto the center of the SimPlate base.

11.13 Cover SimPlate with the lid and gently swirl to distribute the sample into the wells. Note: Air bubbles in the wells do not interfere with the test.

11.14 Tip the plate 90-120 degrees to drain the excess sample into the absorbent pad.

11.15 Invert the SimPlate and incubate for 48 h at 35±0.5 °C.

11.16 0.1 ml plus 9.9 ml of media came be added directly to the SimPlate for a 1:10 dilution or appropriate dilutions came be made with sterile buffer prior to adding 1 ml of the diluted sample to the SimPlate by following 11.11.

11.2HPC Unit Dose

11.21 Add 10±0.2 ml of sample to a media tube, recap and shake to dissolve.

11.22 Pour the contents of the tube onto the center of the SimPlate.

11.23 Cover SimPlate with the lid and gently swirl to distribute the sample into the wells. Note: Air bubbles in the wells do not interfere with the test.

11.24 Tip the plate 90-120 degrees to drain the excess sample into the absorbent pad.

11.25 Invert the SimPlate and incubate for 48 h at 35±0.5 °C.

12.0 Data Analysis and Calculations

12.1 HPC Multi Dose

12.11 Count the number wells showing fluorescence by holding a 6 Watt, 365nm, UV light 6-12 inches above the SimPlate. Face the light away from your eyes and towards the sample.

12.12 Refer to the MPN table provided to determine the Most Probable Number/ml bacteria in the original sample. If any dilutions were made, multiply the MPN/ml by the dilution factor to obtain the final MPN/ml value.

12.2 HPC Unit Dose

12.21 Count the number wells showing fluorescence by holding a 6 Watt, 365nm, light 6-12 inches above the SimPlate. Face the light away from your eyes and towards the sample.

12.22 Refer to the MPN table provided to determine the Most Probable Number/ml of bacteria in the original sample.

13.0 Method Performance

13.1 The SimPlate method for HPC was found to be equivalent to the approved USEPA method in a multi-regional evaluation: $Y = 0.99X + 0.06$, $r = 0.95$, $n = 632$ (6)

14.0 Reporting Results

14.1 Report results as heterotrophic bacteria per ml of sample.

15.0 Verification Procedure

Not applicable

16.0 Pollution Prevention

16.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

16.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

17.0 Waste Management

17.1 It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions. Compliance with all sewage discharge permits and regulations is also required.

17.2 Samples, reference materials and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal.

18.0 References

1. 40 CFR 141.72 and 141,74
2. Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, DC.
3. Annual Book of ASTM Standards, Vol. 11.01, American Society for Testing Materials, Philadelphia, PA 19103
4. Bordner, R., J.A. Winter and P.V. Scarpino (eds.), Microbiological Methods for Monitoring the Environment, Water and Wastes, EPA-600/8-78-017. Office of Research and Development, USEPA
5. Clesceri, L.S., A.E. Greenberg, A.D. Eaton (eds.). 1998,Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association, Washington, DC
6. R. W. Jackson et al., Multiregional Evaluation of the SimPlate Heterotrophic Plate Count Method Comapred to the Standard Plate Count Agar Pour Plate Method in Water, Applied and Environmental Microbiology, Jan. 2000, p. 453-454

Attachments: Package insert for SimPlate Multi Dose and Unit Dose

Revised 11/29/00