Rhode Island State Health Laboratories
50 Orms Street, Providence, RI
Section: Environmental
Laboratory: Water Microbiology

ID No.: 10839
Revision 1
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Approved By: Henry Leibovitz

Cyanobacteria Harmful Algal Bloom Identification and Enumeration

1. **Title:** Cyanobacteria Harmful Algal Bloom Identification and Enumeration.

2. **References:**
   2.1. STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 22nd EDITION, 10-17.

3. **Scope and Principle of the Analysis:**
   3.1. Fresh water samples are analyzed for the identification and enumeration of potentially harmful cyanobacteria species. The cyanobacteria genera of interest include: *Anabaena*, *Microcystis*, *Aphanizomenon*, and *Planktothrix*, with a screen for other potentially toxic organisms. Freshwater samples will be taken from drinking water reservoirs and recreational freshwater then identification will be performed using a visual identification key. Cyanobacteria will be identified, and enumerated as units/mL by using a Sedgewick Rafter counting chamber for calculations.

   Our results will supplement those of cyanobacteria toxin monitoring performed by Food Chemistry to be utilized by RIDEM and HEALTH to determine appropriate public health action.

4. **Safety Issues:**
   4.1. The samples may contain cyanotoxins. These potent toxins are easily absorbed through the skin and can cause damage to humans. Each sample should be treated as though it contains dangerous levels of cyanotoxin.
   4.2. Gloves should be worn when handling the samples.
   4.3. Samples are disposed of in a histology bucket after the read.

5. **Interferences:**
   5.1. There are "look-alike" species that may be frequently mistaken for certain harmful species and must be distinguished for the count.
   5.2. Photos of harmful species, used for reference, will be taken by the Nikon Eclipse E400 camera microscope. Each photo will be labeled with the location site of the sample and archived digitally in a folder on the L: drive sorted and named by the sample's date and Element work order number.

6. **Glassware:**
   6.1 1.0mL Sedgewick Rafter counting chamber.
   6.2 24x50mm coverslip.

7. **Instrumentation:**
   7.1 Microscope equipped with 10X and 40X lenses.
   7.2 Nikon Eclipse E400 camera.

8 **Sample Collection, Preservation and Storage:**
   8.1 Collect samples in amber 40mL glass vials and transport on ice.
   8.2 Analyze samples immediately.

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8.3 If samples are unable to be analyzed immediately, refrigerate the samples and observe promptly the next morning.

9 Sample preparation:

9.1 Invert sample container gently 5-10 times.

9.2 Place clean coverslip diagonally on top of the well of the Sedgewick Rafter counting chamber.

9.3 Carefully fill the well of the chamber with 1.0 mL of the sample. Twist the coverslip to cover the well completely.

  9.3.1 Be sure that there are no bubbles in the reading area. If there are, re-pipette.

9.4 Let the sample sit 10-15 minutes before reading to allow the bacteria to settle.

10 Procedure:

10.1 Set the counting chamber onto the stage of the microscope.

10.2 Begin at one side of the slide and count how many units there are of potentially toxic organisms in the entire field. One full read across is equal to 100uL.

  10.2.1 Genera of potentially toxic cyanobacteria include: *Anabaena*, *Microcystis*, *Aphanizomenon*, *Planktothrix*.

  10.2.2 Identify an organism if it makes up a large part of the population. If it is identified as being a potentially toxic organism, enumerate.

10.3 If the numbers are too high to count 100uL, count 10 square volumes at random and use the average to determine the value of units/uL, which will later be calculated to units/mL.

11 Expression of Results:

11.1 The final result should be reported as units/mL.

  11.1.1 If the entire slide is read across, the result should be multiplied by 10 to get from units/100uL to units/mL.

  11.1.2 If the numbers are high and an average of units/uL is determined, the value should be multiplied by 1,000 to get to units/mL.

11.2 Refer to section 10-17 of *Standard Methods for the Examination of Water and Wastewater*, *22nd Edition* for more information.

12 Reporting:

12.1 Prior to entering results electronically, worksheets must be reviewed.

  12.1.1 Daily worksheets are accepted or rejected based on correctness and completeness by laboratory scientist. Date and initials are required.

  12.1.1.1 Any worksheets that are rejected are brought to the supervisor's attention for a corrective plan of action.

  12.1.1.2 Acceptable work sheets are ready to be entered into the Element system.

    12.1.1.2.1 Refer to Promium Element LIMS Data Entry for Water Microbiology.

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Document Type: Standard Operating Procedure

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