STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF MICROCYSTINS

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1.0 Identification of Test Method

1.1 This method is the Standard Operating Procedure for the determination of microcystins in the laboratory of the South Florida Water Management District. This document is identified as SFWMD-LAB-SOP-3170-005.

1.2 This method is based on EnviroLogix QuantiPlate™ Kit for microcystins. This method is modified to insure the near same reaction time for each sample by using a pre-mixing step with a glass coated 96 flat well microplate. The non-direct contact technique between hand/fingers and antibody coated wells has been applied during the wash step to prevent the temperature effect on the enzymatic reaction.

2.0 Applicable Matrix or Matrices

2.1 This method can be applied to the determination of microcystins in surface, ground and saline waters collected within the South Florida Water Management District.

3.0 Detection Limit

3.1 The method detection limit (MDL) for this method is determined using the procedure outlined at 40 CFR Part 136, using seven replicates of standard solution with a value in the range of three to five times the expected detection limit. The applicable method detection limit for this method is 0.2 ppb (ug/L)

3.2 The MDL is reviewed annually by the QA officer and is subject to change. The current MDL is noted on the Test Control Parameters sheet and is available from the QA officer.

4.0 Scope and Application

4.1 This method is useful for determining microcystins in surface, saline, and ground waters.

4.2 This method is applicable for ranges from the minimum detection limit (MDL) to 2.5ppb. Making suitable dilutions of over-range samples can extend the upper range.

4.3 The method code for microcystins is Microcystin LR.

5.0 Summary of the Test Method

5.1 The water sample is autoclaved prior to analysis in order to release (lyse) the microcystins from the algal and bacterial cells present. Therefore this method determines the “total” (see 5.3) amount of microcystins present in the sample.

5.2 Microcystins analysis is a competitive enzyme-linked immunosorbent assay (ELISA). The microcystin toxin in the sample competes with enzyme labeled microcystin for antibody binding sites on the test cell wall. Results are determined with a color development step where the color development is inversely proportional to concentration in the sample.

5.3 This QuantiPlate Kit for Microcystins does not distinguish between the microcystin toxin variants, but detects their presence to differing degrees. The accompanying table shows the value for 50% B_0 and the value for 81.3% B_0 limit of detection for four microcystin toxin variants and nodularin toxin. The cross-reactivity is as follows: (concentration is in ppb):

Cross-Reactivity

Compound	50% B_0*	LOD
Microcystin LR	0.50	0.15
Microcystin LA	0.81	0.24
Microcystin RR	0.92	0.27
Microcystin YR	1.42	0.44
Nodularin	0.73	0.21

* 50%B_0: the absorbance of microcystins at the concentrations is 50% of the absorbance of negative control.

5.4 Concentration is measured using an absorbance plate reader at 450 nm.

5.5 The standard and control solutions are prepared from Microcystin LR, therefore the results are expressed as the concentration equivalent of Microcystin LR (MY-LR CE).

6.0 Definitions

6.1 A comprehensive listing of terms and definitions in common use in the SFWMD laboratory can be found in the Laboratory Quality Manual glossary. All acronyms are defined upon first use in this document. Only terms specific to this procedure are defined in this section.

7.0 Interferences

7.1 Humic acid interferes in the assay at concentrations above 100 ppm.

7.2 While ultrasonic lysing of cells is generally effective, it is not useful for this analysis as it can degrade microcystins in solution. For this reason, an autoclave is used to lyse all algal cells in the samples prior to analysis.

7.3 The enzymatic reaction is temperature sensitive. Direct contact between hand/fingers and wells should be avoided during the washing process.

7.4 The enzymatic reaction is time dependent. The time interval (for mixing/transferring step) between each well strip should be near constant.

8.0 Safety

8.1 All personnel conducting this method should be familiar with the SFWMD Chemical Hygiene Plan and should have reviewed any pertinent Material Safety Data Sheets.

8.2 Wear safety glasses and a full-length, long-sleeved laboratory coat.

8.3 No hazardous wastes are generated by this procedure; liquid waste may be disposed of down the drain.

9.0 Equipment and Supplies

9.1 Sun-SRI glass coated 96 flat well microplate, 370 µl, or equivalent.

9.2 BioTek Multi-Mode Microplate Reader Synergy 2 attached to a computer with Gen5 v1.1 program.

9.3 EnviroLogix Quantiplate for microcystins (96 wells) (Catalog # EP 022).

9.4 Eppendorf 100 µL to 1000 µL adjustable pipette (calibrated quarterly).

9.5 Eppendorf 10 µL to 100 µL adjustable pipette (calibrated quarterly).

9.6 Eppendorf 8 channel 30 µL to 300 µL adjustable pipette.

9.7 Rainin 2-20 ml EDP3-plus advanced Electronic Pipette with LTS (calibrated quarterly).

9.8 Eppendorf Centrifuge 5810R.

9.9 Autoclave.

9.10 Nalgene autoclavable polycarbonate rack (catalog #: 5929 0020) or equivalent.

9.11 Fisherbrand Borosilicate glass tubes with cap, 16×100 mm (Catalog #:14-962-26F) or equivalent.

9.12 Class A 10, 25, 50, 100, and 250 ml Volumetric flasks.

9.13 Class A 1, 2, and 5 ml glass pipettes.

9.14 Labnet ORBIT P4 high speed orbital shaker.

9.15 Thermolyne RotoMix Type 48200 low speed orbital shaker.

10.0 Reagents and Standards

10.1 All reagents and standards are supplied by EnviroLogix.

10.2 Laboratory reagent water

10.3 Add ~3 ml laboratory reagent water to the assay diluent bottle and mix well.

10.4 Washer buffer solution: To make 1 L, add the contents of one packet of phosphate-buffered saline-Tween 20 (supplied by EnviroLogix) to 1 L DI water. Mix thoroughly to dissolve the salts. The buffer solution can be stored at room temperature.

10.5 LCS working Stock/spike solution (100 ppb): Using a disposable glass pipette to transfer 1 ml of 10 ppm LCS stock solution (Sigma 33893-1ML-R analytical standard) into a 100 ml volumetric flask. Dilute to mark with Laboratory reagent water and mix. Store in the amber glass bottle at ≤6 °C and not frozen.

10.6 1 ppb LCS solution: Transfer 2 ml of 100 ppb LCS working stock solution into a 200 ml volumetric flask. Dilute to mark with Laboratory reagent waterand mix. Store in the amber glass bottle at ≤6 °C and not frozen.

10.7 Refer to the SFWMD-LAB-SOP-5000 for the instructions on entering the LCS and spike solution into the LIMS system.

11.0 Sample Collection, Preservation, Shipment and Storage

11.1 Samples are collected according to the procedures outlined in the SFWMD Field Sampling Manual and the "Work Plan for Assessment of Cyanobacterial Toxins in the District's Northern Watershed".

11.2 Samples are transported to the lab in coolers containing wet ice or other devices to maintain the temperature of the sample at ≤6 °C and not frozen until delivery to the SFWMD laboratory.

11.3 Samples are stored in the laboratory in a cooler that is maintained at ≤6 °C and not frozen. Samples are removed from the cooler prior to testing.

11.4 Samples must be analyzed within 7 days.

12.0 Quality Control

12.1 Each analytical run should include working standards, method blank, LCS, matrix spike and duplicate.

12.2 An initial method blank is analyzed at the beginning of each run, and a continuing method blank is subsequently analyzed every ten samples. The results must be below the detection limit.

12.3 A spike and duplicate analysis should be conducted for every 10 samples analyzed. The spike recovery, mean and relative percent difference of the duplicate set are determined (using the formula in section 15.0).

13.0 Calibration and Standardization.

13.1 The correlation coefficient (r) of the standard curve must be ≥0.995.

13.2 Matrix spikes are prepared by adding 0.1 mL of spike solution (10.5) to 10 ml sample selected at random.

13.3 All LCS's must be within established limits.

13.4 Method Blanks must be less than the Method Detection Limit.

14.0 Procedure

14.1 Sample Preparation

14.1.1 Create a batch for the samples to be analyzed. Refer to the SFWMD-LAB-SOP-5000 for creating the batch. Download the Batch Worklist file from the LIMS and save the lab ID as a text file in the excel program.

14.1.2 Referring to the batch, complete a autoclave log (refer to section 24.1 example) with the following information: date and time digestion was started, autoclave temperature and pressure, Kit lot # and expiration date, LCS preparation date, and the order in which each sample is processed.

14.1.3 Remove the samples, the QuantiPlate™ kit with the standards/reagents/plate and QC solutions from the refrigerator **(Note: Do not remove well strips from the bag with desiccant until they have warmed up to room temperature)**.

14.1.4 Arrange and label glass tubes in the autoclave rack according to the tray protocol.

14.1.5 Using a calibrated pipette with disposable tips, pipette 10 ml aliquot of blank, LCS and samples into their respectively labeled glass tubes while changing tips between each aliquot. Each solution and sample must be shaken thoroughly before taking an aliquot.

14.1.6 When all tubes are filled, cap the tubes tightly, place a sterilization indicator strip on the rack and place the tray in the autoclave. (see autoclave SOP for operating instructions: SFWMD-LAB-SOP-5300)

14.1.7 When the cycle is finished, the autoclave will automatically stop and the pressure will decrease gradually.

14.1.8 Take the rack out of autoclave and store the samples in the dark by putting the rack in a covered box. Let the samples cool down to room temperature and centrifuge the samples at 1000 RPM for 10 minutes.

14.2 Turn on the microplate reader and allow it to warm up for 15 to 30 minutes.

14.3 Double click on Gen5 to open the program window.

14.4 Click “Experiment” to create a new experiment. Select the protocol “MC.prt”.

14.5 Click “plate 1” and then “sample IDs” at the left side of the toolbar menu. A New window will open. Click “Import” in the new window and select the lab ID text file to open. Click “OK” to close the new window.

14.6 Select “layout” from pull down icon (ᐯ) and click the excel icon on the plate 1 data screen to export the well layout into the excel program. Print the well layout using the excel program.

14.7 Click in “plate layout” at the left side of the toolbar menu to check the plate well layout and the sample dilution factor. The default plate well layout has 14 samples (SPL14) (included the MB, LCS, MS and MSD). If one run requires analyzing more than 14 samples, select “Type” as “sample”, increase the sample ID # by clicking the upper arrow and then click the empty wells. If one run requires analyzing less than 14 samples, select “Type” as “empty” and click to remove the sample # assigned to the wells. If a sample is over ranged and requires the dilution, enter the dilution factor to the “dil.”, select the sample ID# and click the assigned wells.

14.8 Add 156 µl of microcystin assay diluent to wells of a glass coated 96 well plate using a Eppendorf 8 channel adjustable pipette according to the well layout.

14.9 Add 25 µL of each standard, autoclaved and centrifuged LCS, BLANK and sample to respective wells according to the printed template. Put the plate on the fast orbital shaker and shake for 2 min at 450 RPM.

14.10 Adjust the Eppendorf 8 channel adjustable pipette to 145 µL and load the new tips to the pipette. Mix the sample with diluent in the first 8 wells (A1-H1) of glass coated plate by refluxing 5 times with the pipette. Transfer 145 µL mixed solution into the well A1-H1 (first strip) of the QuantiPlate. Change the new pipette tips and repeat the process for the second well strip A2-H2 and so on. The time interval (for mixing/transferring step) between each well strip should be near constant and take about 20~30 seconds.

14.11 Put the plate on the fast orbital shaker and shake for 2 min at 450 RPM, and then cover the plate with parafilm and mix on a low speed orbital shaker for 28 minutes.

14.12 Add 100 µL microcystin-enzyme conjugate to the first strip of wells (A1-H1) and mix by refluxing 5 times with the pipette. Change the new pipette tips and repeat the process for the second well strip (A2-H2) and so on. The time interval (for adding/mixing step) between each well strip should be near the same as the step 14.10.

14.13 Put the plate on the fast orbital shaker and shake for 2 min at 450 RPM, and then cover the plate with parafilm and mix on a low speed orbital shaker for 28 minutes.

14.14 Transfer the first well strip (A1-H1) from the original plate to another well strip hold/plate, empty the well strip into the sink and rinse with wash solution (**Note: don't contact the wells directly using the hand during the washing process**). Transfer the washed well strip to the original plate. Repeat the process for the second well strip (A2-H2) and so on. The time interval (for empting/washing step) between each well strip should be near the same as the step 14.10. Repeat the wash step 5 times. Carefully tap the plate on several paper towels and a couple of Kimwipes to remove wash solution as much as possible.

14.15 Add 100 µL substrate to the first strip of wells (A1-H1) using the Eppendorf 8 channel adjustable pipette. After waiting for about 15 seconds, add 100 µL substrate to the second well strip (A2-H2) and so on.

14.16 Put the plate on the fast orbital shaker and shake for 2 minute at 450 RPM, and then cover the plate with parafilm and mix on a low speed orbital shaker for 28 minutes.

14.17 Add 100 µL stop solution to the first strip of wells (A1-H1) and mix by refluxing 5 times with the pipette. Change the new pipette tips and repeat the process for the second well strip (A2-H2) and so on. The time interval (for adding/mixing step) between each well strip should be near the same as the step 14.15.

14.18 Put the plate on the fast orbital shaker and shake for 2 minute at 450 RPM, and then cover the plate with parafilm and mix on a low speed orbital shaker for 10~20 minutes.

14.19 Remove the parafilm, clean the bottom of the microplate using Kimwipes with 1~2 drops of water and check the bottom to insure it is clean.

14.1 Place the plate in the plate reader. Click on the "Read Plate" icon on the top of the icon toolbar menu. The Plate reading/Runtime Prompts screen appears. In the analyst field put in the user name of the analyst. In the batch filed, enter the batch #.

14.20 Click "Read" and then save the experiment file as the batch #.

14.21 When the run is done, save the experiment file name as the batch # by clicking the save icon on the top icon toolbar menu**.** Check the correlation coefficient, MB, LCSs and duplicate result.

14.22 Click the "Print" icon on the top icon toolbar menu to print the report

14.23 To edit a batch Refer to SFWMD-LAB-SOP-5000.

14.24 To send the data (saved file) and transfer analytical run documentation (autoclave log and printout) to LIMS Refer to SFWMD-LAB-SOP-5000.

14.25 To post data using the autopost pipe refer to SFWMD-LAB-SOP-5000

14.26 Consult your supervisor before making any major changes, adjustments, and/or repairs to the instrumentation.

14.27 Samples that are over range (higher than the highest standard) should be diluted and reanalyzed in the following run. Enter the dilution factor to the program (See 14.7). Dilute the autoclaved and centrifuged sample 5, 25, 100, or 500 times with Laboratory reagent waterdepending on the "estimated" concentration from the undiluted sample during the initial run according to the following table:

Dilution factor	Sample (ml)	Final volume (ml)
5	2	10
25	1	25
100	1	100
500	0.1	50

15.0 Calculations

15.1 The raw data are transformed into B/Bo before the linear regression of the standard curve and sample results calculation by the instrument software.

$$B/B_o = \frac{\text{Absorbance of standard or sample}}{\text{Absorbance of Negative Control}}$$

15.2 The standard curve fits to the linear regression between B/B_0 and logarithm of the standard concentration.

15.3 The recovery of the LCS is calculated as follows:

$$\%\ Recovery = \frac{ObservedValue}{TrueValue} \times 100$$

15.4 The spike recovery is calculated as follows:

$$\%\,\text{Recovery} = \frac{Observed\ \ Spike\ value - (\text{sample conc.} \times 0.99)}{\text{Spike Conc.}} \times 100$$

15.5 The percent relative standard deviation (RPD) is calculated as follows:

$$\%RPD = \frac{|Value\ 1 - Value\ 2|}{(Value\ 1 + Value\ 2)/2} \times 100$$

16.0 Method Performance

16.1 This procedure was validated during method development to performance equivalent to the referenced method. Additional quality controls have been incorporated to assure consistent performance.

17.0 Pollution Prevention

17.1 The reagents and standards used in this method pose little threat to the environment when disposed down the sink with excess tap water.

17.2 Standards and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards and reagents to be disposed.

18.0 Data Assessment and Acceptance Criteria for Quality Control Measures

18.1 The sample holding time is 7 days. Notify your supervisor if any samples are out of the holding time.

19.0 Corrective Actions for Out-of-Control Data.

19.1 See Section 24.2 for corrective actions for out-of control laboratory quality controls. The recommended corrective actions in Table 24.2 are intended as general guidelines to initiate the analytical trouble shooting process. In all cases, if the out-of-control condition persists beyond the second step in troubleshooting, notify your supervisor of the condition and request assistance.

20.0 Contingencies for Handling Out-of-Control or Unacceptable Data

20.1 If the out-of-control situation cannot be resolved by the corrective actions described in Section 24.2, the sample result(s) should be flagged with the appropriate qualifier code.

20.2 It may be necessary to obtain technical service to make repairs to instruments that are producing unacceptable data. See your supervisor for assistance in obtaining this service.

21.0 Waste Management

21.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

21.1 No hazardous wastes are generated by this method. All reagents and standards can be disposed in the sink with running water.

21.2 The samples collected for analysis by this method are not hazardous. They are disposed according to SFWMD-LAB-SOP-5030.

22.0 Instrument Maintenance

22.1 A maintenance logbook for each piece of instrumentation identified in this procedure shall be maintained by the analyst. The maintenance logbook includes the following items:

1. The name or laboratory designation of the instrument and the associated software;
2. The manufacturer’s name, type identification, serial number or other unique identification, and the SFWMD inventory control number;

3. Initial calibration records or other checks to confirm that the instrument complies with the method requirements;
4. The current location of the instrument;
5. Manufacturer's instructions, owner's manuals, or reference to their location;
6. Dates, results and copies of reports and certificates of all calibrations, adjustments, acceptance criteria, and the due date of the next calibration;
7. The maintenance plan as appropriate, and documentation of all maintenance carried out to date, including all routine and non-routine maintenance activities and reference material verifications;
8. Records of any damage, malfunction modification or repair to the instrument;
9. The date received and condition when first placed into service.

23.0 References

23.1 EnviroLogix QuantiPlate™ Kit for Microcystins. Rev.09-11-03.

23.2 Bio-Tek Instruments, Inc. Synergy 2 Mulyi-Mode Microplate reader Operator's Manual.

23.3 Bio-Tek Instruments, Inc. Gent Getting Started Guide Microplate Data Collection & Analysis Software.

23.4 SFWMD-LAB- SOP-5000.

23.5 SFWMD-LAB-SOP-5300

23.6 SFWMD-LAB-SOP-5030.

23.7 SFWMD Comprehensive Quality Assurance Manual, current version.

23.8 Song W., T. Teshiba, K. Rein, and K. E. O'Shea. 2005. Ultrasonically Induced Degradation and Detoxification of Microcystin-LR (Cyanobacterial Toxin). Environ. Sci. Technol. 39, 6300-6305.

24.0 Tables, Diagrams, Flowcharts and Validation Data.

24.1 Tables

Table 1.0 Autoclave Log

TEMP:_____°C

Mycrocystin Sample Autoclave Log

PRESSURE:_____PSI

	Autoclaved By	Date/Time Autoclave Started:
date:	Analysis date:	QC Prep. Date/By:
:	Autoclave:	Analyzed By:

RACK NAME				
Sample ID	**Note**	**Tube #**	**Sample ID**	**Note**
MB		21	sample14	
LCS		22	sample 15	
sample 1		23	sample 16	
MS		24	sample 17	
sample 2		25	sample 18	
sample 3		26	sample19	
sample 4		27	sample 20	
sample 5		28	MSD	
sample 6		29	LCS	
sample 7		30	MB	
sample 8		31	sample 21	
sample 9		32	sample 22	
sample 10		33	sample 23	
MSD		34	sample 24	
LCS		35	sample 25	
MB		36	sample 26	
sample 11		37	sample 27	
MS		38	sample 28	
sample 12		39	sample 29	
sample 13		40	sample 30	

TABLE 2.0 Standard plate tamplate (96-well plate)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bo	Bo	SPL5 Sample2 1	SPL5 Sample2 1	SPL13 Sample10 1	SPL13 Sample10 1	SPL21 Sample14 1	SPL21 Sample14 1	SPL29 LCS 1	SPL29 LCS 1	SPL37 Sample26 1	SPL37 Sample26 1
B	STD1 0.16	STD1 0.16	SPL6 Sample3 1	SPL6 Sample3 1	SPL14 MSD 1	SPL14 MSD 1	SPL22 Sample15 1	SPL22 Sample15 1	SPL30 MB 1	SPL30 MB 1	SPL38 Sample27 1	SPL38 Sample27 1
C	STD2 0.6	STD2 0.6	SPL7 Sample4 1	SPL7 Sample4 1	SPL15 LCS 1	SPL15 LCS 1	SPL23 Sample16 1	SPL23 Sample16 1	SPL31 Sample21 1	SPL31 Sample21 1	SPL39 Sample28 1	SPL39 Sample28 1
D	STD3 2.5	STD3 2.5	SPL8 Sample5 1	SPL8 Sample5 1	SPL16 MB 1	SPL16 MB 1	SPL24 Sample17 1	SPL24 Sample17 1	SPL32 MS 1	SPL32 MS 1	SPL40 Sample29 1	SPL40 Sample29 1
E	SPL1 MB 1	SPL1 MB 1	SPL9 Sample6 1	SPL9 Sample6 1	SPL17 Sample11 1	SPL17 Sample11 1	SPL25 Sample18 1	SPL25 Sample18 1	SPL33 Sample22 1	SPL33 Sample22 1	SPL41 Sample30 1	SPL41 Sample30 1
F	SPL2 LCS 1	SPL2 QC1 1	SPL10 Sample7 1	SPL10 Sample7 1	SPL18 MS 1	SPL18 MS 1	SPL26 Sample19 1	SPL26 Sample19 1	SPL34 Sample23 1	SPL34 Sample23 1	SPL42 MSD 1	SPL42 MSD 1
G	SPL3 Sample1 1	SPL3 Sample1 1	SPL11 Sample8 1	SPL11 Sample8 1	SPL19 Sample12 1	SPL19 Sample12 1	SPL27 Sample20 1	SPL27 Sample20 1	SPL35 Sample24 1	SPL35 Sample24 1	SPL43 LCS 1	SPL43 LCS 1
H	SPL4 MS 1	SPL4 MS 1	SPL12 Sample9 1	SPL12 Sample9 1	SPL20 Sample13 1	SPL20 Sample13 1	SPL28 MSD 1	SPL28 MSD 1	SPL36 Sample25 1	SPL36 Sample25 1	SPL44 MB 1	SPL44 MB 1

24.2 Corrective actions for out-of control laboratory quality controls.

Lab QC Type	Acceptance Criteria	Recommended Corrective Action
Initial Instrument Blank Method Reagent Blank	<MDL response & value	Trouble shoot the instrument and determine cause of error (reagents, calibration standards, environment, equipment failure, etc), correct the problem, then reanalyze.
Initial Calibration Standards	Correlation coefficient ≥0.995	Re-analyze standards. If same response is obtained, trouble shoot the instrument & re-start analysis. If same response is obtained, prepare new standards & re-start analysis.
Quality Control or Check Standards	Accuracy within established limits. See Quality Manual	Re-analyze LCS check standard. If same response is obtained, trouble shoot the instrument & re-analyze. If same response is obtained, prepare new LCS & re-start analysis.
Matrix Spikes	Accuracy within established limits. See Quality Manual	Re-analyze and re-make if needed. If results are not acceptable, spike a different sample.

Note: The recommended corrective actions in Table 24.2 are intended as general guidelines to initiate the analytical trouble shooting process. In all cases, if the out-of-control condition persists beyond the second step in troubleshooting, notify your supervisor of the condition and request assistance.

24.3 Corrective actions for out-of-control field quality control samples.

Field QC Type	Acceptance Criteria	Recommended Corrective Action
Equipment Blank(EB) Trip Blank(TB)	<MDL or less than established limits	Laboratory should re-analyze positive field blanks to confirm results.
Field Duplicates (FD) Replicate Samples (RS) Split Samples (SS)	Precision within limits See Quality Manual (only if values >PQL)	Laboratory should re-analyze failing field QC samples to confirm results.

24.4 Instrument parameters setting in the Gen5 software

Tab	Item	Setting
Reading Procedure	Plate Type	96 well
	Set Temperature	Temperature: Setpoint 26 ºC
	Read	Read: (A) 450
Instrument Sets	Step Label	<default>
	Detection Method	Absorbance
	Read Type	Endpoint
	Read Speed	Normal

CHECKLIST

Parameter / SOP #

Microcystins/SFWMD-LAB-SOP-3170-005

Analysis Date

HBN #

Analyst Review	ANALYST YES	ANALYST NO	SPVR YES	SPVR NO
Initial Calibration and Quality Control criteria met				
Continuing Quality Control and Calibration criteria met				
Sample Analysis				
Sample holding times met				
Reported Data readings within the calibration range				
Samples analyzed out of designated bottle set (if not note in comments)				
Other				
Required forms completed and submitted				
Above cited SOP read and followed				
All reagents and standards documented as required				
Electronic Content				
Post Run Annotations				
All required documents printed / scanned into LIMS				

Comments: _____

_____

_____

_____

_____

Analyst: _____ **Date:** _____

Supervisor: _____ **Date:** _____

QA: _____ **Date:** _____

25.0 **SOP Addendums and Changes**

26.0 Document History

Document Version	Date Issued	Description	Author
3170-002	4/24/09	New revision of SOP	M. Zhou SFWMD
3170-003	3/24/10	New revision of SOP	M. Zhou SFWMD
3170-004	11/9/10	New revision of SOP	M. Zhou SFWMD
3170-005	5/29/13	Updated SOP	M. Zhou SFWMD