

Laboratory
Certification HAB
Rule Update Total
Microcystins
2016

Overview

1. Changes to the method
2. MDL Study
3. Transition from Acceptance to Certification
4. Applications
5. Looking Forward
6. Reporting Requirements

Method Changes

Current method:

Ohio EPA Total (Extracellular and Intracellular) Microcystins -
ADDA by ELISA Analytical Methodology

Version 2.0

January 2015

New Method:

Ohio EPA Total (Extracellular and Intracellular) Microcystins -
ADDA by ELISA Analytical Methodology

Ohio EPA DES 701.0

Version 2.2

November 2015

Sampling

**Version 2.0
January 2015**

**Version 2.2
November 2015**

- 100 mL collected in glass or PETG

- No change

- Quenched immediately with 10mg sodium thiosulfate or ascorbic acid

- Ascorbic Acid removed as an optional quenching agent

- Test 5% of cleaned containers (<RL)

- No change

- Store on ice (0-4°C)

- No change

- Analyze or freeze within 5 days of collection

- Analyze as soon as practical but no later than 5 days from time of collection

- Field duplicate

- No field duplicate

Equipment

Version 2.0
January 2015

- pH Test Strips (pH 0-14)
 - pH Meter not listed

- Chlorine meter
 - Test Strips not listed

- Automated analysis not listed

Version 2.2
November 2015

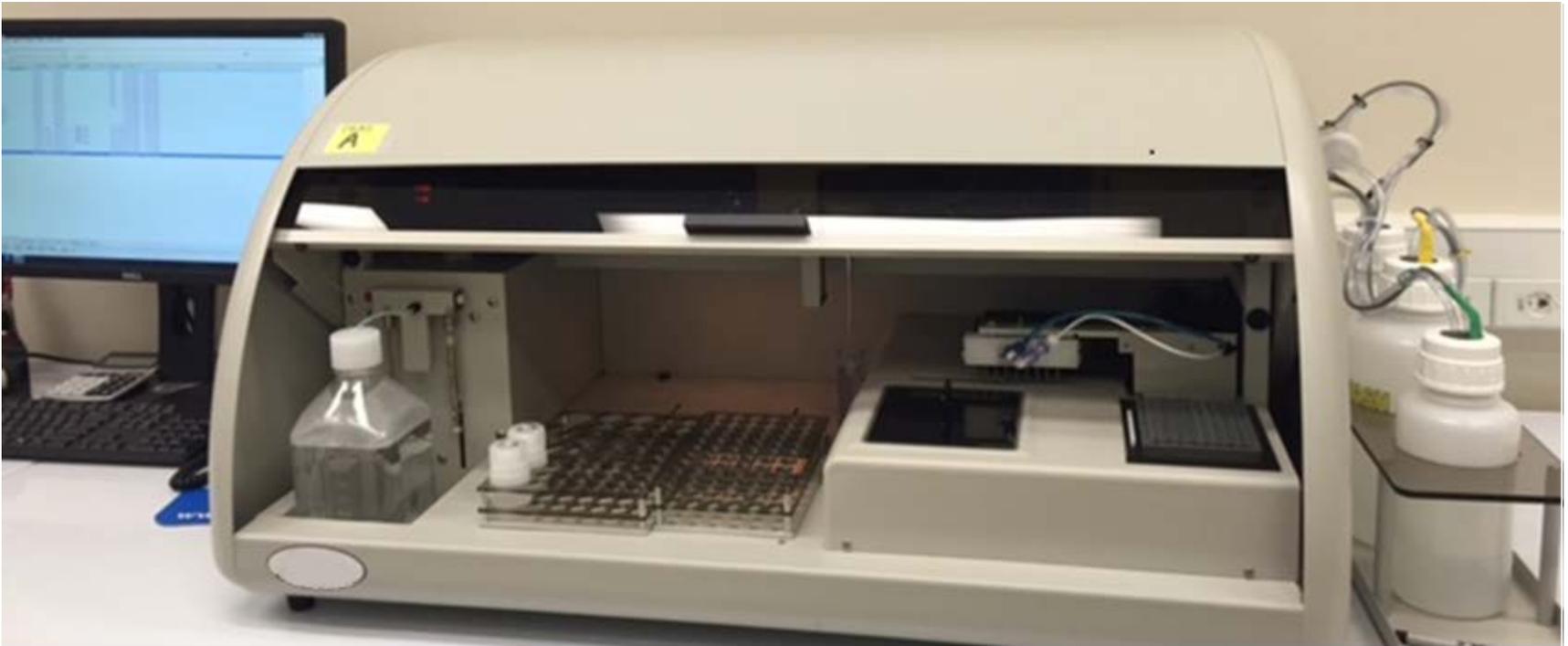
- pH Meter
 - Test Strips: may be used demonstration of capability

- Chlorine meter
 - Test Strips: may be used demonstration of capability

- Automated analysis
 - alternative to manual analysis

Equipment

- Automated analysis – optional



Sample Preparation

Version 2.0
January 2015

Version 2.2
November 2015

- Check pH and adjust as necessary to a pH of 5 to 11

- No Change

- Samples treated with chlorine or other oxidizer:
 - Check for sufficient quenching If residual >0.1 mg/L add 10mg quenching agent
 - Use qualifier (CL) If still not sufficiently quenched and note in report

- Samples treated with chlorine or other oxidizer → Check for residual chlorine:
 - Finished Drinking Water Samples
 - >0.1 mg/L do not analyze
 - **No Qualifier**
 - Insufficiently quenched raw or treatment train samples may be analyzed if qualified (CL)
 - Note: Potassium Permanganate Interference with DPD Methods

Analysis

Version 2.0
January 2015

Version 2.2
November 2015

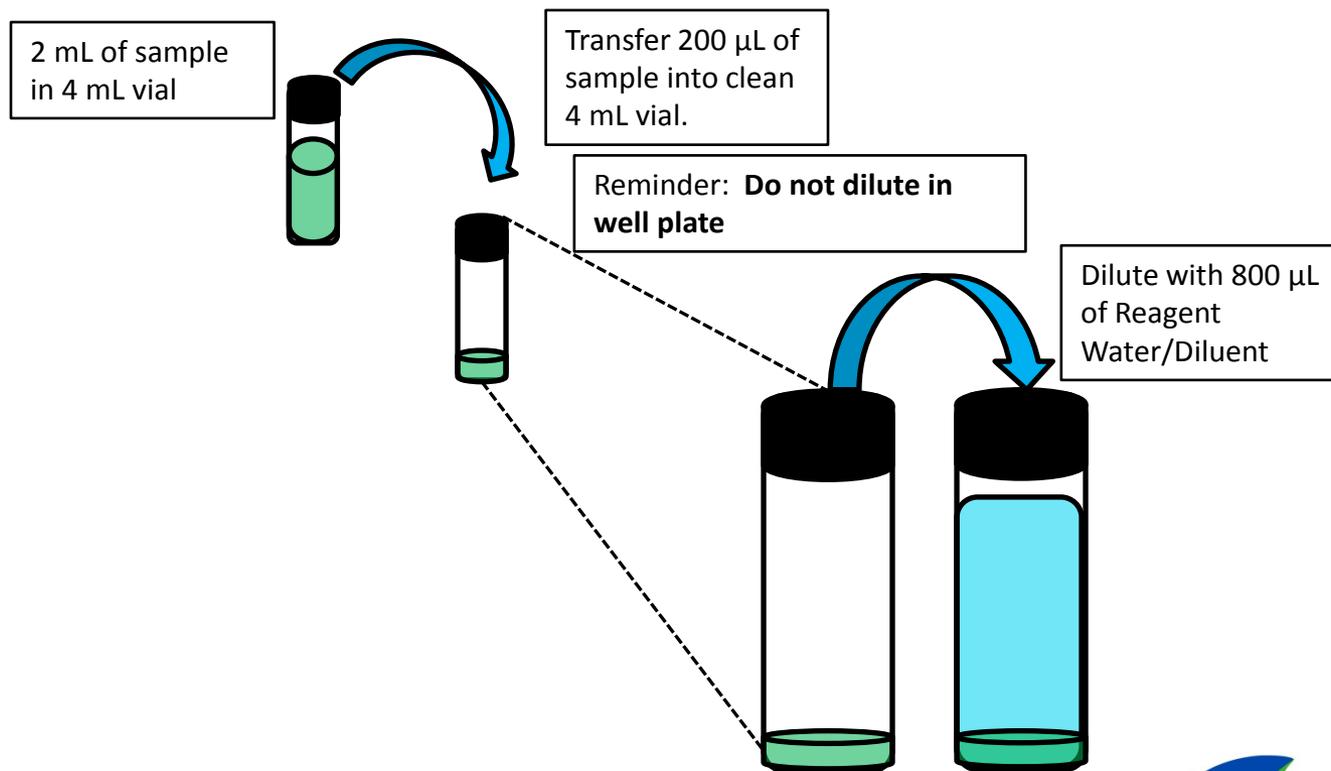
No Major Changes

Updates

- Follow manufacturer's instructions for automated systems
- Perform dilution in vials **NOT** well plates
- **No** laboratory or field duplicates required
- **No** Past Holding Time Qualifier (PT)

Analysis-Using Dilution Example

This sample requires a 1:5 dilution.



Analysis-Using Dilution

Example (Continued)

- Test Report Result for diluted sample = 0.503 $\mu\text{g/L}$
- What result do I report?
 - 0.503 $\mu\text{g/L}$?
 - 5.03 $\mu\text{g/L}$?
 - 2.52 $\mu\text{g/L}$?

Answer

$$\begin{aligned}\text{Reported result} &= 0.503 \mu\text{g/L} \times \text{dilution factor} \\ &= 0.503 \mu\text{g/L} \times 5 \\ &= \underline{2.52 \mu\text{g/L}}\end{aligned}$$

Quality Control and Data Reporting

Version 2.0
January 2015

Version 2.2
November 2015

- Calibration/QC standards and samples analyzed in at least two well replicates
- Curve includes calibration point \leq reporting limit ($0.30\mu\text{g/L}$)
- $(R) > 0.990$
- $(R^2) > 0.980$
- %CV
 - Calibration and QC Standards $< 10\%$
 - Acceptable for one calibration standard $> 10\%$ but $< 15\%$
 - Samples $< 15\%$
 - Use qualifier (J) if $> 15\%$ and note in report

▪ No Change



Quality Control and Data Reporting

Version 2.0
January 2015

Version 2.2
November 2015

- **Laboratory Reagent Blank (LRB)** with each batch must be \leq RL
 - If $LRB > RL \rightarrow$ Reanalyze batch or use Qualifier (B) and note in report

- **Low Calibration Range Check (LCRC)** with each batch
 - If outside acceptance limits ($\pm 40\%$) \rightarrow corrective action/reanalyze batch or use Qualifier (J) and note in report

- **Quality Control Standard (QCS)** with each batch
 - If outside of acceptance limits ($\pm 25\%$) \rightarrow corrective action/reanalyze or use qualifier (J) and note in report

- **Laboratory Reagent Blank (LRB)** with each batch must be \leq RL
 - If $LRB > RL \rightarrow$ Reanalyze batch/Corrective

- **No qualifier**

- **Low Calibration Range Check (LCRC)** with each batch
 - If outside of acceptance limits and reanalysis is not possible use Qualifier (J) for all sample results less than an acceptable Quality Control Standard (QCS)

- **Quality Control Standard (QCS)** with each batch
 - If outside of acceptable limits and reanalysis not possible use Qualifier (J) for all sample results greater than an acceptable LCRC
 - If **both** LCRC and QCS exceed acceptable limits reanalyze or use Qualifier (J) for all sample results.

Quality Control and Data Reporting

Version 2.0
January 2015

Version 2.2
November 2015

- **Laboratory Duplicate** on 5% of samples required
- Field Duplicate on 5% of samples recommended

- Excluded from the method

- **Use Qualifier (PT)** on samples not analyzed within holding time

- Recollect samples past holding time

- **Qualifiers:**
 - (B) Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples
 - (CL) Analytical result is estimated due to ineffective quenching
 - (J) Analyte was positively identified; the associated numerical value is estimated
 - (PT) The reported result is estimated because the sample was not analyzed within required holding time

- (CL) Analytical result is estimated due to ineffective quenching
- (J) Analyte was positively identified; the associated numerical value is estimated

MDL Study

- Determine analyst capability to perform method
- Mathematical representation of instrument sensitivity
- Must be performed annually by each analyst
 - Submit results to OEPA Laboratory Certification Office
- Calculate MDL using 7 replicate aliquots of a standard with a concentration between 1 and 10 times the reporting limit ($0.30\mu\text{g/L}$)
 - Analyze each aliquot in at least two well replicates and report the average of each set
 - Recommend using the $0.40\mu\text{g/L}$ standard on the curve for MDL study
 - MDL for automated system performed initially by each new analyst, then once per year for each instrument

MDL Study

Total Microcystin-ADDA												
	Laboratory Name:				Analysis Date:			Analyst:				
	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 5	LCRC	MDL 4								
B	STD 1	STD 5	LCRC	MDL 4								
C	STD 2	STD 6	MDL 1	MDL 5								
D	STD 2	STD 6	MDL 1	MDL 5								
E	STD 3	LRB	MDL 2	MDL 6								
F	STD 3	LRB	MDL 2	MDL 6								
G	STD 4	QCS	MDL 3	MDL 7								
H	STD 4	QCS	MDL 3	MDL 7								

Report the average for each set of replicates

Microcystins ADDA OH - Assay Calibration Report

Assay Information

Name: Microcystins ADDA OH
 No.: 0.300 - 5.000
 # of decimals: 3
 Assay Substances:

Assay Mode: 4-Parameter Logistic
 Units: µg/L
 Assay Description: ELISA

Standards:
 Std1, Concentration = 0.000, Minimum number to use: 2
 Std2, Concentration = 0.150, Minimum number to use: 2
 Std3, Concentration = 0.400, Minimum number to use: 2
 Std4, Concentration = 1.000, Minimum number to use: 2
 Std5, Concentration = 2.000, Minimum number to use: 2
 Std6, Concentration = 5.000, Minimum number to use: 2
 Curve valid interval: 7 days 0 hours
 Axis Mode: Y = Abs, X = Log(Conc)

Assay Calibration

Current Calibration Status: "

Name	Absorbance	Concentration	Interpretation	Position
29/01/2015 11:18:34				
Std1	1.120 Abs	< 0.000 µg/L		A01
Std1	1.076 Abs	0.016 µg/L		B01
Std2	0.857 Abs	0.137 µg/L		C01
Std2	0.853 Abs	0.139 µg/L		D01
Std3	0.525 Abs	0.503 µg/L		E01
Std3	0.581 Abs	0.406 µg/L		F01
Std4	0.391 Abs	0.923 µg/L		G01
Std4	0.398 Abs	0.889 µg/L		H01
Std5	0.306 Abs	1.605 µg/L		A02
Std5	0.291 Abs	1.825 µg/L		B02
Std6	0.201 Abs	> 5.000 µg/L		C02
Std6	0.205 Abs	> 5.000 µg/L		D02

St	CV	Absorbance	Concentration
Std1 [MEAN]	1.098	1.120	< 0.000
Std1 [SD]	0.031	1.076	0.016
Std1 [%CV]	2.83	1.076	0.016
Std2 [MEAN]	0.855	0.857	0.138
Std2 [SD]	0.003	0.853	0.001
Std2 [%CV]	0.33	0.853	0.001
Std2 [%DIFF]			-8.00
Std3 [MEAN]	0.553	0.525	0.455
Std3 [SD]	0.040	0.581	0.069
Std3 [%CV]	7.16	0.581	0.069
Std3 [%DIFF]			15.09
Std4 [MEAN]	0.395	0.391	0.906
Std4 [SD]	0.005	0.398	0.024
Std4 [%CV]	1.25	0.398	0.024
Std4 [%DIFF]			2.65
Std5 [MEAN]	0.299	0.306	1.715
Std5 [SD]	0.014	0.291	0.156
Std5 [%CV]	4.55	0.291	0.156
Std5 [%DIFF]			-9.40
Std6 [MEAN]	0.203	0.201	1.715
Std6 [SD]	0.003	0.205	0.007
Std6 [%CV]	1.39	0.205	0.007
Std6 [%DIFF]			-100.00

Ascending Order

Descending Order

The %CV for Standard 1 -6 should be <10%.

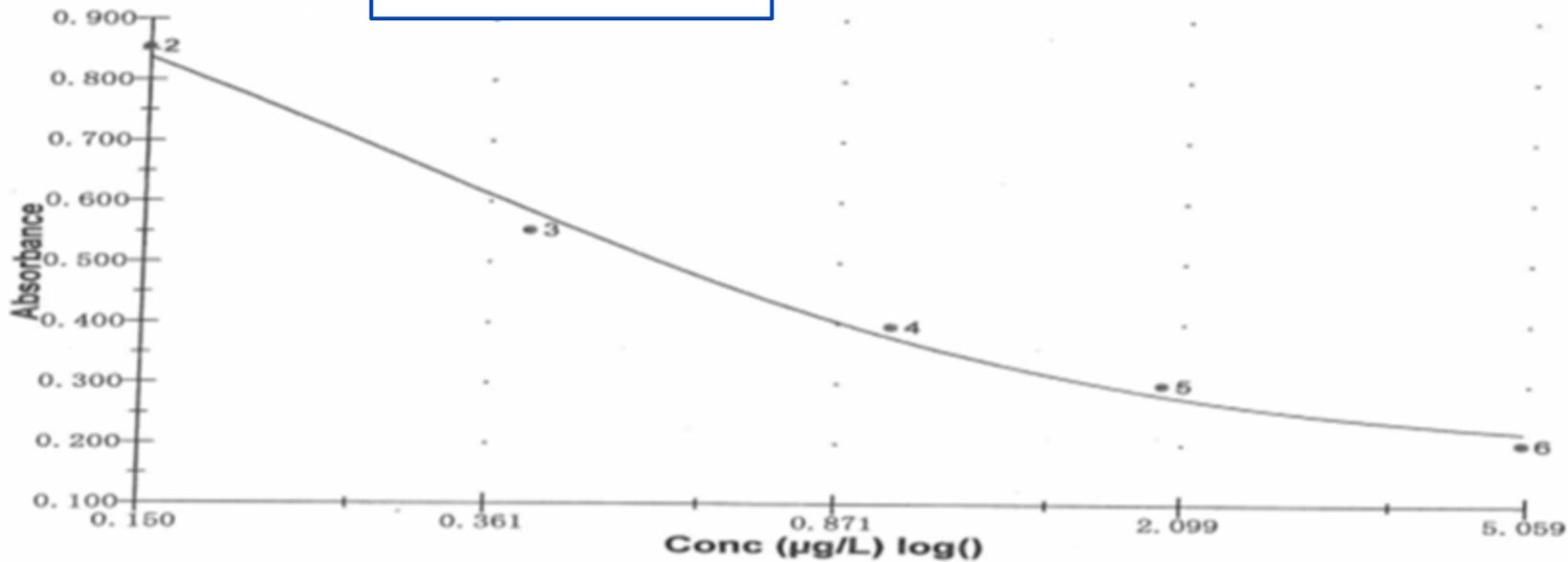
- Blank standard need not be <10%

NOTE: It is acceptable for a single standard to be >10% but it must be <15%.

Assay Curve

$y = (A-D)/(1+(x/C)^B) + D$
A = 0.18730
B = -1.1806
C = 0.31984
D = 1.1022
R2 coef = 0.99606

R^2 must be >0.980
or
R must be >0.990



Test Report

Test Information

29/01/2016 11:18:34

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference
Std1	Microcystins ADDA OH	1.120 Abs	< 0.000 µg/L		0.000
Std1	Microcystins ADDA OH	1.076 Abs	0.016 µg/L		0.000
Std2	Microcystins ADDA OH	0.857 Abs	0.137 µg/L		0.150
Std2	Microcystins ADDA OH	0.853 Abs	0.139 µg/L		0.150
Std3	Microcystins ADDA OH	0.525 Abs	0.503 µg/L		0.400
Std3	Microcystins ADDA OH	0.581 Abs	0.406 µg/L		0.400
Std4	Microcystins ADDA OH	0.391 Abs	0.923 µg/L		1.000
Std4	Microcystins ADDA OH	0.398 Abs	0.889 µg/L		1.000
Std	Microcystins ADDA OH	0.306 Abs	1.605 µg/L		2.000
Std	Microcystins ADDA OH	0.291 Abs	1.825 µg/L		2.000
Std	Microcystins ADDA OH	0.201 Abs	> 5.000 µg/L		5.000
Std	Microcystins ADDA OH	0.205 Abs	= 5.000 µg/L		5.000
LRE (0.000-0.300)	Microcystins ADDA OH	1.137 Abs	< 0.000 µg/L	Out(LR)	0.300 - 5.000
LRE (0.000-0.300)	Microcystins ADDA OH	1.194 Abs [1.1655 (3.5 CV)]	< 0.000 µg/L (< 0.000)	Out(LR) [Out(LR)]	0.300 - 5.000
QCS (0.563-0.937)	Microcystins ADDA OH	0.477 Abs	0.613 µg/L		0.300 - 5.000
QCS (0.563-0.937)	Microcystins ADDA OH	0.515 Abs [0.4960 (5.4 CV)]	0.524 µg/L ((0.567))	11.	0.300 - 5.000
LCRC (0.240-0.560)	Microcystins ADDA OH	0.623 Abs	0.347 µg/L		0.300 - 5.000
LCRC (0.240-0.560)	Microcystins ADDA OH	0.627 Abs [0.6250 (0.5 CV)]	0.342 µg/L ((0.344))	11.0	0.300 - 5.000
MDL 0.4 - 1	Microcystins ADDA OH	0.585 Abs	0.400 µg/L		0.300 - 5.000
MDL 0.4 - 1	Microcystins ADDA OH	0.850 Abs [0.6175 (7.4 CV)]	0.314 µg/L [0.354] (17.1)		0.300 - 5.000
MDL 0.4 - 2	Microcystins ADDA OH	0.553 Abs	0.451 µg/L		0.300 - 5.000
MDL 0.4 - 2	Microcystins ADDA OH	0.609 Abs [0.5810 (5.8 CV)]	0.365 µg/L [0.406] (14.1)		0.300 - 5.000
MDL 0.4 - 3	Microcystins ADDA OH	0.565 Abs	0.431 µg/L		0.300 - 5.000
MDL 0.4 - 3	Microcystins ADDA OH	0.547 Abs [0.5560 (2.3 CV)]	0.462 µg/L [0.446] (4.9)		0.300 - 5.000
MDL 0.4 - 4	Microcystins ADDA OH	0.623 Abs	0.347 µg/L		0.300 - 5.000
MDL 0.4 - 4	Microcystins ADDA OH	0.608 Abs [0.6155 (1.7 CV)]	0.366 µg/L [0.356] (3.8)		0.300 - 5.000
MDL 0.4 - 5	Microcystins ADDA OH	0.643 Abs	0.322 µg/L		0.300 - 5.000
MDL 0.4 - 5	Microcystins ADDA OH	0.556 Abs [0.5995 (10.3 CV)]	0.446 µg/L [0.379] (22.1)		0.300 - 5.000
MDL 0.4 - 6	Microcystins ADDA OH	0.605 Abs	0.371 µg/L		0.300 - 5.000
MDL 0.4 - 6	Microcystins ADDA OH	0.549 Abs [0.5770 (6.9 CV)]	0.458 µg/L [0.412] (14.1)		0.300 - 5.000
MDL 0.4 - 7	Microcystins ADDA OH	0.550 Abs	0.456 µg/L		0.300 - 5.000
MDL 0.4 - 7	Microcystins ADDA OH	0.528 Abs [0.6390 (2.9 CV)]	0.498 µg/L [0.477] (6.2)		0.300 - 5.000

The % CV of all QC samples must be <10%.

All other samples must be <15%.

The mean value of the LRB, QCS and LCRC must fall within the acceptance criteria

Acceptance Criteria	True Value
(0.000-0.300)	0.0
(0.563-0.937)	0.75
(0.240-0.560)	0.40



Reminder: All samples including QC must be analyzed in at least two well replicates

Lot No 15A5759 Exp 31 July 2016

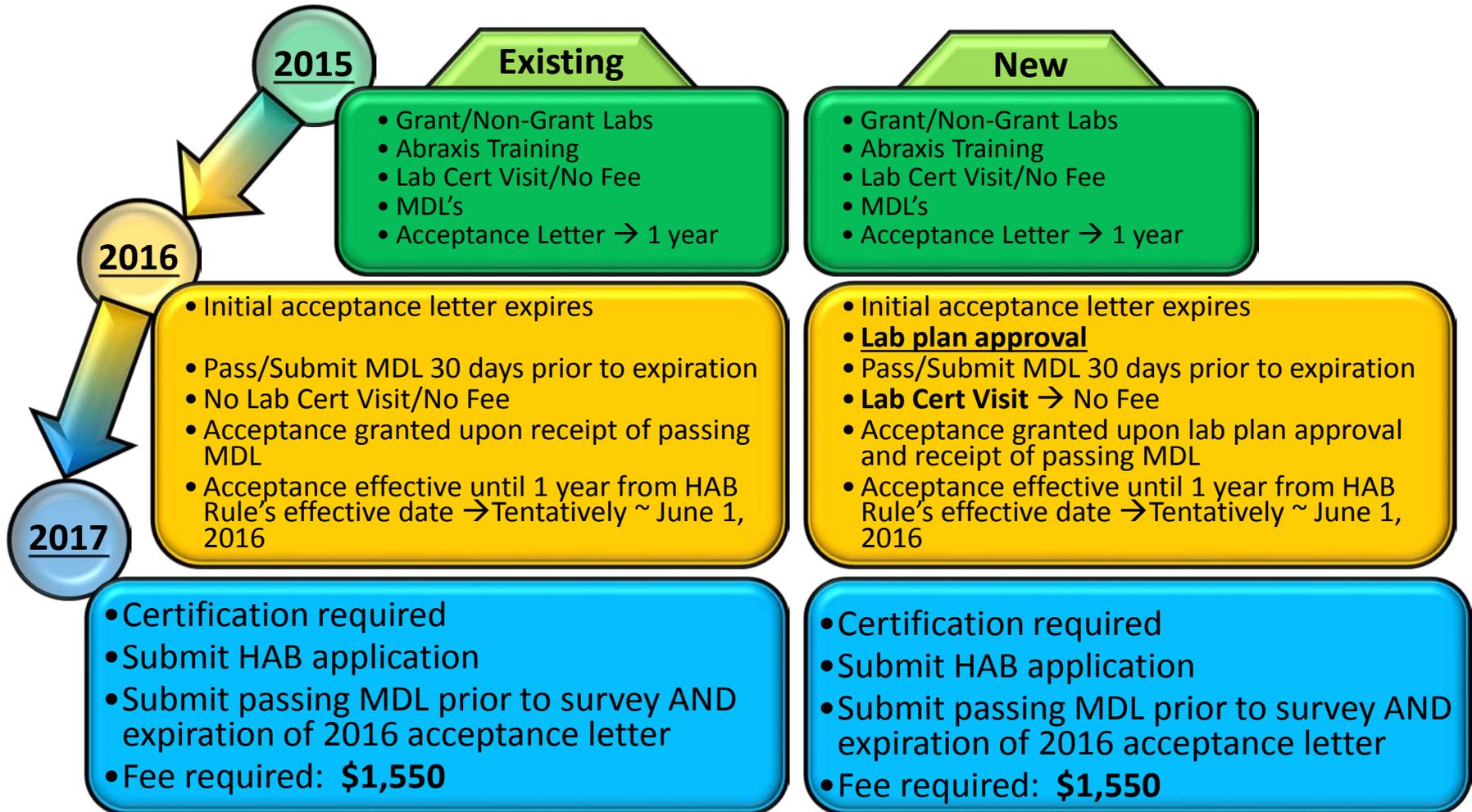
MDL Study

HAB Method Detection Limit Report	
Laboratory Name:	
Date:	
Analyst:	
Sample ID	Aliquot/Reporting Limit Result (ppb or $\mu\text{g/L}$)
MDL 1	0.334
MDL 2	0.305
MDL 3	0.435
MDL 4	0.316
MDL 5	0.360
MDL 6	0.329
MDL 7	0.378
Spike Concentration	0.400
Average	0.351
STD Deviation	0.045
MDL Result	0.140
(must be < 0.295 $\mu\text{g/L}$)	
MDL Acceptable	YES

TRANSITION

Acceptance Letter to Certification

Timeline: Existing Labs vs New Labs



Interim Authorization Approval

- Microcystin **ONLY**
- Submit the following:
 - IA Application
 - MDL Study
 - Raw Data Report
 - Calibration Curve
 - MDL result
- Lab Cert review
- Acceptance - until laboratory's current expiration date



Ohio Environmental Protection Agency

OEPA Office Use Only

Application ID: _____
Received: _____ Approved: _____

Interim Authorization Application for Cyanotoxin Analysis

The applicant affirms the right of the Ohio Environmental Protection Agency (Ohio EPA) to inspect the laboratory, its operations and pertinent records. The applicant agrees that the personnel seeking Interim authorization will fully comply with the policies of the Ohio EPA contained herein. Interim authorization grants acceptance until the accepted laboratory's current expiration date. An on-site evaluation will not be required.

Name of Laboratory: _____

Laboratory Certification Number: _____

Mailing Address: _____

Laboratory Address: _____

Phone Number: _____ Fax Number: _____

E-Mail Address: _____

County: _____ Ohio EPA District: _____

Name of primary contact for the Laboratory: _____
First Middle Initial Last

Fill in the date the acceptance expires: _____

Looking Forward

- Send samples for cyanobacteria screening to Ohio EPA DES – one year from effective date of the rule
 - qPCR Testing performed by DES at no cost to facility
 - Possible facility expenses
 - Collection
 - Shipping – Ohio EPA working on potential options
 - Bottles
 - Preservative if applicable
 - Etc.
- 2017 - qPCR certification possible
 - Send samples to DES until sufficient capacity in state

Total Microcystins Reporting

Total Microcystins Reporting

- Via eDWR
- Analyte Code = MT70
- Routine microcystin sample results must be reported by the 10th day of the following month **IF** the results do not trigger further actions
- The following results must be reported by the **end of the next business day** after analysis:
 - Detections of microcystin in finished water
 - Resample, repeat, daily, and distribution microcystin samples collected because of an action level exceedance
 - Raw microcystin concentrations >5 ppb $\mu\text{g/L}$

Record ID	<NEW>
Lab Sample Number*	12345
PWS ID Number*	OH2599912
Water Facility State Code*	2562342
Sample Point ID*	LT2001
Sample Collection Date*	2/1/2016
Sample Collection Time (HHMM)	630
Lab Receipt Date	2/1/2016
Sample Rejection Reason	
Sample Type*	Routine-Compliance
Pb/Cu Location Type	
Collection Address	Raw tap
Comments	
Analyte Code*	MT70 MICROCYSTINS TOTAL
Analysis Completion Date	2/2/2016
Analysis Completion Time (HHMM)	930
Data Quality	Accepted
Data Quality Reason	
Analysis Method Code	DES 701.0
Less Than Indicator	<
Result	1
Result Unit Code	µg/L
Analytical Lab	8000
Analyst #	1122
QC Date	2/2/2016

- Enter results in eDWR as a Chemical SSR
- Sample Point: EP001 or LT2001
- Analyte Code: MT70| MICROCYSTINS TOTAL
- Facility Code will be the corresponding Facility/STUID
- Method Codes: DES 701.0
Result Units: µg/L
- For specific questions, contact Brian Tarver (614) 644-2752 or Brian.Tarver@epa.ohio.gov

Questions?

Contact

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Charles Vasulka	Charles.Vasulka@epa.ohio.gov	(614) 644-4266

Send all applications to the Laboratory Certification
Mailbox: DWLabCert@epa.ohio.gov