2015 NC DWR Chlorophyll *a* Round Robin

Currently, 94 miles and 15,700 acres of surface waters in North Carolina are impaired due to elevated chlorophyll *a*, a chemical parameter used to assess algal productivity (2014 Final 303(d) List). These are impairments that require Total Maximum Daily Load (TMDL) development and increased regulation, often at significant costs to both the State and the stakeholders in the affected watershed. It is important that the North Carolina Division of Water Resources (NC DWR) understands the quality of the data used to make these decisions.

Because of the lack of performance evaluation samples to test the entire chlorophyll *a* analysis method, NC DWR began a chlorophyll *a* round robin in August 2007 involving the State's certified laboratories as well as other academic and government laboratories. Seventeen participating laboratories in 2007 analyzed eight freshwater samples for chlorophyll *a* concentrations. The first round robin results indicated significant inconsistencies with the quality of the data. The Division used the results of that round robin to work with laboratories and improve analyses.

The data presented within this report represents the ninth chlorophyll *a* round robin on August 27, 2015. Nineteen laboratories participated, each analyzing eight samples. All eight samples were collected from Raleigh-area waterbodies.

Methodology

Sample Collection

On August 27, 2015, NC DWR staff collected a batch of eight surface water grab samples. Samples were placed in light protected carboys and transported on ice to NC DWR's Water Sciences Section (WSS).

At WSS, each of the eight samples was split into nineteen 500 mL subsamples using a churn splitter. Every sample was churned for two minutes prior to splitting and was continually churned during the split. The splitter faucet was purged prior to sample collection. The order in which the subsamples were split from the main sample was randomized in an effort to control bias. Subsamples were put into amber HDPE bottles, then placed on ice and were either delivered to laboratories by NC DWR staff (in-state laboratories) or shipped overnight (out-of-state laboratories) to meet holding times.

Analysis

Participating laboratories were asked to analyze the eight samples according to their Standard Operating Procedures for chlorophyll *a* analysis and to complete a questionnaire concerning the analysis. The answers to most of the questionnaire's questions and the data from the study are found on pages 3 through 9. Analyses of the data are presented graphically on pages 10 and 11. Final interpretation of the data is presented on page 12.

Participating Laboratories

Participating laboratories were referred to by random letter identification throughout the round robin. The order of letters are alphabetical and do not represent the order of the following list.

ALS Environmental – Jacksonville
ALS Environmental – Rochester
Charlotte Water – Environmental Laboratory Services
Environment 1, Inc.
Environmental Conservation Laboratories, Inc. – Orlando
Environmental Research Laboratory, Department of Biology, East Carolina University
EPA Region IV
Florida Department of Environmental Protection
Meritech, Inc.
NC Division of Water Resources Chemistry Laboratory
NCSU Center for Applied Aquatic Ecology
NOAA Center for Coastal Fisheries and Habitat Research
Raleigh, E. M. Johnson Water Plant
Research & Analytical Laboratories
SC Department of Health and Environmental Control
St. Johns River Water Management District
UNC Institute of Marine Sciences
UNCW Center for Marine Sciences – Aquatic Ecology Lab
US Geological Survey National Water Quality Laboratory

NC DWR appreciates the time and cooperation of each participating laboratory.

Chlorophyll *a* Round Robin Analysis Details

Answers from Participants' Questionnaires

Lab ID	Method used	Date samples received	Temperature samples received	Temperature samples stored prior to filtering	Date samples were filtered
B	EPA Method 445.0	08/28/15	3.1°C	4°C	08/28/15
C	SM 10200 H-2011 (Spectrophotometric)	08/27/15	3.1C	<4C	08/27/15
D	SM 10200H	08/28/15	1.2 C	0-6 C	08/28/15
E	EPA 445.0 Rev. 1.2	08/27/15	5.5 deg. C	5 deg. C	08/27/15
F	EPA 445.0	8/28/2015	3.2°C	0.5°C (8/28/15)	8/28/2015
H	fluorometric (non-acidification)	8/27/2015	4 degrees C	4 degrees C	8/28/2015
I	SM10200H	8/28/2015	1.1 degrees C	0-6 degrees C	8/28/2015
J	SM10200H Spectrophotometer	8.28.15	2.4	room temperature	8.28.15
L	SM 10200H-2001	08/28/15	3.1°C	25°C	08/28/15
M	EPA 445Rev. 2.1, modified option (In vitro determination of Chl A- uncorrected)	08/27/15	2.1 C°	23 C°	08/27/15
P	EPA 445.0	08/27/15	on ice	on ice	08/27/15
R	EPA Method 445.0 modified	8/27/2015	1	Filtered immediately, Not Stored	8/27/2015
S	EPA 445.0, Rev. 1.2 (Fluorometric)	08/27/15	1.5°C	3.1°C	08/27/15
T	SM 21st Ed 10200H Chlorophyll	8/28/2015	2.8 C	~1 C	2/28/2015
U	EPA 445.0	08/27/15	Site:Temp°C 662:1.3 232:1.8 802:1.3 585:2.8 167:1.6 333:2.4 779:2.3 472:2.0	0.1-4.4°C	08/28/15
V	Standard Methods 10200H (2011)	08/27/15	On ice	1 to 4˚C	08/28/15
W	EPA Method 445.0 Rev 1.2 Uncorrected	08/28/15	2.6	3.0-4.0	08/28/15
Y	EPA Method 445	08/27/15	on ice	on ice, in walkin coldroom at 4C	08/28/15
Z	EPA 445.0	08/27/15	5.7 deg C	4 deg C	08/27/15

Lab ID	Type of filters used	Brand of filters used	Pressure at which filtered	Volume of sample filtered
B	glass fiber	Whatman (GF/F)	≤6 in Hg	50-100 mL
C	A/E glass fiber 47mm	Millipore	4-6 inches of mercury	250ml
D	47mm glass fiber	VWR	Not measured	100 mL
E	GF/F Glass Microfiber filters 47mm	Whatman	4.5 in. Hg	100ml - 150ml
F	Glass Fiber	Whatman GF/F	-5 kPa	50-100 mL
H	GF/F (glass fiber) 25mm circles	Whatman	5 inches Hg	25-50mL (attached on data sheet next tab)
I	61631	Pall	N/A	250-500mL
J	GF/C	Whatman	not measured	150-480 mL
L	1 micron	Whatman	50 cm Hg	200 to 500 mL
M	GF/F-47mm,Cat#1825-047	GE-Whatman®	< 20 Kpa	50-150 mL
P	Glass Fiber	Millipore	≤6 mm Hg	25 mL
R	25 mm GF/F	Whatman	6 in Hg	25-50 mL
S	GF/C 42.5mm	Whatman	<5 in Hg	53 - 74mL
T	GF/C 42.5 mm Glass Microfiber Filters	Whatman	We do not measure pressure at which samples are filtered	100 - 350 mL
U	47mm Glass Fiber GF/F	Whatman	<6 in Hg	Site:Vol.(mL) 662:200mL 232:100mL 802:50mL 585:100mL 167:100mL 333:50mL 779:100mL 472:100mL
V	glass fiber	Whatman GF/F - 0.7µm porosity, 47 mm diameter	600 to 700 mbar	100 to 200 ml
W	Glass microfiber filter, 25mm, pore size 1.0µm	Pall type A/E	<2 in. Hg	15 ml
Y	Whatman GF/F	Whatman	~7.0 in Hg	95ml-250ml
Z	GF-75, 47 mm	Advantec	< 3 in Hg	100 mL

Lab ID	Describe filtering technique (how were sample volumes measured? were sides rinsed? Etc.)
B	measured in 100 mL glass graduated cylinder; poured into plastic filter tower; cylinder and filter towers sides both rinsed with DI water
C	measured with 250ml graduated cylindar, vaccum filtered, cylinder & funnel rinsed between uses
D	Sample volume measured in graduated cylinder. Poured onto filter. Grad cylinder rinsed and poured onto filter. After filtration, filter folded in half twice and wrapped in aluminum foil and placed in plastic bag with label prior to storage.
E	Volume measured with graduated cylinder. Funnel was not rinsed.
F	50mL aliquots filtered in graduated cyliner. When filtration slows , final volume recorded and sides of cylinder rinsed 3x with DI water
H	samples were poured into 100mL graduated cylinder and then into filter manifold. The cylinder and manifold was rinsed with deionized water between each sample
I	Measured with 500mL measuring cylinder. Cylinder and filter flask rinsed three times with DI water.
J	After being mixed, sample is poured into a 500 mL Class A graduated cylinder to be measured before filtration. Sample is vacuum filtered as quickly as possible. When filtration is nearing the end, 1-2 mL saturated MgCO3 solution is added. Funnel is rinsed thoroughly with DI Water. Filters are folded and wrapped in aluminum foil. Cylinder is thoroughly rinsed after each sample with DI water.
L	Sample volumes were measured in class A volumetric cylinder and poured slowly into the filter funnel. Cylinder and funnel are rinsed well.
M	By Using class A- 250 mL plastic volumetric cylinders.
P	Volume measured in a graduated cylinder, filter funnel sides not rinsed down
R	duplicate aliquots of 50 ml were measured using a 50 ml graduated cylinder, sides of filter towers were not rinsed (we typically measure estuarine samples and do not rinse due to possible osmotic shock and cell lysis)
S	After mixing, sample is poured into a graduated cylinder and volume is recorded. After pouring sample into filter funnel, the sides of graduated cylinder are rinsed twice and poured in funnel. The inside of the funnel is rinsed as the last step.
T	500 mL of sample measured in class A graduated cylinder. Sample poured slowly to determine greastest volume that will filter within 20 minutes. If less than 500 mL filtered, volume calculated by subtracting amount remaining in cylinder. Sides of filtration cups not rinsed.
U	Measured in a TD graduated cylinder, sides not rinsed
V	Samples were measured using a polypropylene graduated cylinder; rinsed cylinder twice with dilute MgCO3 solution
W	Samples filtered using a graduated 15 ml disposable into a filtration manifold (Millipore multi-sample manifold with hand pump capable of maintaining a vacuum up to 6 in. Hg). The blank filter has 15 ml of DI water run through, and all samples filtered using a hand pump. The sample contact surfaces of the filtration manifold is rinsed with DI water between each set of samples
Y	sample volume was measured in a graduated cylinder. Sides of cylinders and filter apparatus were rinsed with deionized water. Samples were filtered to dryness.
Z	Measured volume in a graduated cylinder. Filtered with a hand pump. Rinsed sides of filtration unit and graduated cylinder with DI water. Filtered the DI water through the filter.

Lab ID	Homogenization technique prior to filtering	How long were samples filtered?	Lighting conditions during filtering
B	shaking bottles several times prior to each aliquot measured	about 30-60 seconds per sample	dimmed fluorescent (25% of full lab lighting)
C	Shaken	<5 mins.	Darkroom w/ green light
D	Shaking sample bottle	Approximately 35 seconds	Ambient lab lighting, in hood with light off.
E	shake vigorously several times	1- 5 minutes	dark room with green lights
F	Samples inverted 4 times	Approx. 3 minutes	Dark Room with Red Lights
H	n/a	3 to 7 minutes	sunlight through the windows, lab lights were turned off
I	Shaken	2.75-3 minutes	Fluorescent lighting
J	Sample bottle is vigorously shaken by hand before filtration.	1min54sec-8min46sec	Filtration is done with regular overhead lighting. (Intensity Range 20-30 ft-candles)
L	Samples are shaken well before filtering.	15 to 26 mins	Adequate and bright room lighting
M	Lightly inverted back and forth approx. 20 times.	4-9 min	Low intensity Green light
P	Gentle shaking for 10 secs	10-30 seconds	low ambient light from windows
R	briskly inverted bottle ~10 times	1-2 minutes	lights turned off, blinds closed
S	Samples gently inverted 10 times	5 - 10 sec	All overhead lights off, two small lamps with 25 watt green bulbs.
T	mix sample volume 3 times between sample bottle and 500 mL graduated cylinder	5 - 15 minutes	normal/ subdue lighting of room
U	Sample bottle shaken by hand for 5-10 seconds	3 minutes or less	Dark room with subdued green LED lighting
V	Gently invert 4 to 6 times	1 to 2 minutes	lights off, blinds down and closed, door closed
W	Sample bottles inverted gently 12-15 times	30-45 seconds	Dark room with subdued yellow lighting
Y	sample bottle inverted 3x	typically less than 1 minute/sample; some up to 3 minutes.	ambient outside light; blinds closed as well, lights off
Z	Gently inverted the bottle several times	1-2 minutes	Overhead fluorescent lights

Lab ID	Extraction solvent, purity, and volume used	Length of time samples were stored after filtering	Steeping time	Was grinding used?
B	90% HPLC-grade acetone, 25 mL	12 days	20 hours	yes
C	90% acetone 10% DI water Water Purity 99.7% @10 mls used	Ground Immediately	Overnight 1600 to 0900	Yes
D	10 mL of acetone:DI (90:10)	4 days	at least 2 hours	yes
E	90% Acetone, Baker analyzed-ACS reagent grade, 25ml	14 days	20 hrs.	Yes
F	90% Acetone, Type 1 Water	13 Days	20.2 Hours	Yes
H	90% Acetone : 10% water 7.5mL for each sample	3 days	24 hours	yes
I	90/10 Acetone/MgCO3. The acetone is chromatography grade and the MgCO3 is reagent grade and filtered through a 0.45um filter. 10mL of Acetone/MgCO3 solvent was used to extract the sample.	11 days	4hrs 30min	yes
J	90% Acetone with 10% MgCO3 solution. Extract has a final total volume of 8 mL.	5days 23hrs 5mins	23hrs 40 mins	yes
L	90% Acetone	10 days	4.5 hours	Yes
M	90% Acetone- 25 mL	18 min.	6:03:00 hours	Yes
P	Acetone,90%, 10 mL	15 days	6.5 hrs	yes
R	90% reagent grade acetone	21 days	24h	yes
S	90% acetone, Fisher Scientific Certified ACS, 14mL	6 days	20 hours	Yes
T	5 mL of 90% acetone (HPLC grade)/ 10% saturated MgCo3	~65 hours	~2 hours 15 minutes	yes
U	90% Acetone, Optima grade, 25 mL	5 Days	18.5 Hours	Yes
V	90:10 Acetone:saturated MgCO3; reagent grade	13 days	2 hours	Yes
W	90% Acetone HPLC grade, with DI water, 12 ml	6 days	22-23 hours	Yes
Y	90% acetone, 12ml	15 days	2 hours	Yes
Z	90 % Acetone/ 10 % Water Solution,	6 days	Approx: 22 hrs	yes

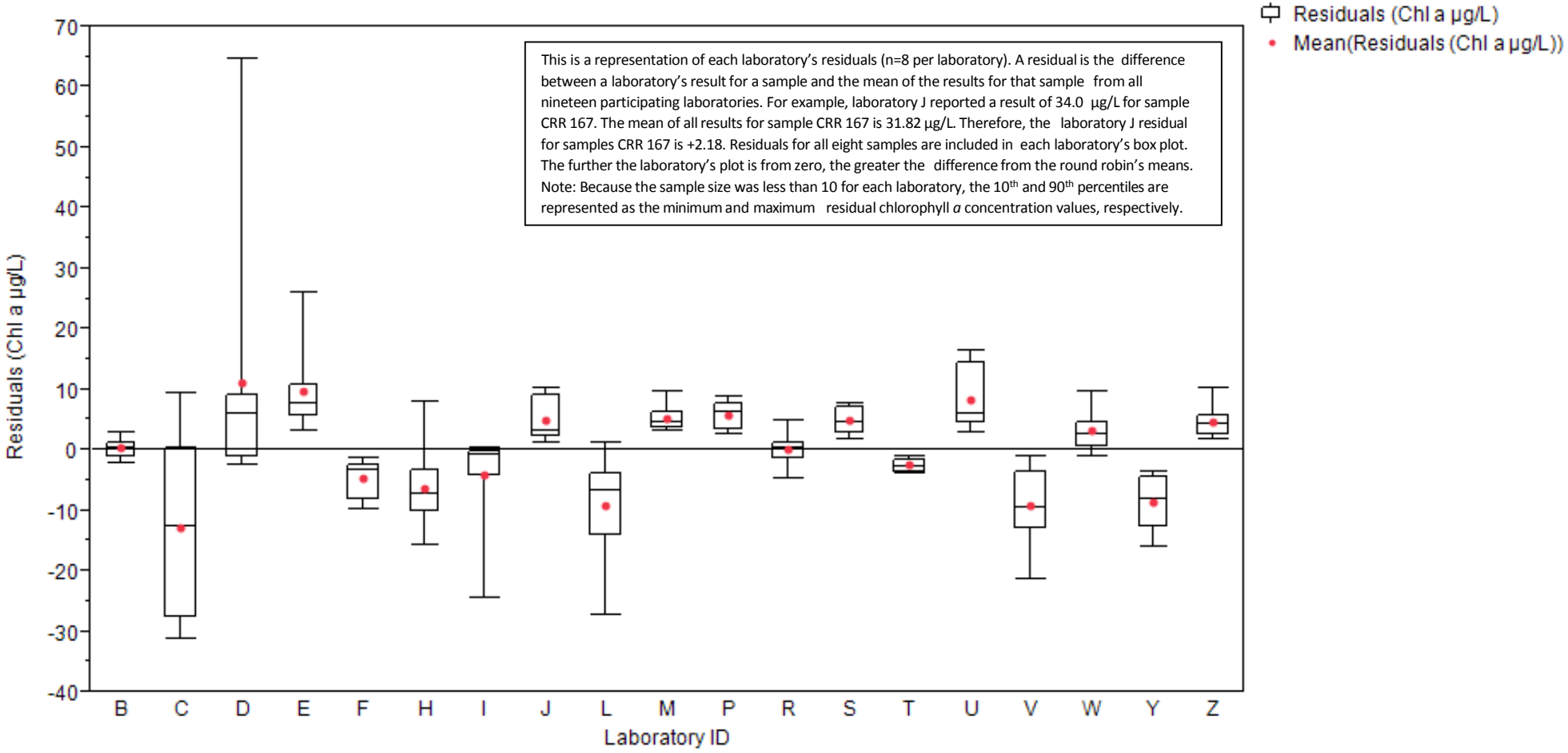
Lab ID	Description of grinding setup
B	teflon grinding pestle attached to rotor; glass test tube; temperature controlled by feel
C	Arrow 850 motor 1/10 hp Kontes tissue grinder pestle SZ 24 and matching tube No temperature control
D	Tissue Grinder with Teflon tip in glass vessel (wrapped in foil). Temperature not controlled. Slurry transferred to centrifuge tube.
E	Teflon pestle with radial serrations on tip; temperature controlled by touch/feel
F	Tissue Grinder. Sample in plastic centrifguge tube. Temperature controlled to prevent evaporation
H	Teflon tissue grinder was used with a drill to grind the filter and 7.5mL of acetone completely (30seconds) Temperature was not controlled
I	Drill press with a teflon grinding tip. Not temperature controlled, ambient temp.
J	Filter is rolled up and placed in a 30 mL glass tube that is kept on ice between samples (to minimize heat from friction). An Eberbach power unit with a Wheaton Tissue grinder is used to grind sample down with solvent. The slurry is added to a centrifuge tube. The 30 mL grinding tube is rinsed with solvent until clean and added to the centrifuge tube. The grinder is rinsed with solvent a second time and added to the centrifuge tube. If slurry in centrifuge tube is less than 8 mL, the volume is brought up to 8 mL with solvent. If the slurry in the centrifuge tube is greater than 8 mL, solvent is used to bring the volume up to the nearest whole number. Samples are steeped in refrigerator.
L	Samples are ground using a teflon tip in a glass test tube for 1 minute with 3mL of 90% Acetone solution. Then sample was transferred into a 25mL screw top centrifuge tube with an additional 7mL of 90% Acetone. Grinding occurs at room temp and was not temperature controlled.
M	Teflon® pestle (50 mm X 20 mm) with grooves in the tip with ¼" stainless steel rod long enough to chuck onto a suitable drive motor and 30 ml capacity plastic grinding tubes- temperture controled (No built in thermometer)
P	A teflon tip tissue grinder is attached to a motor and the filter is ground in a 50 mL centrifuge tube till completely macerated. Temperature does not rise signifcantly as felt when holding the tube.
R	Teflon (PTFE) tissue grinder, temperature was not controlled however grinding time was very short ~ 15 seconds per sample to prevent heating of the acetone/ filter slurry
S	Pro Scientific stainless steel tip homogenizer, glass grinding vessel, temperature was not controlled
T	Pro DPS 20 Homogenizing System, in 15 mL vial, not temperature controlled
U	Ground in a glass mortar. Pestle has round, serrated Teflon pestle. Unit powered by an electric drive motor. Temperature monitored by feel, sample was not allowed to heat.
V	Tear filter into quarters and place into grinding tube (Kimble # 886000-0023) with PTFE grooved tip; samples mascerated using electric lab mixer (Arrow Engineering - Model 1750); temperature not controlled
W	Tissue grind pestle (Kontes®size 21), on stainless steel shaft fit into drive motor, with grooves in the Teflon tip. Round-bottom glass grinding tubes (Kontes®size 21) that match pestle. Temperature was not controlled, but monitored by touch.
Y	Teflon (PTFE) tissue grinder with radial serrations on tip, powered by electric drill. Temperature not controlled - samples were removed from -20oC freezer, ground for approximately 30 seconds, and placed in dark box with ice packs.
Z	drill with a Teflon® pestle with grooves, 50-mL polypropylene conical tubes

Lab ID	Were samples acidified? If so, what type, concentration, and volume?	Type of calibration standard and source
B	no	chlorophyll *a* from Anacystis (Sigma C6144); chlorophyll a from spinach (Sigma C5753)
C	No	N/A
D	0.06 mL 0.1N HCl into a 2 mL extract. Measured with and without acidification.	Initial Calibration: Turner Designed foil wrapped sealed ampules at nominal concentrations of 20 and 200 ug/L, diluted as needed for a range of 4-200 ug/L. Daily cal check: Solid Secondary Standard Turner P/N 8000-952
E	No	Fluorometric Chlorophyll standard, Turner Designs
F	No	Turner Designs Chlorophyll A and B Standard
H	no	chla pigment standard (sigma aldrich)
I	0.1mL of 0.1N HCL	N/A
J	Samples are acidified with 100 uL of 0.1 N HCl, mixed with a mini-mixer, and timed for 90 seconds.	A 0.20 mg/L concentration of chlorophyll-a standard is read at the beginning of each batch. The standard is made from Sigma Chlorophyll-a from spinach 5 mg powder (Cat# C5753-5MG). The standard read at 112% recovery.
L	Yes, with 0.1mL of 0.1N HCl	N/A
M	N/A	Sigma chemicals®- Anacystis Nudulans Algae.
P	no	Liquid Standard made with purified Chla from Anacystis, Sigma Aldrich C6144-1mg
R	no	Primary standard was pure chl-a extracted from Anacystis nidulans (Sigma) dissolved in HPLC grade 90% acetone. This standard was used to determine chl-a equivalent of Turner Designs solid secondary standard which is used to account for instrument drift (should be minimal with LED excitation source). Solid standard is run immediately before measurement of environmental chl-a extracts.
S	No	Chl a from Anacystis nidulans, Sigma C6144
T	Yes, samples acidified with 100uL 0.1 M HCl	quarterly check: PerkinElmer Secondary Spectrometric Calibration Standards daily check: Chlorophyll a std made using Anacystis nidulans algae (Sigma-Aldrich)
U	Not Applicable	Turner Designs Fluorometric Chlorophyll Standard
V	Yes, 0.1 N HCl - used 0.1 ml per 3 ml of extract	Turner Designs P/N 10-950 (20 ml ampule of known concentration)
W	No	Turner Designs Fluorometric Chlorophyll Standard. Daily verification standards used: Turner Designs solid secondary standard (10-AU-904)
Y	No	chlorophyll powder isolated from Anacystis nidulans dissolved in 90% acetone and spectrophotometrically analyzed using Jeffrey Method (1997) to determine concentration; purchased from Turner Designs
Z	0.1 N HCl solution, 135 uL to 4.5 ml of sample	Chlorophyll a free of chlorophyll b Neat, Sigma

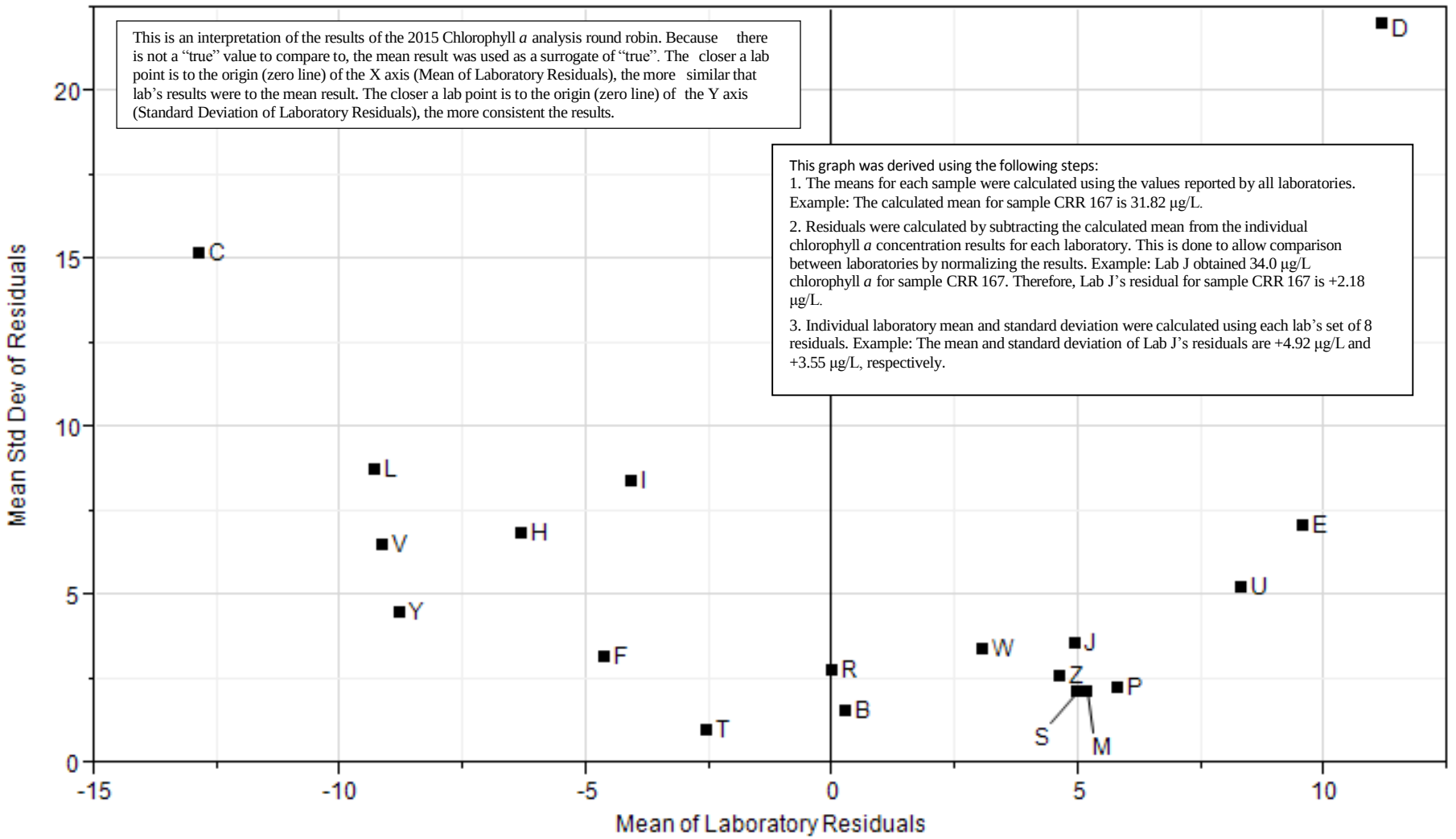
Notes:

1. Answers to the questionnaire are entered as the laboratory presented them unmodified, except for spacing.
2. Additional information obtained from participating laboratories but not included in this report: time samples were filtered, make and model of instrument, instrument bandwidth(s) and wavelength(s), time between acidification and analysis by instrument, and notable differences between samples.

2015 Chlorophyll *a* Round Robin Laboratory Box Plots of Laboratory Residuals



2015 Chlorophyll *a* Round Robin Laboratory Residual Mean vs. Standard Deviation



August 2015 Chlorophyll a Round Robin Results

Values reported by laboratories participating in the Round Robin, as well as the mean, median, and standard deviation (Std Dev) for each sample, are displayed in the Table below, in µg/L. Acceptance ranges (PT Min to PT Max) were calculated using NELAC Proficiency Testing (PT) methods[1] for microbiological parameters in non-potable water.

Lab ID	CRR 167	CRR 232	CRR 333	CRR 472	CRR 585	CRR 662	CRR 779	CRR 802
B	32.9	54.5	68	22.1	33.1	21.7	56.1	69.2
C	26.2	34.1	36.7	25.1	28.3	33.3	27.7	41.3
D	31.6	60.6	77.7	22.1	39.5	21.3	58.3	134
E	38.8	61.96	93.87	29.31	37.84	26.88	64.33	79.18
F	29.3	48.5	58.1	20.8	27.9	22.6	50.7	60.5
H	24.41832	43.13141	59.26273	16.14975	25.81714	21.34092	37.56722	77.24821
I	30.9	52.5	67.8	23.7	26.9	23.7	52.4	45
J	34	64	75	26	35	25	63	73
L	25	55	53	20	27	17	42	42
M	36	59	73	27	36	27	60	79
P	35	60	76	26	39	28	62	76
R	33.14	54.57	67.74	21.96	32.77	22.65	58.04	64.54
S	36.3	61	74.7	25.9	36.6	25.7	60.8	74.2
T	30.061	52.718	64.184	20.774	30.604	20.066	50.408	66.208
U	37.6	64	84.4	28	36.9	26.6	59.4	85.1
V	30.7	40.1	57.4	20	22	20	44.1	48.1
W	33.06	58.45	77.51	22.26	33.27	24.26	57.73	73.51
Y	24.89	43.03	51.82	19.92	25.59	20.16	43.65	56.26
Z	34.63	56.53	73.62	27.53	36.84	25.67	63.43	74.44
Mean **Median**	31.82 32.90	53.88 55.00	67.88 68.00	23.40 22.26	32.15 33.10	23.84 23.70	53.25 57.73	69.41 73.00
Std Dev	4.34	8.52	13.13	3.47	5.26	3.74	10.09	20.64
PT Min	20.60	31.57	35.20	14.65	19.01	14.80	27.03	28.68
PT Max	48.23	89.51	125.79	36.59	52.95	37.53	100.66	155.61

Note: Data values are shown with significant figures as reported by laboratories.

[*] EPA/600/R-04/003, table available at http://nelac-institute.org/fopt.php, full document available at http://nelac-institute.org/docs/2003nelacstandard.pdf