2016 NC DWR Chlorophyll *a* Round Robin

Currently, 4 miles and 95,145 acres of surface waters in North Carolina are impaired due to elevated chlorophyll *a*, a chemical parameter used to assess algal productivity (2016 Draft 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 tenth chlorophyll *a* round robin. Twenty laboratories participated, each analyzing eight samples. All eight samples were collected from Raleigh-area waterbodies.

Methodology

Sample Collection

On June 28, 2016, NC DWR staff collected a batch of eight surface water grab samples from six local waterbodies. 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 twenty 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 and 12. Statistical analyses and results of the data are presented graphically on pages 10 and 11.

Participating Laboratories

Participating laboratories were referred to by random letter identification throughout the round robin. The order of letters is alphabetical and does 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
ETT Environmental, Inc.
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
Santee Cooper Public Service Authority
SC Department of Health and Environmental Control
St. Johns River Water Management District
UNC Institute for 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 Participant's Questionnaires

Lab ID	Method used	Date samples received	Temperature samples received	Temperature samples stored prior to filtering	Date samples were filtered
B	SM10200H	06/29/2016	2.5°C	Not stored	06/29/2016
D	EPA Method 445.0 REV 1.2 uncorrected	6/29/2016	2.1°C	3.0°C	6/29/2016
E	SM10200H	6/29/2016	3.0 degrees C	0-6 degrees C	6/29/2016
F	EPA 445.0	6/28/2016	4.8'°C	4.0°C	6/28/2016
G	EPA Method 445.0	6/29/2016	1.7°C	4°C	6/29/2016
H	EPA 445.0	6/28/2016	4 degrees C	4 deg C	6/28/2016
J	EPA 445.0	6/28/2016	on ice	on ice	6/28/2016
K	EPA 445.0 Rev. 1.2	6/28/2016	2.2 deg. C	4.0 deg. C	6/28/2016
M	EPA Method 445.0 modified	6/28/2016	4.5 C	4 C	6/28/2016
N	EPA 445.0, Rev. 1.2 (Fluorometric)	6/28/2016	no temp blank provided, portion taken from sample #551 read 10.6°C	2.6 C	6/28/2016
O	SM 10200H-2001	06/29/2016	-0.4°C	25°C	06/29/2016
P	EPA 445	6/28/2016	< 6.0 Celsius	0.8 Celsius	6/28/2016
Q	SM10200H-2011	6/28/2016	3.1C	Filtered immediately	6/28/2016
R	SM 21st Ed 10200H Chlorophyll	6/29/2016	2.7 C	< 6 C	6/29/2016
S	EPA Method 445	6/29/2016	1.3 c	N/A	6/29/2016
T	Fluorometric (non-acidification)	6/28/2016	4 degrees C	4 C	6/29/2016
V	SM10200H	6/29/2016	0.6	4.1	6/29/2016
X	Standard Methods 10200H (2011)	6/28/2016	On Ice	Not Stored	6/28/2016
Y	SM10200H Spectrophotometer	6/29/2016	2.4° C	Room temperature	6/29/2016
Z	EPA 445.0	6/28/2016	15:20	0.1-4.4°C	06/29/16

Lab ID	Type of filters used	Brand of filters used	Pressure at which filtered	Volume of sample filtered
B	47mm glass fiber	VWR	Not measured	100 mL
D	Glass fiber filter, 25mm, pore size 1.0 µm	Pall type A/E	<6 in. Hg	15 mL
E	61631	Pall	N/A	50-300mL
F	Glass Fiber	Whatman GF/F	-5 kPa	50-200 mL
G	glass fiber	Whatman (GF/F)	≤6 in Hg	25-100 mL
H	GF-75, 47 mm	Advantec	< 3 in Hg	100 mL
J	Glass Fiber	Millipore	< 6 mm Hg	25 mL
K	GF/F Glass Fiber filters 47mm	Whatman	4.5 in. Hg	150 ml
M	25 mm GF/F	Whatman	6 in Hg	25-50 mL
N	GF/C 42.5mm	Whatman	< 5 in Hg	27 – 93mL
O	1 micron	Whatman	50 cm Hg	100 to 480mL
P	GF/F- 47mm, Cat#18 25-047	GE-Whatman®	< 20 Kpa	50 – 150 mL
Q	A?E glass fiber 47mm	Millipore	4-6 inches of mercury	250mls of all samples except ID#CRR 230. 125mls of that one.
R	GF/C 42.5 mm Glass Microfiber Filters	Whatman	We do not measure pressure at which samples are filtered	100-250 mL
S	Glass Micro Filters Diameter 25 mm	Whatman	5 in-hg	20ml-50ml
T	GF/F (glass fiber) 25mm circles	Whatman	5 inches Hg	25-50mL (attached on data sheet next tab)
V	HAWP 04700 nitrocellulose 0.45u	Millipore	pressure not measured	175-300 mL
X	Glass Fiber	Whatman GF/F – 0.7 um porosity, 47mm diameter	600 to 700 mbar	100 to 200 ml
Y	GF/C	Whatman	Not Measured	115-355 mL
Z	47mm Glass Fiber GF/F	Whatman	<6 in Hg	50 mL – 100 mL filtered

Lab ID	Describe filtering technique (how were sample volumes measured? were sides rinsed? Etc.)
B	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
D	Samples were filtered using a 15 mL graduated disposable syringe fit to a syringe dispenser (Nichiryo Model 8100). Samples were filtered through a filtration manifold (Millipore multi-sample manifold) using a hand pump capable of maintaining a vacuum up to 6 in. Hg. A laboratory reagent blank was filtered last using 15 mL of DI water. The surfaces of the filtration manifold that were contacted by the samples were thoroughly rinsed with DI water between each set of samples.
E	Measured with 500mL measuring cylinder. Cylinder and filter flask rinsed three times with DI water.
F	50mL aliquots filtered in graduated cylinder. When filtration slows, final volume recorded and sides of cylinder rinsed 3x with DI water
G	measured in 100 mL glass graduated cylinder; poured into plastic filter tower; cylinder and filter towers sides both rinsed with DI water
H	Measured volume in a graduated cylinder. Filtered with a hand pump. Rinsed sides of filtration unit and graduated cylinder with DI water.
J	Volume measured in a graduated cylinder, filter funnel sides not rinsed down
K	Volume measured with graduated cylinder. Funnel was not rinsed.
M	duplicate aliquots of 25-50 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)
N	After mixing, sample is poured into a 100mL glass 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.
O	Sample volumes were measured in class A volumetric cylinder and poured slowly into the filter funnel. Cylinder and funnel are rinsed well.
P	Filter was put on the filter base, funnel on the top. Sample inverted several times, then measured using a class A graduated cylinder. Cylinder and filter were rinse with deionized water. Pour in to funnel. Once everything passed through the filter. The filter is fold in half then placed in 50 mL centrifuge tube which is cover with aluminum foil and put in freezer.
Q	measured with 250 ml graduated cylinder, vacuum filtered, cylinder and funnel rinsed between uses
R	500 mL of sample measured in class A graduated cylinder. Sample poured slowly to determine greatest 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.
S	Use a disposable volumetric pipet to transfer the subsample into the filter tower of the filtration apparatus and apply vacuum. A sufficient volume has been filtered when a visible green or brown color is apparent on filter. Rinse filter tower thoroughly after each sample with laboratory grade DI water.
T	volumes measured with graduated cylinder, sides of filter funnels were rinsed with deionized water
V	filtrate volume measured by graduated cylinder after filtration
X	Samples were measured using a polypropylene graduated cylinder, rinsed cylinder twice with $MgCO_3$ solution
Y	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 $MgCO_3$ 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.
Z	Measured in a class A graduated cylinder, sides not rinsed

Lab ID	Homogenization technique prior to filtering	How long were samples filtered?	Lighting conditions during filtering
B	Shaking sample bottle	~30 seconds	Ambient lab lighting, in hood with light off.
D	Sample bottles gently inverted 12-15 times	30-45 seconds	Dark room with subdued yellow lighting
E	Shaken	~1min	Fluorescent lighting
F	Sampled inverted 4 times	Up to 6 minutes	Darkened Room with Red Light
G	Shaking bottles several times prior to each aliquot measured	About 30-60 seconds per sample	Dimmed fluorescent (25% of full lab lighting)
H	Gently inverted the bottle several times	1-2 minutes	Overhead fluorescent lights
J	Gentle shaking for 10 secs	10-30 seconds	Low ambient light from windows
K	Shake vigorously several times	1 – 5 minutes	Dark room with green lights
M	briskly inverted bottle ~10 times	1-2 minutes	Lights turned off, blinds closed
N	Samples gently inverted 10 times	5 – 10 sec	All overhead lights off, two 25 watt green bulbs
O	Samples are shaken well before filtering.	7 to 21 mins	Adequate and bright room lighting
P	Lightly inverted back and forth approx. 20 times.	4-9 min	Low intensity Green light
Q	shaken	<5 mins	Darkroom w/ green light
R	Mix sample volume by inversion of sample container 7 times.	2-23 minutes	normal/subdue lighting of room
S	Thoroughly but gently invert the sample container	30 sec-1min 30sec	Subdued light
T	n/a	3 to 7 minutes	sunlight through the windows, lab lights were turned off
V	shaking of sample bottle	10 min	Low light; 40 watt amber light
X	Gently invert 4 to 6 times	1 to 2 minutes	Lights off, blinds down and closed, door closed
Y	Sample bottle is vigorously shaken by hand before filtration.	2min 36sec – 9min 47sec	Filtration is done with regular overhead lighting. (Intensity Range 20-30ft-candles)
Z	Sample bottle inverted by hand from 5-10 seconds	3 minutes or less	Dark room with subdued green LED lighting

Lab ID	Extraction solvent, purity, and volume used	Length of time samples were stored after filtering	Steeping time	Was grinding used?
B	acetone:DI (90:10)	8 days	2 or more hours	yes
D	90%-HPLC grade Acetone/10% DI Water, 12 mL	12 days	23 hours	yes
E	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.	13 days	5 hours	yes
F	90% Acetone, Type 1 Water	7 Days	14.5 Hours	Yes
G	90% HPLC-grade acetone, 25 mL	15 days	20 hours	yes
H	90 % Acetone/ 10 % Water Solution	24 hrs	Approx: 21 hrs	Yes
J	Acetone, 90%, 10 mL	23 days	4.25 hours	yes
K	90% Acetone, Baker analyzed-ACS reagent grade, 25ml	15 days	23.5 hrs.	Yes
M	90% reagent grade acetone	40 h	24h	yes
N	90% acetone, Fisher Scientific Certified ACS, 14mL	20 days	21 hours	yes
O	90% Acetone	12 days	17 hours	Yes
P	90% Acetone- 25 mL	7 days	2.06 hours	Yes
Q	90% Acetone 10% deionized water. Purity =99.7% @ 10 mls used	6 days	22 hours	yes
R	5 mL of 90% acetone (HPLC grade)/ 10% saturated MgCO3	~ 24 hours	~16 hours	yes
S	90% Acetone Solution, Extract volume 10 ml	8 days	2 hr 3 min	yes
T	90% Acetone : 10% water 7.5mL for each sample	1 day	24 hours	yes
V	10 mL of 90% acetone	23 hrs	23 hrs	yes
X	90:10 Acetone: saturated	14 days	2.5 hours	yes
Y	90% Acetone with 10% MgCO3 solution. Extract has a final total volume of 8 mL.	12 days 16 hrs 20 mins – 13 days 16 hrs 20 mins	3 hrs 55 mins and 3 hrs 40 mins	Yes
Z	90% Acetone, Optima grade, 25 mL	Approximately 13 days and 4 hours	Approximately 19.25 hours	Yes

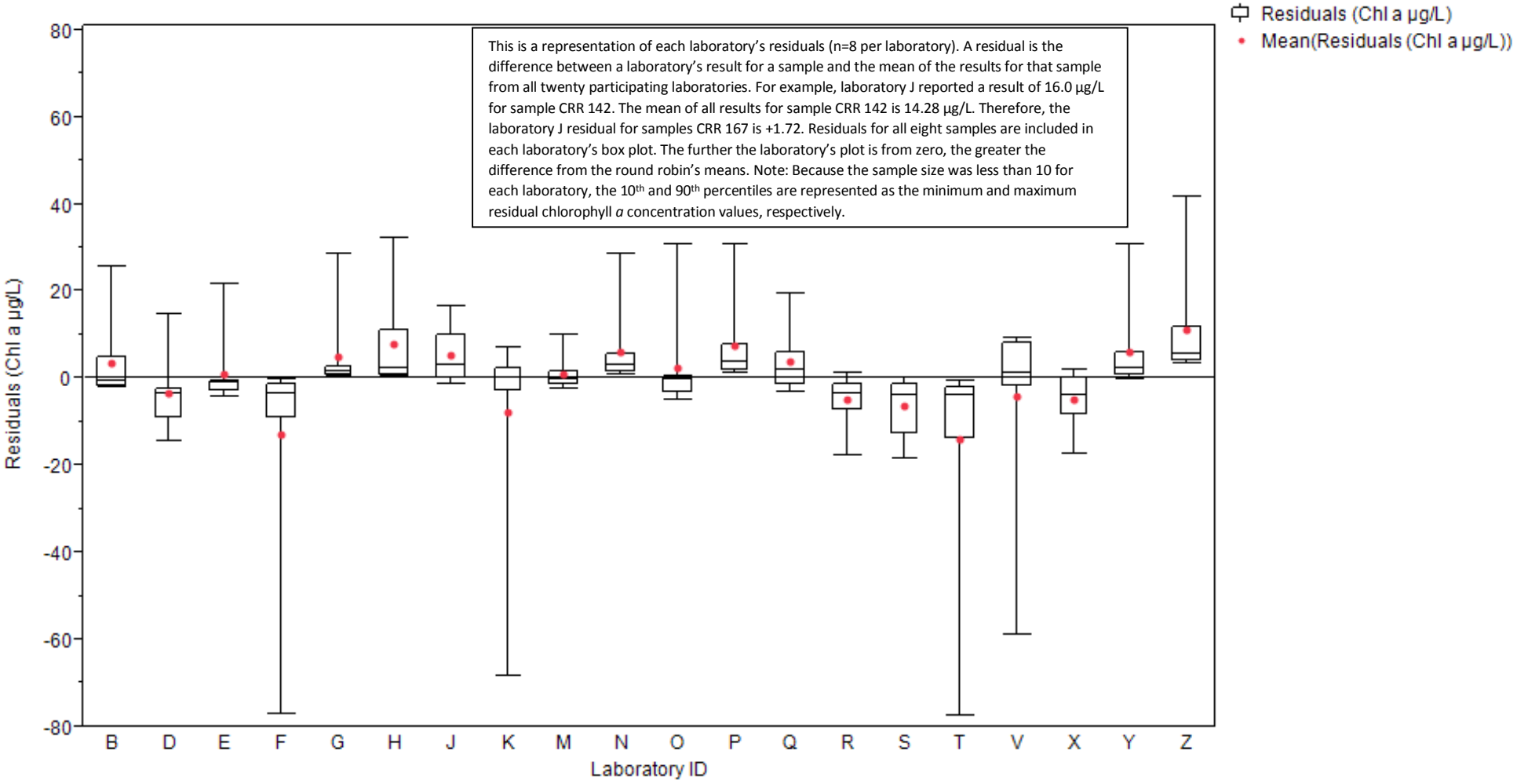
Lab ID	Description of grinding setup
B	Tissue Grinder with Teflon tip in glass vessel (wrapped in foil). Temperature not controlled. Slurry transferred to centrifuge tube.
D	Tissue grind pestle(Kontes® size 21), on stainless steel shaft fit into a drive motor with a grooved Teflon tip. Round-bottom glass grinding tubes (Kontes® size 21) that match pestle. Temperature was not controlled, but it was monitored by touch.
E	Drill press with a teflon grinding tip. Not temperature controlled, ambient temp.
F	Tissue Grinder. Sample in plastic centrifuge tube. Temperature controlled to prevent evaporation
G	teflon grinding pestle attached to rotor; glass test tube; temperature controlled by feel
H	Drill with a Teflon® pestle with grooves, 50-mL polypropylene conical tubes
J	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 significantly as felt when holding the tube.
K	Teflon pestle with radial serrations on tip; temperature controlled by touch/feel
M	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
N	Pro Scientific stainless steel tip homogenizer, glass grinding vessel, temperature was not controlled
O	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.
P	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- temperature controlled (No built in thermometer)
Q	Arrow 850 motor 1/10 hp Kontes tissue grinder pestle SZ24 and matching tube. No temperature control.
R	Pro DPS 20 Homogenizing System, in 15 mL vial, not temperature controlled
S	Tissue grinder, Teflon pestle with grooves in the tip with 1/4 stainless steel rod long enough to chuck on to a suitable drive motor, glass centrifuge tube, room temperature controlled by thermostat.
T	samples were poured into 100mL graduated cylinder and then into filter manifold. The cylinder and manifold was rinsed with deionized water between each sample
V	piston type teflon pestle with glass tissue grinder - manual -30 mL tube
X	Tear filters into quarters and place into grinding tube (Kimble #886000-0023) with PTFE grooved tip; samples macerated using electric lab mixer (Arrow engineering - Model 1750); temperature not controlled but grinding time was short to avoid heating of sample
Y	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.
Z	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.

Lab ID	Were samples acidified? If so, what type, concentration, and volume?	Type of calibration standard and source
B	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
D	No	Turner Designs Fluorometric Chlorophyll Standard used quarterly. Chlorophyll a from spinach (Sigma C5753) used bimonthly. Turner Designs solid secondary standard (10-AU-904) used daily.
E	0.1mL of 0.1N HCL	N/A
F	No	Turner Designs Chlorophyll A and B Standard
G	no	chlorophyll *a* from Anacystis (Sigma C6144); chlorophyll a from spinach (Sigma C5753)
H	0.1 N HCl solution, 135 uL to 4.5 ml of sample	Chlorophyll a free of chlorophyll b Neat, Sigma
J	no	Pure Chla in 90% acetone, from Anacystis, Sigma Chemical
K	No	Fluorometric Chlorophyll standard, Turner Designs
M	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.
N	no	Chl a from Anacystis nidulans, Sigma C6144
O	Yes, with 0.1mL of 0.1N HCL	N/A
P	N/A	Sigma chemicals®- Anacystis Nudulans Algae.
Q	No	
R	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)
S	no	Commercially Prepared Chlorophyll a Std Turner Designs
T	no	chla pigment standard (sigma aldrich)
V	no	zeroed at each wavelength with 90% acetone; Turner Designs 149-01
X	Yes, 0.1N HCL used 0.1 ml per 3ml of extract	Turner Designs P/N 10-950 (20 ml ampule of known concentration)
Y	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 102% and 105% recovery.
Z	Not Applicable	Turner Designs Fluorometric Chlorophyll Standard

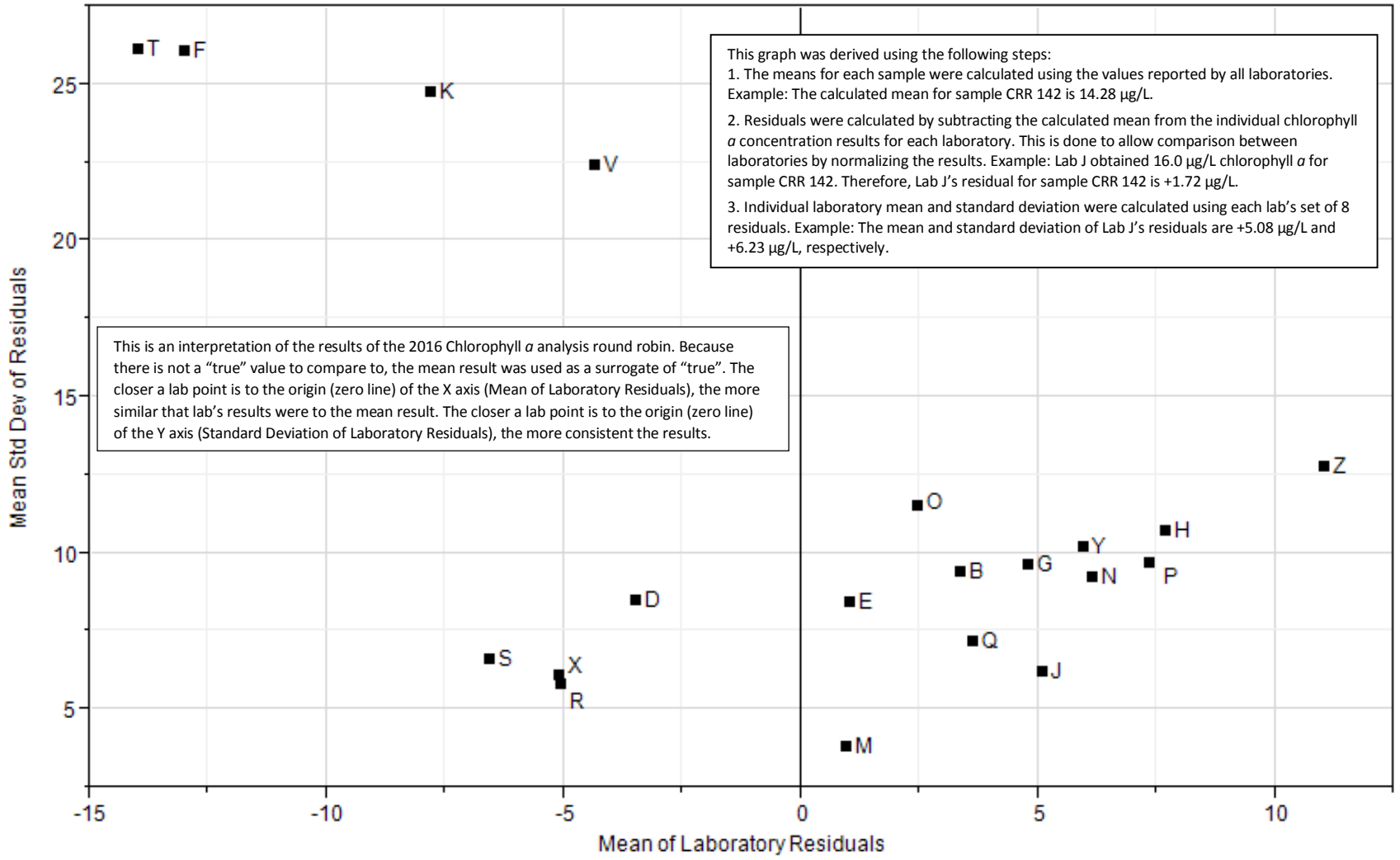
Notes:

1. Answers to the questionnaire are entered as the laboratory presented them unmodified, except for spacing.
2. Additional information obtained from participating laboratories: 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.

Chlorophyll *a* Round Robin Box Plots of Laboratory Residuals



2016 Chlorophyll *a* Round Robin Laboratory Residual Mean vs. Standard Deviation



2016 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 and PT Max) were calculated using NELAC Proficiency Testing (PT) methods* for microbiological parameters in non-potable water.

Lab ID	CRR 142	CRR 230	CRR 396	CRR 418	CRR 551	CRR 674	CRR 707	CRR 995
B	12.5	195	62.8	11.8	57.9	39.2	30.3	12.6
D	11.86	184.05	41.94	11.06	46.34	34.69	26.02	11.41
E	13.2	191	54.0	13.3	52.4	35.8	30.5	13.4
F	13.1	92.4	49.9	12.0	46.8	33.6	31.2	12.4
G	15	198	59	15	59	40	33	15
H	15.58	201.42	67.58	14.46	67.2	39.72	33.77	17.05
J	16	186	57	14	65	43	42	13
K	14.85	100.81	58.32	16.46	54.01	45.96	28.52	14.06
M	13.860	179.192	56.195	14.196	58.791	36.407	30.069	14.2242
N	15.7	198.0	62.8	14.8	60.1	43.1	34.2	15.8
O	14	200	57	14	54	34	28	14
P	16	200	63	15	65	43	35	17
Q	17.2	189	57.6	14.8	63.4	36.7	28.3	17.5
R	12.6021	151.7484	49.0850	12.3970	50.1904	35.1633	28.1271	15.5534
S	10.6	155.5	38.2	14.15	47.5	35	28.10	13.8
T	12.641	91.920	52.567	13.350	40.195	35.346	28.626	8.919
V	13.3	110.6	65.4	12.0	56.2	48.2	37.2	17.5
X	15.4	152.2	48.1	10.7	58.7	34.7	24.0	10.7
Y	14	200	61	15	63	40	35	15
Z	18.4	211	68.9	18.7	67.0	43.8	38.0	17.8
Mean	14.28	169.39	56.52	13.84	56.63	38.86	31.51	14.34
Median	14.00	187.50	57.30	14.08	58.30	37.95	30.40	14.14
Std Dev	1.91	39.86	8.10	1.88	7.62	4.38	4.46	2.43
PT Min	9.48	71.03	35.34	9.16	36.79	27.77	20.58	8.22
PT Max	21.17	378.17	88.51	20.55	85.60	53.76	47.36	24.27

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