**2020 NC DWR Chlorophyll *a* Round-Robin**

Currently, 60.3 miles and 131,105 acres of surface waters in North Carolina are impaired due to elevated levels of chlorophyll *a*, a chemical parameter used to assess algal productivity (2018 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.

The NC DWR initiated the first chlorophyll *a* round-robin in August 2007 because commercially available Proficiency Testing Samples did not include the extraction component required in chlorophyll *a* analysis methods. The round-robin involved 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 Thirteenth chlorophyll *a* round-robin. Twenty-one laboratories participated, each analyzing eight samples. All eight samples were collected from Raleigh-area waterbodies.

**Methodology**

Sample Collection

On July 29, 2020, 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 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. Statistical analyses and results of the data are presented graphically on pages 10 and 11. Individual laboratory results, along acceptance limits can be found on page 12.

**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

Charlotte Water – Environmental Laboratory Services

Environment 1, Inc.

Environmental Chemists, Inc. (Envirochem)

Environmental Conservation Laboratories, Inc. – Orlando

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

S.C 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

US Geological Survey Oregon Water Science Center

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

**Chlorophyll *a* Round-Robin Analysis Details**

**Answers from Participant’s Questionnaires**

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| --- | --- | --- | --- | --- | --- | --- |
| **Lab ID** | **Method Used** | **Date Samples Received** | **Temperature Samples Received** | **pH of Samples Received** | **Temperature Samples Stored Prior to Filtering** | **Date Samples were Filtered** |
| A | EPA Method 445.0 modified | 7/29/2020 | 4C | not measured | not stored, filtered immediately | 7/29/2020 |
| C | Standard Methods 22nd edition 10200 H Chlorophyll, sections 1 and 2 | 7/30/2020 | 2.4 °C | We do not test pH on Chlorophyll samples as part of our SOP | < 6.01 °C | 7/30/2020 |
| D | fluorometric(non-acidification) | 7/29/2020 | 5 degrees C | n/a | 4C | 7/30/2020 |
| E | EPA 445.0 | 29-Jul | on ice | N/A | on ice | 30-Jul |
| F | EPA 445.0, Rev 1.2 (Fluorometric) | 7/29/2020 | 1.5°C | ranged from 7.31 to 8.49 | 3.4°C | 7/29/2020 |
| H | SM10200 H | 7-30-20 | 1.2 C | Not measured | 1.2 C | 7-30-20 |
| I | EPA 445.0 Rev 1.2 (Fluorometric) (Aqueous) | 7/29/2020 | 2.7°C | 07292020 CRR281 6.87 SU 07292021 CRR828 6.79 SU 07292022 CRR615 6.89 SU 07292023 CRR933 7.19 SU 07292024 CRR840 6.85 SU 07292025 CRR435 6.88 SU 07292026 CRR917 7.29 SU 07292027 CRR753 7.82 SU | 5.0°C | 7/30/2020 |
| J | EPA method 445.0 | 7/29/2020 | 1.8C | 6.5su | 5.9C | 7/29/2020 |
| K | EPA 445.0 Rev. 2.0-1997, modified option (Invitro Determination of Chlorophyll a ) | 7/30/2020 | 22.2 Celsius | 6.5 TO 7.0 S.U. | 3.6 to 3.9 Celsius | 7/31/2020 |
| L | SM10200H | 7/30/2020 | 1.0oC | 6.5 | 0-6 degrees C | 7/31/2020 |
| M | SM10200H Spectrophotometer | 7/30/2020 | 10.5°C | 6.84-8.54 | 21°C | 7/30/2020 |
| N | SM 10200H-2011 | 7/30/2020 | 1.3°C | >6 | 25C | 7/30/2020 |
| O | U.S. EPA method 445.0 | 7/30/2020 | 2.8° C | Not measured | 2.8° C | 7/31/2020 |
| P | Standard Methods 10200H (2011) | 7/29/2020 | 1.6°C | >6.0 | Not Stored | 7/29/2020 |
| Q | EPA 445.0 Rev. 1.2 | 7/29/2020 | 0.5°C | pH of Samples at Time Received > pH 6.0 | 0.1-4.4° C | 7/30/2020 |
| R | EPA Method 445.0 | 7/30/2020 | 1.0°C | not measured | 4.0°C | 7/30/2020 |
| T | EPA Method 446 (non-acidic method) | 7/30/2020 | 1.8 C | Did not measure | 1.0 C | 7/30/2020 |
| V | EPA 445.0 Rev. 1.2 | 7/29/2020 | 1.2 Deg. C | all samples > 6 | 4 Deg. C | 7/30/2020 |
| W | EPA 445.0 Revision 1.2 | 7/30/2020 | 1.3 C | 6.55 - 8.04 S.U. | On Ice | 7/30/2020 |
| X | EPA 445.0 | 7/29/2020 | 2.4 deg C | 6.97 - 8.15 | 4 deg C | 7/29/2019 |
| Y | SM 10200 H Rev. 2011 | 7/29/2020 | 2.8C | 7.03 to 7.27 | <4C | 7/29/2020 |

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| --- | --- | --- | --- | --- |
| **Lab ID** | **Pressure at which Samples were Filtered (if not measured, please state that)** | **Volume of sample filtered (if not same for all samples, please state range)** | **Describe technique for homogenization of samples prior to filtering** | **How long were samples filtered? (if not same for all samples, please state range)** |
| A | 6 in Hg | 50 mL | briskly inverted bottle ~10 times | 1-5 minutes |
| C | We do not measure pressure at which samples are filtered | 180-450 mL | Mix sample volume by inversion of sample container at least 7 times | 1-30 minutes |
| D | 5 inches Hg | 15mL | inverting sample bottle several times before measuring in graduated cylinder | 3-5 minutes |
| E | <6 mm Hg | 20 mL | Gentle shaking for 10 secs | 10-15 secs |
| F | <5 in Hg | ranged from 64 to 82mL | Samples gently inverted 10 times | 5-10 sec |
| H | 23 in Hg | 300-318 mL | container shaken | 10 min |
| I | -5 kPa | 100 ml | Sample inverted 4-5 times | approximately 3 minutes |
| J | < 20 KPA | 50ml | INVERSION | <1 min |
| K | Less than 20 KPA | 50 -150 mL | Gently shake bottle several times | all samples are filtered for less than 10min. If it takes longer a smaller volume is used. |
| L | N/A | 250-450mL | Bottles inverted at least three times | ~1-2min |
| M | not measured | 200mL-400mL | Sample bottle is shaken vigorously by hand before filtration. | 1:17-8:15 |
| N | 10 cm Hg | 290 to 500mL | Samples are shaken well before filtering | <30 min |
| O | Not measured | 150mL to 350mL | The sample bottles were inverted numerous times for 15 seconds prior to filtering. | Between 2-5 minutes. |
| P | 600 to 700 mbar | 200 ml | Gently invert sample 4 to 6 times | 1 to 2 minutes |
| Q | <6 in Hg | 100-250mL | Sample bottle inverted by hand for 5-10 seconds | 1-8 minutes |
| R | ≤6 in Hg | 100-150 mL | shaking bottles several times prior to each aliquot measured | about 30-60 seconds per sample |
| T | Not measured | 15 mL per filter | Samples were mixed by gently inverting the bottles back and forth about 12-15 times. | Samples were filtered for approximately 1-3 minutes |
| V | 5 in. Hg | 150ml for all samples | shake vigorously several times | < 35 seconds |
| W | 5 in Hg | 30 mL | Placed on magnetic stir plate with small stir bar, gently stirred for one minute before pulling sample with pipet. | Roughly 30 seconds. |
| X | < 3 in Hg | 100 mL | Gently inverted the bottle several times | 1-2 minutes |
| Y | 4-6 inches of mercury | 250mls | shaken | <5mins |

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| --- | --- |
| **Lab ID** | **Describe filtering technique (how were sample volumes measured, were sides rinsed, etc.)** |
| A | 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) |
| C | 500 mL of sample measured in Class A graduated cylinder. Sample poured slowly to determine greatest volume that will filter within 30 minutes. If less than 500 mL filtered, volume calculated by subtracting amount remaining in cylinder. Sides of filtration cups not rinsed; filter apparatus rinsed in between samples |
| D | volumes measured with graduated cylinder, sides of filter funnels were rinsed with deionized water |
| E | Volume measured in a graduated cylinder, filter funnel sides not rinsed down into filter, funnel rinsed to clean b/t samples |
| F | Samples are vacuum filtered. The filter flask is connected with Tygon tubing to a manifold. The manifold is connected with Tygon tubing to the vacuum pump. After mixing, the 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. Filter is folded in half and wrapped in opaque cover. Samples are stored in the freezer. |
| H | measured with graduated cylinder, rinsed |
| I | 50 ml aliquots filtered in a graduated cylinder. When filtration slows, final volume recorded and sides of cylinder rinsed 3 times with type 1 DI water. |
| J | By graduated cylinder, sides were rinsed |
| K | Sited filter by adding about 5.0 mL , Reagent water , volume measured using class A 250 mL graduated plastic cylinder, Cylinder is rinsed sides , sides of filter funnel are rinsed. |
| L | Measured with 500mL Class A graduated cylinder. Cylinder and filter flask rinsed three times with DI water. |
| M | After mixing, the sample is poured into a 500mL Class To Deliver graduated cylinder to be measured for filtration. Then, the sample is vacuum filtered as quickly as possible . When the filtration is nearing its end, 1-2mL of saturated MgCO₃ solution is added. The funnel is then rinsed thoroughly with DI water. The filters are then folded into quarters and wrapped in foil and frozen. The cylinders are thoroughly rinsed with DI water in between samples. |
| N | Sample volumes were measured in class A volumetric cylinder and poured slowly into the filter funnel. Cylinder and funnel were rinsed well. |
| O | A cleaned graduated cylinder was rinsed with DI and native water three times before measuring the samples. |
| P | Samples were measured using a polypropylene graduated cylinder, rinsed cylinder twice with MgCO3 solution |
| Q | Measured in a class A graduated cylinder, sides not rinsed |
| R | measured in 100 mL glass graduated cylinder; poured into plastic filter tower; cylinder and filter towers sides both rinsed with DI water |
| T | Samples were filtered for approximately 1-3 minutes |
| V | Volume measured with graduated cylinder. Funnel was not rinsed. |
| W | Samples were measured and pulled using disposable volumetric pipets. Funnel and screen on manifold were rinsed with distilled water and wiped clean with Kimwipes between each sample. The fiberglass filter was placed on the screen, then the funnel was attached, the vacuum valve opened, then 30mL aliquots were pipetted into each funnel. 30 mL produced a sufficient green stain for each sample. |
| X | Measured volume in a graduated cylinder. Filtered with a hand pump. Rinsed sides of filtration unit and graduated cylinder with DI water.  |
| Y | measured with a 250ml graduated cylinder, vacuum filtered, graduated cylinder and funnel rinsed between samples |

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| --- | --- | --- | --- | --- |
| **Lab ID** | **Describe lighting conditions during filtering** | **List the extraction solvent, its purity and the volume used** | **Length of time samples were stored after filtering** | **Length of steeping time?** |
| A | lights turned off, blinds closed | 90% reagent grade acetone | 14 days | 24h |
| C | Room ceiling lights were on; window blinds were closed; subdued lighting | 10% Saturated MgCO3 / 90% Acetone mixture; HPLC Grade; 5-mL | 7 days | 21 hours |
| D | ambient light from window | 90% Acetone : 10% water 7.5mL for each sample | 5 days | 24 hours |
| E | low ambient light from windows | Acetone,90%, 10 mL | 20 days | 20 hrs |
| F | All overhead lights off, two small lamps with 25 watt green bulbs. | 90% acetone, Fisher Scientific Certified ACS, 14mL | 12 days | 21 hours |
| H | amber light | 90% acetone, 10 mL | 24 hrs | 24 hrs |
| I | Darkened room w/ Red Light | Acetone, 90% (w/ type 1 DI water) 10ml volume | 12 Days | 19.0 Hours |
| J | DARK ROOM WITH GREEN LIGHTING | 90% ACETONE, 10 MLS | 21 days | 23 hours, 22 minutes shook once during this time |
| K | Analysis takes place in a dark room with green lighting. | 25 mL of 90% Acetone | 7 days | 21:45 hours |
| L | Fluorescent lighting; filter funnel covered with tin foil | 90/10 Acetone/MgCO3. The acetone is chromatography grade; the MgCO3 is reagent grade and filtered through a 0.45um filter. 10mL of Acetone/MgCO3 solvent was used to extract the sample. | 24 days | 18.4 hrs |
| M | Filtration is done under regular overhead lighting. (Intensity Range 20-30 ft-candles) | 90% HPLC grade acetone & 10% filtered MgCO3 solution extracted up to 12mL | 3 Days 23 hours 50 minutes | 4 hours 6 minutes |
| N | Room Lighting | 90% Acetone | 26 hours | 3 hrs |
| O | Well lit laboratory room. Samples were then placed in a petri dish and covered with aluminum foil | 10mL of 90% acetone | 3 hours | 3 hours |
| P | lights off, blinds down and closed, door closed | 90:10 Acetone: Saturated | 15 Days | 24 hours |
| Q | Dark room with subdued green LED lighting | 25 mL of 90% Acetone, Optima grade | 6 Days | Approx: 21:15 hours |
| R | dimmed fluorescent (25% of full lab lighting) | 90% HPLC-grade acetone, 25 mL | 18 days | 20 hours |
| T | Lights were off during filtering with the exception of a lamp using a subdued yellow bulb. | Filters were extracted using 90% acetone. The extracted filter was placed in a centrifuge tube and then filled up with 90% acetone until it reached 12.5 mL | Samples were stored for 22 days after filtering | Samples were steeped for 3 hours |
| V | dark room with green lights | 90% Acetone, Baker analyzed-ACS reagent grade, 25ml | 22 hours | 3.5 hours |
| W | Dimmed, no outside light | 90% Acetone, 10mL | 4 days | 2.5 hrs |
| X | Overhead fluorescent lights | 90 % Acetone/ 10 % Water Solution | 20 Days | Approx: 21 hrs |
| Y | Darkroom w/ green light | 90% acetone, 10% DI water, Purity-99.7% @10mls used | ground immediately | 17 hours |

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| **Lab ID** | **Grinding used? (yes or no)** | **Samples acidified? If so, please state type, concentration, and volume of acid used** | **Type of calibration standard used and source** |
| A | yes | 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. |
| C | yes | yes; 0.1M HCl; 100 uL | Photometric and Wavelength Accuracy Secondary Reference Material Set traceable to NIST (3 neutral density glass filters and a holmium oxide glass filter) for PM and Quarterly Check; Daily Check: Chlorophyll A standard made using Anacystis nidulans algae (Sigma-Aldrich) |
| D | yes | no | chla pigment standard (sigma aldrich) |
| E | yes | no | Chlorophyll a free of chlorophyll b Neat, Sigma |
| F | yes | no | Chlorophyll a from Anacystis nidulans, Sigma C6144 |
| H | yes | samples acidified as second part pheophytin step; 10 uL per 2 mL extract of 0.1 N HCl | Turner Designs Std |
| I | Yes | No | Turner Designs Chlorophyll A & B Standard |
| J | yes | No | Sigma 1mg Chlorophyll A |
| K | Yes | not acidified | Make a calibration curve standards from a stock solution |
| L | Yes | 0.1mL of 0.1N HCL | N/A |
| M | Yes | Samples are acidified with 100 uL of 0.1 N HCl, mixed with a mixing paddle, and timed for 90 seconds. | 200ug/L chl-a from spinach |
| N | Yes | Yes, with 0.1 mL of 0.1N HCl | N/A |
| O | Yes | 0.1 N HCL | Concentrated solutions (8-22 mg/L chlorophyll-a) in 90% acetone, produced at the USGS National Water Quality Laboratory using Sigma concentrated pigment |
| P | yes | Yes, 0.1N HCL used 0.1 ml per 3ml of extract | Not Required - only check the instrument performance with a QC |
| Q | Yes | Not Applicable | Anacystic Nidulans Algae, Sigma Aldrich |
| R | yes | no | chlorophyll a from Anacystis (Sigma C6144) |
| T | Yes | No | Calibration standard used was from Turners Solution (known chlorophyll in 90% acetone). Additional calibration standards were used by diluting dried chlorophyll a from spinach in 90% acetone and confirming on a spectrophotometer. |
| V | yes | No | Fluorometric Chlorophyll standard, Turner Designs |
| W | Yes | No | Commercially Prepared Chlorophyll a Std Turner Designs |
| X | Yes | 0.1 N HCl solution, 90uL to 3.0ml of sample | Chlorophyll a free of chlorophyll b Neat, Sigma |
| Y | yes | No | N/A |

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| --- | --- |
| **Lab ID** | **Description of grinding setup** |
| A | 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 |
| C | Dremel Tissue-Tearor Model 985370 BioSpec products, Inc.; 7 mm probe, 8.3 cm in length; 10-mL plastic conical bottom with skirt tube vessel; Not temperature-controlled |
| D | "A Teflon tissue grinder was used attached to a drill to grind the filter and 7.5mL of 90% acetone: water completely (~30 seconds). Grinding was done with tube in a bucket of ice." |
| E | 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. |
| F | Pro Scientific homogenizer with stainless steel saw tooth generator, 5mL glass grinding vessel, temperature was not controlled, transferred to 15mL graduated centrifuge tube to steep in refrigerator overnight. |
| H | high speed rotating glass macerator |
| I | Tissue Grinder, sample in plastic centrifuge tube. Temperature controlled to prevent evaporation. |
| J | TEFLON TISSUE GRINDER IN GLASS TUBE. TEMPERATURE DID NOT RISE |
| K | Tissue grinder tip on a drill press. |
| L | Drill press with a teflon grinding tip. Not temperature controlled, ambient temp. |
| M | 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 up to 14 mL. Samples are steeped in refrigerator. |
| N | Samples were grinded using a teflon tip in a glass test tube for 1 minute with 3mL of 90% Acetone solution. Then samples were transferred into a 25mL screw top centrifuge tube with an additional 7mL of 90% Acetone solution. Grinding occurs at room temperature and was not temperature controlled. |
| O | A clean and dry Teflon pestle is placed into the grinding tube. This Teflon pestle is connected to a drill with a base for stability. |
| P | 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 the sample |
| Q | 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. |
| R | teflon grinding pestle attached to rotor; glass test tube; temperature controlled by feel |
| T | Grinding was completed in a mortar and pestle. |
| V | Teflon pestle with radial serrations on tip; temperature controlled by touch/feel |
| W | Glass centrifuge tube, tissue grinder; teflon pestle with grooves in the tip with 1/4 stainless steel rod long enough to chuck on to a IKA RW 20 Overhead Stirrer, spun at 1170 rpm. Rm temp controlled by thermostat: 19.0°C  |
| X | Drill with a Teflon® pestle with grooves, 50-mL polypropylene conical tubes |
| Y | Arrow 850 motor 1/10hp kontes tissue grinder pestle SZ24 matching tube No temperature control |

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| --- | --- | --- |
| Lab ID | Were samples acidified? If so, what type, concentration, and volume? | Type of calibration standard and source |
| A | 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. |
| C | yes; 0.1M HCl; 100 uL | Photometric and Wavelength Accuracy Secondary Reference Material Set traceable to NIST (3 neutral density glass filters and a holmium oxide glass filter) for PM and Quarterly Check; Daily Check: Chlorophyll A standard made using Anacystis nidulans algae (Sigma-Aldrich) |
| D | no | chla pigment standard (sigma aldrich) |
| E | no | Pure Chla in 90% acetone, from Anacystis, Sigma Chemical |
| F | no | Chlorophyll a from Anacystis nidulans, Sigma C6144 |
| H | samples acidified as second part pheophytin step; 10 uL per 2 mL extract of 0.1 N HCl | Turner Designs Std |
| I | No | Turner Designs Chlorophyll A & B Standard |
| J | No | Sigma 1mg Chlorophyll A |
| K | not acidified | Make a calibration curve standards from a stock solution |
| L | N/A | 0.1mL of 0.1N HCL |
| M | Samples are acidified with 100 uL of 0.1 N HCl, mixed with a mixing paddle, and timed for 90 seconds. | 200ug/L chl-a from spinach |
| N | Yes, with 0.1 mL of 0.1N HCl | N/A |
| O | 0.1 N HCL | Concentrated solutions (8-22 mg/L chlorophyll-a) in 90% acetone, produced at the USGS National Water Quality Laboratory using Sigma concentrated pigment |
| P | Yes, 0.1N HCL used 0.1 ml per 3ml of extract | Turner Designs P/N 10-950 (20ml ampule of known concentration) |
| Q | Not Applicable | Anacystic Nidulans Algae, Sigma Aldrich |
| R | no | chlorophyll a from Anacystis (Sigma C6144) |
| T | No | Calibration standard used was from Turners Solution (known chlorophyll in 90% acetone). Additional calibration standards were used by diluting dried chlorophyll a from spinach in 90% acetone and confirming on a spectrophotometer. |
| V | No | Fluorometric Chlorophyll standard, Turner Designs |
| W | No | Commercially Prepared Chlorophyll a Std Turner Designs |
| X | 0.1 N HCl solution, 90uL to 3.0ml of sample | Chlorophyll a free of chlorophyll b Neat, Sigma |
| Y | No | N/A |

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, pH of samples at receipt, and notable differences between samples.

Chlorophyll a Round-Robin Box Plots of Laboratory Residuals



This is a representation of each laboratory’s residuals (n=8 per laboratory). A residual is the difference between a laboratory’s result for a sample and the mean of the results for that sample from all nineteen participating laboratories. For example, laboratory Q reported a result of 8.4 μg/L for sample CRR 281. The mean of all results for sample CRR 281 is 7.95 μg/L. Therefore, the laboratory Q residual for sample CRR 281 is 0.45. Residuals for all eight samples are included in each laboratory’s box plot. The further the laboratory’s plot is from zero, the greater the difference from the round robin’s means. Note: Because the sample size was less than 10 for each laboratory, the 10th and 90th percentiles are represented as the minimum and maximum residual chlorophyll *a* concentration values, respectively.

**2020 Chlorophyll *a* Round-Robin Laboratory Residual Mean vs. Standard Deviation**



**W**

This is an interpretation of the results of the 2020 Chlorophyll *a* analysis round-robin. Because there is not a “true” value to compare to, the mean result was used as a surrogate of “true”. The closer a lab point is to the origin (zero line) of the X axis (Mean of Laboratory Residuals), the more similar that lab’s results were to the mean result. The closer a lab point is to the origin (zero line) of the Y axis (Standard Deviation of Laboratory Residuals), the more consistent the results.

**A**

**T**

**I**

**O**

**R**

**K**

**X**

**N**

**C**

**M**

**E**

**Q**

F

**V**

**J**

**L**

This graph was derived using the following steps:

1. The means for each sample were calculated using the values reported by all laboratories. Example: The calculated mean for sample CRR 281 is 7.95 μg/L.

2. Residuals were calculated by subtracting the calculated mean from the individual chlorophyll *a* concentration results for each laboratory. This is done to allow comparison between laboratories by normalizing the results. Example: Lab Q obtained 8.4 μg/L chlorophyll *a* for sample CRR 281. Therefore, Lab Q’s residual for sample CRR 281 is 0.45 μg/L.

3. Individual laboratory mean and standard deviation were calculated using each lab’s set of 8 residuals. Example: The mean and standard deviation of Lab Q’s residuals are 2.77 μg/L and 1.03 μg/L, respectively.

**P**

**H**

**Y**

**D**

**2020 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. *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>

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 2020Lab ID | 281 | 435 | 615 | 753 | 828 | 840 | 917 | 933 |
| V | 10.08 | 4.05 | 54.20 | 60.32 | 15.81 | 17.36 | 61.85 | 57.55 |
| Y | 14.3 | 8.46 | 45.6 | 52.1 | 14.8 | 16.6 | 44.5 | 13.4 |
| J | 10 | 4 | 59 | 65 | 18 | 18 | 67 | 60 |
| I | 8.44 | 3.98 | 40.0 | 45.9 | 13.3 | 13.4 | 49.1 | 46.5 |
| Q | 8.4 | 4.1 | 46.9 | 56.3 | 14.3 | 13.8 | 51.9 | 53.6 |
| N | 8.0 | 3.9 | 44 | 49 | 15 | 14 | 53 | 47 |
| P | 5.3 | 4.0 | 26.7 | 46.7 | 8.0 | 9.3 | 46.7 | 36.7 |
| F | 8.5 | 3.40 | 49.7 | 55.2 | 14.1 | 14.4 | 59 | 52.9 |
| L | 5.8 | 2.9 | 36.4 | 37.3 | 11.0 | 9.8 | 44.7 | 42.9 |
| E | 8 | 4 | 46 | 56 | 15 | 14 | 59 | 49 |
| X | 6.451 | 2.462 | 44.698 | 51.341 | 12.776 | 11.455 | 53.294 | 45.416 |
| H | 11.5 | 3.7 | 35.6 | 41.2 | 13.0 | 11.1 | 37.9 | 21.9 |
| K | 7.32 | 3.29 | 45.6 | 51.3 | 13.6 | 12.6 | 54.6 | 50.4 |
| R | 7.4 | 3.2 | 46 | 50 | 13 | 13 | 53 | 50 |
| M | 7.5 | 3.4 | 48 | 51 | 14 | 14 | 54 | 49 |
| D | 4.5 | 2.8 | 18.6 | 22.6 | 8.6 | 7.1 | 23.3 | 21.1 |
| T | 4.6 | 1.4 | 39.3 | 50.4 | 11.1 | 11.3 | 51.7 | 46.7 |
| W | 8.23 | 3.97 | 51.7 | 56.0 | 15.9 | 14.7 | 59.7 | 55.3 |
| C | 7.36136 | 4.01814 | 45.9566 | 52.0388 | 12.7201 | 13.4132 | 52.8282 | 48.8531 |
| A | 7.91 | 3.19 | 41.79 | 46.01 | 11.68 | 12.69 | 46.36 | 43.52 |
| O | 7.4 | 3.1 | 43.8 | 50.7 | 12.3 | 13 | 52.6 | 48.6 |
| **MEDIAN**  | 7.91 | 3.70 | 45.60 | 51.00 | 13.30 | 13.40 | 52.83 | 48.60 |
| **MEAN**  | 7.95 | 3.68 | 43.31 | 49.83 | 13.24 | 13.10 | 51.22 | 44.78 |
| **STD DEV**  | 2.25 | 1.28 | 8.86 | 8.71 | 2.35 | 2.58 | 9.16 | 12.10 |
| **PT Warning Low** | 4.41 | 1.85 | 25.60 | 31.83 | 8.86 | 8.37 | 32.53 | 20.00 |
| **PT Warning High** | 13.34 | 6.63 | 69.61 | 75.12 | 19.13 | 19.67 | 77.56 | 90.08 |
| **PT Minimum Limit** | 3.34 | 1.35 | 19.94 | 25.68 | 7.31 | 6.76 | 26.18 | 13.73 |
| **PT Maximum Limit** | 17.59 | 9.12 | 89.38 | 93.10 | 23.19 | 24.36 | 96.38 | 131.23 |