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EPA Method 445.0 – Chlorophyll a non-acidified

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1 Scope and Application

1.1 This method provides a procedure for low level determination of chlorophyll a (chl a) in marine and freshwater phytoplankton using fluorescence detection. This method uses a set of very narrow band pass excitation and emission filters that nearly eliminates the spectral interference caused by the presence of pheophytin a and chlorophyll b. This procedure follows the Welschmeyer non-acidification modification of the original method. Procedure is modified from the published method by the instrumentation manufacturer.

1.2 Restricted Procedure
This procedure is restricted to use by an analyst experienced in the operation of a Turner Designs Fluorometer. Additionally, the analyst must complete the requirements of the GAEPD Initial Demonstration of Analyst Proficiency prior to the analysis of actual samples. Analysts are further warned that performance of this analysis involves the use of potentially hazardous chemicals; refer to the GAEPD Chemical Hygiene Plan for additional information regarding chemicals required by this method.

2 Definitions

Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions.

3 Interferences

3.1 Any substance extracted from the filter or acquired from laboratory contamination that fluoresces in the red region of the spectrum may interfere in the accurate measurement of chlorophyll a.

3.2 The relative amounts of chlorophyll a, b and c vary with the taxonomic composition

of the phytoplankton. Chlorophylls b and c may significantly interfere with chlorophyll a measurements depending on the amount present. Due to the spectral overlap of chlorophyll b with pheophytin a and chlorophyll a, underestimation of chlorophyll a occurs accompanied by overestimation of pheophytin a when chlorophyll b is present in the sample. The degree of interference depends upon the ratio of a:b.

3.3 Quenching effects are observed in highly concentrated solutions or in the presence of high concentrations of other chlorophylls or carotenoids. Minimum sensitivity settings on the fluorometer should be avoided; samples should be diluted instead.

3.4 Fluorescence is temperature dependent with higher sensitivity occurring at lower temperatures. Samples, standards, and QC must be at the same temperature to prevent errors and/or low precision. Analysis of samples at ambient temperature is recommended in this method. Ambient temperature should not fluctuate more than +/- 3ºC between calibrations or recalibration of the fluorometer will be necessary.

3.5 Sample extracts must be clarified by centrifugation prior to analysis.

3.6 All photosynthetic pigments are light and temperature sensitive. Work must be performed in subdued light and all standards, QC materials and filter samples must be stored in the dark at -20 ºC or -70 ºC to prevent degradation.

4 Safety

4.1 Refer to the EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision (See SOP Reference 13.6).

5 Apparatus and Equipment

5.1 Turner Designs Fluorometer
5.2 Centrifuge
5.3 Tissue grinder-capable of 400 rpm grinding speed
5.4 Fume hood for organic vapors
5.5 50 ml calibrated centrifuge tubes,
5.6 5 ml glass vials with screw tops, Turner Designs PN 7200-938 or equivalent.
5.7 Glass fiber filter, 47-mm
5.8 Volumetric pipettes
5.9 Room Thermometer
5.10 Spectrophotometer
5.11 Tweezers
5.12 Laboratory tissues
5.13 Volumetric flasks, Class A, 25 mL, 50 mL and 100 mL
5.14 Glass rods
5.15 Disposable transfer pipettes
5.16 Grinding tubes
5.17 Laboratory Freezer capable of maintaining a temperature of -20ºC or cooler.
5.18 Laboratory refrigerator capable of maintaining a temperature of 0 - 6ºC.
5.19 Workspace area where lighting can be kept to the minimum necessary

6 Reagents

6.1 Reagent Water – Purified water which does not contain any measurable quantities of target analytes or interfering compounds for each compound of interest. (Deionized, HPLC, Milli-Q water or equivalent. Milli-Q water has a resistivity of 18.2[MΩ.cm]@ 25ºC and a TOC of 50 ug/L or less).

6.2 Acetone

6.2.1 Spectrophotometric grade

6.3 Chlorophyll a Standard

6.3.1 Place 1 mg of the Chlorophyll Standard a (purchased commercially), dissolve and bring to volume in a 100ml volumetric flask with 90% Acetone Solution. The concentration of the solution must be determined spectrophotometrically using a multi-wavelength spectrophotometer and is good for 6 months if kept in the dark and in an airtight container at freezer temperatures.

6.4 Chlorophyll MDLS Stock

6.4.1 Pipette 1.00mL of Chlorophyll A Standard into a 100mL volumetric flask and dilute to volume with 90% Acetone Solution. After determining concentration of Chlorophyll A Standard spectrophotometrically, divide that concentration by 100 to determine concentration of MDLS Stock. Then calculate the amount needed to result in a concentration of 0.200ug/L (or as close as possible). See Section 11.5 for equation.

6.5 90% Acetone Solution

6.5.1 Mix 900 ml of Acetone with 10 ml of reagent water. The volume may be altered as long as the final concentration remains the same.

6.6 Chlorophyll Calibration standards

6.6.1 Prepare five calibration standards as close as possible to the following concentrations: 0.2, 2, 5, 20 and 200 µg chl a/L. Note: Once the Turner Designs standards have been opened and diluted for calibration, they cannot be stored and used again.

6.6.2 The mid-range (approximately 20 µg/L) and the high-range (approximately 200 µg/L) standards are commercially prepared and purchased from Turner Designs. The concentrations of these standards vary and are not known until received in the lab.

6.6.3 The low level standard (approximately 0.2 µg/L) is made from a 100x dilution of the mid-range standard by pipetting 0.5 ml of the mid-range standard into a 50 ml volumetric flask and bringing it to volume with 90% Acetone.

6.6.4 The 2 µg/L standard is prepared by pipetting 0.5 ml of the high-range standard into a 50 ml volumetric flask and bringing it to volume with 90% Acetone.

6.6.5 The 5 µg/L standard is prepared by pipetting 3 ml of the high-range standard into a 100 ml volumetric flask and bringing it to volume with 90% Acetone.

6.7 Solid Secondary Standard, P/No. 8000-952. The solid secondary standard provides a very stable fluorescent signal. It has an adjustment screw so that you can tune the solid standard to provide a signal to match a specific standard.

6.7.1 The Solid Secondary Standard should be set to the reading of the mid-range standard in the curve. It can be used in place of a primary liquid standard once a correlation between a primary standard and the solid standard has been established.

6.7.2 To use, insert the solid standard in the optical module and note the reading. To adjust the standard to match the mid-range standard in the curve, turn the secondary standard adjustment screw clockwise to lower reading, and counterclockwise to increase reading.

6.7.3 Use the setscrew to lock the reading on the solid secondary standard container.(See SOP Section 8.3)

7 Sample Collection

7.1 Samples are collected on filters, wrapped in aluminum foil and kept cool at –20°C.
7.2 Sample holding time is 21 days.
7.3 Preservation is not required.

8 Calibration

8.1 Calibration Standards
The calibration curve consists of five calibration standards.
8.2 Calibration Curve
The Turner Designs Fluorometer is calibrated at least every 2 months. Five standards are used to calibrate the instrument. Results are exported to Excel to show linearity. Minimum acceptable correlation coefficient, r, is $r \geq 0.995$ or $r^2 \geq 0.990$ using a linear regression. All samples above 180 ug/L are to be diluted.
8.3 Calibration Verification
A solid second source standard (Independent Calibration Verification standard (ICV)) must be analyzed prior to sample analysis and at the end of a sample run. The ICV must be within 10% of the true value.
8.4 An MDLS (low level spike) at the concentration of 0.20ug/L must be analyzed with each batch to perform an ongoing MDL study. All batch QC must be valid to report this result.
8.5 An MDLB (MBLK) must be analyzed once per analytical batch to perform an ongoing MDL study. All batch QC must be valid to report this result.

9 Quality Control

9.1 Refer to Table 14. 1 for Reporting Limits (RL's), Refer to Table A.1 for QC Control Limits, Appendix A for Quality Assurance Criteria, and Table 14. 2 for Quality Control Procedures associated with this method.
9.2 For Initial Demonstrations of Capability (IDC), the EPD Lab sets a recovery range of 85% -115% for Method 445.0 (see calculation 11.6).
9.3 The EPD Laboratory uses the most current accuracy and precision control ranges in use for samples for Continuing Demonstrations of Capability (CDC). If 4 replicates are performed (as opposed to two LCS/LCSD pairs) a 20% RSD is required (see calculation 11.5)
9.4 The default control limits from EPA 445.0 are 85 – 115% recovery for Chlorophyll for LCS recoveries as determined by the EPD Laboratory. The EPD Laboratory applies LCS recovery limits to LCSDs. Note, unless specified by method, the EPD Laboratory does not validate batch quality based on LCSD recoveries.
9.5 By default, the EPD Laboratory sets LCS/LCSD precision control limits to be 0 – 20% RPD.
9.6 10% of all routine samples must be analyzed in duplicate.
9.7 See SOP Reference 13.4. for control charting procedures.

9.8 See SOP Reference 13.3 for training procedures.

9.9 MDL (method detection limit) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.

9.9. 1 The actual MDL varies depending on instrument and matrix.

9.9. 2 The MDL must be determined annually for each instrument prior to results being reported for that instrument. The MDL determined for each compound must be less than the reporting limit for that compound.

9.9.3 The Method Detection Limit Study for all analytes must be performed initially on a new instrument and performed after major instrument repairs or changes to procedures. There are two ways to perform the MDL. The first is with 7 samples and 7 blanks over 3 separate days. The second preferred way the MDL is run as a continuous format.

9.9.4 The 7 MDL samples study is performed by preparing 7 spiked vials, MDLSpike, spiked at the lowest calibration point of the curve, and preparing 7 clean blank vials filled with DI water, MDLBlank. These 7 sets of spiked and blank vial "pairs" are analyzed over 3 separate days, there may or may not be a non-analysis day between each of the 3 days. A total of 14 vials are prepared, 7 spiked and 7 blank.

9.9.5 A continuous format MDL study is performed where one vial is spiked as an MDLSpike, at the lowest point of the calibration curve and analyzed with every batch of samples along with the method blank vial as an MDLBlank.

9.9.6 The results of the MDLBlank will be entered into Labworks using the Method Blank test code, B_CHLA_C or B_BENTHIC. The MDLSpike result will be entered using the MLCHLA_C or MLBENTHIC. The MDL Spiked Amount will be entered into the test code MACHLA_C or MABENTHIC. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-CHLA_C or INSTR-BENTHIC.

9.9.7 MDL study must be performed every six months and before the MDL for the instrument expires.

10 Procedure

10.1 Extraction Procedure

10.1.1 Close the Chlorophyll curtain and turn on the low-level light, which is located in the hood, to keep light to a minimum.

10.1.2 Remove filters to be extracted from freezer and keep in a cooler with a freezer pack.

10.1.3 Using forceps transfer a sample filter to the bottom of the tissue grinding tube. A clean glass rod may be used to position filter to bottom of tube.

10.1.4 Add 3 to 5 ml of 90% Acetone solution.

10.1.5 Grind sample for 60 seconds @ 400 RPM. Use a very slow up and down motion with tube to assist in grinding. Also, upward pressure on tube (against grinding pestle) is needed to completely disintegrate the filter.

10.1.6 Carefully transfer mixture from the grinding tube to a 50 ml plastic centrifuge tube. Complete transfer by washing tube and grinding pestle with 90% acetone solution and adding washings to centrifuge tube. Bring solution up to the 25 ml mark on the centrifuge tube with 90 % acetone solution. Record the time of grinding and the total amount of extract in the centrifuge tube on the extraction log. Note: Best to pre-number centrifuge tubes. Watch that you do not wash number off of tube with acetone.

10.1.7 Cap tube as soon as possible and allow it to steep in the dark at 0-6°C (not frozen) for at least

2 hours but not to exceed 24 hours. Samples should be inverted several times during cold storage period.

10.1.8 Thoroughly clean grinding tube and pestle with straight acetone between each sample.

10.2 Quality Control

10.2.1 Batches of samples are limited to 20 field samples per batch plus the following QC:

10.2.1.1 For each batch a QC Sample is selected and a Method Blank, Solvent Blank, LCS and LCSD are analyzed.

10.2.1.2 The Method Blank must be extracted after all other samples to show that there is no contamination of the laboratory equipment.

10.2.1.3 Each batch must have 10% of samples run as duplicate samples (i.e. for batches of 10 samples or less, one sample duplicate is analyzed; for batches of 11-20 samples two sample duplicates are analyzed). If a batch requires two sample duplicates, one should be selected from the first 10 samples in the batch (the QC Sample) and one from the rest of the samples in the batch, if possible.

10.2.1.4 A Solid Secondary Standard ICV (not extracted) is analyzed at the beginning and end of every batch.

10.2.2 To prepare an LCS or LCSD, place a new blank filter into the bottom of the grinding tube. Add 3 to 5 ml of the 90% acetone solution (See SOP section 6.4).

10.2.3 Pipette 0.5 ml of the Chlorophyll a standard (See SOP Section 6.3) into the grinding tube. Then follow steps 10.1.5 – 10.1.8.

10.2.4 To prepare an MDLS, place a new blank filter into the bottom of the grinding tube. Add 3 to 5 mL of the 90% acetone solution (See SOP section 6.5).

10.2.5 Pipette amount determined in Section 6.4.1 into the grinding tube. Then follow steps 10.1.5-10.1.8.

10.2.6 As samples allow, a field sample duplicate should be analyzed for each batch. The sample collectors will provide extra sample filters (i.e. Rep 3 & Rep 4) for these analyses.

10.2.7 For each batch of samples, a laboratory blank should be analyzed. To prepare the blank, place a new blank filter into the bottom of the grinding tube. Add 3 to 5 ml of the 90% acetone solution.

10.2.8 A solvent blank should also be prepared by filling a 50 ml plastic centrifuge tube to the 25 ml mark with 90% acetone solution. No filter should be included. Then follow steps 10.1.5-10.1.7. The laboratory blank should be the last sample extracted to show that there was no contamination from the laboratory environment.

10.2.9 For an ICV, a solid secondary standard should be analyzed. The recovery of this standard is required to be 90-110%. The solid secondary standard is set to a mid-range value, which is chosen and set when a new calibration is performed. The new standard value is logged in and is good for two months or until fluorometer is recalibrated.

10.3 Fluorometer Analysis

10.3.1 Turn on Fluorometer and allow it to warm up for 30 minutes.

10.3.2 Turn on the computer and monitor and plug in printer.

10.3.3 Double click on the SIS Trilogy icon. Choose COM1 for FLUOR01 or COM3 for FLUOR02. Then click on start.

10.3.4 The window will read Opening Excel Worksheet. Both MS Excel and COM1 or COM3 boxes should turn green. Then minimize the window so that the Excel spreadsheet can be viewed.

10.3.5 After steeping is complete, shake the tubes vigorously and centrifuge samples at medium

speed for 15 minutes. Allow samples to stand at room temperature, but protected from light, for 30 minutes before analysis.

10.3.6 Record room temp. Ambient temperature should not fluctuate more than +/- 3ºC between calibrations or recalibration of the fluorometer will be necessary.

10.3.7 On the Fluorometer, touch "Chl-NA" and then "Okay." Next touch Calibrate."

10.3.8 Touch "Used stored calibration." Then touch the calibration curve name you want to use (ex: 052207). Next, touch "View Cal Details" which transfers the calibration curve data to the Excel spreadsheet. Finally touch "Select."

10.3.9 Type in the sample ID by touching the "Sample ID" key in the top left corner of the screen. Using the keypad, enter the sample name into the name field. Touch "Save" to save the sample ID.

10.3.10 Pour up the sample into the 12 x 35 mm capped glass round vial. Open the lid of the Trilogy and insert the vial into the round adaptor. Make sure the vials used for reading the samples are the same type as was used to calibrate the instrument.

10.3.11 Close the lid and touch "Measure Fluorescence" to commence measurement. Enter the volume of filtered sample water (collector provides this information on the collection sheet). This value is usually 250ml. Enter the volume of solvent used as 25 ml. Then press "OK." The Trilogy will measure the sample for 6 seconds and report the average reading for the sample. The Trilogy reports data on the "home" screen and displays the results for the most recent 20 measurements. The data automatically exports to the Excel spreadsheet on the computer. Be sure to manually record absorbance for each sample on an appropriate form.

10.3.12 For the LCS, LCSD, and MDLS, the volume of filtered water is always 250 ml and the volume of solvent used is always 25 ml. For the Secondary Stock Standard, the volume of filtered water is always 1 ml and the volume of solvent used is always 1ml. For the blank, the volume of filtered water is always 1 ml and the volume of solvent used is always 1ml. The same applies for reading the Turner Design standards.

10.3.13 After the reading is complete, remove the vial and empty its contents into the waste acetone container. Rinse the vial with the 90% Acetone solution and then turn upside down and tap to drain. Then proceed to the next sample.

10.3.14 View the Excel form and transfer Fluorescence results to "Chlorophyll Template 5-8-20." This template has a calculation check on it.

10.3.15 Change all results that are below 0.20ug/L to ND, excluding MDLS result. Also make sure that the calibration curve data is on the report as well as the temperature at time of calibration and time of batch analysis.

10.3.16 Make sure to save and print final data and then turn off fluorometer and computer.

10.4 Fluorometer Calibration

10.4.1 Turn on Fluorometer and allow to warm up for 30 minutes.

10.4.2 Turn on the computer and monitor and plug in printer.

10.4.3 Double click on the SIS Trilogy icon. Choose COM1 for FLUOR01 or COM3 for FLUOR02. Then click on start.

10.4.4 The window will read Opening Excel Worksheet. Both MS Excel and COM1 or COM3 boxes should turn green. Then minimize the window so that the Excel spreadsheet can be viewed.

10.4.5 Record room temp on data packet and in maintenance log. Ambient temperature should not fluctuate more than +/- 3ºC between calibrations or recalibration of the fluorometer will be necessary.

10.4.6 Remove standards from freezer. Pour standards into the labeled 12 x 35 mm capped glass

round vials and keep them in the dark and allow them to come to ambient temperature. This can be achieved by letting them stand at room temperature for 30 min. The standards are still light sensitive so they must be kept in a light-blocking container.

10.4.7 On the Fluorometer, touch "Chl-NA" and then "Okay." Next touch "Calibrate."

10.4.8 Select "Run New Calibration." Select the unit of measurement, which is "ug/l."

10.4.9 Insert the calibration "blank" and touch "OK." The calibration blank is the 90% acetone solution.

10.4.10 Enter the concentration for the first Standard. The Trilogy can only go two significant figures past the decimal place. Use Standards in order of increasing concentration. Follow the screen prompt indicating that the standard should be inserted, touch OK.

10.4.11 After the calibration is complete, select "Enter More Standards," in which case, return to step (10.4.10).

10.4.12 After all five standards have been read; select "Proceed with Current Calibration". Save the calibration for future use. Enter the date as the name to save the calibration curve under. Confirm successful completion of the calibration by measuring the same standard. The displayed concentration should equal the value of the standard.

10.4.13 The Solid Secondary Standard can now be adjusted to give the same reading for future calibrations. Make sure to record the value of the Solid Secondary Standard on the case that it is kept in so you will know the true value for future use. To adjust the value of this standard, turn the secondary standard adjustment screw clockwise to reduce the displayed concentration and counterclockwise to increase the concentration. Try to pick a value that is similar to the midrange standard.

10.4.14 Make sure to log in the new value of the Solid Secondary Standard in the Standard Logbook.

11 Calculations

11.1 For "uncorrected chlorophyll a," calculate the chlorophyll a concentration in the extract as:

$$C_{E,u} = R_b \times Fs$$

Where :

$C_{E,u}$ = uncorrected chlorophyll a concentration (ug/L) in the extract solution analyzed,
R_b = fluorescence response of sample extract before acidification, and
Fs = fluorescence response factor for sensitivity setting S.

11.2 Calculate the "uncorrected" concentration of chlorophyll a in the whole water sample as follows:

$$C_{S,u} = \frac{C_{E,u} \times \text{extract volume (L)} \times DF}{\text{Sample volume (L)}}$$

Where

$C_{S,u}$ = uncorrected chlorophyll a concentration (ug/l) in the whole water sample,
Extract volume = volume (L) of extraction prepared before any dilutions,
DF = dilution factor,
Sample volume = volume (L) of whole water sample.

11.3 Calculate the amount of chlorophyll a in the Chlorophyll a standard (SOP Section 6.3) using a multi-wavelength spectrophotometer:

$(Absorbance_{664} - Absorbance_{750}) / (87.67) * 1000 = \mu g/ml$ Chlorophyll

11.4 Calculation for true value of LCS:

$$\frac{(0.5\ ml)\ (\text{Chlorophyll a concentration } (\mu g/ml))}{0.25\ L\ (\text{filtered volume})} = LCS_{\text{True Value}}\ \mu g/L$$

11.5 Calculation for MDLS spike amount:

$$\frac{\text{Chlorophyll A concentration (ug/mL)}}{100} = \text{Concentration of MDLS Stock Solution (ug/L)}$$

$$\frac{(0.200ug/L)(250mL)}{\text{Concentration of MDLS Stock Solution (ug/L)}} = \text{Amount needed to pipette into extraction tube (mL)}$$

11.6 Relative Percent Difference (%RPD or RPD):

$$\left(\frac{|x_1 - x_2|}{\frac{(x_1 + x_2)}{2}}\right) 100$$

11.7 Percent Recovery:

11.7.1 *LCS/LCSD*:

$$\%Recovery = \frac{Conc_{spiked}}{Conc_{expected}} * 100$$

12 Waste Management

12.1 See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating procedures, SOP reference 13.5.

13 References

13.1 EPA Method 445.0, In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence, Rev. 1.2, September 1997.
13.2 EPD Laboratory Quality Assurance Plan, online revision.
13.3 GA EPD Laboratory SOP's – Initial Demonstration of Capability SOP 6-001, online revision or Continuing Demonstration of Capability SOP 6-002, online revision.
13.4 GA EPD Laboratory SOP-EPD Laboratory Procedures for Control Charts and Control Limits SOP, SOP 6-025, online revision.
13.5 GA EPD Laboratory SOP-EPD Laboratory Waste Management SOP, SOP 6-015 online revision.
13.6 GA EPD Laboratory Safety Plan – EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.
13.7 GA EPD Laboratory SOP – Determination of Method Detection Limit, Method Detection Limit SOP 6-007, online revision.

14 Reporting Limits (RLs), Precision and Accuracy Criteria, and Quality Control Approach

Table 14.1 RLs for Method EPA 445.0

Parameter/ Method	Analyte	Matrix (aqueous)	
		RL	Unit
EPA 445.0	Chlorophyll a non-acidified (CHLA_C)	0.20	ug/L
EPA 445.0	Chlorophyll a non-acidified in Benthic Substrate (Benthic)	0.20	ug/L

Table 14.2.1 Acceptance Criteria for Method EPA 445.0 Water

QC Type	Analyte	Accuracy Water (%R)	Precision Water (RPD)
LCS/LCSD	**Chlorophyll a non-acidified**	85-115	20
Sample Duplicate	**Chlorophyll a non-acidified**	--	NA

Table 14.2.2 Acceptance Criteria for Method EPA 445.0 -Benthic Substrate			
QC Type	**Analyte**	**Accuracy Water (%R)**	**Precision Water (RPD)**
LCS/LCSD	**Chlorophyll a non-acidified in Benthic Substrate (Benthic)**	85-115	20
Sample Duplicate	**Chlorophyll a non-acidified in Benthic Substrate (Benthic)**	--	NA

Table 14.3 Summary of Calibration and QC Procedures for Method EPA 445.0

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
EPA 445.0	Chlorophyll a non- acidified	Five- point Initial calibration	Curve regenerated every two months	Correlation coefficient ≥ 0.995 linear regression.	Correct problem then repeat initial calibration	
		Second Source calibration Verification (ICV)	Prior to sample analysis and at the end of a sample sequence	Concentration within 10% of expected value	Correct problem then repeat initial calibration	
		Initial Demonstration: Demonstrate ability to generate acceptable accuracy and precision using four analysis of a QC check sample, a method blank and a blind sample. In addition, the analyst must prepare one standard..	Once per analyst	QC Acceptance Criteria Table, SOP 3-047 Appendix A, Table A.1, A.2 and Initial Demonstration SOP (SOP Reference 13.3)	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		Continuing Demonstration of Capability Continuing Demonstration: Demonstrate ability to generate acceptable accuracy and precision using a variety of analysis options of a QC sample(s)	Required Every Six Months after IDC for each analyst	QC Acceptance Criteria Table, SOP 3-047 Appendix A, Table A.1, A.2 and Initial Demonstration SOP (SOP Reference 13.3)	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		Method Blank (MBLK)	Extracted at the end of the analytical batch	Value must be < 0.20 ug/l.	Correct problem then analyze method blank and all samples processed with the contaminated blank	If unable to re-analyze, flag with a "B"

Table 14.3 Summary of Calibration and QC Procedures for Method EPA 445.0

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
EPA 445.0	Chlorophyll a non- acidified	Solvent Blank	One per analytical batch	Value must be < 0.20 ug/l.	Correct problem then analyze method blank and all samples processed with the contaminated blank	If unable to re-analyze, flag with a "B"
		Sample Duplicate	10% of samples	NA	Evaluate out of control event and reanalyze, if possible	
		Laboratory Control Sample (LCS/LCSD)	One LCS/LCSD per analytical batch	QC Acceptance Criteria Table, SOP 3-047 Appendix A	Flag samples in affected batch	If unable to reanalyze, flag with a "J"
		MDL low level spike (0.20ug/L) (MDLS)	Once per analytical batch	All batch QC must be valid	Correct problem and reanalyze affected batch	
		MDL Blank (MDLB) *can be combined with MBLK	Once per analytical batch	All batch QC must be valid	Correct problem and reanalyze affected batch	
		MDL study	Every six months or after major maintenance of the instrument	All Spiked MDLs must have a value greater than 0. Minimum Detection Limits established shall be < the RLs in Table 14.1	Re-do MDL Study	MDL study
		MDL analysis	Once per batch or as needed to acquire data points per SOP 6-007, online revision	All Spiked MDLs must have a value greater than 0. All other QC in the MDL blank and MDL sample (i.e. Surrogate Spike or Internal Standard, etc. if included) must meet established criteria	Correct problem and re-run the MDL sample or MDL blank once and initiate a corrective action. If the re-run fails a second time, do not use MDL data. Update corrective action, and use associated sample data	MDL analysis

Appendix A, Table A.1 – Quality Assurance Criteria for EPA Method 445.0- Chlorophyll A – Non-Acidified

Table A.1 – Current Control Limits – EPA 445.0 -Water			
QC Type	**Analyte**	**Accuracy (%R)**	**Precision (%RPD)**
LCS/LCSD	**Chlorophyll a non-acidified**	85 - 115	20
Sample Duplicate	**Chlorophyll a non-acidified**	------	NA
Control chart data generated from 01/01/2019 to 01/01/2021			

Table A.2 – Current Control Limits – EPA 445.0 – Benthic Substrate			
QC Type	**Analyte**	**Accuracy (%R)**	**Precision (%RPD)**
LCS/LCSD	**Chlorophyll a non-acidified in Benthic Substrate (Benthic)**	85 - 115	20
Sample Duplicate	**Chlorophyll a non-acidified in Benthic Substrate (Benthic)**	------	NA
*Note: New Analysis test code			

Updates to Previous Version

Appendix A added. Updated for online revision.
Section 9
Section 10
Table 14.1
Table 14.2.1
Table 14.2.2
Table 14.3
Table A.1
Table A.2