DETERMINATION OF TRUE COLOR IN WATER USING THE AQ2 DISCRETE ANALYZER

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1. SCOPE AND APPLICATION

1.1. The test covered by this SOP is W-COLOR. This method is applicable to waters and wastes that have color characteristics derived from naturally occurring materials.

1.2. The calibration range is from the method detection limit (MDL) of 2.5 to 150 Platinum Cobalt Units (PCU). Sample values above 150 PCU may be determined by quantitative dilution.

2. SUMMARY OF THE METHOD

2.1. True sample color is determined spectrophotometrically at a wavelength of 450 nm. Particulate matter is removed prior to color determination. Platinum cobalt standards are used for quantification. One unit of color is that produced by 1 mg/L platinum in the form of the chloroplatinate ion.

2.2. The basic principles of this method are consistent with SM 2120 C-2011.

2.3. Interferences: Turbidity and the presence of colloidal and suspended particles will interfere with this analysis. All samples analyzed are lab filtered before true color is measured. The results are pH dependent. Highly colored industrial wastes may have hues far different from those of the platinum-cobalt standards. Their color characteristics may not be derived from naturally occurring materials such as humic acids.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation

Instrument ID	Component	Manufacturer	Model
AQ2-3	Discrete Analyzer	SEAL Analytical	AQ2
AQ2-2	Discrete Analyzer	SEAL Analytical	AQ2
pH Meter	pH Meter	Thermo Scientific or equivalent	Orion Star A211 or equivalent

3.2 Equipment

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Adjustable Pipette, 100–1000 µL	Eppendorf/Fisher Scientific	Reference 2, 4924000088
Pipette, Volumetric, Glass, 0.5 mL	Fisher Scientific (SR)	13-650-2A
Pipette, Volumetric, Glass, 3 mL	Fisher Scientific (SR)	13-650-2D
Pipette, Volumetric, Glass, 5 mL	Fisher Scientific (SR)	13-650-2F
Pipette, Volumetric, Glass, 10 mL	Fisher Scientific (SR)	13-650-2L
Pipette, Volumetric, Glass, 20 mL	Fisher Scientific (SR)	16-650-SH
Pipette, Volumetric, Glass, 40 mL	Fisher Scientific (SR)	13-650-2R
Pipette, Volumetric, Glass, 75 mL	Fisher Scientific (SR)	13-650-2T

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Volumetric Flask, Class A, Assorted	Fisher Scientific (SR)	10-212B, 10-210-5D, 10-212BB, 10-212A, 10-240B
Or Equivalent [1]**Note:** (SR) designates equipment is typically ordered through the in-house stockroom		

3.3 Supplies/Consumables

Supplies/Consumables	Vendor[1]	Catalog Number
Cup, Sample, 1.2	SEAL Analytical (SR)	5385
Cup, Sample, Reagent	SEAL Analytical (SR)	5000
Pipette tips, Eppendorf, 1000 µL	Fisher Scientific (SR)	05-403-43
Bottle, Wash, 500 mL	Fisher Scientific (SR)	03-409-22C
Sample Beakers, Polystyrene, 20 mL	Fisher Scientific (SR)	08-732-120
Paper, pH, 0.0-6.0	Fisher Scientific (SR)	14-850-114
Paper, pH, 5.0-9.0	Fisher Scientific (SR)	14-850-113
Pipette tips, Eppendorf, 1000 µL	Fisher Scientific (SR)	05-403-43
Reagent Containers	SEAL Analytical	5010
Pipette, Transfer, Polyethylene, 5 mL	Fisher Scientific (SR)	13-711-5AM
Syringe, Polypropylene, 5 cc	Fisher Scientific (SR)	22-204-500
Filters, Syringe, 25mm (0.45 µm Filters)	Fisher Scientific (SR)	05-713-404A
Filters, Syringe, 25mm (0.2 µm Filters)	Fisher Scientific	05-713-403
Cleaning Swabs (20/pk)	SEAL Analytical	AQCSW-20
Lube Pen	SEAL Analytical	AQLP
Peristaltic Pump Tubing	SEAL Analytical	5140
Or Equivalent [1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom		

4. REAGENTS AND STANDARDS (SEE DEP SOP NU-042)

Note: Before preparing standards or running samples, please read the safety notes in the safety/hazardous waste section of this SOP and the MSDS for the chemicals used in this analysis.

Note: Standard preparations must be documented in the Laboratory Information Management System (LIMS) 'Standard Preparation Tracker' module. Unique ID numbers for each standard are generated in the module and documented in the Reagent and Standard Logsheet (NU-RGTSTD). The NU-RGTSTD template is located on the network at \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\Color\. This excel sheet needs to be updated and submitted as part of the analysis report folder. If available, a Certificate of Analysis for each purchased standard or reagent is also maintained in the REAGENT_PREP\Color folder. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: The volumes prepared for the reagents and standards may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

4.1 Chemicals and Purchased Standards

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	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration [2]	Storage
4.1.1	Color Standard Solution 500 Platinum Cobalt Units	Stock	Hach	141453	See CoA	See CoA	Room Temp in the dark
4.1.2	WatR™ Pollution Color	ALTQC, ERA	Environmental Resource Associates	070	See CoA	See CoA	Room Temp
4.1.3	Hydrochloric Acid	HCl	Fisher Chemical (SR)	A144S-212	36.5 to 38.0% (w/w), Certified ACS Plus	5 years (*)	Room Temp
4.1.4	Sodium Hydroxide	NaOH	Fisher Chemical (SR)	S318-3	≥97.0%, Certified ACS	5 years (*)	Room Temp
4.1.5	Deionized (DI) Water	H_2O	In house	N/A	N/A	Daily	N/A
4.1.6	Cuvette Cleaning Solution		SEAL Analytical	5676		See bottle	Room Temp
4.1.7	Bleach, Generic	NaOCl, For Cleaning	Store Brand (no Clorox)	N/A	No additives, ~6%	N/A	Room Temp

Or Equivalent
[1]**Note:** (SR) designates chemical is typically ordered through the in-house stockroom
[2]**Note:** (*) designates manufacturer's suggested retest date. No expiration date given.

4.2. Calibration Standard Preparation

4.2.1. Calibration (Top) standard (150 PCU) - To a 250 mL volumetric flask containing approx. 100 mL of deionized (DI) water, add 75 mL of 500 PCU stock standard (4.1) and bring to volume. Store in the dark when not in use. Hold time: 30 days.

4.2.2. The remaining standards are prepared from the 150 PCU calibration standard (4.1.1.). Using a class-A glass volumetric pipette, place the appropriate volume of top standard in a volumetric flask partially filled with DI water and dilute to the mark with DI water. Use the below table as a guide. Store in the dark when not in use. Hold time: 30 days.

Concentration (PCU)	QC Standard, Alias	Final Volume (mL)	Vol of TOP Standard (150 PCU) added (mL)
120		25	20
90		50	30
60	CCV	200	80
45	LFB	200	60
30		50	10
15		50	5
10.5		50	3.5
6	PQL	100	4

4.3. 1:10 Hydrochloric Acid, HCl, analytical grade (for pH adjustments) – Slowly add 5 mL of concentrated hydrochloric acid (3.13.) to 30mL deionized (DI) water in a

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50mL volumetric flask. Dilute to the mark. Prepare as needed. Holding time, 30 days.

4.4. 1 M Sodium Hydroxide, NaOH, analytical grade (for pH adjustments) – Add 40 mL DI water to a 50 mL volumetric flask. Add 2.0 g NaOH pellets (3.12.) and bring to volume with DI water. Invert to mix. Prepare as needed. Holding time, 30 days.

4.5. Bleach Cleaning Solution - To a 500 mL volumetric flask, add 100 mL of Clorox (approximately 8.25 % sodium hypochlorite) and bring to volume.

5. SAMPLE PRESERVATION, PREPARATION AND HANDLING

Since biological activity may change the color characteristics of a sample, the color determination should be made as soon as possible. Samples are stored refrigerated ≤ 6°C (not frozen) and must be analyzed within 48 hours of sampling.

6. SAMPLE ANALYSIS AND QUANTITATION (SEE DEP SOP NU-033)

Note: Observe routine maintenance as appropriate to keep the instrument in good working condition and enter any maintenance performed in the maintenance logbook.

6.1. Initial Preparation:

Remove standards from their designated storage area and check the volumes and expiration dates. Discard any expired standards and prepare, if necessary. Make sure there is sufficient volume of all standards to complete the analysis.

6.2. Calibration:

6.2.1. The AQ2 is manually calibrated at least every 30 days using the calibration standards in Section 4.1.

6.2.2. The standards in the calibration are as follows:

STD	Conc. (PCU)	Source
S1	0.0	DI water
S2	6.0 (PQL)	See 4.1.2.
S3	10.5	See 4.1.2.
S4	15.0	See 4.1.2.
S5	30.0	See 4.1.2.
S6	45.0 (LFB)	See 4.1.2.
S7	60.0 (CCV)	See 4.1.2.
S8	90.0	See 4.1.2.
S9	120.0	See 4.1.2.
S10	150.0	Top Standard (4.1.1.)
S0	0.0	DI water

6.3. Controls and Calibration Standard Loading:

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6.3.1. The instrument can be auto calibrated using the high calibration standard placed into a reagent wedge in position 5 and select "Auto Calibrate" in the Options dialog box.

6.3.2. Following the calibration or at the start of a new run, the following QC is required: CCV, CCB, ALTQC and a PQL.

6.3.3. CCV and CCB should be poured individually on the sample tray.

6.3.4. Fill two separately labeled AQ2 reagent containers with the following: continuing calibration blank (CCB/DI water) (refill daily) and continuing calibration verification standard (CCV).

6.3.5. The reagent tray, with plastic wedge shaped containers, is located on the right side of the injector arm. Cuvette cleaning solution is in position 1 and DI water is in position 15. The sample tray is loaded starting at cup 1. If calibration is not necessary, the run starts with a CCV and CCB.

6.4. Analysis Setup/Sequence Setup:

6.4.1. Turn on the instrument computer.

6.4.2. Double click on the AQ2 software icon. Select '**Instrument 2 or 3**' as the login name from the drop-down list and enter the password to display the Main Operation Screen. The password is "password".

Note: Some system parameters changes require administrative privileges. In order to access admin settings, the super username: "seal" and password: "toledo" must be used.

6.4.3. Turn on the AQ2 by switching on the reagent cooling switch (small switch) first, and then the main power switch (large switch) located at the rear of the instrument. **Allow the instrument to warm-up for at least 15 minutes before performing the Daily Start-up procedure.**

6.4.4. AQ2 System Flush (performed before the Daily Start-up when necessary):

a. Empty and refill large wash container located at the side of the instrument with DI water.

b. Empty waste containers located below the instrument (as necessary). Check that the pH is within the range of 2-12.5 before emptying in the sink with running water.

Note: pH that is below 2 or above 12.5 is considered hazardous waste and must be disposed of properly in hazardous waste containers.

6.4.4.1. From the maintenance menu perform the following before the daily start-up:

a. Prime the syringe - 10 primes.

b. Verify flow through the probe wash assembly.

c. Wash the cuvette – Run 'Extra Wash' followed by 'Auto Wash' (5 times). Repeat process.

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Note: A bleach cleaning solution (4.6.) can be substituted in step (6.4.4.1) in place of DI water as needed, following the same procedure. An AQ2 system flush (6.4.4.) with DI water must follow a bleach cleaning.

6.4.5. Daily Start-up:

a. Load the instrument with clean reaction well segments. Zero the reaction wells by selecting *Settings→Maintenance* and clicking on the picture of the reaction well.

b. Perform the Daily Start-up procedure by selecting *Settings→Daily Start-Up*.

Note: This procedure primes the syringe, washes the cuvette, and performs water baselines. The water baselines should read 'OK'. If not, contact a supervisor.

6.4.6. Analytical Setup - Select *Scheduling* from the Main Operations Screen. Select a *Tray Number* (**first FREE #**) and *Reagent Set 1*. Enter the tray name which will be the run file name as follows: CTYYMMDDR# for Instrument AQ2 3 and CTYYMMDDQ# for instrument AQ2 2, where # represents a number1, 2 etc. to indicate the 1st, 2nd etc. run of the day. Press *OK*. The sequence screen should appear. Starting at line 1, type the sample sequence in the 'Sample ID' column. Next, highlight the typed lines and select the **MAN_CLR** test. Click on the *Save* icon to save the schedule and the *Print* icon to print the tray report/bench sheet.

6.4.7. Filter samples using the 0.45 µm filter and syringe apparatus into **labeled** disposable beakers. If filtration with a 0.45 µm filter does not remove all particulate matter from a sample, or if the Turbidity result for the sample is >15 NTU (see DEP SOP NU-072), an aliquot filtered with a 0.45 µm filter should additionally be filtered using a 0.20 µm filter. Samples requiring additional 0.20 µm filtration are batched together. Record these steps on the tray report/bench sheet where applicable. Samples with results affected by particulate matter may have to be qualified.

Note: In some rare cases precipitation may occur over time in the already filtered aliquot of the sample. Samples should be analyzed as soon as possible after filtration. Always document visible changes of the sample appearance on the tray report/bench sheet.

6.4.8. Pour filtered samples and batch QC into the 1.2 mL instrument sample cups according to the schedule. Note that each batch begins with an MBLK that is filtered and an LFB filtered from the 45 PCU standard (4.1.2). All samples and QC that appear on the run schedule are poured in individual sample cups.

6.4.9. Start the run by double-clicking on the *Run* icon. A dialog box should appear. Select the tray (run file) to be analyzed and click *OK*. On the *Options* dialog box, check the auto-standardize box **only** if you intend to re-calibrate the instrument. Click *OK*.

6.4.10. After the analysis is complete, determine the pH of the samples including any diluted sample aliquots and record on the NU-COLORpH sheet

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(\\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\AQ2\color\ Templates).

Check the pH of the filtered samples using pH strips with range 5.0 – 9.0 (3.6.2). If the pH of a sample is observed as 5 on this strip range, verify the sample pH using an indicator strip with range 0.0 – 6.0 (3.6.1).

If the pH of a sample aliquot is < 4 or > 9 it must be verified with a pH meter (See DEP SOP NU-026). If the pH is verified outside the 4-10 pH range, adjust a sufficient aliquot of the sample to pH 7 with 1.0 M NaOH or 1:10 HCl. The color measurement must be taken from the pH adjusted aliquot. A test comment is entered in the QC Manager indicating that the sample result was obtained from a sample aliquot adjusted to pH 7.

Note: If pH adjustment is needed, then the volume of solution added to sample aliquot cannot exceed 3%.

If the pH of a sample is < 2, perform a $BaCl_2$ test to check for improper preservation. To perform the Barium Chloride test, place an aliquot of sample into a beaker and drop a small amount of $BaCl_2$ into the sample. If the sample turns cloudy then the sample was acidified with sulfuric acid (see your supervisor if the sample has been preserved with acid).

6.5. Quantitation/Calculations:

6.5.1. The instrument will give the analysis results for Color in PCU.

6.5.2. The QC Manager calculates relative percent differences (RPDs) for duplicate samples as well as recoveries for QC standards. For general calculations including RPDs, see Section 5.9 of the FDEP Quality Manual.

6.5.3. The high calibration standard is prepared according to Section 4.1.1. The other standards used in the linear calibration are prepared from the high standard. The correlation coefficient for the calibration is calculated by the instrument software.

6.6. Note any equipment/field/trip blanks or field duplicates on the backlog. The backlog fields Matrix, Field ID, and Sampling Location are useful for identifying these sample types.

6.6.1. All equipment/field/trip blanks should have a color reading of < 2.5 and > - 2.5 PCU. If the measured color is not in this range, rerun the blank for confirmation on the same run (if possible) and add a comment for the sample in QC Manager.

6.6.2. Salt water matrix may cause results <-2.5 PCU. Dilute sample until result is within the -2.5> and <2.5 PCU range.

6.6.3. The RPD for the field duplicates should be less than 20 % if the color values are above the practical quantitation limit (PQL). If the RPD is 20 % or greater, both field duplicates should be rerun for confirmation on the same run, if possible, and commented in QC Manager. Also note any visible

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differences between the field duplicates on the tray report and in the comments section of QC Manager.

6.7. Post-Run Procedure:

6.7.1. Click on the *Data Review* menu located on the left side of the screen.

6.7.2. Make any necessary changes to the sample list (dilutions, additional samples, etc.). Click on the *Accept All* icon. This adds 'ACC' to the results. A pop-up window appears where the file name needs to be entered. Follow the naming convention described in 6.4.6. Accepting the run in AQ2 creates the excel transfer file used for uploading the data to the Laboratory Information Management System (LIMS). This file should be saved in the current year's subfolder in \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\AQ2\COLOR\.

6.7.3. Print the AQ2 Test Parameters Report from the 'Settings' menu on the top of the screen.

6.7.4. Click on the *Reporting* icon on the left side of the screen. Check the reporting format 'Color Report' and print the run report. . Select the chronological order report function. A browser window will open containing the report with the calibration and all the data.

6.7.5. Convert the excel file into QC Manager raw data. Use instrument 'GenChem: Seal AQ2 3' or 'Nutrients: Seal AQ2 2'.

7. DATA ARCHIVAL

7.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labeled with the test (w-color), filename/s (CTYYMMDDR# (Instrument AQ2 3), or CTYYMMDDQ# (Instrument AQ2 2)), and initials of the analyst.

7.2. Each report folder should contain the following items - AQ2 run report, AQ2 test parameters, AQ2 tray report/bench sheet, NU-RGTSTD_W-Color sheet, NU-COLORpH sheet, backlog, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization.

7.3. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

7.4. Color instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located in: \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\AQ2\COLOR\.

7.5. Current worksheet templates are located in subfolder: \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\AQ2\color\Templates

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8. QUALITY CONTROL

8.1. For method performance and acceptance criteria for QC measures and corrective action for out-of-control data and contingencies for handling out-of-control or unacceptable data, refer to the following DEP SOPs, NU-038 and NU-043.

8.2. A CCV and a CCB are run as check standards at the beginning of the run, every 10 samples, and at the end of each run. After the initial CCV and CCB a PQL level standard and an ALTQC (4.2.) standard are analyzed. Acceptance limits for recoveries of the standards are 70 - 130 % for the PQL and 90 - 110 % for the CCV. The ALTQC recovery should be within the manufacturer's certified limits. Acceptance limits for the CCB are < 2.5 PCU and > -2.5 PCU.

8.3. Analyze an MBLK (filtered), a LFB (filtered, spike code C1), and a duplicate sample for every batch of ten (or less) samples. Acceptance limits for the MBLK (filtered) are < 2.5 PCU and > -2.5 PCU. Acceptance limits for recoveries of the LFB are 90 - 110 %. The RPD for the duplicates should be less than 20 % provided the duplicate results are above the PQL (6 PCU).

8.4. Calibration Range - See QA Manual Appendix B Section 7.1.1, 'Protocol for Determining the Test Method Range of Applicability'. Acceptance limits for recoveries of calibration standards above the PQL are 90 - 110 % and 70 - 130 % at the PQL level. The correlation coefficient of the calibration must be equal to or greater than 0.995 for the calibration to be acceptable.

8.5. The pH of each aliquot analyzed for color is recorded on the NU-COLORpH sheet (\\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\AQ2\color\Templates). If the pH of a sample is verified to be below 4 or above 10 units, it needs to be adjusted to pH 7. The adjusted aliquot is analyzed and a test comment is added in QC Manager indicating that the aliquot analyzed was adjusted (see 6.4.10.).

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1. Review the Laboratory Health and Safety Plan (accessible on the intranet at https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents)

9.2. Safety glasses, gloves and lab coats should be worn at all times whilst handling samples and standards.

9.3. Before performing the color analysis for the first time, read the MSDS sheet on the platinum-cobalt standard used in this analysis.

9.4. All purchased color standards and wastes thereof may be corrosive. Wastes exhibit the characteristic of corrosivity if they are aqueous and have a pH ≤ 2 or ≥ 12.5. Test the pH of any instrument waste before discarding it down the sink. If it is determined to be corrosive it must be discarded as hazardous waste (acidic waste drum in the waste room if the pH is below 2). The standard described in section 4.1 must be discarded as hazardous waste (pH < 2).

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9.5 Neutralize acid and base solutions prepared for pH adjustments prior to disposal through the sink.

10. REFERENCES/DEFINITIONS/VALIDATION DATA

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use. Method validation data is on file with the supervisor.

10.1. Standard Methods Online 2120 C-2011.

10.2. DEP SOP NU-026, Manual Determination of pH.

10.3. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients Workgroups.

10.4. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

10.5 DEP SOP NU-042, Standard Preparation Procedure

10.6. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines.

10.7. Quality Manual, Bureau of Laboratories, FDEP.

10.8. Laboratory Health and Safety Plan.

10.9. DEP SOP LB-006, Standard operating procedure for records maintenance and storage.

Appendix A

Appendix of Significant Changes

11/17/2011	Section 6.4.5: Added Bleach Cleaning Procedure. Section 6.4.10. and 8.4.: Procedure for recording and adjusting the pH.
01/13/2012	MDL was adjusted to 2.5 PCU. Clarified waste disposal procedure. A filtered MBLK is run at the beginning of every batch (6.4.7.).
02/01/2012	Standard preparation was corrected in Section 4. Naming convention corrected in Section 6.4.6.
05/24/2012	Calibration preparation procedure changed, see Section 4. Calibration and analysis procedure edited in Section 6.
01/08/2015	File naming convention was modified for consistency and to specify the instrumentation used (see 6.4.6. and 7.1.). Data archival information was clarified in section 7.

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01/07/2015 Filtering procedure in Section 6.4.7. and pH determination procedure in Section 6.4.10. modified. Appendix C example NU-COLORpH sheet updated.

04/21/2017 Section 6.4 was revised for clarity and passwords were added. Appendix B and C were removed.

06/25/2018 A prepared standard is used as LFB. One LFB is filtered and analyzed for every batch of samples. Updated the reference method to the 2011 version.

01/19/2022 Added tables for instrumentation, supplies, chemicals, and standards. Updated sample filtering procedure. Removed mention of centrifugation as an option for removing particulate matter. Updated QC requirements. Removed SM 2120 B-2011 as reference method.

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