DETERMINATION OF TOTAL PHOSPHORUS IN WATER USING THE AQ2 DISCRETE ANALYZER

1. SCOPE AND APPLICATION

1.1. This method details the determination of phosphorus as total phosphorus (TP) in drinking, surface, saline waters and wastewaters. The applicable range for this method is from the practical quantitation limit (PQL) of 0.050 to 1.0 mg P/L. The method detection limit (MDL) is 0.005 mg P/L. The range may be extended by dilution. All samples with concentrations below the PQL on the AQ2 are analyzed using the segmented flow analyzer (see DEP SOP NU-082).

1.2. The tests covered by this SOP are W-S-A-TP and W-S-A-TP-F.

2. SUMMARY OF THE METHOD

2.1. This SOP is based on EPA Method 365.1, Rev. 2.0 (1993) and SEAL Analytical AQ2 Method EPA-119-A Rev. 5.

2.2. Prior to analysis the samples are acid-persulfate digested according to the DEP SOP NU-049. This process converts inorganic and organic forms of phosphorus to ortho-phosphate.

2.3. Ortho-phosphate reacts with molybdenum and antimony in an acidic medium to form a phosphoantimony/molybdenum complex, which is reduced with ascorbic acid. The AQ2 Discrete Analyzer is used to measure the absorbance of the complex at 880 nm.

2.4. Interferences - Ferric Iron, Copper, and Silica. Also, samples with background absorbance at the analytical wavelength may interfere, see EPA Method 365.1. High conductivity can cause interferences in matrix spike recoveries. To minimize this interference samples having conductivities above 5000 µmhos/cm are batched together and diluted two-fold prior to digestion, see DEP SOP NU049 section 5.3.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation – Seal AQ2 Discrete Analyzer

3.2. Glassware (Class A)

- Various pipettes for reagent solution preparation
- 1- 1 L volumetric flask
- 1- 2 L volumetric flask
- 6- 500 mL volumetric flasks
- 2- 500 mL brown Nalgene bottles
- 2- 500 mL Nalgene bottles

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4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 and NU-042)

Note: Refer to DEP SOP NU-049 for the preparation of all standard solutions.

Note: Wash all glassware with 1:2 HCl and rinse at least three times with deionized (DI) water. Before preparing reagents, 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: All reagent and standard preparations must be documented in the LIMS 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the Reagent and Standard Logsheet (NU-RGTSTD) located in the LLTP Reagent Prep folder on the Nutrients drive at, \\FLDEP1\DEAR\labs\Lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\. It needs to be updated whenever a reagent or standard is newly prepared. The Logsheet must be submitted with each analysis folder. If available, a Certificate of Analysis for each purchased reagent or chemical is maintained in the REAGENT_PREP folder on the Nutrients drive at \\FLDEP1\DEAR\labs\Lab3\Chem_Data\Inorganic\Nutrients\. 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:

4.1.1. Ammonium Molybdate, $(NH_3)_6Mo_7O_{24}*4H_2O$

4.1.2. Antimony Potassium Tartrate, $K(SbO)C_4H_4O_6*1/2\ H_2O$

4.1.3. Ascorbic Acid, $C_6H_8O_6$

4.1.4. DI Water

4.1.5. Potassium Dihydrogen Phosphate, KH_2PO_4

4.1.6. Sodium Hydroxide, NaOH

4.1.7. Sodium EDTA dihydrate, $Na_2EDTA*2H_2O$

4.1.8. Sulfuric Acid, concentrated, H_2SO_4

4.1.9. Sodium hypochlorite, NaOCl (Generic Bleach, 8.25%)

4.2. Reagents:

4.2.1. 5 N Sulfuric Acid - In a 500 mL volumetric flask, slowly add 70 mL of concentrated sulfuric acid (4.1.8.) to approximately 400 mL of DI water. Dilute to volume and invert to mix. Holding time, 180 days.

4.2.2. Stock Ammonium Molybdate, 4 % (w/v) - Using a 500 mL flask, add 20.0 g of ammonium molybdate tetrahydrate (4.1.1.) to 400 mL of DI water. Dilute to volume with DI water. Stir for at least 2 hours. Store this solution in a plastic bottle and refrigerate. Holding time, 60 days. Discard this reagent if it becomes visibly blue or turbid.

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4.2.3. Stock Antimony Potassium Tartrate Reagent, 3 g/L - In a 500 mL volumetric flask, dissolve 1.5 g of antimony potassium tartrate (4.1.2.) in approximately 250 mL of DI water. Dilute to 500 mL with DI water. Store refrigerated in a dark bottle. Holding time, 30 days.

4.2.4. Color Reagent - To a 250 mL volumetric flask, add 100 mL of 5N sulfuric acid (4.2.1.), followed by 15 mL of stock antimony potassium tartrate (4.2.3.) reagent. Swirl the mixture and then add 50 mL of the stock ammonium molybdate reagent (4.2.2.) and dilute to the mark. Invert several times to mix. Transfer to a dark bottle and store in the refrigerator. Holding time, 14 days.

4.2.5. Ascorbic Acid Reagent (40 g/L) – In a 100 mL volumetric flask, dissolve 4.0 g of ascorbic acid (4.1.3.) in approx. 50 mL of DI water. Dilute to volume with DI water. Transfer to a dark bottle and store in the refrigerator. Holding time, 7 days.

4.2.6. Alkaline EDTA Rinse (cuvette wash) - Add approximately 250 mL DI water to a 500 mL volumetric flask. Add 5 g sodium EDTA dihydrate (4.1.7.) and 10 g NaOH (4.1.6.) to the flask and swirl until the NaOH dissolves. Dilute to volume with DI water and mix well. Holding time, 60 days.

4.2.7. Sodium hypochlorite wash solution - Prepare a 1 % v/v bleach solution by adding 1 mL Bleach (4.1.9.) to 100 mL DI water. The bleach solution is made fresh with each use.

4.2.8. Calibration and Quality Control Standards - Refer to DEP SOP NU-049 for the preparation of all standard solutions.

5. SAMPLE PRESERVATION, PREPARATION AND HANDLING (SEE DEP SOP NU-033)

Refer to DEP SOP NU-049 for preparation instructions and sample handling details. The carrier solution and all samples, working standards and QC standard solutions, and blanks are digested prior to analysis.

6. SAMPLE ANALYSIS AND QUANTITATION\CALIBRATION

Note: • Samples containing particulate matter should be filtered through a 0.45 μm filter prior to analysis on the AQ2 Discrete Analyzer. Redigestion will also be necessary - consult supervisor to determine if predilution will be required.

• If the digested sample has color, it must be pre-diluted and redigested.

• Samples with TP concentrations above 5 mg P/L must be prediluted and redigested.

• Observe routine maintenance as recommended by the instrument manufacturer and enter any maintenance performed in the maintenance logbook.

6.1. Initial Preparation:

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6.1.1. Remove reagents from the refrigerator and check their expiration dates. Discard expired reagents and re-prepare, if necessary.

6.1.2. Select digestion trays to be analyzed and vortex samples thoroughly.

6.2. Calibration:

6.2.1. The instrument is calibrated using the 1.0 mg P/L working calibration standard (AQ2 CAL STD; see DEP SOP NU-049, section 4.3.3.).

6.2.2. The diluent for TP is the digested blank, and is used for the continuing calibration blank (CCB) (see DEP SOP NU-049, section 4.2.3.).

6.3. Reagents, Controls, and Stock Standard Loading:

6.3.1. Fill three separately labeled AQ2 reagent vessels with the following, diluent (see DEP SOP NU-049, section 4.2.3.), color reagent (4.2.4.) and ascorbic acid reagent (4.2.5.).

6.3.2. Reagent Location - The reagent tray, with plastic wedge-shaped containers is located on the right side of the injector arm. It holds all reagents and controls (CCV and blank) in the assigned locations. The color reagent is in position 7, the ascorbic acid reagent in position 8, and the stock standard (1.0 mg/L) is in position 9.

6.3.3. Controls - Fill one reagent vessel with check standard (CCV, 0.40 mg/L, see section 4.3.6. in DEP SOP NU-049) and place it in position 11. Fill another vessel with digested blank (CCB, 6.2.2.) and place it in position 10.

6.4. Analysis Setup/Sequence setup:

6.4.1. Turn on the AQ2 Analyzer (large button on the right side behind the Instrument) and the computer. To start the software, double click on the AQ2 Icon on the screen. The username is '**Instrument 1'** and the password is 'password'.

6.4.2. Perform the daily maintenance (Section 9) if it is the first run of the day. Otherwise continue to 6.4.3.

6.4.3. Check that the DI water reservoir is full and empty the waste container.

6.4.4. Replace all used reaction segments.

6.4.5. In the software, go to 'Settings' menu and select maintenance. Zero the reaction segment counter.

6.4.6. Setup of the analytical run - Click on the scheduling icon on the left side of the screen. Choose **Reagent Set 3**. Select a free tray and name the run. The file name follows the TPYYYYMMDD001Q data format, where TP stands for Total Phosphorus, YYYY is the year, MM is the month and DD is the day of the month. Postscripts 001,002 etc. are used to denote the first or second run of the day, and Q indicates the AQ2-2 instrument (K indicates the AQ2-1 instrument). For example, the first run on instrument

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AQ2-2 on Sept 1, 2015 would be named TP20150901001Q. Now the sequence screen appears.

Then using the prep worksheet (see DEP SOP NU-049, section 5.18.), begin typing up the run in order of the batches listed on the worksheet. Starting at line 1, type the sample sequence in the 'Sample ID' column. Include the tray number with the PQL sample line, and the prep batch ID number with each MBLK sample line. Include at least 7, blank, "C" cups at the end of the run to avoid a known timing issue affecting the final CCB/CCV results (see the Seal AQ2 Method EPA-111-A, Rev. 4 for more information).

An example run sequence includes:

- **Calibrants** - auto-diluted from the high standard, 6.2.1.
- **CCV/CCB** - these checks bracket every 10 cups on the run, 6.3.3.
- **PQCK1_tray #** (See DEP SOP NU-049, section 4.3.3.)
- **ERAQC#** (See DEP SOP NU-049, section 4.3.5.)
- **Batched samples,** including MBLKs (indicate prep batch ID), LFBs, and spiked samples
- **CCV/CCB bracket**
- **7 blank "C" cups**
- **Final CCV/CCB bracket**

Next, highlight the typed lines and click on "AutoFill" to populate the cup numbers, than select and "AutoFill" the **LLTP2** test. The test should appear in the requested test column to the right of the sample ID's. Click the 'save' icon. Click on the 'print' icon to print the bench sheet.

Note: LLTP samples are normally initially ran on the AQ2. Sample results above 0.05 mg P/L are reported from the AQ2, and samples with concentrations below 0.05 mg P/L are run on the Bran+Luebbe (see DEP SOP NU-082). Check carefully against the backlog or prep worksheet to make sure that no samples are missed in the process. In rare cases (instrument out of commission, large sample backlogs, etc.) the entire tray may be ran on the Bran+Luebbe instrument.

Note: Multiple trays can be run on the same run sequence using one calibration. Indicate on the report which tray the BLK, CCV, and calibration standard came from. Identify the tray QC in the sequence. The first ALTQC should be identified as the initial calibration verification (ICV). A failing ICV renders the calibration unusable.

6.4.7. Pouring the analytical run - cup positions 1 and 2 are reserved for the calibration standards. Pour the samples according to the cup position on the bench sheet.

6.4.8. Double-click on the 'Run' icon on the left side of the screen. A dialog box appears. Select the tray that contains the sequence to be analyzed. Click 'ok'. Another dialog box appears. Here check the auto-standardize box and click 'ok'. If the previous calibration is to be used for the run do not

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check the auto-standardize option. Select 'ok' on the following dialog box.

6.4.9. The reagent levels are checked by the instrument and are displayed on the upper right corner of your screen. If the levels are sufficient for the entire run the analysis will start. If not, the run will stop and display the reagent which has insufficient volume. Fill up the reagent and re-start the run according to 6.4.8.

6.5. Quantitation/Calculations:

6.5.1. The instrument will give the analysis results for TP in mg P/L.

6.5.2. The QC-Manager calculates recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and spikes. For general calculations including RPDs and spike recoveries see Sections 5.9 of the FDEP Quality Manual.

6.5.3. The high calibration standard is prepared according to section 4.3.3. in DEP SOP NU-049. The standards (seven plus a calibration blank) used in the calibration are auto-diluted by the instrument from the high standard. The linear calibration curve, including the correlation coefficient and the % error between the target value of each calibrant and its calculated value, is documented by the instrument software and is part of the analysis report.

6.6. Post-Run Procedure:

6.6.1. After the run has finished, print the AQ2 Test Parameters Report from the 'Settings' menu on the top of the screen.

6.6.2. Click on the 'Acceptance' menu located on the left side of the screen.

6.6.3. 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 convention described in 6.4.6. Accepting the run in the AQ2 creates the excel transfer file for uploading the data to the Laboratory Information Management System (LIMS), see section 7.4.

6.6.4. Click on the 'Reporting' icon on the left side of the screen. Check the reporting format 'TKN/TP' and print the run report. It contains the calibration and all the data. Select the chronological order report function to print your report.

6.6.5. Convert the excel file created in 6.6.3. into Raw data in the QC Manager. The Instrument used is 'Nutrients: Seal AQ2 2' or 'Nutrients: Seal AQ2 1'.

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/s (W-S-A-TP, W-S-A-TP-F), filename/s

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(TPYYYYMMDD001Q), and initials of the analyst. Submit the folder to a supervisor for review and authorization.

7.2. Each report folder should contain the following items for the AQ2 run - report summary, calibration curve, method parameters, digestion temperature log, Reagent and Standard Logsheet, 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. AQ2 instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located at, \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\AQ2\LLTP\.

7.5. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\fldep1\dear\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

8. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES (SEE DEP SOP NU-038 AND NU-043)

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

8.2. The 0.40 mg P/L standard (CCV; see DEP SOP NU-049, section 4.3.6.) and the MBLK/CCB (digested blank) will be run at the beginning of the run, every 10 samples, and at the end of each run. Acceptance limits for recovery of the CCVs are 90-110 %. Acceptance limits for the MBLK and CCB are < 0.005 and > -0.005 mg P/L.

8.3. A laboratory fortified blank (LFB; 0.4 mg P/L; see DEP SOP NU-049, section 5.9.) and duplicate samples and/or duplicate matrix spikes are analyzed for every batch of 10 samples. Acceptance limits for the LFB and matrix spike recoveries are 90-110 %. The sample/matrix spike reproducibility should be less than 20 % RPD for results above the PQL.

8.4. One PQL level standard, PQCK1 (0.05 mg P/L; see DEP SOP NU-049, section 4.3.3.), is analyzed on every analytical run. Acceptance limits for recovery for PQCK1 are 70-130 %. The MDLSPK (0.020 mg P/L standard; BLCAL6 see DEP NU-049) is analyzed for the ongoing MDL and PQL verification. Acceptance limits for recovery of the MDLSPK are 50-150 %.

8.5. One certified standard reference material, ALTQC, is analyzed on every analytical run. The supplier for the QC stock standard differs from the supplier of the calibration standards. Acceptance limits for the ALTQC are 90-110 %.

8.6. Linear Calibration Range (LCR) - See Quality Manual, Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'. The

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correlation coefficient of the linear calibration must be equal to or larger than 0.995. Acceptance limits for the percent error of the PQL level standard is ±30%. Acceptance limits for the percent error of calibration standards above the PQL are ±10%.

8.7. Every day samples are analyzed a new calibration has to be performed at the beginning of the analysis and the LCR must be verified (See 8.6.). Consecutive runs on one day may use the same calibration.

9. MAINTENANCE/TROUBLESHOOTING

9.1. Prepare a 1% v/v bleach solution, a beaker of DI water, and empty and rinse the DI water reservoir at least three times. Refill it to the top.

9.2. Empty the instrument rinse waste container.

9.3. Place the instrument probe in the bleach solution.

9.4. In the SEAL AQ2 Program, go to ‘Settings’ then ‘Maintenance.’

9.5. In ‘Initialise,’ press ‘Zero Segments.’ **Do not press ‘OK’ yet.**

9.5.1. Check to see if there are enough reaction segments from the day before to do maintenance. 2-3 unused is enough. Then press ‘Cancel.’ The sampler probe will go to the first clean segment instead of Segment 1, spot 1.

9.5.2. If there are not enough clean segments, replace the segments. Double tap clean segments on the bench to knock out excess manufacturing particles, then place in the instrument. Press ‘OK’ to reset the used segments. The sampler probe will go to Segment 1, spot 1.

9.6. Go to ‘Diluter,’ set the number of primes to 10, then press ‘Start Primes.’ Press ‘Start Primes’ twice more for a total of 30 primes in the bleach solution.

9.7. Go to ‘Cuvette Functions,’ set the drop-down number to 5, then press ‘Auto Wash.’ Press ‘Auto Wash’ twice more for a total of 15 washes in the bleach solution.

9.8. Transfer the instrument probe from the bleach cleaning solution to the beaker of DI water.

9.8.1. Repeat the ‘Primes’ three times for a total of 30 primes.

9.8.2. Repeat the ‘Auto Wash’ three times for a total of 15 washes.

9.9. Transfer the instrument probe to the DI water reservoir.

9.9.1. Repeat the ‘Primes’ five times for a total of 50 primes.

9.9.2. Repeat the ‘Auto Wash’ five times for a total of 25 washes.

9.10. Clean the aspiration bath with a Q-tip or cleaning swap.

9.10.1. The particles from manufacturing can get caught in the cuvette. Daily cleaning of the aspiration bath helps prevent this from occurring.

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9.11. In a reagent wedge in position 1, place the alkaline EDTA rinse (4.2.6.). In 'Cuvette Functions,' press 'Extra Wash.'

9.11.1. Watch the glass probe cleaner. A water vortex should be visible when the probe is being washed and liquid should not drip out.

9.11.2. Contamination between samples and reagents can occur if the probe cleaner is not operating properly. Liquid drips out of the glass probe cleaner when the probe creates a wider hole at the bottom of the glass (will look oval). This can occur over time or if the probe is bent. Replace the probe cleaner and probe if necessary.

9.11.3. Watch the syringe piston to see if there are excess bubbles dragging from the top. A worn syringe piston can interfere with the calibration auto-dilution and analysis. Replace if necessary.

9.12. After 'Extra Wash' is done, set the number of 'Auto Wash' to 5. Then press 'Auto Wash.'

9.12.1. Make sure the aspiration bath is filling and emptying each time. Change the two peristaltic tubing going to the aspiration bath if it is not.

9.13. After 'Auto Wash' is done, press 'Extra Wash' again.

9.14. After the second 'Extra Wash' is done, press the 'Auto Wash' again.

9.15. To adjust the aspiration, remove the cover from the top of the cuvette/lamp. Press 'Test Aspiration' and observe where the water line begins. It should be around 2 inches out of the cuvette. Repeat 'Test Aspiration' 2 more times, then check that the 4^{th}, 5^{th}, and 6^{th} repetition are in the same spot.

9.15.1. To make the water line come out of the cuvette further, increase the number of steps. Decrease the number of steps if it is too far out. Then repeat the above test.

9.15.2. If the water line is not around the same location in each repetition, random errors may occur in analysis. Replace the peristaltic tubing on the pump located next to the lamp.

9.16. In 'Initialise,' press 'Zero Segments,' and replace all used segments. Then press 'OK.'

9.17. Close the maintenance window then go to 'Settings' → 'Daily Startup' → 'Continue.' Write down the highest voltage and the corresponding filter in the maintenance book.

9.17.1. The highest voltage filter should remain the same for each instrument. A drastic change in voltage from day to day indicates a lamp going bad or an electrical issue.

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10. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

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

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

10.3. Read the MSDS information for safe reagent and sample handling.

10.4. Sample waste, excess reagents, and waste exiting the flow cell may be poured down the sink while flushing with water.

11. REFERENCES/DEFINITIONS

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.

11.1. AQ2 Operator Handbook and AQ Software guide.

11.2. EPA Method 365.1, Rev. 2.0 (1993)

11.3. SEAL Analytical AQ2 METHOD NO: EPA-119-A Rev. 5

11.4. DEP SOP LB-031, Limit of Detection Verification.

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

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

11.7. DEP SOP NU-041, Reagent Preparation Procedure.

11.8. DEP SOP NU-042, Standard Preparation Procedure.

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

11.10. DEP SOP NU-049, Total Phosphorus (TP) Autoclave Sample Preparation.

11.11. DEP SOP NU-082, Analysis of Low Level Total Phosphorus Using the Bran+Luebbe Segmented Flow Analyzer.

11.12. Quality Manual, Bureau of Laboratories, FDEP.

11.13. Laboratory Health and Safety Plan.

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

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Appendix of Significant Changes

07/15/2010	Section 1.1 and 8.2: Changed MDL from 0.020 mg P/L to 0.005 mg P/L (effective 12/29/2009).
04/14/2011	Section 8: Verification of the LCR was clarified.
03/28/2012	The standard preparation section was removed and added to the sample digestion SOP (NU-049).
08/20/2015	Data archival information added to section 7.
12/27/2018	MDLSPK is analyzed for the ongoing MDL and PQL verification (see section 8.4). Note in section 4 was modified to reflect that all standard certificates are stored in pdf format at the location provided in the link. Instrument startup procedure was modified in section 6.
12/10/2019	Added clarifications to Section 4. Added explanation on running multiple trays on one sequence to Section 6. In Section 8 the acceptance limits for the calibration standards recovery were added. Section 9 Maintenance and Troubleshooting was added. Technical support contact section was removed.

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