MINISTRY OF ENVIRONMENT AND CLIMATE CHANGE
LABORATORY SERVICES BRANCH

METHOD TITLE: THE DETERMINATION OF CHEMICAL OXYGEN DEMAND (COD) IN WATER BY PHOTO-ELECTROCHEMICAL MEASUREMENT

SECTION: INORGANIC CHEMICAL ANALYSIS

METHOD OWNERS: CRISTINA PETRAN AND WILLIAM JENNNINGS

QMS REVIEW BY: ESA MISTRY

APPLICABLE REGULATIONS/PROGRAMS:

MANAGER APPROVAL / CONTACT: _____ROBERT TOOLEY_____

This method is deemed fit for purpose as of the date shown on this cover page by the data provided in section 1.6 (Analytical Performance Summary).

* The approval of this document is valid for two years at which time it will be subject to review to determine if any updates or modifications are warranted.*

Note: **Equivalent suppliers to those stated in the method are acceptable. Reference to a particular brand does not constitute an endorsement by the Ontario Ministry of Environment.***

TABLE OF CONTENTS

THE DETERMINATION OF CHEMICAL OXYGEN DEMAND (COD) IN WATER
BY PHOTO-ELECTROCHEMICAL MEASUREMENT

1.0   SUMMARY

Chemical oxygen demand (COD) is a measure of the oxygen requirement of the oxidizable component of organic matter by a strong chemical oxidant. The test is most widely used in measuring waste loadings of treatment plants and in evaluating the efficiency of treatment processes.

1.1   Principle of Method

The COD is determined following a photo-electrochemical measurement of chemical oxygen demand. The sample enters a microcell which contains UV activated TiO_2 nanoparticles. Under UV light an electrical signal is generated by the TiO_2 nanoparticles, which is directly proportional to the amount of oxidizable species being present. As the organic matter in the cell gets oxidised, electrons are released, with the signal converted into an equivalent COD measurement.

1.1.1   Relationship to Other Methods.

Method E3515 follows ASTM International standard method D8084-17 "Standard Test Method for Photoelectrochemical Oxygen Demand of Freshwater Sources for Drinking Water Treatment Plants and Treated Drinking Water". Method E3515 replaced the standard dichromate methods E3170 and E3246, eliminating the use of harmful and toxic reagents.

1.2   Parameters Measured

The procedure determines the oxygen demand of that portion of the chemical constituents in a sample which are oxidized under the conditions of the test. The LIMS Product Code is COD E3515. Results are reported as mg/L O_2.

1.3   Sample Matrices

This procedure is routinely used on precipitation (WP), groundwater (WG), and surface water (WS).
Drinking water (WD) matrix is validated but not licensed for analysis.

1.4   Sample Requirements

1.4.1   Specifications.

Glass, polyethylene (PET) or polystyrene (PS) containers are acceptable. A minimum sample volume of 100 mL is required for analysis. Samples are to be stored at $5 \pm 3°C$.

Sample holding time is 7 days for unpreserved samples from the sampling date.

All samples are refrigerated at 5°C ± 3°C upon receiving. Before analysis samples are brought to room temperature for a minimum of 2 hours.

NOTE: If samples cannot be analyzed within the required holding time, the samples can be preserved with sulphuric acid to pH≤2 (Use 0.2 mL of acid per 100 mL of sample). Preserved samples have a holding time of 21 days from preservation date (see 1.4.2).

NOTE: An in house stability study was performed using sulphuric acid and nitric acid as preservatives. 0.2 ml of acid was added per 100ml of sample. These samples were held in clear containers for the duration of the study. The study showed that unpreserved samples were stable for 7 days from sampling and preserved samples were stable for 21 days from preservation date (Refer to 1.7.12)

1.4.2   Contingencies.

Unpreserved samples may be preserved with sulphuric acid to pH ≤ 2 and stored up to the holding time and will be adjusted between pH 4 - 9 with 1N sodium hydroxide prior to analysis (see 1.7.9). Check pH of the sample using pH paper. (Pour small amount of pH adjusted sample to check the pH range.)

Acidified samples will have the LIMS remark code PPS (test performed on preserved sample).

Any sample that has been analyzed after the acceptable holding time will have LIMS remark code UAL (Unreliable: Sample Age Exceeds Normal Limit) accompanying the result.

1.5   Shortcomings

1.5.1   Interferences.

The PeCOD® measurement is affected by the presence of chloride at concentrations higher than 400 mg/L.

1.5.2   Biases.

Potassium hydrogen phthalate (KHP) produces significantly biased high results. Samples at pH ≥ 9 may bias results low.

1.5.3   Limitations.

PeCOD® measures total soluble COD, which does not include proteins, macromolecules or cellulose. Dichromate COD provides total COD, so differences may be observed for some samples between E3515 and historical data from E3170 and E3246.

1.6 Analytical Performance Summary

1.6.1 Method Performance, Within-Run Precision (Duplicates)
(November 2017 to January 2018)

Sample duplicates are analysed in every batch to monitor method precision. The performance data covers the period from November 2017 to January 2018.

Table 1.6.1 Duplicates

Number of Data Pairs	Range (mg/L as O_2)	Standard Deviation	% Coeff of Variation
5	0 - 2.5	0.3	33
8	2.51 - 5.0	0.31	8.3
7	5.01 - 12.5	0.12	1.6
8	12.51 - 25.0	0.23	1.2

Note: Duplicates will be re-evaluated as data becomes available.

1.6.2 Instrument Performance, QC Control Standards

Two levels of QC control standards are analysed in every batch to check if the calibration is valid before samples are analysed. Table 1.6.2 shows the instrument performance covering the period from November 2017 to January 2018.

Table 1.6.2 QC **Control Standards.**

Number of Data Points	Expected Concentration (mg/L as O_2)	Mean Concentration (mg/L as O_2)	Mean Bias (mg/L as O_2)	Standard Deviation
17	20	19.8	-0.2	0.42
15	5	4.7	-0.3	0.31

1.6.3 Method Detection Limit (MDL) - Verified January 2018

MDL was established by analysing ten replicates of a low level standard at the lower 5% of the working range. The data is shown in Table 1.6.3 supports an estimated MDL value of 0.65 mg/L. The reporting method detection limit is 1.0 mg/L.

Table 1.6.3 MDL

	Target Value (mg/L)	n	Average mg/L	Standard Deviation mg/L	LIMS Reported MDL (mg/L)
COD	5	10	4.64	0.23	1.0

SD (Standard Deviation); MDL = $t_{(.01,9)}$ x SD = 2.821 x SD = 0.648

1.7 Bibliography

Note: **The most current version of Branch and Section SOPs and Forms are available from Qualtrax.**

1.7.1 ASTM D8084-17, Standard Test Method for Photoelectrochemical Oxygen Demand of Freshwater Sources for Drinking Water Treatment Plants and Treated Drinking Water, ASTM International, West Conshohocke PA, 2017, www.astm.org

1.7.2 Zhang, S., Jiang, D., Zhao, H. Development of chemical oxygen demand on-line monitoring system based on a photoelectrochemical degradation principle. *Environ. Sci. Technol.* **2006**, *40*, 2363–2368.

1.7.3 Zhang, J., Zhou, B., Zheng, Q. Li, J., Bai, J., Liu, Y., Cai, W. Photoelectrocatalytic COD determination method using highly ordered TiO_2 nanotube array. *Water Research*. **2009**, *7*, 1986-1992.

1.7.4 Zhao, H., Jiang, D., Zhang, S., Catterall, K., John, R., Development of a direct photoelectrochemical method for determination of chemical oxygen demand. *Anal. Chem*. **2004**, *76*, 155-160.

1.7.5 Jonsson, S., Validation of mercury free methods for analysis of Chemical Oxygen Demand in municipal wastewater, Uppsala University, Sweden, 2015.

1.7.6 PeCOD® COD Analyser L100 User manual. Mantech Inc.

1.7.7 Aqua Diagnostic. Systematic Comparison of PeCOD® and Dichromate Methods of COD Measurement for a Suite of 34 Organic Species, 2010

1.7.8 Mantech Technical Bulletin No. 2016-011 – Glossary of PeCOD Terms, 2016.

1.7.9 Mantech Technical Bulletin No. 2015-004 – Differences between PeCOD and Dichromate COD.

1.7.10 Mantech Technical Bulletin No. 2011-017 – Overview of PeCOD Technology, 2011.

1.7.11 Mantech Application Note No. 1 – PeCOD – Electrode Block and Sensor Installlation/Storage Instructions, 2016.

LaSB APPROVED   **PAGE 7 OF 24**
February 26 2018   **COD – E3515**
REVISION: 2

1.7.12   Furdui, V. and Cristina P. (2018). Updated Validation Report for Method E3515. (ICA's Internal Report). Ontario Ministry of the Environment, Laboratory Services Branch, Toronto.

1.7.13   List of Supporting Branch/Sectional SOPs

      LSBSOP.035   Staff Training
      SOPICA.001   Procedure for Control Charting
      SOPICA.002   Procedure for Records Management

1.7.14   List of Supporting Forms and Logbooks

      FRM_ICA_010   Preparation of Stock Standard Solution
      FRM_ICA_012   Preparation of Working Standard Solutions
      FRM_ICA_017   Run Review Cover Sheet
      FRM_ICA_021   Analyst Method Proficiency Checklist, ICA

      FRM_LSB_031   Staff Orientation /Training / Updated Training
      FRM_LSB_047   Analyst Method Proficiency Testing
      FRM_LSB_048   Analyst Method Proficiency Summary

      PeCOD® Binder 1   Equipment List, Reagent Preparation
      PeCOD® Logbook 1   Maintenance/Troubleshooting/Corrections

1.8   History of Changes/Revisions

1.8.1   February 2014, Version 1.0
       New method.

1.8.2   February 2016 – Version 2.0

       Section 1.6 performance data updated. Reference to FRM_3515_001 Control Charting for Method E3515, Green Range, not used as posted form was removed. Minor typographical/editorial changes throughout the document applied.

1.8.3   June 2016 – Version 2.1

       In response to a CALA assessment the following updates to the method were applied. QCC (10 mg/L) was added to sections 1.6.2 (as a Note), 5.2.7, 6.1.1 and the acceptance criteria in 6.2.1.
       Section 5.2.10 removed, (Chloride interference check solution) this solution is not used as visual check of white precipitate on electrode is carried out.
       Note following section 6.1 was clarified for observation and action required. .
       The frequency of CRM was defined as quarterly; it is now changed to be run with PT samples in section 5.2.8 and 6.2.4.
       Note added under section 5.0 on the one point calibration

1.8.4   February 2018- Revision 2.0

       Note: Version 2.1 was auto published in Qualtrax as Revision 1.0.
       Updated method to follow standard method ASTM D8084-17.

Reference to ASTM method D8084-17, added in section 1.7
Performance data updated from the revised procedures.

1.9   SAFETY

Refer to LaSB Heath & Safety Manual for 125 Resources Road Complex for General Health and Safety Guidelines.

1.9.1   General

Any analyst working in the laboratory must be thoroughly familiar with proper safe laboratory practices as outlined in the 125 Resources Road Complex Health and Safety Manual (available through the LaSB desktop).

1.9.2   Protective Safety Attire

Lab coats and safety glasses must be worn in the laboratory at all times. Protective gloves shall be worn when handling any chemicals described in this procedure. Apron and face shield must be worn when handling concentrated acid solutions.

1.9.3   Chemicals

Ensure that WHMIS regulations are followed and consult the information available in Safety Data Sheets (SDS) for proper procedures of handling and disposal of the chemicals and stock solutions use in this method. Preparation of toxic and corrosive reagents must always be carried out in a fume hood.

1.9.4   Equipment

Analyst must be proficient before operating the instrument. Refer to the instrument manual for any safety considerations before doing any routine maintenance or repair of the instruments. Always disconnect the electrical power source before undertaking any service work.

1.9.5   Laboratory Cleanliness

Good laboratory practices require that all work areas be kept clean and tidy. Counter tops must be cleared and wiped down regularly. Unused equipment must be properly stored and labelled out of service. Dirty glassware must be cleaned and stored in cupboards. Any chemicals/reagents spilled on and around balances must be removed immediately.

2.0   SAMPLE PREPARATION

2.1   Labwares

2.1.1   Tubes, disposable centrifuge tubes 50 mL plastic rinsed 3 times with deionized water and air dried.
2.1.2   Racks, for centrifuge tubes.
2.1.3   Pipettes, Eppendorf, various sizes, calibrated.
2.1.4   Pipettes, glass, class A, various sizes.
2.1.5   Volumetric Flasks, class A, various sizes.
2.1.6   pH paper (Range 1-14).
2.1.7   Small Beaker or disposable polystyrene tubes.

2.2   Reagents

2.2.1   Pure Water that has been further deionized, Pure-DW.
2.2.2   1 N sodium hydroxide – Expiry one year from preparation date. Dissolve 40 g NaOH in 800 mL Pure-DW. Dilute to 1 L with Pure-DW.
2.2.3   Concentrated Sulfuric Acid (if samples need preservation, add 0.2 mL of concentrated sulphuric acid for every 100 mL of sample).

2.3   Equipment

2.3.1   Centrifuge, set at 1500 RPM.

2.4   Procedure

2.4.1   Create a workgroup file in LIMS for analytical department 5318. Use the DCI (Direct Computer Input) function "enhanced modify workgroup" to sequence samples in ascending order by matrix. Print an Enhanced Workgroup report and a Workgroup Sample Holding Time.

2.4.2   Vigorously shake the samples and pipette 15 mL of sample into clean, dry 50 mL centrifuge tube.

2.4.3   If the samples contain particulates, centrifuge at 1500 RPM for 10 minutes. Enter LIMS Remark Code, FAQ (Fraction of Sample Analyzed: Aqueous Phase only).

2.4.4   Analyse one duplicate for every 20 samples, to a maximum of 3 duplicates. Record the sample numbers of the duplicate samples on the bench sheet.

2.4.5   If samples are suspected to have COD levels higher than 25 mg/L, perform a dilution with Pure-DW and record dilution factor. Pipette 15 mL of diluted samples into clean, dry 50 mL centrifuge tube.

NOTE: Ensure volumetric testing and traceability as per LSBSOP021.

If the sample is preserved, adjust pH using 1N NaOH solution to obtain the pH between pH 4 – 9 (see 1.7.9). Check pH of the adjusted sample using pH paper, by pouring a small aliquot in a small beaker or a disposable polystyrene tube.

3.0   ANALYTICAL PROCESSING

No further processing of the samples is required before introducing them to the Detection System.

4.0   DETECTION SYSTEM

4.1   Labwares

4.1.1.   Volumetric flask, class A, various sizes.
4.1.2.   Pipettes, Eppendorf, various sizes, calibrated.

4.2   Reagents

4.2.1.   Pure Water that has been further deionized, Pure-DW

4.2.2.   PeCOD® Electrolyte. Blue Range (<25mg/L) - Expiry as stated by the manufacturer.
NOTE: Ensure sufficient electrolyte solution for the duration of the entire analytical run (approx. 5 mL per sample).

4.2.3.   Electrolyte Blank Solution – Expiry one year from preparation date (1 part PeCOD Electrolyte with 3 parts Pure-DW).
NOTE: Ensure sufficient electrolyte blank solution for the duration of the entire analytical run (approx. 2.5 mL per sample).

4.3   Equipment

4.3.1.   PC titrate equipment
-Interface Module PC-1055-00
-2 Mantech PC titrate titra-dose / A modules PC-1000-550 (electrolyte and calibrant)
- Mantech pump PC 1000-475
4.3.2.   Autosampler- Mantech, 73 tube capacity
4.3.3.   PeCOD® L 100
4.3.4.   PC-Titrate™ V3.0.0.718 software (automated system)

4.4   Procedure

4.4.1.   In LIMS, create a Workgroup of appropriate length. The run format is 20 samples followed by an in-run QC check (see section 6.1.1)

4.4.2.   Turn ON the automated titration system at the power bars (consisting of the computer, Interface module, 2 Mantech PC titrate titra-dose / A modules, pump, Mantech auto sampler) and PeCOD unit, if the system has been shut down. Set up the reservoir (the 20L container) for the rinsing station with Pure-DW, fill up /check the level of the electrolyte and the electrolyte blank solution. Ensure sufficient electrolyte and electrolyte blank solution for the duration of the entire analytical run.

4.4.3.   On the computer desktop, double click the icon "PC-Titrate V3.exe" to activate the PC-Titrate system. A new window named "PC_titrate For Winodws V3-Main Menu" will open.

4.4.4.　Ensure that the tubing is properly connected; crinkle free and that sufficient solutions are available for the entire run. Examine the positions of the stirrer, dispense tips, sample transfer needle and ensure that they are properly positioned in the probe holder assembly.

4.4.5.　Examine the PeCOD® sensor for any fine white precipitate. This indicates a reduction in chloride tolerance (caused by AgCl adhering to the surface of the sensor which limits the proper functioning) (refer to "PeCOD Troubleshooting Guide" - PeCOD binder 1). If white precipitate is visually noted on the sensor replace the sensor with a new one (see Appendix 1 – sensor routine).

4.4.6.　At the end of the run check the sample tubes and make sure that the level is consistent.

4.5　Shutdown Procedure

4.5.1.　When the analyses have been completed, the results will be displayed on the screen. If a hard copy is desired, press the print icon to print the results. The probe holder assembly will then go back to the home position and remain suspended in air. To return the needle to the rinse station, select Titrator from the PC-Titrate main menu and Manual control. From the auto-sampler tab choose load tray from folder. Select tray file standard tube sampler-73x50.GTY then select Home sampler and go to this location XYZ.

5.0   CALIBRATION

5.1. Labwares

    5.1.1.   Flasks, volumetric, Class A, various sizes as required.
    5.1.2.   Weighing boat, or plastic weighing dishes.
    5.1.3.   Tubes, 50mL plastic disposable rinsed 3 times with DI water and air dried.
    5.1.4.   Pipettes, Eppendorf, various sized as required
    5.1.5.   Analytical Balance, readable to $\pm$ 0.0001 g.

5.2. Standards and Reference Materials

NOTE: Unless otherwise stated all dry chemicals are stored at room temperature for a period of up to three years from the date it is opened.

NOTE: Stock and Working solutions are stored at 5$\pm$3°C, and are prepared every 3 months or sooner if depleted.

5.2.1.   Sorbitol, $C_6H_{14}O_6$ (>98%)
NOTE: The source of the calibration and quality control stock solutions must be from different suppliers. If two different suppliers are not available then two different lot numbers are used for calibration and quality control standards.

5.2.2.   Calibration Sorbitol Stock Solution (1000mg/L as O_2)

Dissolve 0.8758 $\pm$ 0.0001 g sorbitol in 800mL Pure-DW in a 1L volumetric flask and dilute to the mark.

5.2.3.   Working Calibration Standard – 20 mg/L

Working calibration standard is prepared by diluting 20 mL of the Calibration Sorbitol Stock Solution to 1 L with Pure-DW.

5.2.4.   Quality Control Stock Standard Solution (1000 mg/L as O2)

Using different sorbitol source or different lot # independent of the calibration stock standard, dissolve 0.8758 $\pm$ 0.0001 g of sorbitol in a 1 L volumetric flask using Pure-DW and dilute to the mark.

5.2.5.   Working Quality Control Standards

Dilute the following volumes of Quality Control Stock Standard Solution (section 5.2.4) with Pure-DW to 1L using volumetric class A glassware.

QC	Volume of Stock (mL)	COD concentration (mg/L)
QCA	20	20
QCB	5	5

NOTE: New QC standards are verified before existing solutions are depleted or expired (refer to section 5.4).

5.2.6.   CRM (Certified Reference Material)

A CRM is purchased externally as a concentrated solution and is prepared according to the manufacturer's instructions. The expiry date and storage conditions are indicated in the Certificate of Analysis (C of A) of the CRM and on the label.   This is analysed every six months.

5.3. Equipment

5.3.1. Automated PeCOD

5.3.1.1 Priming the pumps

5.3.1.1.1 Priming before the run

From the "PC-Titrate for Windows V3-Main Menu" press "Titrator" and choose "Manual Control".
Press "digital' tab, look for Digital I/O 9-16, place a tube under the electrolyte dispense tip and press I/O 13 to prime the Electrolyte Pump; observe the solution flowing and press back the I/O 13 to stop the priming.
Look for Digital I/O 9-16, place a tube under the calibrant dispense tip press I/O 14 to prime the Calibration Pump; observe the solution flow and press back the I/O 14 to stop priming.
Press "OK" button to finish this step.

5.3.1.1.2 Monthly flow rate checks for Pumps.

Monthly check the calibrant and electrolyte flow rate/volume by placing 50 mL graduated cylinder under the calibrant/electrolyte dispensing tip.
Select "Pump cal- Calibrant" schedule and "Pump cal-Electrolyte" schedule, or choose the dedicated icons from the bottom of the "PCTitrator V3 Main menu" window.
Press Auto-generate Order Number, fill the sample Name column and press Start.
A window will pop-up informing that the solution will be dispensed for 60s.
Measure 3 times the volume, average the value and change accordingly in the "Interface\Hardware Setup\Digital Ampliers under "Flow rate" for Extended Digital Output #13.
Record the value in the Maintenance LogBook.

5.3.1.2 Calibration

Calibration Range:

The Calibration range is covered from method MDL (1.0 mg/L) up to 25 mg/L.
Any samples outside the calibration range of 25 mg/L are diluted with Pure-DW and re-analysed noting the dilution factor.
Also when samples are > 25 mg/L, the instrument will flag with a code to indicate that 'COD out of Range', see the print out from the calibration results.
The instrument will print 'PeCOD Calibration Results' print-out.

5.3.1.2.1   Automatic instrument calibration (calibration from pre-mixed calibrant)

Place two clean dry empty tubes on the tray in the position 1 and 2.

Press "Daily Start Up" button and automatically under "Schedule" section, 2 rows will be populated: 1st row will be PeCOD Cal 2X (the instrument will calibrate automatically 2 times), Order number (year/month/day-order), Sample Name and Vial and 2nd raw will be PeCOD QCcheck (instrument will re-read the calibration solution).

Fill out in the table the empty tube position with number 1 and 2.
Press "Start" button and the calibration will start immediately.

For the next day calibration fill out the Start Date cell in the table (select date and time, am or pm) and press 'Start'.

The software will calibrate maximum 3 times and will re-analyze the same solution as QC.

Check for M, C, Iterm(μA)   and QC criteria, see Table 5.3 below for acceptable ranges.

If the criteria are met continue with the sample analysis; if not try to re-calibrate or start troubleshooting, see Table 5.3 below 'Calibration Acceptable Ranges'.

Note: The following manual procedures can also be followed if pre-mixed calibrant is depleted.

5.3.1.2.2   Manual calibration (calibration from pipetted calibrants in the tubes)

Perform at least 2 calibrations before every sample run to achieve low end resolution and good accuracy.

 Pipette 2 times 15 mL calibration solution (20 mg/l) into 2 clean dry 50 mL tubes and place them on the tray into positions 1and 2.

Under the "Schedule" section choose "PeCOD cal 1XPump Adds ELEC" (the instrument will add 5mL Electrolyte).

Place a 3rd tube on the tray in the position 3 with 15 mL calibration solution in order to re-read the Calibration solution.

Under the "Schedule" section choose " PeCOD Sample 1X". Press "Start" button and the calibration will start.

Check for M, C, Iterm(μA)   and QC criteria, see Table 5.3 below for acceptable ranges.

If the criteria are met, continue with the sample analysis; if not try to re-calibrate or start troubleshooting and see Group Leader.

Table 5.3 - Calibration Acceptable Ranges:

M Value (COD/µC)	C Value (µC) Advanced blue	Iterm (µA)	QC Recovery
0.01<M<0.08	50<C<300	>= 16	17< QC Recovery <23

5.3.1.3 Creating Run List

From the "PC-Titrate for Windows V3-Main Menu" press "Titrator" once and choose "Run Titration".

Under "Schedule" choose "Load Template" (if previously created) or start creating by pressing "X Rows", type the approximate number of rows (including QCs plus samples) and press OK.

Double click under "Schedule" and a new window will pop-up "Schedule Search Form – Select Schedule to use". Choose from the following available schedule:

"PeCOD Cal 1X, 2X, 3X" – calibrates the instrument 1time, 2times or 3times with pre-mixed 20 ppm calibrants.

"PeCOD QCCheck" – re-analyse Calibration Solution as QC
"PeCOD CAL 1X Pump Adds ELEC" – calibrates the instrument from the tube adding 5 mL electrolyte

"PeCOD Sample 1X" – analyse the sample adding 5 mL electrolyte

 "PeCOD Sample 1X No electrolyte" – analyse the sample but the 5 mL electrolyte are added manually (possible used for troubleshooting)

"Wait 30 minutes" – interrupts the analysis for 30 minutes and starts automatically after this interval

"Wait to continue" – interrupts the analysis and wait for pressing "OK" to continue (if there is a need to check QC samples or before last QC if there is a need to check for extra-dilutions). After pressing "OK" press "Priority" within first 10 seconds in order to modify the "run List"

Follow Run Format 6.1.1 and populate the "Schedule", "Order Number", "Sample Name": and "Vials" Columns.

Type "1" in the "Weight" column if there is no dilution or enter dilution factor if dilution was performed.

Press "Check Timetable" for errors. Highlight and press "Delete Highlighted Sample" if there are any empty rows.

Press "Print Existing Timetable", choose "Printer destination" and press "Print".

Verify the tubes on the sampler tray against the printed table and press "Start"

LaSB APPROVED   **PAGE 18 OF 24**
February 26, 2018   **COD-E3515**
RIVISION: 2

button.

5.4. Standards Verification

For working Quality Control (QC) standards (section. 5.2.5), analyze the new standard in triplicate and compare the results to previous QC performance (must be within ± 2SD, warning limits) described in section. 6.2.2.

If the new working standard fails the acceptance criteria, repeat the standard again in triplicate. If it is still outside the acceptable limits, the cause is investigated and the solution is discarded. Prepare a new working solution and record all the results and comments on form FRM_ICA_012.

6.0   RUN PROCESSING AND QUALITY ASSURANCE

6.1.   Run Format

6.1.1. The following Run Format is used

1.   Blank (Pure-DW)
2.   QCA (20 mg/L)
3.   QCB (5 mg/L)
4.   DUP (one for every 20 samples to a maximum of 3 duplicates)
5.   CRM (every 6 months)
6-20. Samples (20)
21.   In-run QCA

Run with additional samples after In-run QCA in position 21.   The number of samples is limited by the available space with the auto sampler. The run is ended by analysing the In-run QCA.

NOTE: If pre-dilution of the sample is performed, enter the dilution factor in the Weight column.

QC A and B	Quality control standards in position 2 and 3 of Run Format.	To verify calibration control standards A,B (s. 5.2.5)
DUP(x)	Duplicate samples	1 to 3 duplicates to monitor within-run precision and repeatability.
In-run QCA	QC standard (20 ppm)	To monitor Calibration status during analysis.

6.2.   Run Control Operations/Limits

6.2.1.   Blank (Pure-DW)

Expectation: The blank reading must be ≤ 0.5 mg/L (MDL=1.0 mg/L O_2).

Action: Values greater than 0.5 mg/L O_2 indicate possible contamination or an erroneous reading. Repeat analysis, change Pure-DW and/or tube containing the blank.

6.2.2.   Calibration Control Standards:

Expectation: The QC standards QCA and QCB verifies the calibration. The limits for QCA and QCB results are calculated from historical data (November 2017 to January 2018). Data are as shown in the table below (section

1.6.2)

Calibration check QC's	Expected Value (mg/L O$_2$)	Warning Limits (mg/L O$_2$)	Control Limits (mg/L O$_2$)
QCA	20	19.2 – 20.8	18.8 – 21.2
QCB	5	4.4 – 5.6	4.1 – 5.9

Action: Values falling outside of the control limits may be identified during the analytical run or data processing. If the control limit exceedance is identified during the analytical run, immediately stop the run, correct the problem (e.q. re-analyze the QC or recalibrate and repeat the samples impacted).If the control limit exceedance is identified during data processing investigate the possible cause, take action and, repeat all the samples if within hold time or qualify data in LIMS with the remark code "UQC" – Unreliable-QC failure. Consult the group leader or manager for instructions.

Values between the warning and control limits may indicate a developing problem. Examine all aspects of the calibration process, reagents, and standards to be sure all are within specification. Carry out QC trend analysis as per SOPICA001.

6.2.3. Duplicates:

Expectation: Samples analyzed in duplicate must agree to within three standard deviations of the selected concentration span. (see 1.6.1)

Concentration Range (mg/L O$_2$)	Control Limits (mg/L O$_2$)
0 - 2.50	$\pm$ 0.9
2.51 - 5.0	$\pm$ 0.9
5.01 - 12.5	$\pm$ 0.4
12.51 - 25.0	$\pm$ 0.7

Action: Regard a single failure as a warning that some part of the process may be out-of-control, and conduct a review of those steps which may lead to such failures, correct any problems identified and repeat analysis of samples. Regard the failure of more than one duplicate as cause to withhold the run results until the cause is identified, corrected and affected samples are identified for re-analysis. Where a cause cannot be identified with confidence, repeat the run.

6.2.4. In-run Quality Control Standards

Expectation: The In-run QCA standard monitors the performance during the analysis of the run. The control limits for the In-run QCA are the same as that for initial QCA analysed after the calibration.

Expected Concentration (mg/L O_2)	Control Limits (mg/L O_2)
20	18.8 – 21.2

Action: If an In-Run QCA is not within the acceptable limits, samples before and after up to the next passing In-run QCA must be repeated.

6.2.5. Certified Reference Material (CRM)

The certified reference material is analyzed every 6 months. The theoretical concentration and limits may change for externally purchased CRMs. Refer to the Certificate of Analysis certificate issued when purchased for theoretical concentration and acceptable control limits.

Expectation: The reference material is used to access accuracy/recovery of COD (mg/L O_2). The CRM result must fall within the stated acceptance limits in the C of A of the reference material.

Action: If the CRM falls outside the acceptance limits, check the calibration results and correct any discrepancies. Repeat analysis of the CRM. If the CRM still falls outside the limits, consult the group leader for instructions.

6.3. Calculation Procedure

6.3.1. The calculations of the results are performed by PC-Titrate™ software and no other additional calculations are required. Report the data from COD Final column (mg/L) (COD value multiplied by dilution factor)

6.3.2. Verify the results with sample order from the print out against the PeCOD® Units – L 100 display.

6.3.3. Plot the calibration control standard results in the Control chart. Examine the results in the context of the control chart for trends which may indicate developing problems or can confirm the identified problems. Take appropriate action to correct any problem identified (This summary must be audited, with appropriate comments and approved by the group leader or manager).

6.4 Reporting

6.4.1   Chemical oxygen demand is reported in mg/L as O_2

MDL = 1.0 mg/L O_2

6.4.2   In LIMS, first create a "Summary Record" under Result menu and press "Commit". Manually entry the results in the "Entry Data" menu. Once the data are in LIMS "move" the workgroup . Flag to Y in "Replicate Data"' any results which are repeated values that require reporting. In "Update Status", set to REDO any samples transmitted with NDRR (No Data: Rerun Requested), or other samples which require repeating. Audit the results from the APP1report. After assuring the quality and transcription of the results, update the status to APP1 and print an APP1 approval report.

6.4.3   Attach together the Run Review' cover sheet (FRM_ICA_017), Control charts print out, LIMS APP1 approval report, results print out, , PeCOD® instrument calibration report, the enhanced work group report, holding time work list, and run sample table. Label the package with the unique run number ("R-workgroup #"), LIMS workgroup number, initial and date, and have the manager or group leader audit and initial the package. Store these records according to SOPICA002.

.

Appendix 1

Changing the sensor

When the calibration no longer is passing and the sensor coating is depleted, the sensor should be changed. Open the PeCOD® unit and remove the sensor. Replace it with a new sensor and close the door
Automated sensor routine

Place 2 empty tubes positions 1 and 2 of the autosampler. Select the new sensor routine and 3 schedules will pop-up in the table ("PeCOD New Sensor Reset" and 2 times "PeCOD CAL 2X). Populate the table with tubes location and select the start icon. Enter the new sensor number (i.e., 11117) and select enter. The calibration solution will be taken automatically from the external bottle. Alternatively, delete the 2 schedules "PeCOD CAL 2X" and replace them with "PeCOD Cal 1X PUMP ADDS ELEC" 4 times where the calibrant solution will be taken form the 4 with 15 mL of calibrant (20 mg/L) tubes placed on the autosampler tray.
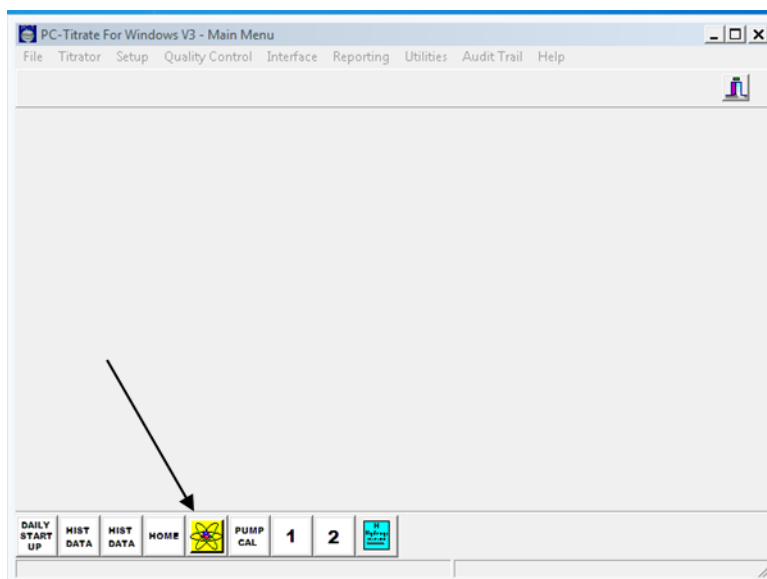
Manually:

Set up the system for manual sample introduction. Mix three parts of calibration solution with one part electrolyte and have ready to introduce into the system.. On the PeCOD® screen select menu, set up, enter, sensor operation, enter, new sensor. Confirm new sensor will appear on the screen. Select yes by pressing enter. The previously prepare electrolyte solution will then introduced into the system.

Troubleshooting

If an LED or over current error occurs the sensor may need to be conditioned. On the PeCOD® unit select menu, setup, enter, sensor operation, enter, set baseline. Reduce the baseline from 20

µA to 10-15 µA. Run a calibrations or quality control solutions at this level. Gradually increase the baseline back to 20µA without getting an error message.

If a communication error occurs reboot the system. If the error continues erase the error logs. On the PeCOD® unit select menu, diagnostics, enter, erase logs, enter, erase event log, and enter. For the system to work an error must exist in the logs so a false error must be created. To create a false error on the PeCOD® unit select menu, operation, enter, run calibration, enter. Once it start select exit to create a false error.