

**METHOD 14XXCOD**  
**Chemical Oxygen Demand (COD)**  
**by Dichromate Oxidation and Photometry**

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### Acknowledgments

This method was prepared under the direction of Gunter Decker, Senior Global Product Manager, Merck KGaA, Darmstadt, Germany, and was updated to current EPA format by Dr. Edward F. Askew, Askew Scientific Consulting LLC, Muscatine, Iowa 52761.

### Disclaimer

This method has been submitted to the U.S. Environmental Protection Agency for use in EPA's water programs. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Merck KGaA welcomes suggestions for improvement of this method. Suggestions and questions concerning this method or its application should be addressed to:

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## **Introduction**

This method is a convenient, ready to use reagent test kit for Chemical Oxygen Demand (COD) testing, which is based on EPA Method 410.4, Revision 2, August 1993. The test kit is suitable for both on-site testing and typical laboratory testing. The test kit consists of pre-measured reagent sets for both sample preparation and analytical determinations. This method's approach with pre-measured reagents reduces analytical errors, the amount of hazardous waste, and increases occupational safety.

The method incorporates multiple COD ranges (spanning 4 to 90000 mg/L). This broad range can help to reduce the amount of sample manipulation, especially in field applications.

**Method 14XXXCOD**  
**Chemical Oxygen Demand by Dichromate**  
**Oxidation and Photometry**

**1. Scope and Application**

1.1. Chemical oxygen demand (COD) is used to measure the oxygen equivalent of the organic matter of treated and untreated sanitary and industrial waste waters, and other waste water matrices. This method determines the oxygen equivalent of materials by oxidizing them in the presence of sulfuric acid and a known excess of potassium dichromate. Summarized data in Table A, below

<b>Table A: Summarized Spectroquant® Tests</b>					
<b>Catalogue Number</b>	<b>Spectroquant® Test kits</b>	<b>Measuring range</b>	<b>Absorbance Change with Concentration of COD</b>	<b>Wavelength (nm)</b>	<b>Chemical Species Detected</b>
1.14560	COD Cell Test	4.0 – 40.0 mg/L COD	Decrease Absorbance	340	Cr <sub>2</sub> O <sub>7</sub> <sup>2-</sup>
1.01796	COD Cell Test	5.0 – 80.0 mg/L COD	Decrease Absorbance	340	Cr <sub>2</sub> O <sub>7</sub> <sup>2-</sup>
1.14540	COD Cell Test	10 – 150 mg/L COD	Decrease Absorbance	445	Cr <sub>2</sub> O <sub>7</sub> <sup>2-</sup>
1.14895	COD Cell Test	15 – 300 mg/L COD	Decrease Absorbance	445	Cr <sub>2</sub> O <sub>7</sub> <sup>2-</sup>
1.14541	COD Cell Test	25 – 1500 mg/L COD	Increase Absorbance	605	Cr <sup>3+</sup>
1.14690	COD Cell Test	50 – 500 mg/L COD	Decrease Absorbance	445	Cr <sub>2</sub> O <sub>7</sub> <sup>2-</sup>
1.14691	COD Cell Test	300 – 3500 mg/L COD	Increase Absorbance	605	Cr <sup>3+</sup>
1.14555	COD Cell Test	500 – 10000 mg/L COD	Increase Absorbance	605	Cr <sup>3+</sup>
1.01797	COD Cell Test	5000– 90000 mg/L COD	Increase Absorbance	605	Cr <sup>3+</sup>
1.17058	COD Cell Test for seawater / high chloride contents	5.0 – 60.0 mg/L COD	Decrease Absorbance	340	Cr <sub>2</sub> O <sub>7</sub> <sup>2-</sup>
1.17059	COD Cell Test for seawater / high chloride contents	50 – 3000 mg/L COD	Increase Absorbance	605	Cr <sup>3+</sup>

1.2. This method is for use in the United States Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Clean Water Act.

- 1.3. The method detection limit (MDL; 40 CFR 136, Appendix B) has been established at 2.0 mg/L (Section 13.2). The Minimum Level (ML) for reporting results is 4.0 mg/L (Section 13.3).
- 1.4. This method is capable of measuring COD in the range of 4 to 90000 mg/L, and may be extended to higher levels by serial dilution.
- 1.5. This method measures organic and inorganic compounds which are oxidizable by dichromate, with the exception of some heterocyclic compounds (e.g., pyridine), quaternary nitrogen compounds, and readily volatile hydrocarbons.
- 1.6. This method is intended for the analysis of COD on treated and untreated sanitary and industrial waste waters, and other waste water matrices.
- 1.7. This method is based on prior Environmental Protection Agency (EPA) and association methods for the determination of COD (references 16.1 and 16.2).
- 1.8. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

## **2. Summary of Method**

- 2.1. Depending upon the measurement range (Section 17, Table 1), an aliquot of sulfuric acid-preserved samples (to pH <2) is measured into Spectroquant® COD cell tests and mixed thoroughly. The pre-measured reagents in the Spectroquant® COD cell tests contain a known excess of potassium dichromate and sulfuric acid. Silver sulfate and mercuric sulfate are included as a catalyst and chloride complexing agent, respectively.
- 2.2. The Spectroquant® COD cell tests are sealed and heated at 148 - 150°C for two hours. Oxidation of inorganic and organic matter results in a production of the green trivalent chromium ion ( $\text{Cr}^{3+}$ ) and remaining chromate.
- 2.3. The photometric determination can be conducted on a colorimeter, photometers or spectrophotometer.
- 2.4. Quality is assured through the use of quality control samples (QCS), calibration of the instrumentation by using calibration test solutions (potassium hydrogen phthalate-KHP), and operation of a formal quality assurance program.

## **3. Definitions**

- 3.1. Definitions for terms used in this method are given in the glossary at the end of the method (Section 18).

## **4. Interferences**

- 4.1. Halogens interfere to produce precipitants that are oxidized only partially. The interferences are largely overcome by complexing with mercuric sulfate. For waters containing over the levels listed in Table 1, the sample must be diluted with deionized

water to overcome this interference.

- 4.2. Volatile straight chain aliphatic compounds are not oxidized appreciably by this method. These compounds are oxidized only to the extent that they remain in contact with the oxidant. These compounds are oxidized more readily through the addition of silver sulfate to the Spectroquant® COD cell test.
- 4.3. Pyridine and related compounds resist oxidation.
- 4.4. Nitrite (NO<sub>2</sub><sup>-</sup>) interferes, but as NO<sub>2</sub> levels in waters rarely exceed one or two milligrams per liter, this is not considered a significant interference. If necessary, high NO<sub>2</sub> levels, and interferences, can be overcome by the treatment of the test sample with sulfamic acid before digestion.

## 5. Safety

- 5.1. This method does not address all safety issues associated with its use. The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical and environmental sample should be regarded as a potential health hazard and exposure should be minimized. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 16.3 and 16.4.
- 5.2. The toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level. It is suggested that the laboratory perform personal hygiene monitoring of each analyst using this method and that the results of this monitoring shall be made available to the analyst.
- 5.3. Samples of unknown origin may possess potentially hazardous compounds. Samples should be handled with care (e.g., under a hood), so as to minimize exposure.
- 5.4. As samples of unknown origin may contain compounds, which could react violently with the reagents, pipette the sample into the cell under a hood, and direct the opening of the cell away from anyone in the area.
- 5.5. Operate the thermoreactor with the safety shield in place (or behind a suitable shield) in case of severe reaction of sample and reagents, which could result in leakage.
- 5.6. The Spectroquant® COD cell tests are extremely hot after digestion, handle with care when transferring them to the cooling rack. Hold the cells by the cap, or using a test tube holder.
- 5.7. This method employs the use of COD cell tests containing pre-measured reagents, which limits the handling of hazardous chemicals.

## 6. Equipment and Supplies

*NOTE: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

- 6.1. Sample collection bottles-1-L borosilicate amber glass or plastic.
- 6.2. Blender.
- 6.3. Analytical balance-Capable of weighing 0.1 mg.
- 6.4. Volumetric flasks-Variou sizes.
- 6.5. Volumetric pipettes-Variou sizes.
- 6.6. Reaction cells-Spectroquant® COD cell test (Section 17.0, Table 1).
- 6.7. Block Digester - Capable of maintaining constant temperatures of 148 - 150°C, Spectroquant® Thermoreactor, or equivalent.
- 6.8. Laboratory timer.
- 6.9. Rack for cells.
- 6.10. Dry cloths for cleaning cell tests.
- 6.11. Photometric device.
  - 6.11.1. Spectrophotometer, photometer or colorimeter for use at 340-nm, 445-nm, and 605-nm wavelength, with cell compartment for tubes 16 x 100 mm.

## 7. Reagents and Standards

- 7.1. Spectroquant® COD cell test appropriate to the concentration range selected (Section 17.0, Table 1).
- 7.2. Deionized water - free of organic matter.
- 7.3. Potassium hydrogen phthalate (KHP) stock solution-Lightly crush and then dry potassium hydrogen phthalate to a constant weight at 120°C.
  - 7.3.1. Stock Solution 1: Dissolve 8.5 g in deionized water and dilute to 1 L. KHP stock has a theoretical COD of 10000 mg of O<sub>2</sub>/L. This solution is stable when refrigerated for up to three months in the absence of biological growth.
  - 7.3.2. Stock Solution 2: Dissolve 76.5 g in deionized water and dilute to 1 L. KHP stock has a theoretical COD of 90000 mg of O<sub>2</sub>/L. This solution is stable when refrigerated for up to three months in the absence of biological growth.
  - 7.3.3. Standard 1: Dilute 50 ml of the KHP Stock Solution 1 to 1-L with deionized water. KHP has a theoretic al COD of 500 mg O<sub>2</sub>/L. This solution is stable when

refrigerated for up to three months.

7.4. Sulfamic acid-ACS grade.

## 8. Sample Collection, Preservation, and Storage

8.1. Collect approximately 1-L, or a minimum of 100 ml, of a representative sample in a plastic or glass bottle following conventional sampling techniques (Reference 16.5).

8.2. Preserve the sample with H<sub>2</sub>SO<sub>4</sub> to a sample pH <2.

8.3. Refrigerate samples at 0 to 4°C from the time of collection until the time of analysis, 40 CFR 136, Table II.

8.4. Analyze the sample within 28 days of collection.

8.5. Collect an additional two aliquots of a sample for each batch (of at least 20 samples) for the matrix spike and matrix spike duplicate.

8.6. Blend samples containing settleable solids in a blender for two minutes. Homogenizing the sample in this way ensures the solids are evenly distributed throughout the sample, thereby increasing accuracy and reproducibility of results.

## 9. Quality Control

9.1. Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the ongoing analysis of laboratory reagent blanks, precision and recovery standards, and matrix-spiked samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data thus generated. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1.1. The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

9.1.2. Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Section 9.3.

9.1.3. Analyses of laboratory blanks are required to demonstrate freedom from contamination. The procedure and criteria for blank analyses is described in Section 9.4.

9.1.4. The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the

analysis system is in control. These procedures are described in Sections 9.5 and 9.6.

9.1.5. The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.7 and 9.6.3.

9.1.6. Accompanying QC for the determination of COD is required per analytical batch. An analytical batch is a set of samples analyzed, to a maximum of 20 samples. Each analytical batch, of up to 20 samples, must be accompanied by a laboratory blank (Section 9.4), and ongoing precision and recovery sample (OPR, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3).

9.2. Initial demonstration of laboratory capability-The initial demonstration of laboratory capability is used to characterize laboratory performance and method detection limits.

9.2.1. Method detection limit (MDL)-The method detection limit must be established for the analyte, using the QC spiking solution (Sections 7.3 and 7.4). To determine MDL values, take seven replicate aliquots of the diluted QC spiking solution and process each aliquot through each step of the analytical method. Perform all calculations and report the concentration values in the appropriate units. MDLs should be determined every year or whenever a modification to the method or analytical system is made that will affect the method detection limit.

9.2.2. Initial Precision and Recovery (IPR) - To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

9.2.2.1. Analyze four samples of the KHP standard or stock (Sections 7.3 and 7.4) according to the procedure beginning in Section 11.

9.2.2.2. Using the results of the four analyses, compute the average percent recovery ( $\bar{x}$ ) and the standard deviation ( $s$ , Equation 1) of the percent recovery for COD.

Equation 1
$S = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$
Where:
n = number of samples
x = % recovery in each sample
s = standard deviation

9.2.2.3. Compare  $s$  and  $\bar{x}$  with the corresponding limits for initial precision and

recovery in Table 3, (which lists EPA's proposed standardized QC and QC Acceptance Criteria for Methods in 40 CFR Part 136, Table IB). If  $s$  and  $x$  meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however,  $s$  exceeds the precision limit or  $x$  falls outside the range for recovery, system performance is unacceptable. In this event, correct the problem, and repeat the test.

9.3. Matrix Spikes-The laboratory must spike, in duplicate, a minimum of five percent of all samples (one sample in each batch of 20 samples). The two sample aliquots shall be spiked with the KHP stock or KHP standard solutions (Sections 7.3 and 7.4).

9.3.1. The concentration of the spike in the sample shall be determined as follows:

9.3.1.1. If, as in compliance monitoring, the concentration of COD in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1 to 5 times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2. If the concentration of COD in a sample is not being checked against a limit, the spike shall be at the concentration of the precision and recovery standard (Sections 7.3 and 7.4), or at 1 to 5 times higher than the background concentration, whichever concentration is higher.

9.3.2. Analyze one sample aliquot out of each set of 20 samples, according to the procedure beginning in Section 11.0, to determine the background concentration (B) of COD.

9.3.2.1. If necessary, prepare a standard solution appropriate to produce a level in the sample at the regulatory compliance limit or at 1 to 5 times the background concentration (per Section 9.3.1).

9.3.2.2. Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking (A).

9.3.3. Calculate the percent recovery (P) of COD in each aliquot using the following equation:

Equation 2
$P = 100 * \frac{(A - B)}{T}$
P = Percent recovery
A = Measured concentration of COD after spiking
B = measured concentration of COD before spiking
T = True concentration of the spike

9.3.4. Compare the percent recovery of the COD with the corresponding QC

acceptance criteria in Table 3, (which lists EPA's standardized QC and QC Acceptance Criteria for Methods in 40 CFR Part 136, Table IB).

- 9.3.4.1. If the results of the spike fail the acceptance criteria, and the recovery of the QC standard in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria in Table 3, an interference is present. In this case, the result may not be reported for regulatory compliance purposes and the analyst must assess the potential cause for the interference. If the interference is attributable to sampling, the site or discharge should be resampled. If the interference is attributable to a method deficiency, the analyst must modify the method repeat the tests required in Section 9.1.2, and repeat the analysis of the sample and the MS/MSD.
- 9.3.4.2. If the results of both the spike and the ongoing precision and recovery test fail the acceptance criteria, the analytical system is judged to be out of control, and the problem shall be identified and corrected, and the sample re-analyzed.
- 9.3.5. Compute relative percent difference (RPD) between the two results (not between the two recoveries) using the following equation:

<p>Equation 3</p> $RPD = 100 * \frac{( D_1 - D_2 )}{\left(\frac{D_1 + D_2}{2}\right)}$ <p style="text-align: center;">where:</p> <p style="text-align: center;">RPD = Relative percent difference</p> <p style="text-align: center;">D<sub>1</sub> = Concentration of COD in the sample</p> <p style="text-align: center;">D<sub>2</sub> = Concentration of COD in the second (duplicate) sample</p>
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- 9.3.6. The relative percent difference for duplicates shall meet the acceptance criteria in Table 3. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected, and the analytical batch re-analyzed.
- 9.3.7. As a part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained. After the analysis of five spiked samples, in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (Pa) and the standard deviation of the percent recovery (sp). Express the accuracy assessment as a percent recovery interval from Pa-2sp to Pa+2sp. For example, if Pa = 90% and sp = 10% for five analyses of COD, the accuracy interval is expressed as 70-110%. Update the accuracy assessment on a regular basis (e.g., after each five to ten new accuracy measurements).

- 9.4. Laboratory blanks-Laboratory reagent water blanks are analyzed to demonstrate freedom from contamination.
- 9.4.1. Prepare and analyze a laboratory blank initially (i.e., with the tests in Section 9.2) and with each analytical batch. The blank must be subjected to the same procedural steps as a sample.
- 9.4.2. If material is detected in the blank at a concentration greater than the ML (Section 1.6), analysis of samples must be halted until the source of contamination is eliminated and a new blank shows no evidence of contamination. All samples must be associated with an uncontaminated laboratory blank before the results may be reported for regulatory compliance purposes.
- 9.5. Calibration verification-Verify calibration of the photometric device per Section 10.0 for each analytical batch of up to 20 samples. If calibration curve linearity differs more than 10%, run a new calibration curve.
- 9.6. Ongoing Precision and Recovery (OPR)-To demonstrate that the analysis system is in control, and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations:
- 9.6.1. Analyze a precision and recovery standard (Sections 7.3 and 7.4) with each analytical batch according to the procedure beginning in Section 11.0.
- 9.6.2. Compare the concentration with the limits for ongoing precision and recovery in Table 3. If the concentration is in the range specified, the analysis may proceed. If however, the concentration is not in the specified range, the analytical process is not in control. In this event, correct the problem, repeat the analytical batch, and repeat the ongoing precision and recovery test.
- 9.6.3. The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for each analyte by calculating the average percent recovery ( $R$ ) and the standard deviation of the percent recovery ( $s_r$ ). Express the accuracy as a recovery interval from  $R - 2s_r$  to  $R + 2s_r$ .
- For example, if  $R = 95\%$ , and  $s_r = 5\%$ , the accuracy is 85 % to 105 %.*
- 9.7. Quality control sample (QCS)—It is suggested that the laboratory obtain a quality control sample from a source different from the source of the KHP used routinely in this method (Sections 7.3 and 7.4).
- 9.8. The standards used for initial precision and recovery (IPR, Section 9.2.2) matrix spikes (MS/MSD, Section 9.3), and ongoing precision and recovery (OPR, Section 9.6) should be identical, so that the most precise results will be obtained.

## 10. Calibration and Standardization

- 10.1. The Spectroquant® instruments are shipped factory calibrated (Reference 16.7), refer to the manufacturer's documents (Reference 16.8). The calibration curve can be verified, and the data from this verification can be stored, modified or re-entered at any time. However, the factory program settings cannot be changed by the user.
- 10.2. For all photometric equipment plot a calibration curve with a minimum of five (5) data points, from standards prepared from a KHP solution appropriate to the range to be tested. The calibration curve should also include a blank.
- 10.2.1. For Spectroquant® COD cell tests in the concentration range 4 to 500 mg/L (Section 17.0, Table 1) prepare a standard curve from the KHP (500 mg/L) solution (Section 17.0, Table 2). The curve should include the lowest and highest concentrations for the range tested.
- 10.2.2. For Spectroquant® COD cell tests in the concentration range 25 to 10000 mg/L (Section 17.0, Table 1) prepare standard curve from stock KHP (10000 mg/L) solution (Section 17.0, Table 2) and from the standard KHP (500 mg/L) solution (Section 17.0, Table 2). The curve should include the lowest and highest concentrations for the range tested.
- 10.2.3. For Spectroquant® COD cell tests in the concentration range 5000 to 90000 mg/L (Section 17.0, Table 1) prepare standard curve from stock KHP (10000 mg/L) solution (Section 17.0, Table 2) and from the stock KHP (90000 mg/L) solution (Section 17.0, Table 2). The curve should include the lowest and highest concentrations for the range tested.
- 10.3. Verify the curve, using a calibration standard (mid-point of the curve), with each analytical batch of samples (Section 9.5).
- 10.4. Run a new calibration curve with each new lot of reagents.
- 10.5. Ongoing Calibration Check:
- 10.5.1. Utilizing the calibration concentration values obtained from the standards, back calculate the true percent difference concentration of each calibration point. The back-calculated and true concentrations shall agree within no more than 10%.

$$\text{Percent Ongoing Calibration Check} = \left| \frac{A - B}{A} \right| \times 100$$

A = True Concentration For Standard

B = Concentration From Instrument

## 11. Procedure

### 11.1. COD Cell Tests

- 11.1.1. Preheat the Spectroquant® Thermoreactor.
- 11.1.2. Test the sample for chloride, to determine if dilution is necessary. Refer to Table 1 for the chloride level which merits dilution of the sample.
- 11.1.3. Choose a Spectroquant® COD cell test concentration range appropriate for the sample matrix to be tested, using prior knowledge of the particular waste stream. For the list of test ranges, see Table 1.
- 11.1.4. Suspend sediment in the Spectroquant® COD cell test by swirling. Do not invert the cell.
- 11.1.5. While directing the cell opening away from anyone in the area (Section 5.4), carefully pipette the appropriate aliquot (Section 17.0, Table 1) of representative, homogenized (Section 8.6) sample into a Spectroquant® COD cell test.
- 11.1.6. Close tightly with the screw cap and mix vigorously. **WARNING**–The Spectroquant® COD cell test becomes very hot (Section 5.6).
- 11.1.7. Heat the COD test cell in the Spectroquant® Thermoreactor at 148 - 150°C for two hours.
- 11.1.8. When digestion is complete, remove the COD test cell from the thermoreactor and place in the cell rack to cool.
- 11.1.9. After 10 minutes, swirl the COD cell, to remove any condensed water which may have adhered to the sides and top of the cell.
- 11.1.10. Replace the COD cell in the rack for cooling to room temperature. Photometric determination can be performed once the COD tube has reached 20 - 40°C
- 11.1.11. Determination using photometric equipment.
  - 11.1.11.1. Warm up the instrument as per manufacturer's suggestion for operation.
  - 11.1.11.2. Set the instrument to the wavelength appropriate for the Spectroquant® COD cell test used (Section 17.0, Table 1).
  - 11.1.11.3. Zero the instrument with a reagent water / blank which has been prepared in the same manner as the standards and samples.
  - 11.1.11.4. Place the cell into the cell compartment/cell holder with the vertical line facing you.
  - 11.1.11.5. Record the absorbance or concentration reading from the instrument.
  - 11.1.11.6. If the instrument does not provide the concentration, plot the absorbance reading on the calibration curve to obtain the concentration COD as mg O<sub>2</sub>/L.

## 11.2.COD Cell Test for seawater / high chloride contents

<b>Table B: 1.17058 Chloride Depletion</b>			
	<b>Sample</b>	<b>Blank <sup>(1)</sup></b>	
Pretreated sample	20 ml	-	Pipette into a 300-ml Erlenmeyer flask with the <b>glass pipette</b> , add the magnetic stirring rod, and cool in the ice bath.
Distilled water <sup>(2)</sup>	-	20 ml	Pipette into a second 300-ml Erlenmeyer flask with the <b>glass pipette</b> , add the magnetic stirring rod, and cool in the ice bath.
Sulfuric acid for the determination of COD	25 ml	25 ml	<b>Add slowly</b> with the <b>glass pipette under cooling and stirring</b> (temperature should not exceed 45 °C).
After the complete addition of sulfuric acid, cool to room temperature in the ice bath.			
Soda lime with indicator	6 - 7 g	6 - 7 g	Fill into separate absorption tubes, close with the glass stoppers, and attach to the top of the cooled Erlenmeyer flasks.
Place the flasks closed with the absorption tubes onto separate magnetic stirrers and stir at 250 rpm for 2 h at room temperature (a turbidity may be formed). Subsequently check the chloride content of the stirred sample. <sup>(3)</sup> In the case of concentrations of chloride exceeding 2000 mg/l, exchange the soda lime in the absorption tube and stir repeatedly until a chloride content < 2000 mg/L is achieved.			

1) Every 6 weeks or upon the first opening of a new bottle of “Sulfuric acid for the determination of COD”.

2) It is recommended to use water for chromatography LiChrosolv®, Cat. No. 115333.

3) Using the MColortest™ Chloride Test, Cat. No. 111132, as per the application instructions.

<b>Table C: 1.17058 COD Procedure</b>			
Suspend the bottom sediment in the reaction cell by swirling.			
	<b>Measuring sample</b>	<b>Blank <sup>(1)</sup></b>	
Homogenized and depleted sample	5.0 ml		<b>Carefully</b> allow to run from the <b>glass pipette</b> down the inside of the tilted reaction cell onto the reagent ( <b>Wear eye protection! The cell becomes hot!</b> ).
Depleted Blank		5.0 ml	Carefully allow to run from the glass pipette down the inside of the tilted reaction cell onto the reagent (Wear eye protection! The cell becomes hot!).
<p>Tightly attach the screw cap to the cell.</p> <p><b>In all subsequent steps the cell must be held only by the screw cap! Vigorously</b> mix the contents of the cell.</p> <p>Heat the cell at 148 °C in the preheated thermoreactor for 120 min.</p> <p>Remove the hot cell from the thermoreactor and allow to cool in a test-tube rack.</p> <p><b>Do not cool with cold water!</b></p> <p>Wait 10 min, swirl the cell, and return to the rack for complete cooling to room temperature (cooling time at least 30 min).</p> <p>Measure in the photometer.</p>			

1) Every 6 weeks or upon the first opening of a new bottle of “Sulfuric acid for the determination of COD”.

<b>Table D: 1.17059 Chloride Depletion</b>			
	<b>Sample</b>	<b>Blank <sup>(1)</sup></b>	
Pretreated sample	20 ml	-	Pipette into a 300-ml Erlenmeyer flask with the <b>glass pipette</b> , add the magnetic stirring rod, and cool in the ice bath.
Distilled water <sup>(2)</sup>	-	20 ml	Pipette into a second 300-ml Erlenmeyer flask with the <b>glass pipette</b> , add the magnetic stirring rod, and cool in the ice bath.
Sulfuric acid for the determination of COD	25 ml	25 ml	<b>Add slowly</b> with the <b>glass pipette under cooling and stirring</b> (temperature should not exceed 45 °C).
After the complete addition of sulfuric acid, cool to room temperature in the ice bath.			
Soda lime with indicator	6 - 7 g	6 - 7 g	Fill into separate absorption tubes, close with the glass stoppers, and attach to the top of the cooled Erlenmeyer flasks.
Place the flasks closed with the absorption tubes onto separate magnetic stirrers and stir at 250 rpm for 2 h at room temperature (a turbidity may be formed). Subsequently check the chloride content of the stirred sample. <sup>(3)</sup> In the case of concentrations of chloride exceeding 2000 mg/l, exchange the soda lime in the absorption tube and stir repeatedly until a chloride content < 2000 mg/L is achieved.			

1) Every 6 weeks or upon the first opening of a new bottle of “Sulfuric acid for the determination of COD”.

2) It is recommended to use water for chromatography LiChrosolv®, Cat. No. 115333.

3) Using the MColortest™ Chloride Test, Cat. No. 111132, as per the application instructions

<b>Table E: 1.17059 COD Procedure</b>			
Suspend the bottom sediment in the reaction cell by swirling.			
	<b>Measuring sample</b>	<b>Blank <sup>(1)</sup></b>	
Homogenized and depleted sample	3.0 ml		<b>Carefully</b> allow to run from the <b>glass pipette</b> down the inside of the tilted reaction cell onto the reagent ( <b>Wear eye protection! The cell becomes hot!</b> ).
Depleted Blank		3.0 ml	Carefully allow to run from the glass pipette down the inside of the tilted reaction cell onto the reagent (Wear eye protection! The cell becomes hot!).
<p>Tightly attach the screw cap to the cell.</p> <p><b>In all subsequent steps the cell must be held only by the screw cap! Vigorously</b> mix the contents of the cell.</p> <p>Heat the cell at 148 °C in the preheated thermoreactor for 120 min.</p> <p>Remove the hot cell from the thermoreactor and allow to cool in a test-tube rack.</p> <p><b>Do not cool with cold water!</b></p> <p>Wait 10 min, swirl the cell, and return to the rack for complete cooling to room temperature (cooling time at least 30 min).</p> <p>Measure in the photometer.</p>			

1) Every 6 weeks or upon the first opening of a new bottle of “Sulfuric acid for the determination of COD”.

## 12. Data Analysis and Calculations

12.1.If no pre-dilution was performed upon the sample, no calculation is necessary.

12.2.If pre-dilution was required, calculate the COD (mg O<sub>2</sub>/L) as follows:

Equation 4
$COD = A * \frac{V_2}{V_1}$
A = Measured concentration of COD from photometer (mg/L)
V <sub>1</sub> = Volume of sample used for dilution (mL)
V <sub>2</sub> = Final total volume of diluted sample (mL)

12.3.Report results to two significant digits for concentrations found above the ML (Section 1.6) in all samples. Report results below the ML (Section 17.0, Table 1–item 14560) as <4.0 mg/L for COD.

## 13. Method Performance

13.1.This method, as equivalent to EPA Method 410.4, Revision 2, August, 1993 (Reference 16.1) should achieve the method performance as cited in Section 6 of that method.

13.2.The method detection limit (MDL) study was performed by a single analyst, and was determined as 2.0 mg/L.

13.3.The minimum level (ML) is determined as 4.0 mg/L, based on the Spectroquant® COD cell test with the lowest concentration (Section 17.0, Table1–item 14560). 4.0 mg/L is the lowest calibration standard used for this range (10.2.1 - 10.2.3).

## 14. Pollution Prevention

14.1.The reagents used in this method pose little threat to the environment, when managed properly.

14.2.Reagents should be ordered consistent with laboratory use, to minimize the amount of expired materials to be disposed.

14.3.Dispose of as per local, State and Federal requirements.

## 15. Waste Management

15.1.It is the laboratory’s responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restriction. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations.

15.2.For further information on waste management, consult “The Waste Management

Manual for Laboratory Personnel” and “Less is Better: Laboratory Chemical Management for Waste Reduction,” both available from the American Chemical Society’s Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

## 16. References

- 16.1. “Methods for the Chemical Analysis of Water and Wastes,” 3rd Edition, Environmental Protection Agency, Environmental Monitoring Systems Laboratory–Cincinnati (EMSL–Ci), Cincinnati, Ohio 45268, EPA–600/4-79-020, Method 410.4.
- 16.2. “Standard Methods for the Examination of Water and Wastewater,” 18th Edition, American Public Health Association, 1015 Fifteenth Street, N.W., Washington, D.C. 20005, Method 5220 D.
- 16.3. “OSHA Safety and Health Standards, General Industry,” (29CFR 1910), Occupational Safety and Health Administration, OSHA 2206, revised January 1976.
- 16.4. “Safety in Academic Chemistry Laboratories,” American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
- 16.5. “Standard Practices for Sampling Water,” ASTM Annual Book of Standards, Part 31, D3370-76, American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103-1187, 1980.
- 16.6. “Handbook of Analytical Quality Control in Water and Wastewater Laboratories,” USEPA, EMSL–Ci, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.
- 16.7. “German Standard Methods for the Examination of Water, Wastewater, and Sludge,” Deutsches Institut für Normung e.V., D-10772, Berlin, DIN Method 38402 Part 51, May 1986.
- 16.8. Spectroquant® Instrument manuals, Merck KGaA, Frankfurter Straße 250, Darmstadt 64271, Germany, Latest version.

## 17. Tables and Validation Data

<b>Table 1. Product Range, Number, and Usage Information</b>				
<u>Range</u> <u>mg/L</u>	<u>Product</u> <u>Number</u>	<u>Sample</u> <u>Volume (ml)</u>	<u>Wavelength</u> <u>nm</u>	<u>Chloride</u> <u>Level (mg/L)</u>
4.0 – 40.0	1.14560	3	340	2,000
5.0 – 80.0	1.01796	2	340	2,000
10 – 150	1.14540	3	445	2,000
15 – 300	1.14895	2	445	2,000
25 – 1500	1.14541	3	605	2,000
50 – 500	1.14690	2	445	2,500
300 – 3500	1.14691	2	605	2,500
500 – 10000	1.14555	1	605	5,000
5000– 90000	1.01797	0.1	605	50,000
5.0 – 60.0	1.17058	5	340	2,000
50 – 3000	1.17059	3	605	250

<b>Table 2. Calibration Standard Calibration Preparation</b>			
<u>Product # Range (mg/L)</u>	<u>KHP Solution</u>	<u>KHP Volumes (ml)*</u>	<u>COD Equivalent (mg/L)</u>
1.14560 (4-40)	Standard 1	0-0.8-2-4-6-8	0, 4, 10, 20, 30, 40
1.01796 (5-80)	Standard 1	0-1-4-8-12-16	0, 5, 20, 40, 60, 80
1.14540 (10-150)	Standard 1	0-2-6-18-24-30	0, 10, 30, 80, 120, 150
1.14895 (15-300)	Standard 1	0-3-15-30-45-60	0, 15, 75, 150, 225, 300
1.14541 (25-1500)	Standard 1	0-5-	0, 25
	Stock 1	3-7-12-15	300, 700, 1200, 1500
1.14690 (50-500)	Standard 1	0-10-25-50-75-100	0, 50, 125, 250, 375, 500
1.14691 (300-3500)	Stock 1	0-3-10-17.5-25-35	0, 300, 1000, 1750, 2500, 3500
1.14555 (500-10000)	Stock 1	0-5-25-50-75-100	0, 500, 2500, 5000, 7500, 10000
1.01797 (5000-90000)	Stock 1	0-50	0, 5000
	Stock 2	10-40-60-100	9000, 36000, 54000, 90000
1.17058 (5-60)	Standard 1	0-1-2-4-8-12	0, 5, 10, 20, 40, 60
1.17059 (50-3000)	Standard 1	0-10	0, 50
	Stock 1	3-10-20-30	300, 1000, 2000, 3000

\* Dilute all working calibration standards to 100 ml in volumetric flasks.

<b>Table 3. Acceptance Criteria for Performance Tests</b>		
<b>Acceptance Criterion</b>	<b>Section</b>	<b>Limit (%)</b>
<u>Initial precision and recovery</u>	9.2.2	
COD Precision (s)	9.2.2.2	30
COD Recovery (X)	9.2.2.2	47 - 153
<u>Matrix spike/matrix spike duplicate</u>	9.3	
COD Recovery	9.3.4	40 - 160
COD RPD	9.3.5	36
<u>Ongoing precision and recovery</u>	9.6	
COD Recovery	9.6	40 - 160

The above-mentioned table was taken from the COD release April 1999 from the ATP Case No. N99-0007. The USEPA confirm here that the COD cell tests are an acceptable version of the EPA-approved method at 40 Code of Federal Regulations (CFR) Part 136 for the determination of chemical oxygen demand (COD) by spectrophotometry [i.e. EPA method 410.4: “Chemical Oxygen Demand (Colorimetric, Automated; Manual)”].

The precision of the method and the recovery from the initial and spiked samples from our COD Cell Tests are much better as described in the above table. Details can be found in the Certificate of Analysis (which is available for each item) and the individual lot certificate which is also available for every produces lot. They can be both found in our internet homepage.

## 18. Definitions

18.1. The definitions and purposes are specific to this method, but have been conformed to common usage as much as possible.

### 18.1.1. Symbols

°C degrees Celsius

< less than

% percent

### 18.1.2. Alphabetical Characters

g gram

L liter

mg milligram

mg/L milligram per liter ml milliliter

nm nanometer

## 18.2. Definitions, acronyms, and abbreviations.

- 18.2.1. Analyte: COD, which is test for by this method.
- 18.2.2. Analytical batch: The set of samples analyzed at the same time, to a maximum of 20 samples. Each analytical batch must be accompanied by a laboratory blank (Section 9.4), and ongoing precision and recovery sample (OPR, Section 9.6), a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), and a reagent blank (Section 9.4).
- 18.2.3. COD: See Chemical oxygen demand.
- 18.2.4. Spectroquant® COD cell test: The pre-measured COD reagents, packaged in 16 x 100 mm tubes.
- 18.2.5. Chemical oxygen demand (COD): The parameter which is tested for by this method.
- 18.2.6. IPR: See initial precision and recovery.
- 18.2.7. Initial precision and recovery (IPR): Four aliquots of the diluted KHP analyzed to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or instrument is modified.
- 18.2.8. KHP: See potassium hydrogen phthalate.
- 18.2.9. Laboratory blank (method blank): An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, and reagents that are used with samples. The laboratory blank is used to determine if analyte or interferences are present in the laboratory environment, or the reagents.
- 18.2.10. Matrix spike (MS) and matrix spike duplicate (MSD): Aliquots of environmental sample to which known quantities of the analyte are added in the laboratory. The MS and MSD are prepared and/or analyzed exactly like a field sample. Their purpose is to quantify any additional bias and imprecision caused by the sample matrix. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for background concentrations.
- 18.2.11. May: This action, activity, or procedural step is neither required nor prohibited.
- 18.2.12. May not: This action, activity, or procedural step is prohibited.
- 18.2.13. Method detection limit (MDL): The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.
- 18.2.14. Minimum level (ML): The lowest level at which the entire analytical system

gives a recognizable signal and acceptable calibration point of the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method- specified sample weights, volumes, and preparation procedures have been employed.

- 18.2.15. Must: This action, activity, or procedural step is required.
- 18.2.16. OPR: See ongoing precision and recovery standard.
- 18.2.17. Ongoing precision and recovery standard (OPR): A laboratory blank spike with known quantities of analyte. The OPR is treated exactly like a sample. Its purpose is to establish performance of the method by the analyst.
- 18.2.18. Potassium hydrogen phthalate (KHP): The standard solution from which the calibration and quality control samples can be prepared.
- 18.2.19. Quality control sample (QCS): A sample containing analyte of interest at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.
- 18.2.20. Reagent water: Water demonstrated to be low or free from organic matter.
- 18.2.21. Shall: This action, activity, or procedural step is required.
- 18.2.22. Should: This action, activity, or procedural step is suggested, but not required.
- 18.2.23. Spectroquant<sup>®</sup> Thermoreactor: The block digester which can operate at a temperature of 148 - 150°C, to effect digestion of the Spectroquant<sup>®</sup> COD cell tests.