Designation: D 1252 – 00

An American National Standard

Standard Test Methods for Chemical Oxygen Demand (Dichromate Oxygen Demand) of Water[1]

This standard is issued under the fixed designation D 1252; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

This standard has been approved for use by agencies of the Department of Defense.

1. Scope

1.1 These test methods cover the determination of the quantity of oxygen that certain impurities in water will consume, based on the reduction of a dichromate solution under specified conditions. The following test methods are included:

Test Method A—Macro COD by Reflux Digestion and Titration

Test Method B—Micro COD by Sealed Digestion and Spectrometry

1.2 These test methods are limited by the reagents employed to a maximum chemical oxygen demand (COD) of 800 mg/L. Samples with higher COD concentrations may be processed by appropriate dilution of the sample. Modified procedures in each test method (Section 15 for Test Method A and Section 24 for Test Method B) may be used for waters of low COD content (< 50 mg/L).

1.3 As a general rule, COD results are not accurate if the sample contains more than 1000 mg/L Cl^-. Consequently, these test methods should not be applied to samples such as seawaters and brines.

1.4 This test method was used successfully on a standard made up in reagent water. It is the user's responsibility to ensure the validity of these test methods for waters of untested matrices.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazard statements, see Section 8 and Note 3 and Note 9.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water[2]

D 1192 Specification for Equipment for Sampling Water and Steam in Closed Conduits[2]

D 1193 Specification for Reagent Water[2]

D 3223 Test Method for Total Mercury in Water

D 3370 Practices for Sampling Water from Closed Conduits[2]

D 3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water[2]

D 4210 Practice for Intralaboratory Quality Control Procedures and a Discussion on Reporting Low-Level Data[2]

D 5905 Practice for the Preparation of Substitute Wastewater[2]

E 60 Practice for Photometric and Spectrophotometric Methods for Chemical Analysis of Metals [3]

E 275 Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near Infrared Spectrophotometers [4]

3. Terminology

3.1 *Definitions*—For definitions of other terms used in these test methods, refer to Terminology D 1129.

3.2 The term "oxygen demand" (COD) in these test methods is defined in accordance with Terminology D 1129 as follows:

3.2.1 *oxygen demand*—the amount of oxygen required under specified test conditions for the oxidation of water borne organic and inorganic matter.

4. Summary of Test Methods

4.1 Most organic and oxidizable inorganic substances present in water are oxidized by a standard potassium dichromate solution in 50 % sulfuric acid standard potassium dichromate solution in 50 % (vol/vol) sulfuric acid. The dichromate consumed (Test Method A) or tri-valent chromium produced (Test Method B) is determined for calculation of the COD value.

4.2 The oxidation of many otherwise refractory organics is facilitated by the use of silver sulfate that acts as a catalyst in the reaction.

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[1] These test methods are under the jurisdiction of ASTM Committee D-19 on Water and are the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] *Annual Book of ASTM Standards,* Vol 11.01.

[3] *Annual Book of ASTM Standards,* Vol 03.05.

[4] *Annual Book of ASTM Standards,* Vol 03.06.

4.3 These test methods provide for combining the reagents and sample in a manner that minimizes the loss of volatile organic materials, if present.

4.4 The oxidation of up to 1000 mg/L of chloride ion is inhibited by the addition of mercuric chloride to form stable and soluble mercuric sulfate complex. The maximum chloride concentration that may be tolerated with the procedure for low COD, Test Method A (15.11), has not been established.

4.5 The chemical reaction involved in oxidation of materials by dichromate is illustrated by the following reaction with potassium acid phthalate ($KC_8H_5O_4$):

$$41\ H_2SO_4 + 10\ K_2Cr_2O_7 + 2\ KC_8H_5O_4 \rightarrow 10\ Cr_2(SO_4)_3 + 11\ K_2SO_4 + 16\ CO_2 + 46\ H_2O$$

Since 10 mol of potassium dichromate has the same oxidation power as 15 mol of oxygen, the equivalent reaction is:

$$2\ KC_8H_5O_4 + 15\ O_2 + H_2SO_4 \rightarrow 16\ CO_2 + 6\ H_2O + K_2SO_4$$

Thus 2 mol of potassium acid phthalate consumes 15 mol of oxygen. The theoretical COD of potassium acid phthalate is 1.175 g of oxygen per gram of potassium acid phthalate (Table 1).

TABLE 1 Test Method A, Recovery of Theoretical COD for Various Organic Material

Component	Reactivity, Percent of Theoretical				
	1 [A]	2 [B]	3 [C]	4 [D]	5 [E]
Aliphatic Compounds					
Acetone	98	...	96	94	...
Acetic acid	92	92	98	...	...
Acrolein	62	...	...	...	...
Butyric acid	89	93	...	...	...
Dextrose	95	...	...	...	...
Diethylene glycol	93	...	...	70	...
Ethyl acetate	95	...	...	85	...
Methyl ethyl ketone	98	...	...	90	...
Aromatic Compounds					
Acetophenone	89	...	...	...	...
Benzaldehyde	...	...	...	80	...
Benzene	60–98	...	41	...	...
Benzoic acid	98	...	...	100	...
Dioctyl phthalate	83	...	...	...	...
Diphenyl	81	...	...	...	...
o-cresol	95	...	...	95	...
Toluene	83	...	...	45	...
Potassium acid phthalate	100	...	...	...	...
Nitrogen Compounds					
Acrylonitrile	48	...	...	44	...
Adenine	...	...	...	...	59
Aniline	80	...	...	74	...
Butyl amine	57	...	...	...	...
Pyridine	0	...	1	...	2
Quinoline	...	...	...	...	87
Trimethylamine	1	...	...	...	...
Tryptophane	...	...	...	...	87
Uric acid	...	...	...	...	61

[A] Hamilton, C. E., unpublished data.

[B] Moore, W. A., and Walker, W. W., *Analytical Chemistry*, Vol 28, 1956, p 164.

[C] Dobbs, R. A., Williams, R. T., *ibid.*, Vol 35, 1963 p. 1064.

[D] Buzzell, J. C., Young, R. H. F., and Ryckman, D. W.," Behaviors of Organic Chemicals in the Aquatic Environment; Part II, Dilute Systems," *Manufacturing Chemists Association*, April 1968, p. 34.

[E] Chudoba, J., and Dalesicky, J., *Water Research*, Vol 7, No. 5, 1973, p. 663.

5. Significance and Use

5.1 These test methods are used to chemically determine the maximum quantity of oxygen that could be consumed by biological or natural chemical processes due to impurities in water. Typically this measurement is used to monitor and control oxygen-consuming pollutants, both inorganic and organic, in domestic and industrial wastewaters.

5.2 The relationship of COD to other water quality parameters such as TOC and TOD is described in the literature. [5]

6. Interference and Reactivity

6.1 Chloride ion is quantitatively oxidized by dichromate in acid solution. (1.0 mg/L of chloride is equivalent to 0.226 mg/L of COD.) As the COD test is not intended to measure this demand, concern for chloride oxidation is eliminated up to 1000 mg/L of chloride by complexing with mercuric sulfate.

6.2 Oxidizable inorganic ions, such as ferrous, nitrite, sulfite, and sulfides are oxidized and measured as well as organic constituents.

7. Reagents

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. All reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. [6]

7.2 *Purity of Water*—Unless otherwise indicated, reference to water shall mean reagent water conforming to Specification D 1193, Type II.

8. Hazards

8.1 Exercise extreme care when handling concentrated sulfuric acid, especially at the start of the refluxing step (15.7).

8.2 Silver sulfate is poisonous; avoid contact with the chemical and its solution.

8.3 Mercuric sulfate is very toxic; avoid contact with the chemical and its solution.

9. Sampling

9.1 Collect the sample in accordance with Practices D 3370.

9.2 Preserve samples by cooling to 4°C if analyzed within 24 h after sampling, or preserve for up to 28 days at 4°C and at pH < 2 by addition of concentrated sulfuric acid. The addition of 2 mL of concentrated sulfuric acid per litre at the time of collection will generally achieve this requirement. The actual holding time possible without significant change in the COD may be less than 28 days, especially when easily oxidizable substances are present. It is the responsibility of the users of the test method to ensure the maximum holding time for their samples.

[5] *Handbook for Monitoring Industrial Wastewater*, U.S. Environmental Protection Agency, Aug. 1973, pp. 5-10 to 5-12.

[6] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

TEST METHOD A—MACRO COD BY REFLUX DIGESTION AND TITRATION

10. Scope

10.1 The amount of dichromate consumed in Test Method A is determined by titration rather than the spectrophotometric procedure used in Test Method B. This test method is appropriate where larger sample volumes would provide better precision and better representativeness of where equipment or space limitations exist.

10.2 The precision of this test method in standard solutions containing low-volatility organic compounds has been examined in the range of approximately 10 to 300 mg/L.

11. Summary of Test Method

11.1 The sample and standardized dichromate solution, in a 50 % by volume sulfuric solution, is refluxed for a 2-h digestion period.

11.2 Excess dichromate after the digestion period is titrated with a standard ferrous ammonium sulfate solution using ortho-phenanthroline ferrous complex as an internal indicator.

12. Interferences

12.1 The test method does not uniformly oxidize all organic materials. Some compounds, for example, are quite resistant to oxidation, while others, such as carbohydrates, are easily oxidized. A guide to the behavior of various types of organic materials is provided in Table 1.

12.2 Volatile organics that are difficult to oxidize may be partially lost before oxidation is achieved. Care in maintaining a low-solution temperature (about 40°C) and permitting oxidation to proceed at the lower temperature for a period of time before reflux is initiated will result in higher recoveries of theoretical COD of volatile organics.

13. Apparatus

13.1 *Reflux Apparatus*—The apparatus consists of a 500-mL Erlenmeyer or a 300-mL round-bottom flask, made of heat-resistant glass connected to a 300-mm (12-in.) Allihn condenser by means of a ground-glass joint. Any equivalent reflux apparatus may be substituted, provided that a ground-glass connection is used between the flask and the condenser, and provided that the flask is made of heat-resistant glass.

13.2 *Sample Heating Apparatus*—A heating mantle or hot plate capable of delivering sufficient controlled heat to maintain a steady reflux rate in the reflux apparatus is satisfactory.

13.3 *Apparatus for Blending or Homogenizing Samples*—A household blender is satisfactory.

14. Reagents

14.1 *Ferrous Ammonium Sulfate Solution (0.25 N)*—Dissolve 98.0 g of ferrous ammonium sulfate solution ($FeSO_4 \cdot (NH_4)SO_4 \cdot 6H_2O$) in water. Add 20 mL of sulfuric acid (H_2SO_4, sp gr 1.84), cool and dilute to 1 L. Standardize this solution daily before use. To standardize, dilute 25.0 mL of 0.25 *N* potassium dichromate solution ($K_2Cr_2O_7$) to about 250 mL. Add 20 mL of sulfuric acid (sp gr 1.84) and allow the solution to cool. Titrate with the ferrous ammonium sulfate solution to be standardized, using the phenanthroline ferrous sulfate indicator as directed in 15.10. Calculate the normality as follows:

$$N = (A \times B)/C$$

where:

N = normality of the ferrous ammonium sulfate solution,
A = potassium dichromate solution, mL,
B = normality of the potassium dichromate solution, and
C = ferrous ammonium sulfate solution, mL.

14.2 *Ferrous Ammonium Sulfate Solution* (0.025 *N*)—Dilute 100 mL of 0.25 *N* ferrous ammonium sulfate solution to 1 L. Standardize against 0.025 *N* potassium dichromate solution as in 14.1. This solution is required only if COD is determined in the range of 10 to 50 mg/L.

14.3 *Mercuric Sulfate*—Powdered mercuric sulfate ($HgSO_4$).

14.4 *Phenanthroline Ferrous Sulfate Indicator Solution*—Dissolve 1.48 g of 1,10-(ortho)-phenanthroline monohydrate, together with 0.70 g of ferrous sulfate ($FeSO_4 \cdot 7H_2O$), in 100 mL of water. This indicator may be purchased already prepared.

14.5 *Potassium Acid Phthalate Solution, Standard* (1 mL = 1 mg COD)—Dissolve 0.851 g of potassium acid phthalate ($KC_8H_5O_4$), primary standard, in water and dilute to 1 L.

14.6 *Potassium Dichromate Solution, Standard* (0.25 *N*)—Dissolve 12.259 g of potassium dichromate ($K_2Cr_2O_7$) primary standard grade, previously dried at 103°C for 2 h, in water and dilute to 1 L in a volumetric flask.

14.7 *Potassium Dichromate Solution, Standard* (0.025 *N*)—Dilute 100.0 mL of 0.25 *N* potassium dichromate solution to 1 L. This solution is necessary only for determination of COD in the range of 10 to 50 mg/L.

14.8 *Sulfuric Acid-Silver Sulfate Solution*—Dissolve 15 g of powdered silver sulfate (Ag_2SO_4) in 300 mL of concentrated sulfuric acid (sp gr 1.84) and dilute to 1 L with concentrated sulfuric acid (sp gr 1.84).

15. Procedure

15.1 Homogenize the sample by blending if necessary. Place 50.0 mL of the sample in a reflux flask. If less than 50 mL of the sample is used, make up the difference in water, then add the sample aliquot and mix. Samples containing more than 800 mg/L COD are diluted and mixed precisely with water and 50.0 mL of the diluted sample are placed in a reflux flask.

NOTE 1—If the sample is diluted, it must consume at least 5 mL of dichromate. Dilute the sample if more than 20 mL of the titrant is needed to reach the endpoint.

15.2 Place 50 mL of water in a reflux flask for the blank determination.

15.3 Place the reflux flasks in an ice bath and add 1 g of powdered mercuric sulfate, 5.0 mL of concentrated sulfuric acid, and several glass beads or boiling stones. Mix well to complete dissolution.

15.4 With the flasks still in the ice bath, add slowly and with stirring, 25.0 mL of 0.25 *N* standard potassium dichromate solution.

15.5 With the flasks still in the ice bath, add 70 mL of sulfuric acid-silver sulfate solution slowly such that the solution temperature is maintained as low as possible, preferably below 40°C.

NOTE 2—If a particular waste is known to contain no volatile organic substances, the acid mixture may be added gradually, with less precaution, while the flask is immersed in the iced bath.

15.6 Attach the flasks to the condensers and start the flow of cold water.

NOTE 3—**Caution:** Take care to ensure that the contents of the flask are well mixed; if not, superheating may result and the mixture may be expulsed from the open end of the condenser.

15.7 Apply heat to the flasks and reflux for 2 h. Place a small beaker or other cover over the open end of each condenser to prevent intrusion of foreign material.

15.8 Allow the flasks to cool and wash down the condensers with about 25 mL of water before removing flasks. If a round-bottom flask has been used, transfer the digestate to a 500-mL Erlenmeyer flask, washing out the reflux flask three or four times with water. Dilute the acid solution to about 300 mL with water and allow the solution to cool to about room temperature.

15.9 Add 8 to 10 drops of phenanthroline ferrous sulfate solution and titrate the excess dichromate with 0.25 *N* ferrous ammonium solution. The color change at the end point will be sharp, changing from a blue-green to a reddish hue. If the solution immediately turns a reddish-brown upon the addition of the indicator, repeat the analysis on a smaller sample aliquot.

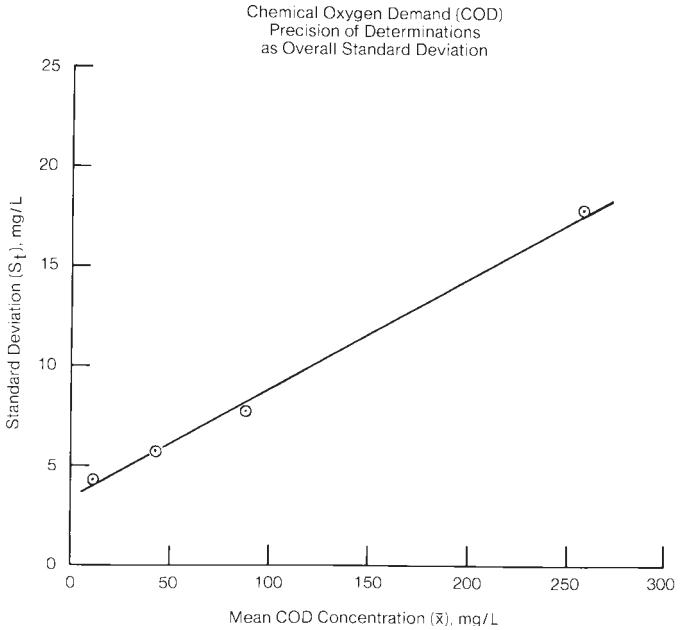

FIG. 1 Test Method A, Chemical Oxygen Demand (COD) Precision of Determination as Overall Standard Deviation

NOTE 4—To avoid unnecessary pollution of the environment, dispose of mercury-containing waste solution properly. Refer to Test Method D 3223, Appendix XI for instructions.

15.10 For waters of low COD (10 to 50 mg/L), use 0.025 *N* potassium dichromate and ferrous ammonium sulfate solutions (14.2 and 14.7). If the COD is determined to be higher than 50 mg/L after using these reagents, reanalyze the sample, using the more concentrated reagents.

16. Calculation

16.1 Calculate the COD in the sample in milligrams per litre as follows:

$$\text{COD, mg/L} = ((A - B)N \times 8000)/S$$

where:

- A = ferrous ammonium sulfate solutions required for titration of the blank, mL,
- B = ferrous ammonium sulfate solution required for titration of the sample, mL,
- N = normality of the ferrous ammonium sulfate solution, and
- S = sample used for the test, mL.

17. Precision and Bias [7]

17.1 The overall precision of Test Method A within the range from 10 to 300 mg/L varies with the quantity being tested according to Fig. 1.

17.2 The data used in the calculation of precision are from EPA "Method Research Study 3" (1971) that involved two levels of COD, 12.3 mg/L (86 laboratories) and 270 mg/L (82 laboratories), and EPA" Water Pollution Laboratory Performance Evaluation, No. 8" (1982) that involved two levels of COD, 40.2 mg/L (65 laboratories) and 92 mg/L (67 laboratories).

17.3 The test data were obtained on reagent grade water and these precision and bias values may not be applicable to more complex water matrices. It is the user's responsibility to ensure the validity of this test method to waters of untested matrices.

17.4 The precision obtained by the interlaboratory study is overall, S_t. Since very carefully standardized samples in very pure water were used rather than natural samples collected by usual sampling procedures, the estimates do not include the increase in precision statistics and the potential change in bias that may be attributed to the sample collection activities.

17.5 The trend of the approximately 5 % negative bias is shown in Fig. 2.

17.6 *Prepared Standards*—Recoveries of known amounts of COD in the series of prepared standards (previously described) were as shown in Table 2.

[7] Supporting data were taken from "Method Research Study 3" (1971) and "Water Pollution Laboratory Performance No. 8" (1982), Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, OH. These documents are available from ASTM Headquarters as RR:D 19-1044.

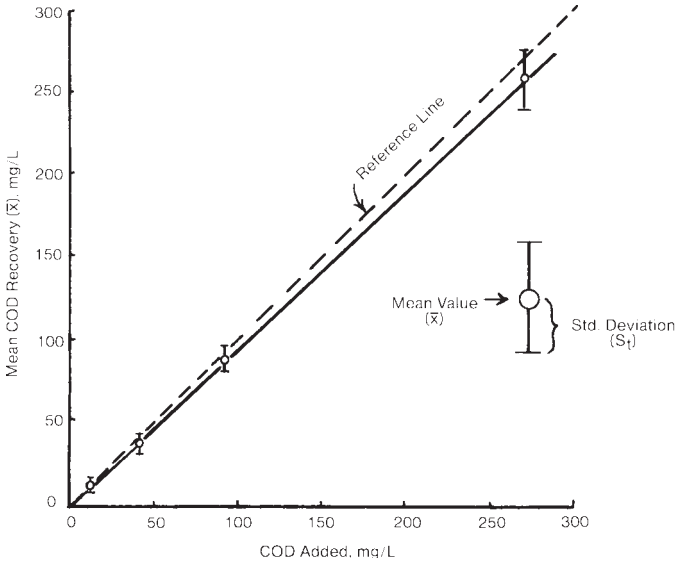

FIG. 2 Test Method A, Chemical Oxygen Demand (COD) Bias of Determinations

TABLE 2 Test Method A, Recovery and Precision Data

Prepared COD, mg/L	Recovered COD, mg/L	Bias, mg/L	% Bias	Statistically Significant
12.30	12.34	+0.04	+0.33	no
40.2	37.9	−2.3	−5.7	yes
92.0	88.6	−3.4	−3.7	yes
270	257	−13	−4.8	yes

TEST METHOD B—MICRO COD BY SEALED DIGESTION AND SPECTROMETRY

18. Scope

18.1 This test method is essentially equivalent to Test Method A, but it utilizes micro volumes of the same reagents contained in a sealable ampule or a screw-top culture tube and a spectrophotometer or filter photometer to measure absorbance or transmittance at selected wavelengths. This test method is applicable where only small sample volumes are available and where large numbers of samples need to be analyzed. This test method requires less space per analysis and uses less of the reagents, minimizing costs and volume of wastes discharged.

18.2 This test method was tested on Type II reagent water. It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

19. Summary of Test Method

19.1 The dichromate reagent and silver catalyst used in this test method are similar to those used in Test Method A, but the volumes employed are 1⁄20 th of those in Test Method A.

19.2 A sample aliquot is introduced carefully into an ampule or screw-top tube so that the sample is layered on top of previously introduced reagents and remains there until the ampule or tube is sealed. This technique limits evolution of heat of solution until the container is sealed, minimizing the loss of volatile organics.

19.3 After sealing, the ampule or tube is heated in an oven, sand bath, or heated block at 150 ± 2°C for 2 h. The COD concentration is determined spectrophotometrically after digestion. In the low COD range (5 to approximately 50 mg/L), the loss of hexavalent chromium is measured at 420 nm, while for the high range (50 to approximately 800 mg/L), the increase in trivalent chromium is measured at 600 nm. The ampule or tube serves as the absorption cell.

20. Interferences

20.1 Interferences identified in Section 6 are also applicable to the micro procedure.

20.2 Volatile materials will be lost if the sample is mixed with the reagents before the ampule or tube is sealed. Volatile materials will also be lost during sample homogenization.

20.3 Potentially, the loss of volatile organics in the micro procedure will be less than that which may occur in Test Method A. Thus, results between the two methods may differ if volatile materials are involved.

20.4 Spectrophotometric interferences may exist due to turbidity of precipitated salts that are too colloidal to settle in a reasonable period of time. Centrifugation may be used to

speed separation of the salts. This test method does not address a titration procedure for the micro-volume, but if the digested samples do not clear or spectrophotometric interference is suspected, the COD result can be determined by titration.[8]

20.5 The ampule or tube must have window areas that are free of scratches or smudges. If a suitable window area is not available, do not consider transfer of the sample. The sample and the blank may be titrated and the results used to calculate a COD value (see 24.10).

21. Apparatus

21.1 *Spectrophotometer or Filter Photometer*, suitable for measurements at 600 nm and 420 nm using the ampules or tubes in 21.3 or 21.3.1 as absorption cells. Filter photometers and photometric practices shall conform to Practice E 60. Spectrophotometers shall conform to Practice E 275. For some spectrophotometers, poor sensitivity at 420 nm has been observed. A suggested minimum sensitivity for the spectrophotometer readout is 0.002 absorbance units per milligram per litre of COD for the low range procedure.

21.2 *Heating Oven*, sand bath, or block heater capable of maintaining a temperature of 150 ± 2°C throughout. If an oven is used and screw-top tubes are employed, ascertain that the caps can withstand the oven temperature and solution pressure. The heating device must be equipped with a high temperature shut-off set at 175 to 185°C.

21.3 *Culture Tubes*, borosilicate glass, 16 by 100 mm, with TFE-fluorocarbon-lined screw caps. Protect the caps and culture tubes from dust contamination.

21.3.1 *Ampules*, borosilicate glass, 10 mL, may be substituted for the culture tubes in 21.3. These ampules are rotated and uniformly sealed with a glass blowing torch after addition of sample and reagent solutions. The nominal path length of these ampules shall be 15 to 20 mm.

21.4 *Apparatus for Blending or Homogenizing Samples*—A tissue homogenizer is recommended. However, a household blender may be used, but a suitable reduction in particle size may not be obtained.

NOTE 5—A partial round robin, using cellulose filter paper as the organic material, demonstrated serious difficulties in achieving a representative subsample. The use of a blender followed by a tissue homogenizer was required.

22. Reagents

22.1 *Silver Sulfate Catalyst Solution*—Dissolve 22 g of silver sulfate (Ag_2SO_4) in a 4.09 kg (9 lb) bottle of concentrated sulfuric acid (H_2SO_4).

22.2 *Potassium Acid Phthalate Solution, Standard* (1 mL = 1 mg/L)—See 14.5.

22.3 *Potassium Dichromate Digestion Solution*:

22.3.1 *High Range*—Add 10.216 g of potassium dichromate ($K_2Cr_2O_7$) dried at 103°C for 2 h, 167 mL of concentrated sulfuric acid (H_2SO_4) (sp gr 1.84) and 33.3 g of mercuric sulfate ($HgSO_4$) to about 750 mL of water, mix, and let cool. Dilute the solution to 1 L with water and mix thoroughly.

22.3.2 *Low Range*—Add 1.022 g of potassium dichromate, ($K_2Cr_2O_7$) (dried at 103°C for 2 h), 167 mL of concentrated sulfuric acid (H_2SO_4) (sp gr 1.84) and 33.3 g of mercuric sulfate ($HgSO_4$) to about 750 mL of water, mix, and cool. Dilute the solution to 1 L with water and mix thoroughly.

22.4 *Ferrous Ammonium Sulfate Solution (0.10 N)*—Dilute 400 mL of 0.25 *N* ferrous ammonium sulfate solution (see 14.1 to 1 L. Standardize against 0.25 *N* potassium dichromate ($K_2Cr_2O_7$) as in 14.1.

22.5 *Ferrous Ammonium Sulfate Solution (0.01 N)*—Dilute 40 mL of 0.25 *N* ferrous ammonium sulfate solution (see section 14.1) to 1 L. Standardize against 0.025 *N* potassium dichromate ($K_2Cr_2O_7$) as in 14.1.

22.6 *Phenanthroline Ferrous Sulfate Indicator Solution*—See 14.4. If desired, the indicator may be diluted 1:5 for use in this test method.

23. Calibration

23.1 *High Range*—Dilute the following volumes of COD standard solution (see 22.2) to 50 mL with water. The high range procedure may be used for COD determination as low as 25 mg/L at the discretion of the analyst.

Potassium Acid Phthalate Standard Solution, mL	COD, mg/L
2.5	50
5	100
10	200
20	400
30	600
40	800

NOTE 6—A typical COD calibration curve for spectrophotometric COD method, ampule technique (Test Method B) is shown in Fig. 3.

23.2 *Low Range*—Dilute the following volumes of potassium acid phthalate standard solution to 200 mL with water. At the discretion of the analyst, the upper limit may be extended to approximately 150 mg/L.

Potassium Acid Phthalate Standard Solution, mL	COD, mg/L
1	5
2	10
4	20
6	30
8	40
10	50

23.3 Use the procedure in Section 24 to analyze the prepared standard solutions and a procedural blank of water. For

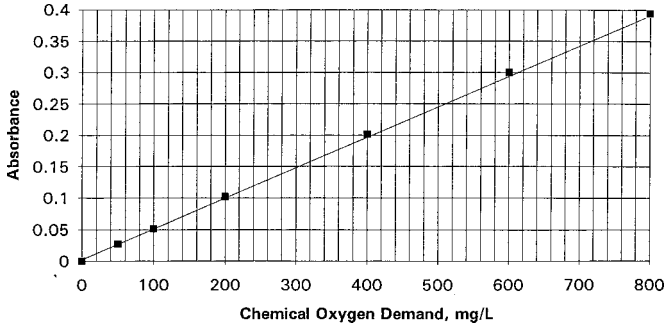

FIG. 3 Typical COD Calibration Curve for Spectrophotometric COD Method, Ampule Technique (Test Method B)

[8] Messenger, A. L., "Comparison of Sealed Digestion Chamber and Standard Method COD Tests," *Journal Water Pollution Control Federation*, Vol 53, Number 2, February 1981, pp. 232–236.

the high COD range, determine the spectrophotometric absorbance of each standard and blank at a wavelength of 600 nm. For the low COD range, determine the spectrophotometric absorbance of each standard and blank at a wavelength of 420 nm. Since the change in absorbance for the low range is negative with increasing COD, it may be convenient to read the blank and standards against water and plot the absorbance difference versus COD concentration.

23.4 Prepare calibration curves for each range by plotting the absorbance of each standard on the abscissa and milligrams per litre of COD on the ordinate. For the low range procedure, the correlation will have a negative slope; for the high range procedure, the slope is positive.

24. Procedure

24.1 Place 1.5 mL of digestion solution (22.3.1 for the high range procedure or 22.3.2 for the low range procedure) in a culture tube (21.3) or glass ampule (21.3.1).

NOTE 7—Accurate addition of the digestion volume in the low range procedure is important because the loss of hexavalent chromium is measured.

24.2 Add 3.5 mL of silver sulfate catalyst solution (22.1), mix, and allow to cool. If the mixed reagents are to be stored, store the sealed or capped solution in the dark.

NOTE 8—Several manufacturers offer similar catalyst and digestion solutions already combined in ampules or culture tubes. If the commercial preparations are used, the manufacturers' directions as to sample size should be followed. The analyst should visually inspect any purchased system to determine that reagent volumes are uniform and should develop calibration curves to confirm or replace precalibrated readouts.

24.3 Homogenize the sample if necessary.

24.4 Carefully add 2.5 mL of the sample, standard, or blank down the side of the tube or ampule so that a layer is formed on top of the reagents. Cap the tubes or seal the ampules.

24.5 Mix the sealed ampules or tubes thoroughly. It is feasible to mix tubes by holding the tube by the cap and shaking vigorously. Complete integrity of the TFE-fluorocarbon liner in the screw cap is imperative. The ampule or tube will become hot because of heat of solution.

NOTE 9—**Warning:** If handling the ampule or tube directly, use insulated gloves, or place the ampules or tubes in a rack for mixing. Use normal laboratory precautions for possible contact with the hot, corrosive reagents from broken ampules or tubes.

24.6 After mixing, place the ampules or tubes in an oven or heating device at 150 ± 2°C for 2 h.

24.7 Allow the ampules or tubes to cool at room temperature. After about 5 min, mix the contents of the ampule or tube thoroughly (to mix condensed water into the solution). Thereafter, permit the solution to cool and permit precipitated solids to settle (normally about 30 min). Rapid cooling will generate colloidal precipitates that are difficult to settle.

24.8 Make spectrophotometric readings using the ampules or culture tubes as the absorption cells. Transfer of cooled solution should not be considered because the solution is supersaturated and solids will precipitate that are difficult to settle.

24.9 Measure the absorbance of the low range solutions at 420 nm and the high range solutions at 600 nm. (See Note 4.)

24.10 Precision and bias in this test method has not addressed a titration procedure for the micro-volume, but if a spectrophotometric interference is suspected because of turbidity or possibly high results, the result may be checked by titrating the suspected sample and the blank. Add one drop of phenanthroline ferrous sulfate solution (22.6), and titrate to the color change with 0.1 *N* ferrous ammonium sulfate solution (22.4) for high range samples or with 0.01 *N* ferrous ammonium sulfate solution (22.5) for low range samples. Follow the same procedure with the procedural blank. The titrant volume for the blank will be about 3 mL. If this volume is not available in the ampule or tube, the digested sample must be transferred to a container of suitable volume for titration. Calculate the COD using the equation in Test Method A (16.1).

25. Calculation

25.1 Determine the COD value directly from the respec-tive calibration curves constructed for the purpose. See Section 23.

25.1.1 If the sample was prediluted, apply the appropriate dilution factor to the result.

25.2 Report all results in milligrams per litre.

26. Precision and Bias [9]

26.1 Precision and bias information was developed in a collaborative test by seven laboratories with Type II water. For other matrices, these data may not apply. Each prepared sample was analyzed on three different days by the same operator in each laboratory.

26.2 Test samples were prepared by dissolving weighed amounts of potassium acid phthalate in Type II water. Four sets of samples, two sets for the low COD range and two sets for the high COD range, were submitted to the laboratories.

26.3 The laboratories followed instructions to dilute one sample set in each range with Type II water. The resulting dilutions provided concentrations of 5, 12, 27, and 45 mg/L COD in the low range and 27, 90, 350, and 750 mg/L in the high range.

26.4 The other set of samples in each range was diluted with Type II water plus 1000 mg/L of chloride ion to provide the same COD concentrations in the low and high ranges as identified in 26.3.

26.5 Recovery, overall precision, and bias results for the low range samples, Type II water, are presented in Table 3 and are shown in Fig. 4.

TABLE 3 Test Method B, Recovery, Precision and Bias for Low Range, Type II Water

Amount Added, mg/L	Amount Recovered, mg/L	Standard Deviation, mg/L	Bias, ±%	Statistical Significance (95% confidence level)
5	6.76	4.02	+35	no
12	13.10	3.37	+9	no
27	26.10	2.86	−5	no
45	43.91	3.69	−2	no

[9] Supporting data are available from ASTM Headquarters. Request RR: D19-1044.

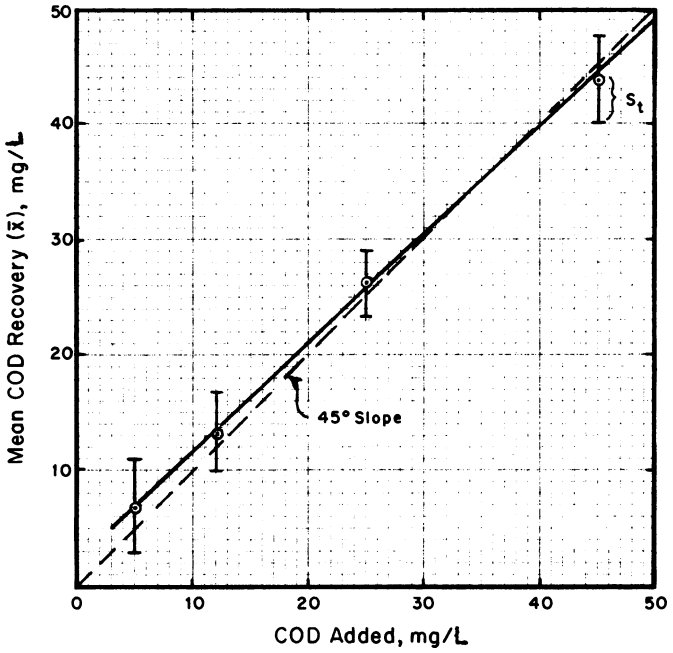

FIG. 4 Test Method B, Correlation of Collaborative Test Data COD Determination by Micro Procedure Type II Water

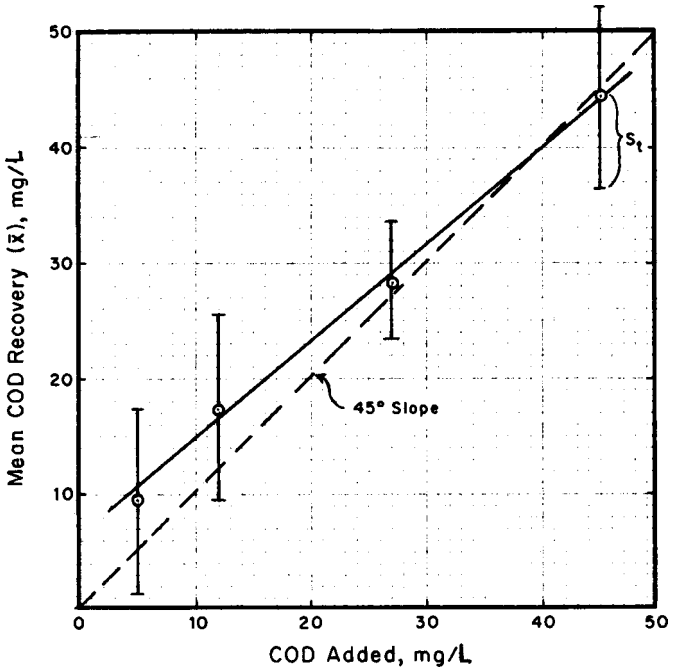

FIG. 5 Test Method B, Correlation of Collaborative Data COD Determination by Micro Procedure Type II Water Plus 1000 mg/L Chloride Ion

26.6 Recovery, overall precision, and bias results for the low range samples, Type II water plus 1000 mg/L of chloride ion, are presented in Table 4 and are illustrated in Fig. 5.

26.7 Recovery, overall precision, and bias results for the high range samples, Type II water, are presented in Table 5 and are illustrated in Fig. 6.

26.8 Recovery, overall precision, and bias results for the high range samples, Type II water plus 1000 mg/L of chloride ion, are presented in Table 6 and are illustrated in Fig. 7.

26.9 The higher positive bias and lower precision at lower concentrations of COD in the presence of chloride ion is not fully understood. All of the bias may not be the result of oxidation of chloride ion to chlorine. Laboratories identified problems with turbidity, but turbidity causes a negative bias in the low range procedure. A secondary source of positive bias may have been organic material adsorbed from laboratory atmosphere on the sodium chloride added to the dilution water.

26.10 The negative bias in results at the 750 mg/L concentration may have been partially a result of incomplete transfer of the sample from the shipment bottle to the prepared dilution. When refrigerated, the potassium acid phthalate, at the shipped concentration, was observed to crystallize from solution on the surface of the sample bottle. Laboratories were notified of the problem.

TABLE 4 Test Method B, Recovery, Precision and Bias for Low Range, Type II Water plus 1000 mg/L Chloride Ion

Amount Added, mg/L	Amount Recovered, mg/L	Standard Deviation, mg/L	Bias, ±%	Statistical Significance (95 % confidence level
5	9.33	8.15	+87	yes
12	17.39	7.89	+45	yes
27	28.65	5.23	+6	no
45	44.56	8.02	−1	no

TABLE 5 Test Method B, Recovery, Precision and Bias for High Range, Type II Water

Amount Added, mg/L	Amount Recovered, mg/L	Standard Deviation, mg/L	Bias, ±%	Statistical Significance (95 % confidence level)
27	26.61	4.55	− 1	no
90	92.00	23.13	+ 2	no
350	329.00	44.15	− 6	no
750	736.07	20.11	− 2	yes

27. Quality Control (QC)

In order to be certain that analytical values obtained using this test method are valid and accurate within the confidence limits of the test, the following QC procedures must be followed when running the test.

Analyses are always performed in a batch. A batch consists of a set of samples accompanied by control samples. Batches must be sized such that the control samples in the batch can be assured to be indicative of the variables affecting the remaining samples in the batch: all variables affecting the batch must affect all samples in the batch in a statistically equivalent manner. The maximum size of a batch is determined by identifying the key variables affecting the batch and assuring that these variables do not vary significantly during a batch. If batch sizes are too large, the user runs the risk of inappropriately rejecting portions of a batch. If batch sizes are too small, the cost of control sample analysis becomes higher.

In addition to other factors limiting batch size indicated in this section, the following variables must remain constant during a batch: analyst, instrument, and day. Recommended maximum batch sizes are specified in the table below

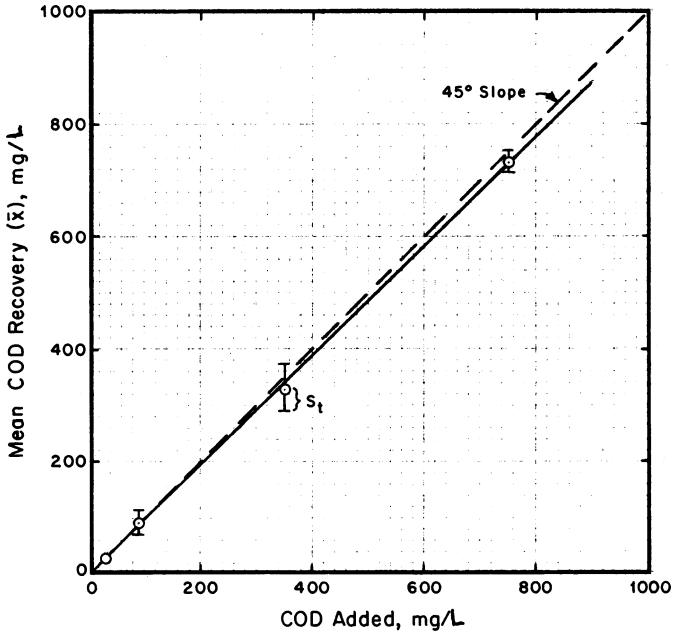

FIG. 6 Test Method B, Correlation of Collaborative Test Data COD Determination by Micro Procedure Type II Water

TABLE 6 Test Method B, Recovery, Precision and Bias for High Range, Type II Water plus 1000 mg/L Chloride Ion

Amount Added, mg/L	Amount Recovered, mg/L	Standard Deviation, mg/L	Bias, ±%	Statistical Significance (95 % confidence level)
27	42.06	7.76	+56	yes
90	92.83	14.18	+3	no
350	331.44	52.56	−5	no
750	686.89	104.00	−8	yes

Batch type	Maximum batch size
Method A	20
Method B	50

27.1 *Calibration and calibration verification*

27.1.1 *Instrument—for Method B.*

27.1.1.1 A calibration curve must be prepared with each batch of samples as specified in section 23. The calibration standards must be digested with the samples in the batch.

27.1.1.2 Calibration must be verified at the end of the batch by checking a mid-range standard. The measured COD must be within 10 % of the rated value of the standard.

27.1.1.3 If the calibration check fails, check for and resolve any spectrophotometer problems. Recalibrate the spectrophotometer and re-measure the absorbance of the ampules or tubes.

27.1.2 *Standardization—for Method A*

27.1.2.1 Ferrous Ammonium sulfate Solution titrant (section 14.1) must be re-standardized with each batch of samples analyzed. The batch must be completed with one preparation of titrant.

27.1.3 *Independent reference material (IRM)*

Analyze a certified reference material following the preparation of stock solutions used to prepare calibration standards. These results will verify the accuracy of the calibration standards.

27.2 *Initial demonstration of laboratory capability*

An initial demonstration of capability must be performed if a laboratory has not performed the test before or reperformed if either the instrument or analyst changes to assure that results equivalent to those obtained in the method collaborative study can be achieved.

27.2.1 For method A and method B, high range, prepare a 100 mg/L standard of primary grade potassium acid phthalate (as in section 23.3). For method B, low range, prepare a 30 mg/L standard (as in section 23.2). Analyze seven replicates of the appropriate standard.

27.2.2 Calculate the mean and standard deviation of the seven values and compare to the acceptable ranges of precision and bias in the following table. The demonstration must be repeated until the single operator precision and the mean recovery are with the limits given.

Method/Level	Acceptable range of recovery	Acceptable range of precision
Method A (100 mg/L)	86–106 mg/L	<6.9 mg/L
Method B, High Range (100 mg/L)	69–135 mg/L	<20 mg/L
Method B, Low Range (30 mg/L)	23–37 mg/L	<5 mg/L

If a concentration other than that specified above is used for laboratory capability testing, refer to D 5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

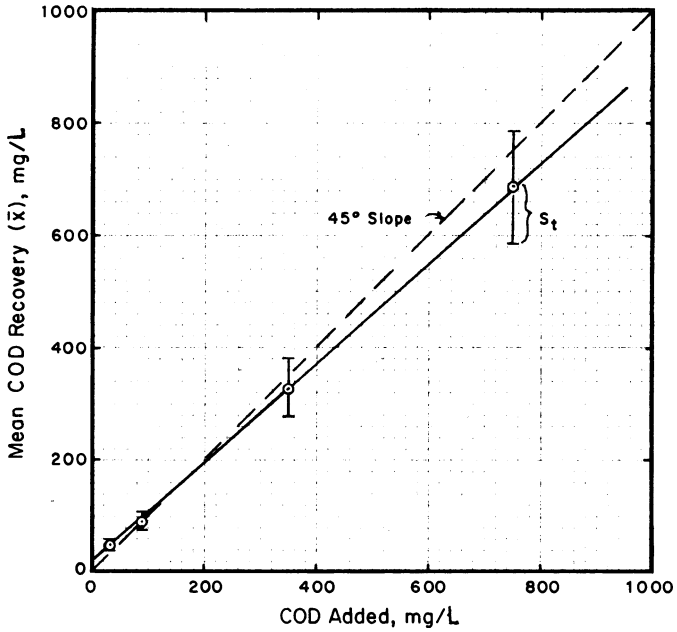

FIG. 7 Test Method B, Correlation of Collaborative Test Data COD Determination by Micro Procedure Type II Water Plus 1000 mg/L Chloride Ion

27.3 *Laboratory control sample (LCS)*

To insure that the performance of the test method is in control, one LCS must be analyzed with each batch of samples to assure continued performance within the limits established by the method collaborative testing.

The LCS will be the same material and concentration used for the initial demonstration of capability and must be taken through all of the steps of the analytical method, including preservation and pretreatment. The result obtained for the LCS must fall within the limits in the table below.

Batch type	LCS acceptance range
Method A	100 mg/L ± 12 mg/L
Method B, high range	100 mg/L ± 30 mg/L
Method B, low range	30 mg/L ± 8 mg/L

If the result does not fall within these limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed, or the results must be qualified with an indication that the method was not performing within acceptance criteria.

27.4 *Method blank (Blank)*

Method A, the amount of titrant needed for the blank is subtracted (blank correction). Analysts should monitor the amount of titrant used for blanks. Any significant change should be investigated.

For Method B, the method blank is used as the "zero" concentration point on the calibration curve. Since the calibration standards are taken through the entire analytical process, any absorbance due to blank levels is automatically subtracted. Analysts should monitor the absorbance of the blank against distilled water, especially when a new lot of reagents is used. Any significant increase in blank absorbance should be investigated.

27.5 *Sample spiking and replicates*

27.5.1 *Spiking*

Chemical Oxygen Demand is a composite, procedurally defined analyte. Recovery of constituents is a composite function of the recoveries of each compound present. For this reason, spiking a sample with a pure material with an experimental COD does not reveal anything about the absolute level of recovery of the constituents in the original sample. Comparison of matrix specific results across various oxygen demand methods and calculations of theoretical COD from constituent analysis may reveal the presence of refractory compounds.

27.5.2 *Replicates*

It is the responsibility of the method user to assure that reported results are of known and acceptable precision. Replicates by matrix and level should be run to establish real world sample precision. This should be done by running duplicates in numerous batches and combining the data to obtain a precision estimate. The collaborative study precision data can be used as a benchmark for these results. If the relative standard deviation of the real world sample results is significantly larger than that from the collaborative study, the results should be annotated for end users.

27.6 *Batch control sample (BCS)*

27.6.1 It is strongly recommended that a challenging control standard be run in duplicate – beginning and end – in each batch. This material is intended to be responsive to critical performance factors of the method, specifically, chloride interference and catalyst effectiveness.

27.6.2 This BCS should be made to have approximately the same COD levels as used in the initial demonstration of capability. 100 % of the COD should come from high purity acetic acid. In addition, the BCS should have 1000 mg/L background chloride level.

Alternatively, diluted substitute wastewater (D 5905), spiked with acetic acid may be used as the BCS. In either case, it is vital that the BCS can be made up routinely and reproducibly.

27.6.3 After performance of the method has been validated through the initial demonstration of capability, collect 20 to 30 pairs of BCS data. Construct Shewhart control charts for precision (range chart) and recovery (X-bar chart). Any out-of-control conditions on a BCS should be investigated and the batch re-analyzed. Up-dating of these control chart limits should never cause the control limits to broaden without sufficient cause.

27.6.4 Once these control charts have been established, they can replace the regular use of the Laboratory Control Sample. The LCS should still be run periodically to assure compliance with the control limits established in the method.

28. Keywords

28.1 chemical oxygen demand; COD; demand; oxygen demand