Designation: D3590 – 17

Standard Test Methods for Total Kjeldahl Nitrogen in Water[1]

This standard is issued under the fixed designation D3590; 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.

1. Scope*

1.1 These test methods cover the determination of total Kjeldahl nitrogen. Section 24 on Quality Control pertains to these test methods. The following test methods are included:

	Sections
Test Method A—Manual Digestion/Distillation	8 – 14
Test Method B—Semiautomated Colorimetric Bertholt	15 – 23

1.2 The analyst should be aware that precision and bias statements included may not necessarily apply to the water being tested.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *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.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D1426 Test Methods for Ammonia Nitrogen In Water
D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D3370 Practices for Sampling Water from Closed Conduits
D5810 Guide for Spiking into Aqueous Samples
D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *continuing calibration blank, n*—a solution containing no analytes (of interest) which is used to verify blank response and freedom from carryover.

3.2.2 *continuing calibration verification, n*—a solution (or set of solutions) of known concentration used to verify freedom from excessive instrumental drift; the concentration is to cover the range of calibration curve.

3.2.3 *total Kjeldahl nitrogen, n*—the sum of the nitrogen contained in the free ammonia and other nitrogen compounds, which are converted to ammonium sulfate $[(NH_4)_2SO_4]$ under the specified digestion conditions.

4. Significance and Use

4.1 These test methods are useful for measuring organic nitrogen and ammoniacal nitrogen, which are essential growth nutrients.

4.2 Nitrogen compounds are widely distributed in the environment. Sources of nitrogen include surface-applied fertilizers, cleaning products, and drinking water treatment aids. Because nitrogen is a nutrient for photosynthetic organisms, it may be important to monitor and control discharge into the environment.

5. Interferences

5.1 Nitrate is known to cause a serious negative interference in the test. Reportedly, a concentration of 250 mg/L NO_3 results in zero recovery of some level of mg/L N added as some nitrogen compound.

5.2 The analyst is cautioned that ammonia in the laboratory may easily become an interference in these test methods from contamination of reagents, caps, or from the laboratory atmosphere. Care should be taken that ammonium hydroxide, either as a reagent or as a cleaning substance, is not used in the same room.

[1] These test methods are under the jurisdiction of ASTM Committee D19 on Water and are the direct responsibility of Subcommittee D19.05 on Inorganic Constituents in Water.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

***A Summary of Changes section appears at the end of this standard**

6. Purity of Reagents

6.1 Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[3] Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, reference to water shall be understood to mean reagent water conforming to Specification D1193, Type I. Other reagent water types may be used provided it is first ascertained that the water is of sufficiently high purity to permit its use without adversely affecting the precision and bias of the test method. Type III water was specified at the time of round-robin testing of this test method.

7. Sampling and Preservation

7.1 Collect the sample in accordance with applicable Practices D3370.

7.2 Samples may be preserved up to 28 days by adding concentrated sulfuric acid to adjust to pH 2 or less and store between 2 and 6°C. The preserved sample should be analyzed as soon as possible; data on decomposition are not available.

TEST METHOD A—MANUAL DIGESTION/DISTILLATION

8.1 This test method covers the determination of total Kjeldahl nitrogen in water. It measures free ammonia or ammonia formed from the conversion of nitrogen components of biological origin such as amino acids and proteins. However, the procedure may not convert the nitrogenous compounds of some wastes to ammonia. Examples of such compounds that may not be measured are nitro compounds, hydrozones, oximes, nitrates, semicarbazones, pyridines, and some refractory tertiary amines.

8.2 Three alternatives are described for the final determination of the ammonia: a titrimetric method, which is applicable to concentrations above 1 mg/L N; a Nesslerization method, which is applicable to concentrations below 1 mg/L N; and a potentiometric method which is applicable to the range from 0.04 to 1000 mg/L N.

8.3 This test method is described for micro and macro systems. Micro determination can be made on sample aliquots containing up to 10 mg of nitrogen.

9. Summary of Test Method

9.1 The sample is heated in the presence of concentrated H_2SO_4, K_2SO_4, and $HgSO_4$, and is digested until SO_3 fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled, diluted, and is treated and alkalized with a hydroxide-thiosulfate solution. The ammonia is distilled into a boric acid solution and total Kjeldahl nitrogen is determined by colorimetry, titrimetry, or potentiometry.

10. Apparatus

10.1 *Digestion Apparatus*—A Kjeldahl digestion apparatus with 800 to 1000-mL flasks and suction takeoff to remove SO_3 fumes and water.

10.2 *Distillation Apparatus*[4]—A macro Kjeldahl flask connected to a condenser and an adaptor so that the distillate can be collected.

10.3 *Spectrophotometer or Colorimeter,* for use at 425 nm with a spectral band path of not more than ±20 nm and a light path of 1 cm or longer.

10.4 *Electrometer* (*pH Meter*), with expanded millivolt scale, or a specific ion meter.

10.5 *Ammonia Selective Electrode.*[5]

10.6 *Magnet Stirrer,* thermally insulated.

11. Reagents and Materials

11.1 *Ammonia Solution Stock,* (1.0 mL = 1.0 mg ammonia nitrogen)—Dissolve 3.819 g of ammonium chloride (NH_4Cl) in water and dilute to 1 L in a volumetric flask with water.

11.2 *Ammonia Solution, Standard* (1.0 mL = 0.01 mg ammonia nitrogen)—Dilute 10.0 mL of the stock solution (see 11.1) with water to 1 L in a volumetric flask.

11.3 *Boric Acid Solution* (2 %)—Dissolve 20 g of boric acid (H_3BO_3) in water and dilute to 1 L with water in a volumetric flask.

11.4 *Mercuric Sulfate Solution*—Dissolve 8 g of red mercuric oxide (HgO) in a mixture of 10 mL of sulfuric acid (H_2SO_4, sp gr 1.84) and 40 mL of water, and dilute solution to 100 mL. (**Warning—**Mercury has been designated by many regulatory agencies as a hazardous material that can cause serious medical issues. Mercury, or its vapor, has been demonstrated to be hazardous to health and corrosive to materials. Caution should be taken when handling mercury and mercury containing products. See the applicable product Safety Data Sheet (SDS) for additional information. Users should be aware that selling mercury and/or mercury containing products into your state or country may be prohibited by law.)

NOTE 1—Alternate catalysts such as copper sulfate ($CuSO_4$) may be used but it is the users responsibility to determine the validity of other catalysts.

11.5 *Mixed Indicator Solution*—Mix 2 volumes of 0.2 % methyl red in 95 % ethanol with 1 volume of 0.2 % methylene blue in ethanol. Prepare fresh every 30 days.

11.6 *Methyl Purple Indicator Solution* (1 g/L)—Dissolve 0.4 g of dimethyl-aminoazobenzene-*o*-carboxylic acid, sodium

[3] *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, and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

[4] Micro Kjeldahl steam distillation apparatus is commercially available.

[5] EIL Model 8002-2 of Electronics Instruments Ltd. (U. S. Representative: Cambridge Instrument Co., 73 Spring St., Ossining, NY 10562) has been found satisfactory for this purpose. Also, Orion Model 95-12 has been found satisfactory for this purpose.

salt, in approximately 300 mL of water. To this solution add 0.55 g of a water-soluble blue dyestuff, dissolve, and dilute to 1 L with water. This indicator is available commercially in a prepared form.

11.7 *Nessler Reagent*—Dissolve 100 g of mercuric iodide (HgI_2) and 70 g of potassium iodide (KI) in a small volume of water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of sodium hydroxide (NaOH) in 500 mL of water. Dilute the mixture to 1 L. This solution is stable for at least one year if stored in a thick amber polyethylene bottle out of direct sunlight. (**Warning—**See 11.4.)

11.8 *Phenolphthalein Indicator Solution*—Dissolve 5 g of phenolphthalein in 500 mL of 95 % ethyl alcohol or isopropanol and add 500 mL of water. Add NaOH (0.8 g/L) solution dropwise until a faint pink color appears.

11.9 *Sodium Hydroxide Solution* (400 g/L)—Dissolve 400 g of NaOH in 800 mL of water, cool, and dilute to 1 L with water.

11.10 *Sodium Hydroxide Solution* (0.8 g/L)—Dilute 2 mL of NaOH solution (400 g/L) (see 11.9) with water to 1 L.

11.11 *Sodium Hydroxide-Sodium Thiosulfate Solution*—Dissolve 500 g of NaOH and 25 g of $Na_2S_2O_3 \cdot 5H_2O$ in water and dilute to 1 L.

11.12 *Sulfuric Acid Solution, Standard* (0.02 N, 1 mL = 0.28 mg ammonia nitrogen)—Prepare a stock solution of approximately 0.1 *N* acid by diluting 3 mL of concentrated H_2SO_4 (sp gr 1.84) to 1 L with water. Dilute 200 mL of this solution to 1 L with water. Standardize the approximately 0.02 *N* H_2SO_4 solution against 0.0200 *N* Na_2CO_3 solution. This last solution is prepared by dissolving 1.060 g of anhydrous Na_2CO_3, oven dried at 140°C, and diluting to 1 L with water.

11.13 *Digestion Solution*—Dissolve 267 g of K_2SO_4 in 1300 mL water and 400 mL of concentrated H_2SO_4. Add 50 mL of mercuric sulfate solution (see 11.4) and dilute to 2 L with water. A digestion packet may be used in place of the digestion solution in the macro Kjeldahl system.

12. Procedure

12.1 Clean the distillation apparatus with steam before use by distilling a 1 + 1 mixture of water and sodium hydroxide-thiosulfate solution (see 11.11) until the distillate is ammonia-free. Repeat this operation each time the apparatus is out of service long enough to accumulate ammonia (usually 4 h or more).

12.2 *Macro Kjeldahl System:*

12.2.1 Place a measured sample into an 800-mL Kjeldahl flask and dilute to 500 mL. The sample size can be determined using the following table:

Kjeldahl Nitrogen in Sample, mg/L	Sample Size, mL
0 to 5	500
5 to 10	250
10 to 20	100
20 to 50	50.0
50 to 500	25.0

Prepare a 500-mL reagent water blank.

12.2.2 Add 100 mL of digestion solution (see 11.13) (see Note 2) and digest the mixture in the Kjeldahl apparatus until SO_3 fumes are given off and the solution turns colorless or pale yellow. Continue heating for an additional 30 min. Cool the residue and add 300 mL of water. Mix well.

NOTE 2—Digesting the sample with a packet and 20 mL of concentrated H_2SO_4 is acceptable. Cut the end of the package and empty the contents into the digestion flask.

12.2.3 Alkalize the digestate by careful addition of 100 mL of sodium hydroxide-thiosulfate solution (see 11.11). Do not mix until the digestion flask has been connected to the distillation apparatus (see 12.2.4).

NOTE 3—Slow addition of the heavy caustic solution down the tilted neck of the digestion flask will cause the heavier solution to underlay the aqueous H_2SO_4 without loss of free ammonia.

12.2.4 Connect the Kjeldahl flask to the condenser with the tip of the condenser (or an extension of the condenser tip) below the level of 50 mL of 2 % boric acid solution (see 11.3) contained in a 500-mL Erlenmeyer flask. Distill 300 mL at the rate of 6 to 10 mL/min.

12.2.5 Transfer the distillate to a 500-mL volumetric flask, dilute to volume with water, and mix. Transfer 250 mL to an Erlenmeyer flask and titrate with H_2SO_4 (see 12.4.1). If the concentration is found to be below 1 mg/L, determine the value colorimetrically. Use the remaining 250 mL for this determination.

12.3 *Micro Kjeldahl System:*

12.3.1 Place 50.0 mL of sample or an aliquot in a 100-mL Kjeldahl flask and add 10 mL of digestion solution (see 11.13). At the same time start a reagent blank. Evaporate the mixture in the Kjeldahl apparatus until SO_3 fumes are given off and the solution turns colorless or pale yellow. Digest for an additional 30 min. Cool the residue and add 30 mL of water.

12.3.2 Alkalize the digestate by careful addition of 10 mL of sodium hydroxide-thiosulfate solution (see 11.11). Do not mix until the digestion flask has been connected to the distillation apparatus (see Note 3).

12.3.3 Connect the Kjeldahl flask to the condenser with the tip of the condenser (or an extension of the condenser tip) below the level of 5 mL of 2 % H_3BO_3 solution (see 11.3) contained in a small Erlenmeyer flask. Distill 30 mL at the rate of 6 to 10 mL/min.

12.3.4 Transfer to a 50-mL volumetric flask, dilute to volume with water, and mix. Pipet 25 mL to an Erlenmeyer flask and titrate with H_2SO_4 (see 12.4.1). If the concentration is found to be below 1 mg/L determine the value colorimetrically. Use 20 mL of the remaining solution for this determination.

12.4 *Determination of Ammonia Distillate*—Determine the ammonia content of the distillate titrimetrically, colorimetrically, or potentiometrically.

12.4.1 *Titrimetric Determination*—Add 3 drops of the mixed indicator (see 11.5) to the distillate and titrate the ammonia with 0.02 *N* H_2SO_4 (see 11.12), matching the end point against a blank containing the same volume of water and H_3BO_3 solution (see 11.3). If a pH meter is preferred, titrate to pH 6.2.

NOTE 4—As an alternative, 2 drops of methyl purple indicator solution

(see 11.6) may be used and the titration carried out to the intermediate gray end point.

12.4.2 *Colorimetric Determination (Samples)*—To a 20-mL aliquot from the macro procedure (see 12.2.5) or micro procedure (see 12.3.4) diluted to 50 mL, add 1 mL of Nessler reagent (see 11.7), and mix. After 20 min, read the absorbance at 425 nm against the blank using 1-cm cells. Read the ammonia nitrogen in milligrams for the samples from the standard curve.

12.4.2.1 *Calibration Curve*—Prepare a series of standards on a daily basis in 50-mL volumetric flasks and dilute as follows:

Millilitres of Standard (see 11.2) 1.0 mL = 0.01 mg NH_3-N	Milligrams of NH_3-N/50.0 mL
0.0	0.0
0.5	0.005
1.0	0.010
2.0	0.020
4.0	0.040
5.0	0.050
8.0	0.080
10.0	0.10

To the standards diluted to 50 mL add 1 mL of Nessler reagent (see 11.7) and mix. After 20 min read the absorbance at 425 nm against the blank using 1-cm cells. From the values obtained for the standards plot a standard curve of absorbance versus milligrams of NH_3-N.

12.4.3 *Potentiometric Determination*—Test Method B of Test Methods D1426 should be used for this determination.

12.4.3.1 It is recommended that at least two standards (a high and a low) be digested, distilled, and compared to similar values on the calibration curve to ensure that the digestion-distillation technique is reliable. If treated standards do not agree with untreated standards, the operator should find the cause of the apparent error before proceeding.

13. Calculation

13.1 If the titrimetric procedure is used, calculate the total Kjeldahl nitrogen in the original sample using Eq 1:

$$\text{total Kjeldahl nitrogen, mg/L} = (A - B)N \times F \times 1000/S \times D/C \quad (1)$$

where:

1000 = 1000 mL/L,
A = standard 0.02 *N* H_2SO_4 solution used in titrating sample, mL,
B = standard 0.02 *N* H_2SO_4 solution used in titrating blank, mL,
N = normality of H_2SO_4 solution,
F = milliequivalent weight of nitrogen (14 mg),
S = sample digested, mL,
C = distillate taken for titration, mL, and
D = final adjusted distillate volume, mL.

If the H_2SO_4 is exactly 0.0200 *N* and exactly one half of the distillate is taken for measurement, the equation is shortened, as shown in Eq 2:

$$\text{total Kjeldahl nitrogen, mg/L} = (A - B) \times 560/S \quad (2)$$

13.2 If the Nessler procedure is used, calculate the total Kjeldahl nitrogen in the original sample using Eq 3:

$$\text{total Kjeldahl nitrogen, mg/L} = E \times 1000/S \times D/C \quad (3)$$

where:

1000 = 1000 mL/L,
E = NH_3-H read from curve, corrected for blank, mg,
D = final adjusted distillate volume, mL,
C = distillate taken for Nesslerization, mL, and
S = sample digested, mL.

13.3 If the potentiometric determination is used, calculate the total Kjeldahl nitrogen in the original sample using Eq 4:

$$\text{total Kjeldahl nitrogen, mg/L} = E \times 1000/S \times D/C \quad (4)$$

where:

E = NH_3-N/L as determined using Test Method B of Test Methods D1426,
S = sample digested, mL,
D = final adjusted volume, mL, and
C = distillate taken for measurement, mL.

14. Precision and Bias[6]

14.1 Thirty-one analysts in 20 laboratories used titration and Nesslerization to analyze natural water samples containing exact increments of organic nitrogen and obtained the following results:

Amount Added as Nitrogen, Kjeldahl, mg/L N	Amount Found as Nitrogen, Kjeldahl, mg/L N	Precision as Standard Deviation, mg/L N	Bias, %
0.20	0.23	0.197	+15.54
0.31	0.33	0.247	+5.45
4.10	4.14	1.056	+1.03
4.61	4.53	1.191	−1.67

14.2 The potentiometric test method has not been validated in conjunction with the digestion-distillation procedure described in this standard. However, since the procedure provides a relatively clean sample, it is thought that the user may be guided by the precision and bias information presented in Test Method B of Test Methods D1426 and by 12.4.3 of this test method.[7]

14.3 The data in Section 14 may not apply to types of water other than those tested. It is the responsibility of the analyst to ensure the validity of this test method for untested matrices.

14.4 Precision and bias for this test method conforms to Practice D2777 – 77, which was in place at the time of collaborative testing. Under the allowances made in 1.4 of Practice D2777 – 13, these precision and bias data do meet existing requirements for interlaboratory studies of Committee D19 test methods.

TEST METHOD B—SEMIAUTOMATED COLORIMETRIC BERTHELOT

15.1 This test method covers the automated determination of total Kjeldahl nitrogen in water and wastewater and is based

[6] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D19-1041. Contact ASTM Customer Service at service@astm.org.

[7] The Results Advisor of Committee D19 on Water has reviewed and approved this statement for conformity with the requirements of Practice D2777 – 85; the Technical Operations Section of Executive Subcommittee D19.90 has supported this approval.