APPENDIX B. DETERMINATION OF TURBIDITY BY NEPHELOMETRY

METHOD 180.1

DETERMINATION OF TURBIDITY BY NEPHELOMETRY

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180.1-1

1.0   SCOPE AND APPLICATION

    1.1   This method covers the determination of turbidity in drinking, ground,
            surface, and saline waters, domestic and industrial wastes.

    1.2   The applicable range is 0 to 40 nephelometric turbidity units (NTU).
            Higher values may be obtained with dilution of the sample.

2.0   SUMMARY OF METHOD

    2.1   The method is based upon a comparison of the intensity of light scattered
            by the sample under defined conditions with the intensity of light scattered
            by a standard reference suspension. The higher the intensity of scattered
            light, the higher the turbidity. Readings, in NTUs, are made in a
            nephelometer designed according to specifications given in sections 6.1 and
            6.2. A primary standard suspension is used to calibrate the instrument. A
            secondary standard suspension is used as a daily calibration check and is
            monitored periodically for deterioration using one of the primary standards.

        2.1.1   Formazin polymer is used as a primary turbidity suspension for
                water because it is more reproducible than other types of standards
                previously used for turbidity analysis.

        2.1.2   A commercially available polymer primary standard is also
                approved for use for the National Interim Primary Drinking Water
                Regulations. This standard is identified as AMCO-AEPA-1,
                available from Advanced Polymer Systems.

3.0   DEFINITIONS

    3.1   CALIBRATION BLANK (CB) - - A volume of reagent water fortified
            with the same matrix as the calibration standards, but without the analytes,
            internal standards, or surrogates analytes.

    3.2   INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) - - A
            solution of one or more method analytes, surrogates, internal standards, or
            other test substances used to evaluate the performance of the instrument
            system with respect to a defined set of criteria.

    3.3   LABORATORY REAGENT BLANK (LRB) - - An aliquot of reagent
            water or other blank matrices that are treated exactly as a sample including
            exposure to all glassware, equipment, solvents, reagents, internal standards,
            and surrogates that are used with other samples. The LRB is used to

determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.4   LINEAR CALIBRATION RANGE (LCR) - - The concentration range over which the instrument response is linear.

3.5   MATERIAL SAFETY DATA SHEET (MSDS) - - Written information provided by vendors concerning a chemical=s toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.6   PRIMARY CALIBRATION STANDARD (PCAL) - - A suspension prepared from the primary dilution stock standard suspension. The PCAL suspensions are used to calibrate the instrument response with respect to analyte concentration.

3.7   QUALITY CONTROL SAMPLE (QCS) - - A solution of the method analyte of known concentrations that is used to fortify an aliquot of LRB matrix. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance.

3.8   SECONDARY CALIBRATION STANDARDS (SCAL) - - Commercially prepared, stabilized sealed liquid or gel turbidity standards calibrated against properly prepared and diluted formazin or styrene divinylbenzene polymers.

3.9   STOCK STANDARD SUSPENSION (SSS) - - A concentrated suspension containing the analyte prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source. Stock standard suspension is used to prepare calibration suspensions and other needed suspensions.

4.0   INTERFERENCES

4.1   The presence of floating debris and coarse sediments which settle out rapidly will give low readings. Finely divided air bubbles can cause high readings.

4.2   The presence of true color, that is the color of water which is due to dissolved substances that absorb light, will cause turbidities to be low, although this effect is generally not significant with drinking waters.

4.3   Light absorbing materials such as activated carbon in significant concentrations can cause low readings.

5.0   SAFETY

5.1   The toxicity or carcinogenicity of each reagent used in this method has not
       been fully established. Each chemical should be regarded as a potential
       health hazard and exposure should be as lows as reasonably achievable.

5.2   Each laboratory is responsible for maintaining 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 (MSDS)
       should be made available to all personnel involved in the chemical analysis.
       The preparation of a formal safety plan is also advisable.

5.3   Hydrazine Sulfate (7.2.1) is a carcinogen. It is highly toxic and may be
       fatal if inhaled, swallowed, or absorbed through the skin. Formazin can
       contain residual hydrazine sulfate. Proper protection should be employed.

6.0   EQUIPMENT AND SUPPLIES

6.1   The turbidimeter shall consist of a nephelometer, with light source for
       illuminating the sample, and one or more photo-electric detectors with a
       readout device to indicate the intensity of light scattered at right angles to
       the path of the incident light. The turbidimeter should be designed so that
       little stray light reaches the detector in the absence of turbidity and should
       be free from significant drift after a short warm-up period.

6.2   Differences in physical design of turbidimeters will cause differences in
       measured values for turbidity, even though the same suspension is used for
       calibration. To minimize such differences, the following design criteria
       should be observed:

       6.2.1   Light source: Tungsten lamp operated at a color temperature
               between 2200-3000°K.

       6.2.2   Distance traversed by incident light and scattered light within the
               sample tube: Total not to exceed 10 cm.

       6.2.3   Detector: Centered at 90° to the incident light path and not to
               exceed $\pm$ 30° from 90°. The detector, and filter system if used,
               shall have a spectral peak response between 400 and 600 nm.

6.3   The sensitivity of the instrument should permit detection of a turbidity
       difference of 0.02 NTU or less in waters having turbidities less than 1 unit.
       The instrument should measure from 0 to 40 units turbidity. Several
       ranges may be necessary to obtain both adequate coverage and sufficient
       sensitivity for low turbidities.

6.4   The sample tubes to be used with the available instrument must be of clear, colorless glass or plastic. They should be kept scrupulously clean, both inside and out, and discarded when they become scratched or etched. A light coating of silicon oil may be used to mask minor imperfections in glass tubes. They must not be handled at all where the light strikes them, but should be provided with sufficient extra length, or with a protective case, so that they may be handled. Tubes should be checked, indexed and read at the orientation that produces the lowest background blank value.

6.5   Balance - - Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.6   Glassware - - Class A volumetric flasks and pipettes as required.

7.0   REAGENTS AND STANDARDS

7.1   Reagent water, turbidity-free: Pass deionized distilled water through a 0.45μ pore size membrane filter, if such filtered water shows a lower turbidity than unfiltered distilled water.

7.2   Stock standard suspension (Formazin):

        7.2.1   Dissolve 1.00 g hydrazine sulfate, $(NH_2)_2.H_2SO_4$, (CASRN 10034-93-2) in reagent water and dilute to 100 mL in a volumetric flask. **CAUTION - - CARCINOGEN**

        7.2.2   Dissolve 10.00 g hexamethylenetetramine (CASRN 100-97-0) in reagent water and dilute to 100 mL in a volumetric flask. In a 100 mL volumetric flask, mix 5.0 mL of each solution (7.2.1 + 7.2.2). Allow to stand 24 hours at $25 \pm 3°C$, then dilute to the mark with reagent water.

7.3   Primary calibration standards: Mix and dilute 10.00 mL of stock standard suspension (7.2) to 100 mL with reagent water. The turbidity of this suspension is defined as 40 NTU. For other values, mix and dilute portions of this suspension as required.

        7.3.1   A new stock standard suspension (7.2) should be prepared each month. Primary calibration standards (7.3) should be prepared daily by dilution of the stock standard suspension.

7.4   Formazin in commercially prepared primary concentrated stock standard suspension (SSS) may be diluted and used as required. Dilute turbidity standards should be prepared daily.

7.5   AMCO-AEPA-1 Styrene Divinylbenzene polymer primary standards are available for specific instruments and require no preparation or dilution prior to use.

7.6   Secondary standards may be acceptable as a daily calibration check, but must be monitored on a routine basis for deterioration and replaced as required.

8.0   SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1   Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with turbidity free water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2   No chemical preservation is required. Cool sample to 4°C.

8.3   Samples should be analyzed as soon as possible after collection. If storage is required, samples maintained at 4°C may be held for up to 48 h.

9.0   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 analysis of laboratory reagent blanks and other solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2   INITIAL DEMONSTRATION OF PERFORMANCE.

9.2.1   The initial demonstration of performance is used to characterize instrument performance (determined of LCRs and analysis of QCS).

9.2.2   Linear Calibration Range (LCR) - - The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by $\pm$ 10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear,

sufficient standards must be used to clearly define the nonlinear portion.

9.2.3 Quality Control Sample (QCS) - - When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analysis of a QCS. If the determined concentrations are not within $\pm$ 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before continuing with on-going analyses.

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1 Laboratory Reagent Blank (LRB) - - The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment.

9.3.2 Instrument Performance Check Solution (IPC) - - For all determinations, the laboratory must analyze the IPC (a midrange check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within $\pm$ 10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within $\pm$ 10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data. NOTE: Secondary calibration standards (SS) may also be used as the IPC.

9.3.3 Where additional reference materials such as Performance Evaluation samples are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Turbidimeter calibration: The manufacturer=s operating instructions should be followed. Measure standards on the turbidimeter covering the range of interest. If the instrument is already calibrated in standard turbidity units, this procedure will check the accuracy of the calibration scales. At least one standard should be run in each instrument range to be used. Some instruments permit adjustments of sensitivity so that scale values will correspond to turbidities. Solid standards, such as those made of lucite blocks, should never be used due to potential calibration changes caused by surface scratches. If a pre-calibrated scale is not supplied, calibration curves should be prepared for each range of the instrument.

11.0 PROCEDURE

11.1 Turbidities less than 40 units: If possible, allow samples to come to room temperature before analysis. Mix the sample to thoroughly disperse the solids. Wait until air bubbles disappear, then pour the sample into the turbidimeter tube. Read the turbidity directly from the instrument scale or from the appropriate calibration curve.

11.2 Turbidities exceeding 40 units: Dilute the sample with one or more volumes of turbidity-free water until the turbidity falls below 40 units. The turbidity of the original sample is then computed from the turbidity of the diluted sample and the dilution factor. For example, if 5 volumes of turbidity-free water were added to 1 volume of sample, and the diluted sample showed a turbidity of 30 units, then the turbidity of the original sample was 180 units.

11.2.1 Some turbidimeters are equipped with several separate scales. The higher scales are to be used only as indicators of required dilution volumes to reduce readings to less than 40 NTU.

NOTE 1: Comparative work performed in the Environmental Monitoring Systems Laboratory B Cincinnati (EMSL-Cincinnati) indicates a progressive error on sample turbidities in excess of 40 units.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Multiply sample readings by appropriate dilution to obtain final reading.

12.2 Report results as follows:

NTU	Record to Nearest
0.0 – 1.0	0.05
1 – 10	0.1
10 – 40	1
40 – 100	5
100 – 400	10
400 – 1,000	50
> 1,000	100

13.0   METHOD PERFORMANCE

13.1   In a single laboratory (EMSL-Cincinnati), using surface water samples at
        levels of 26, 41, 75 and 180 NTU, the standard deviations were ± 0.60, ±
        0.94, ± 1.2 and ± 4.7 units, respectively.

13.2   The interlaboratory precision and accuracy data in Table 1 were developed
        using a reagent water matrix. Values are in NTU.

14.0   POLLUTION PREVENTION

14.1   Pollution prevention encompasses any technique that reduces or eliminates
        the quantity or toxicity of waste at the point of generation. Numerous
        opportunities for pollution prevention exist in laboratory operation. The
        EPA has established a preferred hierarchy of environmental management
        techniques that places pollution prevention as the management option of
        first choice. Whenever feasible, laboratory personnel should use pollution
        prevention techniques to address their waste generation. When wastes
        cannot be feasibly reduced at the source, the Agency recommends recycling
        as the next best option.

14.2   The quantity of chemicals purchased should be based on expected usage
        during its shelf life and disposal cost of unused material. Actual reagent
        preparation volumes should reflect anticipated usage and reagent stability.

14.3   For information about pollution prevention that may be applicable to
        laboratories and research institutions, consult "Less is Better: Laboratory
        Chemical Management for Waste Reduction," available from the American
        Chemical Society=s Department of Government Regulations and Science
        Policy, 1155 16th Street, N.W., Washington, DC 20036, (202) 872-4477.

15.0   WASTE MANAGEMENT

15.1   The U.S. Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. 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 waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel," available from the American Chemical Society at the address listed in Sect. 14.3.

16.0   REFERENCES

1.   American Society for Testing and Materials (ASTM). 1993. *Annual Book of ASTM Standards, Volume 11.01*. Water (1), Standard D1889-88A, p. 359. West Conshohocken, PA,

2.   Standard Methods. 1992. *Standard Methods for the Examination of Water and Wastewater*. Eighteenth Edition, pp. 2-9, Method 2130B. APHA, AWWA, and WEF. Port City Press, Baltimore, MD.

17.0   TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

Table 1. Interlaboratory Precision And Accuracy Data

Number of Values Reported	True Value (T)	Mean (X)	Residual for X	Standard Deviation (S)	Residual for S
373	0.450	0.4864	0.0027	0.1071	-0.0078
374	0.600	0.6026	-0.0244	0.1048	-0.0211
289	0.65	0.6931	0.0183	0.1301	0.0005
482	0.910	0.9244	0.0013	0.2512	0.1024
484	0.910	0.9919	0.0688	0.1486	-0.0002
489	1.00	0.9405	-0.0686	0.1318	-0.0236
640	1.36	1.3456	-0.0074	0.1894	0.0075
487	3.40	3.2616	-0.0401	0.3219	-0.0103
288	4.8	4.5684	-0.0706	0.3776	-0.0577
714	5.60	5.6984	0.2952	0.4411	-0.0531
641	5.95	5.6026	-0.1350	0.4122	-0.1078

Regressions: $X = 0.955T + 0.54$, $S = 0.074T + 0.082$