

## DETERMINATION OF TURBIDITY BY NEPHELOMETRY

### Thermo Scientific Orion Method AQ4500

*Revision 5 3/12/09*

Accepted by U.S. EPA on March 31, 2009 for wastewater compliance monitoring.

#### 1.0 Scope and Applications

- 1.1 This method is a convenient packaging of the technology in “EPA Method 180.1 (*Ref 16.1*). The present method is based on the use of Thermo Scientific Orion AQUAfast Turbidimeter Model AQ4500, Primary Formazin Stock Solution Cat. No. AQ45FZ, and the associated calibration kit, Cat. No. AQ45ST, or suitable equivalents. The applicable range is 0.6-40 nephelometric turbidity units (NTU).
- 1.2 This method covers the determination of turbidity in drinking, ground, surface, and saline waters, domestic and industrial wastes.
- 1.3 This method is for use in the Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.
- 1.4 The MDL for this method was determined to be 0.02 NTU. This value was derived from the analysis of seven aliquots, using two instruments, with a concentration of approximately 0.15 NTU.
- 1.5 Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the quality control procedure in Section 9.2.

#### 2.0 Summary of Method

The method is based upon a comparison of the intensity of light scattered by the sample at 90° to the beam path, with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity. The transmitted beam is used as a reference beam to correct for small amounts of color, if present in the sample. 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 a primary standard.

### 3.0 Definitions

- 3.1 Turbidity In water, turbidity is caused by suspended matter, such as clay, silt, finely divided organic and inorganic matter, soluble colored organic compounds, and plankton and other microscopic organisms. Turbidity is an expression of the optical property that causes light to be scattered rather than transmitted in straight lines through the sample. Correlation of turbidity with the weight concentration of suspended matter is difficult because the size, shape, and refractive index of the particulates also affect the light-scattering properties of the suspension.
- 3.2 Material Safety Data Sheet (MSDS)- Written information provided for each chemical reagent or standard about a chemical's toxicity, health hazards, physical properties, flammability, and reactivity. It also includes storage, spill, and handling precautions.
- 3.3 Method Detection Limit (MDL)-The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.4 Calibration Blank (CB)- A volume of low turbidity reagent water from the same source used to prepare standards
- 3.5 Calibration Standard (CAL)- A solution prepared from the primary dilution standard or stock standards . Used to calibrate the instrument response with respect to analyte concentration
- 3.6 Instrument Performance Check Solution (IPC)- A solution of the analyte used to evaluate the performance of the instrument system with respect to a defined set of criteria.
- 3.7 Laboratory Fortified Blank (LFB)- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like the sample and is used to determine whether the methodology is in control, and if the laboratory is capable of making accurate and precise measurement.
- 3.8 Laboratory Reagent Blank (LRB)- An aliquot of reagent water or other blank matrices that are treated as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. Use the LRB to determine if method analytes or other interferences are present in the laboratory environment, reagents or apparatus.
- 3.9 Quality Control Sample (QCS)- A solution of method analytes of known concentration that is used to fortify an aliquot of LRB Obtain the QCS

from a source external to the laboratory that is different from the source of calibration standards . It is used to check laboratory performance with externally prepared test materials.

- 3.10 Linear Calibration Range The concentration range over which the instrument response is linear.
- 3.12 Calibration Range The range of concentration of analyte for which the use of the method has been approved , as given in the Method Scope, Section 1.
- 3.13 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.
- 3.14 Secondary Calibration Standards (SCAL) Commercially prepared, stabilized sealed liquid or gel turbidity standards calibrated against properly prepared and diluted formazin primary standards.
- 3.15 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. Also, this interference is minimized by the use of a ratiometric optical design which compares the transmitted and scattered signals.
- 4.3 Light absorbing materials such as activated carbon in significant concentrations can cause low readings.

#### **5.0 Safety**

- 5.1 Use good laboratory practices throughout the test procedure. Follow the test procedure carefully and observe all precautionary measures.

- 5.2 An updated Material Safety Data Sheet (MSDS) is available for the reagents used in this method, which contains all known toxicological information. Detailed information is available on the Internet, as well as by toll-free telephone 1- 800-225-1480, or [www.thermo.com/water](http://www.thermo.com/water).
- 5.3 Formazin can contain residual hydrazine sulfate, which is a carcinogen. Proper protection should be employed, since it can be absorbed through the skin.
- 5.4 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of all chemicals. Additional information on laboratory safety can be found in *Ref. 16.2*.

## **6.0 Equipment and Supplies**

- 6.1 Thermo Scientific Orion AQUAfast Turbidimeter Model AQ4500, or equivalent.
- 6.2 Silicon Oil and Polishing Cloth Kit, Thermo Scientific Orion Cat. No.AQ45S1, or equivalent
- 6.3 Replacement Measuring Vials, Thermo Scientific Orion Cat. No. AC2T24
- 6.4 Glassware. Class A volumetric flasks and pipettes as required.

## **7.0 Reagents and Standards**

- 7.1 Reagent water, turbidity free. Use deionized water which has filtered through a 0.45 micron filter, or reverse osmosis water, if these give a lower turbidity than unfiltered distilled water.
- 7.2 Formazin Primary Standard, 4000 NTU, Thermo Scientific Orion Cat. No.AC45FZ
- 7.3 Polymer Primary Standards, 1, 10, 100, 1000 NTU. Thermo Scientific Orion Cat. No. AC45ST

## **8.0 Sample Collection 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. The volume collected should be sufficient to insure a representative sample, allow for replicate analyses (if required), but 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 hours.
- 8.4 Details on sampling techniques from conduits may be found in *Reference 16.3*.

## **9.0 Quality Control**

- 9.1 Each laboratory using this method for compliance reporting is required to operate a formal quality control (QC) program (*see Reference 16.4*). The minimum requirements of this program are initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks, fortified samples, duplicates and other laboratory solutions as a continuing performance check. The laboratory must maintain performance records that define the quality of the data that are generated. See Section 17.1 for QC Performance Criteria.

### **9.2 Initial Demonstration Of Performance**

- 9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.
- 9.2.2 Calibration Range Verification (CRV) - The calibration range must be verified at least every six months or whenever a significant change in instrument response is observed. The verification must use a minimum of a blank and three standards that cover the entire calibration range. Any verification data must determine that the instrument is within  $\pm 10\%$  of calibration for each concentration used. If any verification data exceeds  $\pm 10\%$  of calibration, recalibrate the instrument and repeat the procedure. If the problem is not resolved, call the instrument manufacturer for assistance.
- 9.2.3 Quality Control Sample (QCS) - Obtain an independent quality control standard for turbidity available from commercial sources (preferably verified against American Association for Laboratory Accreditation or National Institute of Standards and Technology reference materials, if available). If needed, dilute the standard according to the directions supplied to obtain standard concentrations within the linear calibration range.

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 analyses of a QCS. If the determined concentrations are not within 10 % of the stated values, performance is unacceptable. Identify and correct the source of the problem before proceeding with the initial determination of MDLs or continuing with on-going analyses.

- 9.2.4 Method Detection Limit (MDL) - An MDL must be established for turbidity using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values (40 CFR, Part 136, Appendix B), take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$\text{MDL} = t \times S$$

Where:

t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom]. S = the standard deviation of the replicate analyses. [t= 3.14 for seven replicates]

MDLs should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response

- 9.2.5 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 +/- 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.

### 9.3 Assessing Laboratory Performance

9.3.1 Laboratory Reagent Blank (LRB) - The laboratory must analyze at least one LRB with each batch of samples. Perform a reagent blank determination according to Section 11.0, *Procedure*, substituting reagent water (as defined in Section 7.1) for the sample. 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 is not within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms the calibration is outside the limits, halt sample analysis, and determine the cause. In the case of drift, recalibrate the instrument, or contact the instrument manufacturer. Reanalyze all samples following the last acceptable IPC solution. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.3.3 If reference materials are available, analyze them to provide additional performance data. Analyzing reference samples is valuable for demonstrating the ability to perform the method acceptably.

## 10.0 Calibration

### 10.1 General Precautions

10.1.1 Thoroughly clean vials and caps after each analysis in order to prevent carry-over errors. Even minute residues could lead to incorrect measurements.

10.1.2 Ensure that the outer walls of the vials are dry and clean before performing the analysis. Fingerprints or water droplets on the light entry surfaces of the vials lead to incorrect measurements.

- 10.1.3 Bubbles on the inside walls of the vial lead to incorrect measurements. To prevent this, cap the vial and remove the bubbles by swirling the vial before performing the test.
- 10.1.4 Soiling of the lens (LED and photo sensor) in the sample chamber leads to incorrect measurements. Do not attempt to clean the lenses. Contact the equipment manufacturer if dirty lenses are suspected.
- 10.1.5 Major temperature differentials between the photometer and the environment can lead to incorrect measurements, due to the formation of condensate in the area of the lens or on the vial.
- 10.1.6 The use of the vial cover is required to prevent stray light from affecting the measurement. If waterproof integrity is desired, the vial cover can be screwed down, by turning it clockwise, thus making the Model AQ4500 instrument waterproof to IP67 standards.
- 10.1.7 The Model AQ4500 uses 4 alkaline or lithium AA batteries. With alkaline batteries, the expected life is 2500 hours. With lithium batteries, the expected life is 10,000 hours. When the battery voltage becomes low, the battery icon will light.
- 10.1.8 Pressing the self-test button in the setup menu on the Model AQ4500 will initiate a built-in program to verify instrument operation.
- 10.1.9 Although it is believed that the instrument and primary standards will remain stable for at least a year, semi-annual calibration with primary formazin standards should be done until the analyst has established that longer time periods between primary calibrations may be justified.
- 10.1.10 Samples should be swirled to disperse the solids, but not so vigorously as to introduce air bubbles, which will interfere with the analysis,

## 10.2 Instrument Calibration

Note: See meter manual for calibration procedure details.

10.2.1. Select the “EPA 180” measurement mode.

10.2.2. Press the CAL key. “H2O INSERT” will be displayed.

- 10.2.3 Insert the vial containing pure water, and press the YES key. “H2O WAIT” will be displayed
- 10.2.4 When the reading has been internally entered, the display will read “1.00 YES?”
- 10.2.5 If the standard is 1.00 NTU, insert the standard vial and press YES. If another standard is to be used, press the “UP” or “DOWN” symbol key. “CHANGE?” will appear. Enter the value of the standard using the numerical keypad. Check the instrument manual for the allowed range at each calibration point.
- 10.2.6 Repeat 10.2.5 for each standard.
- 10.2.7 When the calibration is complete, the AQ4500 will proceed to the measurement mode.

## **11.0 Procedure**

- 11.1 Select “EPA 180” as the measurement mode.
- 11.2 Place the sample in a clean, dry turbidity vial. Cap securely. Wipe off excess liquid or fingerprints with a soft cloth.
- 11.3 Place into the AQ4500 sample chamber and cover with vial cap.
- 11.4 Press MEASURE key.
- 11.5 The result will be displayed on the instrument, and can be printed out or logged for future use.
- 11.6 If the result is less than 40 NTU, repeat procedure for the next sample.
- 11.7 If the result is greater than 40 NTU, 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. Since the AQ4500 is auto-ranging, the required dilution can be estimated quite easily.

## 12.0 Data Analysis and Calculations

12.1 The AQ4500 display resolution is user-adjustable from the “Digits” option in the “Set-up” menu. The resolution should always be greater than the requirement in 12.2

12.2 Report results as follows:

0-1 NTU	to nearest 0.05
1-10 NTU	to nearest 0.1
10-40 NTU	to nearest 1
40-100 NTU	to nearest 5
100-400 NTU	to nearest 10
400-1000 NTU	to nearest 50
>1000 NTU	to nearest 100

## 13.0 Method Performance

13.1 The method detection limit (MDL) study was performed by one analyst, and was determined to be 0.02 NTU.

13.2 The minimum level (ML) for this method is 0.06 mg/L.

## 14.0 Pollution Prevention

14.1 There are no reagents used in this procedure. The small quantity of standards should not present any pollution problems, but the standards are considered health hazards. See Section. 5.3

## 15.0 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 restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 For further information on waste management, consult *Ref. 16.5*

## 16.0 References

16.1 USEPA Guidance Manual, Turbidity Provisions, Appendix B, “Determination Of Turbidity By Nephelometry”. Method 180.1, EMSL, Office of Research and Development, USEPA, Cincinnati, Ohio 45268, April 1999.

- 16.2 "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).
- 16.3 "Standard Practices for Sampling Water," ASTM Annual Book of Standards, Part 31, D3370-76, American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.
- 16.4 40 CFR part 136, Appendix A, Methods 1624 and 1625. See also, "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA, EMS-CI, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.
- 16.5 "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.

## 17.0 Tables and Validation Data

### 17.1 QC Performance Criteria

<i>Type</i>	<i>Frequency</i>	<i>Acceptance Criteria</i>
Laboratory Reagent Blank (LRB)	Daily	Equal to, or less than 0.10 NTU
Instrument Performance Check (IPC)	Immediately after any calibration; after every 10th sample and at the end of a sample run	90-110% of the initial calibration
Independent Standard (QCS)	Initially, or quarterly, and as required to meet data quality needs	90-110% recovery