



**Analytical Services  
4747 East 49<sup>th</sup> Street  
Cuyahoga Hts., OH 44125**

---

## SOP 5001-2 Quality Assurance Manual

*Effective Date: March 15, 2007*

This manual is applicable to the Quality Assurance System governing the Analytical Services Department of the Northeast Ohio Regional Sewer District Analytical Services Department.

---

Approvals:

Superintendent of  
Environmental Services: Richard Connelly Date: 03/14/07

---

Manager of Analytical Services: Mark Citriglia Date: 03/14/07

---

Quality Assurance Specialist: Carol Turner Date: 03/14/07

---

Supervising Chemist: Eva Hatvani Date: 03/14/07

---

Supervising Chemist: Cheryl Soltis-Muth Date: 03/14/07

---

Supervising Chemist: Kristen Greenwood Date: 03/14/07

---



**Analytical Services  
4747 East 49<sup>th</sup> Street  
Cuyahoga Hts., OH 44125**

**Quality Assurance Manual  
Table of Contents**

1.0	Quality Assurance Policy.....	1
2.0	Organization and Management Structure .....	1
3.0	Documents Control .....	4
4.0	Critical Staff Positions .....	6
5.0	Traceability of Analytical Measurements .....	7
6.0	Methods Performed.....	8
7.0	Capabilities Review for Addition to Methods Performed .....	9
8.0	Traceability of Calibration and Method Validation.....	10
8.1	Method Linearity Studies.....	10
8.2	Method Specificity .....	10
8.3	Method Accuracy .....	10
8.4	Method Precision .....	10
8.5	Reagents and Standards .....	11
9.0	Sample Receipt and Handling.....	11
10.0	Facility and Equipment.....	12
11.0	Equipment Calibration and Maintenance.....	12
12.0	Data Verification and Internal Quality Control Activities.....	13
13.0	Corrective Actions .....	14
14.0	Control of Data Generated from non-Conforming Activities.....	14
15.0	Complaints .....	15
16.0	Confidentiality and Public Access .....	15
17.0	Data Review and Audits .....	15
18.0	Training and Demonstration of Capability .....	15
19.0	Ethical Conduct.....	16
20.0	Reporting of Data.....	16

NEORSO Laboratory SOP No.: 5001	Revision: 2	Title: Quality Assurance Manual	Page 1 of 17
------------------------------------	----------------	------------------------------------	--------------

## Quality Assurance Policy

- 1.1 The Analytical Services Department provides performs analytical testing for the various departments within the Northeast Ohio Regional Sewer District (NEORSO). The Analytical Services Department also performs work for external sources on a limited basis. The analytical information generated is used for daily operation of the wastewater treatment facilities, and provides compliance monitoring for the treatment facilities as required by the Ohio Environmental Protection Agency and the District's National Pollution Discharge Elimination System (NPDES) permits. Additionally the laboratory monitors materials introduced into the collection system and monitors water quality throughout the service area form samples submitted from the Water Quality and Industrial Surveillance Department.
- 1.2 The management staff of Analytical Services is committed to operating the laboratory in a safe, professional and proficient manner. To attain these goals, management is committed to and has adopted policies and procedures in accordance with the National Environmental Laboratory Accreditation Conference (NELAC).
- 1.3 The goal of management is to generate information of the highest quality that is legally defensible and presents the laboratory and its employees as ethical and competent. The management staff is responsible for ensuring that policies and objectives are communicated to, understood and implemented by all laboratory personnel.
- 1.4 The Quality System is documented and defined in the Quality Assurance Manual. The Quality Assurance Manual, Standard Operating Procedures and supplemental instructions for the performance of duties are available to the laboratory personnel. Every employee of the NEORSO Analytical Services department is responsible to read, understand and follow the policies defined in the Quality Assurance Manual.

## 2.0 Organization and Management Structure

- 2.1 The *Superintendent of Environmental Services* is the final authority for laboratory operations. The *Superintendent* has assigned daily management of the laboratory to the *Manager of Analytical Services*.
- 2.2 The *Manager of Analytical Services* reports directly to the *Superintendent of Environmental Services*. The manager is responsible for addressing the technical issues of the laboratory and assuring that the technical operations of the laboratory are conducted within the guidelines of the Quality Assurance System. *Manager of Analytical Services* is responsible for implementing actions necessary to bring operations into compliance with the Quality Assurance System.
- 2.3 The *Quality Assurance Specialist* reports directly to the *Manager of Analytical Services*. The *Quality Assurance Specialist* is responsible for monitoring laboratory compliance with those requirements set forth in this Quality Assurance Manual. The *Quality Assurance Specialist* has the authority to issue requests for

NEORS Laboratory SOP No.: 5001	Revision: 2	Title: Quality Assurance Manual	Page 2 of 17
-----------------------------------	----------------	------------------------------------	--------------

corrective action on items or activities found to be out of compliance with the Quality Assurance System. The *Quality Assurance Specialist* has the final authority on issues dealing with the quality of the data. The *Quality Assurance Specialist* has the authority to suspend analyses or require re-analyses.

- 2.4 The *Supervising Chemist* is considered the technical director of the areas under his/her direct supervision. Responsibilities include assisting and training of laboratory personnel with the various approved EPA methods utilized within the laboratory. Management of the day to day analytical activities of chemists, biologist and wastewater analysts. Evaluation, review and approval of data, and quality control statistics for the analyses performed in the laboratory. The Supervising Chemists report directly to the Manager of Analytical Services. A detailed job description for this position is on file with the Employee Resources Department.
- 2.5 The *Logistic Chemists* assists the Manager of Analytical Services and the QA/QC Specialist with coordination of administrative and operational functions including chemical inventory, disposition of laboratory equipment and supplies, data reporting, Chain of Custody procedures, project management, and scheduling. The Logistics Chemist reports directly to the QA/QC Specialist. A detailed job description for this position is on file with the Employee Resources Department.
- 2.6 The *Advanced Instrumentation Chemist* (AI Chemist) performs qualitative, and quantitative chemical analyses utilizing advanced instrumentation such as ICP, GFAA, Automated Analyzers, TOC and other instrumentation. The AI Chemist is responsible for troubleshooting and training on the advanced instrumentation. The AI Chemist reports directly to a Supervising Chemist. A detailed job description for this position is on file with the Employee Resources Department.
- 2.7 *Chemists* are responsible for the analysis of water samples such as municipal and industrial wastewater and sludge sample for various chemical analyses, including wet chemistry, physical properties and instrumental analyses. Chemist follow defined laboratory standard operating procedures and utilize good analytical techniques. Chemists report directly to the Supervising Chemist. A detailed job description for this position is on file with the Employee Resources Department.
- 2.8 **Biologist** analyze water samples such as municipal and industrial wastewater and sludge sample for various bacteriological and microbiological components, bioassay, and physical and chemical, including wet chemistry, physical properties and instrumental analyses. Follow standard methods and good analytical techniques. Biologist follow defined laboratory standard operating procedures and utilize good analytical techniques. The biologist report directly to the supervising chemist. A detailed job description for this position is on file with the Employee Resources Department
- 2.9 **Wastewater Analysts III** analyze water samples such as municipal and industrial wastewater and sludge sample for various chemical analyses, including wet chemistry, physical properties and instrumental analyses. Analysts follow

---

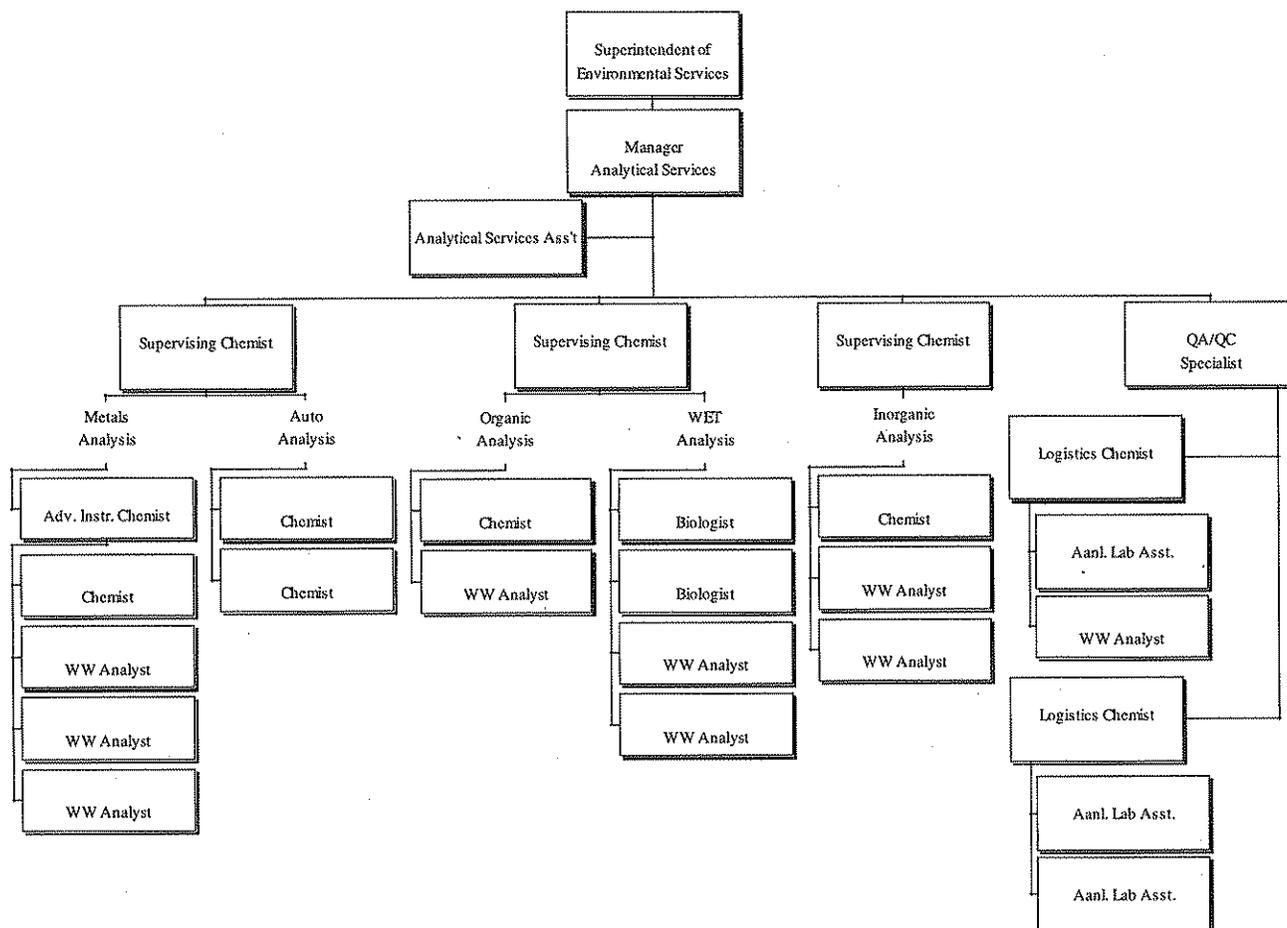
Property of NEORS. This is a date sensitive document and may not be current after 3/15/07.

**This is valid for use only if stamped "Controlled".**

defined laboratory standard operating procedures and utilize good analytical techniques. A detailed job description for this position is on file with the Employee Resources Department

- 2.10 *Wastewater Analyst (II)* analyzes water samples such as municipal and industrial wastewater and sludge sample for various chemical analyses, including wet chemistry, physical properties. Analysts follow defined laboratory standard operating procedures and utilize good analytical techniques. Wastewater Analyst will also collect samples and transport samples utilizing chain of custody procedures defined by the laboratory. A detailed job description for this position is on file with the Employee Resources Department.
- 2.11 *Wastewater Analyst (I)* analyzes water samples such as municipal and industrial wastewater and sludge sample for various chemical analyses, including wet chemistry, physical properties. Analysts follow defined laboratory standard operating procedures and utilize good analytical techniques. Wastewater Analyst will also collect samples and transport samples utilizing chain of custody procedures defined by the laboratory. A detailed job description for this position is on file with the Employee Resources Department.
- 2.12 The *Sample Control Specialist*, administers, coordinates, documents, and participates in the chain of custody program controlling wastewater, sludge, industrial, and surface water samples submitted to Analytical Services. The Sample Control Specialist reports directly to the QA/QC Specialist. A detailed job description for this position is on file with the Employee Resources Department.
- 2.13 The *Analytical Laboratory Assistant*, cleans, organizes and maintains laboratory glassware, sampling equipment, vehicles, refrigerator and general areas within the laboratory facilities. The lab assistant distributes and disposes of supplies and samples as directed. The Analytical Laboratory Assistant reports directly to the Logistic Chemist. A detailed job description for this position is on file with the Employee Resources Department
- 2.14 The *Analytical Services Assistant* assists the management staff of Analytical Services with the coordination of administrative tasks, operational functions, data reporting, document management and storage for compliance reports. A detailed job description for this position is on file with the Employee Resources Department

## 2.15 Table of Organization



## 3.0 Documents Control

- 3.1 Maintenance and management of the document control system is the responsibility of the Quality Assurance Specialist. Documents related to analysis, calibration, calculations and reports are maintained to allow for historical reconstruction of data.
- 3.2 The following documents are considered controlled documents and are to be maintained by the document control system
- 3.2.1 Quality Assurance Manual
  - 3.2.2 Standard Operating Procedures
  - 3.2.3 Analytical Data Sheets, Forms and Notebooks
  - 3.2.4 Instrument Printouts and Run Logs

Property of NEORS. This is a date sensitive document and may not be current after 3/15/07.

**This is valid for use only if stamped "Controlled".**

NEORSD Laboratory SOP No.: 5001	Revision: 2	Title: Quality Assurance Manual	Page 5 of 17
------------------------------------	----------------	------------------------------------	--------------

- 3.2.5 Batch records
- 3.2.6 Calibration curves and records
- 3.2.7 Method detection limits records
- 3.2.8 Training Records
- 3.2.9 Instrument Maintenance logs
- 3.3 Document Control System
  - 3.3.1 Controlled documents are maintained by the document control system. Controlled documents exist as procedures or forms. Logs are maintained of both types of documents to prevent duplication, for reference and organization. Controlled documents must be issued and revised by use of the document control system.
  - 3.3.2 Controlled documents must be approved by the Quality Assurance Specialist and must have a unique identifying number and reflect revision and or effective date.
  - 3.3.3 All controlled copies distributed to laboratory employees will be stamped or have a watermark on the copy that states “**Controlled Copy**”
  - 3.3.4 The document control system is designed so only the current revision of each document is available for use. The document control system is a computerized system. The Quality Assurance Specialist maintains the control of documents on the computer network via password protection. Documents are available on the laboratory information management system as read only documents. Approved copies of controlled operating procedures are distributed throughout the laboratory. These documents are managed by the Quality Assurance Specialist. All analysts receive approved controlled copies of pertinent operating procedures that are stored in the Analytical Services Handbook. These operating procedures are managed by the Quality Assurance Specialist.
  - 3.3.5 When a new revision is issued the original signed hard copy is marked obsolete. The obsolete document is retained in the historic record to provide for reconstruction of laboratory activities. A new controlled copy of the analytical procedures will be placed in the area and the original copy will be destroyed. When a new revision of pertinent operating procedures has been issued, the Quality Assurance Specialist will collect any old version, and distribute the new controlled version of the operating procedure. The Quality Assurance Specialist will maintain the documentation needed for tracking of controlled copies of any operating procedures. Operating procedure that must be distributed to all Analytical Services Personnel will be performed during general meetings.
  - 3.3.6 Support activities are documented on forms and maintained as controlled documents. Support activities include Quality Assurance assignments such

---

Property of NEORSD. This is a date sensitive document and may not be current after 3/15/07.

**This is valid for use only if stamped “Controlled”.**

NEORSD Laboratory SOP No.: 5001	Revision: 2	Title: Quality Assurance Manual	Page 6 of 17
------------------------------------	----------------	------------------------------------	--------------

as reagent standardization, equipment maintenance and thermometer and balance calibrations.

- 3.3.7 Records will reflect the dates, times, observations and identify the individual making the entries and observations. All controlled documents and records are retained for five years unless alternative arrangements are made.

#### 4.0 Critical Staff Positions

- 4.1 The Manager of Analytical Services has authorized the establishment of the Quality Assurance System for the purpose of developing, monitoring and continually improving the quality control and documentation systems used within the laboratory. The Manager of Analytical Services will be informed of any non-compliance of the requirements of the Quality Assurance System. Enforcement of the requirements of the Quality Assurance System ultimately is the responsibility of the Manager of Analytical Services.
- 4.2 The Supervising Chemist of Analytical Services exercises actual day-to-day supervision of laboratory operations and reporting of results. These include:
- 4.2.1 Monitoring standards of performance in quality control and quality assurance.
- 4.2.2 Monitoring the validity of the analyses performed and data generated in the laboratory to assure reliable data.
- 4.2.3 Provide support to laboratory in the review and response to corrective actions.
- 4.2.4 Provide technical support for development and improvement of methodologies.
- 4.2.5 Provide the focal point for technical training of employees.
- 4.3 Quality Assurance Specialist manages the Quality Assurance System as follows:
- 4.3.1 Reviewing Standard Operating Procedures for analytical and Quality Assurance procedures, assuring conformance with document control procedures.
- 4.3.2 Planning and conducting, if necessary, the training of analysts in good laboratory practices and test method requirements.
- 4.3.3 The analysis of trends in the laboratory precision and accuracy that are demonstrated by the results of analysis of quality control samples.
- 4.3.4 Serving as a focal point for the reporting and disposition of non-conformances.
- 4.3.5 Coordinating responses to Corrective Action Requests.

NEORSD Laboratory SOP No.: 5001	Revision: 2	Title: Quality Assurance Manual	Page 7 of 17
------------------------------------	----------------	------------------------------------	--------------

- 4.3.6 Suggesting actions to be taken in order to correct a problem with an analytical procedure.
- 4.3.7 Informing the Manger of Analytical Services of out-of-control situations This includes the authority to require the laboratory to discontinue a procedure until corrective action brings the analysis back into control.
- 4.3.8 Maintaining the laboratory quality files and preparing routine quality control reports for review by the Manager and Superintendent.

4.4 Laboratory personnel are responsible to follow the Quality Assurance Manual and the related Standard Operating Procedures (SOP) as written. All laboratory personnel must adhere to issued quality control practices and procedures as stipulated by management and dictated by good laboratory practices. It is the responsibility of all laboratory personnel to advise management of observations that may result in the laboratory performance not attaining the objectives of the Quality Assurance System.

## 5.0 Traceability of Analytical Measurements

- 5.1 Analytical testing performed within the Analytical Services Department is performed on a batch basis. Each batch will be identified with a unique number for traceability. SOP-5010-1 Sample Batch Determination defines the procedures for creating preparation and analytical batches.
- 5.2 Samples are grouped based on matrix and time. Influent, effluent and pretreatment samples are all classified as a wastewater matrix. Sludge, grits and soils are classified as solid matrix. Each batch of samples is monitored by specified quality control activities.
- 5.3 Analytical measurements are recorded on controlled forms, or entered directly into the laboratory's Information Management System (LIMS) that collects all measurements and quality control activities associated with the batch. The date and time the analysis was performed, measurements obtained and calculations used to obtain the result are recorded.
- 5.4 Calibration curves are part of the document control system. Calibration dates are recorded thus analytical data can be traced to specific calibration curves.
- 5.5 Following data review the batch records become part of the record retention and filed for future retrieval. All records are stored on site for two years and then moved to off-site storage as defined in the District's record retention policy.
- 5.6 All recording and data corrections will be documented according to generally recognized good laboratory practices. These practices include recording in ink, dating, initialing entries and all correction will be made with a single-line through the old data and dated and initialed. The correction must not obscure the original entry.

## 6.0 Methods Performed

- 6.1 Analytical procedures are performed according to issued Standard Operating procedures derived from Standard Method for the Examination of Water and Wastewater, 20th ed and Methods for Chemical Analysis of Water and Wastes EPA 600/4-79-020

TEST NAME	TEST METHODOLOGY
pH 150.1	EPA 150.1
Bisulfite-B601-93	AWWA-B601-93
Hypochlorite-B300-64	AWWA-B300-64
Alkalinity-310.2	EPA-310.2
TSS-160.2 + TVSS-160.4	EPA -160.4
TS 160.3	EPA 160.3
TDS - 160.1	EPA 160.1
Alkalinity-310.1	EPA 310.1
TDS-160.1 +TDVS - 160.4	EPA 160.4
Chloride-325.3	EPA 325.3
TS 160.3 + TVS - 160.4	EPA 160.3 & 160.4
Sulfate 375.4	EPA 375.4
%TVS-SM 2540E	Standard Methods 20th Ed.
Hexavalent Chrome-SM 3500	Standard Methods 20th Ed.
Settleable Solids-160.5	EPA 160.5
%TS-SM 2540B	Standard Methods 20th Ed.
Phenol EPA 420.4	EPA 420.4
Oil & Grease -1664	EPA 1664
Fecal Coliform -9222 D.	Std Methods 19th 9222 D.
COD-EPA 410.4	EPA 410.4
E. Coli-9213 D	Std Methods, 20 ed. 9213 D
Total Coliform - 9222 B.	Std Methods 20th, 9222 B.
Fecal Strep - 9230 C.	Std Methods 20th, 9230 C
Total Chlorine Residual - 4500-CI G.	Std Method 20th, 4500-CI G.
Total Chlorine Residual -4500-CI F.	Std Methods 20th, 4500-CI F.
BOD-Total - 5 Day - 405.1	EPA 405.1
Conductivity SM 2510B	SM 2510B
Turbidity SM 2130B	SM 2130B
Fluoride-9214	EPA 9214
Phosphorus Total-365.2	EPA 365.2
Phosphorus-Soluble EPA 365.2	EPA 365.2
Phosphorus Ortho-365.2	EPA 365.2
Acidity-305.1	EPA 305.1
Field Conductance SM 2510A	SM 2510A
Cyanide Total-335.2	EPA 335.2
BOD-Carbonaceous - 5 DAY - 405.1	EPA-405.1
BOD-Soluble - 5 Day - 405.1	EPA-405.1

Property of NEORS. This is a date sensitive document and may not be current after 3/15/07.

**This is valid for use only if stamped "Controlled".**

TEST NAME	TEST METHODOLOGY
Cyanide WAD-335.2	EPA - 335.2
Nitrogen NH3-350.1	EPA-350.1
Nitrogen-NO3 + NO2 353.2	EPA 353.2
Nitrogen Nitrite354.1	EPA 354.1
Field D.O. SM 4500-0 G	SM 4500-0 G
Field Temperature EPA 170.1	EPA 170.1
Field Turbidity EPA 180.1	EPA 180.1
Field TDS EPA 160.1	EPA 160.1
GFAA As EPA 206.2	EPA 206.2
GFAA Ag-EPA 272.2	EPA 272.2
Cyanide- Amenable SM 4500 CN G	SM 4500 CN G
ICP-Daily Metals-200.7	EPA-200.7
ICP-Total Metals-200.7	EPA-200.7
ICP-Total Metals-6010B	EPA SW846
Cyanide 1677	EPA 1677
Mercury 245.2	EPA 245.2
Mercury 1631	EPA 1631
Nitrogen TKN-351.1	EPA-351.1
BOD-Winkler-Azide Modification Method	STM 20th edition 4500-O C.
TSS 160.2	EPA 160.2
Mercury 1631-Dissolved	EPA 1631
Oil & Grease -SGT HEM 1664	EPA 1664
GFAA Se EPA 270.2	EPA 270.2
Phosphorus-Soluble-Automated	EPA 365.2

## 7.0 Capabilities Review for Addition to Methods Performed

- 7.1 Tests may be added to methods performed after a review of resources and capabilities.
- 7.2 The Manager of Analytical Services is to review equipment, space and personnel resources to determine the capabilities of the laboratory to add methods.
- 7.3 The Quality Assurance Specialist is to review the method for proper quality control activities to be instituted for routine method performance evaluation.
- 7.4 The Quality Assurance Specialist and the Supervising Chemist are to review method validation requirements such as calibration requirements, method detection limit determination, training needed, accuracy and precision of the method for desired use of the data.
- 7.5 Following a determination that resources are satisfactory for successful performance, a test may be added. Standard Operating Procedures and method detection limit studies are to be added to the appropriate documentation.

## 8.0 Traceability of Calibration and Method Validation

### 8.1 Method Linearity Studies

- 8.1.1 Linearity studies are performed where appropriate to define the working range of the method and demonstrate that the response is linearly proportional to the analyte concentration.
- 8.1.2 Standards traceable to the National Institute of Standards are used for linearity studies. Vendor certification is retained for reference.
- 8.1.3 The correlation coefficient of the calibration curve must be 0.995 or better unless specified in individual Standard Operating Procedure. The linearity studies will also define the working range of the method.
- 8.1.4 The reporting level of a method must be included in the calibration curve, or must be verified each day of use with a control sample at the reporting level with 70%-130% recovery.

### 8.2 Method Specificity

- 8.2.1 Methods used at Northeast Ohio Regional Sewer District Laboratory are approved for monitoring and reporting to the Ohio Environmental Protection Agency.
- 8.2.2 Specificity is not monitored directly. Method bias is monitored by performing duplicate and spike analysis. Individual Standard Operating Procedures define the frequency and limits for variability and recoveries.

### 8.3 Method Accuracy

- 8.3.1 Method accuracy is monitored by the analysis of standards with each batch of samples. Individual Standard Operating Procedures define the acceptable performance.
- 8.3.2 The laboratory participates in proficiency test programs where sample are analyzed without prior knowledge of certified concentrations. Results are evaluated after the completion of the studies and any problem identified are addressed with corrective actions.
- 8.3.3 Controls charts are generated for long term tracking of analytical trends. Method specific quality control limits superseded system generate quality control limits unless specified in the specific standard operating procedure.

### 8.4 Method Precision

- 8.4.1 Method precision may be evaluated by the use of control samples of known concentration.
- 8.4.2 Sample matrix effects may create a positive bias or a negative bias. Method precision on specific samples is measured by the used of duplicates, spikes and spike duplicates. The accuracy is measured by the recovery and reproducibility of the recoveries.
- 8.4.3 Controls charts are generated for long term tracking of analytical trends. Method specific quality control limits superseded system generate quality control limits unless specified in the specific standard operating procedure.

## 8.5 Reagents and Standards

- 8.5.1 The type and purity of chemicals, reagents and solvents shall be dictated by the analytical method. Chemicals, reagents, and reference standards are purchased based upon the method specifications for each analysis regarding the purity of the material to be used in the analytical procedure. If a method does not specify the purity, then reagent grade (or better) chemicals, reagents and reference standards are purchased.
- 8.5.2 A reagent or chemical that does not meet the method specifications or is beyond the expiration date shall not be used.
- 8.5.3 The purity of reagents and solvents shall be monitored through reagent blanks that are analyzed with each set of samples.
- 8.5.4 Reference materials (standards) used to calibrate instruments or validate and monitor analytical methods must be National Institute of Standards Technology (NIST) traceable or equivalent.
- 8.5.5 When the laboratory receives a chemical the chemical is labeled with the following information:
- 8.5.5.1 Date of receipt
  - 8.5.5.2 Open date
  - 8.5.5.3 Expiration date
  - 8.5.5.4 Analyst initials
  - 8.5.5.5 Unique Trace ID
- 8.5.6 Reagents are prepared in a controlled room for most analytical procedures. All procedures are documented and reagents are labeled prior to use in the laboratory.
- 8.5.7
- 8.5.8 Buffers are discarded 6 months after being opened or after the manufacturer's expiration date. All other chemical reagents are maintained for six years after receipt, or according to manufacturer's expiration date, which ever comes first.

## 9.0 Sample Receipt and Handling

- 9.1 Samples analyzed at the within the Analytical Services Departments are not limited to pretreatment samples, plant influent, plant effluent, plant process control samples and receiving water from the treatment facility. Other sample types include sludge, soils, sediments and industrial wastes.
- 9.2 Samples are collected in designated containers, labeled with the date and delivered to the laboratory.
- 9.3 Chain of Custody procedures are defined in **SOP-5005-X Chain of Custody**.
- 9.4 Laboratory personnel track samples by the sampling location, sample ID and the sampling date. A unique sample identifier is assigned by the LIMS.

NEORS Laboratory SOP No.: 5001	Revision: 2	Title: Quality Assurance Manual	Page 12 of 17
-----------------------------------	----------------	------------------------------------	---------------

- 9.5 If analysis is delayed samples are preserved and/or stored in refrigeration units until processed. Individual standard operating procedures specify preservation and holding times. The hold time for grab samples starts from the time of sampling. The hold time for composite samples is measured from the time the sampling was completed.
- 9.6 Samples transferred to contract laboratories will be collected in bottles provided by the contract laboratory, and chain of custody forms. Sample storage will be performed at the instruction of the contracting laboratory.

## 10.0 Facility and Equipment

- 10.1 The laboratory facility is heated and cooled to maintain stable conditions throughout the year. Thermostats are programmable and provide control for laboratory and office spaces.
- 10.2 Hot and cold water are provided throughout the laboratory. Sinks are located throughout the laboratory to accommodate need. Laboratory water consists of a main Deionization water system and an ultra-pure DI water system utilized for trace metals and mercury analysis.
- 10.3 Laboratory areas are limited access areas. Safety design was given top priority in the facility. Emergency showers, eye wash stations, and fire extinguishers are located throughout the laboratory.
- 10.4 Exhaust hoods are located in the laboratory for use when fume or odors are of concern. General fume hoods and local dedicated venting systems are located throughout the laboratory to provide adequate space for safe handling of materials and prevent exposure.

## 11.0 Equipment Calibration and Maintenance

- 11.1 Preventive maintenance is a scheduled program of actions taken to maintain analytical instruments and equipment and is performed whether or not the performance of the equipment indicates a need for. This maintenance is designed to eliminate the downtime that might occur from instrument failure.
- 11.2 The Management Staff of Analytical Services is responsible for ensuring all preventive maintenance is performed according to laboratory procedures. Instrument specific-standard operating procedures detail the maintenance program that is in place at the Northeast Ohio Regional Sewer District Laboratory.
- 11.3 Analytical balances are serviced under contact with the manufacturer. The calibration records are maintained as specified in the document control system.
- 11.4 Thermometers are calibrated annually and traceable to the National Institute of Standard. The maintenance and calibration of thermometers is addressed in specific standard operating procedure(s).
- 11.5 Ovens, refrigerators and incubators are monitored daily for acceptable performance. Adjustments are made as needed to meet specifications. Equipment

NEORSD Laboratory SOP No.: 5001	Revision: 2	Title: Quality Assurance Manual	Page 13 of 17
------------------------------------	----------------	------------------------------------	---------------

needing continual adjustment is scheduled for servicing. The Quality Assurance Officer is responsible for reviewing records for performance compliance.

## 12.0 Data Verification and Internal Quality Control Activities

- 12.1 Each analyst is responsible for verifying the correctness of the data produced by any method. This verification includes reviewing the acceptability of produced data with respect to:
  - 12.1.1 Correctness of numerical input
  - 12.1.2 Numerical correctness of calculations
  - 12.1.3 Acceptability of quality assurance/quality control data
  - 12.1.4 Instrument operation according to method specifications (calibrations, performance checks, etc.)
  - 12.1.5 Documentation of dilutions, standard concentrations, etc.
- 12.2 The analyst is further required to perform data review for each batch of samples. This review includes the prescribed QC activities, calculations and supporting documentation as specified by internal procedures. If changes are made to data or reports the changes will be clearly marked to show that they are to replace previously submitted data.
- 12.3 Data will be archived to allow the easy retrieval for submittal when requested. Raw data shall be kept with batch records. All files will be archived for five years, unless previous arrangements have been made with the customer.
- 12.4 Method Blanks (MB) are processed and analyzed with each analytical batch. Method blanks are used in the evaluation of contamination control practices. Method blanks with values  $\pm$  the method reporting level are considered in control and related data can be reported without qualifiers. Data associated with methods blanks that do not meet acceptance criteria can only be reported as specified in specific procedures.
- 12.5 Initial Calibration Verification (ICV) standards are analyzed with each batch in order to evaluate stability of the calibration curve. This standard must be from an independent source.
- 12.6 Continuing Calibration Verification Standards (CCV) are analyzed with each batch in order to evaluate stability of the calibration curve. The acceptance criteria for each analytical method are specified in individual SOPs.
- 12.7 Laboratory Control Standard (LCS) is analyzed with each batch as required by standard operating procedures. An LCS is used to evaluate the methodology. If an LCS is in control it is considered evidence that the procedure was in control when performed. The limits for the control standard are specified in individual method SOPs. An LCS may not be available for some methods such as dissolved oxygen. Individual SOPs will specify activities to be performed.
- 12.8 Matrix Spikes and Matrix Spike Duplicates (MS/MSD) are analyzed in order to determine matrix effect and to evaluate precision. Alternatively, a duplicate and a spike, if appropriate, are performed per batch. The limits for spike recovery and

---

Property of NEORSD. This is a date sensitive document and may not be current after 3/15/07.

**This is valid for use only if stamped "Controlled".**

precision are dependent on the analyte and method. Individual SOPs specify limits and actions to be taken. Methods such as pH and suspended solids cannot be spiked. Individual SOPs will specify activities to be performed.

- 12.9 Raw analytical data are recorded, dated, initialed, or signed on analytical data sheets. Data from instrument output is dated and initialed. Analytical data sheets include provisions for the QC data, including calibration data, method blank data, duplicate data, spike data, and laboratory control standard data, as appropriate for each analytical procedure.
- 12.10 On-going quality control data generated is tracked per standard operating procedures. Generation of control charts is the responsibility of the analysts. Review of the control charts is the responsibility of the Supervising Chemist. When anomalies or out of control conditions arise, the Quality Control Specialist is contacted to initiate required corrective action as prescribed in individual standard operating procedures. Control limits are used for trend analysis of data. Method control limits supersede laboratory control limits for data validation.
- 12.11 Reagents and chemicals used are of the purity specified in the procedure. Method blanks are carried through analysis procedures as an evaluation of contamination and stability of reagents.

### 13.0 Corrective Actions

- 13.1 The Quality Assurance Specialist is responsible for the administration of the corrective action system. The system is to be used to assign responsibility, document action taken and to track activities in order to ensure completion of assignments and meeting of deadlines.
- 13.2 Method specific corrective action is specified in individual procedural SOPs. Method specific corrective actions mainly address quality control activities that do not meet acceptance criteria specified in the individual standard operating procedures. If these actions fail to correct the observed non-compliance then the corrective action system is to be followed.
- 13.3 The corrective action system can be used to respond to findings of internal, customer or regulatory audits. The corrective action system can be used to respond to adverse events in the processing of materials. Corrective action may be used to respond to customer complaints. The corrective action system is used whenever departures from documented policies or procedures occur. Changes in the Quality System are documented using the Corrective Action System.
- 13.4 Completed corrective action are documented and maintained by the Quality Assurance Specialist. Records are maintained with the other controlled documents for 5 years.

### 14.0 Control of Data Generated from non-Conforming Activities

- 14.1 The Quality Assurance Specialist is responsible for responding to activities (i.e. calibration, analysis) that are non-conforming to policy and specifications. The Quality Assurance Specialist is to be responsible for the gathering of information

needed to assess the impact of the non-conformance on data and laboratory performance.

- 14.2 The Manager of Analytical Services, the Quality Assurance Specialist and addition individuals at the discretion of the Superintendent are to evaluate the significance of the non-conformance and the corrective action.
- 14.3 The review must include if client notification is necessary, if work must be recalled and when work can resume.
- 14.4 The response to the non-conformance is to be documented and handled through the corrective action system.

## 15.0 Complaints

- 15.1 Complaints are to be directed to the Manager of Analytical Services or the Quality Assurance Specialist. The Manager of Analytical Services or the Quality Assurance Specialist will determine if the complaint merits a response.
- 15.2 When a complaint raises doubt concerning the laboratory's compliance with the laboratory's policies or procedures or with the quality of the laboratory's results, those areas involved will be audited.
- 15.3 When the complaint meets the criteria above the corrective action system will be used to initiate, track and respond to the complaint and its findings.

## 16.0 Confidentiality and Public Access

- 16.1 Northeast Ohio Regional Sewer District Laboratory is part of a public entity and as such the information generation by the laboratory may be public information.
- 16.2 All external requests for laboratory data from agencies not currently working with the District must be directed to the Districts Legal Department. All other request can be directed to the Manager of Analytical Services for resolution.

## 17.0 Data Review and Audits

- 17.1 The Quality Assurance Specialist will be responsible for audits. Northeast Ohio Regional Sewer District Laboratory personnel may perform audits or an outside auditor may be contracted to perform audits.
- 17.2 The audits are to verify if the laboratory is in compliance with the requirements of the laboratory's quality system as defined in the Quality Manual and standard operating procedures. The results of the audits are considered internal information and not released during audits or inspections.
- 17.3 Response to findings during an audit is handled through the Corrective Action System.

## 18.0 Training and Demonstration of Capability

- 18.1 The Quality Assurance Specialist is responsible for an annual review of the performance records of the laboratory personnel.

- 18.2 A review of the performance on required quality control activities on each analytical procedure will be used to evaluate an analyst's capability. If the last four laboratory control samples are in control this will be considered sufficient evidence that the analyst is capable of performing the procedure.
- 18.3 If one the last four laboratory control samples do not meet the method acceptance criteria then training may be required by the Quality Assurance Specialist. Required training is to be documented as corrective action.
- 18.4 Demonstration of capability to add a new method will be accomplished by analyzing a laboratory control sample four times. The average recovery and standard deviation will be calculated and if the laboratory values are within the published limits the procedure can be performed in the laboratory. Corrective action must be performed and the analysis repeated until it can be demonstrated that the laboratory can generate the expected performance data.

## 19.0 Ethical Conduct

- 19.1 It is the policy of Northeast Ohio Regional Sewer District Laboratory to perform our duties in a manner that will reflex our commitment to highest possible ethical standard. We will perform and report our work in a manner that accurately reflects the results obtained in the laboratory.
- 19.2 Management will provide and document training on the ethical conduct expected in the performance of laboratory duties. Ethics training includes examples of unacceptable conduct, how to report observed misconduct and possible penalties.
- 19.3 It is the responsibility of every employee to report only his or her own data and to report it accurately. Every employee has the responsibility to notify management when they become aware of unethical conduct by another employee.

## 20.0 Reporting of Data

- 20.1 Northeast Ohio Regional Sewer District Laboratory provides service to the Director of Operations and Maintenance for regulatory reporting, and facility operation. Report services for the pretreatment and stream monitoring programs are provided to the Manager of Water Quality and Industrial Surveillance. Reports will be in a format that will allow the Manager of Water Quality and Industrial Surveillance to meet business objectives. Release of information to a third party is at the instruction of the Superintendent of Environmental Services and the District's Legal Department.
- 20.2 Reports will clearly reflect the sample identification; date sampled, results obtained and reporting units.

## 21.0 Revision History

- 21.1 Signature page changed.
- 21.2 Modified Section 2.0 Organization and Management Structure: removed the reference to sample collection was removed for the Biologist, Chemist and Wastewater Analyst II. (MEC 3/14/2007)

- 21.3 Modified Section 2.0 Organization and Management Structure: separated the Wastewater I and Wastewater II responsibilities. (MEC 3/14/2007)
- 21.4 Added section 3.3.3: All controlled copies distributed to laboratory employees will be stamped or have a watermark on the copy that states "Controlled Copy"
- 21.5 Add reference to EPA Method Total Metals by 6010B(MEC 3/14/2007)
- 21.6 Added Section 8.3.3 Controls charts are generated for long term tracking of analytical trends. Method specific quality control limits superseded system generate quality control limits unless specified in the specific standard operating procedure. (MEC 3/14/2007)
- 21.7 Added Section 8.4.3 Controls charts are generated for long term tracking of analytical trends. Method specific quality control limits superseded system generate quality control limits unless specified in the specific standard operating procedure. (MEC 3/14/2007)
- 21.8 Added to section 10.2 reference to the DI water system in the clean room area. (MEC 3/14/2007)

## **Appendix H**



Northeast Ohio Regional  
**Sewer District**

*Protecting Your Health and Environment*

---

**Analytical Services**  
**4747 East 49<sup>th</sup>. Street**  
**Cuyahoga Hts., OH 44125**

---

Title  
**Bacterial Counting Methods**  
***SOP-2016-00***

***Effective Date: 07/12/2005***

---

Approvals

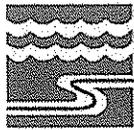
---

Prepared By: Eva Hatvani Date: 07/10/2005

Approved By Supervisor: Mark Citriglia Date: 07/10/2005

Reviewed By QA Specialist: Carol Turner Date: 07/12/2005

Approved By Manager: Mark Citriglia Date: 07/12/2005



**Analytical Services**  
**4747 East 49<sup>th</sup>. Street**  
**Cuyahoga Hts., OH 44125**

*Table of Contents*

1.0 SCOPE AND APPLICATION ..... 1

2.0 SUMMARY OF METHOD ..... 1

3.0 DEFINITIONS..... 1

4.0 INTERFERENCES ..... 2

5.0 SAFETY ..... 2

6.0 EQUIPMENT AND SUPPLIES ..... 2

7.0 COUNTING COLONIES ..... 2

8.0 MANUALLY COUNTING..... 3

9.0 QUALITY CONTROL..... 5

10.0 COUNTING RULES ..... 5

    10.1 Blanks ..... 5

    10.2 Samples..... 5

    10.3 Example Calculations ..... 6

11.0 METHOD PERFORMANCE ..... 7

12.0 DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES 7

13.0 METHOD SPECIFIC CORRECTIVE ACTIONS ..... 7

14.0 CONTINGENCIES FOR OUT-OF-CONTROL/UNACCEPTABLE DATA..... 8

15.0 REFERENCES ..... 8

16.0 REVISION HISTORY ..... 8

<b>Title:</b> SOP Number: 2016	<b>Revision</b> <b>00</b>	<b>Title:</b> Bacterial Counting Methods	Page 1 of 8
--------------------------------------	------------------------------	---	-------------

## 1.0 Scope and Application

- 1.1. This SOP describes the procedure for the determination of the concentration of bacteria based on USEPA methods.
- 1.2. This SOP is for counting Total coliform, Fecal coliform, *E. coli* and Fecal Streptococcus bacteria.
- 1.3. This SOP describes the method for using the aColyte Automated Colony Counter.
- 1.4. The ideal counting range for the Total coliform analysis is 20 to 80 colonies per plate.
- 1.5. The ideal counting range for the Fecal coliform analysis is 20 to 60 colonies per plate.
- 1.6. The ideal counting range for the *E. coli* analysis is 20 to 80 colonies per plate.
- 1.7. The ideal counting range for the fecal streptococcus analysis is 20 to 100 colonies per plate.

## 2.0 Summary of Method

- 2.1 After an appropriate volume of a water sample or its dilution is passed through a 0.45 micron membrane filter and is transferred to the appropriate selective media, the sample is incubated according to methodology. The plates that are in the ideal counting range are counted manually or placed onto the aColyte Automated Colony Counter.

## 3.0 Definitions

- 3.1 Method Blank – A sample containing no detectable analyte processed simultaneously with and under the same conditions as the samples.
- 3.2 Fecal Coliform (FC) Bacteria – Part of the total coliform group. Aerobic and many facultative anaerobic, gram-negative, non-spore forming, rod shaped bacteria that develop a blue colony within 24 hours at a temperature of 44.5°C on an M-FC medium.
- 3.3 *E. coli* (EC) Bacteria – Part of the fecal coliform group. *E. coli* are defined based on the growth under specified conditions and media. The method must be performed under these specified conditions in order to properly enumerate the coliform bacteria present.
- 3.4 Fecal Streptococcus (FS) Bacteria – Include the following species, *S. farcalis*, *S. faecalis subsp. liquefaciens*, *S. faecalis subsp. zymogenes*, *S. faecium*, *S. bovis* and *S. equines* Grow on KF Streptococcus agar and produce red and pink colonies..
- 3.5 NEORSD – Northeast Regional Sewer District May - This action, activity, or procedural step is neither required nor prohibited.

SOP Number: 2016	Revision 00	Title: Bacterial Counting Methods	Page 2 of 8
---------------------	----------------	--------------------------------------	-------------

- 3.6 May not – This action, activity, or procedural step is prohibited.
- 3.7 Must - This action, activity, or procedural step is required.
- 3.8 Shall – This action, activity or procedural step is required.
- 3.9 Should – This action, activity or procedural step is suggested but not required.
- 3.10 Total Coliform (TC) – All of the aerobic and facultative anaerobix, gram-negative, nonspore forming, rod shaped bacteria that ferment lactose in 24-48 hours at 35 C. The definition includes the genera: *E. coli*, *Citrobacter*, *Enterobacter* and *Klebsiella*.
- 3.11 CHP –Chemical Hygiene Plan

## 4.0 Interferences

- 4.1 The aColyte Automated Counter counts colonies based on a dark versus light basis. This may count colonies may be non-coliform colonies, algal filaments or detritus.

## 5.0 Safety

- 5.1 Proper protective equipment is to be worn at all times.
  - 5.1.1 Lab coat
  - 5.1.2 Rubber Gloves
  - 5.1.3 Safety Glasses
- 5.2 Follow the approved chemical hygiene plan.

## 6.0 Equipment and Supplies

- 6.1 Microscope and Light Source, Capable of 10-15X wide field magnification
- 6.2 Hand Tally or Counter
- 6.3 aColyte Automated Colony Counter
- 6.4 Computer

## 7.0 Counting Colonies

- 7.1 The bacterial plates are removed from their source of incubation.
- 7.2 The plates are placed in order of dilution plated.
- 7.3 The plates are examined one by one, starting with the plate plate with the lowest number of colonies formed.

SOP Number: 2016	Revision 00	Title: Bacterial Counting Methods	Page 3 of 8
---------------------	----------------	--------------------------------------	-------------

- 7.4 The plates are either counted manually using a microscope and a hand tally or placed onto the aColyte Automated Colony Counter.
- 7.5 Refer to Section 10 for specific counting rules.

## 8.0 Manually Counting

- 8.1 Turn on the light source of the microscope.
- 8.2 The plate to be counted is placed onto the stage of the microscope.
- 8.3 The hand tally is pressed once per colony as they are counted in the view of the microscope.
- 8.4 The colony count is manually entered into the workbook.
- 8.5 The colonies/100 mL are calculated using the counting rules in Section 10.
- 8.6 Starting up the Software
- 8.6.1 To start the aColyte software, double-click on the aColyte icon on the Windows desktop.
- 8.6.2 When the window opens, the aColyte assistant will appear at the top of the left-hand corner of the screen. Press the “F1” key if you would like the tutorial. To hide the assistant, click on the a in the aColyte panel.
- 8.7 Creating Batches
- 8.7.1 Batches are created by pressing the “**New Batch**” button.
- 8.7.2 Enter a name for your batch. Each batch name must be unique.
- 8.7.3 Enter a “**Comment**” to document the Batch.
- 8.7.4 Select a “**Configuration**” from the drop-down menu.
- 8.7.5 Choose a *default* “**Dilution**” to be used in calculating results for the Batch. You will be able to enter a different dilution for individual results if required.
- 8.7.6 Choose whether to “**Save Image**” of the plate with each result.
- 8.7.7 Enter the “**Dish Diameter**”.
- 8.7.8 Enter the “**Sample Volume**”.
- 8.7.9 Choose “**Pour Plate**” type.
- 8.7.10 Choose *default* frame shape as “**Full Circle**”.
- 8.7.11 Choose “**Dark**” against a light background for “**Colony Appearance**”.
- 8.7.12 Choose the *default* “**Exposure**”. You can choose values between 0 and 100. You will be able to adjust the exposure for each plate if required.
- 8.7.13 Choose the *default* “**Sensitivity**”. You can choose values between 0 and 100. You will be able to adjust the exposure for each plate if required.
- 8.7.14 Enter a default size (as a percentage of the dish area) for the counting frame in the “**Area Counted**” box. Only colonies within the counting frame will be counted. This can be changed for each plate.
- 8.7.15 Press the “**Save Batch**” button to save the new Batch. This will return you to the main screen.

SOP Number: 2016	Revision 00	Title: Bacterial Counting Methods	Page 4 of 8
---------------------	----------------	--------------------------------------	-------------

8.7.16 The name of the batch will appear in the upper right-hand corner of the screen to show it is the current batch.

## 8.8 Counting Colonies

8.8.1 There are two options for counting colonies, the “**Supercount**” and the “**Click ‘n’ Count**” Methods.

### 8.8.1.1 “**Supercount**” Method

8.8.1.1.1 Turn on light box of unit.

8.8.1.1.2 Place the plate on the plate holder in the aColyte System box. An image of the plate will appear in the center of the main screen.

8.8.1.1.3 If needed, change the size and position of the frame. To re-position, click and drag the frame to re-size click on the circle to select it, and drag the drag handles.

8.8.1.1.4 Select the proper dilution of the sample from the drop-down in the Dilution box.

8.8.1.1.5 If necessary adjust the lighting. There are two lamps, one on the top and one on the bottom. The lamp button is pressed until the optimum viewing is achieved.

8.8.1.1.6 If necessary you can slide the colony detection sensitivity and controls to optimize the image appearance.

8.8.1.1.7 Press the “**Supercount**” button. The count will appear in the panel below the Dilution button and the count will appear in the panel below the count panel.

### 8.8.1.2 “**Click ‘n’ Count**” Method

8.8.1.2.1 Follow procedures in 9.3.1.1.1 – 0.3.1.1.6.

8.8.1.2.2 Press the “**Click ‘n’ Count**” button which appears as a hand.

8.8.1.2.3 Left click on the colonies to count them. They will be marked as they are counted.

8.8.1.2.4 To remove a counted colony from the count, move the pointer over the counted colony and hold the shift key down while clicking on the colony.

8.8.1.2.5 Press the count Completed button which appears as a hand.

8.8.1.2.6 The result will be shown in the panels below the dilution button.

### 8.8.1.3 Viewing, printing, exporting and loading the image for results.

8.8.1.3.1 Press the “**Display Batch Results**” button. This will display the results on the screen.

8.8.1.3.2 You can select options in the Results screen to print or export the file.

SOP Number: 2016	Revision 00	Title: Bacterial Counting Methods	Page 5 of 8
---------------------	----------------	--------------------------------------	-------------

8.8.1.3.3 If “**Save Image**” was set when the batch was created, you can reload the image for a result.

8.8.1.3.3.1 To load the saved image for a specific result, click on the result in the Results screen table to select it.

8.8.1.3.3.2 Press the “**Load Result**” button. The results screen will close and the image will be loaded into the main screen.

## 9.0 Quality Control

- 9.1 Periodically confirm the effectiveness of the aColyte system by comparing manually counted plates with those counted on the aColyte Automated Counter.
- 9.2 Maintain a logbook for the comparison.

## 10.0 Counting Rules

### 10.1 Blanks

- 10.1.1 Blanks are counted first.
- 10.1.2 Blanks should have no colony counts on them. If they do, refer to Section 12 for data assessment and acceptance criteria. Investigate sources of contamination and apply corrective action for subsequent batches.

### 10.2 Samples

- 10.1.3 Refer to Section 10.3 for specific examples.
- 10.1.4 Samples are counted in the order of plating.
- 10.1.5 Select plates with colony counts in the ideal range of the method. Apply the formula in Section 10.2.3 for calculating. If colony counts do not fall into the ideal range, apply the counting rules below in Sections 10.2.6-10.2.9.
- 10.1.6 Formula for calculating colonies/100 mL

$$\text{Colonies/100 mL} = \frac{(\text{Colonies counted})(100)}{\text{mL of Sample Filtered}}$$

- 10.1.7 If confluent growth occurs and colonies are not discrete, report result as an estimate and note “confluent growth.”
- 10.1.8 If the total number of acceptable bacterial colonies exceeds 200 per plate, report results as >200 for the three highest dilutions plated and count the plate of the lowest dilution. Calculate the number of colonies /100 mL on the lowest dilution.
- 10.1.9 If all of the plates exhibit counts that are below the acceptable range, the colonies are totaled for all of the plates from the lowest plate showing a

count and all higher dilution plates. This total is then divided by the total volume of the plates that are used.

10.1.10 If all colony counts are zero, report the value as less than the MDL based on the highest dilution.

10.1.11 Mark the result as EC (Estimated Count) if not within the ideal range for WQIS samples only.

### 10.3 Example Calculations

	Volume filtered	Count per plate	Number/100 mL
1.	100	<1	<u>For all Bacterial Species</u>
	50	<1	<1 or AA col/100 mL
	20	<1	
	5	<1	
2.	50	<1	<u>For all Bacterial Species</u>
	20	<1	<2 col/100 mL
	5	<1	
	1	<1	
3.	50	<1	<u>For all Bacterial Species</u>
	20	<1	<2 col/100 mL
	5	<1	
	1	<1	
4.	100	100	<u>For all Bacterial Species</u>
	50	55	$77/70 \times 100 = 110$ col/100 mL
	20	22	
	5	4	
5.	100	>200	<u>For all Bacterial Species</u>
	50	>200	$251/5 \times 100 = 5020$ col/ 100 mL
	20	>200	Mark as EC for WQIS Samples
	5	>200	(Actual Count = 251)
6.	50	99	FS $234/90 \times 100 = 260$ col/100 mL
	25	68	TC/EC $135/40 \times 100 = 338$ col/ mL
	10	45	FC $67/15 \times 100 = 447$ col/mL
	5	22	
7.	100	5	<u>For all Bacterial Species</u>
	50	0	$6/170 \times 100 = 4$ col/100 mL

SOP Number: 2016	Revision 00	Title: Bacterial Counting Methods	Page 7 of 8
---------------------	----------------	--------------------------------------	-------------

	20	1	
	5	0	
8.	100	15	<u>For all Bacterial Species</u>
	50	6	21/170 X 100 = 12 col/100 mL
	20	0	
	5	0	
9.	100	5	<u>For all Bacterial Species - Duplicate</u>
	100	3	8/200 X 100 = 4 col/100 mL
	50	0	
	50	0	

## 11.0 Method Performance

- 11.1 The detection limit is dependent upon the volume of water filtered. One colony unit can be detected.
- 11.2 The ideal counting range for Total coliform is 20 to 80 colonies.
- 11.3 The ideal counting range for Fecal coliform is 20 to 60 colonies.
- 11.4 The ideal counting range for *E. coli* is 20 to 80 colonies.
- 11.5 The ideal counting range for Fecal streptococcus is 20 to 100 colonies.

## 12.0 Data Assessment and Acceptance Criteria for Quality Control Measures

### 12.1 Method Blank

- 12.1.1 If the method blank has 0 colonies per 100 mL results can be reported.
- 12.1.2 If the method blank has coliform colonies present results can be reported for membrane filters that have no coliform colonies present.
- 12.1.3 If the method blank has coliform colonies present results can be reported for samples that are 10 times the method blank value or higher and are below regulatory limit or permit levels.
- 12.1.4 If the method blank has coliform colonies present, sample results greater than 0 and less than 10 times the blank may be reported as estimated values.

## 13.0 Method Specific Corrective Actions

- 13.1 If the method blank does not meet requirements the glassware may be contaminated and in need of cleaning and sterilization.
- 13.2 Cross-contamination may be controlled by grouping samples and performing analysis on low level samples first.

SOP Number: 2016	Revision 00	Title: Bacterial Counting Methods	Page 8 of 8
---------------------	----------------	--------------------------------------	-------------

## 14.0 Contingencies for Out-Of-Control/Unacceptable Data

14.1 If colony counts do not meet requirements data may be reported as estimates if the data quality objectives allow.

## 15.0 References

- 15.1 *Standard Methods for the Examination of Water and Wastewater*, 18<sup>th</sup> edition, 1992.  
15.2 *Microbiological Methods for Monitoring the Environment Water and Wastes*, EPA-600/8-78-017, December 1978, USEPA, Cincinnati, OH.

## 16.0 Revision History

16.1 No revisions.

## **Appendix I**



Northeast Ohio Regional  
**Sewer District**

*Protecting Your Health and Environment*

**Analytical Services**  
**4747 East 49<sup>th</sup>. Street**  
**Cuyahoga Hts., OH 44125**

---

Title

**Determination of *E. Coli* and Enterotube Verification**  
***SOP-2014-01***

***Effective Date: 04/25/2006***

---

Approvals

---

Prepared By: Eva Hatvani Date: 07/12/2005

Revised By: Eva Hatvani Date: 04/04/2006

Reviewed By QA Specialist: Carol Turner Date: 04/25/2006

Approved By Manager: Mark Citriglia Date: 04/25/2006



**Analytical Services**  
**4747 East 49<sup>th</sup>. Street**  
**Cuyahoga Hts., OH 44125**

*Table of Contents*

1.0	SCOPE AND APPLICATION .....	1
2.0	SUMMARY OF METHOD .....	1
3.0	DEFINITIONS.....	1
4.0	INTERFERENCES .....	2
5.0	SAFETY .....	2
6.0	EQUIPMENT AND SUPPLIES .....	2
7.0	REAGENTS AND STANDARDS.....	3
8.0	SAMPLE COLLECTION, PRESERVATION, STORAGE, AND SHIPMENT.....	5
9.0	QUALITY CONTROL.....	5
10.0	PROCEDURE.....	6
11.0	PROCEDURE.....	7
12.0	CALCULATIONS .....	8
13.0	METHOD PERFORMANCE.....	8
14.0	<i>E. COLI</i> VERIFICATION USING THE BBL ENTEROTUBE II .....	8
15.0	DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES.....	9
16.0	METHOD SPECIFIC CORRECTIVE ACTIONS .....	9
17.0	CONTINGENCIES FOR OUT-OF-CONTROL/UNACCEPTABLE DATA .....	9
18.0	REFERENCES .....	10
19.0	REVISION HISTORY.....	10

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 1 of 10
---------------------	----------------	--	--------------

## 1.0 Scope and Application

- 1.1. This SOP describes the method for the determination of *Escherichia coli*.
- 1.2. This SOP describes the method for membrane filtration and Enterotube verification.
- 1.3. This method is applicable to saline waters, treated wastewater, stream and surface water, and drinking water.
- 1.4. Methods are based on the growth under specified conditions in order to properly enumerate the coliform bacteria present.
- 1.5. The ideal counting range for this analysis is 20 to 80 colonies per plate.

## 2.0 Summary of Method

- 2.1 An appropriate volume of a water sample or its dilution is passed through a 0.45 micron membrane filter that retains bacteria that is present in the sample
- 2.2 The filter is transferred to a culture dish containing selective media and incubated at  $35 \pm 0.5^{\circ}\text{C}$  for 2 hours to rejuvenate injured bacteria.
- 2.3 Plates are transferred to be incubated at  $44.5 \pm 0.2^{\circ}\text{C}$  for 22 hours.
- 2.4 The mTec method membrane filters are transferred to pads with urea substrate for approximately 15 minutes. The modified mTec method does not require this step.
- 2.5 Following incubation the plate is examined for colonies that are yellow or yellow-brown under fluorescent light for the mTec method and deep purple for the modified mTec method.
- 2.6 *E. coli* colonies can be verified as *E. coli* using the Enterotubes.

## 3.0 Definitions

- 3.1 Method Blank – A sample containing no detectable analyte processed simultaneously with and under the same conditions as the samples through all steps of the analytical procedure.
- 3.2 Batch – A batch will consist of a maximum of 20 samples.
- 3.3 *E. coli* - is a type of fecal coliform bacteria commonly found in the intestines of animals and humans. The presence of *E. coli* in water is a strong indicator of sewage contamination. Sewage may contain many types of disease-causing organism.

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 2 of 10
---------------------	----------------	--	--------------

- 3.4 CHP - Chemical Hygiene Plan
- 3.5 May - This action, activity, or procedural step is neither required nor prohibited.
- 3.6 May not – This action, activity, or procedural step is prohibited.
- 3.7 Must - This action, activity, or procedural step is required.
- 3.8 Shall – This action, activity or procedural step is required.
- 3.9 MDL – Method Detection Limits as defined by 40 CFR Part 136.

## 4.0 Interferences

- 4.1 Using the membrane filtration method, suspended material may inhibit the filtration of sample volumes sufficient to produce significant results
- 4.2 Low estimates may be caused by the presence of high numbers of non-coliform bacteria or toxic substances.

## 5.0 Safety

- 5.1 Proper protective equipment is to be worn at all times.
  - 5.1.1 Lab coat
  - 5.1.2 Rubber Gloves
  - 5.1.3 Safety Glasses
- 5.2 Follow the approved CHP.
- 5.3 High temperatures and steam are used for sterilization; care must be taken to avoid burns.

## 6.0 Equipment and Supplies

- 6.1 Incubator, Capable of maintaining temperature of  $35.0 \pm 0.5^{\circ}\text{C}$
- 6.2 Incubator Bath, Capable of maintaining temperature of  $44.5 \pm 0.2^{\circ}\text{C}$
- 6.3 Microscope and Light Source, Capable of 10-20X wide field magnification
- 6.4 Hand Tally or Counter
- 6.5 aColyte Automated Colony Counter
- 6.6 Filtration Units –Autoclavable and designed to hold filters used for filtration of samples. (Like Pall 4238 can be used.)
- 6.7 Graduated Cylinder – Autoclavable in various sizes

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 3 of 10
---------------------	----------------	--	--------------

- 6.8 Vacuum source or pump
- 6.9 Safety Trap Flask (between filter flask and the vacuum source)
- 6.10 Forceps – Smooth Tipped, without corrugations on the inner side of the tip
- 6.11 Flame Source
- 6.12 Erlenmeyer Flasks, various sizes
- 6.13 Plasti Bottles – Autoclavable in various sizes
- 6.14 Sterile Disposable Mohr Pipets, in various sizes
- 6.15 Sterile Plastic Petri Plates, Not Padded (Like Millipore PDF0047SO can be used.)
- 6.16 Absorbent Pads
- 6.17 Membrane Filters, Preferably Pre-Sterilized, 47 mm in Diameter, 0.45 + 0.2 micron pore size. (like Millipore EZHAWG474 can be used.)
- 6.18 Filtering Flask, 4-Liter
- 6.19 Waterproof Plastic Bags
- 6.20 Autoclave, Capable of maintaining a temperature approximately 121°C and approximately 15 psi.
- 6.21 Hot Plate
- 6.22 Aluminum Foil
- 6.23 Oven, Capable of maintaining 180 +/- 2 °C

## 7.0 Reagents and Standards

- 7.1 Deionized Water
- 7.2 95% Methanol, Ethanol, or propanol (for sterilizing forceps)
- 7.3 Sodium Hydroxide Solution, 1N
  - 7.3.1 Dissolve 40 g of sodium hydroxide (NaOH) in 600 mL of deionized water. Caution: solution will be hot. Cool and dilute to 1-L with deionized water.
- 7.4 Stock Phosphate Buffer Solution
  - 7.4.1 Dissolve 34.0 g of potassium dihydrogen phosphate,  $\text{KH}_2\text{PO}_4$ , in 500 mL of deionized water in a volumetric flask. Adjust the pH to 7.2 + 0.5 with 1N NaOH then dilute to 1-L with deionized water.
- 7.5 Magnesium Chloride Solution

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 4 of 10
---------------------	----------------	--	--------------

7.5.1 Dissolve 81.1 g of magnesium chloride hexahydrate,  $MgCl_2 \cdot 6H_2O$ , or 38.0 g of magnesium chloride,  $MgCl_2$  in 500 mL of deionized water in a volumetric flask. Dilute to 1-L with deionized water.

7.6 Phosphate Buffered Dilution Water

7.6.1 Add 1.25 mL of Stock Phosphate Buffer Solution (prepared in Section 7.1.1) and 5.0 mL of Magnesium Chloride Solution (prepared in Section 7.2.1) to 500 mL of deionized water in a volumetric flask. Dilute to 1-L with deionized water.

7.6.2 Dispense into 1 L wash bottles. Without delay, transfer the bottles containing the dilution water to the autoclave and sterilize at appropriate temperature, pressure for 15-20 minutes.

7.6.3 Dilution water may be stored for 6 months as long as there is no evidence of bacterial growth (turbidity).

7.7 Dilution Bottles, marked at 99 mL volume, (Commercially purchased sterile dilution water, 99 mL like HACH Cat. 14305-98 can be used.

7.8 Sodium Thiosulfate Solution, 10%

7.8.1 Dissolve 0.1 g of sodium thiosulfate ( $Na_2S_2O_3$ ) in 500 mL of deionized water. Dilute to 1 liter with deionized water.

7.9 EDTA Solution, 15%

7.9.1 Dissolve 0.375 g of EDTA, disodium salt in 500 mL of deionized water. Dilute to 1 liter with deionized water. Adjust the pH to 6.5 +/- 0.2 before using.

7.10 Urea Substrate

7.10.1 Dissolve 2 g of urea, 10 mg of phenol red in 100 mL of deionized water. Adjust to pH 3.0 – 4.0. Store at 4°C for one week.

7.11 Modified m-Tec Agar

7.11.1 Dissolve 45.6 g of modified m-Tec Agar in 1L of DI water. Mix thoroughly. Heat with frequent agitation and boil for one minute. Cover with aluminum foil and autoclave at appropriate temperature and pressure for 15-20 minutes. Cool to 45-50°C. Add sterile NaOH to achieve a pH of 7.3±0.2 SU. Pipette 5ml into a petri dish. Allow to cool. Cover and store upside down at 4 +/- 2°C.

7.12 m-Tec Agar

7.12.1 Dissolve 45.3 g of m-Tec Agar in 1L of DI water. Mix thoroughly. Heat with frequent agitation and boil for one minute. Cover with aluminum foil and autoclave at the appropriate temperature and pressure for 15-20 minutes. Pipette approximately 5ml of the hot solution into petri dishes. Allow to cool. Cover and store upside down at 4 +/- 2°C. Good for 2 weeks.

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 5 of 10
---------------------	----------------	--	--------------

### 7.13 BBL Enterotube II – for the verification of E. coli.

#### 7.13.1 Kovacs' Reagent

- 7.13.1.1 Dissolve 5 g p-dimethylaminiobenzaldehyde in 75 ml of amyl or isoamyl alcohol and then slowly add 25 ml concentrated hydrochloric acid.

## 8.0 Sample Collection, Preservation, Storage, and Shipment

### 8.1 Preparation of Sample Bottles

8.1.1 Treated Bottles: Use for samples when residual chlorine may be present (ex. plant effluents, drinking waters)

8.1.2 Untreated Bottles: Use for samples where no residual chlorine is expected (ex. lake and stream waters)

- 8.2 Collect samples that are representative of the water being tested, flush or disinfect sample ports, and use the aseptic technique to avoid sample contamination.
- 8.3 When the sample is collected leave ample air space in the bottle to allow for mixing and shaking prior to analysis.
- 8.4 Keep the sample bottle closed until it is to be filled. Remove the cap as a unit.
- 8.5 Fill the container without rinsing, replace the cap, and label the sample container. Information should include the location, date, the sampler, the time of sampling and any unusual observations.
- 8.6 All samples must be kept at 4 +/- 2°C. A cooler with refrigerant is to be used in transport of the samples.
- 8.7 All samples must be analyzed within 6 hours from the time of collection.

## 9.0 Quality Control

- 9.1 Confirm the effectiveness of the sterilization and selectivity of the method on each batch.
- 9.1.1 Analyze a blank at the beginning, middle, and end of plating.
- 9.2 Analyze one duplicate per batch of samples to confirm precision of the method.
- 9.3 Verify each lot of mTec media using a pure culture of *E. coli* bacteria.
- 9.4 *E. coli* should be verified using the BBL Enterotubes once a week or as needed for verification of suspect colonies.
- 9.5 Maintain logbooks for temperatures (incubator oven, water bath, and refrigerator), media preparation, and sterilization of equipment and solutions and pH meter calibration.

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 6 of 10
---------------------	----------------	--	--------------

## 10.0 Procedure

### 10.1 Using the Autoclave – Moist Sterilization

*NOTE: Attaining proper temperature, pressure and time of sterilization will be confirmed with the use of heat indicating tape or heat indicating strips.*

- 10.1.1 Place a piece of indicating tape onto each piece of equipment to be sterilized. Mark the date on the tape. Record the items in the logbook for sterilization.
- 10.1.2 Place all of the equipment that you want to sterilize into the autoclave.
- 10.1.3 Move the toggle switch to **Fast** for instruments or **Slow** for liquids to the proper position.
- 10.1.4 Fill the cavity of the autoclave with deionized water up to the bottom lip of the autoclave door.
- 10.1.5 Place one heat indicating strip with the date and the analyst's initials on the shelf of the autoclave. This strip will indicate whether the autoclave has reached the appropriate temperature at the completion of the cycle.
- 10.1.6 Close the autoclave door and secure the latch. Turn the timer to 15-20 minutes.
- 10.1.7 Wait for the cycle to complete. Open the door only when the pressure has dropped. Allow the equipment to cool. Place equipment into the designated areas for use.

### 10.2 Preparing Bottles

- 10.2.1 To prepare the Treated Bacti Bottles add the following to the respective size bottle.

Bottle Size mL	Sodium Thiosulfate mL	EDTA mL
250	0.2	0.6
500	0.4	1.2
1 L	0.6	2.4

- 10.2.2 Place a piece of autoclavable tape on the side of the bottle. Put caps on loosely and autoclave at proper temperature and pressure for 15 – 20 minutes. Allow to cool and tighten caps. Mark each bottle with a "T" and mark the date that the bottles were autoclaved on the tape. To prepare the Untreated Bacti Bottles autoclave bottles as in 8.1.1.1 but do not add additional chemicals.

### 10.3 Using the Sterilizing Oven – Dry Sterilization

- 10.3.1 Place the equipment you want to sterilize into the oven. Mark the type and quantity of each piece in the Sterilization logbook.

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 7 of 10
---------------------	----------------	--	--------------

10.3.2 Leave the equipment in the oven for at least 2 hours. At the end of this time, shut off the oven and allow the equipment to cool. When the equipment has cooled. Label the glassware with the date and place into the designated areas for use.

## 11.0 Procedure

11.1 The working area should be wiped down with propanol before beginning any work in the area. Using aseptic technique throughout the entire process.

### 11.2 Sample Size Selection

11.2.1 Select the sample size to be filtered based on the expected bacterial density or the detection level that must be attained. An ideal sample volume will yield about 20-80 *E. coli* colonies and not more than 200 total colonies

11.2.1.1 Analyze drinking waters by filtering 100 mL.

11.2.1.2 Analyze other waters by filtering four different volumes (diluted or undiluted) depending on the expected bacterial density.

11.2.1.3 Use the appropriate sample volume to obtain colony counts within the ideal range.

11.2.1.4 Perform at least 4 dilutions. When unfamiliar with the sample, more dilutions should be added.

### 11.3 Sample Filtration

11.3.1 Secure the base filtration unit to the filtration flask.

11.3.2 Immerse the tips of a pair of forceps in alcohol. Shake off the excess alcohol and ignite. Wait until the flame is completely extinguished.

11.3.3 Using the forceps place a sterile membrane filter (grid side up) over the base and lock into place.

11.3.4 Dispense a small amount of phosphate buffer dilution water into each funnel, approximately 30 mL.

11.3.5 Pour 100 mL of the phosphate buffer dilution water into one of the funnels. This will serve as a check of the sterility of the phosphate buffer dilution water and glassware.

11.3.5.1 Repeat for blanks at the middle and end of the batch.

11.3.6 Shake the sample vigorously.

11.3.7 Measure the appropriate volume of sample into the respective funnel with the vacuum off.

11.3.8 Filter the sample under vacuum. With the vacuum still on, rinse the funnel walls and filter with three 20-30 mL portions of sterile dilution water.

11.3.9 Discontinue the vacuum, remove the funnel, and remove the membrane filter with sterile forceps. Immediately place the

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 8 of 10
---------------------	----------------	--	--------------

membrane filter on the selected media with a rolling action to avoid entrapment of air, replace the top of the dish and label.

11.3.10 Place a new filter on the funnel base and repeat the filtration process for the remaining sample aliquots.

11.3.11 The same filtration apparatus may be used for additional samples in a filtration series.

#### 11.4 Sample Incubation

11.4.1 Place the plated Petri dishes into waterproof plastic bags. Invert the culture dishes and incubate for 2 hours at  $35 \pm 0.5^{\circ}\text{C}$ .

11.4.2 After 2 hours transfer the culture dishes in the plastic bags to an incubator bath at  $44.5 \pm 0.2^{\circ}\text{C}$  for 22 hours.

11.4.3 After incubation, read the modified mTec plates directly for purple colonies. If using the mTec agar, saturate an individual filter pad with urea solution for each culture dish.

11.4.4 Transfer the membrane filter to the urea pad and allow to sit for 15 minutes.

11.4.5 Examine the filter under fluorescent light with magnification for yellow or yellow-brown colonies.

## 12.0 Calculations

12.1 Refer to the SOP 2016-00 on Bacterial Counting Methods.

## 13.0 Method Performance

13.1 The detection limit is dependent upon the volume of water filtered. One colony unit can be detected.

13.2 The ideal counting range is 20 to 80 colonies.

## 14.0 *E. coli* Verification Using the BBL Enterotube II

14.1 Remove both caps of the BBL Enterotube II.

14.2 Pick a well-isolated colony directly with the tip of the BBL Enterotube II inoculating wire.

14.3 Inoculate the BBL Enterotube II by twisting the wire as it is pulled through all 12 compartments of medium using a turning motion.

14.4 Reinsert the wire using a turning motion through all 12 compartments, until the notch on the wire is aligned with the opening of the tube. Break the wire at the notch by bending.

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 9 of 10
---------------------	----------------	--	--------------

- 14.5 Punch holes with the broken off part of the wire through the foil covering the air inlets of the last 8 compartments in order to support aerobic growth in these compartments.
- 14.6 Replace both caps and incubate at 35 +/- 0.5°C for 18-24 hours with the BBL Enterotube II lying on its flat surface.
- 14.7 Interpret and record all reactions with the exception of indole test on the provided BBL Entertube II Results Pad. Circle the appropriate numbers.  
Note: The addition of additional chemicals could alter the results of the other compartments.
- 14.8 Indole test if required– Add one or two drops of Kovacs' reagent through plastic film of H<sub>2</sub>S/indole compartment using either a needle or syringe. Allow the reagent to contact the surface of the medium or the inner surface of the plastic film. A positive test is indicated by development of a red color in the added reagent on surface of media or plastic film within 10 sec.
- 14.9 Total the circled numbers in each bracketed section and enter the sum in the space provided below the arrow.
- 14.10 Locate the five digit number in the interpretation guide provide with the Enterotubes. Note: Should more than one organism be listed, the confirmatory test is required to correctly identify the organism.

## 15.0 Data Assessment and Acceptance Criteria for Quality Control Measures

### 15.1 Method Blank

- 15.1.1 If the method blank has 0 colonies per 100 mL results can be reported.
- 15.1.2 If the method blank has coliform colonies present results can be reported for membrane filters that have no coliform colonies present.
- 15.1.3 If the method blank has coliform colonies present results can be reported for samples that are 10 times the method blank value or higher and are below regulatory limit or permit levels.

## 16.0 Method Specific Corrective Actions

- 16.1 If the method blank does not meet requirements the glassware may be contaminated, and in need of cleaning and sterilization.
- 16.2 Cross-contamination may be controlled by grouping samples and performing analysis on low level samples first.

## 17.0 Contingencies for Out-Of-Control/Unacceptable Data

- 17.1 If colony counts do not meet requirements data may be reported as estimates if the data quality objectives allow.

SOP Number: 2014	Revision 01	Title: Determination of E. Coli and Enterotube Verification	Page 10 of 10
---------------------	----------------	--	---------------

## 18.0 References

- 18.1 *Standard Methods for the Examination of Water and Wastewater*, 18<sup>th</sup> edition, 1992.
- 18.2 *Method 1603: Escherichia coli (E. coli) in Water by Membrane Filtration Using Modified membrane-Thermotolerant Escherichia coli Agar (Modified mTec)*, USEPA, Washington, D.C., September 2002.

## 19.0 Revision History

- 19.1 Section 3.2 changed definition of batch to 20 samples (E. Hatvani 4/4/06).
- 19.2 Section 7.2 added propanol as a reagent (E. Hatvani 4/4/06).
- 19.3 Added Section 11.3.5.1 (E. Hatvani 4/4/06).

## **Appendix J**

## Edgewater Daily Beach Data

Location	Collection Date	E. coli <i>cfu/100ml</i>	pH <i>S.U.</i>	Temp <i>°C</i>	Turbidity <i>NTU</i>	ISM/ATP <i>RLU</i>
Edgewater Beach East	5/22/2006 10:52:00 AM	82.5	7.69	14.6	64	
Edgewater Beach West	5/22/2006 11:18:00 AM	83	7.4	12.8	61	
Edgewater Beach West	5/23/2006 9:15:00 AM	26	8.2	15.5	18	
Edgewater Beach East	5/23/2006 9:30:00 AM	27	8.2	16	18	
Edgewater Beach East	5/24/2006 9:20:00 AM	13	7.7	17	6	
Edgewater Beach West	5/24/2006 9:30:00 AM	19.5	8.1	16.7	4	
Edgewater Beach East	5/25/2006 9:40:00 AM	21	8.25	19.5	6	
Edgewater Beach West	5/25/2006 9:54:00 AM	17.5	8.39	20	6	
Edgewater Beach East	5/26/2006 9:05:00 AM	105	8.2	17.1	5.48	
Edgewater Beach West	5/26/2006 9:15:00 AM	74	8.3	17.2	4.77	
Edgewater Beach East	5/29/2006	AH	AH	AH	AH	AH
Edgewater Beach West	5/29/2006	AH	AH	AH	AH	
Edgewater Beach East	5/30/2006 9:30:00 AM	15	8.32	22	3	
Edgewater Beach West	5/30/2006 9:40:00 AM	29	8.45	23.4	4	
Edgewater Beach Composite	5/30/2006 12:00:00 PM	AE				231739
Edgewater Beach Composite	5/31/2006 10:20:00 AM	24			4	22570
Edgewater Beach East	5/31/2006 10:20:00 AM	24	8.7	23	4	
Edgewater Beach West	5/31/2006 10:25:00 AM	9	8.7	22.4	3	
Edgewater Beach East	6/1/2006 9:30:00 AM	45	8.5	21.4	3.62	
Edgewater Beach West	6/1/2006 9:40:00 AM	48	8.5	21.1	5.16	
Edgewater Beach Composite	6/1/2006 10:32:00 AM	40			4.54	369551
Edgewater Beach East	6/2/2006 9:20:00 AM	230	8.2	20.9	8	
Edgewater Beach West	6/2/2006 9:30:00 AM	364	8.2	21.1	16	
Edgewater Beach Composite	6/2/2006 10:30:00 AM	275			12	57052
Edgewater Beach East	6/5/2006 9:56:00 AM	140	8.3	20.3	20	
Edgewater Beach West	6/5/2006 10:05:00 AM	250	8.53	19.7	15	
Edgewater Beach Composite	6/5/2006 10:05:00 AM	220			18	6988
Edgewater Beach East	6/6/2006 9:23:00 AM	16	8.4	22.5	7	
Edgewater Beach Composite	6/6/2006 9:33:00 AM	21			7	78553
Edgewater Beach West	6/6/2006 9:33:00 AM	32	8.6	23.1	7	
Edgewater Beach East	6/7/2006 9:41:00 AM	9	8.54	22.7	2.3	
Edgewater Beach West	6/7/2006 9:51:00 AM	9	8.48	23.1	2.9	
Edgewater Beach Composite	6/7/2006 9:51:00 AM	13			3.2	16411



## Daily Beach Data

<i>Location</i>	<i>Collection Date</i>	<i>E. coli</i> <i>cfu / 100ml</i>	<i>pH</i> <i>S.U.</i>	<i>Temp</i> <i>°C</i>	<i>Turbidity</i> <i>NTU</i>	<i>ISM/ATP</i> <i>RLU</i>	<i>Wave Ht.</i> <i>inches</i>
<b>Edgewater Beach</b>							
Edgewater Beach West	6/22/2006 9:42:0	14	8.29	24.5	4.04		4
Edgewater Beach East	6/22/2006 9:32:0	20	8.19	23.1	4.27		4
Edgewater Beach Composite	6/22/2006 9:42:0	9			4.66	50168	
<b>Euclid Beach</b>							
Euclid Beach East	6/22/2006 10:15:	420	7.91	24.4	5.33		10
Euclid Beach West	6/22/2006 10:21:	375	7.89	24.5	4.63		
<b>Huntington Beach</b>							
Huntington Beach Composite	6/22/2006 8:49:0	174			11.4	47007	
<b>Villa Angela Beach</b>							
Villa Angela-West	6/22/2006 10:05:	220	7.7	24.1	5.13		8
Villa Angela-East	6/22/2006 9:56:0	240	7.7	24.7	5.24		8
Villa Angela-Composite	6/22/2006 10:05:	220			5.01	26620	
Euclid Creek 30ft N. Brid	6/22/2006 9:43:0	6800	7.29	22.5	144		
Euclid Creek Site 0.5	6/22/2006 9:31:0	6800	6.8	21.4	149		



## Daily Beach Data

<i>Sample ID</i>	<i>Collection Date</i>	<i>Location</i>	<i>E. coli</i> <i>cfu / 100ml</i>	<i>pH</i> <i>S.U.</i>	<i>Temp</i> <i>°C</i>	<i>Turbidity</i> <i>NTU</i>
VABE0606260001	6/26/2006 9:49:00 AM	Villa Angela-East	580	7.87	22.8	2.42
EUBE0606260001	6/26/2006 10:02:00 AM	Euclid Beach East	62	8.03	22.6	1.8
EDGE0606260001	6/26/2006 10:06:00 AM	Edgewater Beach East	37	8.73	23.3	17.7