



**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 4**

**Laboratory Services & Applied Science Division
980 College Station Road
Athens, Georgia 30605-2720**

August 14th, 2023

MEMORANDUM

SUBJECT: Caney Creek WWTP Final Report
Pippa Passes, KY
LSASD Project ID: 23-0274

FROM: Chris McHugh
Environmental Engineer
Water Quality Section

THRU: Derek Little, Acting Supervisor
Water Quality Section

TO: Jairo Castillo-Valenzuela, P.E., Supervisor
Wastewater Enforcement Section Enforcement
& Compliance Assistance Division

Attached is the Final Report for the Caney Creek WWTP NPDES NCI Reconnaissance Sampling Inspection.

If you have any questions concerning the sampling event, please call me at 706-410-4802 or email me at mchugh.christopher@epa.gov.

Attachments:
cc (via email): Sandra Aker, LSASD
Shelia Holiman, ECAD

Mission: To provide sound Science to our customers through superior environmental evaluation

Vision: To be a solutions-oriented organization, and seen as a leader in sound science through innovation, responsive customer service, and cutting-edge expertise

LSASD Project ID: 23-0274

National Pollutant Discharge Elimination System National Compliance Initiative Reconnaissance Inspection

Caney Creek Wastewater Treatment Plant

**7034 KY 899
PIPPA PASSES, KY. 41844**

Project Date: June 13-14th, 2023

Report Date: August 14th, 2023

Project Leader: Chris McHugh
Water Quality Section
 Field Services Branch
 Laboratory Services & Applied Science Division
 USEPA – Region 4
 980 College Station Road
 Athens, Georgia 30605-2720

The ANSI National Accreditation Board attests that U.S. EPA Region 4 Laboratory Services and Applied Science Division fulfills the requirements of ISO/IEC 17025:2017 ANAB Forensic Testing & Calibration AR 3125:2019 in the field of Forensic Testing. The activities contained in this report fall within the scope of accreditation, Certificate Number: AT-2628. Expires 08 June 2024.



LSASD
 LABORATORY SERVICES & APPLIED SCIENCE DIVISION

Project Requestor:

Jairo Castillo-Valenzuela, Supervisor
Wastewater Enforcement Section
Enforcement & Compliance Assurance Division
USEPA – Region 4
61 Forsyth Street, SW
Atlanta, GA 30303-8960

Analytical Support:

Inorganic Chemistry Section
Laboratory Services Branch
Laboratory Services and Applied Science
Division
980 College Station Rd Athens, GA 30605

Waypoint Analytical
2790 Whitten Rd.
Kingsport, TN

Approvals:

Chris McHugh
Water Quality Section
Field Services Branch

Date

Approving Officials:

Jon McMahan, Technical Reviewer
Water Quality Section
Field Services Branch

Date

Derek Little, Acting Section Supervisor
Water Quality Section
Field Services Branch

Date

Introduction

On June 13th – 14th 2023, representatives of the U.S. Environmental Protection Agency (U.S. EPA) Region 4 Laboratory Services and Applied Science Division (LSASD) conducted a National Pollutant Discharge Elimination System (NPDES) reconnaissance sampling inspection at the Caney Creek Wastewater Treatment Plant (WWTP) in Pippa Passes, Kentucky. The evaluation was requested by the Enforcement & Compliance Assurance Division (ECAD) Water Enforcement Branch as part of the agency's goal to address significant non-compliance facilities. The inspection followed the procedures listed in the LSASD Project No. 23-0274 Sample and Analysis Plan (SAP). There were no deviations from the SAP throughout the course of this inspection. All standard operating procedures used to conduct this assessment were followed as prescribed and are listed in the project's SAP. Table 1 lists the sampling participants and facility staff.

Table 1 – Inspection Personnel

Name	Position Organization	Dates on site
Chris McHugh	Inspector U.S EPA Region 4 LSASD	June 13 th -14 th , 2023
Asher Sampong	Inspector U.S EPA Region 4 LSASD	June 13 th -14 th , 2023

Background

The Caney Creek WWTP is located at 7034 KY899 Pippa Passes, KY 41844. The plant operates under NPDES permit KY0042854 to discharge treated domestic wastewater into Caney Fork.

Currently, this facility is listed on the EPA Office of Enforcement and Compliance Assurance (OECA) significant/category 1 non-compliance for a variety of effluent discharge limit violations. The facility is also located in proximity to the area that was impacted by significant flood events in the Fall of 2022.

Study Area and Methodology

The study area for the sampling evaluation was the effluent discharge basin after chlorine disinfection. A 24-hr composite sample was collected with an automatic composite sampler and analyzed for Total Suspended Solids (TSS), Ammonia (NH₃), Nitrate/Nitrite (NO₃, NO₂), and Total Kjeldahl Nitrogen (TKN), Phosphorus (P), and Carbonaceous Biochemical Oxygen Demand (CBOD₅). E. coli was collected as a grab sample. In – situ parameters of pH and dissolved oxygen (DO) were measured with a multi – parameter meter and total residual Chlorine (TRC) with a pocket chlorimeter. Analytical methods used during this study are provided in this project's SAP.

Sample Analysis Results

Below are the analytical results for the sample collected at EFF001 after final chlorine disinfection stage in the contact chamber. Note the CBOD₅ and TSS were just below the weekly max permitted limits. NH₃, DO, E. coli, and TRC results exceeded the permitted limits. Note the Nitrate/Nitrite analytical result was impacted by an unknown cause. The result is an estimate. The full analytical results listed below in table 3 are included in the final report PDF portfolio.

Table 2: EPA Sample Results vs. Permitted Limits (Highlighted Exceedances)

Analyte	EPA Analytical Results	Permitted Limit	Sample Location	Sample Date and Time
Total Suspended Solids (TSS)	36.0 mg/L	≤ 45 mg/L Weekly Max	EFF001	6/14/23 10:45 am
CBOD ₅	39.0 mg/L	≤ 40 mg/L Weekly Max	EFF001	6/14/23 10:45 am
NH ₃ as N	8.2 mg/L	≤ 6 mg/L Weekly Max	EFF001	6/14/23 10:45 am
Nitrate/Nitrite	0.062mg/L** J, D-2, QC-1, QC-6, QL-2, QM-1, QM-3, QR-2	Report only mg/L	EFF001	6/14/23 10:45 am
Total Phosphorus	1.4 mg/L	Report only mg/L	EFF001	6/14/23 10:45 am
Total Kjeldahl Nitrogen (TKN)	12 mg/L	Report only mg/L	EFF001	6/14/23 10:45 am
E Coli – grab sample	579 MPN/100mL	<240 of colonies/100 ml Weekly Max	EFF001	6/14/23 10:45 am
Total Nitrogen(sum of NH ₃ , Nitrate/Nitrite, TKN)	20.2 mg/L	Report only mg/L	EFF001	6/14/23 10:45 am
Dissolved Oxygen (DO) – In-situ	< 2.0 mg/L*	≥ 7.0 mg/L Weekly Min	EFF001	6/14/23 10:45 am
pH – In-situ	7.6 S.U.	6.0 S.U. < pH < 9.0	EFF001	6/14/23 10:45 am

Total Residual Chlorine - Grab	0.73 mg/L	<0.019 mg/L	EFF001	6/14/23 10:45 am
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* Less than 2.0 mg/L probe was pulled out to protect probe from fouling.

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D-2 Due to Matrix Interference, the sample cannot be accurately quantified. The reported result is estimated.

J The identification of the analyte is acceptable; the reported value is an estimate.

QC-1 Analyte concentration low in continuing calibration verification standard

QC-6 Calibration check standard greater than method control limits.

QL-2 Laboratory Control Spike Recovery greater than method control limits

QM-1 Matrix Spike Recovery less than method control limits

QM-3 Matrix Spike Precision outside method control limits

QR-2 MRL verification recovery greater than upper control limits.

Table 3: Analytical Results (See attached documents in PDF portfolio)

Analysis	Total Pages
TSS, CBOD ₅	7
Nitrate/Nitrite, Phosphorus, TKN	10
E. coli	35

Quality Assurance/ Quality Control

Chemistry data for analyses conducted by LSASD Laboratory Services Branch (LSB) were verified and qualified based on Laboratory Operations and Quality Assurance Manual (LOQAM) found on www.epa.gov/region4/sesd/asbsop. Quality control for the analyses: TSS, CBOD₅, Nitrate/Nitrite, Phosphorus TKN and Total Nitrogen can be found in the quality control section of each attached analytical data package (E232409 CNA FINAL 06 28 23 and E232409 CNA FINAL 07 14 23).

E Coli analysis performed by Waypoint Analytical was analyzed using Standard Method 9223B – 2016 using EPA – Approved IDEXX Colilert. All provided certifications and accreditations are valid and unexpired. Copies of the certifications and quality control are included in the attached analytical package 23-166-0055.

The E. Coli analytical package was reviewed by a senior LSB chemist and found to have no errors and acceptable. The project requestor was contacted and agreed on the level of data validation.

Summary

Caney Creek WWTP is a single clarifier package plant, with a chlorine contact chamber for disinfection. The headworks consist of a manually cleaned bar screen, and the effluent discharge is measured with a 45 degree v-notch weir before leaving the plant via a straight pipe to the Caney Fork receiving stream.

According to the plant operator (John Pearl), who spoke via phone call during the inspection, the facility experienced catastrophic flooding last fall during the Eastern Kentucky flood event. This event flooded the clarifier and damaged the mechanical pumps that allow the clarifier to properly rotate and operate. Additionally, the floods deposited silty material from the Caney Fork basin into the clarifier and chlorine contact chamber. The operator stated the facility was trying to secure funding to remediate the clarifier and bring the plant back to fully operational status. Based on observations, analytical data from effluent samples, and in-situ measurements made during inspection, this facility is not properly operating and meeting the limits listed in its NPDES permit. The most significant deficiencies impacting the facility to properly operate are listed below.

Operational Deficiencies

- The primary clarifier/aeration unit was not operational.
- The chlorine contact chamber was impacted with sediment deposits.

Sampling and Flow Measurement Deficiencies (See Appendix A for Photo Documentation)

- The facility's composite sample tubing was brittle and black colored.
 - The composite sample refrigerated storage area was lacking a NIST-traceable thermometer and did not have any refrigeration operating while it was collecting a sample during the inspection.
- The facility's flow totalizer chart was not operational.

EPA collected 24-hr Composite Sampling Results

- Total Ammonia (8.2 mg/L) was above the maximum permitted limit (6.0 mg/L).
- Dissolved oxygen levels (< 2.0 mg/L) were below the minimum permitted limit (6.0 mg/L).
- The total residual Chlorine (0.73 mg/L) exceeded the permit limit of (< 0.019 mg/L).
- The E. coli result (579 MPN/100mL) exceeded the limit of (< 240 MPN/100 mL).

Appendix A Photo Log



Figure 1: Facility grounds/non-operational clarifier photo: Taken 6/13/23 10:09 am



Figure 2: Facility grounds Chlorine contact chamber, effluent discharge photo: Taken 6/13/23 10:10 am



Figure 3: Effluent Discharge Weir photo: Taken 6/13/23 10:12 am



Figure 4: Effluent composite sampler photo: Taken 6/13/23 10:12 am



Figure 5: Effluent Flow Totalizer non-operating photo: Taken 6/13/23 10:18 am

End of Report

6/22/2023

EPA Region IV
Mr. Chris McHugh
980 College station Road
Athens, GA, 30605

Ref: Analytical Testing
Lab Report Number: 23-166-0055
Client Project Description: Analytical Testing

Dear Mr. Chris McHugh:
Waypoint Analytical, LLC. received sample(s) on 6/15/2023 for the analyses presented in the following report.

The above referenced project has been analyzed per your instructions. The analyses were performed in accordance with the applicable analytical method. Where the laboratory was not responsible for the sampling stage (refer to the chain of custody) results apply to the sample as received.

The analytical data has been validated using standard quality control measures performed as required by the analytical method. Quality Assurance, method validations, instrumentation maintenance and calibration for all parameters (NELAP and non-NELAP) were performed in accordance with guidelines established by the USEPA (including 40 CFR 136 Method Update Rule May 2021) and NELAC unless otherwise indicated. Any parameter for which the laboratory is not officially NELAP accredited is indicated by a '~' symbol. These are not included in the scope because NELAP accreditation is either not available or has not been applied for. Additional certifications may be held/are available for parameters, where NELAP accreditation is not required or applicable. A full list of certifications is available upon request.

Certain parameters (chlorine, pH, dissolved oxygen, sulfite...) are required to be analyzed within 15 minutes of sampling. Usually, but not always, any field parameter analyzed at the laboratory is outside of this holding time. Refer to sample analysis time for confirmation of holding time compliance.

The results are shown on the attached Report of Analysis(s). Results for solid matrices are reported on an as-received basis unless otherwise indicated. This report shall not be reproduced except in full and relates only to the samples included in this report.

Please do not hesitate to contact me or client services if you have any questions or need additional information.

Sincerely,



Connie Cook
Project Manager

Laboratory's liability in any claim relating to analyses performed shall be limited to, at laboratory's option, repeating the analysis in question at laboratory's expense, or the refund of the charges paid for performance of said analysis.



Certification Summary

Laboratory ID: WP MTN: Waypoint Analytical, LLC., Memphis, TN

State	Program	Lab ID	Expiration Date
Alabama	State Program	40750	02/29/2024
Arkansas	State Program	88-0650	02/07/2024
California	State Program	2904	06/30/2023
Florida	State Program - NELAP	E871157	06/30/2023
Georgia	State Program	C044	11/14/2025
Georgia	State Program	04015	06/30/2023
Illinois	State Program - NELAP	200078	10/10/2023
Kentucky	State Program	80215	06/30/2023
Kentucky	State Program	KY90047	12/31/2023
Louisiana	State Program - NELAP	LA037	12/31/2023
Louisiana	State Program - NELAP	04015	06/30/2023
Mississippi	State Program	MS	11/14/2025
North Carolina	State Program	47701	07/31/2023
North Carolina	State Program	415	12/31/2023
Pennsylvania	State Program - NELAP	68-03195	05/31/2024
South Carolina	State Program	84002	06/30/2023
Tennessee	State Program	02027	11/14/2025
Texas	State Program - NELAP	T104704180	09/30/2023
Virginia	State Program	00106	06/30/2023
Virginia	State Program - NELAP	460181	09/14/2023



Sample Summary Table

Report Number: 23-166-0055

Client Project Description: Analytical Testing

Lab No	Client Sample ID	Matrix	Date Collected	Date Received
87136	EFF001-0614	Aqueous	06/14/2023 10:45	06/15/2023

10536

EPA Region IV

Mr. Chris McHugh

980 College station Road

Athens , GA 30605

Project Analytical Testing

Information :

Report Date : 06/22/2023

Received : 06/15/2023

Report Number : **23-166-0055**

REPORT OF ANALYSIS

Lab No : **87136**

Sample ID : **EFF001-0614**

Matrix: **Aqueous**

Sampled: **6/14/2023 10:45**

Test	Results	Units	MQL	DF	Date / Time Analyzed	By	Analytical Method
E-coli	579	MPN/100mL	1	1	06/14/23 15:40	KSS	9223B-2016

**Qualifiers/
Definitions**

DF

Dilution Factor

MQL

Method Quantitation Limit

L688077

Analyst	1655
Date/Time Batch Started	6-14-73 1540

Calculate

Incubation Started:	6-14-23 1540		
Start Temperature °C:	35.2	Initial	165
4-hr temp check date / time:	6-14-23 2015		
Temperature °C:	35.2	Initial	165
Incubation Ended:	6-15-23 1600		
Ending Temperature °C:	35.2	Initial	165

Incubator ID: JCO35

Temp range 34.5°C - 35.5°C

[illegible]

Media ID:	EW476	Quanti-tray ID:	FU023.T	DPD Reagent ID:	A9189
Colilert-18 (18 - 22hrs.) OR Colilert (24 - 28 hrs.)		Anti - foam ID:	NA	Comparator Lot # CR342	KS

EU278

QUALITY CONTROL CERTIFICATE

Colilert*

Product and Company Contact Information

Product Catalog Number: WP200I
Part Number: 98-12973-00

Lot Number: EU476
Expiration date: 08 July 2023
Store At: 2-30 °C

Technical Support Inquiries:

1-207-556-4496
1-800-321-0207 (US/CAN)
00-800-4339-9111 (Europe)
Email: water@idexx.com

Manufacturer:

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, ME 04092 USA
Fax: 1-207-556-4630
idexx.com/water

Physical Properties

Powder Appearance** White to off-white, free flowing, granulated powder, free of foreign particles.
pH Tested for pH range 7.0 - 7.6

Results

Passed
Passed

Product Performance

Representative samples of this lot have been tested as received with the organisms listed below. Ten samples per organism are incubated at 35 ± 0.5 °C with reads at 24 hours for target organisms and 28 hours for non-target organisms. The inoculum level for target bacteria is approximately 20-50 cfu/100mL. Quantitative testing is performed on all target organisms using IDEXX Quanti-Tray* MPN compared to spread plate counts using Tryptic Soy Agar with or without 5% Sheep's Blood. Productivity ratio is the average MPN / average plate count. The passing range is ≥ 0.5 to ≤ 1.4 (i.e. greater than or equal to 0.5 to less than or equal to 1.4).

Note: Testing performed in accordance with ISO 11133:2014.

Target Bacteria	P/A Result	Quantitative Result	Productivity Ratio
<i>Escherichia coli</i> ATCC 25922 (WDCM 00013) ATCC 11775 (WDCM 00090)	Yellow color change and fluorescence $\geq$ comparator	Confirmation plate counts within 95% confidence limits of Quanti-Tray MPN	Passed
<i>Klebsiella variicola</i> † ATCC 31488 (WDCM 00206)	Yellow color change $\geq$ comparator, no fluorescence	Confirmation plate counts within 95% confidence limits of Quanti-Tray MPN	Passed

† *Klebsiella pneumoniae* ATCC 31488 has been renamed to *Klebsiella variicola* ATCC 31488

Rec'd 8/31/22 LCS

*Colilert and Quanti-Tray are trademarks or registered trademarks for IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries.

**Parameter does not fall under the IDEXX ISO/IEC 17025:2017 Scope of Accreditation



IDEXX Water QC Lab is accredited to
ISO/IEC 17025:2017

frm-QA-132_C, CO #129106, Effective Date: 02/01/2021

IDEXX

ISO 9001 CERTIFIED
ISO 14001 CERTIFIED

Page 1 of 2

QUALITY CONTROL CERTIFICATE

Colilert*

Product and Company Contact Information

Product Catalog Number: WP200I
Part Number: 98-12973-00

Lot Number: EU476
Expiration date: 08 July 2023
Store At: 2-30 °C

Technical Support Inquiries:

1-207-556-4496
1-800-321-0207 (US/CAN)
00-800-4339-9111 (Europe)
Email: water@idexx.com

Manufacturer:

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, ME 04092 USA
Fax: 1-207-556-4630
idexx.com/water

Non-Target Bacteria

Pseudomonas aeruginosa
ATCC 10145 (WDCM 00024)

P/A Result

No color change, no fluorescence at inoculation level of approximately 20,000 cfu/mL.

Coliform-free

Lot has been subjected to gamma irradiation. In accordance with ISO 11137-02, post-irradiated product has a minimum sterility assurance level (SAL) of 10^{-3} for coliforms. Colorless and non-fluorescent after 28 hours incubation at 35 ± 0.5 °C in sterile water. Frequency on a per lot basis: random cross lot production testing (minimum 20 replicates).

Note: The World Data Center for Microorganisms (WDCM) number refers to the same bacterial strain as the culture collection number listed for the American Type Culture Collection (ATCC).

This product was performance tested and has met all quality control specifications required for release.

This information is released by IDEXX Quality Assurance:

Name: Paula Guerrette, Quality Associate Sr II

Signature:

Date: 22 August 2022

*Colilert and Quanti-Tray are trademarks or registered trademarks for IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries.

**Parameter does not fall under the IDEXX ISO/IEC 17025:2017 Scope of Accreditation



IDEXX Water QC Lab is accredited to
ISO/IEC 17025:2017

frm-QA-132_C, CO #129106, Effective Date: 02/01/2021

IDEXX

ISO 9001 CERTIFIED
ISO 14001 CERTIFIED

Page 2 of 2

QUALITY CONTROL CERTIFICATE

Quanti-Tray*/2000

Product and Company Contact Information

Product Catalog Number: WQT-2K
Part Number: 98-21675-00

Lot Number: FU023J
Expiration date: 20 July 2025

Technical Support Inquiries:

1-207-556-4496
1-800-321-0207 (US/CAN)
00-800-4339-9111 (Europe)
Email: water@idexx.com

Manufacturer:

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, ME 04092 USA
Fax: 1-207-556-4630
idexx.com/water

Physical Properties

Product Sterility: This lot was sterilized with Ethylene Oxide.

No growth after 48 hours incubation at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ with sterile Tryptic Soy Broth.

Representative samples of this lot have been tested by IDEXX Laboratories for the following requirements and passed:

1. Volume of 96 wells: 98 ± 2.0 mL
2. Average volume of overflow well: > 10.0 mL
3. Volume ratio large wells to small wells: between 11:1 and 13:1
4. Dye Test: sealed with 100 mL dye and checked for absence of leaks
5. Seal Integrity: trays sealed with 100 mL water, Colisure*, and anti-foam: wells withstood 18 PSI of pressure without leaking

Rec'd 8/31/22 KS

This product was performance tested and has met all quality control specifications required for release.

This information is released by IDEXX Quality Assurance:

Name: Wilma Ledoux, Quality Associate Sr I

Signature:

Wilma B. Ledoux

*Quanti-Tray and Colisure is a trademark or registered trademark of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries.

WAYPOINT ANALYTICAL, LLC.
MICROBIOLOGY: Media Control Check

Instructions: Test each lot of Colilert media for performance by inoculation with three control bacteria minimally quarterly. Three control bacteria are used: EC Positive: *Escherichia coli*, TC Positive: *Klebsiella variicola* and a Negative Control: *Pseudomonas aeruginosa*. Prepare and process as a sample. Record the results of the controls. Also process a sterile water control. If the sterile water control exhibits faint fluorescence or faint positive coliform result, discard and use a new batch of media. Incubate these controls at 35 + 0.5°C for 24 hours. Read and record the results.

Expectations of Controls and Blank		Yellow Color Observed	Fluorescence Observed
	E. coli	Yes	Yes
	Total Coliform	Yes	No
	Negative Control	No	No
	Blank (Sterile Water)	No	No

Incubator ID:	Micro JCO35	Thermometer ID: T1				
Colilert ID:		Yellow Color Observed	Fluorescence Observed	Accept	Reject	Date/Initials
Colilert Lot#	MTN84					
Date/Time In:	4/8/22 1630					
Incubator Temp °C	35.0					
E. coli ID:	465-165-4	Yes	Yes	✓		4/9/22 KS
Total Coliform ID:	KV 261-59-4	Yes	No	✓		I
Negative Control ID:	PA 484-1426-4	No	No	✓		I
Sterile Water ID:	03020	No	No	✓		I
Date/Time Out:	4/9/22 1640					
Incubator Temp °C	35.0					

Incubator ID:	Micro JCO35	Thermometer ID: T1				
Colilert ID:		Yellow Color Observed	Fluorescence Observed	Accept	Reject	Date/Initials
Colilert Lot#	EU476					
Date/Time In:	9/15/22 1640					
Incubator Temp °C	35.0					
E. coli ID:	465-165-4	Yes	Yes	✓		9/16/22 KS
Total Coliform ID:	KV 261-59-4	Yes	No	✓		I
Negative Control ID:	PA 484-1426-4	No	No	✓		I
Sterile Water ID:	34021	No	No	✓		I
Date/Time Out:	9/16/22 1645					
Incubator Temp °C	35.0					

WAYPOINT ANALYTICAL, LLC.
MICROBIOLOGY: Media Control Check

Instructions: Test each lot of Colilert media for performance by inoculation with three control bacteria minimally quarterly. Three control bacteria are used: EC Positive: *Escherichia coli*, TC Positive: *Klebsiella variicola* and a Negative Control: *Pseudomonas aeruginosa*. Prepare and process as a sample. Record the results of the controls. Also process a sterile water control. If the sterile water control exhibits faint fluorescence or faint positive coliform result, discard and use a new batch of media. Incubate these controls at $35 \pm 0.5^{\circ}\text{C}$ for 24 hours. Read and record the results.

Expectations of Controls and Blank		Yellow Color Observed	Fluorescence Observed
	E. coli	Yes	Yes
	Total Coliform	Yes	No
	Negative Control	No	No
	Blank (Sterile Water)	No	No

Incubator ID:	Micro JCO35	Thermometer ID: T1				
Colilert ID:		Yellow Color Observed	Fluorescence Observed	Accept	Reject	Date/Initials
Colilert Lot#	EU476					
Date/Time In:	3/23/23 1700					
Incubator Temp °C	35.0					
E. coli ID:	465-165-4	Yes	Yes		✓	
Total Coliform ID:	KV 261-59-4	Yes	No		✓	
Negative Control ID:	PA 484-1424-4	No	No		✓	
Sterile Water ID:	34021	No	No		✓	
Date/Time Out:	3/24/23 1715					
Incubator Temp °C	35.0					

Incubator ID:	Micro	Thermometer ID:				
Colilert ID:		Yellow Color Observed	Fluorescence Observed	Accept	Reject	Date/Initials
Colilert Lot#						
Date/Time In:						
Incubator Temp °C						
E. coli ID:						
Total Coliform ID:						
Negative Control ID:						
Sterile Water ID:						
Date/Time Out:						
Incubator Temp °C						

Instructions: Place 100 mL of sterile Tryptic Soy Broth, TSB in a Quanti - Tray from each new lot obtained. Incubate at $35.0 \pm 0.5^{\circ}\text{C}$ for 48 hours. Check for cloudy growth. If cloudy growth is present, the lot of Quanti-Tray is rejected.

Quanti - Tray Sterility Verification

[illegible]

Instructions: For each new lot of Quanti-Trays received, select one at random to test. Open a bottle containing 90mL of sterile water. Aseptically add 10mL sterile water from the same lot to get 100mL, add one packet of Colilert media, close and shake to dissolve media. Pour entire contents into Quanti Tray. Incubate at $35.0 \pm 0.5^{\circ}\text{C}$ for 24 hours. Check for color change and fluorescence. If either color or fluorescence are present, the lot of Quanti-Tray is rejected.

Quanti - Tray Sterility Verification

[illegible]

Procedure: Aseptically add 50 mL of sterile, unbuffered dilution water to 50 mL Double Strength Tryptic Soy broth, incubate at 35.0± 0.5 °C. Check for growth after 24 and 48 hours. Cloudy growth indicates unsterile rinse/dilution water and therefore the lot is rejected.

[illegible]



Document Revision Form

Date	01/25/2022	
DRF #	012522A	
Reviewer's Name	Blake Smith	
Original SOP Document Title	9223B E.coli and Total Coliform 070121	
Original SOP Effective Date	07/01/2021	
Reason for Revision	☐ : Addendum ☐ : Correction ☒ : Minor Update	
Internal or External Audit ID		
CA Report ID		
Other		
Describe Changes Below		
1	9.0 EZ DPD Dispenser for Total Chlorine	
	9.0 Plastic paint pallets (to use with DPD Dispenser) -- (remove white printer paper)	
2	10. HACH (IDEXX) DPD Free Chlorine Powder - pillows, bulk (vial to use with dispenser)	
	12.12 samples will be checked before analyzing using DPD powder (bulk) by dispensing a small amount of powder in a	
3	plastic paint pallet well and dropping sample onto the powder using a sterile, disposable pipette.	
	14.1 Check for excess Cl ₂ by dispensing a small amount of DPD powder in a plastic paint pallet well....	
Approvals	Signature	Date
Chief Quality Officer		
Technical Officer	Jimmy R Ferguson II	Digitally signed by Jimmy R Ferguson II Date: 2022.01.26 10:04:13 -06'00'
Supervisor	Blake (Andres) Smith	Digitally signed by Blake (Andres) Smith Date: 2022.01.25 11:33:39 -06'00'
Analyst		
Other		
Quality Assurance Section Only		
Revised SOP or Document Title	9223B E.coli and Total Coliform 070121A	
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Notes or Conditions	This DRF is effective until said SOP or document undergoes its annual or subsequent full review.	

Standard Operating Procedure
For
The Determination of E. coli and Total coliforms
by SM 9223 B -2004 and SM 9223B - 2016
Using EPA-Approved IDEXX Colilert®
With Addendum for Colilert-18® and P/A Method.

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1. Identification of Test Method

This test method is identified as the determination of E. coli and Total coliforms by Standard Methods 9223 B. The laboratory uses the IDEXX Colilert® system approved by the EPA in monitoring for E. coli and Total coliforms in drinking water and E. coli only in wastewater. An addendum is also included for the analysis of Fecal coliforms, Total coliforms, and E. coli in wastewater and drinking water using Colilert-18®. Another addendum is also included for the Presence/Absence of Total coliforms and E. coli in drinking water.

2. Applicable Matrix or Matrices

This method is applicable to drinking water, source waters, ground waters and wastewaters. It is not applicable for solid samples.

3. Detection Limits

The current 40 CFR Part 141 and DW Manual 5th Edition approves the use of the IDEXX Colilert® for the analysis of Total coliforms and E. coli in drinking water. The current 40 CFR Part 136 approves the Colilert® method for E. coli analysis in wastewater. The Colilert® test detects total coliforms and E. coli at 1 MPN/100 mL.

4. Scope and Application

Method 9223 B is a method that utilizes Colilert® to simultaneously detect coliforms and E. coli in water samples. This method allows the monitoring of various waters to determine sanitary quality. The method is intended to indicate the degree of Total coliform/E. coli contamination in water.

5. Summary of Test Method

This enzyme substrate test utilizes hydrolyzable substrates for the simultaneous detection of total coliforms and E. coli enzymes. When total coliforms are present they hydrolyze Colilert's nutrient indicator, OPNG, and the sample will exhibit a color change to yellow. Further, if E. coli is present the fluorogenic substrate MUG is hydrolyzed and the sample will fluoresce when exposed to a UV light source at 365 nm, as specified by the manufacturer. Total coliform results are compared to a reference comparator when a yellowish color is questionable. Both Total coliform and E. coli are quantified using the manufacturer's Most Probable Number Table and results are quantified as MPN/100 mL.

Drinking water samples can be analyzed as either Present / Absent or quantified as MPN/100mL

5.1 Method Modifications from Reference

Samples are exposed to a UV light source at 365 nm as specified by the manufacturer (IDEXX). Standard Methods recommends a UV light source at 366 nm. The current DW Manual indicates 365-366 nm.

6. Definitions

The laboratory's Quality Manual contains the definitions of standard terms used in this SOP as applicable.

An analytical batch is defined as either 20 samples, or a full day, whichever occurs first.

7. Interferences

Sterile/dilution water that may be used for analyzing wastewater must prove to be sterile. Likewise, sample containers and media must prove to be sterile and contaminant free.

8. Safety

The analyst must consult the SDS for the reagents and chemicals that are used in this analysis. The analyst is responsible for following the safety procedures defined in the SDS. The use of personal protective equipment is provided and its use is required in the laboratory. Personal protective equipment includes eye protection and protective clothing. Wear appropriate gloves while handling media, reagents and samples. Special considerations must be taken when handling samples with the potential to harbor coliform group of bacteria.

Handle reagents, media and samples that release toxic or irritating vapors and dust under a properly functioning hood. The consumption of food, beverages, etc. is strictly prohibited in the laboratory except for designated lunch areas. Smoking is prohibited in all areas of the laboratory.

Clean up any spills immediately, and thoroughly clean all work areas following each analysis. Report any accidents or spills immediately to the Laboratory Safety Officer. Other safety precautions should be conducted in accordance with the laboratory Safety Manual. Other actions can also be applied if deemed necessary. A reference file of safety data sheets (SDS) is available to all personnel involved in an analysis using reagents and or media. Due to the harmful effects of coliforms on humans, food and drink shall be prohibited from the laboratory work area.

9. Equipment and Supplies

Thermometer: Verified against a NIST-Traceable reference. It shall be replaced if it differs by more than 1°C from the reference thermometer.

Refrigerator: Capable of maintaining a temperature of just above freezing to <10°C. The temperature of the refrigerator must be checked and recorded daily.

Incubator: Capable of maintaining a temperature of the method requirement of $35.0 \pm 0.5^\circ\text{C}$. The temperature of the incubator shall be recorded at the start of incubation, at approx. 4-hours and at end of the analysis to demonstrate consistent conditions.

Incubator (Colilert-18®): Capable of maintaining a temperature of the method requirement of $44.5 \pm 0.2^\circ\text{C}$ for Fecal coliform and $35.0 \pm 0.5^\circ\text{C}$ for Total coliforms and E. coli. The temperature of the incubator shall be recorded at the start of incubation, at approx. 4-hours and at end of the analysis to demonstrate consistent conditions.

IDEXX Quanti-Tray Sealer, Model 2X

Ultraviolet Hand Lamp, Spectroline E-Series or similar, equipped with a 6-watt, 365-nm UV light

Reference Comparator: One for enumeration, and one for presence/absence. Store at 2-30°C in the dark. Observe the manufacturer's expiration date. Replace before the expiration date.

Sterile 100-mL sample bottles, QEC, Idexx, or similar. The bottles shall be verified for sterility and volume prior to distribution to clients or use. Additionally, bottle stock lot numbers shall be recorded in the appropriate log.

Sterile 150-mL containers. The bottles shall be verified for sterility prior to use. Additionally, bottle stock lot numbers shall be recorded in the appropriate log.

Class A graduated cylinder

Dark box for use with UV light

IDEXX Quanti-Trays. The trays shall be verified for sterility prior to use. Additionally, Quanti-Tray stock lot numbers shall be recorded in the appropriate log.

Autoclave: Capable of maintaining a sterilization temperature of 121 °C for 30 minutes at a minimum of 15 PSI.

Holding Bins

Buckets

Sterile, disposable pipettes

White printer paper

pH Meter

10. Reagents, Media and Stock Solutions

The laboratory shall ensure that the quality of the reagents and media used is appropriate for this test.

Sodium Thiosulfate: 0.1N, prepare by dissolving 2.5 g of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in 100 mL deionized water. Note: The laboratory purchases 125 mL pre-preserved sterile containers from IDEXX or similar

IDEXX Colilert® and Colilert-18® Snap Packs Test Kit: Store at 2 - 25°C protected from light and observe the manufacturer's expiration date up to 12 months from the date of manufacture.

Non-selective Growth Medium: Double Strength Tryptic Soy Broth (for water sterility) and Single Strength Tryptic Soy Broth (Quanti – tray and bottle sterility)

Sterile Water: 90 ml and 99ml, Purchased from Edge Biologicals, or similar.

Butterfields Buffer, 90 ml and 99ml, GVS Life Sciences or similar, COAs maintained.

15 mg/L Chlorine Solution: Place 0.019 mL of Clorox™ Bleach into 100 mL deionized water.

IDEXX Anti-Foam Agent.

HACH DPD Free Chlorine Powder – pillows and bulk.

Clorox® Germicidal Bleach

pH Buffers 7.0, 8.0, 10.0

E coli Positive Control: *E coli*, Microbiologics 0465L 6/kit. Derived from ATCC 11775. Prepare stock solution by adding one dehydrated bacteria pellets to 90 or 99 mL Butterfield buffer. Prepare E coli positive control by adding 1 - 10 mL of this stock solution into 90 or 99 mL of sterile water. Process as routine sample. Use this control to assess the media at a minimum quarterly or when a new lot of media is obtained.

Total coliform Positive Control: *Klebsiella variicola*, Microbiologics 0261L 6/kit. Derived from ATCC 31488, formerly *K. pneumoniae*. Prepare stock solution by adding one dehydrated bacteria pellet to 90 or 99 mL Butterfield buffer. Prepare Total coliform control by adding 1 - 10 mL of bacteria solution into 90 or 99 mL of sterile water. Process as routine sample. Use this control to assess the media at a minimum quarterly or when a new lot of media is obtained.

Negative Control: *Pseudomonas aeruginosa*, Microbiologics 0484L 6/kit. Derived from ATCC 9027. Prepare stock solution by adding one dehydrated bacteria pellet to 90 or 99 mL Butterfield buffer. Prepare negative control by adding 1 - 10 mL of bacteria solution into 90 or 99 mL of sterile water. Process as routine sample. Use this control to assess the media at a minimum quarterly or when a new lot of media is obtained. This control will also be used with each analytical batch, or at the end of the work day, whichever occurs first.

Instructions for labeling and recordkeeping of reagents and standards are contained in the laboratory's Quality Manual and the Solution Validation Quality Assurance Program. All documentation for media purchased includes manufacturer, lot number, amount of media received, and date of receipt and expiration date. Discard any commercially prepared media by the manufacturer's expiration date.

11. Sample Collection, Preservation, Shipment and Storage

11.1 Sample Collection

Samples shall be collected in wide-mouth sterilized plastic or glass bottles capable of holding 100 mL of sample for each required tests and having adequate headspace (2.5 cm) to facilitate mixing by shaking. Do not collect less than 100 mL, or overfill to the top. Collect samples that are representative of water being tested. Flush or disinfect sample ports, and use aseptic techniques to avoid sample contamination.

Keep sample bottles closed until sampling. Do not contaminate inner surface of cap or neck of sample container. Fill sample container without rinsing and cap immediately.

For drinking water samples, 100 mL of sample must be collected for analysis.

11.2 Sample Preservation

Samples containing chlorinating agent will be removed by the sodium thiosulfate present in the sample bottle. The sample must be cooled to < 10°C at the time of collection. The laboratory sample custodian will verify that the sample was collected in an appropriate container and received at < 10°C.

11.3 Sample Shipment and Handling

Ship samples at a temperature < 10°C assuring that the containers will not break or samples may become contaminated.

11.4 Sample Storage

Hold samples below 10°C during transport and until time of analysis. Wastewater samples being tested for E. coli or Fecal coliform must be in the incubator within eight (8) hours of collection. Drinking water total coliform samples must be in the incubator within 30 hours of collection. Food safety related Total Coliform and E. coli samples must be in the incubator within 24 hours of collection.

12. Quality Control

The results of these quality control samples are used to assess the acceptability of data. See Sections 19 and 20 of this SOP for acceptance criteria as well as a description of corrective action and contingency requirements.

12.1 Colilert® and Colilert-18® Media Check

Colilert® and Colilert-18® media is purchased pre-made as single-use sterile packs. Each new lot of media must be tested for auto-fluorescence and sterility using a 365-nm ultraviolet light by placing one packet per lot under the laboratory's UV light. This test is preformed prior to use. If the media fluoresces it may not be used and no LMS number will be assigned. The analyst will use another lot that does not fluoresce. If any media fails fluorescence test, it will not be used for testing.

Each new lot of media must also be tested to ensure the proper pH of the media. Empty a packet of Colilert® or Colilert-18® to a 100mL sterile water and mix the media until contents are dissolved. Test the pH of the water containing the media. The appropriate pH is 7.0 – 7.6, if this requirement is not met the lot of media is rejected and a new lot is obtained. Results are maintained in the appropriate log.

12.2 Water Sterility Check

Each lot of sterile water used must be verified for sterility. Water sterility is performed to confirm the sterility of water used in this procedure. Water sterility consists of one media packet added to a 100 mL container of sterile water. The sample is sealed in a Quanti-tray and incubated at the appropriate time and temperature. This sample should show no growth of any kind. There should be no color change and no UV fluorescence. Additionally, a second sterility check must be performed using a non-selective growth medium, such as double strength Tryptic Soy Broth. The Sterile Water/TSBx2 mix should be incubated and monitored at 24 and 48 hours. Any reaction indicates contamination and media should be discarded and a new lot obtained and tested. Record water sterility check in the appropriate logs. COA's and LMS reagent ID bench sheets are maintained in the Sterile and Buffer Water Lot Log.

For each new lot of Butterfield's Buffered Water's, the COA shall be acquired and the sterility information shall be reviewed and verified prior to use. Verification shall be documented on the COA and maintained in the Sterile and Buffer Water Lot Log. Any lot of buffered water that does not pass the method criteria for sterility shall not be used and a new lot obtained and verified prior to use.

12.3 E. coli Positive Control

A reference E. coli control is analyzed to demonstrate that the medium supports the growth of target organisms. An E. coli positive control shall be analyzed for each new lot or minimally quarterly. An E. coli positive control consists of 1-10 mL of the EC bacteria stock added to 90 or 99 mL sterile water. Colilert® or Colilert-18® media is added and sample is treated as a routine sample. Sample should show a yellow color change as well as UV fluorescence. If both reactions are not observed, the lot is not valid and another must be acquired and retested.

12.4 Total coliform Positive Control

A reference total coliform control is analyzed to demonstrate that the medium supports the growth of target organisms. A Total coliform other than E. coli is used to demonstrate the ability of the medium to differentiate between the two groups. A Total coliform positive control shall be analyzed for each new lot or minimally quarterly. The Total coliform positive control consists of 1-10 mL of *Klebsiella variicola* bacteria stock added to 90 or 99 mL sterile water. Colilert® or Colilert-18® media is added and sample is treated as a routine sample. Sample should show a yellow color change but no UV fluorescence. If appropriate reactions are not observed, the lot is invalid and another lot must be acquired and retested.

12.5 Negative Control

A reference negative culture control is analyzed to demonstrate that the medium does not support the growth of non-target organisms or does not demonstrate the typical positive reaction of the target organisms. A negative control shall be analyzed for each analytical batch, or at the end of the work day, whichever occurs first. Using the Negative Control Check Log, the analyst shall maintain records of the samples analyzed (20 max) while analyzing the negative control. If less than 20 samples are received in a work day, the analyst will run a negative control at the end of the day. All records will be kept by the supervisor and scanned into the appropriate folder. A negative control consists of 1-10 mL of *Pseudomonas aeruginosa* bacteria stock added to 90 or 99 mL sterile water. Colilert® or Colilert-18® media is added and sample is treated as a routine sample. Sample should show no yellow color change and no UV fluorescence. If appropriate reactions are not observed, the lot is invalid and another lot must be acquired and retested.

12.6 Test Performance

The laboratory shall demonstrate performance through the use of analyzing performance testing samples. When warranted by an Accrediting Authority, the lab shall analyze a minimum of two wastewater performance testing samples per year with acceptable results of two of the last three performance testing samples analyzed. For the drinking water program, the laboratory shall analyze a performance testing sample annually. The laboratory shall maintain documentation of this test performance for a minimum of 5 years for wastewater and 6 years for drinking water. A2LA requires two PTs per program annually.

12.7 Test Variability/Reproducibility

When a duplicate sample is supplied by a client, analyze the duplicate with each batch of samples processed. Results must be within 5% RPD.

12.8 Sample Bottle Sterility

A sterility test shall be performed on each lot of bottles purchased by the laboratory using a non-selective growth medium such as single strength Tryptic Soy Broth and monitored at 24 and 48 hours. Results are documented in the appropriate log indicating box and bottle lot numbers, TSB lot number, sterility results, analyst and date/time.

12.9 Quanti-Tray Sterility Check

A sterility and fluorescence test shall be performed on each lot of Quanti – Trays purchased by the laboratory using sterile water. As such, add 100mL sterile deionized water and a snap pack of Colilert® or Colilert-18® media to a Quanti-Tray and incubate at $35\pm0.5^{\circ}\text{C}$ for 24 hours. Assess for auto fluorescence / color. Results are documented in the appropriate log book documenting the Quanti-Tray lot numbers, sterile water lot numbers, Colilert® or Colilert-18® ID, color/fluorescence results, analyst and date/time. Reject any lots where fluorescence/color is observed.

Additionally, a sterility test shall be performed on each lot of Quanti-Trays purchased by the laboratory using a non-selective growth medium, single strength Tryptic Soy Broth. As such, 100mL Tryptic Soy Broth will be added to a Quanti-Tray and incubated at $35\pm0.5^{\circ}\text{C}$ for 48 hours. At the end of 48 hours, the Quanti-Tray will be checked for turbidity. If cloudy growth is present, the lot will be rejected and a new lot will be tested. Results are documented in the appropriate log book documenting the Quanti-Tray lot numbers, Tryptic Soy Broth lot numbers, sterility results, analyst and date/time.

12.10 Sealer Check

The sealer should be checked monthly by adding a dye (e.g., food coloring) to 100-mL of water and poured into a tray and sealed. If the dye is observed outside the wells, another sealer must be obtained.

12.11 Sample Bottle Volume Check

Sample containers sent to the client by the laboratory will be pre-verified for the required volume of 100 mL and documented in the appropriate log. For sample containers received by the laboratory which were not issued by the laboratory or previously verified will be recorded in the appropriate column on the batch sheet by the analyst.

For **Waste Water Samples** received by the laboratory which do not hold 100 mL of sample, perform the following procedure:

If over-filled, pour the entire contents of sample into a larger (150 mL), sterile container and shake to mix the sample, then pour back into the original container to 100 mL and proceed with analysis as normal.

If under-filled, mark the meniscus line of sample, and add enough Sterile Water to bring the volume to 100 mL. Proceed with analysis as noted. After the sample has been poured into the Quanti-Tray, fill the original container with water to the meniscus line marked. Then pour the water into a Class A graduated

cylinder and measure the volume. Record the volume of the sample on the batch sheet. Calculations in LMS have been programmed to account for sample volume.

For **Drinking Water Samples** Drinking Water samples MUST analyze 100 mL of sample. Samples received by the laboratory which do not hold 100 mL of sample, perform the following procedure:

If over-filled, pour the entire contents of sample into a larger (150 mL), sterile container and shake to mix the sample, then pour back into the original drinking water container to 100 mL and proceed with analysis as noted.

If under-filled, the client must be contacted and the sample will be rejected.

If the client wishes to proceed with analysis out of compliance the analyst will add sterile water to reach 100mL. The amount of sample will be recorded in LMS and the report will be flagged as non – compliance for drinking water standards and for A2LA standards.

12.12 Excess Chlorine Check

Each lot of sample containers will undergo a dechlorination procedure for neutralizing up to 15 mg/L residual chlorine for wastewater samples. The 15 mg/L Chlorine Solution is made by placing 0.019 mL of Clorox™ Bleach into 100 mL deionized water. Place the solution in a sample container, add Colilert® or Colilert-18® media and watch for a blue flash upon shaking. Additionally, place 10 mL of this solution in an appropriate graduated cylinder and add one packet of DPD Free Chlorine Reagent Powder Pillow. In 3-5 minutes if the solution has turned pink, the sodium thiosulfate did not sufficiently remove residual chlorine and the lot of bottles is rejected. If the solution does not exhibit a color change, the lot of bottles is accepted. Results are documented in the appropriate log book indicating bottle lot numbers, DPD Powder Pillow reagent number, Blue and pink color change results, analyst and date/time.

Samples will be checked before analyzing using DPD powder (bulk) by placing a small amount of powder on a sheet of white printer paper and dropping sample onto the powder using a sterile, disposable pipette. For samples that remain the same color (not pink) they will be marked as A for an absence of excess chlorine. For samples that turn pink with the DPD powder, they will be marked as P for a presence of excess chlorine. Samples with excess chlorine will include a narrative in the final lab report that the sample is not in compliance.

NOTE: Laboratory-supplied bottles are purchased pre-preserved with Sodium Thiosulfate and are verified that the amount of this dechlorination agent is sufficient to neutralize 15 mg/L Chlorine. Document this verification procedure in the appropriate logbook. Laboratory-supplied containers are noted on the batch sheet.

12.13 Sample Bottle Auto-fluorescence check

Each lot of sample containers will undergo an auto-fluorescence check. This will be performed by placing one container from each new lot under the laboratory's 365-nm UV light. If the bottle fluoresces it may not be used and the lot will be rejected. The analyst will use another lot that does not fluoresce. This test is done prior to use.

13. Calibration

The following calibration procedures apply to this test method.

13.1 Thermometers

On a scheduled basis, calibrate all working thermometers against an NIST-traceable thermometer. Digital thermometers shall be calibrated quarterly. Record all such calibrations on an Annual/Quarterly Thermometer Calibration Log sheet. Temperature monitoring devices should be of sufficient graduation to monitor the temperatures of the incubators and refrigerators. Note any correction factors and assure they

are applied when recording temperatures. Replace the thermometer when it differs by more than 1°C from the reference thermometer.

13.2 Refrigerator

Store samples, media and reagents in a refrigerator capable of maintaining a temperature of < 10°C. Monitor and document the refrigerators temperature daily to assure sample storage temperature is maintained. Do not store volatile solvents, food or beverages in a refrigerator with media or samples.

13.3 Incubator

The incubator must maintain a constant and uniform temperature at all times. Maintain a temperature reading device that has been calibrated against an NIST-traceable thermometer. Record temperature readings three times daily preferably first thing in the morning, at noon, and before the working day is over on the applicable batch sheet and temperature log. Likewise, record any maintenance performed on the incubator in the instrument's maintenance log.

13.4 Incubator Uniformity of Temperature

Perform and document a one-time uniformity of temperature study on the incubator used in this procedure. Study shall verify that each shelf and position used for incubation maintains a uniform temperature consistent with the temperature range of noted for this method.

14. Procedure

14.1 Set-up and Analysis Preparation

Obtain enough IDEXX reagent snap packs to match the number of samples and applicable quality control samples for each analytical batch.

Ensure that the Quanti-Tray Sealer is on and the incubator is at the appropriate temperature.

Label all Quanti-Trays with the corresponding sample IDs. Do not label near the opening to prevent contamination. Handle all media and trays as aseptically as possible.

It is not necessary to allow Colilert® media to come to room temperature prior to analysis. According to the manufacturer the specified incubation period allows for the time needed for the sample to reach incubation temperature.

If a sample container is filled too full to allow for proper mixing, (100 mL for sample analysis) pour the entire sample into a larger (150 mL) sterile container, mix properly. Pour sample back into original 120 mL container to the 100 mL line, proceed with analysis.

Check for excess Cl₂ by placing a small amount of DPD powder on a white sheet of printer paper. Using a sterile pipette, aseptically pull sample from container and place 1 drop of sample onto the designated DPD powder. If the sample and powder turns any shade of pink to dark red the sample has excess Cl₂ and must be noted on the batch sheet. If no color change, proceed to sample prep.

When checking for excess chlorine, if the sample container is filled too full, use the remaining sample left in the larger container to pull sample from. If the sample container is not filled too full, aseptically pull only a drop of sample from the container.

14.2 Sample Preparation

- Snap open the contents of one packet Colilert® and add contents to a 100mL sample.
 - Be careful not to touch the score mark on the packet and only handle the bottom and top portions.

- Using a container that has been pre-preserved with sodium thiosulfate for chlorinated samples
- Cap sample vessel and shake until the contents are dissolved.
- Pour sample/media mixture into a pre-labeled Quanti-Tray and seal using the IDEXX Quanti-Tray Sealer.
 - (If the sample plus media exhibits color changes before incubation discard the media and use another lot.)
 - If no color changes occur before incubation continue as normal.
- Place the sealed tray in a $35.0 \pm 0.5^{\circ}\text{C}$ incubator for 24 hours.
 - Note the time and date the incubation period started.
 - Approximately 4 hours after incubation has started, check the temperature of the incubator and document this check on the batch sheet in the appropriate place.

14.2.1 Interpretation

After 24 hours, remove the sample from the incubator, note the date, time and temperature removed from the incubator and compare to the IDEXX Comparator for Total coliform and the UV light for E. coli. Samples are read immediately after the incubation period and results documented on the batch sheet.

If the sample is clear, or no color change is noted, then the sample is negative for Total coliforms, including E. coli. If the sample exhibits a yellow color that is lighter than the comparator, then incubate the sample for an additional 4 hours at $35.0 \pm 0.5^{\circ}\text{C}$. After a total of 28 hours, if the sample is lighter than the comparator then the sample is negative for Total coliforms and E. coli. Do not incubate longer than 28 hours.

If the sample has turned a yellow equal to or darker than the comparator, then the sample is positive for Total coliforms. The sample can now be checked for E. coli using the UV light. Record the number of yellow wells on the batch sheet.

In a dark box, use the UV lamp to detect the presence of E. coli. If yellow wells fluoresce, the sample is positive for E. coli. Do not count wells that did not turn yellow. Record the number of wells where fluorescence was observed.

Results interpretation after incubation is summarized according to the Table 1:

Table 1

Appearance	Result
Less yellow than the comparator	Negative for Total coliforms and E. coli
Yellow $\geq$ the comparator	Positive for Total coliforms
Yellow $\geq$ the comparator and fluorescence	Positive for E. coli

14.3 Results

Colilert® results are definitive at 24 hours. Additionally, positives for both total coliforms and E. coli observed before 24 hours and negatives observed after 28 hours are also valid.

15. Calculations

Use the manufacturer's comparator to determine positive for total coliforms and then use the MPN Table, for the quantitation of Total coliform and for E. coli when the sample fluoresces. Record the number of small and large wells exhibiting yellow color or fluorescence.

16. Instrument Maintenance

As warranted, perform instrument maintenance as described in this standard operating procedure for the sealer and UV lamp.

17. Method Performance

The following method performance procedures shall be performed:

17.1 Demonstration of Capability

The laboratory analyst shall initially demonstrate their capability by analyzing 4 QC samples obtained from a reputable vendor. Record the results in the appropriate laboratory records. Results must demonstrate acceptability as noted on the certificate of compliance or documents forwarded by the vendor. Ongoing demonstrations of capability must be performed annually thereafter using a Performance Testing sample. The laboratory shall maintain documentation of these test performances for a minimum of 5 years.

18. Pollution Prevention

The preparation of excessive volumes of laboratory reagents and standards shall be avoided so that waste and potential for pollution are minimized. Samples, reagents and standards shall be disposed in compliance with the laboratory waste disposal program and applicable waste disposal regulations.

19. Corrective Actions for Out-Of-Control Data

19.1 Positive Controls

If a positive control does not exhibit the predicted result or anticipated outcome, reanalyze with new fresh media. If the results are the same, (no fluorescence) then the control organisms may be suspect. Obtain new organisms and reanalyze. If the result exhibited is different (anticipated results are confirmed) then the initial media was suspect.

19.2 Negative Controls

Negative controls demonstrate that the media used does not support the growth of non-target organisms. If fluorescence is exhibited, the media may be suspect. Obtain new media and reanalyze the negative control.

19.3 Media Check

All growth media must be checked to assure that the target organisms respond in an acceptable and predictable manner. If the analysis of the blank, positive and negative controls do not conform to predictable or acceptable results, discard the media and obtain new media. Analyze the media using the controls for acceptability. All new lots of media must be verified for compliance before use and again Quarterly if the same lot is still in use.

19.4 Dilution Water Sterility Check

If the sterile dilution water control checks exhibit faint fluorescence or faint positive coliform results, the dilution water is not appropriate for use in the analysis using this method. Obtain new dilution water and perform the dilution water check. The process for handling corrective actions is found in the laboratory Quality Manual.

20. Contingencies for Handling Out-Of-Control or Unacceptable Data

The process for handling unacceptable and out of control data is found in the laboratory's Quality Manual. The reporting of data that is out of control must be approved and documented by the Technical Director.

21. Waste Management

The laboratory's Waste Management Plan identifies proper waste management practices for the chemicals, reagents and byproducts generated during its normal operations. Additionally, samples are stored and discarded accordance with procedures indicated in the laboratory's Waste Management Program.

All negative Total coliforms and E. coli samples may be poured down the sanitary sewer system and sample containers are crushed before being placed in the dumpster.

All positive (Quanti – Tray) samples will be autoclaved for a minimum of 30 minutes in an autoclave set at 121°C until sterile.

All positive (Drinking Water) samples will be disinfected with bleach for a minimum of 5 minutes.

Refer to SOP: Sterilization of Total Coliforms, E.coli, Fecal Coliforms, and Heterotrophic Bacteria Samples Using an Autoclave and Disinfection of Total Coliforms and E.coli in Drinking Water Bleach (Sodium Hypochlorite)

22. References

Standard Methods for the Examination of Water and Wastewater, 23rd.

SM9223B-2004

SM9223B-2016

IDEXX Laboratories, Inc, Colilert® Method and Colilert-18®, Quanti-Tray.

40 CFR Parts 136 and 141, Current Edition.

Manual for the Certification of Laboratories Analyzing Drinking Water, 5th Edition, EPA 815-R-05-004, January 2005.

Supplement 1 to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water, EPA 815-F -08-006, June 2008.

23. Tables, Diagrams, Flowcharts and Validation Data

Refer to the attachments in the Appendix of this standard operating procedure.

24. Revision History

Date	Revision	Comments
01/30/09	MS.9223B.01	Initial Document
05/12/14	9223B Colilert EC TC SOP 051214	Revised SOP
08/21/14	9223B Colilert EC TC SOP 082114	Revisions based on Internal Audit
07/20/15	9223B Colilert EC TC SOP 082114A	Name Change DRF 072015A
12/07/15	9223B Colilert EC TC SOP 120715	Annual Review
12/21/16	9223B Colilert EC TC SOP 122616	Annual Review
03/09/17	9223B Colilert EC TC SOP 030917	Amendments to Document
05/30/17	9223B Colilert EC TC SOP 030917A	Amendments to Document
07/26/17	9223B Colilert EC TC SOP 072617	Amendments based on A2LA-LELAP Audit July 2017
10/03/17	9223B Colilert EC TC SOP 072617A	DRF 100317A
12/06/17	9223B Colilert EC TC SOP 120617	Update and Annual Review
06/29/18	9223B Colilert EC TC SOP 120617A	Minor Updates



9223 B Determination of E. coli and Total coliforms 070121

Effective Date: 7/1/21

08/14/18	9223B Colilert EC TC SOP 081418	A2LA Surveillance Audit Updates
08/31/18	9223B Colilert EC TC SOP 081418A	DRF 083118A
05/08/19	9223B Colilert EC TC SOP 050819	Updates and Annual Review
06/06/19	9223B Colilert EC TC SOP 050819A	DRF 060619A
07/11/19	9223B Colilert EC TC SOP 071119	A2LA Audit Updates
02/28/20	9223B Colilert EC TC SOP 022820	Addition for Presence / Absence Testing
06/30/20	9223B Colilert EC TC SOP 022820A	DRF 063020A
07/01/21	9223B Colilert EC TC SOP 070121	Update Standard Method 2016

25. Addendum 1

Analysis of Fecal Coliforms using Colilert-18® in Wastewater

Background: The EPA Region 4 Science and Ecosystem Support Division et al., approves the use of IDEXX Colilert-18® for the detection and enumeration of E coli including fecal coliforms in wastewater samples as an Alternative Test Procedure (ATP) until it has been formally promulgated nationally as part of 40 CFR Part 136 under the Clean Water Act.

This laboratory has contacted EPA Region 4 and 6 in seeking interim use of this product and has submitted a proposal letter to Region 4. See letter in QA files.

Procedure: This ATP is only for Colilert-18® for the analysis of fecal coliforms in wastewater.

Summary of Procedure: A 100-mL wastewater sample is mixed with Colilert-18® media and incubated at $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ for 18 hours. If fecal coliforms are present they will metabolize the nutrient indicator ONPG producing a yellow color. This method can detect these bacteria at 1 MPN/100 mL. Fecal coliform samples are quantified using the manufacturer's Most Probable Number Table and results are quantified as MPN/100mL.

Procedure: For the quantification of Fecal coliforms perform the following procedure:

1. Aseptically add one Colilert-18® packet to 100-mL wastewater sample that is at room temperature and mix well.
2. If foaming is observed add IDEXX Antifoam reagent to reduce foaming or allow sample to sit for a few minutes to allow the foam to dissipate.
3. Pour the entire sample/reagent mixture into a Quanti-Tray and seal with the IDEXX Quanti-Tray Sealer.
4. Incubate the sealed tray in a $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ water bath for 18 hours but no more than 22 hours. Record both the start date/time and temp on the batch sheet. At approximately 4 hours after incubation has started, check the temperature of the water bath and record both the date/time and temp on the batch sheet. Also record the end date/time and temp on the batch sheet.
5. After the incubation time period is over, read results according to the MPN Table. Yellow wells indicate positive fecal coliforms. Count the number of positive wells and refer to the table provided by IDEXX for quantification. Less yellow than the comparator is negative for fecal coliforms. Fluorescence verification is not required.

Quality Control: Follow the QC procedures detailed in the Colilert® procedure found with in this operating procedure.

If the sample has turned a yellow equal to or darker than the comparator, then the sample is positive for Fecal coliforms. Record the number of yellow wells on the batch sheet.

Results interpretation after incubation is summarized according to the Table 2:

Table 2

Appearance	Result
Less yellow than the comparator	Negative for Fecal coliforms
Yellow $\geq$ the comparator	Positive for Fecal coliforms

26. Addendum 2

Analysis of Total coliforms and E. coli in water using Colilert-18®

Procedure: This option will only be used if a sample is delivered to the lab late in the day and the analyst will not be available to read the results in 24-28 hours using the Colilert® media.

Summary of Procedure: A 100-mL sample is mixed with Colilert-18 ® media and incubated at $35.0 \pm 0.5^{\circ}\text{C}$ for 18 hours. If Total coliforms are present they will metabolize the nutrient indicator, OPNG, and the sample will exhibit a color change to yellow. Further, if E. coli is present the fluorogenic substrate MUG is metabolized and the sample will fluoresce when exposed to a UV light source at 365 nm, as specified by the manufacturer. This method can simultaneously detect these bacteria at 1 MPN/100 mL within 18 hours.

Procedure: For the quantification of Total coliforms and E. coli perform the following procedure:

1. Aseptically add one Colilert-18® packet to 100-mL sample and mix well.
2. If foaming is observed add IDEXX Antifoam reagent to reduce foaming or allow sample to sit for a few minutes to allow the foam to dissipate.
3. Pour the entire sample/reagent mixture into a Quanti-Tray and seal with the IDEXX Quanti-Tray Sealer.
4. Incubate the sealed tray in a $35.0 \pm 0.5^{\circ}\text{C}$ dry air incubator for 18 hours but no more than 22 hours. Record both the start date/time and temp on the batch sheet. At approximately 4 hours after incubation has started, check the temperature of the incubator and record both the date/time and temp on the batch sheet. Also record the ending date/time and temp on the batch sheet.
5. After the incubation time period is over, read results according to the MPN Table.
 - a. Yellow wells indicate positive total coliforms.
 - b. Fluorescent wells indicate positive E. coli
6. Count the number of positive wells and refer to the table provided by IDEXX for quantification. Less yellow/fluorescence than the comparator is negative for total coliforms and E.coli.

In addition, positives for both total coliforms and E. coli observed before 18 hours and negatives observed after 22 hours are also valid.

If the sample has turned a yellow equal to or darker than the comparator, then the sample is positive for Total coliforms. The sample can now be checked for E. coli using the UV light. Record the number of yellow wells on the batch sheet.

In a dark box, use the UV lamp to detect the presence of E. coli. If yellow wells fluoresce, the sample is positive for E. coli. Do not count wells that did not turn yellow. Record the number of wells where fluorescence was observed.

Quality Control: Follow the QC procedures detailed in the Colilert® procedure found with in this operating procedure.

Results interpretation after incubation is summarized according to Table 1:

Table 1

Appearance	Result
Less yellow than the comparator	Negative for Total coliforms and E. coli
Yellow $\geq$ the comparator	Positive for Total coliforms
Yellow $\geq$ the comparator and fluorescence	Positive for E. coli

27. Addendum 3

Analysis of Total coliforms and E. coli in drinking water using Presence/Absence.

Procedure: All drinking water samples, unless requested otherwise by the client, will be analyzed for Total coliforms and E. coli using P/A.

Summary of Procedure: A 100-mL sample is mixed with Colilert® media and incubated at $35.0 \pm 0.5^\circ\text{C}$ for 24 hours. If Total coliforms are present they will metabolize the nutrient indicator, OPNG, and the sample will exhibit a color change to yellow. Further, if E. coli is present the fluorogenic substrate MUG is metabolized and the sample will fluoresce when exposed to a UV light source at 365 nm, as specified by the manufacturer. Samples will be recorded as either Present or Absent for Total coliforms and E. coli.

Procedure: For the P/A analysis of Total coliforms and E. coli in drinking water perform the following procedure:

1. Aseptically add one Colilert® packet to 100-mL sample and mix well.
 - a. If using Colilert-18®, the sample must be pre-warmed to room temperature before analysis.
 - i. Pre-warm samples after adding Colilert-18® but before placing them in the incubator.
 1. To pre-warm the samples place them in a $44.5 \pm 0.2^\circ\text{C}$ water bath for 7 to 10 minutes to bring it to incubator temperature.
 - ii. If foaming is observed add IDEXX Antifoam reagent to reduce foaming or allow sample to sit for a few minutes to allow the foam to dissipate.
2. Incubate the sample in a $35.0 \pm 0.5^\circ\text{C}$ dry air incubator for 24 hours but no more than 28 hours. Record both the start date/time and temp on the batch sheet.
 - a. If using Colilert-18®, incubate for 18-22 hours
3. At approximately 4 hours after incubation has started, check the temperature of the incubator and record both the date/time and temp on the batch sheet. Also record the ending date/time and temp on the batch sheet.
4. After the incubation time period is over, read results according to the Table below.
 - a. Yellow sample indicate positive total coliforms.
 - b. Fluorescent sample indicate positive E. coli
 - c. Less yellow/fluorescence than the comparator is negative for total coliforms and E. coli.
5. Record results as either Present or Absent for Total coliforms and E. coli.

In addition, positives for both total coliforms and E. coli observed before 24 hours and negatives observed after 28 hours are also valid.

The same applies for Colilert-18® at 18 and 22 hours.

If the sample has turned a yellow equal to or darker than the comparator, then the sample is positive for Total coliforms. The sample can now be checked for E. coli using the UV light. Record the results on the batch sheet.

In a dark box, use the UV lamp to detect the presence of E. coli. If yellow samples fluoresce, the sample is positive for E. coli. Do not count samples that did not turn yellow. Record the results based on if fluorescence was observed.

Quality Control: Follow the QC procedures detailed in the Colilert® procedure found with in this operating procedure for Drinking Water Samples.

Results interpretation after incubation is summarized according to Table 1:

Table 1

Appearance	Result
Less yellow than the comparator	Negative for Total coliforms and E. coli
Yellow $\geq$ the comparator	Positive for Total coliforms
Yellow $\geq$ the comparator and fluorescence	Positive for E. coli

Addendum to Standard Operating Procedure

When conducting compliance analyses the following essential QA/QC elements, as detailed in 40 CFR 136.7 effective August 28, 2017, are required in this standard operating procedure unless it has been check-marked in this addendum that the specific element 1) Does not apply to the analytical method or 2) Is inappropriate for the method or is impossible to perform. These elements do not apply to Part 136 approved methods previously containing or referencing QA/QC procedures:

- ☐ Demonstration of Capability (DOC)
- ☒ Method Detection Limit (MDL)
- ☐ Laboratory Reagent Blank (LRB)/Method Blank (MB)
- ☒ Laboratory Fortified Blank (LFB)/Laboratory Control Sample (LCS)
- ☒ Matrix Spike/Matrix Spike Duplicate
- ☒ Internal Standards, Surrogates or Tracers (radiochemistry)
- ☒ Calibration
- ☒ Control Charts
- ☐ Corrective Action
- ☐ QC Acceptance Criteria
- ☐ Preparation and Analytical Batches
- ☐ QC Frequency

Laboratory Additional Requirement

- ☐ Sample Duplicate and Frequency

MPN Table

# Large Wells Positive	IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)																								
	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.7	76.7	78.7	80.7	82.7	84.7	86.8	88.9	91.1	93.2	95.3	97.4	99.6	101.7
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.3	133.1	137.9	143.0	148.3	153																		

# Large Wells	IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)																																															
	# Small Wells Positive																																															
	Positive	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48																							
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5																								
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2																								
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8																								
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5																								
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3																								
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1																								
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9																								
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8																								
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8																								
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8																								
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9																								
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1																								
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4																								
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7																								
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1																								
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6																								
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2																								
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9																								
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8																								
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7																								
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8																								
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1																								
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5																								
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1																								
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9																								
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.1	110.0	111.9																								
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2																								
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7																								
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6																								
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8																								
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4																								
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5																								
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1																								
33	100.0	102.2	104.4	106.6	108.8	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4																								
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.3	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4																								
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3																								
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3																								
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7																								
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7																								
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0																								
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1																								
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.6	251.5	257.2	263.1																								
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5																								
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7																								
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4																								
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4																								
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.6	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5																								
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0																								
48	344.1	360.9	378.4	396.6	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2																								
49	481.1	488.4	517.2	547.5	579.6	613.1	648.8	686.7	727.0	770.1	816.4	866.6	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	-2419.6																								

19-63235-01

Shipment Receipt Form

Customer Number: **10536**

Customer Name: **EPA Region IV**

Report Number: **23-166-0055**

Shipping Method

☐ Fed Ex	☐ US Postal	☐ Lab	☐ Other :	
☐ UPS	☐ Client	☒ Courier	Thermometer ID:	NA

Shipping container/cooler uncompromised?	☒ Yes	☐ No	
Number of coolers/boxes received	1		
Custody seals intact on shipping container/cooler?	☐ Yes	☐ No	☒ Not Present
Custody seals intact on sample bottles?	☐ Yes	☐ No	☒ Not Present
Chain of Custody (COC) present?	☒ Yes	☐ No	
COC agrees with sample label(s)?	☒ Yes	☐ No	
COC properly completed	☒ Yes	☐ No	
Samples in proper containers?	☒ Yes	☐ No	
Sample containers intact?	☒ Yes	☐ No	
Sufficient sample volume for indicated test(s)?	☒ Yes	☐ No	
All samples received within holding time?	☒ Yes	☐ No	
Cooler temperature in compliance?	☒ Yes	☐ No	
Cooler/Samples arrived at the laboratory on ice. Samples were considered acceptable as cooling process had begun.	☒ Yes	☐ No	
Water - Sample containers properly preserved	☒ Yes	☐ No	☐ N/A
Water - VOA vials free of headspace	☐ Yes	☐ No	☒ N/A
Trip Blanks received with VOAs	☐ Yes	☐ No	☒ N/A
Soil VOA method 5035 – compliance criteria met	☐ Yes	☐ No	☒ N/A
☐ High concentration container (48 hr)	☐ Low concentration EnCore samplers (48 hr)		
☐ High concentration pre-weighed (methanol -14 d)	☐ Low conc pre-weighed vials (Sod Bis -14 d)		
Special precautions or instructions included?	☐ Yes	☒ No	

Comments:

Signature:

Carleen Qualls

Date & Time:

06/15/2023 10:57:06

CHAIN OF CUSTODY RECORD

Site #: 23-0274

Cooler #:

CarrierName:

Contact Name:

Lab: Region 4 Lab

AirbillNo:

Contact Phone:

Lab Phone: 706-355-8856

[illegible]

Special Instructions:

SAMPLES TRANSFERRED FROM

CHAIN OF CUSTODY #

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	CL M82	6/14/23	KL Stoney/WPA	6/14/23 1808	Good on ice
	KL Stoney	6/14/23		6/15/23 1610	



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Region 4 Laboratory Services and Applied Science Division
980 College Station Road, Athens, Georgia 30605-2700
D.A.R.T. Id: 23-0274
Project: 23-0274, Knott County WWTP Recon - Reported by Kristin Trapp

June 28, 2023

MEMORANDUM

SUBJECT: FINAL Analytical Report
Project: 23-0274, Knott County WWTP Recon

FROM: Kristin Trapp
LSB Inorganic Chemistry Section Chief

THRU: Stacie Masters, Chief
Laboratory Services Branch

TO: Christopher McHugh

Attached are the final results for the analytical groups listed below. This report shall not be reproduced except in full without approval of the Region 4 laboratory. These analyses were performed in accordance with the Laboratory Services Branch's Laboratory Operations and Quality Assurance Manual (LSB LOQAM) found at www.epa.gov/region4/sesd/asbsop. Any unique project data quality objectives specified in writing by the data requestor have also been incorporated into the data unless otherwise noted in the Report Narrative. Chemistry data have been verified based on the LSB LOQAM specifications and have been qualified by this laboratory if the applicable quality control criteria were not met. Verification is defined in Chapter 5 of the LSB LOQAM. For a listing of specific data qualifiers and explanations, please refer to the Data Qualifier Definitions included in this report. The reported results are accurate within the limits of the method(s) and are representative only of the samples as received by the laboratory.

Analyses Included in this report:

Method Used:

Accreditations:

Classical/Nutrient Analyses (CNA)

Demand
Solids

SM 5210B (Water)
USGS I-3765-85 (Water)

ISO
ISO



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Sample Disposal Policy

Due to limited space for long term sample storage, LSB's policy is to dispose of samples on a periodic schedule. Air samples collected in summa canisters will be disposed of 30 days following the issuance of this report. All other sample media including original samples, sample extracts and or digestates will be disposed of, in accordance with applicable regulations, 60 days from the date of this report.

This sample disposal policy does not apply to criminal samples which are held until the laboratory is notified by the criminal investigators that case development and litigation are complete.

These samples may be held in the laboratory's custody for a longer period of time. If samples require storage beyond the 60-day period, please contact the Sample Control Coordinator by e-mail at R4SampleCustody@epa.gov.



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SAMPLES INCLUDED IN THIS REPORT

Project: 23-0274, Knott County WWTP Recon

Sample ID	Laboratory ID	Matrix	Date Collected	Date Received
EFF001-0614	E232409-01	Wastewater	6/14/23 10:45	6/15/23 12:05



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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D.A.R.T. Id: 23-0274

Project: 23-0274, Knott County WWTP Recon - Reported by Kristin Trapp

DATA QUALIFIER DEFINITIONS

None

ACRONYMS AND ABBREVIATIONS

CAS Chemical Abstracts Service

Note: Analytes with no known CAS identifiers have been assigned codes beginning with "E", the EPA ID as assigned by the EPA Substance Registry System (www.epa.gov/srs), or beginning with "R4-", a unique identifier assigned by the EPA Region 4 laboratory.

MDL Method Detection Limit - The minimum concentration of a substance (an analyte) that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero.

MRL Minimum Reporting Limit - Analyte concentration that corresponds to the lowest demonstrated level of acceptable quantitation. The MRL is sample-specific and accounts for preparation weights and volumes, dilutions, and moisture content of soil/sediments.

TIC Tentatively Identified Compound - An analyte identified based on a match with the instrument software's mass spectral library. A calibration standard has not been analyzed to confirm the compound's identification or the estimated concentration reported.

ACCREDITATIONS:

ISO Accredited to ISO/IEC 17025:2017 and accreditation requirements for Forensic Science Testing Laboratories.

Refer to the certificate and scope of accreditation FT-0330 at:

<http://www.epa.gov/aboutepa/about-region-4s-science-and-ecosystem-support-division-sesd>

NR Not accredited for this test.

DW Accredited for conformance with ISO/IEC 17025:2017 and testing elements in the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water, EPA 815-R-05-004, 2005.

Refer to the certificate and scope of accreditation AT-2628 at:

<http://www.epa.gov/aboutepa/about-region-4s-science-and-ecosystem-support-division-sesd>

ISO/DW Accredited to ISO/IEC 17025:2017 and accreditation requirements for Forensic Science Testing Labs, and conformance with ISO/IEC 17025:2017 and testing elements in the Manual for the Certification of Laboratories Analyzing Drinking Water.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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D.A.R.T. Id: 23-0274
Project: 23-0274, Knott County WWTP Recon - Reported by Kristin Trapp

Classical/Nutrient Analyses

Project: 23-0274, Knott County WWTP Recon

Sample ID: EFF001-0614

Lab ID: E232409-01

Station ID: EFF001

Matrix: Wastewater

Date Collected: 6/14/23 10:45

<i>CAS Number</i>	<i>Analyte</i>	<i>Results</i>	<i>Qualifiers</i>	<i>Units</i>	<i>MRL</i>	<i>Prepared</i>	<i>Analyzed</i>	<i>Method</i>
E1640614	BOD, 5 Day, Carbonaceous	39		mg/L	2.0	6/15/23 13:57	6/20/23 11:00	SM 5210B
E1642818	Total Suspended Solids	36		mg/L	5.0	6/20/23 7:27	6/20/23 7:27	USGS I-3765-85



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 D.A.R.T. Id: 23-0274
 Project: 23-0274, Knott County WWTP Recon - Reported by Kristin Trapp

Classical/Nutrient Analyses (CNA) - Quality Control

US-EPA, Region 4, LSASD

Analyte	Result	Reporting Limit	Units	Spike Level	Source Result	%REC	%REC Limits	RPD	RPD Limit	Notes
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Batch 2306030 - C SM5210 BOD

Blank (2306030-BLK1)

Prepared: 06/15/23 Analyzed: 06/20/23

SM 5210B

BOD, 5 Day, Carbonaceous	U	2.0	mg/L							U
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LCS (2306030-BS1)

Prepared: 06/15/23 Analyzed: 06/20/23

SM 5210B

BOD, 5 Day, Carbonaceous	150.80	2.0	mg/L	170.00		88.7	47.5-152.3			A
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Batch 2306032 - C 2540 Solids

Blank (2306032-BLK1)

Prepared & Analyzed: 06/20/23

USGS I-3765-85

Total Suspended Solids	U	5.0	mg/L							U
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LCS (2306032-BS1)

Prepared & Analyzed: 06/20/23

USGS I-3765-85

Total Suspended Solids	100.90	5.0	mg/L	100.00		101	77.1-110			
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LCS Dup (2306032-BSD1)

Prepared & Analyzed: 06/20/23

USGS I-3765-85

Total Suspended Solids	103.00	5.0	mg/L	100.00		103	77.1-110	2.06	10	
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Duplicate (2306032-DUP1)

Source: E232409-01

Prepared & Analyzed: 06/20/23

USGS I-3765-85

Total Suspended Solids	33.600	5.0	mg/L		35.900			6.62	10	
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MRL Verification (2306032-PS1)

Prepared & Analyzed: 06/20/23

USGS I-3765-85

Total Suspended Solids	3.9000	5.0	mg/L	5.0000		78.0	57.1-130			MRL-2, U
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Region 4 Laboratory Services and Applied Science Division
980 College Station Road, Athens, Georgia 30605-2700

D.A.R.T. Id: 23-0274

Project: 23-0274, Knott County WWTP Recon - Reported by Kristin Trapp

Notes and Definitions for QC Samples

- U The analyte was not detected at or above the reporting limit.
- A The analyte was analyzed in replicate. Reported value is an average value of the replicates.
- MRL-2 MRL verification for Non-Potable Water matrix

**KENTUCKY POLLUTANT
DISCHARGE ELIMINATION
SYSTEM****FACT SHEET****KPDES No.:** KY0042854**AI No.:** 2527

Caney Creek WWTP

7034 KY 899

Pippa Passes, Knott County, Kentucky

Date: May 15, 2019**Public Notice Information**

Public Notice Start Date: March 20, 2019

Comment Due Date: April 19, 2019

General information concerning the public notice process may be obtained on the Division of Water's Public Notice Webpage at the following address:

<https://eec.ky.gov/Environmental-Protection/Water/Pages/Water-Public-Notices-and-Hearings.aspx>.

Public Notice Comments

Comments must be received by the Division of Water no later than 4:30 PM on the closing date of the comment period. Comments may be submitted by e-mail at: DOWPublicNotice@ky.gov or written comments may be submitted to the Division of Water at 300 Sower Blvd, Frankfort, Kentucky 40601.

Reference Documents

A copy of this proposed fact sheet, proposed permit, the application, other supporting material and the current status of the application may be obtained from the Department for Environmental Protection's Pending Approvals Search Webpage:

http://dep.gateway.ky.gov/eSearch/Search_Pending_Approvals.aspx?Program=Wastewater&NumDaysDoc=30.

Open Records

Copies of publicly-available documents supporting this fact sheet and proposed permit may also be obtained from the Department for Environmental Protection Central Office. Information regarding these materials may be obtained from the Open Records Coordinator at (502) 782-6849 or by e-mail at EEC.KORA@ky.gov.

THIS KPDES FACT SHEET CONSISTS OF THE FOLLOWING SECTIONS:

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SECTION 1

FACILITY SYNOPSIS

1. FACILITY SYNOPSIS**1.1. Name and Address of Applicant**

Knott County Water and Sewer District
7777 Big Branch Rd
Vicco, Kentucky 41773

1.2. Facility Location

Caney Creek WWTP
7034 KY 899
Pippa Passes, Knott County, Kentucky

1.3. Description of Applicant's Operation

Publicly-Owned Treatment Works (POTW) and associated collection system.

1.4. Wastewaters Collected and Treatment

The following table lists the actual average flow reported, the facility's approved long-term average design treatment capacity, the wastewater types collected, and the treatment type for each outfall:

TABLE 1.				
Outfall No.	Avg. Flow (MGD)	Design Capacity (MGD)	Wastewater Types Collected	Treatment Type
001	0.114	0.100	Domestic (Sanitary) Wastewater	Mixing, aeration, clarification, chlorine disinfection, dechlorination, and discharge to surface water. Solids are aerobically digested, dewatered and landfilled.

1.5. Permitting Action

This is a reissuance of a minor KPDES permit for an existing POTW [SIC Code 4952]. This renewal removes monitoring requirements for Hardness, Calcium, Copper, Lead and Zinc.

SECTION 2

RECEIVING/INTAKE WATERS

2. RECEIVING / INTAKE WATERS

2.1. Receiving Waters

All surface waters of the Commonwealth have been assigned stream use designations consisting of one or more of the following designations: Warmwater Aquatic Habitat (WAH), Primary Contact Recreation (PCR), Secondary Contact Recreation (SCR), Domestic Water Supply (DWS), Coldwater Aquatic Habitat (CAH) or Outstanding State Resource Water (OSRW)[401 KAR 10:026].

All surface waters of the Commonwealth are assigned one of the following antidegradation categories: Outstanding National Resource Water (ONRW), Exceptional Water (EW), Impaired Water (IW) or High Quality Water (HQ)[401 KAR 10:030].

Surface waters categorized as an IW are listed in Kentucky's most recently approved Integrated Report to Congress on the Condition of Water Resources in Kentucky - Volume II. 303(d) List of Surface Waters.

The following table lists the stream use classifications associated with this permit.

TABLE 2.				
Receiving Water Name	Use Designation	Antidegradation Category	7Q10 Low Flow (cfs)	Harmonic Mean Flow (cfs)
Caney Fork	WAH, FC, PCR, SCR	IW	0.0	0.49
This segment of Caney Fork (mile point 7.5 to 11.6) is listed as impaired in the 2016 303(d) List of Waters for Kentucky. Impaired uses are Warm Water Aquatic Habitat (nonsupport). The pollutants of concern are Organic Enrichment (Sewage) Biological Indicators, Nutrient/Eutrophication Biological Indicators, Specific Conductance, and Total Dissolved Solids. The suspected sources are Municipal Point Source Discharges and Coal Mining, Petroleum/natural Gas Activities. Caney Fork (mile point 0.0 to 7.5) is listed as impaired in the 2016 303(d) List of Waters for Kentucky. Impaired uses are Warm Water Aquatic Habitat (nonsupport). The pollutants of concern are Nutrient/Eutrophication Biological Indicators, Specific Conductance, and Total Dissolved Solids. The suspected sources are Package Plant or Other Permitted Small Flows Discharges and Coal Mining, Package Plant or Other Permitted Small Flows Discharges, Petroleum/natural Gas Activities. A TMDL was issued in 2010 for e. Coli in the Beaver Creek Watershed which includes this segment and includes a wasteload allocation for this WWTP.				

2.2. Intake Waters – Nearest Downstream Intake

TABLE 3.						
Intake Water Name	Public Water Supply Name	Latitude (N) Decimal Degrees	Longitude (W) Decimal Degrees	Miles Downstream	7Q10 Low Flow (cfs)	Harmonic Mean Flow (cfs)
Prestonsburg WTP	Prestonsburg City Utilities	37.667489°	82.743964°	46.3	169	683

SECTION 3

OUTFALL 001

3. OUTFALL 001

3.1. Outfall Description

The following table lists the outfall type, location, and description:

TABLE 4.				
Outfall Type	Latitude (N)	Longitude (W)	Receiving Water	Description of Outfall
External	37.350556°	82.862583°	Caney Fork	Domestic Wastewater from a Publicly Owned Treatment Works

3.2. Reported Values

The following table summarizes the reported values for Outfall 001:

TABLE 5.							
Reported Parameters	Units	EFFLUENT					
		Loadings (lbs./day)		Concentrations			
		Monthly Average	Maximum Weekly Average	Minimum	Monthly Average	Maximum Weekly Average	Maximum
Flow, Effluent	MGD	0.114	0.180 ⁸	N/A	N/A	N/A	N/A
pH	SU	N/A	N/A	6.05	N/A	N/A	8.75
CBOD ₅ ² , Effluent	mg/l	9.1	16.4 ⁸	N/A	8.9	15.0 ⁸	N/A
CBOD ₅ ² , Influent	mg/l	N/A	N/A	N/A	138.0	224.2 ⁸	N/A
CBOD ₅ ² , Percent Removal	%	N/A	N/A	N/A	93.1	N/A	N/A
TSS ³ , Effluent	mg/l	13.0	21.9 ⁸	N/A	12.1	19.4 ⁸	N/A
TSS ³ , Influent	mg/l	N/A	N/A	N/A	119.0	198.1 ⁸	N/A
TSS ³ , Percent Removal	%	N/A	N/A	N/A	86.3	N/A	N/A
Ammonia (as mg/l NH ₃ N)							
May 1 – October 31	mg/l	N/A	N/A	N/A	7.5	11.8 ⁸	N/A
November 1 – April 30	mg/l	N/A	N/A	N/A	6.7	11.2 ⁸	N/A
Dissolved Oxygen	mg/l	N/A	N/A	4.20	N/A	N/A	N/A
E. Coli ⁴	#/100 ml	N/A	N/A	N/A	205 ⁵	8197 ⁶	N/A
Total Residual Chlorine	mg/l	N/A	N/A	N/A	0.010	.058 ⁸	N/A
Total Nitrogen ⁷ , Effluent	mg/l	N/A	N/A	N/A	10.9	17.4 ⁸	N/A
Total Phosphorus, Effluent	mg/l	N/A	N/A	N/A	1.43	2.49 ⁸	N/A
Hardness (as mg/l CaCO ₃)	mg/l	N/A	N/A	N/A	178.9	248.6 ⁸	N/A

TABLE 5.							
Reported Parameters	Units	EFFLUENT					
		Loadings (lbs./day)		Concentrations			
		Monthly Average	Maximum Weekly Average	Minimum	Monthly Average	Maximum Weekly Average	Maximum
Cadmium, total recoverable	mg/L	N/A	N/A	N/A	0.000085	0.000085 ¹	N/A
Copper, total recoverable	mg/L	N/A	N/A	N/A	0.00365	0.00365 ¹	N/A
Lead, total recoverable	mg/L	N/A	N/A	N/A	0.0012	0.0012 ¹	N/A
Zinc, total recoverable	mg/L	N/A	N/A	N/A	0.0186	0.0186 ¹	N/A
¹ Daily Maximum							
² CBOD ₅ – Carbonaceous Biochemical Oxygen Demand, 5-day							
³ Total Suspended Solids							
⁴ E. Coli – Escherichia Coli Bacteria							
⁵ Thirty (30) day Geometric Mean							
⁶ Seven (7) day Geometric Mean							
⁷ Total Nitrogen is the summation of the analytical results for Total Nitrates, Total Nitrites, and Total Kjeldahl Nitrogen							
⁸ Weekly Average							

The above values are based on 5-year DMR averages from 2/28/2014 to 01/31/2019.

3.3. Effluent Limitations and Monitoring Requirements

The following table summarizes the effluent limitations and monitoring requirements for Outfall 001:

TABLE 6.									
EFFLUENT LIMITATIONS								MONITORING REQUIREMENTS	
Effluent Characteristic	Units	Loadings (lbs/day)		Concentrations				Frequency	Sample Type
		Monthly Average	Maximum Weekly Average	Minimum	Monthly Average	Maximum Weekly Average	Maximum		
Flow, Effluent	MGD	Report	Report ¹	N/A	N/A	N/A	N/A	Continuous	Recorder
Flow, Influent	MGD	Report	Report ¹	N/A	N/A	N/A	N/A	Continuous	Calculated
pH	SU	N/A	N/A	6.0	N/A	N/A	9.0	1/Week	Grab
CBOD ₅ ² , Effluent	mg/l	21	34	N/A	25	40	N/A	1/Week	24-Hr Composite ³
CBOD ₅ ² , Influent	mg/l	N/A	N/A	N/A	Report	Report	N/A	1/Week	24-Hr Composite ³

TABLE 6.

EFFLUENT LIMITATIONS								MONITORING REQUIREMENTS	
Effluent Characteristic	Units	Loadings (lbs/day)		Concentrations				Frequency	Sample Type
		Monthly Average	Maximum Weekly Average	Minimum	Monthly Average	Maximum Weekly Average	Maximum		
CBOD ₅ ² Percent Removal ⁴	%	N/A	N/A	N/A	85	N/A	N/A	1/Month	Calculated ⁴
TSS ⁵ , Effluent	mg/l	26	38	N/A	30	45	N/A	1/Week	24-Hr Composite ³
TSS ⁵ , Influent	mg/l	N/A	N/A	N/A	Report	Report	N/A	1/Week	24-Hr Composite ³
TSS ⁵ , Percent Removal ⁴	%	N/A	N/A	N/A	85	N/A	N/A	1/Month	Calculated ⁴
Ammonia (as mg/l NH ₃ N)									
May 1 – October 31	mg/l	N/A	N/A	N/A	4	6 ¹	N/A	1/Week	24-Hr Composite ³
November 1 – April 30	mg/l	N/A	N/A	N/A	10	15 ¹	N/A	1/Week	24-Hr Composite ³
Dissolved Oxygen	mg/l	N/A	N/A	7.0	N/A	N/A	N/A	1/Week	Grab
E. Coli ⁶	#/100 ml	N/A	N/A	N/A	130 ⁷	240 ⁸	N/A	1/Week	Grab
Total Residual Chlorine	mg/l	N/A	N/A	N/A	0.011	0.019 ¹	N/A	1/Week	Grab
Total Nitrogen ⁹ , Effluent	mg/l	N/A	N/A	N/A	Report	Report ¹	N/A	1/Week	24-Hr Composite ³
Total Nitrogen ⁹ , Influent	mg/l	N/A	N/A	N/A	Report	Report ¹	N/A	1/Week	24-Hr Composite ³
Total Phosphorus, Effluent	mg/l	N/A	N/A	N/A	Report	Report ¹	N/A	1/Week	24-Hr Composite ³
Total Phosphorus, Influent	mg/l	N/A	N/A	N/A	Report	Report ¹	N/A	1/Week	24-Hr Composite ³
Chronic WET ¹⁰	TU _c	N/A	N/A	N/A	N/A	N/A	1.00	1/Quarter	(¹¹)

¹Daily Maximum

²CBOD₅ – Carbonaceous Biochemical Oxygen Demand, 5-day

³A 24-hour composite is a sample collected using an automated sampler set to collect equal volume aliquots of at least 100 ml each every 15 minutes over a 24 hour period. The sample must be maintained at between 0° C and 6° C at all times.

⁴Percent Removal is calculated using the following equation: $\text{Percent Removal} = \left[\frac{(\text{Monthly Average Influent} - \text{Monthly Average Effluent})}{\text{Monthly Average Influent}} \right] \times 100$

⁵TSS – Total Suspended Solids

⁶E. Coli – Escherichia Coli Bacteria

⁷Thirty (30) day Geometric Mean

⁸Seven (7) day Geometric Mean

⁹Total Nitrogen is the summation of the analytical results for Total Nitrates, Total Nitrites, and Total Kjeldahl Nitrogen

TABLE 6.									
EFFLUENT LIMITATIONS								MONITORING REQUIREMENTS	
Effluent Characteristic	Units	Loadings (lbs/day)		Concentrations				Frequency	Sample Type
		Monthly Average	Maximum Weekly Average	Minimum	Monthly Average	Maximum Weekly Average	Maximum		
¹⁰ WET – Whole Effluent Toxicity									
¹¹ Three (3) 24-hour composite samples with one each collected every other day for a period of five (5) days, i.e. days 1, 3, & 5									

3.4. Pertinent Factors

The effluent limitations for this outfall were developed in accordance with DOW's General Procedures for Limitations Development located on DOW's webpage at:

<https://eec.ky.gov/Environmental-Protection/Forms%20Library/General%20Procedures%20for%20Limitations%20Development.pdf>

3.4.1. Secondary Treatment Standards

Discharges from POTWs are subject to the technology-based effluent limitations (TBELs) known as the Secondary Treatment Standards. Both state and federal regulations establish the requirements for secondary treatment [401 KAR 5:045 and 401 KAR 5:080, Section 8(3) – 40 CFR 133.102 – respectively].

TABLE 7.		
State Defined Secondary Treatment Standards		
Pollutant or Pollutant Characteristic	30-day average	7-day average
BOD ₅ (mg/l)	30	45
TSS (mg/l)	30	45

TABLE 8.				
Federal Defined Secondary Treatment Standards				
Pollutant or Pollutant Characteristic	Minimum	30-day average	7-day average	Maximum
BOD ₅ (mg/l)	N/A	30	45	N/A
BOD ₅ Percent Removal (%)	N/A	85	N/A	N/A
CBOD ₅ (mg/l)	N/A	25	40	N/A
CBOD ₅ Percent Removal (%)	N/A	85	N/A	N/A
TSS (mg/l)	N/A	30	45	N/A
TSS Percent Removal (%)	N/A	85	N/A	N/A
pH (standard units)	6.0	N/A	N/A	9.0

3.4.2. Water Quality-Based Effluent Limitations

The following table lists those pollutants and/or pollutant characteristics of concern that DOW has determined exhibit reasonable potential to cause or contribute to an excursion of a water quality-based criterion, and the basis of DOW's determination. These determinations are consistent with the DOW's reasonable potential analysis (RPA) procedures outlined in *Permitting Procedures For Determining "Reasonable Potential"* Kentucky Division of Water May 1, 2000.

TABLE 9.	
Pollutant or Pollutant Characteristic	Basis
Total Residual Chlorine	The POTW uses a chlorine-based disinfection process on this outfall
Whole Effluent Toxicity	The POTW discharges nutrients at a level which have the reasonable potential to cause or contribute to an excursion above any State water quality standard, including State narrative criteria for water quality.

3.4.3. Calculation of Water Quality-Based Effluent Limitations

These calculations were performed using a Microsoft EXCEL based workbook developed by DOW. The workbook is designed to compare effluent data to the applicable water quality standards while also incorporating the characteristics of the receiving water and any regulatory ZID and/or MZ. The following table summarizes the results of these calculations for this outfall:

TABLE 10.									
Effluent Characteristic	Units	Reported Avg	Reported Max	Average Limitation	Maximum Limitation	Average Discharge %	Maximum Discharge %	MZF	Data Source
Cadmium	µg/L	0.085	0.085	0.416584542	3.85548346	20.40	2.20	0	DMR
Copper	µg/L	3.65	3.65	15.34241432	24.22911251	23.79	15.06	0	DMR
Lead	µg/L	1.2	1.2	6.676154385	171.3215381	17.97	0.70	0	DMR
Zinc	µg/L	18.6	1.86	196.2269688	196.2269688	9.48	0.95	0	DMR

3.4.4. Nutrients

These effluent characteristics are generally associated with sanitary wastewaters and organic effluents. While this facility treats wastewater that includes domestic wastewater and/or wastewater from organic sources, these constituents are present in quantities that demonstrate reasonable potential to cause or contribute to an excursion of the narrative water quality standard. As discussed in the DOW's General Procedures for Limitations Development, the applicable factors in 40 CFR 122.44(d)(1) and the designated uses established in 401 KAR 10:026 were considered in making this determination.

The DOW has considered whether this facility was designed for and was previously issued a construction permit requiring achievement of a numerical limit.

Accordingly, this permit contains an effluent limitation for Whole Effluent Toxicity.

3.5. Justification of Requirements

The Title 401 Chapters 5 and 10 of the Kentucky Administrative Regulations (KARs) and Title 40 of the Code of Federal Regulations (CFR) cited in the following have been duly promulgated pursuant to the requirements of Chapter 224 of the Kentucky Revised Statutes (KRSs) and the Clean Water Act (CWA) respectively.

At a minimum all permits shall contain technology-based effluent limitations (TBELs) [401 KAR 5:065, Section 2(4) – 40 CFR 122.44(a)]. When necessary to achieve water quality standards all permits shall contain WQBELs [401 KAR 5:065, Section 2(4) – 40 CFR 122.44(d)]. The WQBELs included in this permit are based upon the KYWQS [401 KAR 10:031].

3.5.1. Aliquot Size

All sampling for reportable monitoring is required to be representative of the discharge. The federal Environmental Protection Agency has found that collection of aliquots of less than 100 ml can lead to sediment bias in the sample. The minimum aliquot size required by the permit is consistent with the NPDES Compliance Inspection Manual, No. 305-K-17-001 Revised Jan 2017 and with 401 KAR 5:065 Section 2 [40 CFR 122.41 (j)].

3.5.2. Flow (Effluent & Influent)

The monitoring requirements for these parameters are consistent with the KPDES permit program requirements for establishing effluent limitations, standards, and permit conditions [401 KAR 5:065, Section 2(4) – 40 CFR 122.44(i)(1)(ii)] and requirements for recording and reporting of monitoring results [401 KAR 5:050, Section 4 – 40 CFR 122.48].

3.5.3. CBOD₅ (Effluent)

The limitations for this parameter are the secondary treatment standards for POTWs as defined in both state and federal regulations [401 KAR 5:045, Section 3, 401 KAR 5:080, Section 8(3) – 40 CFR 133.102]. DOW found that it was necessary to impose WQBELs for this parameter in order to achieve water quality standards [401 KAR 5:065, Section 2(4) – 40 CFR 122.44(d)]. These effluent limitations are also consistent with KYWQS [401 KAR 10:031, Section 4(1)(e) & (i) respectively]. The EPA's River and Stream Water Quality Model (QUAL 2E/K) was used to develop these limitations.

3.5.4. TSS (Effluent)

The limitations for this parameter are the secondary treatment standards for POTWs as defined in both state and federal regulations [401 KAR 5:045, Section 3, 401 KAR 5:080, Section 8(3) – 40 CFR 133.102 respectively]. These effluent limitations are also consistent with KYWQS [401 KAR 10:031, Section 4(1)(f)].

3.5.5. CBOD₅ (Influent) and TSS (Influent)

The monitoring requirements for these parameters are consistent with the KPDES permit program requirements for establishing effluent limitations, standards, and permit conditions [401 KAR 5:065, Section 2(4) – 40 CFR 122.44(i)(1)(i)] and requirements for recording and reporting of monitoring results [401 KAR 5:050, Section 4 – 40 CFR 122.48].

3.5.6. CBOD₅ (Percent Removal) and TSS (Percent Removal)

The limitations for these parameters are the secondary treatment standards for POTWs as defined in federal regulations. [401 KAR 5:080, Section 8(3) – 40 CFR 133.102]

3.5.7. Ammonia and Dissolved Oxygen

The limitations for these parameters are WQBELs developed using the EPA's River and Stream Water Quality Model (QUAL 2E/K) [401 KAR 10:031, Section 4(1)(e) & (i)].

3.5.8. E. Coli

The limitations for E. Coli are consistent with the KYWQS [401 KAR 10:031, Section 7].

3.5.9. pH

The limitations for this parameter are both TBELs and WQBELs. The limitations are consistent the secondary treatment standards for POTWs as defined in federal regulations and the KYWQS [401 KAR 5:080, Section 8(3) – 40 CFR 133.102, and 401 KAR 10:031, Sections 4(1)(b) and 7 – respectively].

3.5.10. Total Residual Chlorine

The limitations for this parameter are consistent with Kentucky's Water Quality Standards [401 KAR 10:031, Sections 4(1)(k)].

3.5.11. Hardness, Cadmium, Copper, Lead, and Zinc (Removal of Limitations)

The removal of limitations for these parameters is consistent with Kentucky's Water Quality Standards [401 KAR 10:031, Section 6]. The limitations have been removed from the permit because the reasonable potential analysis performed did not indicate that the discharge concentrations of these pollutants exceed 70% of the calculated chronic/acute water quality-based effluent limitations (WQBELs) for these pollutants.

3.5.12. Chronic WET

The limitations for this parameter are consistent with Kentucky's Water Quality Standards [401 KAR 10:031, Sections 4(1)(j)].

3.5.13. Total Phosphorus and Total Nitrogen (Effluent & Influent)

Excess nutrients impact water quality by causing adverse effects on natural water chemistry and indigenous aquatic community. The Division of Water has concluded that reasonable potential exists for exceedance of a narrative water quality criterion for nutrients for which no numeric limit has been promulgated. Per 401 KAR 10:031 and 40 CFR 122.44 (d)(1)(v) Whole Effluent Toxicity limitations have been applied.

The monitoring requirements for this parameter are consistent with the KPDES permit program requirements for establishing effluent limitations, standards, and permit conditions [401 KAR 5:065, Section 2(4) – 40 CFR 122.44(i)(1)(i)] and requirements for recording and reporting of monitoring results [401 KAR 5:050, Section 4 – 40 CFR 122.48].

SECTION 4

COLLECTION SYSTEM REQUIREMENTS

4. COLLECTION SYSTEM REQUIREMENTS

4.1. General Prohibitions

The following prohibitions apply to the collection system and its users:

- (1) There shall be no sanitary sewer overflows (SSOs);
- (2) No user shall introduce any pollutant or pollutants that will cause pass through or interference with the operation of the POTW and the collection system; or
- (3) No user shall introduce any of the following pollutants:
 - a. Pollutants which create a fire or explosion hazard, including but not limited to, wastestreams with a closed cup flashpoint of less than 140 °F (60 °C);
 - b. Pollutants which will cause corrosive structural damage or have a pH less than 5.0 standard units unless the POTW is designed to accommodate such pH levels;
 - c. Solid or viscous pollutants in amounts that would obstruct the flow to the POTW thus resulting in interference;
 - d. Any pollutant released in a discharge at such a volume or strength as to cause interference in the POTW;
 - e. Heat in such quantities that the temperature at the POTW treatment plant exceeds 104 °F (40 °C) unless the POTW requests and the Approval Authority grants alternate temperature limits;
 - f. Petroleum oil, non-biodegradable cutting oil, or products of mineral oil origin in amounts that will cause interference or pass-through;
 - g. Pollutants which result in the presence of toxic gases, vapors, or fumes within the POTW in a quantity that may cause acute worker health and safety problems; and,
 - h. Any trucked or hauled waste except, at discharge points designated by the POTW.

All POTWs, in cases where pollutants contributed by user(s) of the collection system are likely to result in reoccurring interference or pass-through, shall develop and enforce specific effluent limits for industrial user(s), and all other users, as appropriate, which, together with appropriate changes in the POTW treatment plant's facilities or operation, are necessary to ensure renewed and continued compliance with the POTW's KPDES permit or sludge use or disposal practices.

These prohibitions are consistent with Kentucky's general prohibition against water pollution, the Combined Sewer Overflow Control Policy of 1994 (CSO Policy), and the national pretreatment standards prohibited discharges applicable to all POTW collection systems [KRS 224.70-110, 33 U.S.C. 1342 (q) and 401 KAR 5:055, Section 11 – 40 CFR 403.5 respectively]

4.2. Capacity, Management, Operation and Maintenance (CMOM) Programs

The permittee shall develop and implement CMOM programs that: (1) better manages, operates, and maintains collection systems, (2) investigates capacity constrained areas of the collection system, (3) proactively prevents or minimizes SSOs, and (4) responds to SSO events.

Guidance for the development of effective CMOM programs is available at the following EPA web address: http://www.epa.gov/npdes/pubs/cmom_guide_for_collection_systems.pdf

This requirement replaces the requirement to develop and implement a Best Management Practices (BMP) plan imposed in prior permits. The imposition of this requirement is consistent with the standard conditions applied to all permits regarding the proper operation and maintenance of all facilities and systems of treatment and control including all related appurtenances [401 KAR 5:065, Section 2(1) – 40 CFR 122.41(e)].

5. OTHER CONDITIONS

5.1. Schedule of Compliance

The permittee will comply with all effluent limitations by the effective date of the permit except as specified below [401 KAR 5:050, Section 3 – 40 CFR 122.47].

All conditions of the Agreed Order in *Commonwealth of Kentucky Energy and Environment Cabinet, Division of Enforcement in re Knott Co. Water & Sewer District – Caney Creek WWTP*, Case No. DOW-150080, filed January 5, 2016, are hereby incorporated as enforceable conditions of this KPDES permit, including the submission of all required reports and plans by the dates specified by the Agreed Order.

5.2. Antidegradation

The conditions of Kentucky's Antidegradation Policy have been satisfied [401 KAR 10:029, Section 1]. This permitting action is a reissuance of a KPDES permit that does not authorize an expanded discharge from a POTW. The POTW has developed an approved regional facility plan in accordance with state wastewater planning requirements for regional planning agencies [401 KAR 5:006]. This approved plan constitutes compliance with socioeconomic demonstration and alternatives analysis of the Antidegradation Policy Implementation Methodology [401 KAR 10:030, Section 1(3)(b)2b].

5.3. Sludge Disposal

The disposal or final use of sewage sludge generated during the treatment of domestic sewage by a POTW shall be disposed of in accordance with state and federal requirements [401 KAR Chapter 45 and 40 CFR 503].

5.4. Standard Conditions

The conditions listed in the Standard Conditions Section of the permit are consistent with the conditions applicable to all permits [401 KAR 5:065, Section 2(1) – 40 CFR 122.41].

5.5. Sufficiently Sensitive Analytical Methods

Analytical methods utilized to demonstrate compliance with the effluent limitations established in this permit shall be sufficiently sensitive to detect pollutant levels at or below the required effluent limit, i.e. the Method Minimum Level (ML) shall be at or below the effluent limit. In that instance where a 40 CFR 136 approved method does not exist that has a ML at or below the established effluent limitation, the permittee shall: (1) use the method specified in the permit; or (2) the 40-CFR 136 approved method with a ML that is nearest to the established effluent limit [401 KAR 5:065, Section 2(4) – 40 CFR 122.44(i)].

5.6. Certified Laboratory

All environmental analysis to be performed by a certified laboratory is consistent with the certified wastewater laboratory requirements [401 KAR 5:320, Section 2].

5.7. Certified Operators

Wastewater treatment plants and wastewater collection systems that accept wastewaters containing domestic sewage are to be operated by a certified operator [401 KAR 5:010].

5.8. Application Monitoring

POTWs are required to complete application Form A which requires a minimum of three (3) samples to be collected and analyzed. To ensure that sufficient samples are collected and analyzed, DOW placed sampling requirements within the permit. The results of the application monitoring shall be submitted on an annual DMR and summarized on the renewal application [401 KAR 5:065, Section 2(1) – 40 CFR 122.41(j) and 401 KAR 5:050, Section 4 – 40 CFR 122.48].

Additional application monitoring, if any, is for parameters not listed on Form A. The state has an applicable water quality standard for this parameter or has concluded that the monitoring is appropriate for specific parameters on a case-by-case basis [401 KAR 5:060, Section 2 (3) – 40 CFR 122.21 (j) (4)(iv)]

5.9. Monthly Operating Reports (MORs)

In addition to the monitoring of effluent as specified by the permit, the permittee shall conduct process control monitoring on a daily basis. Process control monitoring is that monitoring performed by the operators of the wastewater treatment plant to determine if the wastewater system is operating at its optimum efficiency. This monitoring includes but is not limited to influent and effluent quality and quantity monitoring, chemical usage, sludge monitoring including volume produced, wasted, and disposed, and monitoring of internal units such as aeration basins and oxidation ditches.

The data is recommended to be recorded using the Microsoft EXCEL-based Monthly Operating Report (MOR) workbook available on the Department for Environmental Protection's Forms webpage at:

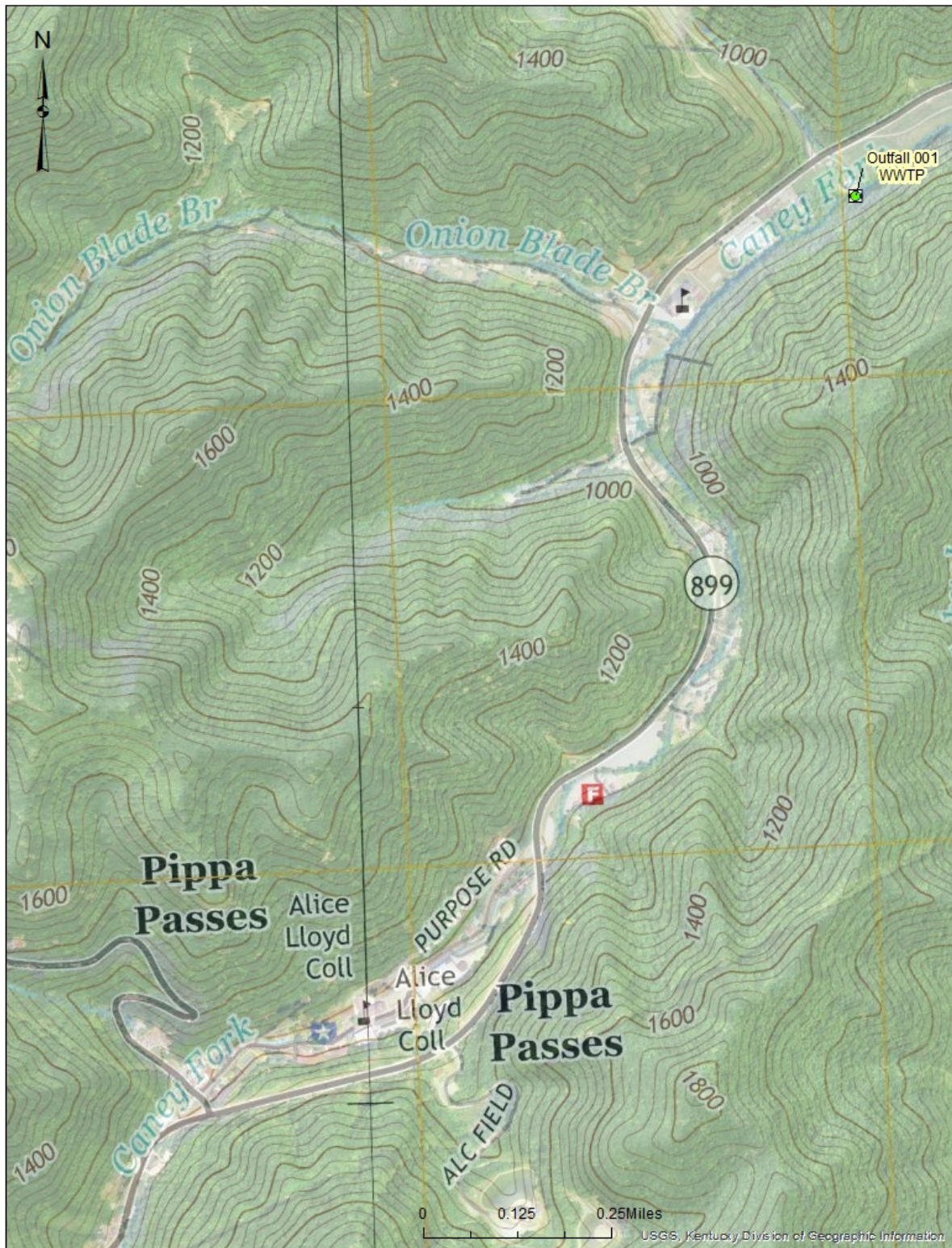
<https://eec.ky.gov/Environmental-Protection/Forms%20Library/MOR-eMOR.xls>

Alternatively, the permittee may choose to use their own electronic MOR workbook, as long as it includes the information required by the above form and/or is approved by the Division's Regional Field Office Supervisor.

The updated workbook shall be maintained on-site and made available upon request by Cabinet personnel.

These additional monitoring requirements are consistent with state and federal regulations that require the permit to include as appropriate monitoring requirements to assure compliance with the permit limitations [401 KAR 5:050, Section 4 – 40 CFR 122.48].

5.10. Location Map





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Region 4 Laboratory Services and Applied Science Division
980 College Station Road, Athens, Georgia 30605-2700
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Project: 23-0274, Knott County WWTP Recon - Reported by Kristin Trapp

July 14, 2023

MEMORANDUM

SUBJECT: FINAL Analytical Report
Project: 23-0274, Knott County WWTP Recon

FROM: Kristin Trapp
LSB Inorganic Chemistry Section Chief

THRU: Stacie Masters, Chief
Laboratory Services Branch

TO: Christopher McHugh

Attached are the final results for the analytical groups listed below. This report shall not be reproduced except in full without approval of the Region 4 laboratory. These analyses were performed in accordance with the Laboratory Services Branch's Laboratory Operations and Quality Assurance Manual (LSB LOQAM) found at www.epa.gov/region4/sesd/asbsop. Any unique project data quality objectives specified in writing by the data requestor have also been incorporated into the data unless otherwise noted in the Report Narrative. Chemistry data have been verified based on the LSB LOQAM specifications and have been qualified by this laboratory if the applicable quality control criteria were not met. Verification is defined in Chapter 5 of the LSB LOQAM. For a listing of specific data qualifiers and explanations, please refer to the Data Qualifier Definitions included in this report. The reported results are accurate within the limits of the method(s) and are representative only of the samples as received by the laboratory.

Analyses Included in this report:

Method Used:

Accreditations:

Classical/Nutrient Analyses (CNA)

Ammonia as N	EPA 350.1 (Water)	ISO
Ammonia/TKN	EPA 351.2 (Water)	ISO
Nitrate and/or Nitrite	EPA 353.2 (Water)	ISO/DW
Phosphorous	EPA 365.1 (Water)	ISO



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Sample Disposal Policy

Due to limited space for long term sample storage, LSB's policy is to dispose of samples on a periodic schedule. Air samples collected in summa canisters will be disposed of 30 days following the issuance of this report. All other sample media including original samples, sample extracts and or digestates will be disposed of, in accordance with applicable regulations, 60 days from the date of this report.

This sample disposal policy does not apply to criminal samples which are held until the laboratory is notified by the criminal investigators that case development and litigation are complete.

These samples may be held in the laboratory's custody for a longer period of time. If samples require storage beyond the 60-day period, please contact the Sample Control Coordinator by e-mail at R4SampleCustody@epa.gov.



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SAMPLES INCLUDED IN THIS REPORT

Project: 23-0274, Knott County WWTP Recon

Sample ID	Laboratory ID	Matrix	Date Collected	Date Received
EFF001-0614	E232409-01	Wastewater	6/14/23 10:45	6/15/23 12:05



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DATA QUALIFIER DEFINITIONS

D-2	Due to Matrix Interference, the sample cannot be accurately quantified. The reported result is estimated.
J	The identification of the analyte is acceptable; the reported value is an estimate.
QC-1	Analyte concentration low in continuing calibration verification standard
QC-6	Calibration check standard greater than method control limits.
QL-2	Laboratory Control Spike Recovery greater than method control limits
QM-1	Matrix Spike Recovery less than method control limits
QM-3	Matrix Spike Precision outside method control limits
QR-2	MRL verification recovery greater than upper control limits.



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ACRONYMS AND ABBREVIATIONS

CAS Chemical Abstracts Service

Note: Analytes with no known CAS identifiers have been assigned codes beginning with "E", the EPA ID as assigned by the EPA Substance Registry System (www.epa.gov/srs), or beginning with "R4-", a unique identifier assigned by the EPA Region 4 laboratory.

MDL Method Detection Limit - The minimum concentration of a substance (an analyte) that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero.

MRL Minimum Reporting Limit - Analyte concentration that corresponds to the lowest demonstrated level of acceptable quantitation. The MRL is sample-specific and accounts for preparation weights and volumes, dilutions, and moisture content of soil/sediments.

TIC Tentatively Identified Compound - An analyte identified based on a match with the instrument software's mass spectral library. A calibration standard has not been analyzed to confirm the compound's identification or the estimated concentration reported.

ACCREDITATIONS:

ISO Accredited to ISO/IEC 17025:2017 and accreditation requirements for Forensic Science Testing Laboratories.

Refer to the certificate and scope of accreditation FT-0330 at:
<http://www.epa.gov/aboutepa/about-region-4s-science-and-ecosystem-support-division-sesd>

NR Not accredited for this test.

DW Accredited for conformance with ISO/IEC 17025:2017 and testing elements in the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water, EPA 815-R-05-004, 2005.

Refer to the certificate and scope of accreditation AT-2628 at:
<http://www.epa.gov/aboutepa/about-region-4s-science-and-ecosystem-support-division-sesd>

ISO/DW Accredited to ISO/IEC 17025:2017 and accreditation requirements for Forensic Science Testing Labs, and conformance with ISO/IEC 17025:2017 and testing elements in the Manual for the Certification of Laboratories Analyzing Drinking Water.



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Classical/Nutrient Analyses

Project: 23-0274, Knott County WWTP Recon

Sample ID: EFF001-0614

Lab ID: E232409-01

Station ID: EFF001

Matrix: Wastewater

Date Collected: 6/14/23 10:45

CAS Number	Analyte	Results	Qualifiers	Units	MRL	Prepared	Analyzed	Method
7664-41-7	Ammonia as N	8.2		mg/L	0.10	7/07/23 8:42	7/07/23 14:53	EPA 350.1
E17148461	Total Kjeldahl Nitrogen	12		mg/L	0.50	6/23/23 8:40	6/27/23 9:55	EPA 351.2
E701177	Nitrate/Nitrite as N	0.062	J, D-2, QC-1, QC-6, QL-2, QM-1, QM-3, QR-2	mg/L	0.050	7/06/23 11:06	7/06/23 16:25	EPA 353.2
7723-14-0	Total Phosphorus	1.4		mg/L	0.050	6/28/23 10:36	7/05/23 15:06	EPA 365.1



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Classical/Nutrient Analyses (CNA) - Quality Control

US-EPA, Region 4, LSASD

Analyte	Result	Reporting Limit	Units	Spike Level	Source Result	%REC	%REC Limits	RPD	RPD Limit	Notes
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Batch 2306023 - C 351.2 TKN

Blank (2306023-BLK1)

Prepared: 06/23/23 Analyzed: 06/27/23

EPA 351.2

Total Kjeldahl Nitrogen	U	0.050	mg/L							U
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LCS (2306023-BS1)

Prepared: 06/23/23 Analyzed: 06/27/23

EPA 351.2

Total Kjeldahl Nitrogen	1.4590	0.050	mg/L	1.4600		99.9	90-110			
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Matrix Spike (2306023-MS1)

Source: E232402-42

Prepared: 06/23/23 Analyzed: 06/27/23

EPA 351.2

Total Kjeldahl Nitrogen	1.2780	0.050	mg/L	1.0030	0.27100	100	90-110			
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Matrix Spike (2306023-MS2)

Source: E232409-01

Prepared: 06/23/23 Analyzed: 06/27/23

EPA 351.2

Total Kjeldahl Nitrogen	13.400	0.50	mg/L	1.0030	12.140	126	90-110			XM-1
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Matrix Spike Dup (2306023-MSD1)

Source: E232402-42

Prepared: 06/23/23 Analyzed: 06/27/23

EPA 351.2

Total Kjeldahl Nitrogen	1.3280	0.050	mg/L	1.0030	0.27100	105	90-110	3.84	20	
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Matrix Spike Dup (2306023-MSD2)

Source: E232409-01

Prepared: 06/23/23 Analyzed: 06/27/23

EPA 351.2

Total Kjeldahl Nitrogen	13.300	0.50	mg/L	1.0030	12.140	116	90-110	0.749	20	XM-1
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MRL Verification (2306023-PS1)

Prepared: 06/23/23 Analyzed: 06/27/23

EPA 351.2

Total Kjeldahl Nitrogen	0.050000	0.050	mg/L	0.050000		100	70-130			MRL-2
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Batch 2306039 - C 350.1 Ammonia

Blank (2306039-BLK1)

Prepared & Analyzed: 07/07/23

EPA 350.1

Ammonia as N	U	0.050	mg/L							U
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LCS (2306039-BS1)

Prepared & Analyzed: 07/07/23

EPA 350.1

Ammonia as N	0.96100	0.050	mg/L	1.0030		95.8	90-110			
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Classical/Nutrient Analyses (CNA) - Quality Control

US-EPA, Region 4, LSASD

Analyte	Result	Reporting Limit	Units	Spike Level	Source Result	%REC	%REC Limits	RPD	RPD Limit	Notes
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Batch 2306039 - C 350.1 Ammonia

Matrix Spike (2306039-MS1) **Source: E232409-01** Prepared & Analyzed: 07/07/23

EPA 350.1
Ammonia as N 9.1240 0.10 mg/L 1.0030 8.2280 89.3 90-110 XM-1

Matrix Spike Dup (2306039-MSD1) **Source: E232409-01** Prepared & Analyzed: 07/07/23

EPA 350.1
Ammonia as N 9.0540 0.10 mg/L 1.0030 8.2280 82.4 90-110 0.770 10 XM-1

MRL Verification (2306039-PS1) Prepared & Analyzed: 07/07/23

EPA 350.1
Ammonia as N 0.037000 0.050 mg/L 0.050000 74.0 70-130 MRL-2,
U

Batch 2306058 - C 365.1 TPhos

Blank (2306058-BLK1) Prepared: 06/28/23 Analyzed: 07/05/23

EPA 365.1
Total Phosphorus U 0.010 mg/L U

LCS (2306058-BS1) Prepared: 06/28/23 Analyzed: 07/05/23

EPA 365.1
Total Phosphorus 0.39700 0.010 mg/L 0.39300 101 90-110

Matrix Spike (2306058-MS1) **Source: E232409-01** Prepared: 06/28/23 Analyzed: 07/05/23

EPA 365.1
Total Phosphorus 1.9350 0.050 mg/L 0.50000 1.4350 100 90-110

Matrix Spike Dup (2306058-MSD1) **Source: E232409-01** Prepared: 06/28/23 Analyzed: 07/05/23

EPA 365.1
Total Phosphorus 1.9250 0.050 mg/L 0.50000 1.4350 98.0 90-110 0.518 10

MRL Verification (2306058-PS1) Prepared: 06/28/23 Analyzed: 07/05/23

EPA 365.1
Total Phosphorus 0.012000 0.010 mg/L 0.010000 120 70-130 MRL-2

Batch 2307009 - C 353.2 NO3-NO2

Blank (2307009-BLK1) Prepared & Analyzed: 07/06/23

EPA 353.2



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Classical/Nutrient Analyses (CNA) - Quality Control
US-EPA, Region 4, LSASD

Analyte	Result	Reporting Limit	Units	Spike Level	Source Result	%REC	%REC Limits	RPD	RPD Limit	Notes
Batch 2307009 - C 353.2 NO3-NO2										
Blank (2307009-BLK1)				Prepared & Analyzed: 07/06/23						
Nitrate/Nitrite as N	U	0.050	mg/L							U
LCS (2307009-BS1)				Prepared & Analyzed: 07/06/23						
EPA 353.2										
Nitrate/Nitrite as N	0.75180	0.050	mg/L	0.50000		150	90-110			QL-2
Matrix Spike (2307009-MS1)		Source: E232409-01		Prepared & Analyzed: 07/06/23						
EPA 353.2										
Nitrate/Nitrite as N	0.48630	0.050	mg/L	0.50000	0.061600	84.9	90-110			QM-1
Matrix Spike Dup (2307009-MSD1)		Source: E232409-01		Prepared & Analyzed: 07/06/23						
EPA 353.2										
Nitrate/Nitrite as N	0.23590	0.050	mg/L	0.50000	0.061600	34.9	90-110	69.3	10	QM-1, QM-3
MRL Verification (2307009-PS1)				Prepared & Analyzed: 07/06/23						
EPA 353.2										
Nitrate/Nitrite as N	0.074400	0.050	mg/L	0.050000		149	70-130			MRL-2, OR-2



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Notes and Definitions for QC Samples

U	The analyte was not detected at or above the reporting limit.
MRL-2	MRL verification for Non-Potable Water matrix
QL-2	Laboratory Control Spike Recovery greater than method control limits
QM-1	Matrix Spike Recovery less than method control limits
QM-3	Matrix Spike Precision outside method control limits
QR-2	MRL verification recovery greater than upper control limits.
XM-1	Sample background/spike ratio higher than method evaluation criteria