

# Purpose

This TSOP provides instructions on *E. coli* field sampling and analysis using IDEXX Colilert. Specifically, this TSOP covers the processes of:

- 1. sample collection
- 2. sample processing
- 3. sample reading and quantification.

Colilert is used for the simultaneous detection, specific identification, confirmation, and quantification of total coliforms and *E. coli* in water. The method used for this testing is Method 9223-SM Enzyme Substrate Coliform Test. Colilert may be performed in a fixed or mobile laboratory setting.

## Scope

This TSOP applies to agency staff in the Office of Water Quality (OWQ), Watershed Assessment and Planning Branch (WAPB) responsible for collecting *E. coli* water samples from rivers and streams as part of IDEM's ambient water quality monitoring program.

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# **Authorizing Signatures**

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This technical standard operating procedure is consistent with agency requirements.

Quality Assurance Staff Office of Program Support

 3/15/2023	
Date	
3/20/2023	

Date

3/14/2023

Date

3/14/2023

Date

3/14/2023 Date

3/27/2023

Date

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## 1.0. Overview Flowchart



## 2.0. Procedure

### 2.1. Procedural Flowchart

Procedural Flowcharts are shown in Section 2.2, followed by a step-bystep description.

### 2.2. Procedural Steps



- Step 1. Sample collection
  - A. Using a combination of the Indiana Atlas & Gazetteer, aerial maps, topographic maps, cell phone navigation application, and a hand-held Global Positioning System (GPS) unit, navigate to the designated parking spot as described on the Site Reconnaissance Form (Appendix 1), or site description for a fixed station.
  - B. Sanitize hands and put on sterile nitrile gloves before handling the sample bottle.
  - C. Label sample bottle prefilled with sodium thiosulfate preservative with Assessment Information Management System (AIMS) sample number or site ID number for a fixed station.
  - D. Using the hand-held GPS unit and the Site Reconnaissance Form, the crew chief and field crew proceed to the sampling site with the clipboard, backpacks, and any other equipment required to complete the sampling.
  - E. Determine if the site can be sampled. In order to be sampled, water must be present in the streambed. If the site can be sampled, proceed to step 1.G. If the site cannot be sampled, proceed to step 1.F.
  - F. If the site cannot be sampled, fill out the "Sample Collectors", "Date", and "Time", and check the appropriate "No,..." box under "Sample Taken?" on the Stream Sampling Field Data Sheet (Appendix 2). For a fixed station, fill in the date and time on the corresponding site ID row on the Fixed Station Route Field Data Sheet (Appendix 3). Proceed to step 1.N.
  - G. If the site can be sampled, approach the sampling site by entering downstream of the actual sampling point to avoid stirring up stream bed sediment which can contaminate the sample.
  - H. Complete the in-situ sample of field water chemistry and record the information onto the Stream Sampling Field Data Sheet or the Fixed Station Route Field Data Sheet for a fixed station. See the manufacturer's manual for data sonde operation instructions (Hach 2021, YSI 2020).
  - While facing upstream, remove the bottle cap and collect the water sample by inserting the bottle directly into the stream, just below the water surface. Ensure that collected sample measures to the 100 ml fill line. Replace the sample bottle cap.

- J. Return to vehicle with sample bottle and all equipment taken to the sampling site.
- K. Place sample bottle on ice in a cooler for the duration of additional sampling and transportation.
- L. Remove and discard gloves.
- M. Record the date and time on the Stream Sampling Field Data Sheet or the Fixed Station Route Field Data Sheet.
- N. If there are more sites to sample and time allows, locate next site, and repeat steps 1.A through 1.M. If there are no more sites or time, proceed to step 1.O.
- O. If fixed station, proceed to step 1.P. Otherwise, proceed to step 1.Q.
- P. Drop fixed station samples off at the Indiana Department of Health (IDOH) lab with the IDEM OWQ Chain of Custody Form (Appendix 4). End of fixed station sample process.
- Q. Return to the mobile lab and proceed to Step 2 for sample processing.



- A. Using an extension cord, connect the mobile laboratory to the outlet of the hotel.
- B. Turn on the incubator.
- C. Sanitize the laboratory bench tops.
- D. Turn on the Quanti-Tray Sealer (IDEXX 2021). A green light on the device indicates its ready status.
- E. Put on sterile gloves.

- F. Remove samples from the cooler and place on bench and allow to warm.
- G. Log samples into laboratory bench sheets or electronic equivalent using AIMS assigned sample number.
- H. Determine if dilutions are needed based on the sample(s) being excessively turbid (≥ 50 NTU) or recent *E. coli* counts have been high. Dilutions are only performed for watershed characterization projects, unless determined to be necessary for other projects. If dilutions are needed, proceed to step 2.I. If dilutions are not needed, proceed to step 2.K.
- I. For samples requiring dilution, pipet 1mL of sample into a sterile sample bottle prefilled with sodium thiosulfate preservative and labeled with the sample number and "1:100". Add 99 mL of sterile deionized water.
- J. Record dilutions on the laboratory bench sheets or electronic equivalent.
- K. Shake sample containers for about 30 seconds without adding the Colilert reagent to the sample.
- L. Place a Colilert reagent snap pack on top of each sample container.
- M. Add the contents of the Colilert snap pack to the sample container, replace the lid, and mix the sample by shaking for about 30 to 60 seconds (IDEXX 2022).
- N. Label all Quanti-Trays with the AIMS sample number and dilution information, if applicable.
- O. Pour the sample or diluted sample containing the Colilert into the properly labeled Quanti-Tray.
- P. Firmly seat the Quanti-Tray into the rubber template, making sure that the tab is tucked between the tray and the rubber template, to avoid crushing the tray.
- Q. Slide the tray through the sealer.
- R. Properly dispose of the used Colilert snap packs and sample bottles in a biohazard container. Other sample processing waste (i.e., used gloves, paper towels) should not go into the biohazard container.
- S. Incubate sample trays at  $35^{\circ}C \pm 0.5^{\circ}C$  for 24 hours. See Step 3 for sample reading and quantification.
- Step 3. Sample Reading and Quantification



- A. Remove trays from the incubator.
- B. For each tray, count the number of large wells and small wells that are yellow for total coliforms.
- C. Record the total coliforms count on the lab bench sheet (Appendix 5).
- D. Using an ultraviolet (UV) light and UV viewing cabinet or UV safety glasses, count the number of large wells and small wells that fluoresce for *E. coli*.
- E. Record the *E. coli* count on the lab bench sheets.
- F. Properly dispose of the used Quanti-Trays in a biohazard container.

## 2.3. Related Technical Issues

- A. Health and Safety Warnings
  - Safety issues are the responsibility of all crew members; however, any questions in the field should be directed to the crew chief. The crew chief is responsible for the completion of all work listed in the TSOP, the health and safety aspects of the sampling event, and successful interactions with landowners and members of the public.
  - 2. Due to the remoteness of sampling locations and possibility of a mishap, all field staff are required to complete basic first aid and cardiopulmonary resuscitation training.

- 3. According to the memorandum "Change in status of Water Assessment Branch staff in accordance with the Agency training policy", dated November 29, 2010, OWQ WAPB staff are exempt from initial and annual training requirements set forth in Section 6.0 of the IDEM Health and Safety Training Policy (IDEM 2010). The memorandum also states, "as an alternative to the training requirements of the policy, the Branch will conduct in-service training at a minimum of four hours per year on topics directly related to duties performed by staff." New hires or those changing job responsibilities without the minimum four-hour training must be accompanied in the field by a staff member who has met the training requirements.
- 4. Due to the hazards related to sampling on surface waters, safety consciousness of staff members and the use of specialized equipment are required. Thus, staff will comply with the IDEM Personal Protective Equipment (PPE) Policy (IDEM 2008). If an injury or illness arises in the field, staff will follow the IDEM Injury and/or Illness Resulting from Occupational Exposure Policy (IDEM 2016).
- 5. To prevent drowning during sample collection, personnel will wear appropriate clothing and PPE when operating boats or sampling in deep water or swift currents. According to the memorandum "Use of Personal Flotation Devices (PFDs) by Branch Personnel", dated February 29, 2000, staff must wear U.S. Coast Guard approved Type I, II, or III PFDs whenever:
  - a. The planned work requires them to enter the water and the maximum water depth at any place at the work site is over their knee (note that this depth depends on the employee, but it will usually be between 12 to 20 inches or 300 to 500 mm).
  - b. The employee is retrieving, launching, or operating watercraft of any kind on surface water.
  - c. The employee must work from docks, launches, or nearby water structures without guard rails and are over or alongside water where the water depth is, or could reasonably be expected to be, 3 feet or more.
  - d. Working in boats on boundary waters, as defined by Indiana Code (IC) 14-8-2-27, or between sunset and sunrise on any waters of the state, all personnel in the watercraft must wear a high intensity whistle and Safety of Life at Sea certified strobe light.

- Field personnel collecting *E. coli* samples will follow policies and procedures established in the Hazard Communication (HazCom) Plan (IDEM 2019) and Office of Water Quality Watershed Assessment and Planning Branch Laboratory Safety Plan (IDEM 2021).
- 7. Due to possible hazards presented by the chemicals listed below, review the safety data sheets (SDS), for proper handling and required PPE. SDS are stored in a binder in a cabinet under the fume hood in the laboratory in Building 41.
  - a. Laboratory grade cleanser hazards and safety:
    - I. Corrosive
    - II. Causes severe burns.
  - b. Required PPE:
    - I. Chemical resistant latex or nitrile gloves
    - II. UV Viewing Cabinet or UV Safety Glasses
- B. Cautions
  - 1. Shake sample thoroughly before inoculation with Colilert.
  - 2. Do not transfer colonies or cultures pregrown in any enrichment media to Colilert. The suppressant reagents in Colilert may be overloaded by the transfer of heavy inocula of certain noncoliforms. This does not apply to dilute pure cultures used in controls.
  - 3. Do not dilute samples in buffered water before addition to Colilert. Samples may be diluted with sterile laboratory pure water.
- C. Interferences
  - 1. Do not prefilter samples and place the membrane in Colilert.
  - 2. The time from sample collection to initiation of analysis must not exceed six hours.
  - 3. To prevent loss or corruption of data, always record the correct sample number and site information legibly during field collection and processing procedures.
  - 4. Avoid prolonged exposure of Colilert to sunlight because it may cause a false positive in the sample.
  - 5. The mobile laboratory setting requires a standard outdoor AC electrical outlet, or an AC/DC power inverter powered by a DC battery or DC battery pack.
- D. Calibration
  - 1. The NIST traceable digital thermometer is used to test the temperature of the incubator. The digital thermometer is used

simultaneously with the incubator while samples are being processed.

- E. Troubleshooting
  - 1. Not applicable

## 3.0. Roles

- 3.1 Responsibilities
  - A. Crew chief
    - 1. Preparation for sample collection
    - 2. Field collection of E. coli water samples
    - 3. Sample preparation, processing, and reading
    - 4. Cleanup, paperwork, and sample delivery (if necessary)
  - B. Field crew
    - 1. Preparation for sample collection
    - 2. Field collection of E. coli water samples
    - 3. Sample preparation, processing, and reading
    - 4. Cleanup, paperwork, and sample delivery (if necessary)
- 3.2 Training requirements
  - A. Preparation for sample collection
    - 1. Crew chief
    - 2. Field crew
  - B. Field collection of *E. coli* water samples
    - 1. Crew chief
    - 2. Field crew
  - C. Sample preparation, processing, and reading
    - 1. Crew chief
    - 2. Field crew
  - D. Cleanup, paperwork, and sample delivery
    - 1. Crew chief
    - 2. Field crew
  - E. E. coli Sampling and Analysis TSOP
    - 1. Crew chief
    - 2. Field crew

# 4.0. Required Forms, Equipment, or Software List

#### 4.1 Forms

- A. Site Reconnaissance Form (Appendix 1)
- B. Stream Sampling Field Data Sheet (Appendix 2)
- C. Fixed Station Route Datasheet (Appendix 3)
- D. OWQ Chain of Custody Form (Appendix 4)
- E. Lab Bench Sheet (Appendix 5)
- F. E. coli Sampling Checklist (Appendix 6)

#### 4.2 Equipment

- A. Incubator set at 35.0°C ± 0.5°C
- B. 6-watt, long wavelength UV lamp (365 nm)
- C. Sterile, nonfluorescent container
- D. Onset InTemp data logger NIST traceable
- E. IDEXX sealer with rubber template
- F. Hand sanitizer
- G. Paper towels
- H. Biohazard trash receptacle
- I. 10 mL disposable pipets
- J. Sterile deionized water in carboy
- K. Laboratory bench sheets or electronic equivalent
- L. Sterile nitrile gloves
- M. UV safety glasses
- N. UV viewing cabinet
- O. Permanent markers
- P. Pencils
- Q. 120 mL plastic bottles pre-preserved with sodium thiosulfate
- R. Clipboard
- S. Personal floatation device
- T. Waders

- U. Cooler with ice
- V. Quanti-trays
- W. Colilert enzyme substrate
- X. Bench sanitizer
- 4.3 Software
  - A. AIMS database

## 5.0. Records Management

- 5.1. Site Reconnaissance Form
  - A. Information recorded on the Site Reconnaissance Form is entered into the AIMS database.
  - B. The original hard copy is kept in the site folder and stored in a file cabinet in the WAPB library at the IDEM Shadeland office until it is scanned and uploaded to AIMS or the Virtual File Cabinet (VFC).
- 5.2. E. coli Laboratory Bench Sheet
  - A. Information recorded on the bench sheet is entered into the AIMS database.
  - B. The original hard copy is kept in the IDEM *E. coli* mobile laboratory until all samples have been read. It is then stored in a file cabinet in the WAPB library at the IDEM Shadeland office until it is scanned and uploaded to AIMS or VFC.
- 5.3. Stream Sampling Field Data Sheet
  - A. Information recorded on the Stream Sampling Field Data Sheet is entered into the AIMS database. The field data sheets are scanned and stored as attachments in AIMS.
  - B. The original hard copy is kept in the site folder and stored in a file cabinet in the WAPB library at the IDEM Shadeland office until it is scanned and uploaded to AIMS or VFC.
- 5.4. Fixed Station Route Data Sheet
  - A. Information recorded on the Fixed Station Route Data Sheet is entered into the AIMS database. The Fixed Station Route Data Sheets are scanned and stored as attachments in AIMS.

- B. The original hard copy is kept in the site folder and stored in a file cabinet in the WAPB library at the IDEM Shadeland office until it is scanned and uploaded to AIMS or VFC.
- 5.5. Chain of Custody Form
  - A. The chain of custody forms are scanned and stored as attachments in AIMS.
  - B. Original hard copies are kept in the site folder and stored in a file cabinet in the WAPB library at the IDEM Shadeland office until they are canned and uploaded to VFC.
- 5.6. AIMS Database
  - A. http://aims.idem.in.gov/AIMS/Pages/Login/

### 6.0. Definitions

- 6.1. "AA, AB, or AC number" A number assigned to each individual watershed sampling event conducted by Indiana Department of Environmental Management (IDEM) field crews. This number is used to identify the sampling event in the Assessment Information Management System (AIMS) database.
- 6.2. "Ambient" Surrounding environmental conditions.
- 6.3. "Assessment Information Management System database (AIMS database)" IDEM database containing information related to water chemistry, aquatic habitat, macroinvertebrate, fish and algae communities, fish tissue analyses, sediments, and *E. coli* bacteria data collected by agency staff from watershed sampling events.
- 6.4. "Carboy" A large container, generally of five-gallon capacity, used to store water.
- 6.5. "Colilert" An enzyme-substrate medium, used for the detection of both total coliforms and *E. coli*.
- 6.6. "Deionized water" Water that has had its mineral ions removed through a process called ion exchange.
- 6.7. "Environmental Protection Agency (EPA) site ID" The identification number given to the 100 random probabilistic sites drawn yearly. Example: INRB23-001.

- 6.8. "*Escherichia coli (E. coli)*" Are indicator organisms (organisms indicating presence of pathogens) of bacteria associated with the digestive tract.
- 6.9. "Indiana Department of Environmental Management (IDEM)" An agency of Indiana State Government whose mission is to implement federal and state regulations to protect human health and the environment while allowing the environmentally sound operations of industrial, agricultural, commercial, and government activities vital to a prosperous economy.
- 6.10. "Indiana Department of Health (IDOH)" An agency of Indiana State Government responsible for the analysis of the environmental sample.
- 6.11. *"Klebsiella pneumoniae"* A bacteria used as the positive control for total coliforms, but negative for *E. coli*.
- 6.12. *"Pseudomonas aeruginosa"* A bacteria used as a negative control for total coliforms and *E. coli*.
- 6.13. "Quanti-Tray" A specially designed disposable incubation tray used for the quantification of total coliforms and *E. coli* in a water sample.
- 6.14. "Safety Data Sheet (SDS)" A sheet containing data regarding the properties of a particular substance or product. It is intended to provide workers and emergency personnel with procedures for handling or working with that substance or product in a safe manner.
- 6.15. "Site folder" A folder for a specific site that contains all pertinent paperwork concerning the site. Site reconnaissance forms, field data sheets, chain of custody forms, etc. are all stored in this folder which is located in a file cabinet in the WAPB library at the IDEM Shadeland office.
- 6.16. "Site Reconnaissance Form" Form used to gather information such as landowner, equipment needed to complete sampling, and the access route to take to the site (Appendix 3).
- 6.17. "Technical standard operating procedure (TSOP)" A standard operating procedure that involves environmental data generation, manipulation, or compilation of an analytical process.
- 6.18. "Total coliforms" –A normally harmless type of bacteria that resides in the intestinal tract of humans and other animals and whose presence in water is an indicator that the water may be contaminated with other disease-causing organisms found in untreated human and animal waste.

6.19. "Virtual File Cabinet (VFC)" – The agency's electronic digital image document repository system, that stores, files, indexes, redacts, reassembles, and securely accesses electronic documents of all types both received and created by the various program areas within the agency.

# 7.0. Quality Assurance and Quality Control

- 7.1. The analysis of the procedural control culture samples will be performed on each lot of Colilert. Procedural controls consist of:
  - A. E. coli for a positive total coliform and positive E. coli
  - B. *Klebsiella pneumoniae* for positive total coliform and negative *E. coli*
  - C. *Pseudomonas aeruginosa* for negative total coliform and negative *E. coli*
  - D. All procedural control culture samples are obtained from IDEXX Laboratories
- 7.2. A field blank analysis and a field duplicate analysis are required for every 1 to 20 water samples analyzed in each batch.
- 7.3. Lab blank (sterility control) Prepare and analyze a sterile deionized water blank (100mL of sterile deionized water) daily as you would a sample. Results must be negative for both total coliform and *E. coli*.
- 7.4. If dilutions are done for a sample, the result (original sample or diluted sample) falling within the detection range is recorded on the bench sheet. Results either above or below the detection range are rejected.

# 8.0. References

8.1 Indiana Codes (IC) or Indiana Administrative Codes (IAC)

A. <u>IC 14</u>-8-2-27: Boundary Waters

- 8.2 Agency Policies
  - A. IDEM. 2008. <u>Personal Protective Equipment Policy</u>, A-059-OEA-08-P-R0, May 1 2008. Indiana Department of Environmental Management, Office of External Affairs, Indianapolis, Indiana.
  - B. IDEM. 2010. <u>Health and Safety Training Policy</u>, A-030-OEA-10-P-R2, revised October, 1 2010. Indiana Department of Environmental Management, Office of External Affairs, Indianapolis, Indiana.

- C. IDEM. 2016. <u>Injury and/or Illness Resulting from Occupational</u> <u>Exposure Policy</u>, A-034-AW-16-P-R3, revised February 12, 2016. Indiana Department of Environmental Management, Office of External Affairs, Indianapolis, Indiana.
- D. IDEM. 2019. IDEM Hazard Communication (HazCom) Plan. Indiana Department of Environmental Management, Office of Program Support, Indianapolis, Indiana.
- E. IDEM. 2021. Office of Water Quality Watershed Assessment and Planning Branch Laboratory Safety Plan. Indiana Department of Environmental Management, Office of Program Support, Indianapolis, Indiana.
- 8.3 Other Guidance
  - A. Hach Company. 2021, 2100Q: Portable Turbidimeter. Loveland, Colorado.
  - B. YSI Incorporated/Xylem Incorporated. 2020, ProDSS: Multiparameter Digital Water Quality Meter. Yellow Springs, Ohio.
  - C. IDEXX Laboratories, Inc. 2022, Colilert Product Brochure. Westbrook Maine.
  - D. IDEXX Laboratories, Inc. 2021, IDEXX Model 2X: Quanti-Tray Sealer. Westbrook, Maine.
  - E. Standard Methods Online Standard Methods for the Examination of Water and Wastewater. <u>http://standardmethods.org/</u>

# 9.0. Appendices

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Ite Rating By Category (1=easy, 10=difficult)       Circle Reconnaissance Decision       Equipment Selected       Circle Equipment Selected         Access Route       No. Indower denied access. No. Dry No. Physical transfers       No. Indower denied access. No. Dry No. Physical transfers       No. Indower denied access. No. Dry No. Physical transfers       Image: Circle Equipment Selected       Image: Circle Equipment Selected         Safety Factor       No. Physical transfers       No. Indower denied access. No. Dry No. Physical transfers       Image: Circle Equipment Selected       Image: Circle Equipment Selected         Safety Factor       No. Physical transfers       No. Dry No. Breimparted stem No. Breimparted by backwater       Image: Circle Equipment Selected       Image: Circle Equipment Selected         mments       No. Breimparted by backwater       No. Other       Image: Circle Equipment Selected       Image: Circle Equipment Selected         etch of Stream & Access Route - Indicate Flow, Direction, Obstacles, & Land Use (Use back of page, if necessary)       Image: Circle Equipment Selected       Image: Circle Equipment Selected		Rating, Results, Comme	ents, and Planning		
Access Route       Approved Site         Safety Factor       No. Dry         No. Dry       No. Stream channel missing         No. Physical barriers       No. Impounded stream         Safety Factor       No. MarkWvelland         Sampling Effort       No. Unsafe due to traffic or location         No. Dry       No. Steem channel missing         No. Physical barriers       No. MarkWvelland         No. Steem channel missing       No. MarkWvelland         No. Unsafe due to traffic or location       No. Steem channel missing         No. Other       No. Other         mments       No. Other	ite Rating By Category (1=easy, 10=difficult)	Circle Reconnaissance Decisio Pre-Recon Recon in process	n Equ Selo	upment ected	Circle Equipment Needed Backnack
Safety Factor       No. Stream channel missing       Scance         Sampling Effort       No. Mush/Wetland       Seine         No. Bridge gone or not accessible       No. Stream Subject on the accessible       Seine         No. Unsafe due to traffic or tocation       No. Stream Subject on the accessible       Seine         mments       No. Other       Stream Subject on the accessible       Seine         setch of Stream & Access Route - Indicate Flow, Direction, Obstacles, & Land Use (Use back of page, if necessary)       Stream Subject on the accessible	Access Route	Approved Site No, Landowner denied access No, Dry		<.	Boat Totebarge Longline
Sampling Effort       No, MashWetland         No, Bridge gore on chaccessible       No, Mashers         No, Site impacted by backwater       No, Other         mments       No, Other	Safety Factor	No, Stream channel missing No, Physical barriers No, Impounded stream		-	Scanoe Seine Weighted Handline
etch of Stream & Access Route - Indicate Flow, Direction, Obstacles, & Land Use (Use back of page, if necessary)	Sampling Effort	No, Marsh/Wetland No, Bridge gone or not accessible No, Unsafe due to traffic or location No, Site impacted by backwater			Waders Gill Net
retch of Stream & Access Route - Indicate Flow, Direction, Obstacles, & Land Use (Use back of page, if necessary)	nmonte	No, Other			
etch of Stream & Access Route - Indicate Flow, Direction, Obstacles, & Land Use (Use back of page, if necessary)					
	tch of Stream & Access Route	Indicate Flow, Direction, Obstacles, &	Land Use (Use back of	page, if necessary)	

## Appendix 1 Blank Site Reconnaissance Form

			1- 1			••••					3 -	_				-			
	З'	$\Gamma$	<u>s</u>	trear	n San	np	ling F	ielo	1 D	ata S	hee	t	Analysis	Set #		EPA S	itte ID	Ra	ank
Sample (			Site	:			Sample N	lediur	m			Sam	ole Type		Du	olicati	ə Sam	ole 1	
																			_
Stream Nan	10:				•					River Mile	e -			Cou	my:				
Site Descript	tion:																		
Survey	S	ampl	e Colle	ctors	Sam	ple C	ollected	Н	ydrola	ib Dent	Water MGage I	Ht	Water Flo	w	Flow		Mase:	, Aqu	uatic
Crew Chief	1	2	3	4	Date		Time		*		(ft)		(cf/sec)	Es	timat	ed? '	-9	LI LI	le?
						_			_										
Samp	Netak	INO: I	Frozen			4	Wa	Dry	w Ty	pe Istannant	Clear	Wate	or Appears	Ince Shee		Can	opy C	0890	%
No; Stream	Dry 🗌	No; C	Other		120	24	Pool [	Run	Ē	Flood	Murky	ļ	Black	- oth	er i	20-	40% C	80-1	00%
LI No; Owner	refused	Acce	66		72 ∐ A8-FI	ow	LIGIIde L	L Eddy	/ L	Other	Brow	n l	Gray (Sep	tio/Sev	iage)	40-	80%		
Special Notes:																			
	Stream San           strest           strest <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>																		
Field Dat	Stream Samplin         Sample 2010/2         Sample Collectors       Sample Collectors         Sample Collectors       Sample Collectors         Sample Collectors       Sample Collectors         Sample Taken?       Allquots         set (Internet Participation         Sample Taken?       Allquots         set (Internet Participation         Sample Taken?       Allquots         set (Internet Participation         Sample Zathr Time       D.O.         pecial         Collectors       Sample Collectors         Sample Taken?       Allquots         set (Internet Participation         pecial         Option:         Massurement       Set (Internet Participation         Internet Participation         Measurement        All Measurement          Internet Participation         Internet Participation         Internet Participation													-			lasiba	- 0	10.0
(m/d/yy)	24-hr (hh:	mm)	9 D.O (mgi	ŋ PH	Temp (%	c) (	pec Cond pohme/om)	TURD (NT	iaity rU)	% Sat.	(mg/l)		(mg/l)	Chior (IT	opinyi 19/1)	S	C WD	WS	AT
Commente																			
Comments						Т						Т				Т		Г	г
Comments						-						+				+	-	-	-
Comments	└──																		
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Comments					1	<b>—</b>						T				-		-	-
Comments																			L
Comments	_		<b>—</b>						-			We	after Cod	e Defi	nifion	8			
			Meas	urement	> >N	lax. M	eter Measurer leter Measure	ment	ŀ			Γ	WD			we		•	
			L '	Flags	R Rej	ected	(See Comme (See Comme	ents) nts)		Sky Con	ditions		Wind Dire	ction	Wir	nd Str	ength	AIL	emp
Field Cali	ibrati	ions								1 Clear 2 Scattered	8 Rain 9 Snow	00	North (0 de) East (90 de)	rees)	00	aim Iobt		1<3	2
Date	Tim	8	Calibra	itor	C	alibr	ations			3 Partly	10 Sleet	18	South (180	degrees	0 2 0	Aod./Li	ht	346-	-60
(m/d/yy)	(hh:n	nm)	Initia	IS Ty	pe Me	ter #	E Value	Uni	lts	5 Mist		-	mest (270 c	cyrees)	4	Aod./St	ong	576-	-85
							+	+	-	7 Shower					60	iale		0-0	
				-11															
		c	alibrati Type	on DO															
Preserva	tives	/Bo	ttle L	ots:	ay .					Group	: Prese	vati	Ves		В	ottie '	Турев		
Group: Pre	servati	ve	Preser	vative Lo	# Bottle	Туре	Bottle L	.ot#	GC	General (	hemistry:	ice		2000P	2000	mL Pla	stic, Na	rrow M	outh
									Meta	is Metals: H	NO3			500P	500m	L Plas	IC, Nan	ow Mo	nuth
		_			—				OSG	OII & Gre	ase: H280	4		1000G	1000	mL Gla	ss, Nar	row Me	outh
									Ecol	Bacteriok	gy: Ice	CL #	Thiosutate	250G	250m	L Glas	s, Wide	Mouth	
									Pest	Pesticide	s: Ice	ion a	mosanate	40GV	40mL	Glass	Vial	-	
									Sed	Sedment	: Ice	<b>1</b>		1000PF	1000	mL Pla	stic, Co	ming F	iter
									He	Mercury(	631): HC	Maie		60P	50ml	Plasti	c, con c	ang Pl	
									MeH	g Methyl M	ercury(163	(0): H	ici	500T	500m	iL Tello	n		
									<u> </u>					1231	1250		ani i		

# Appendix 2 Blank Stream Sampling Field Data Sheet

Data Entered By: \_\_\_\_\_ QC1: \_\_\_\_\_ QC2: \_\_\_\_\_

Stream Sampling Field Data Sheet

## Appendix 3 Example Fixed Station Route Field Data Sheet

#### West Route Field Data - January 2017

#### Collector: David Arnold

#### Analysis Set: 17FSW009

ID	Sample #	FSite	Site	Date	Time	DO	% Sat	pН	Temp	Cond	Turb	Weather
76	AB27831	SC-57	WSU020-0003	01/04/2017	10:10 AM	10.85	83.3	8.24	4.03	600	46	3021
77	AB27832	WB-303	WLV030-0003	01/04/2017	11:30 AM	12.12	87.8	8.32	1.95	645	40	32731
78	AB27833	PC-21	WLV040-0003	01/04/2017	11:50 AM	11.84	86.9	8.11	2.51	358	59	32731
79	AB27834	WB-284	WLV080-0003	01/04/2017	12:10 PM	12.25	88	8.36	1.65	651	47	32741
80	AB27835	SC-39	WSU050-0002	01/04/2017	01:00 PM	12.08	89.8	8.34	2.95	606	92	3031
81	AB27836	RC-46	WLV160-0001	01/04/2017	01:45 PM	12.24	91.2	8.43	3.04	562	82	32721
82	AB27837	SC-25	WSU050-0005	01/04/2017	02:10 PM	12.35	91.8	8.43	2.98	598	109	32721
83	AB27838	WB-256	WLV140-0001	01/04/2017	02:30 PM	12.48	90.8	8.41	2.15	636	43	32741
84	AB27839	V-0.8	WVE100-0001	01/04/2017	02:45 PM	12.71	93.7	8.54	2.61	663	34	32731
85	AB27840	SC-2	WSU060-0004	01/05/2017	02:15 PM	11.26	85.1	8.11	2.1	530	51	42721
86	AB27841	WB-240	WLV-16-0001	01/05/2017	02:30 PM	12.51	90.6	8.21	1	561	155	42741
87	AB27842	RC-5	WLV190-0012	01/05/2017	01:45 PM	13.45	92.4	8.48	0	548	48	42911
87D	AB27848	RC-5	WLV190-0012	01/05/2017	01:45 PM							92711
88	AB27843	WB-230	WLV200-0001	01/05/2017	01:15 PM	12.46	89.2	8.45	1.54	631	57	92711
89	AB27844	WB-172	WBU-13-0001	01/05/2017	11:50 AM	12.41	90	8.38	2.03	630	46	92741
90	AB27845	EEL-38	WWE080-0001	01/05/2017	10:50 AM	12.08	94.5	8.42	2.23	517	34	42721
91	AB27846	BWC-4	WWE040-0001	01/05/2017	10:15 AM	13.4	104	8.32	4.51	350	33	42711
92B	AB27847	BLANK	BLANK	01/05/2017	01:15 PM							12711

#### Field Calibrations:

pH DO

Turbidity

Calibrati on Type

Sample #	Туре	Meter #	Value	Units
AB27831	DO		12.0	mg/L
AB27831	TUR	2	505.0	NTU
AB27831	Temp	39	2.6	°C
AB27831	pН	39	8.5	SU

Sky 1 Cle 2 Sca 3 Par 4 Clo 5 Mis	<mark>y Condit</mark> ar 8 attered 9 tly 10 udy	ions Rain Snow Sleet	00 09 18	Wind Direction North (0 degrees) East (90 degrees) South (180	↓ 0 1 2	Vind Strength Calm Light Mod /Light	A 1 2 3	<ul> <li>32</li> <li>33-45</li> </ul>
1 Cle 2 Sca 3 Par 4 Clo 5 Mis	ar 8 attered 9 tly 10 udy	Rain Snow Sleet	00 09 18	North (0 degrees) East (90 degrees) South (180	0 1 2	Calm Light Mod /Light	1 2 3	< 32 33-45
2 Sca 3 Par 4 Clo 5 Mis	attered 9 tly 10 udy	Snow Sleet	09 18	East (90 degrees) South (180	1 2	Light Mod /Light	2	33-45
3 Par 4 Clo 5 Mis	tly 10 udy	) Sleet	18	South (180	2	Mod /Light	3	10.00
4 Clo 5 Mis	udy					mouseigne	12	46-60
5 Mis			27	degrees)	3	Moderate	4	61-75
	t			West (270 degrees)	4	Mod./Strong	5	76-85
6 Fog	3				5	Strong	6	> 86
7 Sho	ower				6	Gale		

#### Preservatives/Bottle Lots:

Group: Preservative	Preservative Lot #	Bottle Lot #		Groups: Preservatives
GC: Ice	N/A	319-1000	GC	General Chemistry: Ice
Nx: H2SO4	SA-4349040	319-1000	Nx	Nutrients: H2SO4
Metals: HNO3	NA-5159180	319-1000	Metals CN	Metals: HNO3 Cyanide: NaOH
			O&G	Oil & Grease: H2SO4
			Toxics	Toxics: Ice
			VOA	Volatile Organics: HCI & Thiosulfate
			Pest	Pesticides: Ice
			Phen	Phenols: H2SO4
			Sed	Sediment: Ice
			Gly	Glyphosate: Thiosulfate
			Hg	Mercury(1631): HCI
			Cr6	ChromiumVI(1636): NaOH
			MeHa	Methyl Mercury(1630); HCI

Data Entered By: \_\_\_\_\_ QC1: \_\_\_\_\_

QC2: \_\_\_\_\_

## Appendix 4 Blank OWQ Chain of Custody Form



Indiana Department of Environmental Management OWQ Chain of Custody Form Project:

OWQ Sample Set or Trip #:

I Certify that the sample(s) listed below was/were collected by me, or in my presence. Date:\_\_\_\_\_

Signature:									Se	ction:			
Sample Media (🗆 )	Water, 🗖 Alga	le,□ Fisl	h, 🗆 Ma	acro, 🗆 🤇	Cyanob	acteria/I	Microcy	stin, 🗆	Sedime	nt)			
Lab Assigned	IDEM	iple rpe		M.	E .		l ml lact)	0 ml ene	ml ene	ml Iss	Date and Ti	me Collected	One check
Number / Event ID	Control Number	Sarr		1000 P.N.	1000 G.N.	40 Ki	120 P (E	2000 Nalg	250 Nalg	125 Gla	Date	Time	present
-													
									-	· · · · · · · · ·			
P = Plastic	G = Glass	N.I	M. = Na - Dupli	rrow Mo	buth	Bact =	Bacter	iologica	l Only		Should sample:	s be iced?	Y N

**Carriers** 

I certify that I have received the above sample(s).					
Signature	Date	Time	Seals	Intact	Comments
Relinquished By:			v	N	
Received By:				N	
Relinquished By:			v	N	
Received By:				N	
Relinquished By:			v	N	
Received By:			I	N.	
IDEM Storage Room #					

#### Lab Custodian

I certify that I have received the above sample(s), which has/have been recorded in the official record book. The same sample(s) will be in the custody of competent laboratory personnel at all times, or locked in a secured area.

Signature:\_\_\_\_\_

\_\_\_\_\_

Date:\_\_\_\_\_ Time:\_\_\_\_\_

Lab:

Address:\_\_\_\_\_

Revision Date: 4/27/2016

Appendix 5 Blank Lab Bench Sheet

					Quanti T	ray 2000 \	Nork Sheet						Batch #	DATE	TIME	Analysts			Date Complete
Analysis Set Nu	mber	Sample Medium	LAB name	loh #		Date Received by		Time Received by Lab		Samples Collected By		RUN							
Analysis See Hu	inder	w		500 #		Lab		by Lab		conceted by	Ţ	DRED							
						1		1				THEF		TA D	L - ( Ob +0	X / N	DAOE	4 -4	L
Project	t Name	and Des	cription		Large	Small							DA	Large	Small	Y/N	PAGE	1 OT	<u> </u>
EDI QA Data	Par	ameter			Cubes	Cubes	Measured							Cubes	Cubes	Measured			
Sample #	Me	ethod	Q/C Type	Lab Internal #	Positive	Positive	Value	UNITS	Dil. Mult.	Lab Q/A Flag		Paramet	er Method	Positive	Positive	Value	UNITS	Dil. Mult.	Lab Q/A Flag
	E. coli /	SM9223B	Eq Blank					cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		<b> </b>
	E. coli/s	SM9223B	EC					cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B	КР					cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B	PA					cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B	Eg Blank					cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
<b>IDEXX</b> Supplie	es		Media Lot #																
			Q Tray Lot #																
EDI Lab Data			1	-	Large	Small			1					Large	Small				
Sample #	Par	ameter		I ab Internal #	Cubes	Cubes Positive	Measured Value	UNITS	Dil Mult	Lab O/A Elag		Daramot	er Method	Cubes	Cubes Positive	Measured Value	UNITS		Lab O/A Elag
campie #	E coli/	MODOD	Field Plank	Lub intorna #			Tuluo	cfu/100ml	Dii. Mart.	Lub G/A Hug		Coliform					cfu/100ml	Dit. Mart.	Lub and Hug
	E. conv	01400000	Tield Diatik					-6./100-1				Collionn,	T/01/02200				-6.(100-1		
	E. COII /	SM9223B						CITA LOOME				Coliform,	1/SM9223B				CTU/ TUUML		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform.	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		
	E. coli/s	SM9223B						cfu/100mL				Coliform,	T/SM9223B				cfu/100mL		

Appendix 5 Blank Lab Bench Sheet (continued)

PAGE 2 of\_\_\_\_\_

Y / N

EDI Lab Data	1		1	Large	Small							Large	Small				
Commits #	Parameter	CompTime		Cubes	Cubes	Measured	UNITO					Cubes	Cubes	Measured	LINUTO		
Sample #	Method	SampType	Lab Internal #	Positive	Positive	value	UNITS	Dil. Mult.	Lab Q/A Flag	Parameter	Method	Positive	Positive	value	UNITS	Dil. Mult.	Lab Q/A
	E. coli/SM9223B						cfu/100mL		- I	Coliform, T/	SM9223B				cfu/100mL		
	E. coli /SM9223B						cfu/100mL		I	Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli /SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E coli/SM9223B						cfu/100mL			Coliform T/	SM9223B				cfu/100mL		
	E coli/SM0223B						cfu/100ml			Coliform T/	SM0222B				cfu/100ml		
	E coli/SM0223B						cfu/100ml			Coliform T/	SM02220D				cfu/100ml		
	E coli/SM0222B						chu/100ml			Coliform T/	SM02220D				cfu/100ml		
	E. coli/SM0222B						chu/100ml			Coliform T/	SM02220D				cfu/100mL		
	E. coli/SM0222B						chu/100ml			Coliform T/	SM0223D				ofu/100mL		<u> </u>
	E. coli/SM0223B						chu/100mL			Coliform T/	SM0223D				stu/100ml		
	E. coli/SM9223B						city forme			Coliform T/	SM0223D				city 100mL		
							ciurioumL			Coliform T	SIV19223B				sturioomL		
	E. coll/SM9223B						ctu/100mL			Coliform, 1/	5M9223B				ciu/100mL		
	E. COIVSM9223B						cfu/100mL			Contorm, 1/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		
	E. coli/SM9223B						cfu/100mL			Coliform, T/	SM9223B				cfu/100mL		1

DATA on Back of Sheet?

### Appendix 6 E. coli Sampling Checklist

Front Seat	Back of Truck	<u>E. coli Mobile Lab</u>
Electronics-	Hydrolab Bucket–	Chain of Custodies – Blank
Phone – Charger	Hydrolab (calibrated)	Bench Sheets – Blank
GPS - Charger	· · · · · ·	Bottles
Camera (batteries)	QA Equipment-	Trays
Photos Taken Sheet	Turbidity Meter (cal)	Media
	pH/Temp Meter (cal)	Gloves
Field Box–	Winkler Kit (refill)	UV Lamps
Pens/Pencils	( )	Sealer
Markers	PPE Bags-	Power Cords
E, coli bottles (>40)	Life Jackets	Trash
Sampling Schedule	Yellow Vest	Pinettes
IDEM Field Sheets	Rain Suit	Bench Sanitizer
Site Folders	Flashlight	Hand Sanitizer
Example Data Sheets	Thushinght	Paper Towels
Blank Data Sheets	Loose Equipment_	Markers
Dialik Data Sheets	Lanton Computer	Ice Cooler
Vehicle Binder	Pole Sampler	Ice Scoop
Gazetteer	Hin Waders	Dark Box
Clipboard	WD40	Durk Dox
Hand Sanitizer	Tana	
	Tape	Luggaga
Bug Spray		Luggage
Under Seet		
<u>Under Seat</u> Maghata		
Internete		
Jack – The from Einst Aid Vit		
FIRST AIU KIT		
Middle Seet		
Lee Cooler		
Dettles		
None Prov Carbon		
Nano-Pure Carboy		
Cloves		
Salety Glasses		
Eyewash Solution		