DYE TRACE INVESTIGATION WORK PLAN

Lakeshore Recycling Systems (LRS)

NEW LANDFILL

BAXTER COUNTY, ARKANSAS

SCS ENGINEERS

27222139.01 | January 2023

11219 Richardson Drive

North Little Rock, Arkansas 72213

501-812-4551

Table of Contents

FIGURES

APPENDICES

1 INTRODUCTION

Using fluorescent dye to trace groundwater movement is a cost-effective and reliable method for documentation of point-to-point pathways in karst groundwater flow systems, delineation of spring basin boundaries, and determination of time-of-travel of groundwater between known points in the subsurface (Crawford Hydrology Lab, 2010; Aley, 2003; Quinlan, 1989). Dye-tracing methodology has been used successfully in the past in evaluating potential flow routes in the vicinity of NABORS landfill (Grubbs, Garner, Hoskyn Inc. 1987). Conservative tracer dyes are introduced into groundwater, where they are advectively transported downgradient to points of discharge and can be sampled using passive receptors at potential points along the flow system. These nonhazardous dyes fluoresce at distinct wavelengths and can be detected with a scanning spectrofluorophotometer. As a result, dye tracing provides distinctive, reproducible evidence of groundwater flow connections that would otherwise remain oblique. This dye-tracing study will be used to supplement the dye-tracing study conducted in 1987 (Grubbs, Garner, & Hoskyn, Inc.). See Appendix F for a synthesis of previous tracing studies.

The former NABORS Landfill is located on 56 acres near the community of Three Brothers in Baxter County, Arkansas. The permitted Class 1 area lies within a portion of the Southwest quarter of the Southeast quarter of Section 26, Township 21 North, Range 14 West; and the Northwest corner of the Northeast quarter of Section 35, Township 21 North, Range 14 West. The property has historically been used and operated as a solid waste disposal site (See FIGURE 1).

The site geology consists of a sandy silt to silty clay residuum overlying Cotter Dolomite bedrock. The residuum, which contains chert fragments, occurs with greatest thickness at ridge tops, narrowing to 6' or less in areas of lower elevation. The Cotter Dolomite consists of two types of dolostone: one fine-grained and white to light gray locally called "cotton rock," the other darker gray, medium-grained, and more massive. Chert, minor shale beds, and infrequent thinly interbedded sandstone also characterize the Cotter formation.

In the Cotter Dolomite, groundwater flow tends to occur along joints, fractures, bedding planes, or within cherty zones in areas that are often widened by solutional weathering. Boring logs and previous excavations indicate the presence of a weathered zone in the uppermost 10 feet of bedrock. The presence of this epikarstic layer suggests seasonal groundwater flow in this area of the unit primarily occurs within this weathered zone. Although the bedrock is primarily competent, karst features are present at the site at and below ground surface, including the following: springs present on the landfill property; voids that have been recorded in boring logs of investigative drilling; and possible fast conduit flow that has been inferred by previous investigations.

2 PREVIOUS TRACER INVESTIGATIONS

In 1987, a limited dye trace was performed at the landfill site as part of a larger geotechnical and hydrogeologic investigation at landfill no. 3 (Grubbs et al., 1987). The purpose of the dye tracing in the 1987 investigation was to evaluate the source of groundwater flow to a series of springs identified at or near the site. Dye was introduced into a borehole approximately 165 feet north of “Spring A” located on the northern portion of the project site within a ravine. The drilled borehole was approximately 19 feet in depth. A weathered zone of broken chert and clay with some voids was encountered in the borehole from 14 to 18 feet below ground surface (bgs) with bedrock consisting of hard gray dolomite encountered at a depth of 18 feet to the total depth of 19 feet bgs. Fluorescein dye was introduced into the borehole and flushed with 500 gallons of water. Dye was visually observed at Spring A two hours following the dye introduction and visually observed at Spring B approximately 24 hours later. Analysis of passive charcoal packet samplers indicated dye detections in Springs A, B and C.

No other tracer investigations are known to have been performed at the site.

3 PURPOSE OF TRACER INVESTIGATION

The purpose for conducting the dye trace investigation is to identify points of interconnected groundwater flow in order to characterize groundwater flow and discharge at the landfill site. This information will be utilize to evaluate and revise if necessary, the groundwater monitoring system for the landfill site.

4 DYE TRACE STUDY METHODOLOGY

In order to evaluate groundwater flow at the site, a fluorescent tracer dye will be introduced into a borehole IW-1 in the area near monitoring wells MW-1 and MW-1R. Two rounds of background monitoring will be performed prior to the dye introduction to identify and quantify any potential background fluorescence. Monitoring wells, geotechnical boreholes, and springs/seeps will be monitoring for the tracer dye over the course of several months following the dye introduction to characterize groundwater flow at the site.

4.1 Background Sampling

Background samples will be collected at all available monitoring locations prior to dye introduction in order to identify and quantify any fluorescence background at or near the emission fluorescence wavelengths of tracer dyes that might potentially be used in the investigation. Two rounds of sampling will be conducted. The first round of background sampling results will be used to assist in final dye type and quantity selection. The second round of background sampling assists with quantifying variability of the fluorescence in the background and helps to create a continuous monitoring record during the investigation.

A field reconnaissance was performed at the site on December 20, 2022. Activated carbon samplers were placed in existing monitoring wells, exploratory borings installed in November 2022, and springs and seep locations identified previously as well as additional locations identified on that date. The first round of background samples was collected on December 28-29, 2022 and analyzed by the Ozark Underground Laboratory on January 3, 2023. No fluorescence was detected in the range of standard tracer dyes in any of the sampling locations.

4.2 Dye Introduction

4.2.1 Dye Type and Quantity

The tracer dye will be selected from one of the most commonly used fluorescent tracer dyes: fluorescein, eosine, and rhodamine WT. All three of these dyes are safe for human, animals, and aquatic life when used in professionally directed studies (Field et al, 1995). These fluorescent dyes are highly detectable in grab samples of water or in elutants from activated carbon samplers.

Based on our preliminary understanding of the site and the results of the first round of background monitoring, 10 pounds of fluorescein dye is proposed. Fluorescein is the most mobile tracer dye of the commonly used fluorescent tracer dyes and has been successfully used at the site in the past. The Safety Data Sheet (SDS) for fluorescein is included in Appendix C.

Powdered fluorescein dye will be mixed into water for best results during the dye introduction. Dye mixing will be performed before arriving at the site to minimize potential cross-contamination issues at sampling locations.

4.2.2 Dye Introduction Methodology

A four-inch open borehole was drilled in November 2022 in the area near monitoring wells MW-1 and MW-1R (see FIGURE 2). This dye introduction borehole, identified as IW-1, is 91.29 feet in total depth. Groundwater was encountered at a depth of 62.76 ft bgs. A general description of the borehole lithology is summarized as follows:

0 to 4 ft bgs	silty clay with gravel
4 to 91 ft bgs	dolomite bedrock

A clean water test was performed in November 2022 to verify the borehole will transmit water and dye into the subsurface at a rate adequate to introduce the dye to the groundwater system. Flush water will be obtained from a nearby residential water well.

During the tracer dye introduction, a small amount of water will be initially flushed into the well to wet the interior of the borehole. Liquid dye will then be poured directly into the borehole followed by clean water to flush the dye out of the borehole and into the aquifer. Flush water will be added to the borehole for up to 48 hours or until a minimum of 600 gallons of water are introduced following the dye. Flush water will be added by tremie to the bottom of the borehole to ensure the dye is flushed into the aquifer and prevent the denser dye solution from settling in the bottom of the borehole.

4.3 Sampling for Tracer Dye

Primary sampling reliance will be performed with passive activated carbon samplers (also known as charcoal packets, dye traps or “bugs”). These samplers consist of nylon screen mesh packets approximately one and half inches by four inches (1.5”x4”) in size and containing approximately 4.25 grams of granular activated carbon. These samples adsorb and concentrate the tracer dye over the sampling period. Grab samples of water will also be collected where available during sampling events and archived at the laboratory. Water samples will be analyzed if a positive detection is found in the corresponding activated carbon sampler, if the activated carbon sampler is missing, or if the results will aid in the overall interpretation of the tracer investigation.

A comprehensive round of water levels in the monitoring wells and specific conductance measurements at all sampling locations will be made during at least 3 sampling events to assist in the evaluation of groundwater flow at the site.

4.3.1 Dye Monitoring Locations

Sampling locations will consist of the following monitoring wells, geotechnical borings, springs and seeps previously identified at the site, as well as sampling locations identified on the field reconnaissance performed on December 20, 2022.

Table 1 Dye Trace Monitoring Locations

Location Number	Location Name	Description
001	IW-1	Dye introduction borehole – background sampling only
002	Flush water	Background sampling only
101	EB-1	Recently installed geotechnical borings
102	EB-2	
103	EB-4	
104	EB-5	
201	MW-1	Existing site monitoring well
202	MW-1R	
203	MW-2	
204	MW-3	
205	MW-4	
206	MW-5	
207	MW-6	
208	MW-7	
209	CAO-1	
210	CAO-2	
211	CAO-3	

Location Number	Location Name	Description
212	*Number not used (well damaged).*	
213	NAB-2	
214	NAB-3	
215	NAB-4	
216	NAB-7	
217	NAB-8	
218	MW-509D	
219	MW-577	
220	MW-633D	
221	MW-689D	
222	NE-2	
223	NE-3	
224	NE-4	
225	NE-6	
301	Entrance seep	Springs, seeps, and surface streams
302	Spring A	
303	Spring B	
304	TSP-1 *(previously identified temporary sampling point)*	
305	TSP-2 *(previously identified temporary sampling point)*	
306	TSP-3 *(previously identified temporary sampling point)*	
307	TSP-4 *(previously identified temporary sampling point)*	
308	Class 1 Draw	
309	Class IV Draw	
310	SP-4 *(previously identified spring)*	
311	SP-5 *(previously identified spring)*	
312	SP-7 *(previously identified spring)*	
313	Spring near NE-3	
314	Spring near CAO-3	
315	Seep below southwest pond	
316	SW-1 *(stream near NE-6)*	
317	Seep near MW-6	
318	Spring at culvert downstream of TSP-1 and TSP-2	
319	Seep near MW-577	
320	SW-2 *(stream near NE-3)*	
321	East pond discharge	
322	Seep- below east pond	
323	SW-3 *(stream from north pond)*	

Location Number	Location Name	Description
324	Spring Branch from entrance gate	

4.3.2 Sampling Procedures

Properly trained personnel will retrieve the samplers from wells, springs, and other sampling sites. Receptors will be individually sealed in plastic bags for transport to the lab. The sample number, time and date of collection, and the name of the collector will be recorded on the sampling bag at the time of collection. Disposable gloves will be worn during sample collection. A new pair of gloves will be used at each site to avoid cross-contamination of samples. The samples will be stored in a cool, dark place until analysis is performed.

4.3.3 Sample Frequency and Duration

Sampling following the dye introduction is proposed on the following schedule:

Table 2 Sample Collection Frequency and Duration

Week Number	Sampling Frequency	Number of Sampling Events
Weeks 1 – 2	2 sampling events per week	4
Weeks 3 – 10	1 sampling event per week	8
Weeks 11 - 18	1 sampling event every 2 weeks	4
Total Sampling Events following Dye Introduction		**16**

The proposed sampling duration is 4 months following the dye introduction, with the sampling frequency beginning with two sampling events per week and extending to weekly after two weeks, then one event every other week during weeks 11 through 18. This graduated monitoring schedule allows for determination of fast groundwater discharge at springs at the site, while also allowing for monitoring of slower groundwater movement between monitoring wells over an extended period.

Ongoing monitoring may be continued on a two-to-four-week sampling interval if the preliminary results suggest that continued monitoring is warranted.

4.4 Laboratory Analysis

Laboratory analyses will be conducted by the Ozark Underground Laboratory in Protem, Missouri, following the detailed laboratory procedures presented in Appendix A.

Positive dye detections in activated carbon samplers will be based on the following criteria, as summarized from Appendix A:

1) Fluorescence peak present in the normal range for the dye as analyzed by the RF-5301 (see Table 3);
2) Dye concentration associated with the fluorescence peak must be at least 3 times the detection limit;

3) Dye concentration must be at least 10 times greater than any other concentration reflective of background; and
4) Shape of the fluorescence peak must be typical of the dye standard. Much background fluorescence yields low, broad, and asymmetrical fluorescence peaks rather than the more narrow and symmetrical fluorescence peaks typical of fluorescent tracer dyes.

Table 3. Ozark Underground Laboratory Spectrofluorophotometer Analysis: Normal Emission Wavelength Ranges and Detection Limits for Eosine, Fluorescein, and Rhodamine WT in water and elutant samples.

Dye and Matrix	Normal Acceptable Emission Wavelength Range nanometers (nm)	Detection Limit parts per billion (ppb)
Eosine in elutant	540.0 to 545.8	0.050
Eosine in water	532.8 to 537.8	0.015
Fluorescein in elutant	514.5 to 519.6	0.025
Fluorescein in water	506.8 to 510.6	0.002
Rhodamine WT in elutant	565.2 to 571.8	0.170
Rhodamine WT in water	572.4 to 577.7	0.015

4.5 Quality Assurance/Quality Control (QA/QC)

QA/QC procedures will be followed both in the field and in the laboratory. Careful attention will be paid by all field personnel to verify that the dye samplers only come in contact with water in the monitoring well or spring. The sampling string and weighted bailers will be dedicated to each well to prevent the danger of cross-contamination between wells. Isolating the receptors within sealed bags and using gloves will further preclude contamination. At no time except when the receptor is submerged in the well or spring will it come into contact with a source of dye, other than through groundwater transport from injection sites.

A QA/QC sample of dedicated sampling materials will be analyzed to verify these materials do not contribute outside fluorescence to the study. One sample of each of the following materials is anticipated: bailer, string, and twist tie wire. In addition, a sample of the flush water will be analyzed to verify it is free from background fluorescence.

Sampling supplies provided by the laboratory (i.e., activated carbon samplers, water sample vials, and sample collection whirl-pak® bags) are routinely sampled to verify they are free from background fluorescence.

Laboratory QA/QC is detailed in the laboratory methodology detailed in Appendix A.

Two field blank dye samplers, immersed only in distilled water, will be submitted to the Ozark Underground Lab for analysis. These will not be identified as special samples.

Duplicate samples will be submitted to laboratory for analysis, representing approximately 5% of the entire suite of samples.

The security and preservation of all samples will be recorded by a chain of custody document. This document will accompany all samples and will be signed upon relinquishing and accepting the samples.

5 SCHEDULE

SCS Engineers is prepared to perform the dye introduction within 7 days of the approval of this work plan by ADEQ. The following schedule is tentatively proposed, and will be adjusted according to ADEQ approval of this work plan:

Week Number	Approximate Date	Activity
-3	December 20	Field Reconnaissance, place background samplers
-2	December 28-29	Collect first background samplers, replace samplers
-1	Week of January 3	First background results available; final dye selection; Work plan submittal to ADEQ
0	Week of January 16	Collect second background samplers; Dye introduction.
1	Week of January 23	Two sampling events per week. Collect samplers and grab samples of water at each location; place sampler for next round of sampling.
2	Week of January 28	
3	Week of February 6	One sampling event per week. Collect samplers and grab samples of water at each location; place sampler for next round of sampling.
4	Week of February 13	
5	Week of February 20	
6	Week of February 27	
7	Week of March 6	
8	Week of March 13	
9	Week of March 20	
10	Week of March 27	
11	Week of April 3	One sampling event every-other week frequency.
12	Week of April 10	
13	Week of April 17	One sampling event every-other week frequency.
14	Week of April 24	
15	Week of May 1	One sampling event every-other week frequency.
16	Week of May 8	
17	Week of May 15	One sampling event every-other week frequency.
18	Week of May 22	

At the end of the eighteen weeks, we will assess all available tracing data and discuss them with ADEQ. The investigation will be concluded only by mutual agreement.

The dye test is planned to be monitored for 18 weeks. This is an adequate timeframe given the mass of dye proposed for injection, coupled with large volumes of supplemental recharge water to flush the dye into the dynamic part of the flow system. Ongoing monitoring after the 18-week interval will continue if the data on tracing are incomplete due to low flow, hydrologic, or other natural conditions, based on mutual agreement between relevant parties. If mutually determined, sampling will continue on a two- to four-week sampling interval between sampler replacement until connectivity between injection points and potential monitoring points can be established. A final report will then be completed.

This is not a steady-state dye test; rather, it will create a pressure buildup around the injection well; and it will perturb the natural water level, increasing groundwater velocity. Seeing as the winter season often exhibits low flow conditions, it is appropriate to use these methods to facilitate moving the dye into active parts of the flow system. These approaches have been used by numerous dye-tracing scientists (Aley, 1997; Ralph Ewers, personal communications, 2012), and although they may result in greater observation of radial flow, the benefits they offer justify the understanding of the system.

We feel that careful selection and clean water verification of the dye introduction location to accept supplemental recharge will allow a successful, meaningful test.

During the time period that the dye test is being conducted, it is requested that groundwater monitoring be suspended in those wells included in the dye test until the conclusion of the test. This will allow the monitoring wells to remain undisturbed until the conclusion of the test, and it will minimize the potential for contamination.

6 REPORTING

A summary report will be provided at the conclusion of the investigation and will include the following elements:

1) All dye analysis results;
2) An identification of sampling locations where dye was detected;
3) Other relevant data; and
4) A discussion of the results.

7 REFERENCES

Aley, Thomas, 2003, Groundwater tracing handbook: Ozark Underground Laboratory, Protem, Missouri, 35 p.

Aley, Thomas, 1997, Groundwater tracing in the epikarst: in Beck, B.F. and Stephenson, J. Brad, eds., Sixth Multidisciplinary Conference on Engineering Geology and Hydrogeology of Karst Terranes, A.A. Balkema, Rotterdam, p. 207-211.

Crawford Hydrology Lab, 2012, Karst groundwater investigation research procedures.

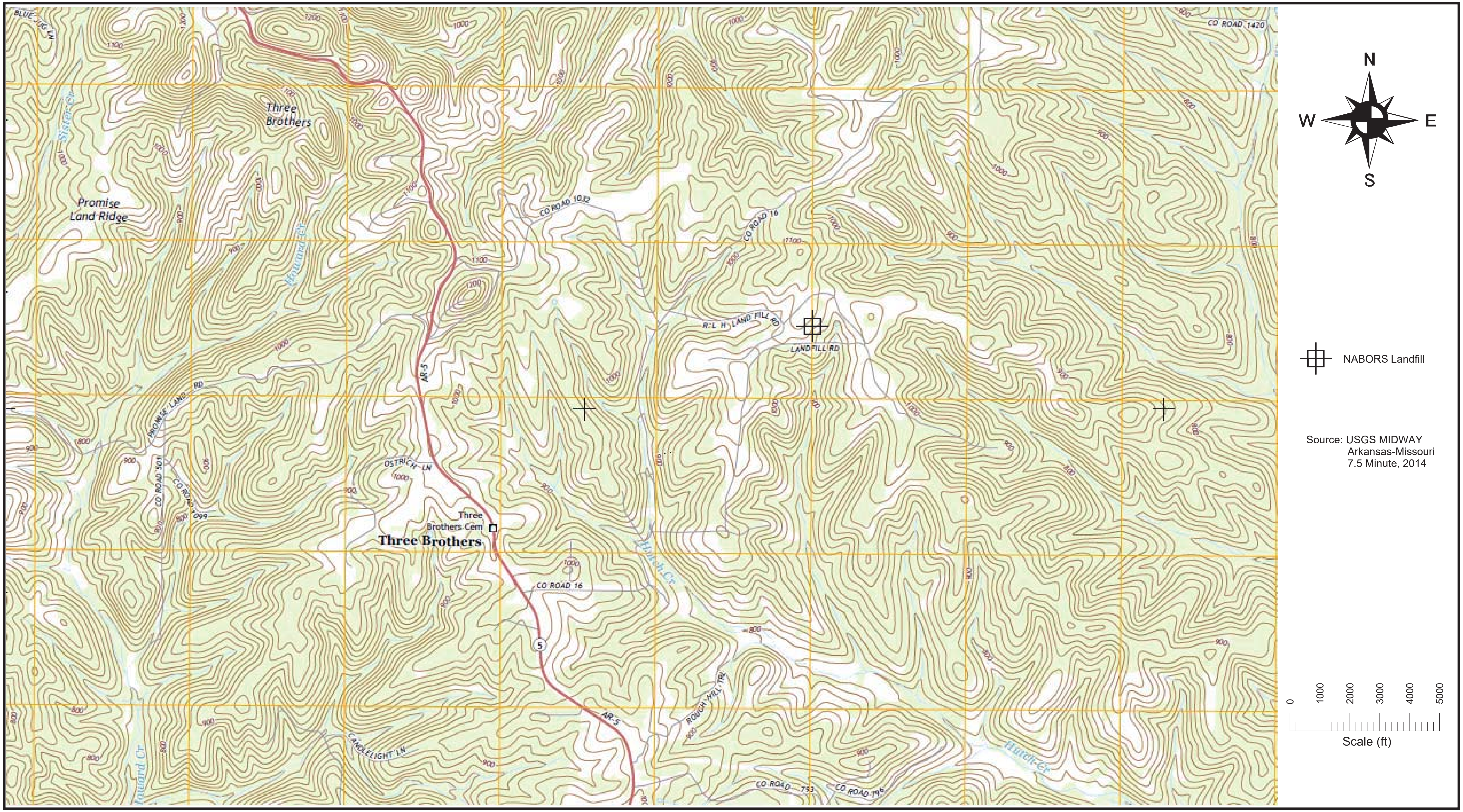
Ewers, Ralph, 2012. Personal Communication.

Field, Malcolm S.; Wilhelm, R.G.; Quinlan, J.F.; and Aley, T.J., 1995, An Assessment of the Potential Adverse Properties of Fluorescent Tracer Dyes Used for Groundwater tracing. Environmental Monitoring and Assessment, Vol. 38, pp. 75-96. Kluwar Academic Publishers.

Grubbs, Garner, Hoskyn Inc., 1987, Geotechnical and Hydrogeologic Study R-L-H Sanitary Landfill No. 3 Near Three Brothers, Arkansas. 229 p.

Quinlan, J.F., 1989, Ground-water monitoring in karst terranes: Recommended protocols and implicit assumptions: U.S. Environmental Protection Agency, Environmental Monitoring Lab, Las Vegas, Nevada. EPA/600/X-89/050. 78 p.

Figures



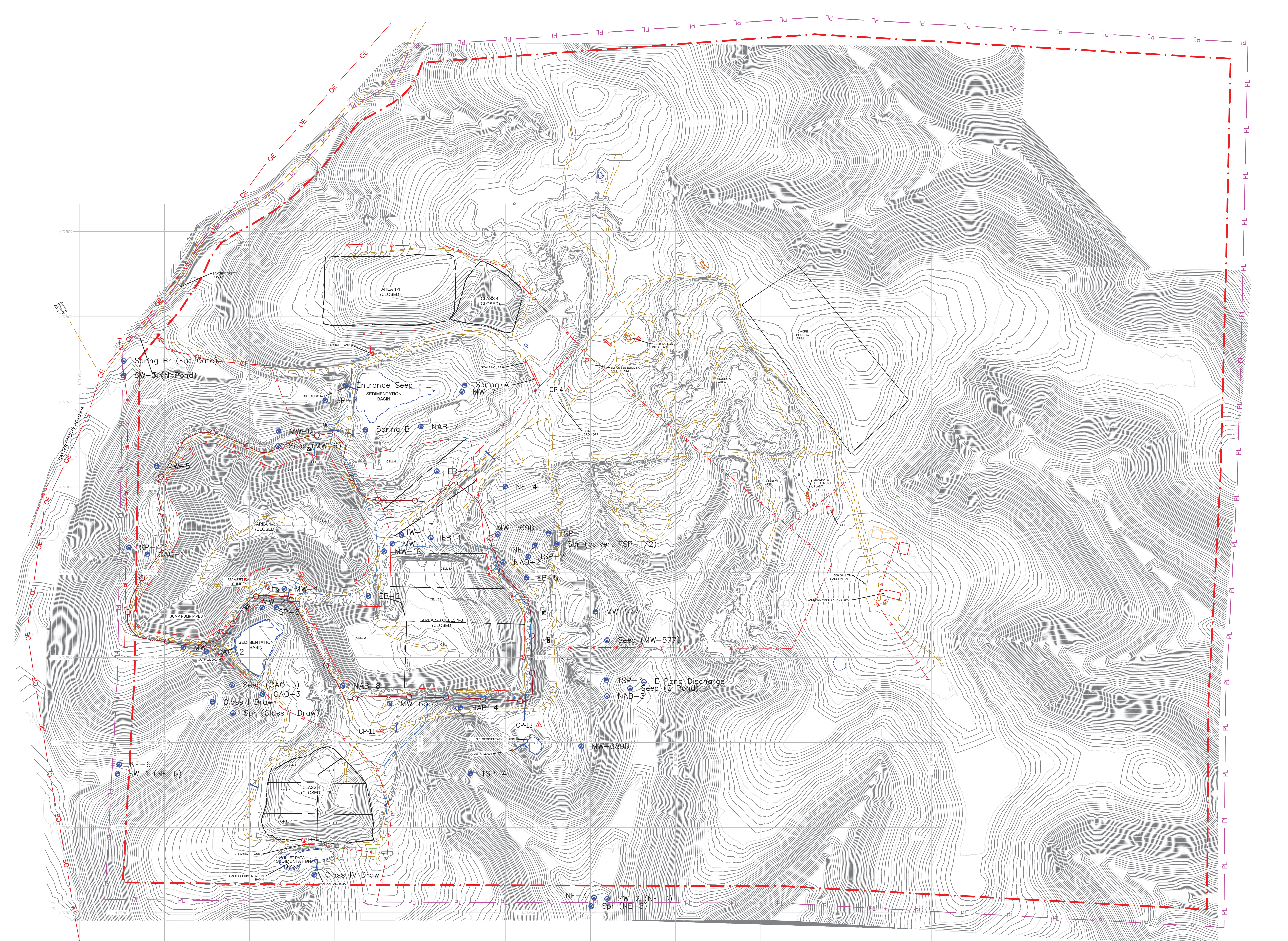
SCS ENGINEERS
www.scsengineers.com

Site Location Map
NABORS Landfill
Baxter County, Three Brothers, AR

FIGURE 1

Date: 1/5/23

DWM



300 0 300 600

SCALE FEET

LEGEND:

- EXISTING 2' MINOR CONTOUR
- 1000 — EXISTING 10' MAJOR CONTOUR
- PL — PROPERTY BOUNDARY
- 100' PROPERTY BOUNDARY OFFSET
- FENCE
- OE — EXISTING OVERHEAD ELECTRIC
- EXISTING ROAD
- OHE — EXISTING OVERHEAD ELECTRIC
- DISPOSAL BOUNDARY (APPROXIMATE)
- EXISTING BODY OF WATER
- EXISTING DRAINAGE PIPE/CULVERT
- MW-2 EXISTING MONITORING WELL
- GP-16 EXISTING GAS PROBE
- CP-5 EXISTING CONTROL POINT

WELL LOCATION TABLE

Point #	Northing	Easting	Description
1	774621.16	1180787.62	MW-1R
2	774665.59	1180834.95	MW-1
4	774057.04	1179608.33	MW-3
5	774400.39	1180200.85	MW-4
9	774603.37	1179397.41	CAO-1
10	774031.04	1179743.71	CAO-2
11	773782.77	1180074.51	CAO-3
12	774557.30	1181484.26	NAB-2
13	773771.34	1182094.69	NAB-3
14	773703.13	1181234.18	NAB-4
16	773831.32	1180543.89	NAB-8
18	774266.10	1182027.19	MW-577
20	774290.12	1180071.70	MW-2
21	775121.22	1179450.96	MW-5
22	775325.87	1180168.40	MW-6
23	775557.84	1181245.10	MW-7
24	775354.05	1181002.60	NAB-7
25	774721.71	1181454.46	MW-509D
26	773726.78	1180820.05	MW-633D
27	773476.20	1181943.37	MW-689D
28	774655.80	1181670.38	NE-2
29	772588.55	1182018.89	NE-3
30	775000.66	1181498.01	NE-4
31	773369.70	1179231.93	NE-6
32	774701.35	1181061.50	EB-1
33	774358.91	1180694.47	EB-2
34	775091.15	1181096.52	EB-4
35	774465.66	1181622.96	EB-5
36	774716.18	1180890.13	IW-1
37	775593.22	1180561.06	Entrance Seep
38	775595.39	1181259.16	Spring A
39	775334.34	1180678.11	Spring B
40	774726.62	1181751.93	TSP-1
41	774591.43	1181632.73	TSP-2
42	773863.46	1182091.40	TSP-3
43	773315.15	1181292.86	TSP-4
44	773736.82	1179779.47	Class I Draw
45	772721.93	1180377.04	Class IV Draw
46	774644.25	1179288.84	SP-4
47	774290.92	1180154.40	SP-5
48	775507.67	1180441.59	SP-7
49	772537.06	1182002.10	Spr (NE-3)
50	773670.57	1179900.08	Spr (Class 1 Draw)
51	773835.53	1179894.66	Seep (CAO-3)
52	773314.09	1179221.68	SW-1 (NE-6)
53	775239.68	1180164.30	Seep (MW-6)
54	774662.85	1181799.36	Spr (culvert TSP-1/2)
55	774098.65	1182095.10	Seep (MW-577)
56	772579.97	1182095.82	SW-2 (NE-3)
57	773853.81	1182311.80	E Pond Discharge
58	773816.56	1182230.18	Seep (E Pond)
59	775653.67	1179258.38	SW-3 (N Pond)
60	775739.07	1179259.31	Spring Br (Ent Gate)

REV. DATE DESCRIPTION CK. BY

SHEET TITLE

DYE STUDY WELL LOCATION MAP

PROJECT TITLE

DYE STUDY

CLIENT

LAKESHORE RECYCLING SYSTEMS OZH

1320 LANDFILL RD.
THREE BROTHERS, AR. 72653

SCS ENGINEERS
STEARNS, CONRAD AND SCHMIDT CONSULTING ENGINEERS, INC.
11219 RICHARDSON DRIVE
NORTH LITTLE ROCK, ARKANSAS 72113
PH. (501) 812-4551

PROJ. NO. 27222230.0 | DSN. BY: KHJ | DWN. BY: KHJ | CHK. BY: FEC | Q/A RVW BY: FEC | APP. BY: FEC

CADD FILE: 001

DATE: JANUARY 2023

SCALE: AS SHOWN

DRAWING NO. 1

C:\Users\5360khj\Desktop\27222139.01\AutoCAD\Sheets\Dye Study\DYE STUDY WELL LOCATION MAP.dwg

Appendix A

Ozark Underground Laboratory, Inc.

Procedures and Criteria Document

Ozark UNDERGROUND LABORATORY 1572 Aley Lane • Protem, MO 65733 • (417) 785-4289 • fax (417) 785-4290 • contact@ozarkundergroundlab.com

PROCEDURES AND CRITERIA
ANALYSIS OF FLUORESCENT DYES
IN WATER AND CHARCOAL SAMPLERS:

FLUORESCEIN, EOSINE, RHODAMINE WT, AND SULFORHODAMINE B DYES

Revision Date:
March 3, 2015

Thomas Aley, PHG, PG
President

and

Shiloh L. Beeman, PG
Sr. Hydrogeologist

Ozark Underground Laboratory, Inc.
1572 Aley Lane
Protem, Missouri 65733
417-785-4289
contact@ozarkundergroundlab.com

INTRODUCTION

This document describes standard procedures and criteria currently in use at the Ozark Underground Laboratory (OUL) as of the date shown on the title page. Some samples may be subjected to different procedures and criteria because of unique conditions; such non-standard procedures and criteria are identified in reports for those samples. Standard procedures and criteria change as knowledge and experience increases and as equipment is improved or up-graded. The OUL maintains a summary of changes in standard procedures and criteria.

TRACER DYES AND SAMPLE TYPES

Dye Nomenclature

Dye manufacturers and retailers use a myriad of names for the dyes. This causes confusion among dye users and report readers. The primary dyes used at the OUL and described in this document are included in Table 1 below.

Table 1. Primary OUL Dye Nomenclature.

OUL Common Name	Color Index Number	Color Index Name	Other Names
Fluorescein	45350	Acid Yellow 73	uranine, uranine C, sodium fluorescein, fluorescein LT and fluorescent yellow/green
Eosine	45380	Acid Red 87	eosin, eosine OJ, and D&C Red 22
Rhodamine WT	None assigned	Acid Red 388	fluorescent red (but not the same as rhodamine B)
Sulforhodamine B	45100	Acid Red 52	pontacyl brilliant pink B, lissamine red 4B, and fluoro brilliant pink

The OUL routinely provides dye for tracing projects. Dyes purchased for groundwater tracing are always mixtures that contain both dye and an associated diluent. Diluents enable the manufacturer to standardize the dye mixture so that there are minimal differences among batches. Additionally, diluents are often designed to make it easier to dissolve the dye mixture in water, or to produce a product which meets a particular market need (groundwater tracing is only a tiny fraction of the dye market). The percent of dye in “as-sold” dye mixtures often varies dramatically among manufacturers and retailers, and retailers are sometimes incorrect about the percent of dye in their products. The OUL subjects all of its dyes to strict quality control (QC) testing. Table 2 summarizes the as-sold dye mixtures used by the OUL.

Table 2. As-Sold Dye Mixtures at the OUL.

OUL Common Name	Form	Dye Equivalent
Fluorescein	Powder	75% dye equivalent, 25% diluent
Eosine	Powder	75% dye equivalent, 25% diluent
Rhodamine WT	Liquid	20% dye equivalent, 80% diluent
Sulforhodamine B	Powder	75% dye equivalent, 25% diluent

Analytical results are based on the as-sold weights of the dyes provided by the OUL. The use of dyes from other sources is discouraged due to the wide variability of dye equivalents within the market. However, if alternate source dyes are used, a sample should be provided to the OUL for quality control and to determine if a correction factor is necessary for the analytical results.

Types of Samples

Typical samples that are collected for fluorescent tracer dye analysis include charcoal samplers (also called activated carbon or charcoal packets) and water samples.

The charcoal samplers are packets of fiberglass screening partially filled with 4.25 grams of activated coconut charcoal. The charcoal used by the OUL is Calgon 207C coconut shell carbon, 6 to 12 mesh, or equivalent. The most commonly used charcoal samplers are about 4 inches long by 2 inches wide. A cigar-shaped sampler is made for use in very small diameter wells (such as 1-inch diameter piezometers); this is a special order item and should be specifically requested in advance when needed. All of the samplers are closed by heat sealing.

In specialized projects, soil samples have been collected from soil cores and analyzed for fluorescent tracer dyes. Project-specific procedures have been developed for projects such as these. For additional information, please contact the OUL.

FIELD PROCEDURES

Field procedures included in this section are intended as guidance, and not firm requirements. Placement of samplers and other field procedures require adjustment to field conditions. Personnel at the OUL are available to provide additional assistance for implementation of field procedures specific to specialized field conditions.

Placement of Samplers

Charcoal samplers are placed so as to be exposed to as much water as possible. Water should flow through the packet. In springs and streams they are typically attached to a rock or other anchor in a riffle area. Attachment of the packets often uses plastic tie wires. In swifter water galvanized wire (such as electric fence wire) is often used. Other types of anchoring wire can be used. Electrical wire with plastic insulation is also good. Packets are attached so that they extend outward from the anchor rather than laying flat against it. Two or more separately anchored packets are typically used for sampling springs and streams. The placement of multiple packets is recommended in order to minimize the chance of loss during the sampling period. The use of fewer packets is discouraged except when the spring or stream is so small that there is not appropriate space for placing multiple packets.

When pumping wells are being sampled, the samplers are typically placed in sample holders made of plastic pipe fittings. Brass hose fittings can be at the end of the sample holders so that the sample holders can be installed on outside hose bibs and water which has run through the samplers can be directed to waste through a connected garden hose. The samplers can be unscrewed in the middle so that charcoal packets can be changed. The middle portions of the samplers consist of 1.5 inch diameter pipe and pipe fitting.

Charcoal packets can be lowered into monitoring wells for sampling purposes. In general, if the well is screened, samplers should be placed approximately in the middle of the screened interval. Due to the typically lower volume of water that flows through a well, only one charcoal sampler should be used per well. However, multiple packets can be placed in a single well at depths to test different depth horizons when desirable. A weight should be added near the charcoal packet to ensure that it will not float. The weight should be of such a nature that it will not affect water quality. One common approach is to anchor the packets with a white or uncolored plastic cable tie to the top of a dedicated weighted disposable bailer. We typically run nylon cord from the top of the well to the charcoal packet and its weight. ***Do not use colored cord*** since some of them are colored with fluorescent dyes. Nylon fishing line should not be used since it can be readily cut by a sharp projection in the well.

In some cases, especially with small diameter wells and appreciable well depths, the weighted disposable bailers sink very slowly or may even fail to sink because of friction and floating of the anchoring cord. In such cases a weight may be added to the top of the disposable bailer. Stainless steel weights are ideal, but are not needed in all cases. All weights should be cleaned prior to use; the cleaning approach should comply with decontamination procedures in use at the project site.

Optional Preparation of Charcoal Samplers

Charcoal packets routinely contain some fine powder that washes off rapidly when they are placed in water. While not usually necessary, the following optional preparation step is suggested if the fine charcoal powder is problematic.

Charcoal packets can be triple rinsed with distilled, demineralized, or reagent water known to be free of tracer dyes. This rinsing is typically done by soaking. With this approach, approximately 25 packets are placed in one gallon of water and soaked for at least 10 minutes. The packets are then removed from the water and excess water is shaken off the packets. The packets are then placed in a second gallon of water and again soaked for at least 10 minutes. After this soaking they are removed from the water and excess water is shaken off the packets. The packets are then placed in a third gallon of water and the procedure is again repeated. Rinsed packets are placed in plastic bags and are placed at sampling stations within three days. Packets can also be rinsed in jets of water for about one minute; this requires more water and is typically difficult to do in the field with water known to be free of tracer dyes.

Collection and Replacement of Samplers

Samplers are routinely collected and replaced at each of the sampling stations. The frequency of sampler collection and replacement is determined by the nature of the study. Collections at one week intervals are common, but shorter or longer collection frequencies are acceptable and sometimes more appropriate. Shorter sampling frequencies are often used in the early phases of a study to better characterize time of travel. As an illustration, we often collect and change charcoal packets 1, 2, 4, and 7 days after dye injection. Subsequent sampling is then weekly.

The sampling interval in wells at hazardous wastes sites should generally be no longer than about a week. Contaminants in the water can sometimes use up sorption sites on the charcoal that would otherwise adsorb the dye. This is especially important if the dye might pass in a relatively short duration pulse.

Where convenient, the collected samplers should be briefly rinsed in the water being sampled to remove dirt and accumulated organic material. This is not necessary with well samples. The packets are shaken to remove excess water. Next, the packet (or packets) are placed in a plastic bag (Whirl-Pak® bags are ideal). The bag is labeled on the outside with a black permanent type felt marker pen, such as a Sharpie®. ***Use only pens that have black ink***; colored inks may contain fluorescent dyes. The notations include station name or number and the date and time of collection. Labels must not be inserted inside the sample bags.

Collected samplers are kept in the dark to minimize algal growth on the charcoal prior to analysis work. New charcoal samplers are routinely placed when used charcoal packets are collected. The last set of samplers placed at a stream or spring is commonly not collected.

Water Samples

Water samples are often collected. They should be collected in either glass or plastic; the OUL routinely uses 50 milliliter (mL) research-grade polypropylene copolymer Perfector Scientific vials (Catalog Number 2650) for such water samples. No more than 30 mL of water is required for analysis. The sides of the vials should be labeled with the project name, sample ID, sample date and time with a black permanent felt tip pen. ***Do not label the lid only***. The vials should be placed in the dark and refrigerated immediately after collection, and maintained under refrigeration until shipment. The OUL supplies vials for the collection of water samples.

Sample Shipment

When water or charcoal samplers are collected for shipment to the OUL they should be shipped promptly. We prefer (and in some studies require) that samples be refrigerated with frozen re-usable ice packs upon collection and that they be shipped refrigerated with frozen ice packs by overnight express. ***Do not ship samplers packed in wet ice*** since this can create a potential for cross contamination when the ice melts. Our experience indicates that it is not essential for samplers to be maintained under refrigeration; yet maintaining them under refrigeration clearly minimizes some potential problems. A product known as "green ice" should not be used for maintaining the samples in a refrigerated condition since this product contains a dye which could contaminate samples if the "green ice" container were to break or leak.

We receive good overnight and second day air service from both UPS and FedEx. The U.S. Postal Service does not typically provide next day service to us. DHL does not provide overnight service to us. FedEx is recommended for international shipments. The OUL does not receive Saturday delivery.

Each shipment of charcoal samplers or water samples ***must be accompanied by a sample custody document***. The OUL provides a sheet (which bears the title "Samples for Fluorescence Analysis") that can be used if desired. These sheets can be augmented by a client's chain-of-custody forms or any other relevant documentation. OUL’s custody document works well for charcoal samplers because it allows for both the placement date and time as well as the collection date and time. Many other standard chain-of-custody documents do not allow for these types of samples. Attachment 1 includes a copy of OUL’s Sample Collection Data Sheet.

Please write legibly on the custody documents and ***use black ink***. Check the accuracy of the sample sheet against the samples prior to shipment to identify and correct errors that may delay the analysis of your samples following receipt at the laboratory.

Supplies Provided by the OUL

The OUL provides supplies for the collection of fluorescent tracer dyes. Supplies provided upon request are charcoal packets, Whirl-Pak® bags (to contain the charcoal packets after collection for shipment to the laboratory), and water vials. These supplies are subjected to strict QA/QC procedures to ensure the materials are free of any potential tracer dye contaminants. The charge for these materials is included in the cost of sample analysis. Upon request, coolers and re-freezable ice packs are also provided for return shipment of samples.

The OUL also has tracer dyes available for purchase. These dyes are subject to strict QA/QC testing. All analytical work is based upon the OUL as-sold weight of the dyes.

LABORATORY PROCEDURES

The following procedures are followed upon receipt of samples at the laboratory.

Receipt of Samples

Samplers shipped to the OUL are logged in and refrigerated upon receipt. Prior to cleaning and analysis, samplers are assigned a laboratory identification number.

It sometimes occurs that there are discrepancies between the sample collection data sheet and the actual samples received. When this occurs, a "Discrepancy Sheet" form is completed and sent to the shipper of the sample for resolution. The purpose of the form is to help resolve discrepancies, even when they may be minor. Many discrepancies arise from illegible custody documents. ***Please write legibly*** on the custody documents and ***use black ink***. Check the accuracy of the sample sheet against the samples prior to shipment to identify and correct errors that may delay the analysis of your samples following receipt at the laboratory.

Cleaning of Charcoal Samplers

Samplers are cleaned by spraying them with jets of clean water from a laboratory well in a carbonate aquifer. OUL uses non-chlorinated water for the cleansing to minimize dye deterioration. We do not wash samplers in public water supplies. Effective cleansing cannot generally be accomplished simply by washing in a conventional laboratory sink even if the sink is equipped with a spray unit.

The duration of packet washing depends upon the condition of the sampler. Very clean samplers may require less than a minute of washing; dirtier samplers may require several minutes of washing.

Elution of the Charcoal

There are various eluting solutions that can be used for the recovery of tracer dyes. The solutions typically include an alcohol, water, and a strong basic solution such as aqueous ammonia and /or potassium hydroxide.

The standard elution solution used at the OUL is a mixture of 5% aqua ammonia and 95% isopropyl alcohol solution and sufficient potassium hydroxide pellets to saturate the solution. The isopropyl alcohol solution is 70% alcohol and 30% water. The aqua ammonia solution is 29% ammonia. The potassium hydroxide is added until a super-saturated layer is visible in the bottom of the container. This super-saturated layer is not used for elution. Preparation of eluting solutions uses dedicated glassware which is never used in contact with dyes or dye solutions.

The eluting solution will elute fluorescein, eosine, rhodamine WT, and sulforhodamine B dyes. It is also suitable for separating fluorescein peaks from peaks of some naturally present materials found in may be found in samplers.

Fifteen mL of the eluting solution is poured over the washed charcoal in a disposable sample beaker. The sample beaker is capped. The sample is allowed to stand for 60 minutes. After this time, the liquid is carefully poured off the charcoal into a new disposable beaker which has been appropriately labeled with the laboratory identification number. A few grains of charcoal may inadvertently pass into the second beaker; no attempt is made to remove these from the second sample beaker. After the pouring, a small amount of the elutant will remain in the initial sample beaker. After the transfer of the elutant to the second sample beaker, the contents of the first sample beaker (the eluted charcoal) are discarded. Samples are kept refrigerated until analyzed.

pH Adjustment of Water Samples

The fluorescence intensity of several of the commonly used fluorescent tracer dyes is pH dependent. The pH of samples analyzed for fluorescein, eosine, and pyranine dyes are adjust to a target pH of greater than 9.5 in order to obtain maximum fluorescence intensities.

Adjustment of pH is achieved by placing samples in a high ammonia atmosphere for at least two hours in order to increase the pH of the sample. Reagent water standards are placed in the same atmosphere as the samples. If dye concentrations in a sample are off-scale and require dilution for quantification of the dye concentration, the diluting water used is OUL reagent water that has been pH adjusted in a high ammonia atmosphere. Samples that are only analyzed for rhodamine WT or sulforhodamine B are not required to be pH adjusted.

Analysis on the Shimadzu RF-5301

The OUL uses a Shimadzu spectrofluorophotometer model RF-5301. This instrument is capable of synchronous scanning. The OUL also owns a Shimadzu RF-540 spectrofluorometers that is occasionally used for special purposes.

A sample of the elutant or water is withdrawn from the sample container using a disposable polyethylene pipette. Approximately 3 mL of the sample is then placed in disposable rectangular polystyrene cuvette. The cuvette has a maximum capacity of 3.5 mL. The cuvette is designed for fluorometric analysis; all four sides and the bottom are clear. The acceptable spectral range of these cuvettes is 340 to 800 nm. The pipettes and cuvettes are discarded after one use.

The cuvette is then placed in the RF-5301. This instrument is controlled by a programmable computer and operated by proprietary software developed for dye tracing applications.

Our instruments are operated and maintained in accordance with the manufacturer's recommendations. On-site installation of our first instrument and a training session on its use was provided by the instrument supplier. Repairs are made by a Shimadzu-authorized repairman.

Our typical analysis of an elutant sample where fluorescein, eosine, rhodamine WT, or sulforhodamine B dyes may be present includes synchronous scanning of excitation and emission spectra with a 17 nm separation between excitation and emission wavelengths. For these dyes, the excitation scan is from 443 to 613 nm; the emission scan is from 460 to 630 nm. The emission fluorescence from the scan is plotted on a graph. The typical scan speed setting is "fast" on the RF-5301. The typical sensitivity setting used is "high."

Table 3. Excitation and emission slit width settings routinely used for dye analysis.

Parameter	Excitation Slit (nm)	Emission Slit (nm)
ES, FL, RWT, and SRB in elutant	3	1.5
ES, FL, RWT, and SRB in water	5	3

Note: ES = Eosine. FL = Fluorescein. RWT = Rhodamine WT. SRB = Sulforhodamine B.

The instrument produces a plot of the synchronous scan for each sample; the plot shows emission fluorescence only. The synchronous scans are subjected to computer peak picks using proprietary software; peaks are picked to the nearest 0.1 nm. Instrument operators have the ability to manually adjust peaks as necessary based upon computer-picked peaks and experience. All samples run on the RF-5301 are stored electronically with sample information. All samples analyzed are recorded in a bound journal.

Quantification

We calculate the magnitude of fluorescence peaks for fluorescein, eosine, rhodamine WT, and sulforhodamine B dyes in both elutant and water samples. Dye quantities are expressed in microgram per liter (parts per billion; ppb). The dye concentrations are calculated by separating fluorescence peaks due to dyes from background fluorescence on the charts, and then calculating the area within the fluorescence peak. This area is proportional to areas obtained from standard solutions.

We run dye concentration standards each day the RF-5301 is used. Six standards are used; the standard or standards appropriate for the analysis work being conducted are selected. All standards are based upon the as-sold weights of the dyes. The standards are as follows:

1) 10 ppb fluorescein and 100 ppb rhodamine WT in well water from the Jefferson City-Cotter Formation
2) 10 ppb eosine in well water from the Jefferson City-Cotter Formation
3) 100 ppb sulforhodamine B in well water from the Jefferson City-Cotter Formation.
4) 10 ppb fluorescein and 100 ppb rhodamine WT in elutant.
5) 10 ppb eosine in elutant.
6) 100 ppb sulforhodamine B in elutant.

Preparation of Standards

Dye standards are prepared as follows:

Step 1. A small sample of the as-sold dye is placed in a pre-weighed sample vial and the vial is again weighed to determine the weight of the dye. We attempt to use a sample weighing between 1 and 5 grams. This sample is then diluted with well water to make a 1% dye solution by weight (based upon the as-sold weight of the dye). The resulting dye solution is allowed to sit for at least four hours to ensure that all dye is fully dissolved.

Step 2. One part of each dye solution from Step 1 is placed in a mixing container with 99 parts of well water. Separate mixtures are made for fluorescein, rhodamine WT, eosine, and sulforhodamine B. The resulting solutions contain 100 mg/L dye (100 parts per million dye mixture). The typical prepared volume of this mixture is appropriate for the sample bottles being used; we commonly prepare about 50 mL of the Step 2 solutions. The dye solution from Step 1 that is used in making the Step 2 solution is withdrawn with a digital Finnpipette which is capable of measuring volumes between 0.200 and 1.000 mL at intervals of 0.005 mL. The calibration certificate with this instrument indicates that the accuracy (in percent) is as follows:

At 0.200 mL, 0.90%

At 0.300 mL, 0.28%

At 1.000 mL, 0.30%

The Step 2 solution is called the long term standard. OUL experience indicates that Step 2 solutions, if kept refrigerated, will not deteriorate appreciably over periods of less than a year. Furthermore, these Step 2 solutions may last substantially longer than one year.

Step 3. A series of intermediate-term dye solutions are made. Approximately 45 mL. of each intermediate-term dye solution is made. All volume measurements of less than 5 mL are made with a digital Finnpipette. (see description in Step 2). All other volume measurements are made with Rheinland Kohn Geprufte Sicherheit 50 mL capacity pump dispenser which will pump within plus or minus 1% of the set value. The following solutions are made; all concentrations are based on the as-sold weight of the dyes:

1) 1 ppm fluorescein dye and 10 ppm rhodamine WT dye.

2) 1 ppm eosine.

3) 10 ppm sulforhodamine B dye.

Step 4. A series of six short-term dye standards are made from solutions in Step 3. These standards were identified earlier in this section. In the experience of the OUL these standards have a useful shelf life in excess of one week. However, in practice, Step 4 elutant standards are made weekly, and Step 4 water standards are made daily.

Dilution of Samples

Samples with peaks that have arbitrary fluorescence unit values of 500 or more are diluted a hundred fold to ensure accurate quantification.

Some water samples have high turbidity or color which interferes with accurate detection and measurement of dye concentrations. It is often possible to dilute these samples and then measure the dye concentration in the diluted sample.

The typical dilutions are either 10 fold (1:10) or 100 fold (1:100). A 1:10 dilution involves combining one part of the test sample with 9 parts of water (if the sample is water) or elutant (if the sample is elutant). A 1:100 dilution involves combining one part of the test sample is combined with 99 parts of water or elutant, based upon the sample media. Typically, 0.300 mL of the test solution is combined with 29.700 mL of water (or elutant as appropriate) to yield a new test solution.

All volume measurements of less than 5 mL are made with a digital Finnpipette. All other volume measurements are made with Rheinland Kohn Geprufte Sicherheit 50 mL capacity pump dispenser which will pump within plus or minus 1% of the set value.

The water used for dilution is from a carbonate aquifer. All dilution water is pH adjusted to greater than pH 9.5 by holding it in open containers in a high ammonia concentration chamber. This adjustment takes a minimum of two hours.

Quality Control

Laboratory blanks are run for every sample where the last two digits of the laboratory numbers are 00, 20, 40, 60, or 80. A charcoal packet is placed in a pumping well sampler and at least 25 gallons of unchlorinated water is passed through the sampler at a rate of about 2.5 gallons per minute. The sampler is then subjected to the same analytical protocol as all other samplers.

System functioning tests of the analytical instruments are conducted in accordance with the manufacturer's recommendations. Spiked samples are also analyzed when appropriate for quality control purposes.

All materials used in sampling and analysis work are routinely analyzed for the presence of any compounds that might create fluorescence peaks in or near the acceptable wavelength ranges for any of the tracer dyes. This testing includes approximately 1% of materials used.

Project specific QA/QC samples may include sample replicates and sample duplicates. A replicate sample is when a single sample is analyzed twice. A sample duplicate is where two samples are collected in a single location and both are analyzed. Sample replicates and duplicates are run for QA/QC purposes upon request of the client. These results are reported in the Certificate of Analysis.

Reports

Sample analysis results are typically reported in a Certificate of Analysis. However, specialized reports are provided in accordance with the needs of the client. Certificates of Analysis typically provide a listing of station number, sample ID, and dye concentrations if detected. Standard data format includes deliverables in MS Excel and Adobe Acrobat (.pdf) format. Hard copy of the data package, and copies of the analytical charts are available upon request.

Work at the OUL is directed by Mr. Thomas Aley. Mr. Aley has 45 years of professional experience in hydrology and hydrogeology. He is certified as a Professional Hydrogeologist (Certificate #179) by the American Institute of Hydrology and licenced as a Professional Geologist in Missouri, Arkansas, Kentucky, and Alabama. Additional details regarding laboratory qualifications are available upon request.

Waste Disposal

All laboratory wastes are disposed of according to applicable state and federal regulations. Waste elutant and water samples are collected in 15 gallon poly drums and disposed with a certified waste disposal facilityas non-hazardous waste.

In special cases, wastes for a particular project may be segregated and returned to the client upon completion of the project. These projects may have samples that contain contaminants that the client must account for all materials generated and disposed. These situations are managed on a case-by-case basis.

CRITERIA FOR DETERMINATION OF POSITIVE DYE RECOVERIES

Normal Emission Ranges and Detection Limits

The OUL has established normal emission fluorescence wavelength ranges for each of the four dyes described in this document. The normal acceptable range equals mean values plus and minus two standard deviations. These values are derived from actual groundwater tracing studies conducted by the OUL.

The detection limits are based upon concentrations of dye necessary to produce emission fluorescence peaks where the signal to noise ratio is 3. The detection limits are realistic for most field studies since they are based upon results from actual field samples rather than being based upon values from spiked samples in a matrix of reagent water or the elutants from unused activated carbon samplers. In some cases detection limits may be smaller than reported if the water being sampled has very little fluorescent material in it. In some cases detection limits may be greater than reported; this most commonly occurs if the sample is turbid due to suspended material or a coloring agent such as tannic compounds. Turbid samples are typically allowed to settle, centrifuged, or, if these steps are not effective, diluted prior to analysis.

Table 4 provides normal emission wavelength ranges and detection limits for the four dyes when analyzed on the OUL's RF-5301 for samples analyzed as of March 3, 2015.

Table 4. RF-5301 Spectrofluorophotometer. Normal emission wavelength ranges and detection limits for fluorescein, eosine, rhodamine WT, and sulforhodamine B dyes in water and elutant samples.

Fluorescent Dye	**Normal Acceptable Emission Wavelength Range (nm)**		**Detection Limit (ppb)**	
	Elutant	**Water**	**Elutant**	**Water**
Eosine	539.3 to 545.1	532.5 to 537.0	0.050	0.015
Fluorescein	514.1 to 519.2	505.9 to 509.7	0.025	0.002
Rhodamine WT	564.6 to 571.2	571.9 to 577.2	0.170	0.015
Sulforhodamine B	575.2 to 582.0	580.1 to 583.7	0.080	0.008

Note: Detection limits are based upon the as-sold weight of the dye mixtures normally used by the OUL. Fluorescein and eosine detection limits in water are based on samples pH adjusted to greater than 9.5.

It is important to note that the normal acceptable emission wavelength ranges are subject to change based on instrument maintenance, a change in instrumentation, or slight changes in dye formulation. Significant changes in normal acceptable emission wavelength ranges will be updated in this document as they occur.

Fluorescence Background

Due to the nature of fluorescence analysis, it is important to identify and characterize any potential background fluorescence at dye introduction and monitoring locations prior to the introduction of any tracer dyes.

There is generally little or no detectable fluorescence background in or near the general range of eosine, rhodamine WT, and sulforhodamine B dyes encountered in most groundwater tracing studies. There is often some fluorescence background in or near the range of fluorescein dye present at some of the stations used in groundwater tracing studies.

Criteria for Determining Dye Recoveries

The following sections identify normal criteria used by the OUL for determining dye recoveries. The primary instrument in use is a Shimadzu RF-5301.

EOSINE

Normal Criteria Used by the OUL for Determining Eosine Dye Recoveries in Elutants from Charcoal Samplers

Criterion 1. There must be at least one fluorescence peak in the range of 540.0 to 545.8 nm in the sample.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. The eosine detection limit in elutant samples is 0.050 ppb, thus this dye concentration limit equals 0.150 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of eosine. Much background fluorescence yields low, broad, and asymmetrical fluorescence peaks rather than the more narrow and symmetrical fluorescence peaks typical of eosine. In addition, there must be no other factors which suggest that the fluorescence peak may not be eosine dye from our groundwater tracing work.

Normal Criteria Used by the OUL for Determining Eosine Dye Recoveries in Water Samples

Criterion 1. In most cases, the associated charcoal samplers for the station should also contain eosine dye in accordance with the criteria listed above. This criterion may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be eosine dye from our groundwater tracing work. The fluorescence peak should generally be in the range of 532.8 to 537.3 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our eosine detection limit in water samples is 0.015 ppb, thus this dye concentration limit equals 0.045 ppb.

Criterion 4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

FLUORESCEIN

Normal Criteria Used by the OUL for Determining Fluorescein Dye Recoveries in Elutants from Charcoal Samplers

Criterion 1. There must be at least one fluorescence peak in the range of 514.5 to 519.6 nm in the sample.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. The fluorescein detection limit in elutant samples is 0.025 ppb, thus this dye concentration limit equals 0.075 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of fluorescein. Much background fluorescence yields low, broad, and asymmetrical fluorescence peaks rather than the more narrow and symmetrical fluorescence peaks typical of fluorescein. In addition, there must be no other factors which suggest that the fluorescence peak may not be fluorescein dye from our groundwater tracing work.

Normal Criteria Used by the OUL for Determining Fluorescein Dye Recoveries in Water Samples

Criterion 1. In most cases, the associated charcoal samplers for the station should also contain fluorescein dye in accordance with the criteria listed above. This criterion may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be fluorescein dye from our groundwater tracing work. The fluorescence peak should generally be in the range of 506.8 to 510.6 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our fluorescein detection limit in water samples is 0.002 ppb, thus this dye concentration limit equals 0.006 ppb.

Criterion 4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

RHODAMINE WT

Normal Criteria Used by the OUL for Determining Rhodamine WT Dye Recoveries in Elutants from Charcoal Samplers

Criterion 1. There must be at least one fluorescence peak in the sample in the range of 565.2 to 571.8 nm.

Criterion 2. The dye concentration associated with the rhodamine WT peak must be at least 3 times the detection limit. The detection limit in elutant samples is 0.170 ppb, thus this dye concentration limit equals 0.510 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of rhodamine WT. In addition, there must be no other factors which suggest that the fluorescence peak may not be dye from the groundwater tracing work under investigation.

Normal Criteria Used by the OUL for Determining Rhodamine WT Dye Recoveries in Water Samples

Criterion 1. In most cases, the associated charcoal samplers for the station should also contain rhodamine WT dye in accordance with the criteria listed above. These criteria may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be rhodamine WT dye from the tracing work under investigation. The fluorescence peak should generally be in the range of 572.4 to 577.7 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our rhodamine WT detection limit in water samples is 0.015 ppb, thus this dye concentration limit is 0.045 ppb.

Criterion 4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

SULFORHODAMINE B

Normal Criteria Used by the OUL for Determining Sulforhodamine B Dye Recoveries in Elutants from Charcoal Samplers

Criterion 1. There must be at least one fluorescence peak in the sample in the range of 576.4 to 583.2 nm.

Criterion 2. The dye concentration associated with the sulforhodamine B peak must be at least 3 times the detection limit. The detection limit in elutant samples is 0.080 ppb, thus this dye concentration limit equals 0.240 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of sulforhodamine B. In addition, there must be no other factors which suggest that the fluorescence peak may not be dye from the groundwater tracing work under investigation.

Normal Criteria Used by the OUL for Determining Sulforhodamine B dye Recoveries in Water Samples

Criterion 1. In most cases, the associated charcoal samplers for the station should also contain sulforhodamine B dye in accordance with the criteria listed earlier. This criterion may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be sulforhodamine B dye from the tracing work under investigation. The fluorescence peak should generally be in the range of 580.8 to 584.4 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. The detection limit in water is 0.008 ppb, thus this dye concentration limit equals 0.024 ppb.

Criterion 4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Standard Footnotes

Sometimes not all the criteria are met for a straight forward determination of tracer dye in a sample. For these reasons, the emission graph is scrutinized carefully by the analytical technician and again during the QA/QC process. Sometimes the emission graphs require interpretation as to whether or not a fluorescence peak represents the tracer dye or not. Background samples from each of the sampling stations aid in the interpretation of the emission fluorescence graphs. When the results do not meet all the criteria for a positive dye detection, often the fluorescence peak is quantified and flagged with a footnote to the result as not meeting all the criteria for a positive dye detection. Standard footnotes are as follows:

Single asterisk (*): A fluorescence peak is present that does not meet all the criteria for a positive dye recovery. However, it has been calculated as though it were the tracer dye.

Double asterisk (**): A fluorescence peak is present that does not meet all the criteria for this dye. However, it has been calculated as a positive dye recovery.

Other footnotes specific to the fluorescence signature are sometimes also used. These footnotes are often developed for a specific project.

The quantification of fluorescence peaks that do not meet all the criteria for a positive dye detection can be important for interpretation of the dataset as a whole.

ATTACHMENT 1

Sample Collection Data Sheet