NCKRI REPORT OF INVESTIGATION 11

PHASE 1 DYE TRACE INVESTIGATION OF A CONGLOMERATE KARST AQUIFER, BLACK RIVER BASIN, EDDY COUNTY, NEW MEXICO

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Phase 1 Dye Trace Investigation of a Conglomerate Karst Aquifer, Black River Basin, Eddy County, New Mexico

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Cover photo: NCKRI released uranine dye into Black River 120 m upstream of where it sinks underground. The protective clothing is worn is to prevent accidentally transferring the dye and causing false-positive results. NCKRI photo by Michael Jones.

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NCKRI Organization and Mission

NCKRI was created by the US Congress in 1998 in partnership with the State of New Mexico and the City of Carlsbad. NCKRI is administered by the New Mexico Institute of Mining and Technology (aka New Mexico Tech or NMT).

NCKRI's enabling legislation, the National Cave and Karst Research Institute Act of 1998, 16 USC, §4310, identifies NCKRI's mission as to:

1) further the science of speleology;
2) centralize and standardize speleological information;
3) foster interdisciplinary cooperation in cave and karst research programs;
4) promote public education;
5) promote national and international cooperation in protecting the environment for the benefit of cave and karst landforms; and
6) promote and develop environmentally sound and sustainable resource management practices.

NCKRI Report of Investigation Series

NCKRI uses this report series to publish the findings of its research projects. The reports are produced on a schedule whose frequency is determined by the timing of the investigations. This series is not limited to any topic or field of research, except that they involve caves and/or karst. All reports in this series are open access and may be used with citation. To minimize environmental impact, few or no copies are printed. They may be downloaded at no cost from the NCKRI website at www.nckri.org.

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Phase 1 Dye Trace Investigation of a Conglomerate Karst Aquifer, Black River Basin, Eddy County, New Mexico

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Abstract
Personnel with the National Cave and Karst Research Institute (NCKRI) conducted two dye traces on the Black River in southeastern New Mexico. Previous workers have described spring flow in the Black River as discharging from karstic solution conduits developed within a carbonate-cemented limestone cobble conglomerate aquifer. NCKRI staff injected nontoxic organic dyes into two sinking stream reaches within the upper Black River to trace groundwater flow paths and measure flow velocities to downgradient springs and wells. During the dye trace there were no positive detections at any monitoring sites, including Blue Spring and Castle Spring, the farthest downgradient monitoring locations. Insufficient dye mass could account for these non-detects. However, these results might also arise from groundwater flow velocities through the Quaternary gravel aquifer that are slower than anticipated, and/or groundwater discharge from spring outlets other than Blue Spring and Castle Spring.

Introduction
This report presents the results of a dye trace investigation conducted by National Cave and Karst Research Institute (NCKRI) personnel to quantify groundwater flow parameters through a limestone conglomerate karstic aquifer developed in the upper Black River basin (Figures 1 and 2). The Black River is one of the southernmost tributaries to the Pecos River in southeastern New Mexico and makes an important contribution to meeting New Mexico's interstate compact obligations for sharing surface water with the downstream State of Texas. The Black River also provides the only habitat in New Mexico for the Texas hornshell mussel, *Popenaias popeii*, which the US Fish and Wildlife Service (USFWS) listed as an endangered species (USFWS, 2018). Although the basin's water is fully appropriated by a wide range of competing interests, recent increases in oil and gas development and associated demand for water-intensive hydraulic fracturing point to further possible shifts in water use throughout the region (Montgomery and Smith, 2010).

The primary objective of this study is to demonstrate a hydrologic connection between the upper ephemeral reach of the Black River basin and the lower perennial reach of the Black River where groundwater discharges at Blue Spring, the primary source of water for this longest stretch of continuous critical riparian habitat (Figure 1). This Phase 1 study could serve as a first step toward advanced hydrogeologic characterization of the local karst aquifer. Dye-tracing is widely considered the most effective method for delineating a karst groundwater drainage basin.

The Bureau of Land Management (BLM) contracted NCKRI to conduct this study to identify karst groundwater flow paths and conditions to help BLM identify areas of potential concern and minimize potential adverse impacts on the Black River system.

NCKRI's interest in this study is in part to better understand the groundwater system that is economically important to the region, and essential to the survival of its endangered species of mussel. Additionally, the Pecos Springsnail (*Pyrgulopsis pecosensis*) is endemic to Blue Spring and Castle Spring, which are part of the Black River system (Taylor, 1987). However, NCKRI's scientific interest arises from the fact that while karst aquifers formed in limestone conglomerates have been documented in other areas of the world (e.g., Gasparetto and Talamanca, 2004; Ferrarese and Sauro, 2005), they have seen little study. This project is one of the first dye traces in such an aquifer and is most likely the first of its kind conducted in a semi-arid climate, an environment that presents special challenges and uncertainties when compared to dye-tracing in humid regions (Veni, 2014).

Hydrogeologic Setting
The Black River basin is located within the northern portion of the Chihuahuan Desert, where annual daily low and high temperatures average 3 and 13°C in January and 24 and 34°C in July–August, and annual precipitation has averaged 468 mm over the past 10 years in Whites City, New Mexico, which is roughly in the middle of the study area (WeatherWX.com, 2021). The greatest concentra-

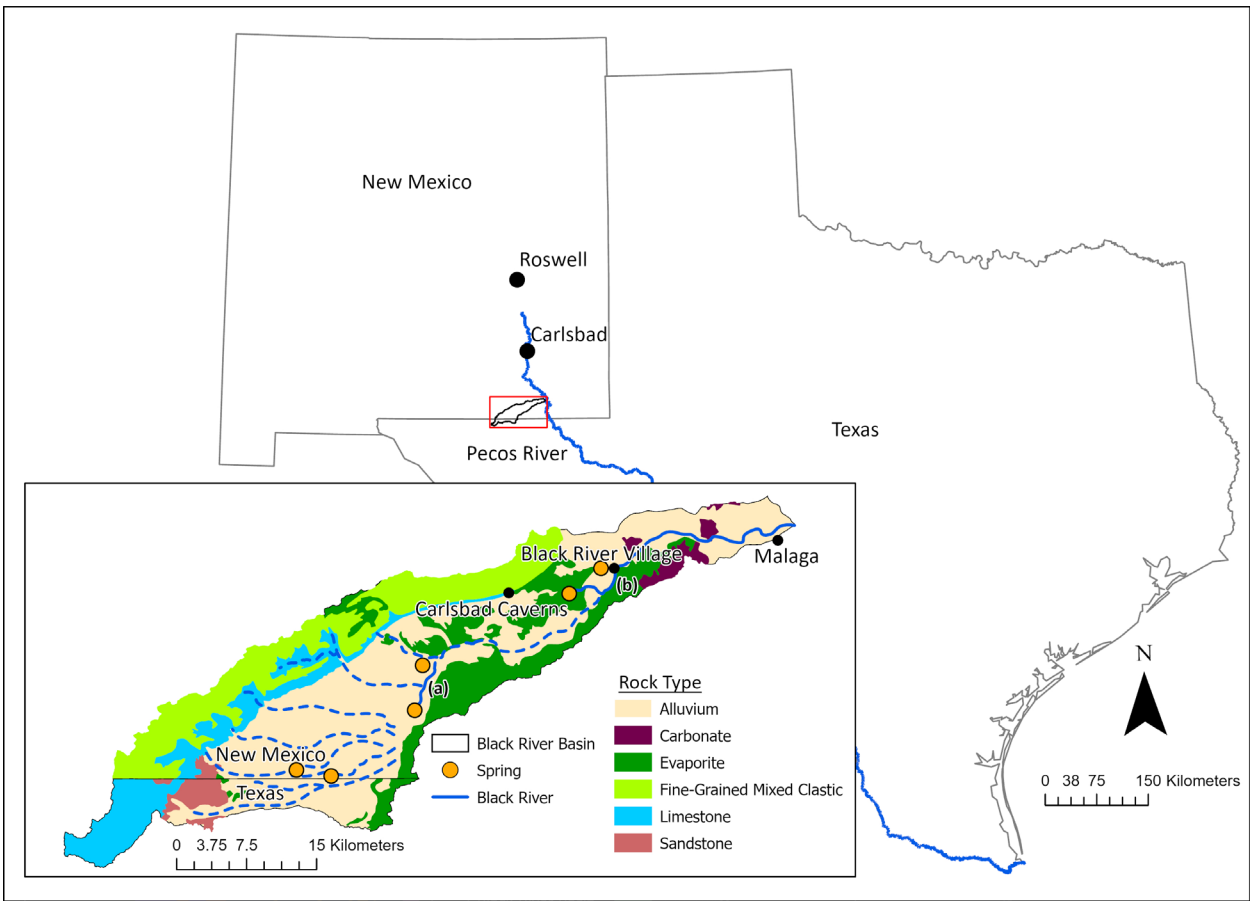


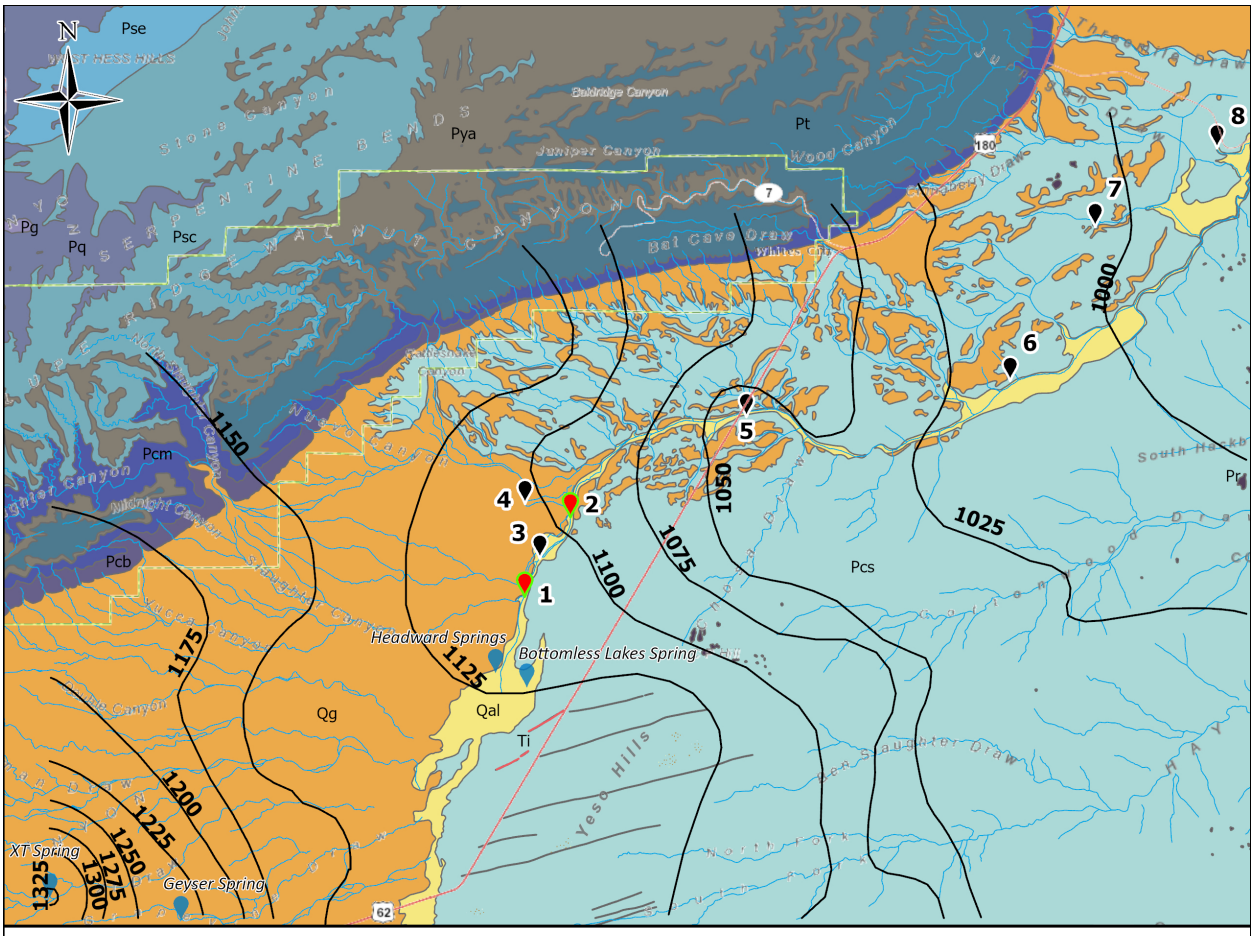
Figure 1. *Simplified geologic map and location of the Black River basin. Solid blue lines are perennial streams, dashed blue lines are ephemeral streams.*

tion of rainfall occurs as high-intensity, short-duration events associated with late-summer monsoonal storms that promote rapid runoff (Stafford, 2013).

The Black River has both perennial and ephemeral reaches (Figure 1). The ephemeral upper reaches and canyon washes that drain the Guadalupe Mountains to the north and west only flow during concentrated storm events. This streamflow infiltrates quickly into the alluvium, often before reaching the valley floor. There are several small perennial springs that discharge into the upper Black River to create a series of pools connected by shallow channels (Cox, 1963). Most of the springs that contribute to flow in the Black River are assumed to discharge from a well-indurated karstic limestone conglomerate that crops out in bluffs that line portions of the Black River valley (Figures 3 and 4; Hale, 1955; Cox, 1963; Bowen, 1998; Love and Land, 2006; Arm et al. 2014). This conglomerate, commonly referred to as the Quater-

Figure 2. *View of the uppermost Black River near its headwater springs. NCKRI photo by George Veni.*

Figure 3. *Limestone conglomerate along the upper Black River. NCKRI photo by George Veni.*



Number	Injection Site	Water Elevation (m)
1	Upper Diversion Dam	1110
2	Lower Road Crossing	1100
Number	**Monitoring Site**	**Water Elevation (m)**
3	Cottonwood Day Use Area Spring Pool	1106
4	Rattlesnake Spring	1101
5	Well C-03458	1038
6	Well C-03285	1004
7	Blue Spring	1007
8	Castle Spring	980

Figure 4. *Geologic map of the upper Black River basin study area, Eddy County, New Mexico, with generalized water-levels, and tracer injection and monitoring sites (modified from Hale 1955; Hayes, 1964). Slaughter Canyon is the furthest southwest canyon dissecting the Guadalupe escarpment, delineated by the Capitan Limestone.*

nary gravel aquifer, was deposited during Pliocene through Pleistocene time in a large alluvial fan originating from Slaughter Canyon (Hale, 1955; Bjorklund and Motts, 1959; Hill, 1996; Bowen, 1998).

Groundwater flow directions generally follow the topographic gradient and slopes from the Guadalupe Mountains eastward toward the Black River, and to the northeast through karstic solution conduits formed in the limestone conglomerate along the valley floor (Figure 4). The most water-productive material in the basin is the limestone conglomerate unit of the Quaternary gravel aquifer (Conover, 1952). Although the details of this conceptual model are not well constrained, sinkholes ranging from high to low width and depth, together with ephemeral and perennial stream swallets in both the alluvial and evaporite landforms, probably contribute recharge to the Quaternary gravel aquifer (Sares, 1984).

Rattlesnake Spring lies ~1.3 km west of the Black River (Figure 4), where it discharges water from the limestone conglomerate and overlying unconsolidated alluvium into a constructed spring pool (Hale, 1955). Bowen (1998) hypothesized that a significant cause of groundwater discharge in the Rattlesnake Spring area is the presence of the Castile gypsum in outcrop and in the shallow subsurface just downstream of the spring on either side of the Black River, forcing northeastward groundwater flow to the surface. The two farthest downgradient springs in the system, Blue Spring and Castle Spring, are located respectively 18 km and 22 km northeast of Rattlesnake Spring, near the distal end of the Slaughter Canyon alluvial fan (Figure 4).

Quaternary Gravel Aquifer

Previous workers (Hill, 1996; Bowen, 1998; Love and Land, 2006) have described the Quaternary gravel unit as a locally important karstic aquifer based on the presence of springs, sinkholes, and solutionally enlarged cavities in the carbonate-cemented gravels. Deposits of different ages are well cemented by calcium carbonate to form extremely durable limestone cobble conglomerates that break across clasts (Horberg, 1949; Sares, 1984). Caves have not been documented in the conglomerate, but its hydrogeological characteristics are comparable to those of typical limestone karst aquifers. Hale (1955) characterized groundwater movement in the upper Black River sub-basin near the vicinity of Rattlesnake Spring as moving through large pores and conduits in conglomeratic units, based on aquifer tests and potentiometric surface mapping (Figure 4). In the mid-20th century, wells developed in the Quaternary gravel aquifer reported yields up to 4,900 L/minute (Hale, 1955). Quaternary gravels contained in the Slaughter Canyon alluvial fan are hydraulically disconnected from the underlying Capitan Reef aquifer, within which water levels are over 100 m lower than in the conglomerate aquifer (Sares, 1984; Hill, 1996).

Recharge to the Quaternary gravel aquifer begins in the upper reaches of the alluvial fan at the mouth of Slaughter Canyon (Figure 5). Within the phreatic zone, groundwater flows downgradient through karstic solution conduits, discharging from springs into the Black River as baseflow (Bowen, 1998). Groundwater-surface water interactions are demonstrated dramatically in the upper Black River where spring runs issue large volumes of water for short distances before sinking back underground.

The development of similar karst phenomena has been observed in other regions of the world with a conglomerate base, such as the Montello Hill in Italy, also referred to as the "classical karst" of conglomerate rocks (Gasparetto and Tartini, 1994; Ferrarese, 1995; Gasparetto and Talamanca, 2004; Ferrarese and Sauro, 2005) and in the Austro-German Alps (Goeppert et al., 2011). However, since qualitative dye-tracing began, few studies have focused on characterizing a karst aquifer formed in carbonate-cemented conglomerates. A dye trace program in carbonate conglomerates conducted in the Molasse zone along the northern margin of the Austro-German Alps measured flow velocities often exceeding 100 m/hour, similar to many limestone karst aquifers (Goeppert and Goldscheider, 2001).

Lower Black River

The lower Black River is defined as that reach of the river below Blue Spring, beyond which the river is a perennial stream. In this area, the Quaternary gravel

Figure 5. *Cobble and gravel alluvial deposit at the mouth of Slaughter Canyon. NCKRI photo by George Veni.*

aquifer merges with an alluvial aquifer related to the Pecos River floodplain (Barroll, 2004). The alluvial aquifer in this area is in hydrologic communication with groundwater stored in fractures, caves, and conduits in the underlying Castile gypsum, referred to by Sares (1984) as the alluvial-evaporite aquifer. This composite aquifer is presumably the source of baseflow to the lower Black River (Gutierrez and Wells, 1979).

The lower Black River has two perennial spring-fed tributaries, Blue Spring Creek and Castle Spring Creek (Figure 4). Blue Spring is the major discharge point of groundwater in the Black River basin. Blue Spring is located near the distal end of the alluvial fan originating from Slaughter Canyon, as indicated by the finer-grained lithologies exposed there, in contrast to much coarser material exposed in bluffs in the upper Black River valley. Groundwater that bypasses Rattlesnake Spring, while moving eastward through the alluvial aquifer, and surface water losses to alluvium in the upper Black River valley, are thought to ultimately discharge at Blue Spring and Castle Spring (Cox, 1963).

Under dry weather baseflow conditions, Blue Spring discharges at a rate of ~226 L/s, although mid-20th century records indicate flow rates as high as 400 L/s. A closer look at nearly 20 years' worth of instantaneous discharge data recorded at USGS Gage 08405450 reveals highly variable discharge typical of a karst aquifer, with peaks following significant precipitation events, usually occurring during the monsoon season during late summer and early fall.

Castle Spring, located 4 km northeast and downgradient of Blue Spring, is lowest in elevation of the major springs. Castle Spring is also sourced from the conglomerate aquifer, with recorded yields of ~11-17 L/s (Hale, 1955). Bjorklund and Motts (1959) report declines in discharge during groundwater pumping for agricultural irrigation, followed by a recovery of discharge volume between irrigation periods. The lower Black River flows into the Pecos River ~20 km downstream from Castle Spring creek, northeast of the village of Malaga.

Alluvial-Evaporite Aquifer

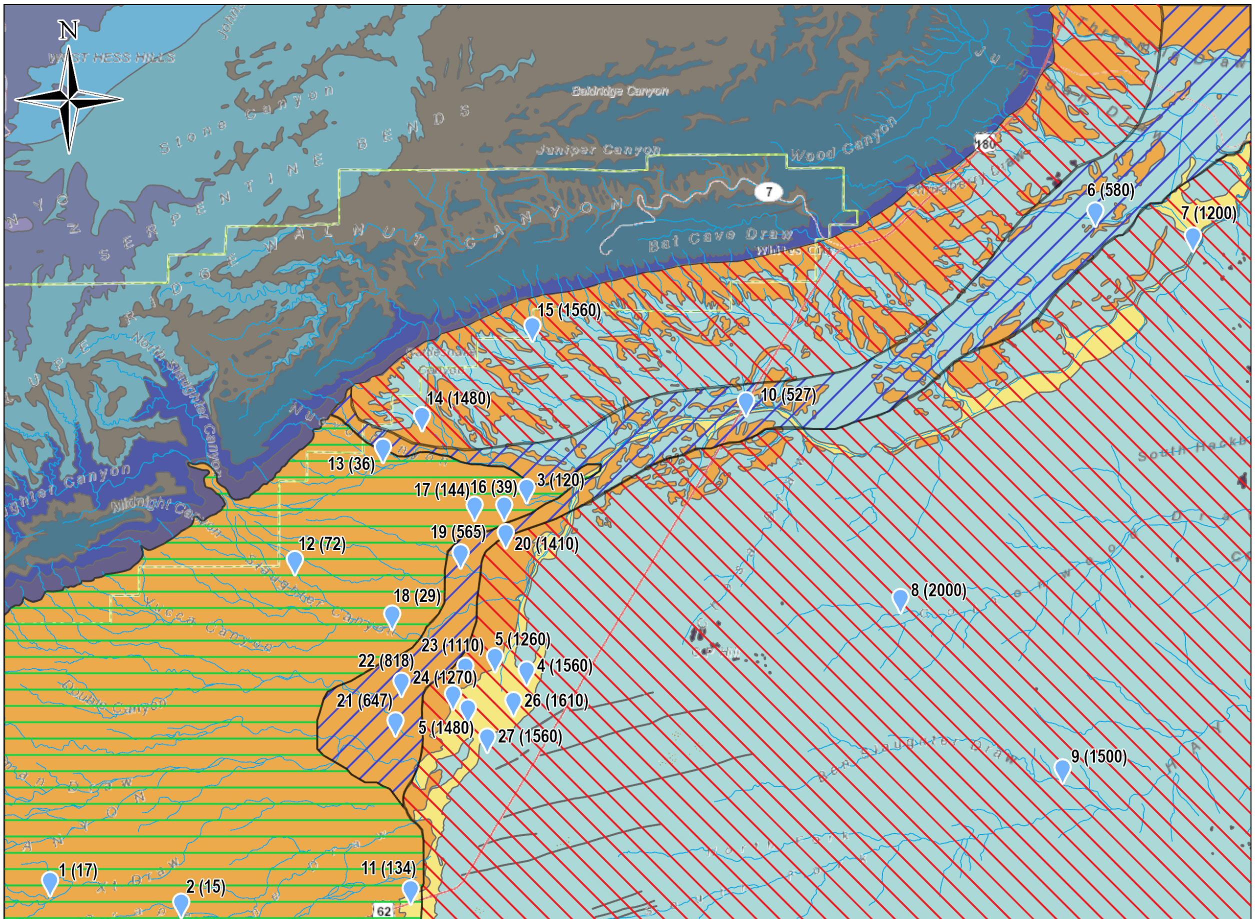
Remnant to continuous exposures of fine-grained, extremely gypsiferous terrace fill crop out along both sides of the Black River upstream from Washington Ranch (1.5 km east of Rattlesnake Spring), at least 7–13 m thick and about that far above the river. Locally, this gypsic material overlies well-cemented Quaternary gravels. The origin of this unit remains in question and perhaps should not be called a terrace because it may not be fluvial in origin (Love and Land, 2006). Sares (1984) suggested that it (or a similar unit in Chosa Draw) is aeolian and presented a granulometric sand sized distribution. Along the Black River, some parts of this unit may be aeolian, parts of it may be piedmont-slope deposits from the Castile dissolution scarp to the east, and some of it appears to have been deposited by gypsum- and carbonate-depositing springs because there is snail-bearing spring tufa overlying it locally. This gypsic material, in hydrologic communication with the underlying Castile gypsum bedrock, forms a shallow secondary aquifer in the upper Black River basin. Spring-fed pools upgradient of Rattlesnake Spring, including the Cottonwood Day Use Area spring pool, Bottomless Lake Sinkhole and Headwater Springs (Figure 4) are assumed to discharge from the alluvial-evaporite aquifer.

The different aquifers are reflected by differences in water chemistry in springs and sinkholes in the upper basin, including the carbonate-rich Quaternary gravel aquifer of Rattlesnake Spring and the gypsic alluvial-evaporite aquifer at Bottomless Lake and the Cottonwood Day Use Area (Figure 4). In those areas of the Black River valley where the Quaternary gravel aquifer is thin or above the water table, the alluvial-evaporite aquifer serves as a secondary source of groundwater. Water moving through gypsite, and underlying gypsum beds of the Castile, is very hard, with sulfate concentrations of 1200-1500 mg/L, distinguishing it from the fresher water stored in the Quaternary gravel aquifer (Figure 6; Hale, 1955; Cox, 1963; Love and Land, 2006).

Methods

The use of fluorescent dyes has been applied in many areas to characterize karst aquifer systems and delineate groundwater drainage basins (e.g., US Environmental Protection Agency, 1996; Johnson et al., 2010). The purpose of this Phase 1 dye trace study was to identify specific point-to-point flow paths, and measure aquifer parameters such as time of travel, dilution, and dispersion. Potential recharge sites, springs, and wells were examined carefully to identify specific locations that would prove most effective for injecting and detecting dyes. Prior to dye injection, all monitoring sites were sampled for any background fluorescence that could be mistaken for the dyes. This Phase I investigation began during baseflow conditions in the spring of 2020. Monitoring lasted seven months through the monsoon season and into the fall of 2020.

Two dye traces were conducted for this study. The first



Number	Site	Sulfate (mg/L)
1	XT Spring	17
2	Geyser Spring	15
3	Rattlesnake Spring	120
4	Bottomless Lakes Spring	1560
5	Headwater Spring	1260
6	Blue Spring	580
7	Black River Spring Run above Mouth of Blue Spring Creek	1200
8	Cottonwood Spring	2000
9	Jumping Spring	1500
10	Well C-03458	527
11	Highway 280 Unnamed Windmill Well	134
12	Well C-02816	72
13	Well C-02815	36
14	Unnamed Well #2	1480
15	Well C-01416	1560
16	Well C-00509	39
17	Well C-02227	144
18	Well C-03027	29
19	Well C-01426	565
20	Well C-00510	1410
21	Unnamed Well #3	647
22	Well C-01423	818
23	Well C-00391	1110
24	Well C-00904	1480
25	Well C-02738	1270
26	Unnamed Well #4	1610
27	Well C-01117	1560

Figure 6. *Sulfate concentrations in the upper Black River Basin.*

was the release of 454 g of eosine where the Black River spills over the Upper Diversion Dam, to enhance thorough mixing, located 1,060 m upstream of the Cottonwood Day Use Area pool—the primary target for detecting the dye. The river sinks normally about 220 m downstream of the dam, reappears after 260 m as a 370-m long pool, before sinking a final time before rising at the pool.

Downstream of the Cottonwood Day Use Area pool, Black River is typically dry for 900 m until reappearing for 550 m before sinking; water is not found again in the Black River system during baseflow conditions until Blue Spring is reached 17.2 km away. A measured mass of 4,436 g of uranine was released into a turbulent section of the Black River, to enhance thorough mixing, about 120 m upstream of where the river sank (Figure 7). Blue Spring was the primary target for detecting the dye.

The dye masses used for injection during this study were calculated using an equation developed by Worthington and Smart (2003) based on data from 185 tracer tests:

$$m = 19\,(DQc)^{0.95}$$

where m = mass of dye injected in grams, Q = output discharge in m^3/s, c = peak recovery dye concentration in g/m^3, and D = distance in meters between injection and recovery points. For the eosine trace, Q was based on the measured flow of the river upstream of the Cottonwood Day Use Area pool and D was the distance from the injection point to the pool. For the uranine trace, Q was the measured flow of Blue Spring and D was the distance to the spring from where the dye sank into the bed of Black River.

The dyes were selected for their high detectability and because they are considered non-toxic. Eosine is approved by the US Food and Drug Administration for internal and external drug and cosmetic use, and uranine for external drug and cosmetic use (FDA, 2021). While the dye masses were calculated for the dyes to be analytically detected at non-visible concentrations at their primary targets, landowners with wells or springs were notified where the dye might have appeared visually, even if those sites were not otherwise involved with this project. NCKRI's quality assurance/quality control procedures for storing, transporting, and injecting dye, and sampling for dye are provided in Appendix B.

Figure 7. *Dr. Lewis Land observes uranine recharging into fractures in the bed of Black River a short distance downstream from where Dr. George Veni (in the background) pours the dye into the stream. NCKRI photo by Michael Jones.*

The dye-tracing monitoring array during this project consisted of four springs and two private wells. The springs were Rattlesnake Spring (Figure 8), the spring pool at the Cottonwood Day Use Area (Figure 9), Blue Spring (Figure 10), and Castle Spring (Figure 11). The wells were completed in the Quaternary gravel aquifer and located on two separate private properties several kilometers upgradient of Blue Spring and Castle Spring (Figure 4).

Activated charcoal dye receptors were deployed at each monitoring site and exchanged weekly over a six-week sampling period, from 2020-05-07 through 2020-06-18, and exchanged biweekly for the remainder of the tracer study, over a 22-week period, from 2020-06-18 through 2020-11-19. Dye receptors were shipped to the Crawford Hydrology Laboratory (CHL) at Western Kentucky University for analysis by use of a spectrofluorophotometer with parts per trillion detection capabilities. The samples were then processed at the laboratory, analyzed for data interpretation, and laboratory reports were delivered to NCKRI personnel by CHL research hydrologists. Dye recovery analysis data were compiled and saved by NCKRI for evaluation (Appendix C).

For a result to be positive, the determined concentration for each dye analyzed in the activated charcoal receptors must be 10 times greater than the initial background fluorescence concentrations, or the practical quantification limit (lowest detection limit) for that dye (Crawford Hydrology Laboratory, 2016). This means that for a dye with a quantification limit of 0.01 parts per billion (ppb), no sample can be designated positive unless its concentration is greater than or equal to 0.10 ppb.

Results

Appendix A shows the detailed results of this investigation. Principal findings are summarized by the following key results:

- During this first phase of groundwater tracing in the upper Black River basin, neither uranine nor eosine dye were recovered with a positive result at any of the spring or well monitoring locations.
- During several monitoring events, charcoal receptors collected at four of the monitoring locations, Rattlesnake Spring, Black River at the Cottonwood Day Use Area, and wells C-03458 and C-03285 (Figure 4), indicated the presence of uranine or eosine dye. However, the dye was only detected at background concentrations (<10 times initial background or lowest detection limit). These detections occurred within the first week of sampling after injection.
- On 11 June 2020, a questionable positive hit of eosine dye was detected at Rattlesnake Spring, which means only one of two consecutive samples met the positive criteria of CHL. Two detections in a row are necessary to equal a positive result. The concentration of the dye eluted from the charcoal should display a rise and fall (the recorded peak of the emission curve must be ±5 nm of a particular dye peak as determined from its laboratory standard), similar to a dye breakthrough, over a period of time. Consequently, no location can be called positive if there is only one occasion when the dye concentration met the above criteria.
- On 8 October 2020, a questionable positive hit of eosine dye was detected at Well C-03285. CHL commented in the Laboratory Report Sheet (Appendix C) that the statistics were "out of range."
- All the background or questionable positive results were at very low concentrations. No detections occurred on charcoal receptors collected at Blue Spring or Castle Spring.

Discussion

Groundwater movement in conglomerate karst aquifers is an under-investigated topic, particularly in semi-arid settings where dye tracing is uncommon. Because our working knowledge of the Quaternary gravel aquifer is limited and based in part on data collected over half a century ago (Hale, 1955; Cox, 1963), there was a relatively high level of uncertainty regarding how successful this tracer investigation would be, especially during what proved to be a period of prolonged drought. According to the US Drought Monitor Classification Scheme, the Black River area experienced abnormally dry to exceptional drought conditions during the entire monitoring period.

The three most common reasons for negative results in a dye trace study are (1) insufficient amount of dye, (2) insufficient monitoring time for dye to move through the system, and (3) not deploying dye detectors in the right locations (Veni, 2014).

Duration of Monitoring

NCKRI personnel made the decision prior to dye injection to continue to collect samples from the springs and monitoring wells until we were satisfied that the dyes had likely traveled out of the aquifer. We initially assumed that the dyes could appear in a matter of days if the injection points and the springs are connected by highly transmissive flow paths. However, relatively low groundwater levels due to the drought at the time and the length of the actual flow path may well have significantly slowed the

dyes' actual or apparent velocity. A convenient way to achieve a positive result and increase dye adsorption is to leave the activated charcoal dye receptors deployed for a longer duration. After the first six weeks of monitoring, we decided to leave the receptors deployed for two weeks at each site before collection for analysis.

The drought during this study resulted in less surface runoff and groundwater recharge, thus reducing baseflow directly to the Black River and springs. These dry conditions inevitably result in more aquifer withdrawals from private wells by farmers and ranchers in the basin when irrigation allotments decrease. Lower groundwater levels equate to less hydraulic pressure in the aquifer and results in slower groundwater velocities. Since no direct velocity measurements are known to pre-date this study, the projected travel times for the dyes to appear, especially during a drought, may have been underestimated.

Volume of Dye

When planning for this tracer study, we used an empirical regression equation developed by Worthington and Smart (2003) for calculating the mass of dye needed and peak recovery concentrations. Numerous subsequent studies have shown that this equation works effectively, but it was not derived from data collected in conglomerate karst aquifers, nor in semi-arid climates, and may not apply as well to such conditions (Veni, 2014).

Our overall results (Appendix A) suggest that the dye could potentially be traveling to our monitoring stations in the Black River basin (Figure 4). However, while the Quaternary gravel aquifer is a well-cemented, low porosity rock at the surface, unobserved below the water table it may present more diffuse flow paths by dissolution around cobbles, potentially resulting in greater dispersion of the dye. Such dispersion could reduce the dyes' concentrations to below detection levels.

Inherent in the equations developed by Worthington and Smart (2003) is the fact that groundwater conduits in karst aquifers are parts of tributary systems, and tracers will be diluted by inflows. It is possible that the upper Black River is contributing a much smaller percentage of the flow volume in the conduit system that resurfaces at the lower Black River than accounted for by the dye mass equations.

Hale (1955) hypothesized that additions to the water in the Quaternary gravel aquifer occur in part via karstic conduits developed by movement of water from adjacent water-bearing beds in the underlying Castile gypsum. Hale stated that recharge to the Castile gypsum aquifer occurs mostly from precipitation where the formation crops out. Water in the Castile gypsum has a high sulfate content, and where this water moves into the Quaternary gravel aquifer it mixes with water of better quality in the limestone conglomerate. This phenomenon probably accounts for the progressive increase in mineral content that occurs as the water moves downgradient through the aquifer in a general northeastern direction down the valley of the upper Black River (Figure 6). The downgradient increase in sulfate content as water moves through the Quaternary gravel aquifer suggests that other sources of groundwater recharge probably exist in addition to flood waters originating in canyons of the bordering Guadalupe Mountains. However, while this may account for the undetected dyes in the lower Black River, it would not account for the undetected dyes at the upper Black River monitoring sites.

In addition to these factors that suggest insufficient dye masses were used, there is something in the groundwater causing an eosine peak shift. A common phenomenon with several dyes is that at very low concentrations, at locations with natural background fluorescence, dye emission curves exhibit peak centers just slightly out of range (more than 5 nm from dye standards is considered background by CHL criteria). Background fluorescence was identified at all the monitoring stations, and while it did not fit into the CHL protocol for reporting as positive detections, it does still affect the results (Crawford Hydrology Lab, personal communication, 2020).

Dye Detector/Injection Locations

An important factor in designing a dye trace is to account for all possible groundwater discharge sites to assure they are monitored. However, sometimes additional sites are discovered after the trace is initiated, which occurred during this study. Some small intermittent and perennial springs occur roughly 10-25 km east and southeast of the dye injection sites, in the Hay Hollow and Ben Slaughter Draw areas, which were not monitored during this investigation. While they do not seem likely outlets for water sinking in the upper Black River, the non-detection of dye at the other sites requires consideration of this possibility.

The lack of detection at the wells suggests that additional wells should be monitored during future tracing efforts. The wells selected were along the groundwater and sulfate concentration troughs

Figure 8. *Michael Jones collecting an activated charcoal dye detector at the outflow channel of Rattlesnake Spring. NCKRI photo by Michael Jones.*

Figure 9. *Michael Jones collecting an activated charcoal dye detector near the downstream end of the Cottonwood Day Use Area spring pool. NCKRI photo by Michael Jones.*

Figure 10. *Michael Jones collecting an activated charcoal dye detector from the spring run immediately downstream of the various Blue Spring outlets. NCKRI photo by Michael Jones.*

Figure 11. *Michael Jones collecting an activated charcoal dye detector from Castle Spring; note limestone conglomerate rock on the right. NCKRI photo by Michael Jones.*

leading downgradient from the upper Black River dye injection sites (Figures 4 and 6). They also had the highest likely permeabilities based on available data. While these factors increase the potential to find dye at a monitored well, because wells are random windows into aquifers and karst aquifers are characterized by discrete flow paths, it is common for dye to not arrive at a well even if it flows near it.

Additional Limiting Factors

The monitoring of wells occurred in stock tanks, which are not the ideal choice if alternatives are available. If dye is recovered from a covered tank, it is possible that residual levels of a selected dye could still be present in the tank from earlier dye-tracing tests. If the dye is recovered at the beginning of the current tracer study, the fluorescent residue might be left in the water tank for the duration of the monitoring period, thus causing an error in the results. But since most stock tanks are uncovered, the opposite situation is more common. The dyes are photodegradable, and the stock tanks monitored in this study are exposed to direct sunlight (Figures 12 and 13). If the dyes arrived at those wells, they may have photodegraded below detection limits. Additionally, the eosine flowed through at least one 220-m long pool before sinking, and possible a 370-m long pool further down the Black River channel where it may have photodegraded.

Data gaps exist in the records for well C-03285, where cattle had eaten the charcoal detector, and for the Cottonwood Day Use Area pool, where the dye receptors were missing during a few site visits. Conducting a study in a popular recreational area presents unique challenges. People may pick up the dye detectors out of curiosity and remove them from the water. Also, securing the detectors to logs or branches is not ideal, since they can be washed away during high flow events. During every site visit when dye detectors were missing from their original location, a new one was deployed with a different camouflaging strategy.

Finally, weekly deployment of charcoal receptors is common in dye tracing, as occurred during the first six weeks of this study. The springs and wells sampled are assumed to issue from solution cavities in a karstic conglomerate aquifer and/or a shallow alluvial-evaporite aquifer. It is thus likely that the hydraulic conductivity of these materials varies considerably, ranging from 0.1 to more than 100 m/day. Consequently, the weekly sampling interval was selected as long enough for dye detection and short enough to produce a rough

Figure 12. *Michael Jones collecting an activated charcoal dye detector from the stock tank fed by Well C-03285; the Guadalupe Escarpment rises in the background along the horizon. NCKRI photo by Michael Jones.*

Figure 13. *Michael Jones collecting an activated charcoal dye detector from the stock tank fed by Well C-03458. NCKRI photo by Michael Jones.*

breakthrough curve. Following the first six weeks of monitoring, the charcoal receptor deployment interval was extended from weekly to every two weeks. If dyes passed through the monitoring sites during the weekly sampling period, there may not have been sufficient time for accumulation of the dyes if they occurred in very low concentrations.

An ongoing question during this study has been whether Blue Spring is the primary source of perennial flow to the lower Black River. Although uranine was not recovered in the charcoal receptors deployed at Blue Spring, discharge measurements indicate that the spring's drainage basin is aerially extensive, and has recharge, storage, and flow characteristics of a karst aquifer. Hydrographs recorded at Blue Spring following storm events over the past 20 years reveal examples when the initial portion of the recession curve signifies fast drainage of large fractures or conduits, ending with a slowly decreasing curve, where the drainage of rock matrix and small fissures might be dominant. Conceivably if the dye trace injections were conducted during baseflow conditions at the

beginning of the monsoon season, and with a larger dye mass, rain events could help push the dye through the groundwater system to achieve a positive result at Blue Spring.

Until successful and unequivocal positive traces are known from each dye injection site, the results of this study should not be overinterpreted. The background "hits" were small and below statistical confidence that they are the injected dyes.

Conclusions and Future Work

There were no unequivocally positive concentrations of dye detected at any of the dye receptor locations during the initial dye trace investigation. These results were unexpected and may be attributed to:

1. groundwater velocities were lower than expected, exacerbated by the drought, and dyes did not reach any of the monitoring sites during the monitored period;
2. insufficient amount of dye to overcome dilution by the aquifer, potentially in part due to the conglomeratic nature of the rock; and/or
3. the dyes traveled from the injection sites to locations that were not monitored.

These results are not surprising given the little and coarse information known about the Quaternary gravel aquifer, that dye tracing in dry climates is not well studied, and that the conglomerate may create a more complex, tortuous, and dispersed conduit flow system.

Based on these results, NCKRI recommends a repeat of the dye traces in the upper Black River with larger dye masses. We suggest a 10-fold increase in the amount of eosine and uranine injected into the sinking stream portions of the upper Black River. We also recommend monitoring locations in the Hay Hollow and Ben Slaughter Draw areas, which were not a part of this study. Furthermore, placement of charcoal receptors at springs and wells should be deployed for a minimum of two weeks to allow sufficient accumulation of dye. Finally, NCKRI recommends monitoring for background prior to any new dye injections for the longest anticipated sampling interval so that baseline conditions can be compared appropriately. Background analyses will be important to any future study.

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Appendix A

Results of Phase 1 Dye Trace

Station no. (Figure 1)	Site Name	Receptor Input Date	Receptor Output Date	Results
3	Cottonwood Day Use Area Spring Pool	2020-05-01	2020-05-07	Fluorescein – No Detection Eosine – No Detection
3	Cottonwood Day Use Area Spring Pool	2020-05-07	2020-05-15	Fluorescein – No Detection Eosine – *Background*
3	Cottonwood Day Use Area Spring Pool	2020-05-15	2020-05-21	Fluorescein – No Detection Eosine – *Background*
3	Cottonwood Day Use Area Spring Pool	2020-05-29	2020-06-05	Fluorescein – No Detection Eosine – *Background*
3	Cottonwood Day Use Area Spring Pool	2020-06-11	2020-06-21	Fluorescein – *Background* Eosine – No Detection
3	Cottonwood Day Use Area Spring Pool	2020-06-18	2020-07-03	Fluorescein – *Background* Eosine – No Detection
3	Cottonwood Day Use Area Spring Pool	2020-08-13	2020-08-27	Fluorescein – No Detection Eosine – No Detection
3	Cottonwood Day Use Area Spring Pool	2020-08-27	2020-09-11	Fluorescein – No Detection Eosine – No Detection
3	Cottonwood Day Use Area Spring Pool	2020-09-11	2020-09-24	Fluorescein – No Detection Eosine – *Background*
3	Cottonwood Day Use Area Spring Pool	2020-09-24	2020-10-08	Fluorescein – No Detection Eosine – *Background*
3	Cottonwood Day Use Area Spring Pool	2020-10-08	2020-10-22	Fluorescein – No Detection Eosine – *Background*
3	Cottonwood Day Use Area Spring Pool	2020-10-22	2020-11-05	Fluorescein – No Detection Eosine – *Background*
3	Cottonwood Day Use Area Spring Pool	2020-11-05	2020-11-19	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-05-01	2020-05-07	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-05-07	2020-05-15	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-05-15	2020-05-21	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-05-21	2020-05-29	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-05-29	2020-06-04	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-06-04	2020-06-11	Fluorescein – No Detection Eosine – *Questionable Positive*
4	Rattlesnake Spring	2020-06-11	2020-06-18	Fluorescein – No Detection 3Eosine – No Detection
4	Rattlesnake Spring	2020-06-18	2020-07-03	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-07-03	2020-07-17	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-07-17	2020-07-30	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-07-30	2020-08-13	Fluorescein – No Detection Eosine – No Detection

4	Rattlesnake Spring	2020-08-13	2020-08-27	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-08-27	2020-09-11	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-09-11	2020-09-24	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-09-24	2020-10-08	Fluorescein – No Detection Eosine – *Background*
4	Rattlesnake Spring	2020-10-08	2020-10-22	Fluorescein – No Detection Eosine – No Detection
4	Rattlesnake Spring	2020-10-22	2020-11-05	Fluorescein – No Detection Eosine – *Background*
4	Rattlesnake Spring	2020-11-05	2020-11-19	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-04-30	2020-05-07	Fluorescein – No Detection Eosine – *Initial Background*
5	Well C-03458	2020-05-07	2020-05-15	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-05-15	2020-05-21	Fluorescein – Background Eosine – *Background*
5	Well C-03458	2020-05-21	2020-05-29	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-05-29	2020-06-05	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-06-05	2020-06-12	Fluorescein – No Detection Eosine – Background
5	Well C-03458	2020-06-12	2020-06-19	Fluorescein – No Detection Eosine – No Detection
5	Well C-03458	2020-06-19	2020-07-03	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-07-03	2020-07-17	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-07-17	2020-07-30	Fluorescein – No Detection Eosine – No Detection
5	Well C-03458	2020-07-30	2020-08-13	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-08-13	2020-08-27	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-08-27	2020-09-11	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-09-11	2020-09-24	Fluorescein – No Detection Eosine – No Detection
5	Well C-03458	2020-09-24	2020-10-08	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-10-08	2020-10-22	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-10-22	2020-11-05	Fluorescein – No Detection Eosine – *Background*
5	Well C-03458	2020-11-05	2020-11-19	Fluorescein – No Detection Eosine – *Background*
6	Well C-03285	2020-05-05	2020-05-07	Fluorescein – No Detection Eosine – No Detection
6	Well C-03285	2020-05-07	2020-05-15	Fluorescein – No Detection Eosine – *Background*

6	Well C-03285	2020-05-15	2020-05-21	Fluorescein – No Detection Eosine – *Background*
6	Well C-03285	2020-05-21	2020-05-28	Fluorescein – No Detection Eosine – *Background*
6	Well C-03285	2020-06-11	2020-06-18	Fluorescein – No Detection Eosine – No Detection
6	Well C-03285	2020-06-18	2020-07-03	Fluorescein – No Detection Eosine – *Background*
6	Well C-03285	2020-07-03	2020-07-16	Fluorescein – No Detection Eosine – No Detection
6	Well C-03285	2020-07-16	2020-07-30	Fluorescein – No Detection Eosine – No Detection
6	Well C-03285	2020-07-30	2020-08-13	Fluorescein – No Detection Eosine – *Background*
6	Well C-03285	2020-08-13	2020-08-27	Fluorescein – No Detection Eosine – No Detection
6	Well C-03285	2020-08-27	2020-09-11	Fluorescein – No Detection Eosine – No Detection
6	Well C-03285	2020-09-11	2020-09-24	Fluorescein – No Detection Eosine – *Background*
6	Well C-03285	2020-09-24	2020-10-08	Fluorescein – No Detection Eosine – *Questionable Positive*
6	Well C-03285	2020-10-08	2020-10-22	Fluorescein – No Detection Eosine – *Background*
6	Well C-03285	2020-10-22	2020-11-05	Fluorescein – No Detection Eosine – *Background*
6	Well C-03285	2020-11-05	2020-11-19	Fluorescein – No Detection Eosine – *Background*
7	Blue Spring	2020-05-01	2020-05-07	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-05-07	2020-05-14	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-05-14	2020-05-21	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-05-21	2020-05-28	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-05-28	2020-06-05	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-06-05	2020-06-11	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-06-11	2020-06-18	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-06-18	2020-07-03	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-07-03	2020-07-20	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-07-20	2020-07-30	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-07-30	2020-08-13	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-08-13	2020-08-27	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-08-27	2020-09-11	Fluorescein – No Detection Eosine – No Detection

7	Blue Spring	2020-09-11	2020-09-24	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-09-24	2020-10-08	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-10-08	2020-10-22	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-10-22	2020-11-05	Fluorescein – No Detection Eosine – No Detection
7	Blue Spring	2020-11-05	2020-11-19	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-05-11	2020-05-14	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-05-14	2020-05-21	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-05-21	2020-05-28	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-05-28	2020-06-04	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-06-04	2020-06-11	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-06-11	2020-06-18	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-06-18	2020-07-03	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-07-03	2020-07-16	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-07-16	2020-07-30	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-07-30	2020-08-13	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-08-13	2020-08-27	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-08-27	2020-09-11	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-09-11	2020-09-24	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-09-24	2020-10-08	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-10-08	2020-10-22	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-10-22	2020-11-05	Fluorescein – No Detection Eosine – No Detection
8	Castle Spring	2020-11-05	2020-11-19	Fluorescein – No Detection Eosine – No Detection

Appendix B

National Cave and Karst Research Institute Quality Assurance/Quality Control Manual for Tracer Testing (version 10 April 2020)

These Quality Assurance/Quality Control (QA/QC) protocols define field and laboratory operations and methods used by the National Cave and Karst Research Institute (NCKRI) for the performance of tracer testing of groundwater in karst terrains using fluorescent dyes. The operations and procedures contained in this manual define a high standard of data collection. However, depending upon the data quality objectives of the project and field conditions, the user may determine that some of the QA/QC methods are not necessary.

This manual is based in part on a manual developed by the Edwards Aquifer Authority by Geary Schindel, Steve Johnson, and others with assistance and revision in 2004 by Dr. George Veni, who later became NCKRI Executive Director. Veni revised it further with Dr. Lewis Land, NCKRI Karst Hydrogeologist, and Michael Jones, NCKRI Cave and Karst Science Specialist. They expanded it to include dye storage, transport, injection, and other procedures, as well as considerations such as scientific advances and specific NCKRI needs.

1.0. DYE HANDLING AND INJECTION

One of the primary purposes of this QA/QC manual is minimize and preferably eliminate the potential for false-positive results. The dyes used for groundwater tracing are established as generally non-toxic, with some approved by the US Food and Drug Administration as safe for use in food, medicines, and other products. However, the dyes are highly concentrated, and the analytical detection methods can identify dye to the parts per trillion level. Consequently, the potential for accidental cross-contamination with the dyes is a serious concern. This section on the use of dye is the most critical to prevent inadvertent transfer of dye onto surfaces and materials that might later be analyzed for dye.

1.1. DYE STORAGE

Dyes will be stored in NCKRI Headquarters in the Dye Room, located off the garage. Dye will only enter and exit the Dye Room through the garage door and not other parts of the building.

All other dye sampling supplies and samples for analysis will enter and exit NCKRI Headquarters through the building's front door and taken directly into the lab. All samples will be stored in a dark, secure area at 4º C unless signed out for analysis by lab personnel.

1.2. DYE AND SAMPLE TRANSPORT

NCKRI designates one truck for the transport of dyes. Dye will be transported in the back of the NCKRI dye truck, which will be neutralized for dye immediately after transport by a thorough washing with common household (sodium hypochlorite) bleach and verified as free of dye by careful inspection with an ultraviolet light.

Other NCKRI vehicles will be used to transport dye sampling supplies and samples. If there is any reasonable possibility that those vehicles, the equipment they contain, or the personal clothing, equipment, and/or bodies of people who may enter the vehicles may have been exposed to any dyes, they will be examined carefully with an ultraviolet (UV) light. If potential dye is discovered by this examination, the dye will be neutralized following the procedures in Section 1.4.

1.3. DYE INJECTION

Whenever possible, only one person will transport, carry, inject, and otherwise handle the dye and dispose of all waste materials. Although this person will be cleaned of dye after handling it, as an added precaution against cross-contamination, this person will not be involved with the collection or processing of dye samples for analysis.

To conduct the dye injection, the person injecting the dye will:

a. Approach the injection site carrying the dye; assistants may carry associated supplies that have not been in contact with the dye containers.
b. Upon reaching the site, place a sheet of heavy-gauge plastic on the ground. The sheet should be large enough to contain all of the dye injection supplies, and any accidentally spilled dye to keep it off the ground surface; this is especially critical for and more sheeting may be needed if the dye is in powder form and needs to be mixed into water on the site.
c. Don a full-body Tyvek suit with hood and booties, as well as a double layer of latex and/or nytril gloves.
d. Confirm the type and mass of dye that will be injected, if packaged from an outside source.

e. Pour the dye into the injection location as carefully as possible to minimize splashing or wind dispersal.
f. Place the empty dye containers into large plastic trash bags; do not set the trash bags on the plastic sheet but on the ground off the plastic.
g. Peel off the Tyvek hood, suit, and booties, with the outside surfaces folding inward, and in that order. In doing so, as each foot is released from the covering, place that foot on the ground off the plastic sheet. Deposit the suit, hood, and booties on the plastic sheet and step away from the sheet.
h. If the gloves are visibly touched by dye, remove the outer set of gloves and deposit them on the plastic sheet. If the inner gloves are also touched by dye, remove and deposit those as well and don a new set of gloves.
i. Roll up the plastic sheet with the suit, hood, and booties on the inside, from the outsides inward, leaving the bottom side of the sheet exposed, and place the plastic roll into a large plastic trash bag.
j. Remove the gloves and add them to the trash bag.
k. Place each trash bag into a second large trash bag and then wash hands and any part of the body potentially touched by dye following the procedures in Section 1.4, including an-examine with a UV light to look for dye that may not be visually apparent.
l. Change into a set new clothing and put the clothing worn during the trace into a separate plastic bag for washing.
m. Place the double-bagged trash bags into a third bag, inspect the outside of the third bag and the clothing bag with a UV light, neutralize any remaining potential dye, and put any final used cleaning materials inside the third bag before sealing.
n. Place the trash bags and clothing bag into the back of the NCKRI dye truck.
o. Dispose of the trash bags into a trash can or dumpster for proper collection and sanitary disposal by the city.
p. Wash personal clothing either in the NCKRI washing machine or home machine, followed by inspecting the clothing, washer, and dryer afterward with a UV light for any residual dye; if found, they will be washed again until no dye is detected.

1.4. DYE NEUTRALIZATION

Common household bleach (~6% sodium hypochlorite) neutralizes fluorescent dyes on contact. Bleach must be used cautiously to not cause physical harm to the people using it, or the equipment being cleaned. Where direct contact with concentrated dye is known or suspected, neutralization of dye on skin will be through one part bleach to three parts water solution while neutralization of dye on equipment will be through an undiluted or 50% diluted application of bleach while wearing latex gloves, depending on the durability of the equipment to bleach. The duration of contact will vary according to the sensitivity to bleach and will be followed by thorough rinsing with water; contact with skin should be kept to less than one minute. Following each neutralization procedure, the potentially affected area will be examined with a UV light. If dye is still apparent, the neutralization treatment will be repeated until no dye is visible.

Equipment that may be sensitive to damage from bleach and/or exposed to low concentrations of dye can use lower concentration bleach solutions with longer contact times, as described below for the neutralization of dye in automatic water samplers.

After each use during a dye trace study, bottles from automatic water samplers will be triple-rinsed with tap water, scrubbed inside if necessary to remove sediment or other materials, and then filled with a solution of 1 part bleach to 10 parts water and allowed to sit 24 hours to neutralize any residual dyes in the bottles. Afterward, the bleach solution is poured out, the bottles are rinsed once with tap water, triple-rinsed with DI water, and allowed to dry before used again.

When an automatic water sampler has completed its sampling program and has been returned to the lab, it will undergo a similar dye neutralization procedure. First, the sampler will collect three samples of tap water to flush itself of materials from the field. Second, it will collect hourly samples of the 1:10 bleach solution to neutralize any residual dyes. Third, it will collect one sample of tap water followed by three samples of DI water to rinse itself and complete the neutralization process. None of these collected samples need to be released into sampling bottles, to avoid needing to clean them, but can be released directly into an underlying tub or drain.

2.0. SAMPLING PROCEDURES

The initial field investigation for tracer test studies will be conducted by NCKRI staff experienced in the identification of karst features. Work will be supervised by the Institute's Karst Hydrogeologist or other qualified personnel. During that investigation, sites will be identified for dye injection and detection.

Sampling for dyes may occur either by collecting water samples for direct analysis of dyes, and/or by

passive charcoal detectors. The date, time, location, tracing project name, and other relevant field data associated with each sample will be recorded per section 3.2 of this manual.

2.1. PROCEDURES FOR COLLECTING GROUNDWATER AND SURFACE WATER FOR DYE

Water samples may be collected either as grab samples (collections at the time when a monitoring site is visited) and/or by automatic water samplers, which collect water at predetermined intervals with no person present.

2.1.1. COLLECTING WATER GRAB SAMPLES

Grab samples of water from springs, cave streams, and surface streams will be collected by submerging a laboratory-supplied container directly into the water. The clean sample bottle will be triple-rinsed with sample water before being used to collect a sample for analysis. When a sample is collected from a spring or stream, the container will be held upstream of the person collecting the sample and oriented in an upstream direction during sample collection.

Samples from groundwater monitoring wells will be collected with pre-cleaned dedicated PVC or Teflon bailers or dedicated submersible pumps. Prior to sampling, the water level in the well will be measured, if possible, with an electronic water level meter, fiberglass tape, or steel tape and recorded. Groundwater will not be purged from the well before the sample is collected.

Water sampling bottles will be handled while wearing a new pair of clean latex or nytril gloves, using one pair of gloves per sampling site. Each bottle will be labeled as to time and place of collection and placed in a sealable plastic bag labeled with the same information. Multiple bottles and charcoal detectors may be placed in the same bag if collected from the same time and location.

2.1.2. COLLECTING WATER WITH AUTOMATIC WATER SAMPLERS

Automatic water samplers will typically be held within a NCKRI-designed security barrel used to prevent tampering or damage to the samplers, and which locks the samplers in place. The barrels have an upper compartment for the sampler and a lower compartment for the marine battery that powers the sampler. This manual does not address the installation, maintenance, security, or programming associated with the sampler or its security barrel.

Water samples will be retrieved from the water sampler when it has been turned off, disconnected from its battery, and the sampling hose is verified visually to not contain any water. The top of the sampler will be lifted vertically off the bottom where the water bottles are held. Care will be used to not jostle the top and cause any residual water to drip or pour into the underlying bottles. After the top is set aside, the bottles will be checked to confirm that sampling began and ended on the preset schedule and that all samples were collected. Bottles in the bottom tray of the sampler will be capped. The tray will then be carried horizontally to prevent accidental spillage and returned to the lab. A replacement bottom with clean bottles will be placed under the top of the sampler to continue sampling if warranted.

At the lab, the bottles are refrigerated to prevent mold growth if analysis is not expected to occur within 24 hours. Each bottle will be removed from the sampler bottom in numerical order and poured into a pre-labeled container for analysis. Any remaining water will be kept in the sampler bottles until analysis is complete and it is clear additional water is not needed. At that time, excess water may be poured down the lab sink drain.

2.1.3. WATER SAMPLE CONTAINERS

Table B-1 lists the sample containers, preservatives, holding times and conditions for groundwater and elutant samples. Except for bottles in the automatic water samplers, only new sample containers will be used for sample collection. For each shipment of containers received, blanks will be taken from the lot and analyzed for the presence of dye. The results will be reviewed before any containers from the lot are used.

All sample containers will be stored in an area isolated from the extraction laboratory. Trip blanks for dye will also be prepared in this area.

2.2. PROCEDURES FOR USE OF CHARCOAL DETECTORS

Dye receptors (detectors or “bugs”) consisting of granular activated coconut carbon (charcoal) will be used to adsorb dye present in surface or groundwater. Approximately 20 grams of charcoal will be placed into a nylon screen mesh or equivalent permeable packet and placed in springs, cave streams, surface streams, and monitoring wells. Charcoal is used to adsorb Uranine,

Table B-1. *Required containers, sample storage techniques and recommended holding times water*

Parameter	Sample Container	Sample Storage/Preservation	Recommended Maximum Holding Times
Uranine (Sodium Fluorescein) (Acid Yellow 73)	13 mm X 100 mm vial with screw top lid or 50-ml plastic culture tube with screw top lid	Store in dark at 4º C.	6 months
Rhodamine WT (Acid Red 388)	13 mm X 100 mm vial with screw top lid or 50-ml plastic culture tube with screw top lid	Store in dark at 4º C.	6 months
Sulforhodamine B (Acid Red 52)	13 mm X 100 mm vial with screw top lid or 50-ml plastic culture tube with screw top lid	Store in dark at 4º C.	6 months
Eosine (Acid Red 87)	13 mm X 100 mm vial with screw top lid or 50-ml plastic culture tube with screw top lid	Store in dark at 4º C.	6 months
Phloxine B (Acid Red 92)	13 mm X 100 mm vial with screw top lid or 50-ml plastic culture tube with screw top lid	Store in dark at 4º C.	6 months
Optical Brightener Solophenyl (Direct yellow 96) Blankophor (F.B.A. 28) Tinopal CBSX (F.B.A. 35)	13 mm X 100 mm vial with screw top lid or 50-ml plastic culture tube with screw top lid	Store in dark at 4º C.	6 months

rhodamine WT, sulforhodamine B, Phloxine B, and eosine.

Charcoal detectors will be suspended in a surface stream, spring, or cave stream using a wire, string, pins, and/or weight. If needed to keep the detectors submerged, they will be attached to an impermeable weight. The detectors will be placed so that they are exposed to any flow that may be present. A rock, brick, or concrete weight ("gum drop") will be used to help maximize the volume of water flowing through the packet and secured with dark colored nylon string to a nearby tree, tree root, rock or pin. The dark colored string is used to blend in with the surroundings to minimize tampering.

If multiple springs flow from the bottom of a pool or lake, a short section of PVC pipe will be inserted into each monitored spring. The length and diameter of the pipe will vary to fit the dimensions of the spring orifice, but each will contain connectors to attach a charcoal detector and/or an automatic water sampler hose to the inside of the pipe. These pipes are "flow isolators," allowing only water from each discrete spring to flow through the pipes and assure the detectors only absorb dye from that spring and not others in the pool.

If charcoal detectors are placed in non-pumping monitoring wells, the detector should be lowered to the middle of the screened interval (if the well is screened), or depth where groundwater-transmitting conduits or fractures occur (if known and if the well is an open hole), to maximize exposure to flowing groundwater. For sampling of pumping water wells where dye detectors cannot be lowered down the well bore, a PVC pipe or bucket may also be fitted with a hose attachment for attachment to a faucet from the well. The pipe or bucket will be constructed as a flow cell, allowing placement of the dye detector within to channel flow through the packet. The flow cell could also be designed to accommodate a hose from an automatic water sampler.

2.3. PROCEDURES FOR USE OF UNBRIGHTENED COTTON

Receptors consisting of unbrightened cotton, polyethersulfone (PES) film, or other absorbent media will be used to absorb dyes and brightening agents, specifically Direct Yellow 96, and F.B.A. 28, and F.B.A. 351. A piece of cotton or filter media will be placed into a screen mesh packet and suspended in water as described in Section 2.2.

3.0. SAMPLE CUSTODY

3.1. FIELD COLLECTION AND SHIPMENT

When samples are transferred/or shipped from the field, they will be accompanied by chain-of-custody records. The records will include the signatures of the relinquisher and the receiver, the date and time of the exchange, and any pertinent remarks. Blank chain-of-custody forms to be used are shown in Figures B-1 and B-2 at the end of this manual.

During sample collection, the following procedures will be observed:

- To maintain the validity of the samples, on-site procedures will be reviewed prior to arrival in the field.
- Sample handling will be minimized in order to reduce the chance of error, confusion, and damage.
- Sample bags will be marked in the field with waterproof ink to prevent misidentification due to illegible labels.
- If the samples are shipped for analysis, the shipping container will be either padlocked or secured with a tamperproof seal.

Samples that are shipped for analysis will be delivered in one of the following ways so chain-of-custody safeguards can be observed.

- Hand carried and delivered;
- Registered mail, so that a return receipt is requested and available for documentation;
- Common carrier, so that a bill of lading can serve this purpose; or
- Air freight collect, for complete documentation.

All samples determined to be hazardous, according to the US Department of Transportation (US DOT) (49 CFR Section 172.1 or 49 CFR 173.3), will be shipped in strict accordance with US DOT regulations.

3.2. DOCUMENT AND SAMPLE CONTROL

Figure B-3, at the end of this manual, is a Sample Data Form that will be used by the person collecting the samples as a permanent record of all activities relating to the sample collection. One form will be completed for each sample or group of samples collected at the same time and location (such as from an automatic water sampler). Upon return from the field, the information will be recorded in a digital spreadsheet for the project.

Identification of samples will be serialized in an alpha-numeric system consistent with the procedures of the study. If a sample is potentially contaminated to yield false-positive results, it will be disposed of properly and noted in the Sample Data Form. Similarly, if a sample is lost, that will be documented in the Sample Data Form. Tags or labels affixed to the sample will include at a minimum, the sample number or code, date of collection, and the name or initials of the person who collected it.

3.3. PACKAGING

Sample packaging for shipment is done such that under normal handling, there is no release or damage of charcoal receptors or water samples, the effectiveness of the packing is not reduced, and there is no internal mixing of substances. The procedures to achieve these objectives are:

- Limit the volume of the sample to the quantity needed for analysis.
- Use plastic containers; if glass containers are used, the glass must be well cushioned.
- Use screw lids whenever possible.
- Place charcoal and cotton detectors in sealed plastic bags with a minimal volume of air.

3.4. SAMPLE RECEIPT

Upon receipt, the Sample Custodian will follow the procedures listed below:

- If samples have been damaged during shipment, the remaining samples will be carefully examined to determine whether they were affected. Any affected samples will also be considered damaged. It will be noted on the chain-of-custody record which specific samples were damaged and that those samples will be removed from the analytical schedule.
- Samples received will be compared against those listed on the chain-of-custody form.
- The chain-of-custody form will be signed and dated and attached to the waybill.
- The samples will be entered in the sample logbook which will contain the following information:
    - Project identification code
    - Sample number
    - Sample location name
    - Type of sample
    - Date and time sampled
    - Date and time received
- The samples will be placed in adequate storage.
- The appropriate Project Manager will be notified of sample arrival.
- The completed chain-of-custody records will be placed in the project file.

If samples arrive either without a chain-of-custody record or an incorrect chain-of-custody record, the

following procedure will be undertaken by the Sample Custodian:

- If the chain-of-custody is incorrect or incomplete, a memorandum to the Project Manager and field personnel will be prepared stating the inaccuracy and necessary correction. The memorandum must be signed and dated by the person originating the chain-of-custody form. The memorandum serves as an amendment to the chain-of-custody. If the information on the chain-of-custody form cannot be corrected by the Project Manager or the field personnel, the affected samples will be removed from the analytical schedule.
- If the chain-of-custody record is not shipped with the samples, the field personnel will be contacted and a memorandum prepared which lists the persons involved in collection, shipment, receipt, and the times, dates, and events of such. Each person involved must sign and date this memorandum. The completed memorandum will be maintained in lieu of the chain-of-custody record.

3.5. CUSTODY DURING TESTING PROGRAM

When chain-of-custody samples are being analyzed or processed, they will be signed out by the appropriate analyst. The individual performing the tests becomes responsible for the samples at that point. The samples will be maintained within sight or in the secure possession of the individual performing the test. When the work is completed, the samples will be returned and logged in to secure them in the proper storage location. During processing, the sample may be split into several fractions, depending upon the analysis required. The chain-of-custody record remains intact, however, for all sample fractions with the corresponding sample number.

After the analytical results have been reported, the chain-of-custody samples remain secured in storage. Restricted access to these samples is maintained.

5.0. QUALITY CONTROL SAMPLES

5.1. FIELD BLANKS

A field blank for water will be obtained by pouring dye-free distilled water into a sample bottle in the field at the first site sampled. One field blank will be collected for each sampling event. The field blank will be used to test for the presence of airborne dye particles as tracer injection artifacts.

5.2. CONTROL BLANKS

A control blank for activated charcoal will consist of an activated-charcoal detector which has been placed in a spring or well located in an area out of the influence of the tracer test. The control blank will have been placed during the previous sampling round and will be collected at the start of the current sampling round. This assures that the control blank will be handled and treated like other charcoal detectors. This protocol better replicates field conditions, thus achieving one of the purposes of using blanks and enhancing the QA/QC program. The term “control blank” is used because, strictly speaking, it is neither a trip blank nor a field blank. A control blank will be utilized during the entire tracer test and will be collected during each charcoal detector collection event.

5.3. FIELD REPLICATES

A field replicate is a second water or charcoal sample collected from a location that is monitored as part of a tracer testing program. The field replicate must be placed, collected and analyzed exactly like the original sample from the site. Replicate samples should be collected from one site in 20 that will be analyzed for the tracer test.

Figure B-1:
NCKRI Automatic Water Sampler Tracking Form

Location Name:	ISCO Sampler ID #:
Personnel:	
Collection Date (yyyy/mm/dd):	Grab Sample?
Start time/date:	End Time/Date:
Water Level:	Datum: Top of Well □ Staff Gauge □
Other comments:	

Bottle #	Date (yyyy/mm/dd)	Sample Time	Other Comments
1	/ /		
2	/ /		
3	/ /		
4	/ /		
5	/ /		
6	/ /		
7	/ /		
8	/ /		
9	/ /		
10	/ /		
11	/ /		
12	/ /		
13	/ /		
14	/ /		
15	/ /		
16	/ /		
17	/ /		
18	/ /		
19	/ /		
20	/ /		
22	/ /		
23	/ /		
24	/ /		
25	/ /		duplicate from bottle #:
26	/ /		rinsate with DI water
27	/ /		stock (tap water used for rinsing)

*Chain-of-Custody information should have signature, date and time

relinquished by:	received by:
relinquished by:	received by:
relinquished by:	received by:
relinquished by:	received by:
relinquished by:	received by:

Figure B-2: Charcoal Detector Sampler Tracking Form

National Cave and Karst Research Institute Tracer Test: Charcoal Detectors

Site:	
Crew:	
Collection Date:	
Start Time/Date (yyyy-mm-dd):	End Time/Date (yyyy-mm-dd):
Datum Type Options (for below) Top of well or Staff Gauge	

Location	Grab Sample	In Time/Date (yyyy-mm-dd)	Out Time/Date (yyyy-mm-dd)	Water Level	Datum Type	Other Comments
Blank						Blank detector
*Chain-Of-Custody information should have signature and date						
Relinquished by:		Received by:				
Relinquished by:		Received by:				
Relinquished by:		Received by:				
Relinquished by:		Received by:				
Relinquished by:		Received by:				
Relinquished by:		Received by:				

Figure B-3
NCKRI SAMPLE DATA FORM

Project name and code:	
Sample name and code:	
Sample location:	
Date (yyyy/mm/dd):	
Time:	
Sampler(s) name & initials:	
Weather conditions:	
Other notable field conditions:	
Problems:	
Other remarks:	
Parameters measured (discharge, stage, temp, pH, etc.):	
Passive detectors collected:	
Water samples collected:	
Split or duplicate samples:	
Blank samples collected:	
Samples labeled/tagged?	

Appendix C

Crawford Hydrology Lab Data Sheet

CRAWFORD HYDROLOGY LAB *

* *Hydrogeologists, Geologists, Environmental Scientists*

* *Karst Groundwater Investigations * Fluorescent Dye Analysis*

Western Kentucky University
Bowling Green, KY 42101
(270) 745-9224
E-mail: Crawford.Hydrology@wku.edu

LABORATORY REPORT SHEET

FLUORIMETRIC ANALYSIS RESULTS

UPPER BLACK RIVER BASIN

Analysis requested by:

MICHAEL JONES- NCKRI

FLUORESCEIN	EOSINE
Color Index:	Color Index:
Acid Yellow 73	Acid Red 87
Dye Receptor:	Dye Receptor:
Activated Charcoal	Activated Charcoal
Analysis by:	Analysis by:
Spectrofluorophotometer	Spectrofluorophotometer

CHARCOAL RECEPTORS										
					FLUORESCEIN PQL in Eluent: 0.005 ppb PQL in Water: 0.010 ppb λ in Eluent: 517.4 nm λ in Water: 510.8 nm			EOSINE PQL in Eluent: 0.005 ppb PQL in Water: 0.010 ppb λ in Eluent: 542.2 nm λ in Water: 536.2 nm			
Lab ID	Event	Date Collected	Feature Name	TIME	PeakFit	Results	Conc in ppb	Peak Center (nm)	Results	Conc in ppb	Peak Center (nm)	Comments
EL-000-0	07	06/22/20	BLANK RECEPTOR	-		ND			ND			
EL-000-0	18	11/19/20	BLANK RECEPTOR	-		ND			ND			
EL-001-0	BG	05/07/20	BLUE SPRING	-		ND			ND			
EL-001-0	02	05/14/20	BLUE SPRING	1708		ND			ND			
EL-001-0	03	05/21/20	BLUE SPRING	-		ND			ND			
EL-001-1	04	05/28/20	BLUE SPRING	-		ND			ND			
EL-001-0	05	06/05/20	BLUE SPRING	-		ND			ND			
EL-001-0	06	06/11/20	BLUE SPRING	-		ND			ND			
EL-001-1	07	06/18/20	BLUE SPRING	-		ND			ND			
EL-001-0	08	07/03/20	BLUE SPRING	-		ND			ND			
EL-001-0	09	07/20/20	BLUE SPRING	-		ND			ND			
EL-001-0	10	07/30/20	BLUE SPRING	-		ND			ND			
EL-001-0	11	08/13/20	BLUE SPRING	-		ND			ND			
EL-001-0	12	08/27/20	BLUE SPRING	1430		ND			ND			
EL-001-0	13	09/11/20	BLUE SPRING	-		ND			ND			
EL-001-0	14	09/24/20	BLUE SPRING	-		ND			ND			
EL-001-0	15	10/08/20	BLUE SPRING	-		ND			ND			
EL-001-0	16	10/22/20	BLUE SPRING	-		ND			ND			
EL-001-0	17	11/05/20	BLUE SPRING	-		ND			ND			
EL-001-0	18	11/19/20	BLUE SPRING	-		ND			ND			
EL-002-0	BG	05/07/20	COTTONWOOD	-		ND			ND			
EL-002-0	02	05/15/20	COTTONWOOD	1000		ND			B	0.049	532.9,POR	PeakFit Peak Pick
EL-002-0	03	05/21/20	COTTONWOOD	-		ND			B	0.049	545.2	PeakFit Peak Pick
EL-002-0	05	06/05/20	COTTONWOOD	-		ND			B	0.016	541.7	PeakFit Peak Pick
EL-002-0	07	06/21/20	COTTONWOOD	-		B	0.039	515.4	ND			
EL-002-0	08	07/03/20	COTTONWOOD	-		B	0.012	515.2	ND			
EL-002-0	12	08/27/20	COTTONWOOD	1530		ND			ND			
EL-002-0	13	09/11/20	COTTONWOOD	-		ND			ND			
EL-002-0	14	09/24/20	COTTONWOOD	-		ND			B	0.111	535.6,POR	PeakFit Peak Pick
EL-002-0	15	10/08/20	COTTONWOOD	-		ND			B	0.020	532.8,POR	PeakFit Peak Pick, Stats out of range
EL-002-0	16	10/22/20	COTTONWOOD	-		ND			B	0.025	544.8	PeakFit Peak Pick, Stats out of range
EL-002-0	17	11/05/20	COTTONWOOD	-		ND			B	0.371	535.6,POR	Only 1.6 nm from +?
EL-002-0	18	11/19/20	COTTONWOOD	-		ND			ND			
EL-003-0	BG	05/07/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	02	05/15/20	RATTLESNAKE SPRING	930		ND			ND			
EL-003-0	03	05/21/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-1	04	05/29/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	05	06/04/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	06	06/11/20	RATTLESNAKE SPRING	-		ND			+?	0.054	539.2	PeakFit Peak Pick
EL-003-1	07	06/18/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	08	07/03/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	09	07/17/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	10	07/30/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	11	08/13/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	12	08/27/20	RATTLESNAKE SPRING	1500		ND			ND			
EL-003-0	13	09/11/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	14	09/24/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	15	10/08/20	RATTLESNAKE SPRING	-		ND			B	0.219	534.6,POR	PeakFit Peak Pick, Stats out of range
EL-003-0	16	10/22/20	RATTLESNAKE SPRING	-		ND			ND			
EL-003-0	17	11/05/20	RATTLESNAKE SPRING	-		ND			B	0.168	534.0,POR	PeakFit Peak Pick
EL-003-0	18	11/19/20	RATTLESNAKE SPRING	-		ND			B	0.052	533.8,POR	PeakFit peak pick
EL-004-0	BG	05/07/20	C-03458	-		ND			IB	0.022	541.0	PeakFit Peak Pick
EL-004-0	02	05/15/20	C-03458	900		ND			B	0.100	537.4	PeakFit Peak Pick
EL-004-0	03	05/21/20	C-03458	-		B	0.006	514.4	B	0.087	538.3	PeakFit Peak Pick
EL-004-1	04	05/29/20	C-03458	-		ND			B	0.125	537.5	PeakFit Peak Pick
EL-004-0	05	06/05/20	C-03458	-		ND			B	0.133	538.4	PeakFit Peak Pick
EL-004-0	06	06/12/20	C-03458	-		ND			B	0.158	538.6	PeakFit Peak Pick
EL-004-1	07	06/19/20	C-03458	-		ND			ND			
EL-004-0	08	07/03/20	C-03458	-		ND			B	0.134	537.9	PeakFit Peak Pick
EL-004-0	09	07/17/20	C-03458	-		ND			B	0.015	537.7	PeakFit Peak Pick
EL-004-0	10	07/30/20	C-03458	-		ND			ND			
EL-004-0	11	08/13/20	C-03458	-		ND			B	0.281	535.6,POR	
EL-004-0	12	08/27/20	C-03458	1445		ND			B	0.110	535.4, POR	
EL-004-0	13	09/11/20	C-03458	-		ND			B	0.170	537.8	PeakFit Peak Pick - stats slightly out of range
EL-004-0	14	09/24/20	C-03458	-		ND			ND			
EL-004-0	15	10/08/20	C-03458	-		ND			ND			
EL-004-0	15	10/08/20	C-03458	-		ND			B	0.009	534.0,POR	PeakFit Peak Pick
EL-004-0	16	10/22/20	C-03458	-		ND			B	0.030	536.8,POR	PeakFit Peak Pick

\+ Positive
++ Very Positive
+++ Extremely Positive

						CHARCOAL RECEPTORS						
						FLUORESCEIN PQL in Eluent: 0.005 ppb PQL in Water: 0.010 ppb λ in Eluent: 517.4 nm λ in Water: 510.8 nm			EOSINE PQL in Eluent: 0.005 ppb PQL in Water: 0.010 ppb λ in Eluent: 542.2 nm λ in Water: 536.2 nm			
Lab ID	Event	Date Collected	Feature Name	TIME	PeakFit	Results	Conc in ppb	Peak Center (nm)	Results	Conc in ppb	Peak Center (nm)	Comments
EL-004-0	17	11/05/20	C-03458	-		ND			B	0.040	533.0,POR	PeakFit Peak Pick
EL-004-0	18	11/19/20	C-03458	-		ND			B	0.079	536.6,POR	Peakfit peak pick
EL-005-0	BG	05/07/20	C-03285	-		ND			ND			
EL-005-0	02	05/15/20	C-03285	800		ND			B	0.364	535.6,POR	
EL-005-0	03	05/21/20	C-03285	-		ND			B	0.267	535.5,POR	PeakFit Peak Pick
EL-005-1	04	05/28/20	C-03286	-		ND			B	0.165	534.7,POR	PeakFit Peak Pick
EL-005-0	07	06/18/20	C-03285	-		ND			ND			
EL-005-0	08	07/03/20	C-03285	-		ND			B	0.013	540.8	PeakFit Peak Pick
EL-005-0	09	07/16/20	C-03285	-		ND			ND			
EL-005-0	10	07/30/20	C-03285	-		ND			ND			
EL-005-0	11	08/13/20	C-03285	-		ND			B	0.226	532.6,POR	
EL-005-0	12	08/27/20	C-03285	1400		ND			ND			
EL-005-0	13	09/11/20	C-03285	-		ND			ND			
EL-005-0	14	09/24/20	C-03285	-		ND			B	0.787	536.8,POR	
EL-005-0	15	10/08/20	C-03285	-		ND			+?	0.080	537.6	PeakFit Peak Pick, Stats out of range
EL-005-0	16	10/22/20	C-03285	-		ND			B	0.414	537.0,POR	Only 0.2 nm from +?
EL-005-0	17	11/05/20	C-03285	-		ND			B	0.583	535.8,POR	Only 1.4 nm from +?
EL-005-0	18	11/19/20	C-03285	-		ND			B	0.158	532.4,POR	Peakfit peak pick
EL-006-0	02	05/14/20	CASTLE SPRING	1630		ND			ND			
EL-006-0	03	05/21/20	CASTLE SPRING	-		ND			ND			
EL-006-1	04	05/28/20	CASTLE SPRING	-		ND			ND			
EL-006-0	05	06/04/20	CASTLE SPRING	-		ND			ND			
EL-006-0	06	06/11/20	CASTLE SPRING	-		ND			ND			
EL-006-1	07	06/18/20	CASTLE SPRING	-		ND			ND			
EL-006-0	08	07/03/20	CASTLE SPRING	-		ND			ND			
EL-006-0	09	07/16/20	CASTLE SPRING	-		ND			ND			
EL-006-0	10	07/30/20	CASTLE SPRING	-		ND			ND			
EL-006-0	11	08/13/20	CASTLE SPRING	-		ND			ND			
EL-006-0	12	08/27/20	CASTLE SPRING	1330		ND			ND			
EL-006-0	13	09/11/20	CASTLE SPRING	-		ND			ND			
EL-006-0	14	09/24/20	CASTLE SPRING	-		ND			ND			
EL-006-0	15	10/08/20	CASTLE SPRING	-		ND			ND			
EL-006-0	16	10/22/20	CASTLE SPRING	-		ND			ND			
EL-006-0	17	11/05/20	CASTLE SPRING	-		ND			ND			
EL-006-0	18	11/19/20	CASTLE SPRING	-		ND			ND			

Approved by: A. Singer on 1/14/21

Comments:

IB = Initial Background
B = Background (<10 times background or lowest detection limit)
POR = Peak Out of Range (>5nm, <10nm from dye peak center)
ND = No Detection
NPI=No Peak Indicated
EL - Eluent Low- High Sensitivity Scan
EH - Eluent High- Low Sensitivity Scan

+ = Positive (10 times background or lowest detection limit)
++ = Very positive (100 times background or lowest detection limit)
+++ = Extremely positive (1000 times background or lowest detection limit)
+? = Questionable Positive, needs two hits in a row to equal +
Q = Lab Duplicate
QA = Quality Assurance/Quality Contol Laboratory Dye Standards
PeakFit Utilized (Statistical Analysis PeakFitting Software)

Criteria for Interpreting Results of Synchronous Scanning

Interpretation of dye tracing data is not the same as interpreting the results of chemical analyses. Background levels of dye are often present above the quantitation limits of the fluorescent dyes used for tracing. Another reason for these background levels is the extremely low detection limits of fluorescent dyes. Crawford Hydrology Laboratory has developed a standard protocol to determine what constitutes background levels, what is positive, and what is negative (non-detect).

Background Samples

In order for background fluorescence to be recorded, it must meet the following conditions:

- The determined concentration for each dye must be greater than or equal to the practical quantitation limit for that dye.
- The shape of the curve from the synchronous scanning must be the characteristic symmetrical shape of each particular dye as determined from its laboratory standard.
- The recorded peak of the emission curve must be +/– 5 nm of a particular dye peak as determined from its laboratory standard. The only times exceptions may be made are:
  - A water smaple collected at the same location verifies the presence of the dye in question.
  - Interference causing a shift in peak position is identified.

Post-Dye Injection Samples

Post-Dye Injection Samples must meet the following criteria for the determination of a positive trace:

- The determined concentration for each dye must be ten times greater than initial background concentrations or the practical quantitation limit for that dye. This means that for a dye with a quantitation limit of 0.01 parts per billion (ppb), no sample can be designated Positive (+) unless its concentration is greater than or equal to 0.100 ppb.

- The shape of the curve from the synchronous scanning must be the characteristic symmetrical shape of each particular dye as determined from its laboratory standard.
- The presence of dye at a particular location must not be attributable to any source other than the dye injected for the purpose of conducting the dye trace.
- The recorded peak of the emission curve must be +/– 5 nm of a particular dye peak as determined from its laboratory standard. The only times exceptions may be made are:
    - A water sample collected at the same location verifies the presence of the dye in question.
    - Interference causing a shift in peak position is identified.
- Two consecutive samples that meet the above criteria. The concentration of the dye eluted from the charcoal should display a rise and fall, similar to a dye breakthrough, over a period of time. Consequently, no location shall be called positive if there is only one occasion when the dye concentration met the above criteria. A minimum of two consecutive positives is needed in order to say that a particular location had a positive trace. If only one sample qualifies for a positive designation, then the location will either be designated as a potential positive, or the trace will be repeated.

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