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**PROCEDURES AND CRITERIA
ANALYSIS OF FLUORESCEIN, EOSINE, RHODAMINE WT,
SULFORHODAMINE B, AND PYRANINE
DYES IN WATER AND CHARCOAL SAMPLERS**

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PROCEDURES

Introduction

This document describes standard procedures and criteria currently in use at the Ozark Underground Laboratory 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 Ozark Underground Laboratory maintains a summary of changes in standard procedures and criteria.

Dye Nomenclature

Fluorescein is C.I. Acid yellow 73, Color Index Number 45350. Rhodamine WT is Acid Red 388; there is no assigned Color Index Number for this dye. Eosine (sometimes called eosin) is Acid Red 87, Color Index Number 45380. Sulforhodamine B is C.I. Acid Red 52, Color Index Number 45100. Pyranine is Solvent Green 7 (also called D&C Green 8), Color Index Number 59040.

Description of the Samplers

The charcoal samplers are packets of fiberglass screening partially filled with approximately 4.25 grams of activated coconut charcoal. The charcoal used by the Ozark Underground Laboratory is Calgon 207C coconut shell carbon, 6 to 12 mesh.

The most commonly used samplers are about 4 inches long by two inches wide. A cigar-shaped sampler is made for use in very small diameter wells (such as 1 inch diameter wells); this is a special order item and should be specifically requested when it is needed. All of the samplers are closed by heat sealing.

Placement of Samplers

Samplers (also called charcoal packets) are placed so as to be exposed to as much water as possible. 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 being flat against it. Two or more separately anchored packets are typically used for sampling springs and streams. 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 placed in sample holders made of PVC pipe fittings. Brass hose fittings are installed 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 consists of 1.5 inch diameter pipe and pipe fitting.

Charcoal packets can also 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. Some sort of weight should be added near the charcoal packet to insure 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. 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 stainless steel weight may be added to the top of the disposable bailer. We have had good success with two to three ounce segments of stainless steel pipe which have an outside diameter of 1.315 inches and an inside diameter of 1.049 inches; such pipe weighs about 1.7 pounds per linear foot. The weight of the stainless steel is approximately 497 pounds per cubic foot. The pipe segments can be attached over the anchoring cord at the top of the bailer. All weights should be cleaned prior to use; the cleaning approach should comply with decontamination procedures in use at the project site.

Placement of samplers requires adjustment to field conditions. The above placement comments are intended as guidance, not firm requirements.

Rinsing of Charcoal Packets Prior to Sampling

Charcoal packets routinely contain some fine powder that washes off rapidly when they are placed in water. Since such material could remain in monitoring wells, charcoal packets to be placed in such wells are 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 from 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.

Where convenient, the collected samplers should be briefly rinsed in the water being sampled. This is typically 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 permanent type felt marker pen. 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.

For most projects the Ozark Underground Laboratory supplies the Whirl-Pak bags. Prior to use, 1% of the new bags are randomly selected. Each bag is soaked in the standard eluting solution and then analyzed for the presence of any of the tracer dyes being used.

Collected samplers are kept in the dark to minimize algal growth on the charcoal prior to analysis work. We prefer (and in some studies require) that samples be placed on "blue ice" or ice upon collection and that they be shipped refrigerated with "blue ice" by overnight express. Do not ship samplers packed in 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.

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 are often collected. They should be collected in either glass or plastic; the Ozark Underground Laboratory routinely uses 50 ml research grade polypropylene copolymer Perfector Scientific vials (Catalog Number 2650) for such water samples. We need no more than 30 ml of water. The vials should be placed in the dark and refrigerated immediately after collection. They should be refrigerated until shipment. For most projects the Ozark Underground Laboratory supplies the vials. Prior to use, 1% of the new vials are randomly selected. Each vial is soaked in the standard eluting solution and then analyzed for the presence of any of the tracer dyes being used.

When water or charcoal samplers are collected for shipment to the Ozark Underground Laboratory they should be shipped promptly. We receive good overnight and second day air service from both UPS and Fed Ex; the Postal Service does not provide next day service to us. DHL works adequately for international shipments.

Each shipment of charcoal samplers or water samples must be accompanied by a sample tracking sheet. These sheets (which bear the title "Samples for Fluorescence Analysis") are provided by the Ozark Underground Laboratory and summarize placement and collection data. These sheets can be augmented by a client's chain of custody forms or any other relevant documentation. Figure 1 is one of our blank sample forms.

[illegible]

Digital cameras can provide an independent verification of the date and time of sample collection. A digital photo can be taken of each sampling location during each sample collection. The photo file has a date and time created. If the camera's clock is set correctly, the photo provides an independent reference of the date and time the sample was collected. It is critical that the photos be taken in the order of sampling; that is, if one has forgotten to take a photo of the previous station and remembers at the current sampling station, do not go back and take the previous station photo.

When we are using a digital camera for sampling documentation we initially take a high resolution photo of each station that shows its context broadly enough for an observer to distinguish it from other sampling station, but narrow enough not to include another sampling station. Subsequently, we download the high-resolution photos into a reference folder and rename the photos to the station number and name. We also make a copy of the photo to another folder and digitally draw arrows to the exact locations of the samplers. During subsequent sampling events a low-resolution digital photo is taken of each sampling station in the order they are visited. It is best to establish a routine of taking the photo upon arrival at the station. We then download these photos into a folder whose name indicates the dates of the photos. We do not rename these photos.

Some sites do not permit cameras. An alternative is to collect a Global Positioning System (GPS) location during each visit. GPS records the date and time each point (sampling station) is visited. While these files are not as easy to review as photographs, they can be used with a base map to show which locations were visited at which dates and times.

Receipt of Samplers

Samplers shipped to the Ozark Underground Laboratory are refrigerated upon receipt. Prior to cleaning and analysis, samplers are assigned a laboratory identification number. All samples are logged in upon receipt.

It sometimes occurs that there are discrepancies between the chain-of-custody sheets and the actual samples received. When this occurs, a "Discrepancy Sheet" form is completed and sent to the shipper of the sample for resolution. A copy of this form is enclosed as Figure 2. The purpose of the form is to help resolve discrepancies, even when they may be minor.

Cleaning of Samplers

Samplers are cleaned by spraying them with jets of clean water. At the Laboratory we use unchlorinated water for the cleansing to minimize dye deterioration. 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.

OZARK UNDERGROUND LABORATORY, INC.					
DISCREPANCIES BETWEEN CHAIN-OF-CUSTODY SHEETS AND ACTUAL SAMPLES RECEIVED					Page _____ of _____
Company & Project Name:			Date Rec'd by OUL:	Wk # _____	
Lab #	Sta #	Station Name	Date Pulled	Problem	Solution
Comments: 					

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, some water, and a strong basic solution such as aqueous ammonia.

The standard elution solution now used at the Ozark Underground Laboratory is a mixture of 5% aqua ammonia and 95% isopropyl alcohol solution and sufficient potassium hydroxide flakes 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 we use will elute fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes. It is also suitable for separating fluorescein peaks from peaks of some naturally present materials found in some 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.

Analysis on the Shimadzu RF-5000U or RF-5301

The Laboratory uses two Shimadzu spectrofluorophotometers. One is a model RF-5000U, and the other is a model RF-5301. Both of these instruments are capable of synchronous scanning. The RF-5301 is the primary instrument used; the RF-5000U is primarily used as a back-up instrument except for tracing studies which were begun using this instrument. The OUL also owns a Shimadzu RF-540 spectrofluorometer which is occasionally used for special purposes.

A sample of the elutant is withdrawn from the sample container using a disposable polyethylene pipette. Approximately 3 ml of the elutant 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 spectral range of the cuvettes is 340 to 800 nm. The pipettes and cuvettes are discarded after one use.

The cuvette is then placed in the RF-5000U or the RF-5301. Both instruments are controlled by a programmable computer. Each instrument is capable of conducting substantial data analysis.

Our instruments are operated and maintained in accordance with the manufacturer's recommendations. On-site installation of the instruments and a training session on the use of spectrofluorophotometers was provided by Delta Instrument Company.

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 "very fast" on the RF-5000U; it is "fast" on the RF-5301. The typical sensitivity setting used on both instruments is "high."

Our typical analysis of an elutant sample where pyranine dye may be present includes a synchronous scanning of excitation and emission spectra with a 35 nm separation between excitation and emission wavelengths. For this dye, the excitation scan is from 360 to 600 nm; the emission scan is from 395 to 635 nm. The emission fluorescence from the scan is plotted on a graph. The typical scan speed setting is "very fast" on the RF-5000U; it is "fast" on the RF-5301. The typical sensitivity setting on both instruments is "high."

Excitation and emission slit width settings vary between the two instruments. The widths vary with the dyes for which we are sampling and for the matrix in which the dyes may be present. Excitation and emission slit width settings are summarized in Table 1.

Table 1. Excitation and emission slit width settings routinely used for dye analysis.
Units are nanometers (nm)

Parameter	RF5000U	RF5301
Excitation slit for Eos, Fl, RWT, and SRB in elutant	5	3
Emission slit for Eos, Fl, RWT, and SRB in elutant	3	1.5
Excitation slit for Eos, Fl, RWT, and SRB in water	5	5
Emission slit for Eos, Fl, RWT, and SRB in water	10	3
Excitation slit for Pyranine in elutant	5	5
Emission slit for Pyranine in elutant	3	3
Excitation slit for Pyranine in pH adjusted water	5	5
Emission slit for Pyranine in pH adjusted water	3	3

Eos = 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; peaks are picked to the nearest 0.1 nm. All samples run on the RF-5000U and

RF-5301 are stored on disk and printed on normal typing paper with a laser printer; sample information is printed on the chart.

All samples analyzed are recorded in a bound journal.

Quantification

We calculate the magnitude of fluorescence peaks for fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes. Dye quantities are expressed in microgram per liter (parts per billion; ppb). On the RF-5000U and RF-5301 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.

Where there are multiple fluorescence peaks it is sometimes necessary to calculate dye concentrations based upon the height of the fluorescence peak rather than the area. The heights of the peaks are also proportional to dye concentrations.

We run dye concentration standards each day the machine is used. Ten separate 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 pyranine in well water from the Jefferson City-Cotter Formation. A sample of the standard is placed for at least two hours in a high ammonia atmosphere to adjust the pH to a value of 9.5 or greater.
- 5) 10 ppb fluorescein and 100 ppb rhodamine WT in elutant.
- 6) 10 ppb eosine in elutant.
- 7) 100 ppb sulforhodamine B in elutant.
- 8) 10 ppb pyranine 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 insure 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, sulforhodamine B, and pyranine. The resulting solutions contain 100 mg/l dye (100 parts per million dye). 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 Finn timer 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. Ozark Underground Laboratory 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 Finn timer. (see description in Step 2). All other volume measurements are made with Rheinland Kohn Geprüfte 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) A solution containing 1 ppm fluorescein dye and 10 ppm rhodamine WT dye.
- 2) A solution containing 1 ppm eosine.
- 3) A solution containing 10 ppm sulforhodamine B dye.
- 4) A solution containing 1 ppm pyranine.

Step 4. A series of eight short-term dye standards are made from solutions in Step 3. These standards were identified earlier in this section. In the experience of the Ozark Underground Laboratory these standards have a useful shelf life in excess of one week. However, in practice, they are kept under refrigeration and new standards are made weekly.

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 dilution is 100 fold. One part of the test sample is combined with 99 parts of water (if the test sample is water) or with 99 parts of the standard elutant (if the test sample is elutant). 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 Finnpiette. 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%

All other volume measurements are made with Rheinland Kohn Geprüfte 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 overnight in open containers in a high ammonia concentration chamber.

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.

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 typically includes approximately 1% of materials used.

Reports

Reports are provided in accordance with the needs of the client. We typically provide copies of the analysis graphs and a listing of stations and samples where dye was detected. The reports indicate dye concentrations.

Work at the Ozark Underground Laboratory 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. Mr. Aley has 40 years of professional experience in groundwater tracing with fluorescent tracing agents.

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 five dyes. 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 2 provides normal emission wavelength ranges and detection limits for the five dyes when analyzed on the OUL's RF-5000U spectrofluorophotometer. Table 3 provides similar data for the OUL's RF-5301. As indicated earlier in Table 1, the analytical protocols used on the two instruments are somewhat different, especially in regard to the widths of excitation and emission slit settings.

Table 2. RF-5000U Spectrofluorophotometer. Normal emission wavelength ranges and detection limits for fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes in water and elutant samples. Detection limits are based upon the as-sold weight of the dye mixtures normally used by the OUL.

Dye and Matrix	Normal Acceptable Emission Wavelength Range (nm)	Detection Limit (ppb)
Eosine in Elutant	533.0 to 539.6	0.035
Eosine in Water	529.6 to 538.4	0.008
Fluorescein in Elutant	510.7 to 515.0	0.010
Fluorescein in Water	505.6 to 510.5	0.0005
Pyranine in Elutant	500.4 to 504.6	0.055
Pyranine in Water*	495.5 to 501.5	0.030
Rhodamine WT in Elutant	561.7 to 568.9	0.275
Rhodamine WT in Water	569.4 to 574.8	0.050
Sulforhodamine B in Elutant	567.5 to 577.5	0.150
Sulforhodamine B in Water	576.2 to 579.7	0.040

* pH adjusted water with pH of 9.5 or greater.

Note: The protocols for the analysis of pyranine dye are substantially different than those for the other dyes. As a result, there is less potential interference between pyranine and fluorescein than might otherwise be indicated by the emission wavelength values shown in the table.

Table 3. RF-5301 Spectrofluorophotometer. Normal emission wavelength ranges and detection limits for fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes in water and elutant samples. Detection limits are based upon the as-sold weight of the dye mixtures normally used by the OUL.

Dye and Matrix	Normal Acceptable Emission Wavelength Range (nm)	Detection Limit (ppb)
Eosine in Elutant	538.1 to 543.9	0.050
Eosine in Water	531.2 to 535.7	0.015
Fluorescein in Elutant	514.0 to 518.1	0.025
Fluorescein in Water	506.2 to 510.0	0.002
Pyranine in Elutant	502.1 to 508.1	0.015
Pyranine in Water*	498.4 to 504.4	0.010
Rhodamine WT in Elutant	565.4 to 572.0	0.170
Rhodamine WT in Water	572.7 to 578.0	0.015
Sulforhodamine B in Elutant	572.8 to 579.6	0.080
Sulforhodamine B in Water	580.1 to 583.7	0.008

* pH adjusted water with pH of 9.5 or greater.

Note: The protocols for the analysis of pyranine dye are substantially different than those for the other dyes. As a result, there is less potential interference between pyranine and fluorescein than might otherwise be indicated by the emission wavelength values shown in the table.

Criteria for Determining Positive Dye Recoveries

The following sections identify normal criteria used by the OUL for determining positive dye recoveries. Beginning January 1, 2001, the primary analytical instrument in use at the OUL was the RF-5301; the RF-5000U was the principal backup instrument. Studies which were in progress prior to January 1, 2001 continued to have samples analyzed on the RF-5000U.

Except for pyranine dye, the analytical protocol used for the RF-5301 provides for the use of narrower excitation and/or emission slit settings than the RF-5000U protocol. This enhances our ability to discriminate between dyes and other fluorescent compounds. The protocol which is possible with the RF-5301 (as contrasted with the RF-5000U) also provides for a better balance in the sizes of the fluorescence peaks associated with an equal concentration of all of the dyes.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Eosine Dye Recoveries in Elutants from Charcoal Samplers.

There is generally little or no detectable fluorescence background in the general range of eosine dye encountered in most groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be eosine dye.

Criterion 1. There must be at least one fluorescence peak at the station in question in the range of 538.1 to 543.9 nm for samples analyzed by the RF-5301. The range must be 533.0 to 539.6 nm for samples analyzed by the RF-5000U.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. For the RF-5301, the eosine detection limit in elutant samples is 0.050 ppb, thus this dye concentration limit equals 0.150 ppb. For the RF-5000U the eosine detection limit in elutant samples is 0.035 ppb, thus this dye concentration limit equals 0.105 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 Ozark Underground Laboratory for Determining Positive Eosine Dye Recoveries in Water Samples.

There is generally little or no detectable fluorescence background in the general range of eosine dye encountered in most groundwater tracing studies. The following three criteria are used to identify fluorescence peaks which are deemed to be eosine dye.

Criterion 1. The associated charcoal samplers for the station should also contain eosine 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 eosine dye from our groundwater tracing work. For samples analyzed on the RF-5301, the fluorescence peak should generally be in the range of 531.2 to 535.7 nm. For samples analyzed on the RF-5000U, the fluorescence peak should generally be in the range of 529.6 to 538.4 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 analyzed on the RF-5301 is 0.015 ppb, thus this dye concentration limit equals 0.045 ppb. For samples analyzed on the 5000U the detection limit is 0.008 ppb, thus this dye concentration limit equals 0.024 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Fluorescein Dye Recoveries in Elutants from Charcoal Samplers.

There is often some fluorescence background in the range of fluorescein dye present at some of the stations used in groundwater tracing studies. We routinely conduct background sampling prior to the introduction of any tracer dyes to characterize this background fluorescence and to identify the existence of any tracer dyes which may be present in the area. The fact that a fluorescence peak is identified in our analytical results is not proof that it is fluorescein dye or that it is fluorescein dye from the trace of concern. The following 4 criteria are used to identify fluorescence peaks which are deemed to be fluorescein dye recoveries from our tracing work.

Criterion 1. There must be at least one fluorescence peak at the station in question in the range of 514.0 to 518.1 nm for samples analyzed by the RF-5301. The range must be 510.7 to 515.0 for samples analyzed by the RF-5000U.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. For the RF-5301, the fluorescein detection limit in elutant samples is 0.025 ppb, thus this dye concentration limit equals 0.075 ppb. For the RF-5000U, the fluorescein detection limit in elutant samples is 0.010 ppb, thus this dye concentration limit equals 0.030 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 Ozark Underground Laboratory for Determining Positive Fluorescein Dye Recoveries in Water Samples.

There is commonly some fluorescence background in the general range of fluorescein dye at some sampling stations used in groundwater tracing studies. The following criteria are used to identify fluorescence peaks which are deemed to be fluorescein dye in water.

Criterion 1. The associated charcoal samplers for the station should also contain fluorescein 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 fluorescein dye from our groundwater tracing work. For samples analyzed on the RF-5301, the fluorescence peak should generally be in the range of 506.2 to 510.0 nm. For samples analyzed on the RF-5000U, the fluorescence peak should generally be in the range of 505.6 to 510.5 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 analyzed on the RF-5301 is 0.002 ppb, thus this dye concentration limit equals 0.006 ppb. For the RF-5000U the detection limit is 0.0005 ppb, thus this dye concentration limit equals 0.0015 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Rhodamine WT Dye Recoveries in Elutants from Charcoal Samplers.

There is generally little or no detectable fluorescence background in the general range of Rhodamine WT dye encountered in most groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be Rhodamine WT.

Criterion 1. For samples analyzed on the RF-5301, there must be at least one fluorescence peak at the station in question in the range of 565.4 to 572.0 nm. For samples analyzed on the RF-5000U, there must be at least one fluorescence peak at the station in question in the range of 561.7 to 568.9 nm.

Criterion 2. The dye concentration associated with the Rhodamine WT peak must be at least 3 times the detection limit. For the RF-5301, the detection limit in elutant samples is 0.170 ppb, thus this dye concentration limit equals 0.510 ppb. For the RF-5000U, the detection limit in elutant samples is 0.275 ppb, thus this dye concentration limit equals 0.825 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 Ozark Underground Laboratory for Determining Positive Rhodamine WT Dye Recoveries in Water Samples.

The following criteria are used to identify fluorescence peaks which are deemed to be Rhodamine WT dye in water.

Criterion 1. 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. For samples analyzed with the RF-5301, the fluorescence peak should generally be in the range of 572.7 to 578.0 nm. For samples analyzed with the RF-5000U, the fluorescence peak should generally be in the range of 569.4 to 574.8 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 analyzed on the RF-5301 is 0.015 ppb, thus this dye concentration limit is 0.045 ppb. For samples analyzed on the RF-5000U the detection limit is 0.050 ppb, thus this dye concentration limit equals 0.150 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Sulforhodamine B Dye Recoveries in Elutants from Charcoal Samplers.

There is generally little or no detectable fluorescence background in the general range of sulforhodamine B dye encountered in most groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be sulforhodamine B.

Criterion 1. For samples analyzed on the RF-5000U, there must be at least one fluorescence peak at the station in question in the range of 567.5 to 577.5 nm. The acceptable range for samples analyzed on the RF-5301 is 572.8 to 579.6 nm.

Criterion 2. The dye concentration associated with the sulforhodamine B peak must be at least 3 times the detection limit. For the RF-5000U, the detection limit in elutant samples is 0.150 ppb, thus this dye concentration limit equals 0.450 ppb. For the RF-5301, 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 Ozark Underground Laboratory for Determining Positive Sulforhodamine B dye Recoveries in Water Samples.

The following criteria are used to identify fluorescence peaks which are deemed to be sulforhodamine B dye in water.

Criterion 1. The associated charcoal samplers for the station should also contain sulforhodamine B dye in accordance with the criteria listed earlier. 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 sulforhodamine B dye from the tracing work under investigation. For samples analyzed with the RF-5000U, the fluorescence peak should generally be in the range of 576.2 to 579.7 nm. For samples analyzed with the RF-5301, the fluorescence peak should generally be in the range of 580.1 to 583.7 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. For samples analyzed on the RF-5301 the detection limit in water is 0.008 ppb, thus this dye concentration limit equals 0.024 ppb. For samples analyzed on the RF-5000U the detection limit in water samples is 0.040 ppb, thus this dye concentration limit equals 0.120 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Pyranine Dye Recoveries in Elutants from Charcoal Samplers.

It must be remembered that the analysis protocol for pyranine dye is different than the protocol for the other four dyes discussed in this document. If the other dyes are present in a sample analyzed for pyranine dye their emission fluorescence peaks (if any) will be appreciably different than the values presented above. Because of this, there is very little analytical interference between fluorescein and pyranine dyes when both are present in a sample.

There is often some detectable fluorescence background encountered in the general range of pyranine dye in groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be pyranine.

Criterion 1. For samples analyzed on the RF-5000U, there must be at least one fluorescence peak at the station in question in the range of 500.4 to 504.6 nm. The acceptable range for samples analyzed on the RF-5301 is 502.1 to 508.1 nm.

Criterion 2. The dye concentration associated with the pyranine dye peak must be at least 3 times the detection limit. For the RF-5000U, the detection limit in elutant samples is 0.055 ppb, thus this dye concentration limit equals 0.165 ppb. For the RF-5301, the detection limit in elutant samples is 0.015 ppb, thus this dye concentration limit equals 0.045 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 pyranine dye. 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 Ozark Underground Laboratory for Determining Positive Pyranine Dye Recoveries in Water Samples.

It must be remembered that the analysis protocol for pyranine dye is different than the protocol for the other four dyes discussed in this document. If the other dyes are present in a sample analyzed for pyranine dye their emission fluorescence peaks (if any) will be appreciably different than the values presented above. Because of this, there is very little analytical interference between fluorescein and pyranine dyes when both are present in a sample.

The fluorescence of pyranine decreases below a pH of about 9.5. Prior to analysis water samples are placed in a high ammonia atmosphere for at least two hours. A pyranine dye in water standard is placed in the same atmosphere as the samples. Prior to analysis samples are tested to insure that their pH is 9.5 or greater. If pyranine dye concentrations in a sample are so great as to require dilution for quantification of the dye concentration the diluting water used is OUL reagent water which has been pH adjusted in a high ammonia atmosphere.

The following criteria are used to identify fluorescence peaks which are deemed to be pyranine dye in water.

Criterion 1. The associated charcoal samplers for the station should also contain pyranine dye in accordance with the criteria listed earlier. 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 pyranine dye from the tracing work under investigation. For samples analyzed with the RF-5000U, the fluorescence peak should generally be in the range of 495.5 to 501.5 nm. For samples analyzed with the RF-5301, the fluorescence peak should generally be in the range of 498.4 to 504.4 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. For samples analyzed on the RF-5301 the detection limit in water is 0.010 ppb, thus this dye concentration limit equals 0.030 ppb. For samples analyzed on the RF-5000U the detection limit in water samples is 0.030 ppb, thus this dye concentration limit equals 0.090 ppb.