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DYE TRACER TESTS IN KARST AREAS

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Dye tracer techniques have evolved from simple labeling experiments used to determine the source of resurgences or the outlet for sinking streams to sophisticated tools for studying the hydraulics of karst aquifers. Karst regions are generally first studied using qualitative methods and passive detectors to monitor springs. Quantitative tests, often using several dyes simultaneously, may then be conducted to study the flow characteristics of the aquifers. The tracer dyes used most often in North America for reconnaissance type studies include fluorescein sodium, direct yellow 96, and optical brighteners. These dyes are readily collected on passive detectors and analyzed without using instruments. Rhodamine WT may be used simultaneously with the above dyes and fluorometric analysis in quantitative studies.

INTRODUCTION

Tracer tests fall into several categories with different data requirements depending on the objectives of the study. The simplest type of test is usually to determine if a water connection exists between two points, as perhaps between two cave passages or between a sinking stream and a spring. After a series of point-to-point tests have been run in an area it may be possible to determine the direction of ground water movement (at least the part flowing through discrete conduits) and the karst ground water catchment areas. Contour maps of the potentiometric surface may be combined with tracer test data to further define the recharge areas (Quinlan, 1982). The time required for the tracer to travel from the injection point (input) to the recovery point (output) may be used to calculate average groundwater velocity and, if velocity data is available for several different flow levels (discharge), inferences can be made about the internal flow characteristics of the aquifer. If the recovery concentration of the tracer is measured at close enough time intervals to characterize the shape of the tracer recovery curve versus time, further insight into the flow characteristics of the aquifer will be gained. The simple point-to-point type of tests are generally conducted as a first step in studying an area and are qualitative in nature. The quantitative dye recovery tests are much more time consuming and expensive and should be based on the results of earlier qualitative works.

The first step in any water tracing program should be to collect all of the available geologic and hydrologic data for the study area and define the objectives of the tracer tests. Aley and Fletcher (1976) suggest that most tracer tests attempted in the United States fail due to: 1) insufficient hydrologic field work before the tracer is injected, 2) failure to allocate sufficient time to the tracing effort, and 3) tracing attempts during low flow conditions. These problems are not directly related to the type of tracer or the techniques employed, but to an insufficient understanding of the hydrogeologic setting and boundary conditions of the study area. Any tracer tests conducted in large or complex karst areas are time consuming and expensive. Researchers who have limited background in karst studies should enlist help from properly qualified individuals before attempting any large scale tracer tests.

The purpose of this paper is to provide detailed directions on the techniques currently used in karst areas for water tracing with fluorescent dyes. The paper begins with a description of basic qualitative procedures and advances to cover some quantitative techniques. The references provide a guide to more advanced techniques. The papers by Wilson (1968) and Smart and Laidlaw (1977) are indispensable references for anyone wishing to go beyond the basic qualitative methods. The works by Jones (1976), Aley and Fletcher (1976), and Milanovic (1981) provide detailed basic

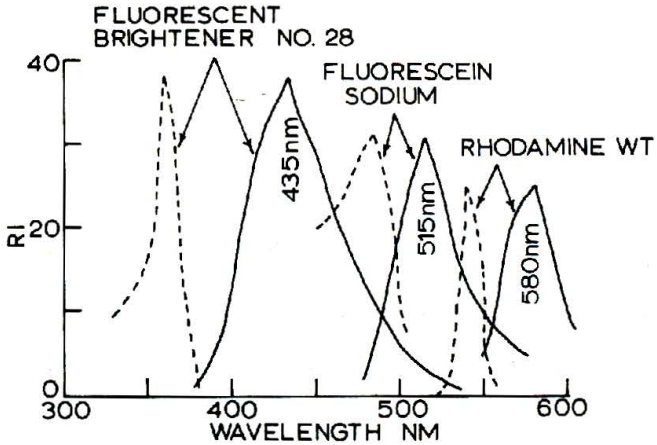

Figure 1. **Excitation (dashed lines) and emission (solid lines) spectra of fluorescent brightener no. 28, fluorescein sodium, and Rhodamine WT. Samples scanned using an American instrument company SPF-125 Spectrofluorometer and 2 mm slit widths for excitation and emission.**

Table 1. Evaluation of Principal Water Tracers Used in North American Karst Studies

Tracer & Color	Passive Detector	Test (elutriant)[1]	Maximum Excitation & Emission nm[2]	Detectable Conc.[3]	Advantages	Disadvantages	Remarks
Fluorescein Sodium $C_{20}H_{10}Na_2O_5$ Yellow-Green Xanthene	Activated cocoanut charcoal 6-14 mesh	Ethyl alcohol and 5% KOH. Visual test or fluorometer & 2A-47B; 2A-12, 65A filters	485 515	0.1 μg/1 Dependent on background levels. "Controls" must be used to determine background.	1) Does not require constant monitoring or any special equipment. 2) Inexpensive.	1) Dye is photochemically unstable. 2) Moderate sorption on clay. 3) pH sensitive.	This is the most popular method used in the USA. Carbon detectors first suggested by Dunn, 1957.
Rhodamine WT $C_{28}H_{31}N_2O_3Cl$ Red-Purple Xanthene	Activated coconut charcoal 6-14 mesh	Ethyl alcohol & 5% KOH or 1-Propanol + NH_4OH. Solution tested using fluorometer and 546-590 filters.	550 580	.01 μg/1. Dependent on background levels and fluctuation.	1) Dye is photochemically stable. 2) Dye may be used in low pH waters.	1) Requires the use of a flurometer. 2) Moderate clay sorption.	Rhodamine has been used extensively in Canada & USA. This is not a suitable method for amateurs without access to a flourometer.
Lycopodium Spores *Lycopodium Calvitum*	Plankton netting nylon-25 micron	Spores & sediment are washed from the nets. Microscopic examination is used to identify spores.	N/A	Dependent on background levels. Several kilograms of spores are usually used.	1) Several simultaneous tests may be conducted using different colored spores 2) No coloring of water occurs.	1) Spores may be prematurely filtered out. 2) Field collection system elaborate. 3) System is generally more expensive.	Spores have not been extensively used in North America.
Optical Brighteners Colorless normal light	Unbleached cotton	Visual examination of detectors under UV light or 7-37; 2A + 47B Filters.	360 435	Dependent on background levels, but generally at least .1 μg/1.	1) Inexpensive. 2) No coloring of water occurs.	1) Background readings may be excessively high. 2) Adsorbed onto organics.	May be used simultaneously with a green & red dye using fluoro-metric separation.
Direct Yellow (DY 96) Low Visibility Stilbene derivative	Unbleached cotton	Visual examination of detectors under UV light or 7-37; 2A + 47B Filters	N/A	1.0 μg/1 on cotton, and with fluorometric analysis.	1) Little natural background. 2) Good stability and low sorption. 3) No coloring of water.	1) Moderate cost. 2) Sensitive to pH.	Has been used extensively in Kentucky.
Salt NaCl Colorless	Recording specific conductance meter or regular sampling	Either a direct test for an increase in chloride, or a substantial increase in specific conductance	N/A	Dependent on background levels. Several hundred kilograms may be needed for larger tests.	1) Generally considered safe for use on public water systems. 2) Useful where fluorescent background conditions exclude other methods	1) Large quantities usually needed. 2) Background specific conductance is often high.	Salt is occasionally used by the US Geological Survey for tests dealing with public water supplies

[1]G. K. Turner Filters for Turner 111 Filter Fluorometer.
[2]Dye is usually most visible in clear water, deep pools, and in bright sunlight. These figures are not exact.
[3]Very dilute dye solutions may be concentrated upon the detector over a period of time.

instruction and hints. Stream tracing is an art and it is not uncommon for experienced researchers to disagree over even basic techniques. Except for the danger of contaminating the dye detectors, qualitative stream tracing using one tracer at a time is fairly certain (if a positive test appears). The quantitative methods using fluorometric separation of different dyes may give somewhat ambiguous results. In short, if a simple qualitative test will answer the question being asked, don't make the test needlessly complicated. Sawdust or computer card chips may be all that's needed to establish the connection between two cave passages. As with working a jigsaw puzzle, the first pieces are the hardest to get, but it becomes more predictable as the pattern begins to emerge.

The fluorescent dyes have an absorbance peak (maxima) at one wavelength and reemit the energy at a longer wavelength (some energy is always lost). Many dyes may be excited at several different wavelengths, but the emission

Table 2. Survey of Groundwater Tracers

NATURAL TRACERS				INJECTED TRACERS			
Stable Isotopes		**Radioactive**		**Activatable**		**Inactive**	
						Ionized Substances	**Drift Material**
Deuterium	^{2}H	Tritium	^{3}H	Bromine	^{35}Br	Salts: Na^+Cl^-	Lycopodium Spores
Oxygen—18	^{18}O	Sodium—24	^{24}Na	Indium	^{49}In	K^+Cl^-	Bacteria
Carbon—13	^{13}C	Chromium—51	^{51}Cr	Manganese	^{25}Mn	Li^+Cl^-	Viruses
Nitrogen—15	^{15}N	Cobalt—58	^{58}Co	Lanthanum	^{57}La	Na^+I^-	Fungi
Strontium—88	^{88}Sr	Cobalt—60	^{60}Co	Dysprosium	^{66}Dy	K^+Br^-	Sawdust
Sulfur—34	^{34}S	Bromine—82	^{82}Br				
Radioactive Isotopes						**Fluorescent Dyes:**	
Tritium—3	^{3}H	Gold—198	^{198}Au			Optical Brighteners	
Carbon—14	^{14}C	Iodine—131	^{131}I			Direct Yellow 96	
Silicon—32	^{32}Si	Phosphorus-32	^{32}p			Fluorescein	
Chlorine—36	^{36}Cl					Lissamine FF	
Argon—37	^{37}Ar					Rhodamine WT	
Argon—39	^{39}Ar						**Physical Characteristics**
Krypton—81	^{81}Kr						Water Temperature
Krypton—85	^{85}Kr						Flood Pulse

wavelength remains constant. Fluorescein always appears green whether it is excited at 365 nm or 485 nm. The optical brighteners (blue dyes) are invisible under "normal" light and generally have peak emission wavelengths around 435 nm. Fluorescein has an emission peak about 515 nm and Rhodamine WT at about 590 nm (Figure 1). Table 1 reviews the characteristics of commonly used tracers in karst studies, and Table 2 categorizes groundwater tracers in general.

Qualitative Techniques Using Fluorescent Dyes

Precautions—Check that no public or private water intakes will be affected by the dyes. Diplomatically notify the state water regulatory agencies of the study. Don't ask permission *per se* to use such and such dye—most agencies will just say "no" to protect themselves. Try to calculate dye concentrations so surface streams will not be colored. Check to make sure no one else is conducting tracer tests in the area.

Planning—Review all the previous tracer tests and hydrogeologic data. Try to locate all possible resurgences. Avoid conducting the initial tests under extremely high or low flow conditions. Measure or estimate discharge at the resurgences and calculate expected travel time for the dye (it may take several tests to get a feel for this). Good experimental control must be planned into the test. The detectors should all be made and handled in the same manner. A few detectors placed upstream of suspected resurgences should provide background values during the testing period.

Dyes—The characteristics of the tracers most frequently used in North American karst studies are summarized in Table 1. Fluorescein sodium (CI45350) is a green dye (Plate 7) which is almost "traditional" for use in karst areas. It is readily adsorbed on activated charcoal and eluted with a basic alcohol solution for visual examination. The dye has a low sorptive tendency, is photochemically unstable, and may lose fluorescence in low pH (< 5) waters.

Direct yellow 96 is a yellow dye which imparts a bright canary yellow to unbleached cotton detectors. The dye is detectable at 10 ppb on cotton examined under ultra-violet (UV) light. No chemical treatment is used. Almost invisible after dilution in water, optical brighteners are fluorescent in the ultraviolet range and impart a blue-white color to unbleached cotton detectors. There may be high natural background levels which interfere with the blue dyes—good "control" detectors must be maintained. These dyes are photochemically unstable and invisible under normal light.

Amount of Dye—The minimum amount of dye which can be detected for the dyes listed above is about .1 ppb (depending on background). The ability of passive detectors to collect dye depends on the length of time they are exposed to the tracer as well as the concentration of the tracer. The average velocity of the water (a function of discharge) is an important factor but hard to estimate. If discharge remains constant and the amount of dye injected is doubled, the peak recovery concentration should more or less double. However, if the amount of dye injected remains constant and the discharge falls by half, the peak recovery concentration will not rise linearly because of greater molecular dispersion of the dye in the lower velocity flow and accompanying longer travel time for the recovery. Almost all of the published formulas overestimate the amount of dye required (especially for long distances or high discharge). Most researchers develop a "standard dosage" based on intuition for their own study area. Perhaps the best empirical formula to-date is presented by Aley and Fletcher (1976) for calculating fluorescein dosages for open channel flow and charcoal detectors

$$Wd = 1.478\,(DQ/V)^{.5}$$

where Wd is the weight of fluorescein dye in Kg, D is the straight line distance in km from sink to resurgence, Q is discharge in m^3 second, and V is estimated velocity of flow in M/hour.

The mean velocity depends on discharge, gradient, and

flow characteristics. Even within a given region, considerable range may be found in groundwater velocities.

If diffuse flow conditions are expected, or if the dye must be injected through a soil cover (such as in the bottom of a doline), the required amount of dye will be considerably greater than needed for open channel flow conditions. Leibundgut and Wernli (1982) present a detailed analysis of the problems of calculating dye injection concentrations.

Injecting the Dye—Generally, the dye should be diluted about 10:1 with water before injection. If possible, the dye should be poured into a rapidly flowing turbulent reach of the stream to be traced. The tracer may be injected in normally dry dolines by using a tank truck to provide at least 2000 l of injection water (Plate 6). About 400 l of water should be dumped before the dye is added. This will help determine that the water drains rapidly from the doline and reduce adsorptive losses from the soil cover. Injecting the dye is often a messy job—place the detectors before injecting the dye.

Figure 2. **Photograph showing sampling equipment and passive detectors. From left to right: "Quinlan Gumdrop" detector holder with cotton ball on left arm and charcoal detector on right arm (railroad spike with charcoal detector), 30 ml glass sample vial, vial holder for use in wells or from bridges, plastic bag for transport of individual detectors, wooden sample holder to transport samples with minimal exposure to light.**

Detectors—Passive activated charcoal detectors are used for fluorescein and unbleached cotton for direct yellow 96 and the optical brightness. The fresh activated coconut charcoal (6–14 mesh) is held in plastic or aluminum window screening folded into "envelopes" about 5 cm x 5 cm. The cotton detectors may be unbleached cotton balls or a small sheet of cotton stretched over a frame. The detectors may be tied to rocks and anchored to the bottom of small streams and springs. The "Quinlan Gumdrop" (Figure 2) may be tied to the shore and is a better anchor for use in larger springs or streams with silty beds.

The changing schedule for the detectors depends somewhat on the sediment, pollution load, and amount of algal growth in the springs. In clear water, the charcoal detectors are sensitive for several weeks, but the ability to adsorb dye and maintain low background interference decreases steadily with time. Once the dye has been adsorbed onto the detector it seems to remain almost indefinitely. Detectors have been retrieved from cave passages over one year following a fluorescein tracer study and tested positive. However, charcoal readily adsorbs and concentrates many compounds over time, and cotton is subject to siltation and algae growth. In most areas the detectors should be changed at least weekly.

The detector should be placed in the main current but shielded from high velocity, turbulent water. The detectors should not be in direct sunlight and may have to be "hidden" to prevent tampering. Smart and Smith (1976) found that charcoal detectors did not work well in tropical regions.

Analysis—The cotton detectors are rinsed under a jet of tap water and air-dried in the dark. They are examined visually with a hand-held ultraviolet lamp (usually at both 254 and 366 nm) and the intensity of dye fluorescence recorded (none, weak, medium, strong). The optical brighteners fluoresce a blue-white; direct yellow 96 fluoresces a bright canary yellow. If both dyes are present, the cotton fluoresces a characteristic white (Quinlan, 1977).

Every researcher seems to use a different procedure for testing charcoal detectors. The charcoal is rinsed in water and shaken into a test tube, jar, or petrie dish (some workers dry the charcoal first). The charcoal is covered with a basic alcohol solution and allowed to sit for from one-half hour to several days. It is then examined under sunlight or a high intensity white light (slide projector beam). The appearance of a yellow-green "glow" at the top of the charcoal signifies a positive test (Plate 3). The principal interference appears to be wastes from livestock which appear as a greenish-yellow color—the eye can tell the difference, but this "interference" can give very high readings on a filter fluorometer using the fluorescien filter combination. Visual examination of the charcoal for fluorescein is probably more reliable than instrumental readings of the elutent (at least using the filter fluorometer). The samples should not be shaken if they are to be examined visually, and should be stored in the dark.

A series of laboratory tests were conducted to evaluate the efficiency of different elutriants for fluorescein determination. Fresh activated coconut charcoal (Fisher catalog no. 5685A) was exposed for .5 hours to a fluorescein solution (10 ppb). The charcoal was rinsed in distilled water and separated into test tubes. The samples were treated with different combinations of alcohol and base elutriants and evaluated visually and with a fluorometer. This test suggested that the greatest intensity of fluorescence was pro-

duced by a solution of approximately 25% distilled water, 25% NH_4OH and 50% l-propanol (Smart and Brown, 1973). The "traditional" mixture of 95% ethanol and 5%KOH (Dunn, 1957) and isopropyl alcohol and NH_4OH (Aley and Fletcher, 1976) both performed satisfactorily for the visual test but ranked well below the l-propanol solution on fluorometric analysis.

In strongly positive tests, the maximum color intensity develops within about one-half hour and then slowly decreases, probably due to readsorption of the dye by the charcoal. However, some weakly positive tests take from several hours to a day to develop a clearly visible color. The detectors should be kept at least 24 hours before a final evaluation is made. Examination under UV light is not usually advantageous—the excitation peak for fluorescein is 485 nm.

There appears to be a complete lack of correlation of detector intensity either with discharge, distance, peak concentration, or length of or time of exposure to the dye (Spangler et al., 1983). Smart (1976) described a method to quantify the peak concentration of optical brightener on cotton detectors using fluorometric procedures, however, the generally high background fluctuations in this wavelength (415 nm) makes quantification of the optical brighteners difficult at best.

The intensity of the dye eluted from charcoal is also a function of the "freshness" of the charcoal, the elutriant mixture, the ratio of elutant to charcoal, and the time of exposure to the elutriant. To achieve maximum elutant fluorescence, Smart and Brown (1973) recommended: 1) dye concentrations should be as high as possible for as long as possible; 2) detectors should be changed often (1 or 2 days); 3) detectors should be dried on removal if analysis is not immediate and; 4) stream flow through the detector should be maximized.

Negative Tests—Negative tests do not necessarily prove that a connection does not exist. Tracer tests may fail for a number of reasons (Jones, 1976): 1) the correct resurgence(s) not monitored; 2) inadequate quantity of dye; 3) sorption losses to clays or organic material; 4) diffuse ground water flow conditions causing very slow movement of the tracer; 5) sufficient dye travel time not allowed; 6) tracer "masked" by high background conditions; 7) insulation (coating) of the detectors by sediments; 8) biochemical decay of the tracers; 9) dilution of the dye from flooding; 10) inadequate time for clearing of the tracers between tests in the same areas.

QUANTITATIVE PROCEDURES

One of the outstanding advantages of fluorescent tracers is the ease with which they may be quantitatively analyzed. The lower limit of detection of any tracer depends on the natural "background" level inherent in the analytical procedure, or more specifically on the fluctuation of this background. In most temperate zone karst areas (and populated areas), the blue wavelengths have the greatest noise-to-signal ratio, the green wavelengths, the intermediate, and red wavelengths the lowest. The red dyes should therefore be detectable at lower concentrations and be the easiest to quantify.

Fluorescent tracers are usually analyzed using a fluorometer. Spectrafluorometers are generally laboratory instruments which can scan (manually or automatically) through the spectrum of interest. Filter fluorometers (Figure 3) are smaller and many are suitable for field use. They are

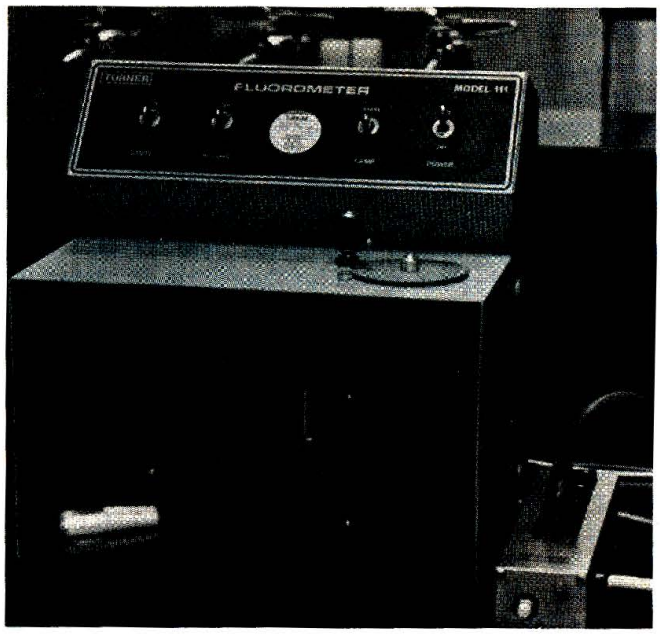

Figure 3. **A filter fluorometer shown with sample door open. Light from the lamp passes through a primary filter to the sample culvet (in door) and is then reflected through the secondary filter to the photomultiplier which sends a signal to the readout devise. Voltage surge protectors may be added at the outlet box (right).**

set at a given wavelength by selecting appropriate filter combinations. For practical purposes, the minimum detectable concentrations of dyes will be a function of the background levels, so both types of instruments have essentially the same sensitivity. The spectrafluorometer's advantage is the ability to scan water samples—a real advantage when some types of interference are present in the sample. The filter unit is more portable, less expensive, and well suited to analyzing large batches of samples. Filter units are the most used instruments in dye-tracing work.

The work by Wilson (1968) is a good basic manual of fluorometric procedures. Also, the instruction manuals provided with the instruments are a good source of information. Several papers describing quantitative procedures are presented in Gospodaric and Habic (1976). Behrens (1982) and Kass (1982) describe techniques for separating and

quantifying samples containing more than one dye. The following comments apply primarily to filter fluorometers.

Filters—The primary filters must be matched to the emission peaks of the lamps (The uneven light output severely limits the ability to do a true double scan with spectrafluorometers.) There is some spectral overlap between the blue, green, and red dyes (Figure 1). Some of this overlap can be eliminated by using "narrow pass" filters to narrow the range of wavelengths transmitted to the photomultiplier. It may be possible to shift the excitation or emission spectrums somewhat away from the maximums for the dye (the sensitivity of the instrument is rarely a limiting factor).

Standards—It may be best to use water collected from the resurgence prior to injecting the dye to prepare the standards. Spangler et. al., 1983 found that the lower pH of distilled water standards quenched some of the fluorescent intensity of fluorescein. Rhodamine WT standards appear to be stable for several months, but standards for fluorscein and the blue dyes should be prepared fresh for each test. Sample standardization curves for fluorescein are shown in Figure 4.

Sampling—The samples should be stored in the dark in glass vials. Figure 2 shows storage boxes for transporting the samples. Automatic samples can save a lot of labor. Several types are available commercially. Crawford (1979) gives a description of a "homemade" sampler.

The sampling interval should be adjusted to the travel time of the tracer. Fifteen minute sampling intervals may be needed for travel times under 24 hours. Tests which are drawn out over a period of months may use 2 or 3 day sampling intervals, although two samples per day is the minumum interval recommended by Milanovic (1981).

Suppression Interference—The samples may sometimes be directly treated to suppress background or selectively enhance fluorescence. The fluorescent intensity of Rhodamine WT is very temperature dependent, so an elevation in sample temperature should have a much greater effect on the fluorescent intensity of Rhodamine WT than on background fluorescence (Calvin Alexander, written communication). Fluorescein is very dependent on the pH of the sample, so (Behrens, 1982) has suggested that acidifying the sample should quench the presence of fluorescein.

Fluorometric analysis of the elutant from charcoal detectors poses several problems, but it's almost a required procedure for using Rhodamine dye. The charcoal concentrates many substances causing background fluoroscence, and some of the elutant solutions may have high background fluorescence. In examining the elutant from charcoal for fluorescein, the unaided eye appears to be more adept at discerning fluorescene dye from livestock waste products than does a filter fluorometer. If the detector is to be tested for both fluoroscein and Rhodamine, Jones (1976) recommended a visual examination for fluorescein and a fluorometric examination for Rhodamine.

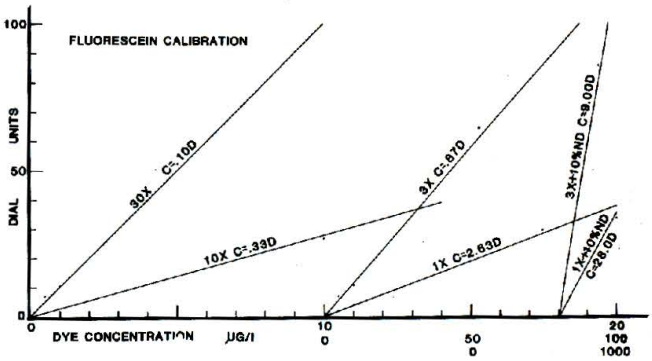

***Figure 4.* Typical set of calibration curves for fluorescein sodium. Each batch of dye, and each instrument requires a unique standardization to convert the dial readings (or relative fluorescent intensity) to concentration.**

CONCLUSIONS

Dye tracing techniques have become increasingly sophisticated in recent years. The skill, however, is still as much an art as a science and technology cannot be substituted for a thorough knowledge of the study area. Tracer tests must be carefully planned and be appropriate to the local hydrologic boundary conditions. The simple coloring of the water can be a great nuisance in the case of a water supply, and amateur speleogists are well advised not to risk tests in areas where public water supply springs are located.

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