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FLUOROMETRY

A Method for Determining A Spray Deposit Using A Fluorescent Dye

by

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Recommendations re: Fluorometry

1. The fluorometer needs calibrating with respect to its magnifying functions. For instance, readings at the 3X power now read four to five times the value of the 1X readings. The 10X and 30X powers also over magnify. If repairs not made, all readings should be taken at the 1X power.
2. 100 unmarked pyrex test tubes 7.5 X 1.0 cm (O.D) for fluorometry should be ordered.
3. To field crew: 2 plates of "indicator" slides should be placed in an open area of the spray plot which is likely to receive a relatively high amount of deposit. These slides will be used to determine the amount of dilution necessary to arrive at fluorometer readings for the sample slides. (each plot).

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Materials:
- pyrex test tubes 7.5 X 1.0 (O.D) cm
- 10% Methanol or 10% Ethanol (suitable solvents)
- 11.0 cm petri dishes
- pasteur pipettes
- micropipettor
- Oxford pipettor
- pipe cleaners
- acetone in squeeze bottle
- fluorometer
- aspirator connected to a running faucet

Methods:

1. Obtaining a standard curve:

Before taking readings of plot samples a curve must be made from dilutions of that plot's standard solution (solution saved from the day of the spray). Since the fluorometer is very sensitive at least a 10,000 fold dilution of the standard is necessary before a reading at its lowest power (IX) can be obtained.

Begin by mixing 1 µl of standard * from a micro pipettor in 10 ml of the chosen solvent in a petri dish. You now have 0.1 µl STD. in 1 ml solvent. The unit ul/ml will serve as the abcissa coordinate in the standard graph. From this solution make ten fold dilutions to 0.0001 ul/ml filling a pyrex test tube 1 cm from the top with a sample of each (See Fig. 1)

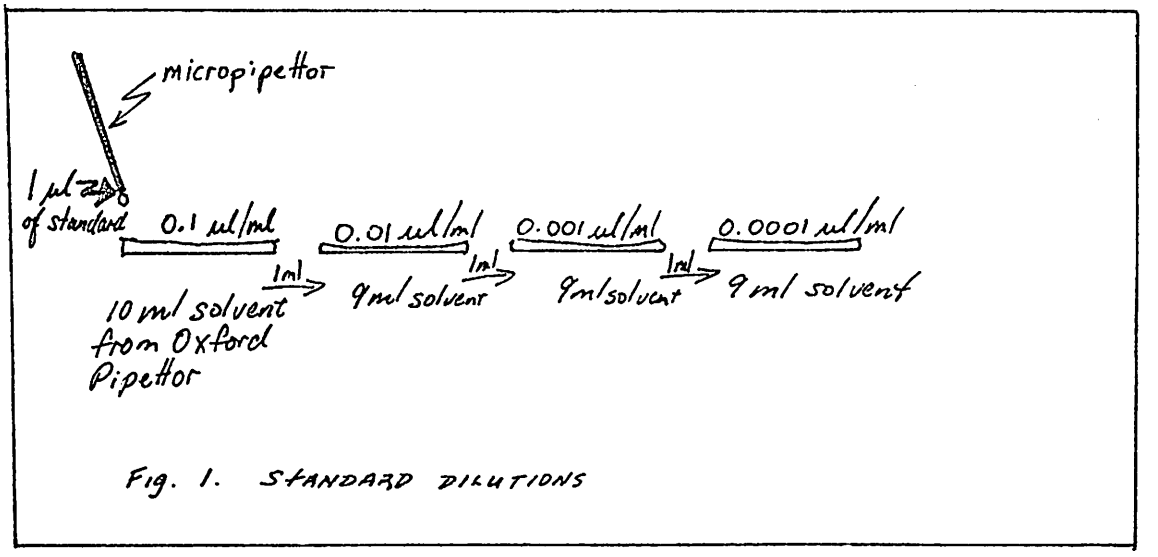

Fig. 1. STANDARD DILUTIONS

* It is absolutely essential to shake the standard bottle to ensure an even distribution of dye.

Readings on the fluorometer: The fluorometer should be warmed up at least 15 minutes before use. See manual for operating instruction and appropriate use of filters (for Uvitex ERN P dye use 7-60 (blue) as the primary filter and 2A (yellow) for the secondary). The diluting solvent is used as a "blank".

Taking the above four samples take readings at each of the four powers of the fluorometer making sure that the sample door is shut and that a few seconds are allowed for the machine to adjust to the sample. A typical result is shown below (table 1).

TABLE ONE. STANDARD CALIBRATIONS

UL STANDARD / ML SOLVENT	FLUOROMETER READINGS			
	1X	3X	10X	30X
0.1	-	-	-	-
0.01	53	-	-	-
0.001	5	32	84	-
0.0001	0	4	27	94

Plotting these figures as in Fig. 2 give linear results. Further readings at intermediate dilutions serve to check that the curves are indeed linear.

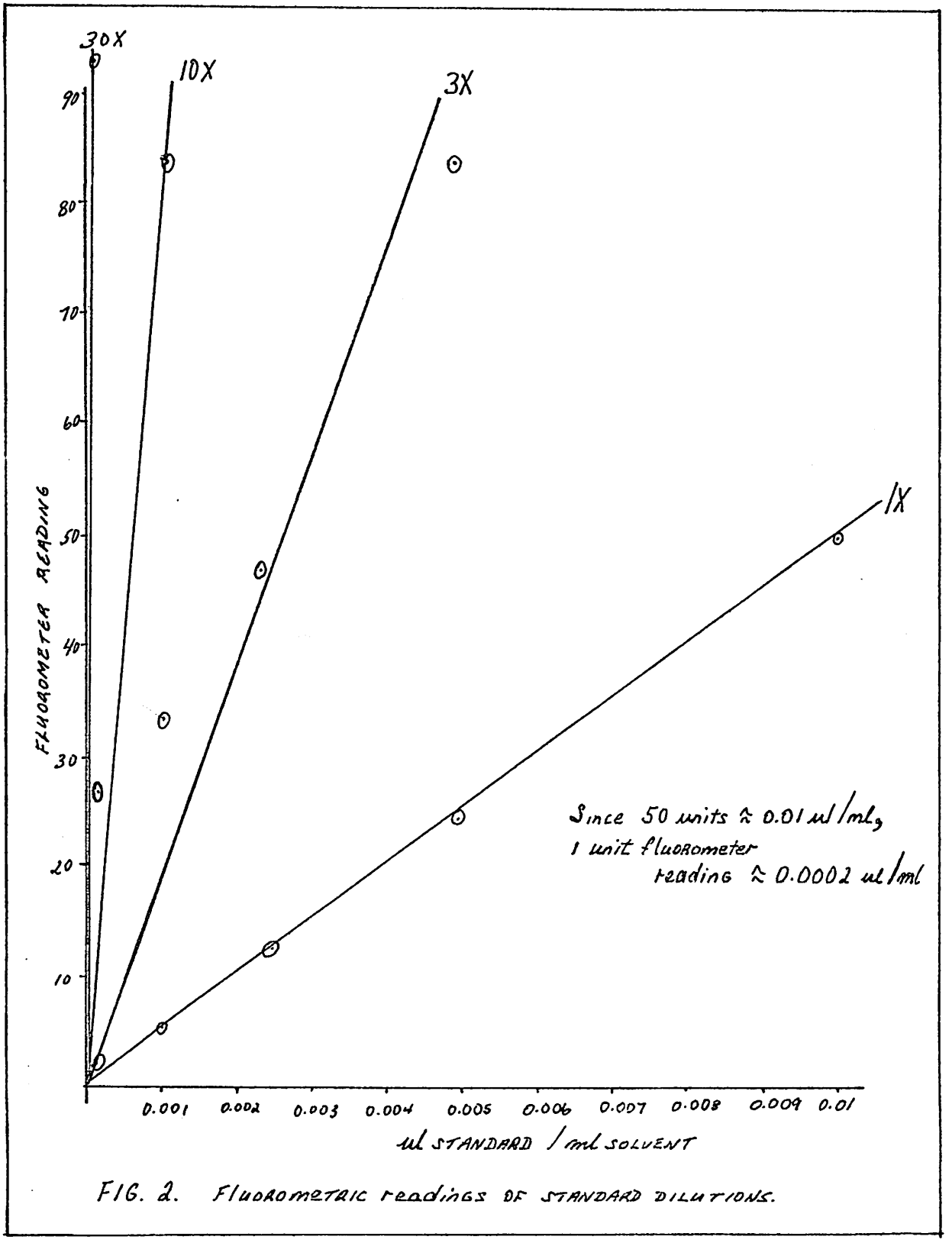

FIG. 2. FLUOROMETRIC readings OF STANDARD DILUTIONS.

Since the 1X line in this case is the most accurate, use this fluorometer power in reading the experimental samples. Determine the unit fluorometer reading from the line. You will need this figure in deposit calculations.

2. Reading the sample slides:

You have a pair of glass slides with an unknown amount of spray on it. The purpose of this experiment is to determine this amount.

Take one of the "indicator"* slides from the spray plot and wash the deposit off in 10 ml of solvent in a petri dish with the aid of a pipe cleaner "hockey stick". Take a reading at 1X on the fluorometer. If the solution is too strong - indicated by a reading greater than 100, make 10 fold dilutions (1:9ml) until a reading is obtained (See Fig. 3).

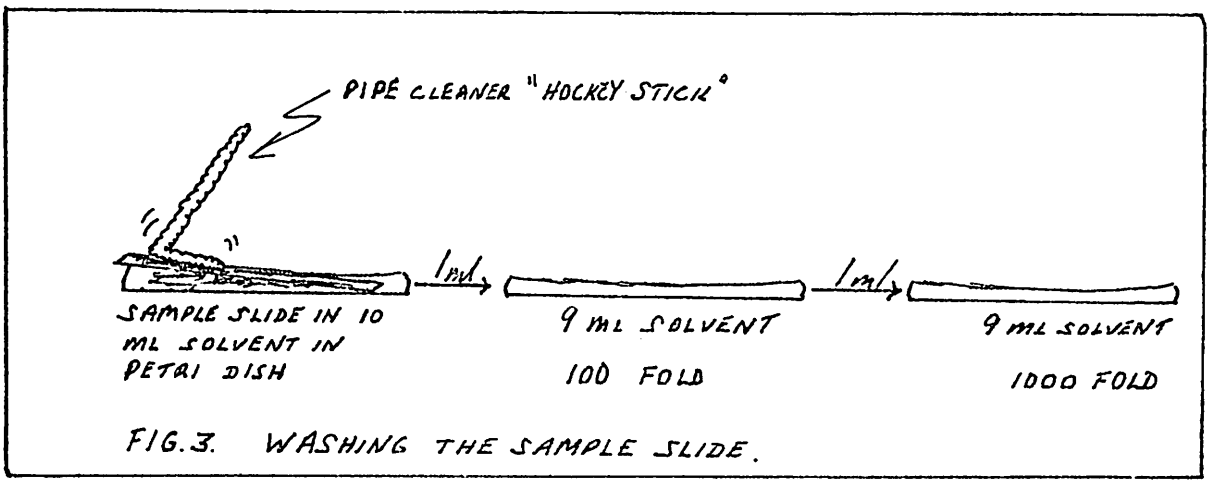

FIG. 3. WASHING THE SAMPLE SLIDE.

* The "indicator" slide is a slide from an open area of the spray plot which ensures a good deposit.

A 1000 fold dilution may be necessary to get a reading in the case of a heavy deposit. Whatever is necessary however with an indicator slide, is also necessary with the rest of the sample slides. In the case of 1000 fold dilutions, the use of two Oxford pipettors, one set at 10 ml with the other at 9 ml, will expedite matters. Take readings for 1 plot of 50 samples checking the "blank" tube after every 10 readings.

3. Washing:

Putting the petri dishes immediately into soapy water to soak after removal of fluorometer sample prevents adhesion of dye with rinsing off only necessary with warm tap water later. As for the fluorometer tubes, they should be washed at least three times in acetone and wiped well with a kleenex on the outer surface. Use an aspirator in the cleaning of the tubes.

Calculations:

The spray deposit in litres/hectare is calculated with the following equation:

$$D = \frac{(F)\ (f)\ (d)}{A}$$

where

D = Spray deposit (ℓ/ha)

F = Fluorometer reading

f = unit fluorometer reading ($\frac{\mu\ell \text{ standard}}{\text{ml sample}} = 10^{-6}\ \ell/\text{ml}$)

d = number of ml used to dilute sample (ml)

A = area of one sample slide = 37.5 cm^2 = 37.5 X 10^{-8} ha

note: 1 $cm^2 = 10^{-4}\ m^2$

1 $m^2 = 10^{-4}$ ha

1 μl = $10^{-6}\ \ell$

Example:

A fluorometer reading of 25, a unit fluorometer reading of 0.0002 $\mu\ell$/ml,

1000 mℓ used to dilute sample, and 1 sample slide used.

$F = 25$

$f = 0.0002\ \mu\ell/\text{mL} = 0.0002 \times 10^{-6}\ \ell/\text{m}\ell$

$d = 1000\ \text{m}\ell$

$A = 37.5 \times 10^{-8}\ \text{ha}$

$$D = \frac{(F)\ (f)\ (d)}{A} = \frac{25 \times 0.0002 \times 10^{+2}\ \ell/\text{m}\ell \times 1000\ \text{m}\ell}{37.5 \times 10^{-8}\ \text{ha}}$$

$$= \frac{25 \times 0.0002 \times 10^{5}}{37.5}\ \ell/\text{ha}$$

$$= \frac{500}{37.5}\ \ell/\text{ha}$$

$$= 13.333\ \ell/\text{ha}$$