# A STABLE LIQUIFLUOR SOLUTION FOR COUNTING <sup>35</sup>S IN PROTEIN-FREE SOLUTION PREPARED FROM PLASMA SAMPLES

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In earlier reports (1,2) we have described a method for counting <sup>35</sup>S in a protein-free filtrate prepared from plasma samples. This method consisted of introducing the assay solution into a specially constructed anthracene cell attached to a photomultiplier tube and scaler system. The efficiency of the anthracene-cell detector system was gradually improved to 6-8% by modifying the construction of the cell (3). Further, a 20-25% efficiency was achieved by replacing anthracene with calcium fluoride (europium-activated) crystals (4).

Liquid scintillation systems for counting weakbeta-emitting nuclides (85S) have definite advantages. Nevertheless, we were confronted with two obstacles: (1) Liquid scintillation counters are expensive and require a substantial outlay of funds for equipment which may be impractical for the occasional user with a limited number of samples to analyze. (2) The protein-free filtrate assay solution containing the beta-emitting nuclide (35S) prepared by adding 50% trichloroacetic acid to plasma samples is an acid (pH = 1.00) aqueous solution with a high crystalloid concentration. All of the standard liquifluor solutions described in the literature proved inadequate. The scintillation medium became discolored and cloudy when the protein-free filtrate was added. Most of the solutions tested were unstable at ambient room temperature.

These obstacles have been overcome, and at present some less expensive, reasonably priced benchtop liquid scintillation counters are available which are stable and perform well at room temperature.

Of the many different combinations of organic solvents with primary and secondary scintillator compounds assayed, solution No. 68 prepared by Dr. Varrone has fulfilled our purpose. This liquifluor scintillation medium is stable at room temperature and miscible in proportions of 0.75-1.5-ml assay solution to 15-ml liquifluor solution. The formula for the Varrone-Albert liquifluor solution No. 68 is 750.0 ml toluene (Nanograde), 250.0 ml Triton X-100, purified for liquid scintillation counting, 5.5 gm PPO and 50.0 mg POPOP. Table 1 compares the response and efficiency of the liquifluor No. 68 with flow cells using anthracene and calcium fluoride (europium-activated) crystals for counting <sup>35</sup>S in acid solution.

#### PROCEDURE

Protein-free filtrate is prepared by adding 1 ml of 50% trichloroacetic acid (TCA) to 3 ml of plasma containing <sup>35</sup>S (sulfate). The protein precipitate is separated by centrifugation. One milliliter of the supernatant fluid is added to 15 ml of the liquifluor solution. The vial is agitated by hand for 15 sec at room temperature and placed in the counter. The vial is allowed to remain in the counting

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EFFICIENCIES OF THREE SCINTILLATION MATERIALS			
Scintillator	Sample volume (ml)	Efficiency (%)	Response (µCi vs. ct. rate)
Anthracene/cell CaFs (europium-	2_3	68	Linear
activated cell) Liquifluor No. 68	34	20–25	Nonlinear
(Varrone-Albert)	1	95-98	Linear

## chamber for 15 sec before actual counting is started.

The advantages for using the liquid-scintillation technique for measuring <sup>35</sup>S in aqueous solutions are obvious. The efficiency of the counting system is higher and the amount of filtrate needed for analysis is much smaller (1 ml). Moreover, the problem of filling of the cell properly with testing solution is eliminated as are the trapping of air bubbles in the flow cell and difficulties encountered in decontaminating the scintillator medium in the cell.

### COMMENTS

The liquifluor solution described here can be used to simultaneously detect two or more weak-beta nuclides. The efficiency for  ${}^{3}H$  is approximately 40-50%; it is 95% for  ${}^{14}C$ .

#### CONCLUSION

An efficient and stable liquifluor solution has been developed for counting weak-beta-emitting nuclides

at room temperature in acid aqueous solutions containing a high concentration of crystalloids.

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