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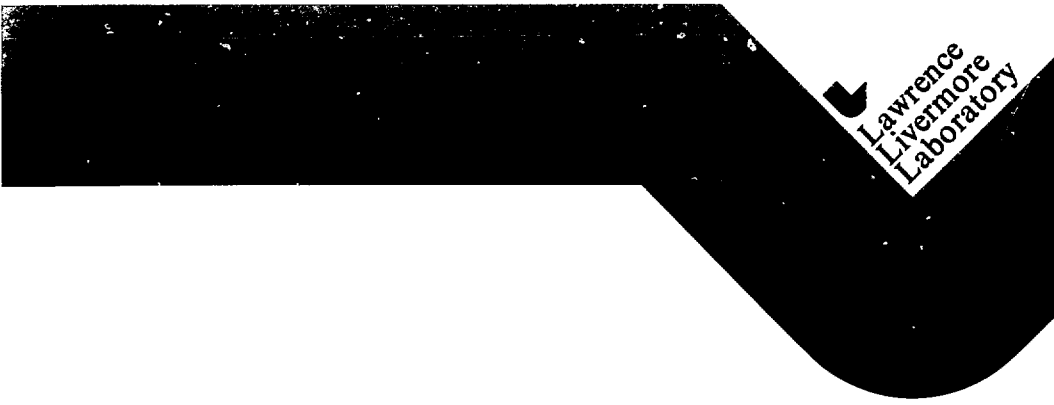
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Evaluation of oxidation techniques for preparing bioassay and environmental samples for liquid scintillation counting

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Lawrence
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EVALUATION OF OXIDATION TECHNIQUES
FOR PREPARING BIOASSAY AND ENVIRONMENTAL SAMPLES
FOR LIQUID SCINTILLATION COUNTING

ABSTRACT

In environmental and biological monitoring for carbon-14 and tritium, the presence of color and chemical quenching agents in the samples can degrade the efficiency of liquid scintillation counting. A series of experiments was performed to evaluate the usefulness, under routine conditions, of first oxidizing the samples to improve the counting by removing the color and quenching agents. The scintillation counter was calibrated for the effects of quenching agents on its counting efficiency. Oxidizing apparatus was tested for its ability to accurately recover the ^{14}C and ^3H in the samples. Scintillation counting efficiencies were compared for a variety of oxidized and unoxidized environmental and bioassay samples. The overall conclusion was that, for routine counting, oxidation of such samples is advantageous when they are highly quenched or in solid form.

INTRODUCTION

In many cases, direct application of liquid scintillation counting techniques to biological and environmental samples is impractical. Aqueous systems are often immiscible with the organic solvents used in scintillation counting, and high concentrations of color and chemical quench can cause large variations in counting efficiency. Combustion techniques have proved useful in reducing samples of diverse origin to carbon dioxide and water, which can be trapped and counted for carbon-14 and tritium, respectively. During the combustion or oxidation of the samples, the quenching agents are effectively removed, and the oxidized sample is automatically dispensed into a counting vial containing highly efficient scintillation solvents unique to the oxidizing apparatus.

To facilitate this technique, various commercial automated combustion units have been developed. At Lawrence Livermore National Laboratory, the

Packard Model B-306 Tri-Carb sample oxidizer is used in monitoring the presence of carbon-14 and tritium in bioassay and environmental samples.

This report describes a series of experiments conducted to evaluate the usefulness of oxidation techniques for preparing bioassay and environmental samples for liquid scintillation counting at LLNL. The series consisted of the following parts:

1. Calibrating the liquid scintillation counter for changes in efficiency due to the presence of quenching agents in the sample.

2. Determining the oxidizer's conformance with the manufacturer's specifications with respect to a) recovery of carbon-14 and tritium from the original sample and b) residual activity in the system after the processing of a sample.

3. Determining for what kind of samples and by what operating procedures prior oxidation of the samples would significantly improve the efficiency of liquid scintillation counting.

SUMMARY AND CONCLUSIONS

The data presented in this report reflects that oxidation of the samples results in an overall improvement in the recovery of tritium and carbon-14 in bioassay and environmental samples containing high concentrations of color or chemical quench. Although statistics show the recovery of tritium in urine to be enhanced 16% by oxidation, the large variability of the data, reflected in the standard deviation of ± 0.25 or 25%, must be considered before applying this technique to a large volume of routine samples.

In general, urine samples prepared directly without oxidation have greater precision, require 50% less time to prepare, and have a 40.5% probability of recovering greater than 90% of the tritium recovered by oxidation. As would be expected, oxidation of tritium samples which have been predistilled before counting, effectively removing quenching agents present, does not show improved recovery. Furthermore, samples which have not been predistilled but nevertheless contain very little quench also show little or no enhancement of tritium detection using the oxidation method of preparation before counting. Some of the data on these samples is incomplete since the channel ratios of the counting data fell below the range of the calibration curves. Additional

curves are being developed over a full range of activities from very low to very high concentrations of tritium and carbon-14 to enable better interpretation of the experimental data.

Oxidation has proved to be a very useful technique when used to prepare solid samples for tritium counting, including samples of tissue, tritium-impregnated plastics, swipes, and tritium pump oils. Oxidation is recommended for these as well as other special samples of this kind, where the physical state of the sample would obviously limit the detection of beta activity.

Performance tests on the oxidizer for carbon-14 recovery show an average recovery of 31.1% for standards in the range of 0.01 $\mu\text{Ci/liter}$, 83.3% for standards containing 12.5 $\mu\text{Ci/liter}$, and 97.6% recovery for standards containing 433 $\mu\text{Ci/liter}$. Recovery of tritium from calibrated standards is also excellent at a value of 98.8% for tritium activity as low as 1.7 $\mu\text{Ci/liter}$.

In the final analysis, certain factors should be considered before applying the oxidation technique to large volumes of routine bioassay and environmental samples. These are: the precision of data, the improvement of counting efficiency, the speed of sample preparation, the relative recovery of isotopes, and the limits of sensitivity of recovery for each method. Based on the data presented in this report, oxidation of bioassay and environmental samples proves to be advantageous only when samples are highly quenched or in solid form.

EXPERIMENTAL PROCEDURE

CALIBRATION OF THE LIQUID SCINTILLATION COUNTER

The channels-ratio technique was used to calibrate the liquid scintillation counter for changes in efficiency due to small amounts of quench still present in the final sample. This technique is based upon the observation that quenching causes attenuation of high-energy pulses and the complete elimination of those pulses barely above the limit of detection (low-energy pulses). This results in a shift in the overall spectrum of pulse heights; see Fig. 1. The extent of this shift, shown by a change in the channels ratio, indicates a

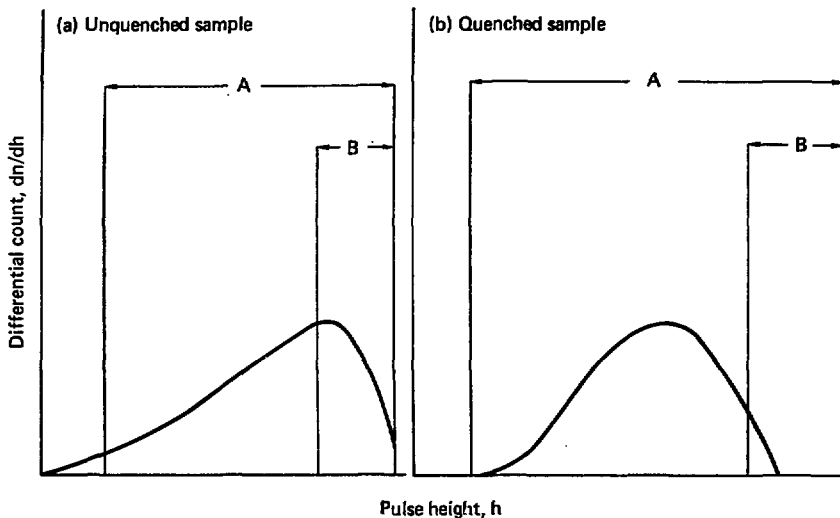


FIG. 1. The presence of quenching agent in a scintillation-counter sample will cause a shift in the spectrum of pulse heights, shown by a change in the ratio of counts in channels A and B.

change in counting efficiency. To do the calibration, a set of standards containing tritium or carbon-14 are counted, each containing the same amount of activity, but with increasing amounts of chemical quench. Portions of the unquenched spectrum of pulses are recorded in two separate channels, denoted A and B in Fig. 1. The ratio of counts in channel A to channel B and the corresponding efficiency for each sample is recorded to produce a quench curve. As the ratios and efficiencies are recorded looking at different portions of the spectrum, a family of curves is generated.

Figure 2 shows a family of quench curves for carbon-14. The best curve is chosen for its maximum slope and minimum curvature. Figures 3, 4, and 5 are the calibration curves chosen to interpret the counting data for tritium and carbon-14. The quench curve in Fig. 3 was used to correlate the tritium oxidation data with the data from duplicate aliquots of tritium not oxidized. Figures 4 and 5 were used to correlate the carbon-14 data in the same manner.

The scintillation counter settings used in this work are given in the appendix.

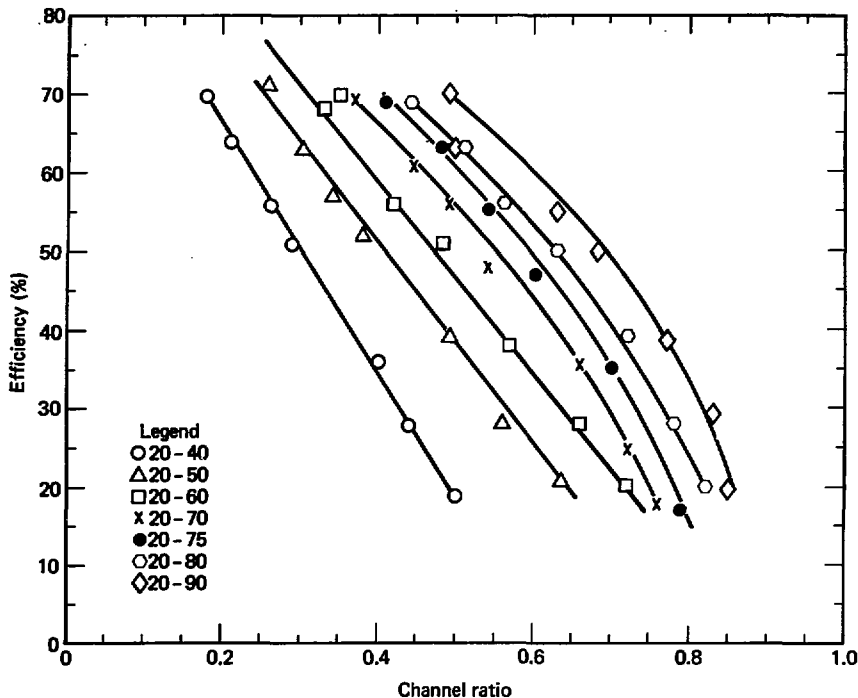


FIG. 2. Quench curve calibration for carbon-14 in oxidizer scintillator. Sample, 0.5 ml. Injected activity, 1.4×10^4 dpm. Gain, 20% (both channels). Optimum window settings, A/B = 20-60/20-750.

EVALUATION OF OXIDIZER PERFORMANCE

In addition to the calibration of the liquid scintillation counter, performance tests were run to assure that the oxidizer operated within manufacturer's specifications. These specifications state that the recovery of tritium or carbon-14 from an oxidized sample is $98 \pm 2\%$. The manufacturer also specifies that the oxidizer "memory," or degree of residual activity remaining in the equipment after oxidation of these nuclides, is 0.05% (maximum) of the activity present in the oxidized sample. Tables 1 and 2 summarize the performance data for tritium and carbon-14. These results indicate good performance for recovery of tritium. In the activity range of 10^5 disintegrations per

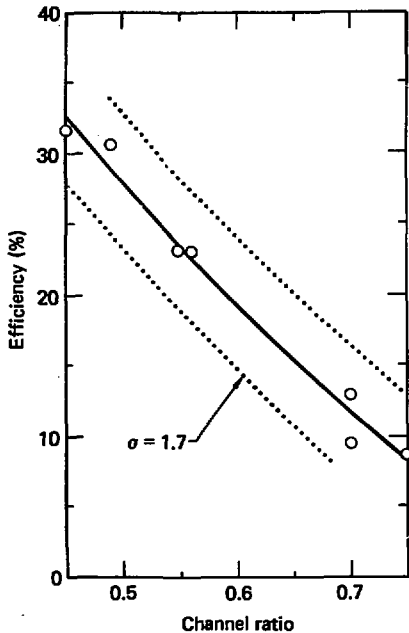


FIG. 3. Counting efficiency curve for tritium in oxidizer scintillator. Sample, 0.5 ml. Injected activity, 2×10^3 dpm.

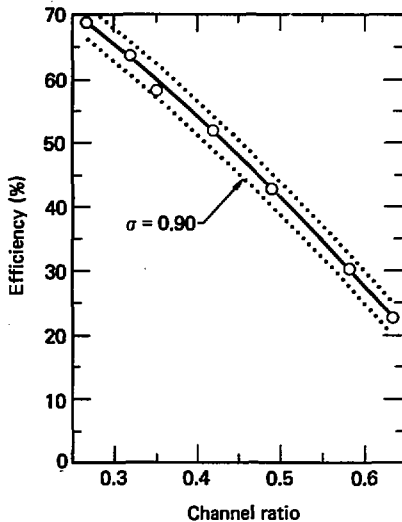


FIG. 4. Counting efficiency curve for carbon-14 in oxidizer scintillator. Sample, 0.5 ml. Injected activity, 1×10^3 dpm.

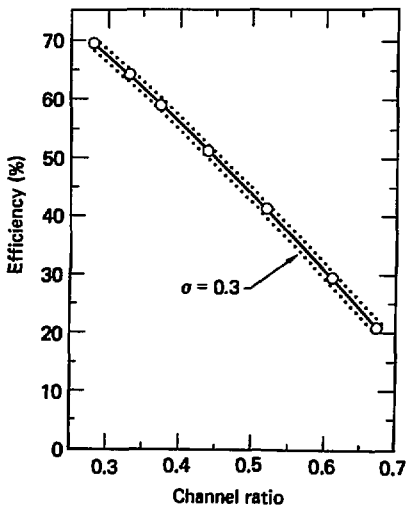


FIG. 5. Counting efficiency curve for carbon-14 in oxidizer scintillator. Sample, 0.5 ml. Injected activity, 1.4×10^4 dpm.

TABLE 1. Oxidizer performance tests: recovery and memory of tritium activity.

Isotope	Sample activity		Recovery (%)	Memory (%)
	(dpm/0.5 ml)	(μ Ci/liter)		
Tritium (Packard standard)	6.484×10^5	584.14	98.2 \pm 1.4	0.11 \pm 0.002
			100.5 \pm 1.4	0.09 \pm 0.002
			8.2 \pm 1.4	0.08 \pm 0.002
			102.1 \pm 1.4	0.00 \pm 0.002
			99.1 \pm 1.4	0.10 \pm 0.002
			102.4 \pm 1.4	0.10 \pm 0.002
			96.9 \pm 1.4	0.10 \pm 0.002
			97.3 \pm 1.4	0.10 \pm 0.002
			94.2 \pm 1.4	0.20 \pm 0.002
			97.8 \pm 1.4	0.24 \pm 0.002
			97.2 \pm 1.4	0.34 \pm 0.002
			96.2 \pm 1.4	0.36 \pm 0.002
Tritium (1:500 standard)	1.909×10^3	1.72	100.00 \pm 3.41	0.00 \pm 0.001
			99.30 \pm 3.39	0.00 \pm 0.001
			97.00 \pm 3.32	0.00 \pm 0.001

minute (dpm) per 0.5-ml sample, average tritium recovery is $98.34 \pm 1.40\%$ with a memory of 0.15%. For tritium activities in the range of 10^3 dpm/0.5-ml sample, the average recovered activity measured $98.77 \pm 3.37\%$ with a memory of 0.00%. The performance data on carbon-14 oxidation indicates lower recovery of carbon-14 activity. The average recovery and memory values for carbon-14 listed in Table 2 are: $97.59 \pm 2.08\%$ recovery and $0.02 \pm 0.002\%$ memory for sample activities in the range of 4×10^5 dpm/0.5 ml; $83.33 \pm 1.92\%$ recovery and $0.36 \pm 0.03\%$ memory for sample activities in the range of 10^4 dpm/0.5 ml; and $24.88 \pm 10.29\%$ recovery and $0.11 \pm 0.05\%$ memory for carbon-14 activities in the range of 11.1 dpm/0.5 ml.

Steps have been taken to improve carbon-14 recovery and reduce memory. Removing the sample vial on the tritium side of the vial carriage after each carbon-14 sample is oxidized will improve recovery and reduce memory of the system. Since the recovered $^{14}\text{CO}_2$ from carbon-14 oxidation must first pass

TABLE 2. Oxidizer performance tests: recovery and memory of carbon-14.

Isotope	Activity level		Recovery (%)	Memory (%)
	(dpm/0.5 ml)	(μ Cl/liter)		
Carbon-14 (Packard standard)	4.805×10^5	432.88	99.80 \pm 2.07	0.03 \pm 0.001
			100.00 \pm 2.15	0.03 \pm 0.001
			100.00 \pm 2.15	0.03 \pm 0.001
			92.92 \pm 2.15	0.01 \pm 0.001
			94.44 \pm 1.82	0.01 \pm 0.001
			98.38 \pm 2.41	0.01 \pm 0.001
Carbon-14 (standard A)	1.3875×10^4	12.50	90.50 \pm 3.51	0.38 \pm 0.03
			86.38 \pm 1.28	0.38 \pm 0.03
			83.87 \pm 1.72	0.38 \pm 0.03
			84.50 \pm 1.88	0.07 \pm 0.02
			77.00 \pm 1.55	0.29 \pm 0.02
			77.70 \pm 1.57	0.66 \pm 0.02
Carbon-14 (standard D)	11.1	0.01	28.50 \pm 11.9	0.00 \pm 0.06
			27.90 \pm 9.20	0.32 \pm 0.03
			28.80 \pm 9.12	0.00 \pm 0.06
			30.00 \pm 12.9	0.00 \pm 0.06
			23.80 \pm 9.37	0.00 \pm 0.06
			16.30 \pm 9.27	0.34 \pm 0.03

through the tritium vial on the way to the carbon exchange and reaction columns, trace levels of carbon-14 activity can become trapped in the waste solvent in this vial. If not removed, the activity contained in the waste solvent in the tritium vial can build up and can then contribute to the memory value for the system. In addition, replacing the tritium vial after each carbon-14 oxidation keeps the volume of waste solvent in the tritium vial to a minimum.

Counting efficiencies for carbon-14, typically 60%, can be improved 20% by reducing the volume of Carbosorb dispensed in the sample vial. Typically the reagent settings used for carbon-14 samples were 15 ml Carbosorb plus 2 ml Permafluor. To determine the optimum reagent settings, one can prepare several samples of maximum size and do a series of oxidations, reducing the amount of Carbosorb until a drop-off in recovery is observed. At this point, there is

not enough Carbosorb to trap the CO_2 produced in the oxidation of the sample. The Carbosorb volume should be set 1-2 ml higher than that used when the drop-off in recovery occurred. The absorption capacity of Carbosorb is approximately 5.8 millimoles of CO_2 per milliliter. If 10 millimoles of CO_2 are produced, one should use approximately 3-4 ml of Carbosorb. If high counting efficiency is a priority over reagent costs, 10 ml Permafluor can be used in addition to the Carbosorb volume.

When summing the values of recovery and memory, we note that the sum does not always equal 100%; see Tables 1 and 2. In the case of clean sample oxidation (no pyrolysis), the remaining activity in the equipment is flushed out of the system with small volumes of water and solvent into the waste bottle. For samples known to contain activities in the millicurie range, several flushes of the system should be made to reduce the memory or background of the instrument before oxidizing low-level environmental or bioassay samples. In the case of sample pyrolysis, the recovery of sample activity is greatly reduced for both tritium and carbon-14; pyrolysis products coat the system, leaving sample activity behind. This problem does not usually increase the memory because the wash-down normally carries off pyrolysis products. Carbon-14 pyrolysis products especially would not show as increased memory because they are trapped in the tritium vial before entering the carbon exchange and reaction columns. Therefore, observing a reduction in recovered carbon-14 activity does not necessarily imply increased memory.¹

Recommended operating parameters for the oxidizer are given in the Appendix.

EVALUATION OF OXIDATION TECHNIQUES

Environmental Samples

In addition to performance tests, a variety of bioassay and environmental samples were oxidized to evaluate the suitability of the oxidation method for routine analysis of these samples. It was suspected that the detection of tritium and carbon-14 in liquid samples which contained little or no chemical or color quench could not be improved using the oxidation technique. The data shown in Table 3 confirms this hypothesis, the system recovering from oxidized samples, on the average, only $89.52 \pm 12.48\%$ of the tritium activity measured without oxidation.

TABLE 3. Recovery of calibrated tritium standard.

Injected activity		Counting efficiency (%)	Activity recovered by oxidation	
(μCi/liter) (dpm/0.5 ml)			(%)	Error (%)
1.73	1920.30	36.0	85.8	+11.3
+ 1%	+ 19.20	36.0	88.9	+11.7
		31.5	91.0	+13.6
		33.8	93.5	+13.1
		31.2	90.3	+13.7
		36.0	87.6	+11.5

Certain environmental samples, such as tritium exhaust stack samples and retention tank samples, undergo distillation before an aliquot is taken for liquid scintillation counting. Therefore, all color and chemical quenching agents have already been effectively removed. Samples of both types were oxidized, and the data in Table 4 shows the measured activity to be greater for those aliquots directly pipetted into Instagel scintillation cocktail than for those undergoing oxidation. The ratio R/R_0 indicates the relative overall efficiency of the two methods, R representing the measured activity using the direct method and R_0 the measured activity using the oxidation method.

Another environmental sample tested for improved sensitivity using the oxidation method included swipes of tritium-contaminated equipment removed for decontamination and waste disposal. A significant improvement in tritium detection was accomplished by oxidation of swipes. The improvement in some cases was dramatic, where the direct method resulted in detecting only 1% of the activity detected by oxidation. However, this improved sensitivity is in part due to a change in technique in sample transfer as well as to oxidizing the sample. Duplicate swipes were collected according to routine procedure and placed in a collection vial with 0.5 ml of distilled water. Normally, when the swipe sample is prepared for counting, the swipe is removed and discarded and the water remaining is poured from the collection vial into a counting vial containing Instagel. In the present case, however, the water from one of the duplicate swipes collected for oxidation was transferred using a disposable pipette, providing a more efficient transfer of the sample. This swipe and the liquid were then both oxidized to produce the results listed in Table 4. This data shows that oxidation plus the complete transfer of the liquid accompanying the swipe may increase the sensitivity of detection of tritium by 75%.

TABLE 4. Measured activities for environmental samples using direct and oxidation techniques for sample preparation.

Type	Sample No.	Activity										
		Net counts/minute		Channel ratio		Efficiency (%)		dpm per sample		uCi/liter		Activity ratio, R/R ₀
		Oxidized	Direct	Oxidized	Direct	Oxidized	Direct	Oxidized	Direct	Oxidized, R ₀	Direct, R	
Tritium stacks	1 HT-05279	4.2 × 10 ⁴	6.0 × 10 ⁴	0.48	0.39	29.9	40	1.4 × 10 ⁵	1.5 × 10 ⁵	127.73	135.94	1.06 ± 0.09
	2 HT-05279	9.2 × 10 ⁴	1.1 × 10 ⁵	.46	.39	31.8	40	2.9 × 10 ⁵	2.0 × 10 ⁵	259.58	254.96	0.98 ± 0.08
	1 HTO-05279	3.1 × 10 ⁴	4.7 × 10 ⁴	.51	.39	29.5	40	1.1 × 10 ⁵	1.2 × 10 ⁵	95.52	106.06	1.14 ± 0.10
	2 HTO-05279	3.9 × 10 ⁴	5.5 × 10 ⁴	.49	.39	29.0	40	1.3 × 10 ⁵	1.4 × 10 ⁵	120.93	123.28	1.02 ± 0.09
	1 HT-4379	8.7 × 10 ⁴	1.1 × 10 ⁵	.47	.40	30.8	40	2.8 × 10 ⁵	2.7 × 10 ⁵	253.06	246.58	0.97 ± 0.08
	2 HT-4379	1.1 × 10 ⁴	2.4 × 10 ⁴	.63	.39	17.0	40	6.4 × 10 ⁴	6.1 × 10 ⁴	58.03	54.87	0.95 ± 0.08
	1 HTO-4379	4.0 × 10 ⁴	5.3 × 10 ⁴	.47	.40	30.8	40	1.3 × 10 ⁵	1.3 × 10 ⁵	118.06	120.43	1.02 ± 0.08
	2 HTO-4379	3.3 × 10 ⁴	4.6 × 10 ⁴	.49	.39	29.0	40	1.1 × 10 ⁵	1.2 × 10 ⁵	103.71	103.67	1.00 ± 0.08
	1 HT-03779	4.5 × 10 ⁵	5.7 × 10 ⁴	.48	.40	29.9	40	1.5 × 10 ⁶	1.4 × 10 ⁶	1.3 × 10 ³	1.3 × 10 ³	0.95 ± 0.08
	1 HTO-03779	2.4 × 10 ⁴	3.0 × 10 ⁴	.46	.39	31.7	40	7.7 × 10 ⁴	7.6 × 10 ⁴	69.11	68.19	0.99 ± 0.08
Retention tanks (tritium)	T-0898	3.53	0.00	.43	.26	a	40	--	0.00	--	0.00	--
	T-0345	3.55	2.50	.35	.28	a	40	--	6.25	--	0.01	--
	T-0376	3.05	16.50	.46	.35	31.7	40	9.62	41.75	0.01	-0.04	1.45 ± 0.28
	T-0377	0.00	7.80	.40	.26	a	40	--	19.50	--	0.02	--
	T-0397	689.50	970.65	.44	.38	33.7	40	2.0 × 10 ³	2.4 × 10 ³	1.84	2.19	1.19 ± 0.10
	T-0550	13.15	2.15	.43	.22	34.5	40	38.12	5.38	0.03	0.01	0.33 ± 0.04
	T-0485	0.00	3.00	.39	.25	a	40	--	7.50	--	0.01	--
	T-0488	0.00	10.00	.39	.25	a	40	--	25.00	--	0.02	--
	T-0489	0.00	10.00	.31	.30	a	40	--	27.00	--	0.02	--
	T-0409	0.00	9.20	.36	.29	a	40	--	23.00	--	0.02	--
Tritium swipes	S-1	23.95	9.30	0.48	0.33	29.9	40	80.10	23.25	0.07	0.02	0.23 ± 0.04

TABLE 4. (Continued.)

Sample Type	No.	Net counts/minute		Channel ratio		Efficiency (%)		dpm per sample		Activity µCi/liter		Activity ratio, R/R ₀	Increase with oxidation (%)
		Oxidized	Direct	Oxidized	Direct	Oxidized	Direct	Oxidized	Direct	Oxidized, R ₀	Direct, R		
Tritium swipes, contami- nated glove box	GB-1	1.4 × 10 ³	873.85	.52	.44	26.3	34	5.1 × 10 ³	2.6 × 10 ³	4.63	2.32	0.50 ± 0.05	
	GB-2	276.25	76.85	.53	.49	25.5	33	992.94	205.61	0.89	0.19	0.20 ± 0.02	
	GB-3	1.5 × 10 ⁵	1.0 × 10 ⁵	.52	.54	26.3	35	5.5 × 10 ⁵	2.9 × 10 ⁵	499.59	262.64	0.60 ± 0.05	
	GB-4	2.8 × 10 ³	230.40	.48	.50	29.9	34	9.2 × 10 ³	677.65	8.33	0.61	0.01 ± 0.001	
	GB-5	1.8 × 10 ⁴	2.6 × 10 ³	.50	.52	28.2	38	6.4 × 10 ⁴	6.8 × 10 ³	57.33	6.10	0.11 ± 0.010	
	GB-6	763.00	168.25	.48	.45	27.9	39	2.8 × 10 ³	431.41	2.23	0.39	0.17 ± 0.016	
	GB-7	251.00	130.10	.49	.44	29.0	29	787.59	448.62	0.71	0.40	0.58 ± 0.055	
	GB-8	2.4 × 10 ³	340.55	.55	.58	23.5	37	1.0 × 10 ⁴	947.43	9.37	0.85	0.09 ± 0.010	
	GB-9	5.2 × 10 ⁵	1.1 × 10 ⁵	.60	.48	19.5	40	2.7 × 10 ⁶	3.5 × 10 ⁴	2.4 × 10 ³	31.67	0.11 ± 0.014	
³ H pump oil ^c	150S-53	456.45	148.55	.52	.32	26.3	40	1735.55	371.38	39.09	8.36	0.21 ± 0.02	
	150S-55	4.7 × 10 ⁵	6.3 × 10 ⁵	.53	.35	25.5	40	1.8 × 10 ⁶	1.6 × 10 ⁶	4.1 × 10 ⁴	3.5 × 10 ⁴	0.86 ± 0.08	17
	150S-56	4.2 × 10 ⁴	5.0 × 10 ⁴	.89	.36	b	40	--	1.3 × 10 ⁵	--	2.8 × 10 ³	--	
	150S-57	7.6 × 10 ⁴	1.2 × 10 ⁵	.46	.36	31.7	40	2.4 × 10 ⁵	3.0 × 10 ⁵	5.4 × 10 ³	6.7 × 10 ³	1.25 ± 0.12	
	150S-54	3.9 × 10 ⁴	4.4 × 10 ⁴	.58	.34	21.2	40	1.0 × 10 ⁵	1.1 × 10 ⁵	4.2 × 10 ³	2.5 × 10 ³	0.59 ± 0.07	68
	150S-54	3.4 × 10 ⁴	5.2 × 10 ⁴	.45	.34	32.8	40	1.0 × 10 ⁵	1.3 × 10 ⁵	2.4 × 10 ³	2.9 × 10 ³	1.23 ± 0.12	
	150S-54	4.0 × 10 ⁴	4.9 × 10 ⁴	.46	.34	31.7	40	1.3 × 10 ⁵	1.2 × 10 ⁵	2.9 × 10 ³	2.8 × 10 ³	1.24 ± 0.12	
	150S-54	2.6 × 10 ⁴	6.0 × 10 ⁴	.57	.35	26.8	40	9.8 × 10 ⁵	1.5 × 10 ⁵	2.2 × 10 ³	3.4 × 10 ³	1.52 ± 0.15	
	150S-54	3.7 × 10 ⁴	4.5 × 10 ⁴	.49	0.34	29.0	40	1.3 × 10 ⁵	1.1 × 10 ⁵	2.9 × 10 ³	2.6 × 10 ³	0.88 ± 0.08 ^d	12
	150S-54	4.0 × 10 ⁴	--	.46	--	31.7	--	1.3 × 10 ⁵	--	2.8 × 10 ³	--	1.00 ± 0.10 ^d	
	150S-54	4.3 × 10 ⁴	--	.46	--	31.7	--	1.4 × 10 ⁵	--	3.1 × 10 ³	--	0.92 ± 0.88 ^d	11
	150S-54	4.6 × 10 ⁴	--	.46	--	31.7	--	1.4 × 10 ⁵	--	3.2 × 10 ³	--	0.89 ± 0.08 ^d	14
	150S-54	3.3 × 10 ⁴	--	.46	--	31.7	--	1.0 × 10 ⁵	--	2.4 × 10 ³	--	1.18 ± 0.11 ^d	
150S-54	2.4 × 10 ⁴	--	0.58	--	21.2	--	1.1 × 10 ⁵	--	2.5 × 10 ³	--	1.14 ± 0.11 ^d		

^aChannel ratio too low to determine efficiency from quench curve.

^bChannel ratio too high to determine efficiency from quench curve.

^cNo cellulose used in oxidation of these samples.

^dAverage of R used to determine ratios.

Tritium samples of oil from diffusion pumps are also submitted to the counting lab for liquid scintillation counting. These samples are highly quenched and therefore pose a problem to the sensitivity of detection of tritium. The volume of oil used for direct analysis was limited to 20 μ l because of the high quenching effect and was kept to 20 μ l for oxidation. Oils are very slow-burning during oxidation. This is because the oxygen available to the system is not enough to support the burn.¹ The addition of approximately 100 mg of cellulose powder in addition to 250-500 μ l of Combustaid should speed up the burn and help prevent partial combustion. Combustaid, a large-molecular-weight alcohol, helps to wet the cellulose and create a better distribution of the sample for burning. It was added to all oxidized samples. Table 4 shows that for oil samples undergoing complete oxidation without cellulose, an 11% to 68% increase in tritium detection was observed. However, 7 of the 13 samples oxidized resulted in incomplete combustion, yielding R/R₀ ratios greater than 1.0; thus the sensitivity of the oxidation method is reduced below that of direct counting. The addition of cellulose powder to the sample improved the combustion of the sample, and soot was not present in the final sample counted. Studies are in progress to determine the effect of cellulose on counting efficiency.

Bioassay Samples

Oxidation was also applied to bioassay samples including spinal chord, liver, muscle, and urine. Tissue samples were obtained from the California Primate Research Center at the University of California, Davis, where research on the uptake of tritium by primates (bonnet and rhesus monkeys) is being studied. Liver, muscle, and spinal chord samples were obtained from the offspring of primates fed tritiated water. Combustion of these samples, using the Packard oxidizer, yielded activities of tritium that were within 20% of the theoretical values calculated. When counted, 30% of the samples oxidized were within 10% of the level of activity expected, based on the concentration of tritium in the feed water.² In one case, incomplete combustion due to improper burn time resulted in the detection of only 50% of the activity expected. In view of the speed and efficiency of the method, oxidation is recommended for samples of this type.

Urine samples analyzed for tritium were obtained from employees routinely monitored for tritium exposure at LLNL. Duplicate samples collected for bioassay were prepared for counting by the oxidation method and the direct method. Urine samples analyzed for carbon-14 were collected from unexposed personnel and later spiked with a known activity of carbon-14 standard. The carbon-14 standard was prepared by diluting carbon-14 labelled methanol with water so as to resemble the biological system.

Figure 6 shows the statistical distribution of the activity ratios R/R_0 for 100 urine samples by both methods studied. The ratio R/R_0 was calculated and the probability of the accuracy of specific ratio values was determined to evaluate the use of oxidation for routine preparation of bioassay urine samples. For each urine sample analyzed, one 0.5-ml aliquot was oxidized with 0.5 ml Combustaid and one 0.5-ml aliquot was pipetted directly into Instagel. The samples were counted and the activity of each was derived using the appropriate quench curve. All associated errors were propagated to the final value of the activity ratio. The average percent error in the value of the ratio

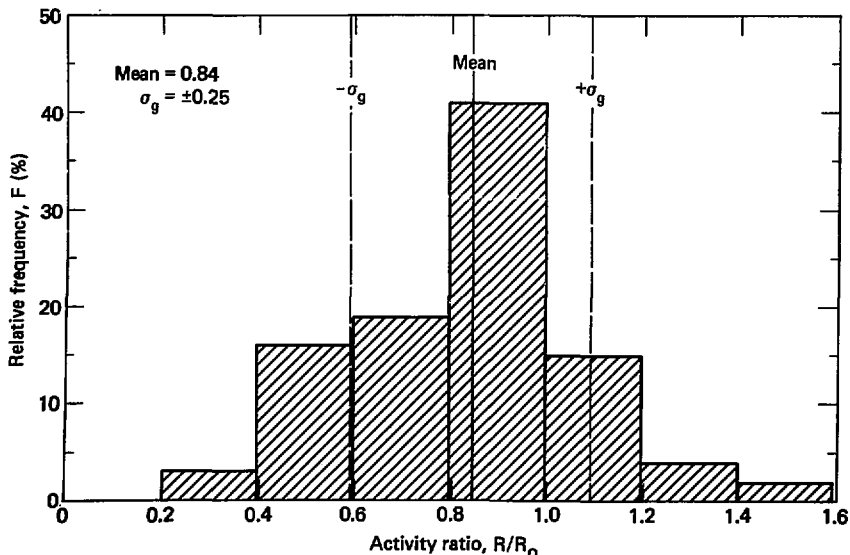


FIG. 6. Frequency of activity ratios for the direct and oxidation methods. Activity ratio, R/R_0 , is the ratio of tritium activity measured directly (R) to tritium activity measured after oxidation (R_0).

itself is 24%. Activity ratios were grouped and a mean value of R/R_o was calculated to be 0.84 with a standard deviation of 0.25. This means that the direct method of sample preparation is 84% as sensitive as the oxidation method, with a fairly large standard deviation of 25%. The relative frequency of the activity ratios for the 100 data points collected is shown in Table 5 and Fig. 6. This data shows that 79% of the samples analyzed had an activity ratio less than 1.0, indicating some enhancement of the measurement of tritium by using oxidation for sample preparation. This data was used to calculate the probability of achieving activity ratios of 0.50, 0.80, 0.90, and 0.95, respectively. From the probability statistics shown in Table 6 we know that

TABLE 5. Relative frequency of activity ratios for tritium in urine (100 data points).

Range of activity ratios, ^a		Rate	Relative frequency (%)
R/R_o			
0	- 0.19	0/100	0
0.20	- 0.39	3/100	3
0.40	- 0.59	16/100	16
0.60	- 0.79	19/100	19
0.80	- 0.99	41/100	41
1.00	- 1.19	15/100	15
1.20	- 1.39	4/100	4
1.40	- 1.59	2/100	2

^aActivity ratio, R/R_o , is the ratio of tritium activity measured directly (R) to tritium activity measured after oxidation (R_o).

TABLE 6. Probability of tritium recovery ratios.

Activity ratio, R/R_o	Probability (%)
0.50	91.3
0.80	56.8
0.90	40.5
0.95	33.0

there is a 40.5% probability that the direct method will detect 90% of the tritium activity present in urine and a 33% probability that the direct method will detect 95% of the tritium activity in urine.

Five sets of urine standards containing various levels of carbon-14 were analyzed to determine the limit of sensitivity of both the direct and the oxidation methods. Carbon-14 activities of 0.22 to 2775.0 dpm/0.5 ml urine were studied. Table 7 shows good recovery of carbon-14 in urine for both methods of sample preparation at activities of 1387.5 dpm/0.5 ml urine (1.25 μ Ci/liter) to 2775.0 dpm/0.5 ml urine (2.50 μ Ci/liter), with calculated counting efficiencies of 40-80% for oxidized samples and 70-80% for samples prepared directly. The channel ratios for some oxidized samples fell below those on the available quench curve; therefore, new quench curves will be generated covering a wider range of carbon-14 activities to allow better interpretation of this data. Analysis of urine samples containing 11.1 dpm/0.5 ml urine was improved with oxidation. This level of carbon-14 activity in many cases fell below the limit of detection for samples prepared directly. Dropping the carbon-14 activity down to 0.22 dpm/0.5 ml urine produced very inconsistent data for both oxidized and directly prepared samples, and that activity level is considered here to be below the limit of sensitivity for either method. The discrepancies between the counting efficiencies calculated and those derived from the quench curves is a matter open to question, and studies are continuing to resolve the differences.

TABLE 7. Recovery data of carbon-14 in urine by oxidation.

Injected activity		Sample ID	Net counts/min		Channel ratio		Calculated counting efficiency (%)		Efficiency from
(dpm/0.5 ml)	(μ Ci/liter)		Oxidized	Direct ^a	Oxidized	Direct ^a	Oxidized	Direct ^a	quench curve (%) ^b Direct ^{a,c}
0.22	0.0002	US3-1	0.00	0.00	0.15	0.13	0.0	0.0	80.5
		US3-2	0.00	0.00	.17	0.14	0.0	0.0	80.1
		US3-3	0.00	0.00	.15	0.13	0.0	0.0	80.5
		US3-4	0.06	0.48	.16	0.13	27.0	218.0	80.5
		US3-5	0.34	0.46	.14	0.13	155.0	209.0	80.5
		US3-6	0.00	0.40	.15	0.13	0.0	182.0	80.5
		US3-7	0.00	0.16	.13	0.12	0.0	73.0	81.0
		US3-8	0.00	0.00	.14	0.12	0.0	0.0	81.0
		US3-9	0.00	0.58	.14	0.13	0.0	264.0	80.5
		US3-10	0.00	0.44	.15	0.13	0.0	200.0	80.5
11.1	0.01	US4-1	5.05	0.00	.15	0.16	45.5	0.0	78.8
		US4-2	4.00	0.52	.17	0.12	36.0	4.7	81.0
		US4-3	2.45	0.00	.1	0.10	22.1	0.0	81.3
		US4-4	2.05	0.07	.14	0.14	18.5	0.6	80.1
		US4-5	2.40	0.00	.14	0.12	21.6	0.0	81.0
		US4-6	7.05	1.87	.17	0.12	63.5	16.8	81.0
		US4-7	2.85	0.00	.12	0.09	25.7	0.0	80.9
		US4-8	0.45	0.00	.13	0.13	4.1	0.0	80.5
		US4-9	5.50	0.00	.14	0.13	49.5	0.0	80.5
		US4-10	1.70	0.72	.14	0.12	15.3	6.5	81.0
		US4-11	9.30	5.65	.16	0.08	83.8	50.9	80.5
		US4-12	5.70	4.70	.19	0.12	51.4	42.3	81.0
		US4-13	8.60	5.45	.13	0.10	77.0	49.1	81.3
		US4-14	7.25	6.95	.17	0.07	65.3	62.6	79.5
		US4-15	3.25	5.20	.17	0.12	29.3	46.8	81.0
		US4-16	5.10	5.40	.16	0.11	45.9	48.6	81.3
		US4-17	11.05	7.35	.15	0.12	99.5	66.2	81.0
		US4-18	9.10	5.05	.17	0.12	82.0	45.5	81.0
		US4-19	5.40	7.45	.16	0.09	48.6	67.1	80.9
		US4-20	8.30	7.25	.16	0.11	74.8	65.3	80.0
US4-21	5.50	--	.18	--	49.5	--	--		
US4-22	7.30	--	.16	--	65.8	--	--		
US4-23	4.10	--	.13	--	36.9	--	--		
US4-24	6.05	--	.18	--	54.5	--	--		
US4-25	4.60	--	.19	--	41.4	--	--		
US4-26	7.35	--	.17	--	66.2	--	--		
US4-27	8.60	--	.19	--	77.5	--	--		
US4-28	4.25	--	.18	--	38.3	--	--		
US4-29	7.10	--	.17	--	64.0	--	--		
US4-30	6.30	--	0.16	--	56.8	--	--		

TABLE 7. (Continued.)

Injected activity		Sample ID	Net counts/min		Channel ratio		Calculated counting efficiency (%)		Efficiency from
(dpm/0.5 ml)	(μ Ci/liter)		Oxidized	Direct ^a	Oxidized	Direct ^a	Oxidized	Direct ^a	quench curve (%) ^b Direct ^{a,c}
1387.5	1.25	US1-1	876.60	1070.57	0.23	0.07	63.2	77.2	79.6
		US1-2	840.05	1087.12	.24	.07	60.5	78.4	
		US1-3	938.75	1072.67	.24	.07	67.7	77.3	
		US1-4	877.80	1076.07	.23	.07	63.3	77.6	
		US1-5	817.10	1098.52	.23	.07	58.9	79.2	
		US1-6	963.30	1076.02	.24	.07	69.4	77.6	
		US1-7	808.95	1072.52	.24	.07	58.3	77.3	
		US1-8	856.35	1078.32	.25	.07	61.7	77.7	
		US1-9	881.25	1077.02	.25	.07	63.5	77.6	
		US1-10	540.80	1079.57	.27	.09	39.0	77.8	
		US1-11	960.40	1050.3	.23	.07	69.2	75.7	
		US1-12	672.80	1054.6	.22	.07	48.5	76.0	
		US1-13	856.60	1066.5	.23	.07	61.7	76.9	
		US1-14	660.60	1036.2	.24	.07	47.6	74.7	
		US1-15	844.60	1068.8	.21	.07	60.9	77.0	
		US1-16	890.00	1104.4	.22	.07	64.1	79.6	
		US1-17	897.60	1066.4	.22	.07	64.7	76.9	
		US1-18	899.10	1092.6	.22	.07	64.8	78.7	
		US1-19	1167.70	1071.0	.21	.07	84.2	77.2	
		US1-20	1650.60	1087.5	.23	.07	61.7	78.4	
1387.5	1.25	US7-1	852.45	—	.21	—	61.4	73.5	79.6
		US7-2	992.70	1019.55	.21	.07	71.5	75.2	
		US7-3	854.15	1043.95	.21	—	61.6	75.3	
		US7-4	817.10	1044.20	.21	—	58.9	75.1	
		US7-5	566.80	1041.70	.22	—	40.9	75.8	
		US7-6	703.65	1051.45	.20	—	50.7	75.3	
		US7-7	589.15	1058.05	.21	—	42.5	76.7	
		US7-8	828.70	1064.10	.21	—	59.7	76.5	
		US7-9	1012.05	1047.95	.21	—	72.9	76.8	
		US7-10	1025.95	1065.45	0.21	0.07	73.9	77.0	

TABLE 7. (Continued.)

Injected activity		Sample ID	Net counts/min		Channel ratio		Calculated counting efficiency (%)		Efficiency from quench curve (%) ^b
(dpm/0.5 ml)	(μ Cl/liter)		Oxidized	Direct ^a	Oxidized	Direct ^a	Oxidized	Direct ^a	Direct ^{a,c}
2775.0	2.50	US2-1	1939.10	2216.02	0.25	0.07	69.9	79.9	79.6
		US2-2	1328.90	2270.27	.25	.07	47.9	81.8	
		US2-3	1627.15	2274.82	.24	.07	58.6	82.0	
		US2-4	1395.00	2273.35	.25	.07	50.3	81.9	
		US2-5	1914.80	2283.90	.24	.07	69.0	82.3	
		US2-6	1117.45	2263.87	.26	.07	40.3	81.6	
		US2-7	1217.20	2287.47	.26	.07	43.9	82.4	
		US2-8	1728.25	2307.72	.24	.07	62.3	83.2	
		US2-9	1727.95	2260.92	.26	.07	62.3	81.5	
		US2-10	1505.60	2228.97	.26	.07	54.3	80.3	
		US2-11	1041.10	2104.60	.21	.07	37.5	75.8	
		US2-12	821.50	2115.90	.22	.07	29.6	76.2	
		US2-13	1547.00	2097.30	.22	.07	55.7	75.6	
		US2-14	1853.10	2131.00	.20	.07	66.8	76.8	
		US2-15	2077.50	2179.90	.21	.07	74.9	78.6	
		US2-16	2102.90	2133.90	.20	.07	75.8	76.9	
		US2-17	2156.60	2153.50	.18	.07	77.7	77.6	
		US2-18	1780.40	2178.90	.20	.07	64.2	78.5	
		US2-19	1884.00	2168.60	.20	.07	67.9	78.1	
		US2-20	1874.20	2174.40	0.21	0.07	67.5	78.4	

^aA dash (—) means there was no sample to evaluate.

^bEvery entry in the efficiency column has an associated error of $\pm 4.1\%$.

^cChannel ratio for oxidized samples was too low to determine efficiency from quench curve. Efficiency estimated to be 70%.

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2. M. S. Singh, Lawrence Livermore National Laboratory, Livermore, CA (1978).

APPENDIX:
OPERATING PARAMETERS FOR OXIDIZER AND SCINTILLATION COUNTER

Oxidizer parameters for combustion of samples.

Type of sample	Volume of sample oxidized	Scintillator settings	Burn time (minutes)
Tritium			
Urine and other liquids	0.5 ml liquid + 0.5 ml Combustaid	15 ml Monophase	1.0
Tissue	0.5 g sample + 0.5 ml Combustaid	15 ml Monophase	2.5
Rubber gaskets	0.05 g sample + 0.5 ml Combustaid	15 ml Monophase	1.5
Plastic fibers	Microgram quantities (~700) + 0.5 ml Combustaid	15 ml Monophase	2.0
Carbon-14			
Urine and other liquids	0.5 ml liquid + 0.5 ml Combustaid	10 ml Carbosorb 12 ml Permafluor	1.0

Suggestions:

- Follow specifications on oxidizer maintenance closely.
- Check gaskets and gas feed systems periodically for leaks.
- Run performance checks periodically to monitor the performance of the oxidizer.
- Rinse the vial carriage with methanol after each burn.
- Always flush out the oxidizer system after oxidizing samples of high activity to reduce background. To flush: push the program button and run through the cycle with no sample.

Suggested settings for the Tri-Carb liquid
scintillation counter.

Tritium	
Window A-B	20-120
Window C-D	20-560
Gain	
Channel A-B	50%
Channel C-D	52%
Carbon-14	
Window A-B	20-60
Window C-D	20-750
Gain (both channels)	50%

The efficiency-vs-gain curve for carbon-14 is shown in Fig. A1, and the calibration quench curve for carbon-14 in oxidizer scintillator at the optimum gain of 50% is shown in Fig. A2.

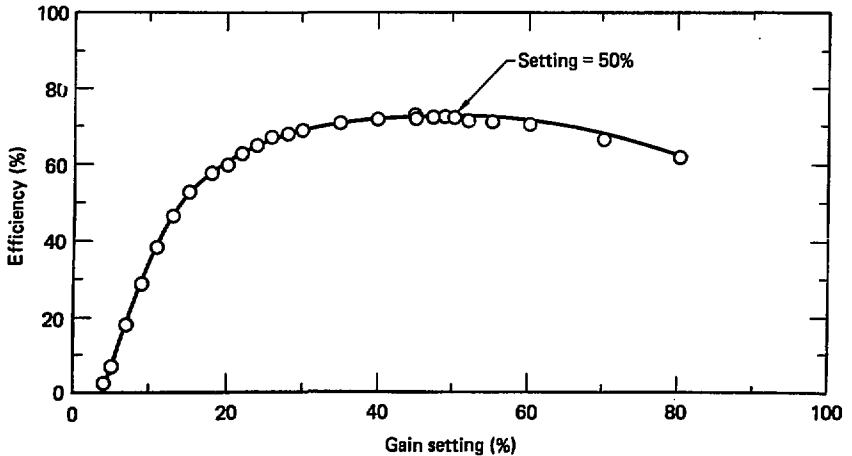


FIG. A1. Efficiency vs gain for carbon-14. Calibration made with MeOH-¹⁴C. Sample, 0.5 ml. Injected activity, 1.4×10^4 dpm. Window settings, 20-60/20-750.

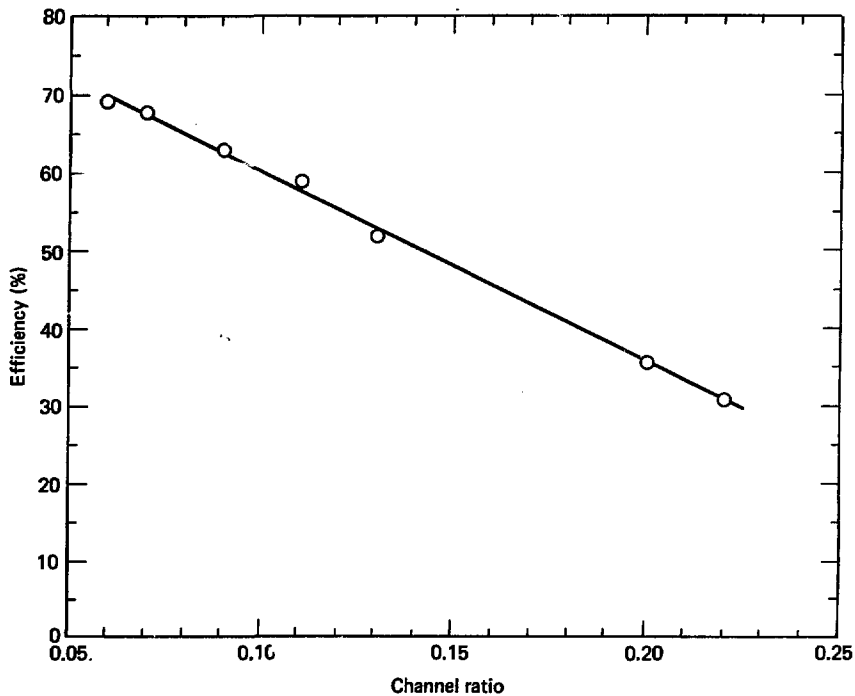


FIG. A2. Optimum carbon-14 quench curve in oxidizer scintillator. Sample, 0.5 ml. Injected activity, 1×10^3 dpm. Gain, 50%. Window settings, 20-60/20-750.