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Liquid scintillation based quantitative measurement of dual radioisotopes (^{3}H and ^{45}Ca) in biological samples for bone remodeling studies

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Abstract

Acute and prolonged bone complications associated with radiation and chemotherapy in cancer survivors underscore the importance of establishing a laboratory-based complementary dual-isotope tool to evaluate short- as well as long-term bone remodeling in an *in vivo* model. To address this need, a liquid scintillation dual-label method was investigated using different scintillation cocktails for quantitative measurement of ^{3}H-tetracycline (^{3}H-TC) and ^{45}Ca as markers of bone turnover in mice. Individual samples were prepared over a wide range of known ^{45}Ca/^{3}H activity ratios. Results showed that ^{45}Ca/^{3}H activity ratios determined experimentally by the dual-label method were comparable to the known activity ratios (percentage difference ~2%), but large variations were found in samples with ^{45}Ca/^{3}H activity ratios in range of 2–10 (percentage difference ~ 20–30%). Urine and fecal samples from mice administered with both ^{3}H-TC and ^{45}Ca were analyzed with the dual-label method. Positive correlations between ^{3}H and ^{45}Ca in urine (R = 0.93) and feces (R = 0.83) indicate that ^{3}H-TC and ^{45}Ca can be interchangeably used to monitor longitudinal *in vivo* skeletal remodeling.

Keywords

^{3}H; ^{45}Ca; liquid scintillator; scintillation cocktail; dual-label; bone remodeling

1. Introduction

1.1 Radioisotopes for Bone Remodeling Studies

Liquid scintillation has been used for the non-invasive study of bone remodeling with multiple radioisotopes (^{3}H, ^{47}Ca, ^{45}Ca) (Bates et al., 1996; Fricke, 1975; Hanes et al., 1999; Mahin and Lofberg, 1966; Shahnazari et al.; Zhao et al., 2010). The method of dual radioisotope labeling in animals is widely used to verify (a) the same physiological process with different methods, or (b) two different physiological processes with respective radioisotopes in a single experiment. Literature is available for determination of dual

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radioisotopes viz. ^{32}P and ^{45}Ca (Bem and Reimschüssel, 1979), ^{3}H and ^{14}C (Los Arcos and Barquero, 1996; Reddy et al., 2009), ^{3}H and ^{125}I (Thibodeau et al., 1981) and ^{90}Sr and ^{90}Y (Lee et al., 2002). ^{3}H-Tetracycline (^{3}H-TC) (DeMoss and Wright, 1997) is individually used to quantify the resorptive phase of bone calcium metabolism while ^{45}Ca is a bone seeking tracer generally used to investigate endocrine metabolism (Shahnazari et al.).

With a single intravenous injection, ^{3}H-TC can best be used for short-term (days) kinetic studies due to its short biological half-life (~2.8 hours) (DeMoss and Wright, 1997), whereas ^{45}Ca can be used for relatively longer-term (several months) kinetic studies due to its longer biological half-life (~187 days) (ICRP). Classically, ^{3}H-TC is used to monitor bone resorption, because it is not readily incorporated into newly formed bone (Muhlbauer and Fleisch, 1990), whereas urinary excretion of calcium tracers is thought to reflect net bone turnover, because calcium tracer is reabsorbed through the kidney. Dual isotope studies have been conducted to compare calcium, the endogenous constituent of bone, with respect to ^{3}H-TC, a known marker for bone resorption. For example, in animal models using ^{3}H-TC and ^{45}Ca (Zhao et al., 2010) and ^{3}H-TC and ^{41}Ca (Cheong et al., 2011), the authors show that both isotopes measured in urine can be used interchangeably to screen dietary and other interventions for beneficial effects on bone (Zhao et al., 2010). Due to the low kidney re-absorption upon turnover in animals, 3H-TC may also be a useful complementary assay technique to 45Ca for studying cancer treatment modalities (example, radiation) that may have a role in kidney damage. Finally, due to the much larger endogenous excretion of 45Ca in feces compared to urine in both mice and rats (Wang and Bhattacharyya, 1993; Zhao et al., 2010), feces can be used for a much longer period than urine to track bone turnover via ^{45}Ca excreta measurements. To date, the kinetics of these two bone markers (^{3}H-TC and ^{45}Ca or ^{41}Ca) have not been correlated in feces and urine.

1.2 Dual-Isotope Approach Using Liquid Scintillation Spectrometry

Liquid scintillation is a simple and expedited technique used for measuring radioisotopes. The important benefits are ease of sample preparation and high counting efficiency with low-levels of nuclide. The dual-isotope liquid scintillation technique can be highly beneficial, but the measurement is critically dependent on an accurate assessment of the actual radioactivity of both radioisotopes (Dodson, 2002). Because the β-particle energy is a continuum extending from zero to some maximum energy, the energy spectrum of a strong β-emitter may overlap the spectrum of a weak βemitter. Depending on the activity of both the radioisotopes and the beta energy difference, this overlap may lead to an error in the measurements. Additional difficulties include variable efficiency of one radioisotope compared to the other radioisotope at a particular energy, which may occur due to quench. Quench reduces the number of photons observed for a given amount of energy. With an increase in quench, the spectra generally shift towards the lower energy. Proper quench determination is crucial for accurate measurement of dual-labeled samples, as it helps in automatic detection of the shift.

Equally important is the use of an appropriate scintillation cocktail. The scintillation cocktails can be categorized by their phasing properties and ability to dissolve aqueous and non-aqueous samples. In the present study, we compared two categories of scintillation cocktails: cocktails that develop a gel phase with the sample (Universol™, Insta-Gel Plus and HI-Fluor) and cocktails that do not develop a gel phase with the sample (Ecolite™, Ecolume™).

Radioisotopes commonly used in biomedical research have low energy and a short range of air or fluid penetration. The liquid scintillation detection efficiency of radioisotopes in biological samples depends on direct contact with the scintillation cocktail and the sample (Medeiros et al., 2003). The liquid scintillation efficiency is in turn highly constrained by

interferences such as inhomogeneity, chemiluminescence, phosphorescence, micro precipitation, adsorption, and chemical (impurities) or color quench, all of which are in a way related to the scintillation cocktail composition (Medeiros et al., 2003) and the sample preparation technique (L'Annunziata et al., 2003).

The three main objectives of the present liquid-scintillation-based study for dual radioisotope estimation in different biological samples were to investigate the **(a)** impact of different scintillation cocktails on dual-label radioisotope counting in different biological samples, **(b)** accuracy of the dual-label method in determining the actual amounts of both radioisotopes at known concentrations and **(c)** association of dual radioisotope excretion in urine and feces after *in vivo* administration of both bone markers to mice. Our aim in optimizing conditions to simultaneously quantitate both isotopes is to apply the dual-isotope approach to evaluate bone remodeling changes that occur in cancer patients after radiation and chemotherapy.

2. Materials and methods

2.1 Reagents and scintillation cocktails

The dual-label samples were prepared from ^{3}H-tetracycline [7-3H(N)] (American Radiolabeled Chemicals Inc., St. Louis, MO) and $^{45}CaCl_2$ (Perkin Elmer, Waltham, Massachusetts) solutions. These are beta emitting nuclides with average and maximum energy being respectively 5.7 keV and 18.6 keV for ^{3}H and 77 keV and 257 keV for ^{45}Ca. Five different types of scintillation cocktails were used, namely Ecolite™, Ecolume™ and UniverSol™ (MP Biomedicals, Irvine CA), and Insta-Gel Plus and HI-Fluor (Perkin Elmer, Boston, MA).

2.2 Apparatus

An alpha/beta liquid scintillation detector (Beckmann Coulter LS 6500) with an energy range of 0–2000 keV was used in the present study. It had a logarithmic amplification, a 32,768 channel multichannel analyzer (MCA) having an effective resolution of 0.0625 keV per channel (2000 keV/ 32,728 channels) and an automated background subtraction option. Different micro-pipettes (Eppendorf, CA) were used for precise measurement of ^{3}H and ^{45}Ca radioisotope solutions. Dilutions were conducted with deionized water (Fischer Scientific, NJ). All the liquid scintillation measurements were carried out in 20ml polyethylene scintillation vials (Perkin Elmer, MA).

2.3 Quench Calibration for ^{3}H and ^{45}Ca

The counting efficiency of ^{3}H and ^{45}Ca was determined by measuring a series of quenched standards for both the radioisotopes having a fixed dpm value. Quenched standards are generally prepared by adding variable amounts of a quenching agent, like nitromethane, to a fixed radioisotope of fixed activity. In the present study, an active stock solution of ^{45}Ca containing 55,000 dpm/0.5 ml was prepared from the standard $^{45}CaCl_2$ of 62.7×10^{6} dpm/µl and deionized water. Then, 0.5ml of this active stock solution was added to 15ml of the Ecolite scintillation cocktail. After an initial precision measurement on 30 samples of ^{45}Ca, a set of 10 samples were selected with less than ±1% deviation from the prepared dpm value of 55,000. A set of samples with a wide quenching range was then obtained by adding increasing volumes of nitromethane (0, 15, 30, 45, 60, 75, 90, 105, 120 and 135 µl) in those ten samples. For ^{3}H radioisotope, the standard Kit (Lot #HGG0608, Perkin Elmer) containing 10 samples of ~50,000 dpm values and varying levels of quench was used.

The quench-indicating parameter used by LSC 6500 is called the Horrocks number or the H#. This parameter is determined by calculation of the difference in channels between

inflection points of the Compton edge of a quenched sample vs. an unquenched sample. The quench curve, or the plot of efficiency of the radioisotope as a function Horrocks number, was used for efficiency determination of both the radioisotopes. In dual-label mode, the quench curves were generated for each individual radioisotope in wide mode and then in two window settings viz. window1 and window2. Window1 mainly ranged from 0–15 keV and consisted of most of the beta spectra of ^{3}H with a small tailing contribution of the ^{45}Ca beta spectra. Window2 mainly ranged from 15–270 keV and consisted of mainly ^{45}Ca beta spectra. LSC 6500 uses H# and a 32,768 channel multi-channel analyzer to provide an automated window adjustment as a function of quench using automatic quench compensation (AQC). This approach reduces the error from spill of the high energy isotope into the low energy isotope. An advantage of the use of H# is that any sample can have only one H#, which reflects the efficiency of counting the two radioisotopes in that sample. Fig. 1 shows the spectral distribution of a typical ^{3}H and ^{45}Ca radioisotope in single- and dual-label mode. The efficiency of ^{3}H and ^{45}Ca in both the single- and dual-label windows vs. H# is shown in Fig. 2.

2.4 Biological Sample Preparation

A total of 18 skeletally mature (15 weeks old) BALB/c mice were injected intravenously with 15μCi of ^{3}H-tetracycline and 15μCi of $^{45}CaCl_2$. This study was approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC). Following the tail vein IV injection, metabolic cages were used to collect 24 hour excreta (feces and urine) from each mouse individually at days 3, 6, 9, 13, and 16. Fecal samples for each mouse on a given collection day were transferred to pyrex beakers. The urine samples were retrieved by rinsing the cage with 15ml of deionized water. Feces digestion was a three day process. The oven-dried (80 °C) feces samples were weighed and powdered, and 0.2 g of that sample was solubilized in 2ml of conc. HNO_3 on the first day, followed by second day addition of 1ml of hydrogen peroxide, and third day addition of 0.5ml 3M HCl and 1ml deionized water; the result was a clear, colorless solution. When assaying for ^{45}Ca and ^{3}H in all experiments, 0.5ml aliquots of feces and 1.0ml aliquots of urine samples were combined with 15ml of scintillation cocktail. The different sample preparation steps are schematically presented in Fig. 3. At least two sets of samples were prepared for each measurement of ^{3}H and ^{45}Ca, and dpm values were corrected for their respective half-life.

3. Experiment

3.1 Effect of different scintillation fluids

To check the activity of ^{3}H and ^{45}Ca in different biological samples, five scintillation cocktails were used, namely Ecolite™, Ecolume™, UniverSol™, Insta-Gel Plus and HI-Fluor. Each of the scintillation vials had 0.5ml of the digested biological sample solution and 15ml of scintillation cocktail. Measurements for different scintillation cocktails were carried out with their respective background sample. For example, while running the samples for Ecolite™, the background was made by adding 0.5ml of deionized water in 15ml of Ecolite™ followed by all the biological samples having only Ecolite™ as a scintillation cocktail. This process was repeated for the other scintillation cocktails. Each sample was counted for a period of 6 minutes.

3.2 Accuracy and sensitivity test for dual-label method

In this phase, the accuracy and sensitivity of the dual-label method was tested to determine the actual activity of ^{3}H and ^{45}Ca using the quench curve. Two groups of samples were prepared having the same or different activity combinations of ^{3}H and ^{45}Ca. For verification of the dual-label method, the samples were run in three different window settings-single, dual and wide window mode. The single-label mode was set for one radioisotope, ^{3}H

or ^{45}Ca i.e. window1 or window2. The dual-label mode had two window settings for both ^{3}H and ^{45}Ca i.e. both window1 and window2 were used. In wide window mode, the full spectrum dpm was taken i.e. the whole scintillator window (0–2000 keV) was used. Steps involved in the verification process were as follows.

3.2.1—Multiple sets of single-isotope samples, containing either ^{3}H or ^{45}Ca, were prepared with activity (dpm) values from 2000–20,000 (e.g. 2000, 5000, 10000, 15000 and 20000 dpm). This range was chosen to make the test samples parallel the activities found in our biological samples. These single-isotope ^{3}H and ^{45}Ca samples were prepared in 10 ml of scintillation cocktail and were measured in both single and dual-label mode. The single mode was used to verify the individual strength, and the dual-label mode was used to determine the contribution (due to overlap) of one radioisotope into the respective window of the other radioisotope i.e. window2 for ^{3}H and window1 for ^{45}Ca. Multiple sets of samples were prepared for each isotope and activity value. These samples were run for ten repeat measurements. All the samples were kept in the same sequence and shaken before putting in the scintillator.

3.2.2—For each activity value, the vials with less than ±2% difference in dpm value were selected from multiple sets of samples. The selected vials were used to measure the dual radioisotope dpm values. The dual-isotope vials were prepared by mixing two single-isotope scintillation vials with different radioisotope solutions. The ^{45}Ca/^{3}H activity ratio in the resulting vials was varied from 0.1 to 10. These samples were run in dual-label and full spectrum modes.

3.2.3—The activity ratios of ^{45}Ca: ^{3}H obtained from the dual-label mode (Step 3.2.2) were compared with the activity ratios obtained by dividing the dpm values of the same single-isotope samples of ^{3}H and ^{45}Ca from the scintillation run conducted in single mode in Step 3.2.1.

3.3 Non-invasive dual radioisotope measurement in mice

As mentioned in section 2.4, 24-hour samples of urine and feces were collected from mice and pre-processed following the steps schematically presented in Fig. 3. The respective background was prepared by adding natural urine and feces samples (without the radioisotope) to the 15ml of Ecolite™. A scatter plot was drawn to measure the association (correlation coefficient) between ^{3}H and ^{45}Ca in the mouse urine and feces samples collected from days 3 to 16 post radiolabelling.

4. Results

In the present work, we measured the efficiency of ^{3}H and ^{45}Ca in single and dual-label mode. This was done by determining the quench curve in two separate window settings for low energy (^{3}H) and high energy (^{45}Ca) radioisotopes. As shown in Fig. 2, for equivalent quenching ranges, the H# and counting efficiency of ^{3}H respectively ranged from 40–315 and 4.5–62%. The maximum efficiency of ^{3}H was 62% in window 1. Likewise, for ^{45}Ca measurements, the H# and counting efficiency respectively ranged from 47–247 and 87–98%. The maximum efficiency of ^{45}Ca was 98% in window 2.

Table 1 shows the comparison of ^{3}H and ^{45}Ca dpm values in urine and feces samples in different scintillation cocktails. In Table 1, the results were compared as percentage difference from the average dpm obtained with Ecolite™. Overall, the ^{45}Ca dpm values were found comparable in all the scintillation cocktails (percentage difference ≤4%) except HI-Fluor (percentage difference ≤16%). The total ^{3}H dpm values of urine samples were also

found to be similar (percentage difference ≤5%). However, for the feces samples, dpm values of ^{3}H were higher in Universol™ (~ 14%) and HI-Fluor (~ 63 %) and lower in UniverSol™ (~ 14%); in the case of Insta-Gel Plus, the feces dpm values were comparable to Ecolite™ (percentage difference ~ 3%).

The single-isotope ^{3}H and ^{45}Ca samples run in dual-label mode showed negligible contribution in their low efficiency window (i.e. the window of the other radioisotope). The feeding of pure ^{45}Ca in the ^{3}H window i.e. window1 was less than 2%, while in the case of pure ^{3}H radioisotope, no contribution was found in the ^{45}Ca window i.e. window 2. Table 2 shows the percentage difference in the activity ratio of isotopes in each dual radioisotope sample from the actual activity ratio, which was calculated from the results of counting the samples individually in single mode before mixing the two radioisotopes. At the same activity value of both the radioisotopes, the percentage difference was found to be ≤ 4%. A slightly larger percentage difference was found at the high dpm value of both ^{3}H and ^{45}Ca (20,000 dpm). For most of the other samples, the percentage difference varied from 2%. However, large percentage differences (~13% to ~28%) were found in the case of samples having higher concentrations of ^{45}Ca in the mixture sample (i.e. higher $^{45}Ca/^{3}H$ ratios). Compared to the case of the higher concentrations of ^{45}Ca, the higher concentrations of ^{3}H (i.e., lower $^{45}Ca/^{3}H$ ratio) gave lesser variations (≤ 7%).

In the mouse study, rapid excretion of ^{45}Ca and ^{3}H was observed early after administration, followed by decreasing excretion in feces and urine. Fig. 4 shows the correlation between ^{3}H and ^{45}Ca dpm values for urine and feces samples collected during day 3 to 16 days. These values were obtained using Ecolite™ scintillation cocktail. The ^{45}Ca:^{3}H ratios ranged from 0.29–0.90, indicating that the dpm values in Fig 4 should be accurate within 7% (Table 2). Finally, results showed a positive linear correlation between ^{3}H and ^{45}Ca, with correlation coefficients (R) of 0.93 and 0.83 for urine and feces, respectively.

5. Discussion

Proper control of certain parameters in liquid scintillation counting, such as the quench curve and the scintillation cocktail, might reduce the error involved in the accurate determination of activity. In the selection of an optimal liquid scintillation cocktail, the important aspects generally taken into consideration are overall cocktail performance and specific laboratory needs (Verrezen et al., 2008). Ecolite™ is generally used for a wide variety of biological samples, Ecolume™ is considered good for high ionic salts while Universol™ is good for insoluble and particulate samples. The other two scintillation cocktails used were Insta-Gel Plus and HI-Fluor. The former has high concentrated salt tolerance, fast chemiluminescence decay and high quench resistance. The latter is considered good for salt samples with high efficiency and low background. Although the dpm values in Insta-Gel Plus and HI-Fluor were higher, we preferred Ecolite™ in the present study because it had additional qualities such as 1) it did not make a gel phase with the sample, 2) it was bio-degradable and 3) it could be used easily for both aqueous and non-aqueous samples. The other scintillation cocktail Ecolume™ would also have been useful for a similar study.

The usual analytic methods for dual radioisotope studies take advantage of large energy differences between the radioisotopes. Although there is a sufficient difference between the β energies emitted by ^{3}H (E_{av} = 5.7 keV, E_{max} = 18.6 keV) and ^{45}Ca (E_{av} = 77 keV, E_{max} = 252 keV), the internal bremsstrahlung effect of the ^{45}Ca spectra (Babu et al., 1976) emitted in the β-decay may lead to sufficient tailing of the peak. This may lead to fictitious peak area measurement in the ^{3}H window and hence lead to inaccurate results, especially for higher activity concentrations of ^{45}Ca compared to ^{3}H. So for better quantification of radionuclides

in the dual-label mode, it is crucial to verify the effect of ^{45}Ca in the ^{3}H regions, especially with increasing quench. In the present measurements, we found that the amount of overlap of the ^{45}Ca β spectra in the ^{3}H region was relatively low except at higher relative concentrations of ^{45}Ca. The present study suggests that, for dual-labeled biological samples having higher activity differences between ^{3}H and ^{45}Ca, initial measurements should be made to evaluate the contribution of ^{45}Ca in the ^{3}H window. The latter consideration is particularly important to the mouse model in this study, because a significant increase in the ^{45}Ca:^{3}H ratio in excreta occurs with time after administration of the two bone markers, due to the fact that the biological half-life for ^{45}Ca (~187 d) is so much longer than for 3H-TC (~2.8 hr). But, for the samples having higher activity of ^{3}H compared to ^{45}Ca, such preliminary measurements should not be needed. The close comparison between the actual and observed dpm values in Table 2 validates the effectiveness of the dual-label mode at other activity ratios.

Finally, applying the dual-isotope method to the *in vivo* experiment, we found a high correlation between ^{3}H and ^{45}Ca in mice excreta, both urine and feces. Therefore, ^{3}H-tetracycline and ^{45}Ca may be interchangeably used to monitor temporal bone remodeling responses, using either feces or urine. Cheong et al. observed a correlation between ^{45}Ca in urine and in bone (Cheong et al., 2009). However, unlike ^{3}H-TC, the majority (80–90%) of endogenous ^{45}Ca excretion occurs via feces in mice (Wang and Bhattacharyya, 1993), making feces an important sample to monitor in that species. It has been recently shown that ^{41}Ca can be used for short- and long-term (6 years) monitoring of bone remodeling in humans (Denk et al., 2007; Fitzgerald et al., 2005; Hui et al., 2007). As shown by Elmore et al. in dogs, ^{45}Ca can serve as a surrogate marker of ^{41}Ca (Elmore et al., 1990). ^{45}Ca is a preferred marker in non-human animal models because it is easy to measure and less expensive.

The *in vivo* method established with this work provides the foundation for highly significant follow-on studies that use this radioisotope model to mimic clinical scenarios. For example, we are preparing to apply this model to gain insight into the acute (short-term) and prolonged (long-term) bone complications associated with radiation and chemotherapy in cancer survivors, which is a growing concern due to the aging population and the increased numbers of cancer survivors.

6. Conclusions

In the present study, we found that Ecolume™ and UniverSol™ are comparable to Ecolite™ for different biological sample digestion processes. Moreover, the dual-label method can be used for accurate estimation of ^{3}H and ^{45}Ca in different biological samples. For high concentrations of ^{45}Ca compared to ^{3}H, it is important to evaluate the contribution of ^{45}Ca in the ^{3}H window. ^{3}H-TC or ^{45}Ca can be used to monitor temporal bone remodeling responses via measurements in murine excreta.

Research Highlight

- Liquid scintillation cocktails support accurate dual-label analysis of ^{3}H and ^{45}Ca
- ^{3}H-tetracycline and ^{45}Ca were highly correlated in mice urine and feces.
- ^{3}H-tetracylcine and ^{45}Ca can be used to monitor *in vivo* temporal bone remodeling

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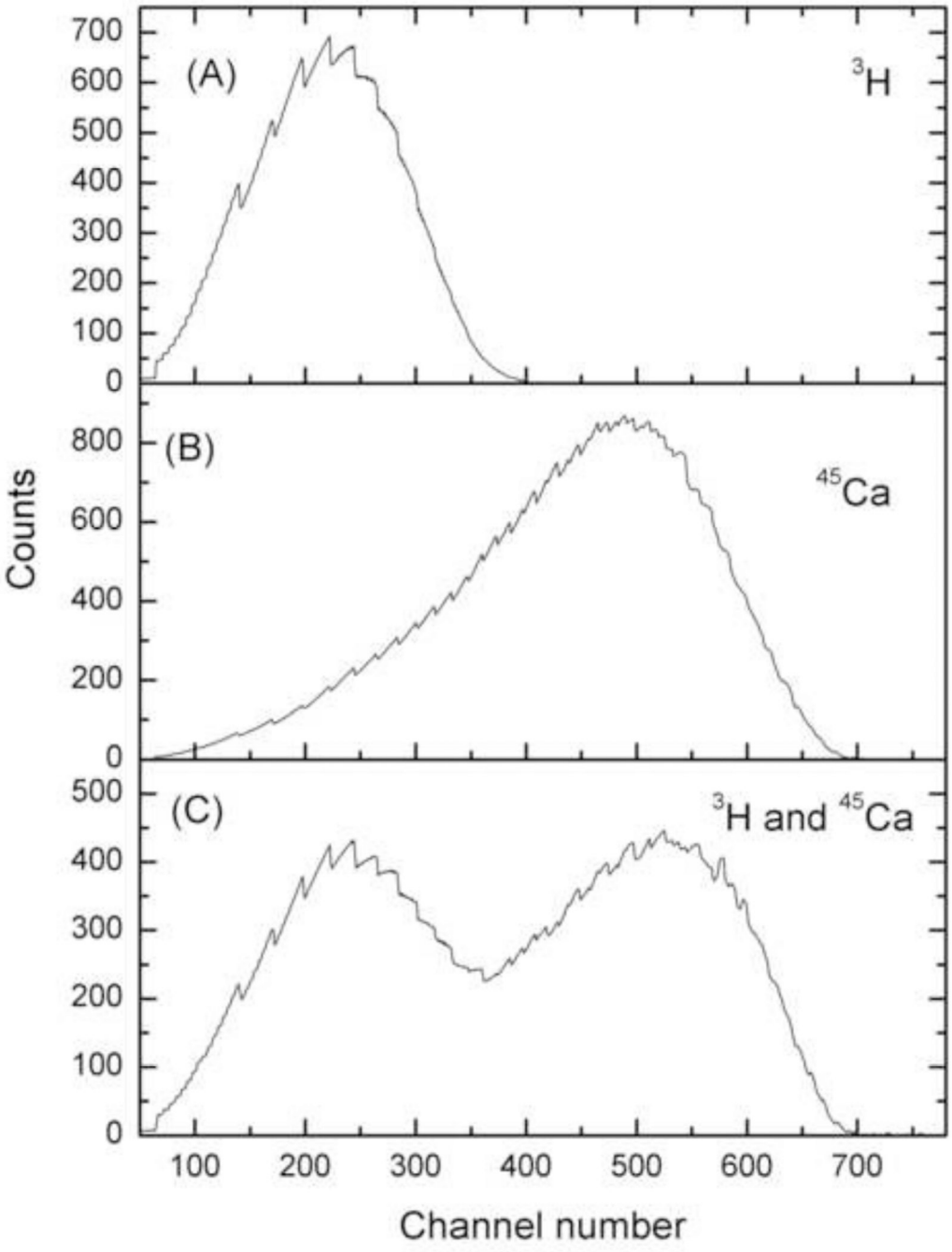

Fig. 1.

The spectra of ^{3}H and ^{45}Ca in single and dual mode. Figure (A) is ^{3}H spectra in single mode, figure (B) is ^{45}Ca spectra in single mode, and figure (C) is ^{3}H and ^{45}Ca spectra in dual mode.

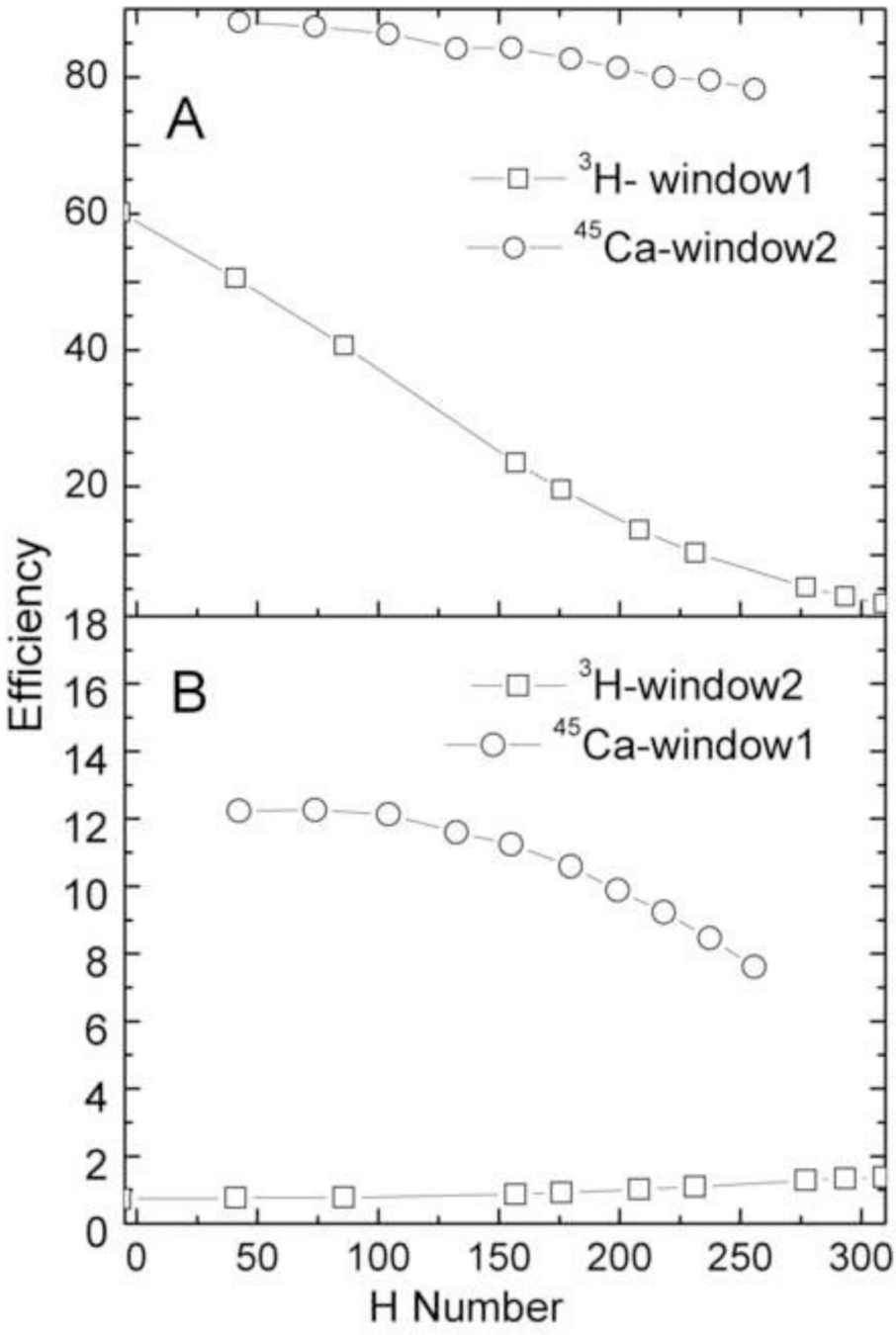

Fig. 2.

Efficiency (in %) of ^{3}H and ^{45}Ca as a function of H#. The upper figure (A) shows higher efficiency channel of the respective isotopes: the efficiency of ^{3}H in window1 and ^{45}Ca in window2. The lower figure (B) shows lower efficiency channel of the respective isotopes: the efficiency of ^{3}H in window2 ^{45}Ca in window1.

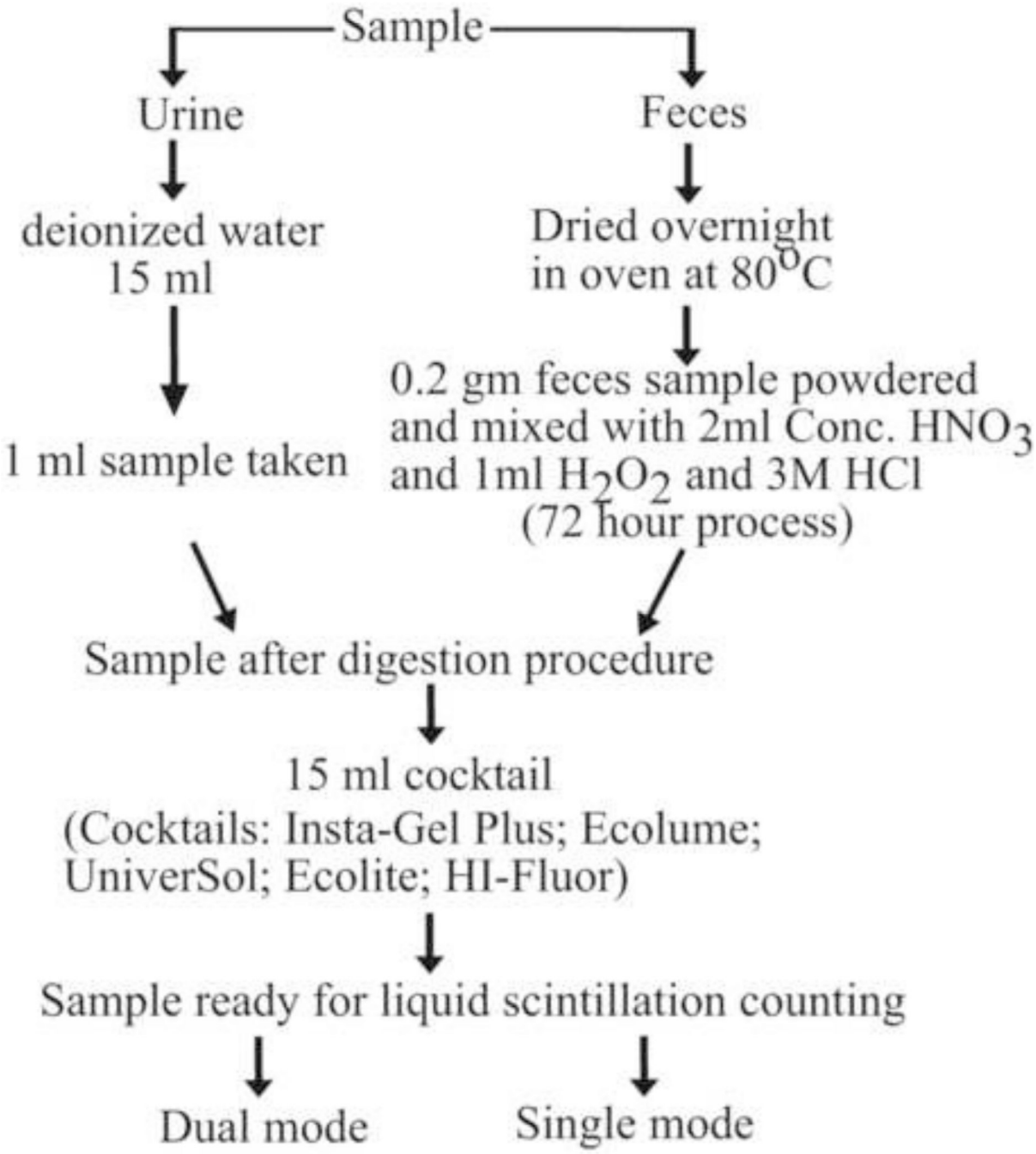

Fig. 3.
The flow diagram shows different sample preparation steps.

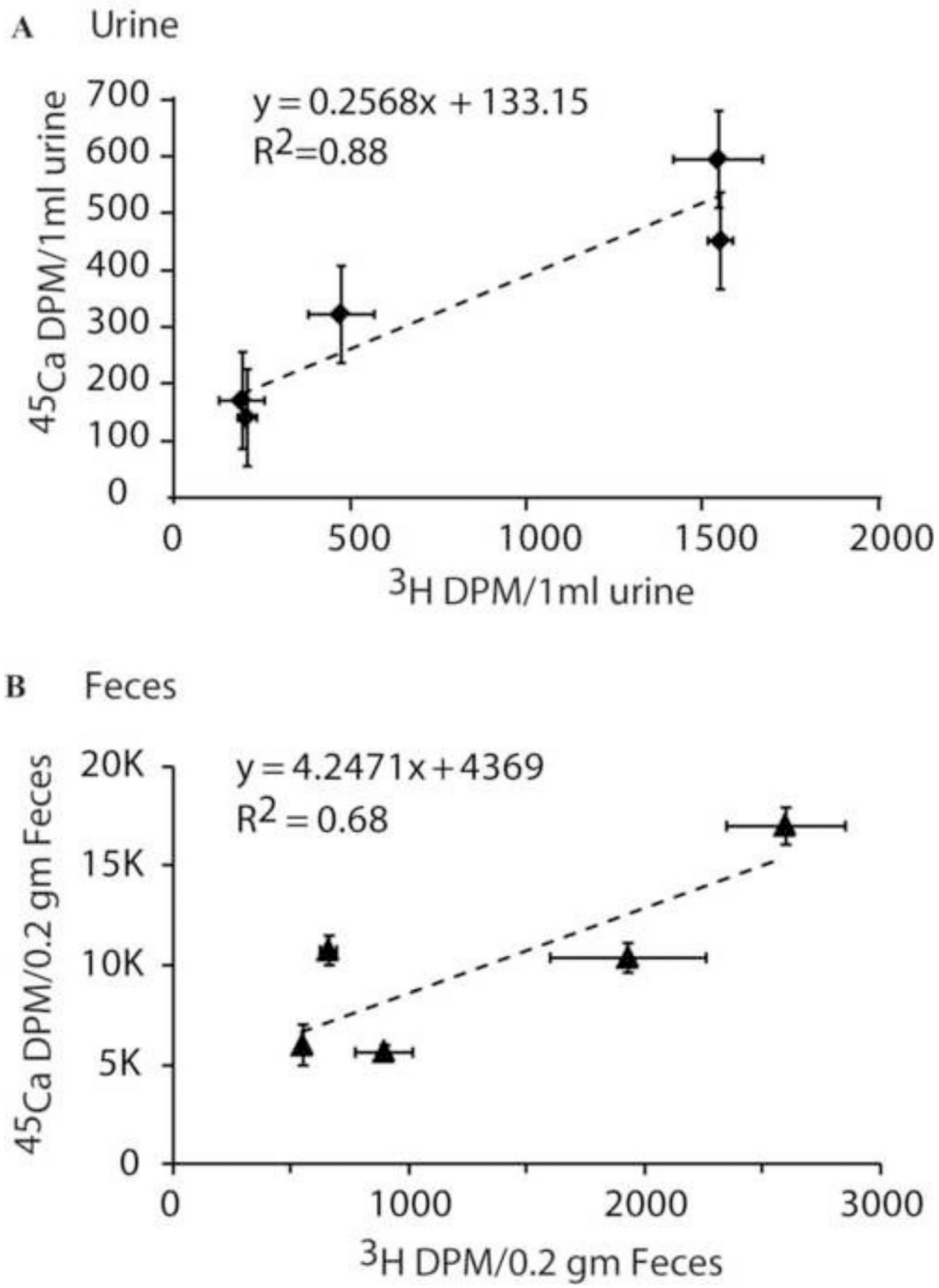

Fig. 4.

The scatter plot of ^{3}H and ^{45}Ca activity in mouse urine/1ml (A) and mouse feces/0.2gm (B) from day 3 to day 19 after administration of isotopes. Error bars show ± SEM for each data point.

Table 1

Comparison between full spectrum dpm values

Comparison between full spectrum dpm values with different scintillation cocktails. The average error in the measurements was ±5%.

Sample	Ecolite	Ecolume	UniverSol (US)	Insta-Gel Plus (IG)	HI-Fluor (HIF)	Percentage difference in Ecolite dpm values from			
						Ecolume	US	IG	HIF
^{3}H (dpm)									
Urine	4746	4554	4799	4631	4445	4.0	−5.4	3.5	4.0
Feces	2811	2425	2760	2848	4650	13.7	−13.8	−3.2	−63.3
^{45}Ca (dpm)									
Urine	805	781	810	805	767	3.0	−3.7	0.6	4.7
Feces	6631	6595	6723	6595	5519	0.5	−1.9	1.9	16.3

Table 2

Comparison of 3H and 45Ca dpm values obtained in single

Comparison of ^{3}H and ^{45}Ca dpm values obtained in single vs. dual-label mode. Pairs of samples containing prepared amounts of either ^{3}H or ^{45}Ca were counted in single-label mode, then mixed and recounted in dual-label mode. Maximum difference in samples with equal activities of the two isotopes was ±3%.

Single-label mode			Dual-label mode			% difference in ^{45}Ca/^{3}H in dual-label mode and single-label mode
^{3}H	^{45}Ca	^{45}Ca/^{3}H	^{3}H	^{45}Ca	^{45}Ca/^{3}H	Dual mode
1961	1958	1.00	1895	1818	0.96	3.90
5034	5025	1.00	4792	4568	0.95	4.51
10093	9925	0.98	9482	8983	0.95	3.66
15222	15278	1.00	14261	13818	0.97	3.46
20136	20286	1.01	19352	18218	0.94	6.56
1915	19938	10.41	2403	17998	7.49	28.0
5030	20107	4.00	5258	18302	3.48	12.9
9945	20365	2.05	11305	18180	1.61	21.5
15114	19912	1.32	12951	18103	1.40	−6.10
20177	2082	0.10	18607	1887	0.10	1.71
19410	4968	0.26	18505	4502	0.24	4.93
20567	9896	0.48	19442	8981	0.46	3.99
19417	15169	0.78	18802	13674	0.73	6.90