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Investigation of select radionuclides stability in urine under various conditions for liquid scintillation counting (LSC)

Olga Piraner[1], **Jonathan Button**[1], **Cynthia D. Ward**[1], **Liza Valentin-Blasini**[1]

[1]Inorganic and Radiation Analytical Toxicology Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, MS S110-5, 30341-3717 Atlanta, GA, Georgia

Abstract

Liquid Scintillation Counting (LSC) gross alpha/beta screening is a valuable tool for providing rapid laboratory response for the analysis of human clinical urine samples during a large-scale radiation incident event. Verification of method performance, as required for clinical laboratory testing, is accomplished by the evaluation of routine, periodic measurements of radioactive spiked samples for quality control, performance testing, and accuracy checks. Radionuclide stability of alpha and beta emitters in urine for LSC analysis is an important consideration. The purpose of this work is to demonstrate optimal preparations and storage conditions of samples used for method verification.

Keywords

Am-241; Sr-90/Y-90; H-3; Stability in urine; Liquid scintillation counting (LSC)

Introduction

The Centers for Disease Control and Prevention's (CDC) Radiation Analytical Toxicology Laboratory designed the urine radionuclide panel to provide rapid response laboratory results used to assess internal contamination from a radiation accident or incident. The complete panel currently consists of 11 Clinical Laboratory Improvement Amendments (CLIA) compliant methods for analyzing 19 priority threat radionuclides. A rapid screen of human urine samples to detect gamma, alpha, and beta activity above the normal population level is an important component of the panel. This allows for the prioritization of samples for analysis on slower throughput identification and quantification analytical methods which are necessary for dose assessment.

✉Olga Piraner, OPiraner@cdc.gov.

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Conflict of interest The authors declare that they have no competing financial interest.

The stability of analytes in matrices plays an important role in any analytical method. Quality control (QC) materials, proficiency testing (PT) samples, and reference material (RM) samples are used for extended periods of time for activities related to method development and the subsequent upkeep of the analytical method. Knowledge about the effect of storage conditions on these types of materials is required. Many articles were published on urine specimens stability, but they are mostly devoted to stability of urine protein and biomarkers [1–4]. The effect of the chemical additives and storage temperature on various trace elements in urine was studied for analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectroscopy (ICP-OES) [5, 6]. It was shown that the appropriate chemical preservation and storage conditions (storage at low temperatures) help stabilize the elements in urine prior to ICP-MS and ICP-OES analysis. Radionuclide stability was partially investigated during the development of the CDC method for urine gross alpha/beta analysis on LSC. However, using previously frozen QC materials, experiments from the prior investigation focused on understanding the effect of multiple freeze–thaw cycles on radionuclide stability in urine and the stability of radionuclides in a liquid scintillation cocktail [7].

The current study is a full investigation of radionuclide stability in urine. We focused on Am-241, Sr-90/Y-90, and H-3 radionuclides because we used them in quality control, reference materials, and proficiency testing samples for the development, validation, and maintenance of the gross alpha/beta LSC screening method. Quality control (QC) materials for the screening method (Am-241, Sr-90/Y-90, and H-3 urine spikes) were analyzed at the beginning and end of each analytical run, which is normally no more than 24 h. The run was considered acceptable if QC activities were within 2 standard deviation (SD) levels. The characterization of QC materials by LSC was presented previously [7, 8]. The results of the investigation are summarized in this article.

Experimental

Reagents and materials

For gross alpha/beta analysis, we used the Ultima Gold™ AB cocktail (UGAB) from Revvity Health Science, Inc (previously part of PerkinElmer Company); 99% nitromethane from ACROS Organics; black tea solution (Lipton™ black tea from any grocery store: One regular tea bag steeped in 200 mL of boiling water for 10 min, the cooled solution was used for quenching); and 99% urea from ACROS Organics. Deionized (DI) water was used for all solutions (≥ 18 MΩ cm, from an Aqua Solutions Ultrapure Water System, Aqua Solutions, Inc.). “Base urine” was collected through anonymous human donations (according to Centers for Disease Control and Prevention Institutional Review Board protocol 3994), refrigerated and decanted. Nonacidified base urine and urine acidified to 1% nitric acid (1%NA) and 2% nitric acid (2%NA) were used in this experiment to prepare urine spiked with high and low levels of activity: three sets for gross alpha/beta (GAB) and three sets for H-3. The gross alpha/beta sets contained Am-241 and Sr-90/Y-90 at low and high levels while the H-3 sets contained H-3 (see Tables 1 and 2). All radioactive source solutions were traceable to the National Institute of Standards and Technology (NIST, Gaithersburg, MD,

USA). Two urine gross alpha/beta quality control materials were purchased from Eckert & Ziegler Analytics, Inc. They were acidified to 1% nitric acid in base urine spiked with Am-241 and Sr-90/Y-90 at low and high levels. QC materials for tritium analysis were prepared in our laboratory by spiking base urine acidified to 1% nitric acid with a H-3 NIST traceable reference solution at low and high levels. All reference solutions used for spiking were purchased from Eckert & Ziegler Analytics, Inc. All QC materials were stored in a freezer at ≤−50°C.

Instrumentation and labware

For this study, we used ultralow level liquid scintillation spectrometers—Quantulus™1220, Tri-Carb®3110 (#1 and #2), Tri-Carb®5110, and Quantulus™ GCT6220 (total 5 instruments, all from Revvity Health Sciences, Inc) for Am-241, Sr-90/Y-90, and H-3, in alpha/beta mode; 20 mL LSC plastic vials (Revvity Health Sciences, Inc.) for LSC analysis; a high precision analytical balance capable of accuracy weighing 0.001 g (Mettler-Toledo, LLC); a bottle top dispenser with a 5 mL to 25 mL capacity (Brinkman Instruments, Inc.) for cocktail dispensing; 250 mL and 125 mL polypropylene bottles (Nalgene, Fisher Scientific) for urine spikes preparation; and four electronic pipettes with total volume ranges from 5 μL to 5 mL (Eppendorf, Inc).

Sample preparation and LSC analysis

The calibration of five instruments was conducted as described in our previous publications [7–10]. It includes pulse shape analysis (PSA) or pulse decay discriminator (PDD) setting determination, quench curves preparation for the listed radionuclides at the chosen PSA/PDD setting, sample analysis time, external standard analysis time, region of interest (ROI) for each nuclide, and sample/cocktail ratio for 20 mL vial geometry.

For sample preparation, 5 mL of well-shaken urine sample was mixed with 15 mL of UGAB cocktail in 20 mL LSC plastic vials until the mixture reached a uniform state. The LSC vials with solutions were placed on the LSC counter tray. LSC analysis was performed in the determined area of interest for each nuclide. We analyzed one aliquot of each urine sample on all 5 instruments and averaged the results. LSC method parameters, including limit of detection data, for each instrument are presented in Table 3.

Results and discussion

There are three primary factors that can impact the stability of elements in solution: acidification, storage temperature, and time. To investigate the effect of these three factors, we used six sets of urine spikes at high and low activity: 3 sets for gross alpha/beta and 3 sets for tritium. The results of these urine spikes initial characterization are presented in the Table 1 (for urine gross alpha/beta spikes) and Table 2 (for tritium spikes).

The Table 1 results show that nonacidified base urine and base urine acidified to 1% with nitric acid gives comparable gross alpha/beta activity results. In contrast, base urine acidified to 2% with nitric acid gives gross alpha results about 6–8% lower, while the gross beta (bias is in the range of −0.38–1.89%) is within the measurement uncertainty. This means that the PSA setting used for the urine LSC analyses, is not optimal for 2% acidified base urine.

The Table 2 displays the H-3 analysis results for urine spikes. The bias between the found and target levels of tritium activity in all matrices ranged from −2 to + 3% which was within the measurement uncertainty. This suggests that tritium, just like Sr-90, was not impacted by acidification, even with 2% nitric acid.

To investigate the effect of acidification, storage temperature and time, we chose low and high urine spikes from each set (BU, 1%NA, and 2%NA) and stored them at different conditions: room temperature (RT), refrigerated (REF) (2–6 °C), and frozen (FR) (lower than −50 °C) for different time intervals. Thus, we studied the effects of acidification (BU versus 1%NA versus 2%NA), storage temperature (RT versus REF versus FR), and time interval (ranging from one day to one year). The results for Am-241 and Sr-90/Y-90 stability are summarized in Table 4.

The data showed that acidification helps with the radionuclide stability while in storage, but temperature plays a key role. The most effective practice is to increase the acidity of urine by using 1% nitric acid, but it is important not to exceed this concentration. Increasing the level of acidification can cause a rise in quenching, which leads to a greater difference between the found and target data. This effect is particularly pronounced for gross alpha, as the PSA setting was optimized for urine without acidification and may not be ideal for urine acidified to 2%.

Both Am-241 and Sr-90/Y-90 are less stable in nonacidified urine at room temperature. The activity of Am-241 decreased by about 9% within a week and about 54–65% within 1 year at room temperature, while the activity of Sr-90/Y-90 decreased by about 5% between 1 and 2 weeks and about 21–32% within a year with the major decrease in activity within the first 2–3 weeks for both alpha and beta nuclides. In urine acidified to 1% with nitric acid and stored at room temperature, the decrease in Am-241 activity started between 6 and 12 months, while Sr-90/Y-90 is stable for 12 months. Am-241 was stable in a refrigerator for at least 6 months in nonacidified urine and almost 12 months in urine acidified to 1% with nitric acid. However, Sr-90/Y-90 stayed stable in refrigerator for a year in all matrices. In a freezer, both the Am-241 and Sr-90/Y-90 were stable for at least one year in all matrices.

Table 5 shows the LSC analysis findings for tritium (H-3). The H-3 nuclide remains stable in both nonacidified and acidified urine. It can be stored at room temperature for up to 6 months without any noticeable decrease in activity. After 12 months, a decrease of approximately 7% in activity was observed in all matrices when stored at room temperature. Tritium also remained stable in all matrices when stored in the refrigerator and freezer for at least one year.

Conclusion

This study showed that acidification and storage conditions play a role in the stability of nuclides such as Am-241, Sr-90/Y-90, and H-3 in urine. Based on the experiments conducted, it was observed that Am-241 exhibited the highest level of sensitivity and experienced a decrease in activity during the initial week when in non-acidified, room-temperature urine. On the other hand, Sr-90/Y-90 displayed a greater degree of stability, with

smaller activity reductions between 1 and 2 weeks under similar conditions. The stability of both these nuclides was enhanced in acidified base urine. Am-241 in 1% acidified urine showed decreases in activity after 6 months at room temperature while Sr-90/Y-90 in 1% acidified urine was stable for 12 months. Am-241 was stable in the refrigerator for at least 6 months, even in nonacidified urine. It has been observed that Am-241 in 1% acidified urine stored in a refrigerator experienced a decrease in activity around 12 months. However, Sr-90/Y-90 in nonacidified and 1% acidified urine stored in a refrigerator remained stable for at least one year. H-3 was stable in nonacidified urine at room temperature for at least the first 6 months. Between six and twelve months, it showed some decrease (about 6–7%) in activity.

Based on the experimental findings, 1% urine acidification helps to increase stability for all these radionuclides. The acidification should not be higher than 1% as results of testing urine samples in 2% nitric acid indicated a consistent negative bias for Am-241 in both low and high nuclide activity levels, regardless of storage temperature and duration.

This proves that PSA/PDD settings, optimal for nonacidified urine, will not be optimal for 2% acidified urine. The storage temperature plays the important role as well: better to keep spiked materials refrigerated for short-term use (no more than 3 months) and frozen for long-term use (more than 3 months).

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Table 1

Urine initial high and low gross alpha/beta (GAB) spikes—LSC analysis (N—number of measurements, N = 5)

Sample ID number	Radionuclide specific activity, Bq/L				Bias, %	
	Found		Target by spiking			
	Am-241 (SD)	Sr-90/Y-90 (SD)	Am-241	Sr-90/Y-90	Am-241	Sr-90/Y-90
GAB-Low-Base	456 (29)	5230 (165)	460	5300	−0.87	−1.32
GAB-High-Base	4700 (380)	51,200 (660)	4600	52,000	2.17	−1.54
GAB-Low-1%NA	379 (36)	5500 (175)	380	5500	−0.26	0.10
GAB-High-1%NA	3800 (275)	52,400 (620)	3800	53,000	0.10	−1.13
GAB-Low-2%NA	348 (38)	5400 (342)	380	5300	−8.42	1.89
GAB-High-2%NA	3590 (333)	52,800 (650)	3800	53,000	−5.52	−0.38

Table 2

Urine initial high and low H-3 spikes—LSC analysis (N—number of measurements, N = 5)

Sample ID number	H-3 specific activity, Bq/L		Bias, %
	Found (SD)	Target by spiking	
H3-low-base	16,960 (598)	17,000	−0.24
H3-high-base	422,000 (4230)	430,000	−1.86
H3-low-1%NA	15,300 (306)	15,000	2.00
H3-high-1%NA	440,000 (10,400)	440,000	0.10
H3-low-2%NA	15,400 (528)	15,000	2.67
H3-high-2%NA	435,000 (9590)	430,000	1.16

Table 3

Liquid scintillation counting method parameters for different instruments [7, 8, 10]

Parameter	Quantulus 1220	Tri-Carb 3110 #1	Tri-Carb 3110 #2	Tri-Carb 5110	Quantulus GCT6220
PSA/PDD setting	90	125	165	135	160
Sample volume (mL)	5	5	5	5	5
Cocktail volume (mL)	15	15	15	15	15
Sample analysis time (min)	5	5	5	5	5
External std analysis time	60 s	2 Ω (15 s)	2 Ω (15 s)	2 Ω (10 s)	60 s
Alpha (Am-241) region of interest (channels or keV)	1–1024 channels	0–300 keV	0–200 keV	0–1000 keV	0–450 keV
High energy beta (Sr-90/Y-90 or P-32 region of interest (channels or keV)	1–1024 channels	0–2000 keV	0–2000 keV	0–2000 keV	0–2000 keV
Low energy beta (H-3) region of interest (channels or keV)	1–250 channels	0–18.6 keV	0–18.6 keV	0–18.6 keV	0–18.6 keV
LOD for gross alpha, Bq/L	5.8	12.6	12.6	12.6	7.6
LOD for gross beta, Bq/L	31.3	44.6	44.6	44.6	40.3

Table 4

LSC analysis of urine spikes stored at room temperature (RT), refrigerated (REF), and frozen (FR) during different time, shown as bias (%) from the target results (N—number of measurements, N = 5)

Sample ID	Nuclide	Storage Time (D-for day, W-for week, M- for month)								
		1D	2D	1W	2W	3W	1 M	2 M	6 M	12 M
GAB-Low-BU-RT	Am-241	1.09	0.00	−8.91	−18.7	−33.0	−31.7	−40.9	−61.3	−54.1
	Sr-90/Y-90	−1.81	−2.58	−3.87	−5.09	−17.1	−17.6	−18.7	−22.1	−21.0
GAB-High-BU-RT	Am-241	2.65	4.11	2.63	−8.98	−53.4	−47.9	−59.3	−76.8	−65.2
	Sr-90/Y-90	−1.12	−1.00	−2.20	−4.00	−31.9	−33.1	−43.1	−54.1	−31.9
GAB-Low-1%NA-RT	Am-241	−0.53	−2.11	−0.26	−4.47	−0.79	−2.63	−3.68	−7.37	−11.1
	Sr-90/Y-90	−0.45	−0.75	0.91	−1.35	0.84	−0.60	0.38	0.35	1.29
GAB-High-1%NA-RT	Am-241	1.26	0.29	−0.29	−1.21	−3.92	−3.34	−3.61	−5.55	−12.0
	Sr-90/Y-90	−0.17	−0.21	−0.73	1.28	−0.48	−0.30	−0.36	0.04	0.37
GAB-Low-2%NA-RT	Am-241	−7.63	−6.32	−12.89	−9.74	−15.0	−13.7	−15.5	−19.5	−22.2
	Sr-90/Y-90	−1.75	−2.60	−2.09	−2.45	−1.62	−0.33	0.12	−1.41	−0.11
GAB-High-2%NA-RT	Am-241	−6.39	−7.97	−8.24	−9.87	−11.1	−11.4	−14.5	−19.5	−23.9
	Sr-90/Y-90	0.05	−0.14	0.28	−0.15	0.33	0.54	0.39	1.04	1.08
GAB-Low-BU-REF	Am-241	1.52	−0.65	2.39	−0.65	−1.30	2.61	−0.22	−6.96	−28.0
	Sr-90/Y-90	−2.25	−1.74	−1.34	−0.40	−0.17	−0.47	−1.04	−1.66	−3.23
GAB-High-BU-REF	Am-241	4.35	1.85	1.89	3.28	1.74	1.30	1.74	0.74	−10.9
	Sr-90/Y-90	−2.42	−4.04	−3.91	−2.30	−1.27	−2.43	−2.13	−2.65	−0.93
GAB-Low-1%NA-REF	Am-241	−0.79	−1.05	−1.32	−1.32	−5.79	0.53	−4.47	−1.32	−9.21
	Sr-90/Y-90	−0.95	−1.18	−0.29	−1.20	0.29	−1.65	0.18	−0.95	0.82
GAB-High-1%NA-REF	Am-241	2.37	2.05	0.24	−0.34	0.08	−0.79	−1.47	−2.24	−4.55
	Sr-90/Y-90	−0.36	−1.34	−1.43	−0.64	−0.68	−0.70	−0.38	−0.34	0.11
GAB-Low-2%NA-REF	Am-241	−7.11	−6.32	−6.58	−7.89	−8.42	−10.5	−8.95	−10.0	−14.2
	Sr-90/Y-90	−1.17	−1.42	−1.98	−1.23	0.57	−1.25	−1.60	−1.17	0.00
GAB-High-2%NA-REF	Am-241	−5.71	−5.26	−6.34	−7.26	−7.92	−8.13	−8.03	−10.9	−13.2
	Sr-90/Y-90	−0.08	−0.78	−0.91	0.09	0.41	0.05	0.16	−0.09	2.41
GAB-Low-BU-FR	Am-241	1.30	1.96	1.74	1.96	4.57	3.70	3.91	4.78	−5.65
	Sr-90/Y-90	−1.00	0.36	0.57	0.53	1.23	0.57	−0.34	0.15	0.74

Sample ID	Nuclide	Storage Time (D-for day, W-for week, M- for month)								
		1D	2D	1W	2W	3W	1 M	2 M	6 M	12 M
GAB-High-BU-FR	Am-241	4.30	5.20	5.78	4.63	4.98	5.50	4.54	5.37	−0.74
	Sr-90/Y-90	−0.12	0.04	0.28	−0.04	0.20	0.64	0.08	0.61	1.01
GAB-Low-1%NA-FR	Am-241	2.89	3.42	0.26	2.63	0.26	2.63	3.42	1.58	0.79
	Sr-90/Y-90	0.40	0.67	0.89	1.02	0.69	0.55	1.09	2.60	2.47
GAB-High-1%NA-FR	Am-241	3.92	2.97	3.95	3.74	4.16	4.05	4.76	3.45	1.08
	Sr-90/Y-90	1.48	1.43	1.36	1.43	1.55	1.37	1.75	1.76	2.33
GAB-Low-2%NA-FR	Am-241	−5.26	−4.74	−2.63	−7.37	−4.74	−4.47	−7.37	−5.79	−9.47
	Sr-90/Y-90	−0.47	−0.04	0.02	−0.53	0.25	0.38	0.66	0.26	0.79
GAB-High-2%NA-FR	Am-241	−4.39	−4.37	−3.16	−3.61	−3.82	−3.66	−3.55	−2.97	−6.79
	Sr-90/Y-90	1.80	1.84	2.43	2.12	2.20	2.74	2.38	2.68	3.21

Table 5

LSC analysis of H-3 urine spikes stored at room temperature (RT), refrigerated (REF), and frozen (FR) during different time, shown as bias (%) from the target results (N—number of measurements, N = 5)

Sample ID	Nuclide	Storage Time (D-for day, W-for week, M- for month								
		1D	2D	1W	2W	2W	1 M	2 M	6 M	12 M
H3-Low-RT	H-3	−0.75	−2.47	−0.59	1.25	0.45	−1.42	−0.75	−1.35	−4.21
H3-High-RT	H-3	−2.15	−2.06	−1.27	0.61	−2.29	−1.65	−0.60	−3.02	−6.17
H3-Low-1%NA-RT	H-3	5.71	2.68	2.70	4.51	9.43	4.43	5.68	3.66	−6.89
H3-High-1%NA-RT	H-3	3.20	5.73	4.96	0.16	3.26	3.77	4.28	−1.18	−6.38
H3-Low-2%NA-RT	H-3	−2.19	−0.83	−0.71	−3.83	−0.85	0.29	1.30	−1.25	−7.62
H3-High-2%NA-RT	H-3	−0.90	2.05	1.93	−1.12	0.10	0.65	3.66	−1.28	−3.16
H3-Low-REF	H-3	−1.06	−0.02	−1.14	−1.56	−1.27	0.14	−0.54	−2.39	0.90
H3-High-REF	H-3	−1.80	−1.56	−1.55	−2.96	−2.31	−2.74	−2.59	−2.46	−3.01
H3-Low-1%NA-REF	H-3	1.07	1.79	0.49	4.54	2.08	1.67	4.23	2.61	−1.84
H3-High-1%NA-REF	H-3	1.49	−0.49	0.06	−0.62	3.27	0.33	1.33	−1.12	−2.01
H3-Low-2%NA-REF	H-3	−0.94	−2.57	−2.19	−1.02	3.36	−3.85	−1.24	−4.71	−4.48
H3-High-2%NA-REF	H-3	−0.22	0.23	−0.30	−0.60	−0.24	−0.78	0.43	−0.20	−3.83
H3-Low-FR	H-3	1.82	0.92	0.94	−0.21	−0.63	−0.27	1.81	−1.50	−3.02
H3-High-FR	H-3	0.56	0.42	0.17	−0.46	−0.07	−0.19	0.10	−0.23	−2.01
H3-Low-1%NA-FR	H-3	4.55	4.62	4.20	4.37	3.15	5.03	5.33	1.07	4.34
H3-High-1%NA-FR	H-3	1.05	3.30	3.20	2.40	1.95	2.54	4.40	1.41	1.36
H3-Low-2%NA-FR	H-3	0.57	−0.14	0.27	−0.29	−0.83	−1.15	−0.51	−1.80	−1.92
H3-High-2%NA-FR	H-3	1.38	0.50	1.18	0.80	0.58	1.99	0.50	0.49	4.70