Is It Possible to Avoid Standards in Studies With Blood Samples?

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Objective: The long-term mutual stability of a tandem radionuclide calibrator-well detector was measured to enable us to omit reference standards in the estimation of blood volume and GFR/ERPF and thus simplify the procedures, and potentially make the results more precise.
Methods: During two months 200 measurements of reference standard aliquots were taken to estimate the stability of a factor of mutual relation (FMR), which provides a conversion constant between a radionuclide calibrator reading and count rate of a diluted aliquot measured in a well detector.
Results: All measurements demonstrated the satisfactory mutual stability of the detectors.
Conclusion: An FMR can be used instead of making up standard aliquots. The activity in the syringe is measured directly in a radionuclide calibrator before injection. The value is then multiplied by the FMR and the result is used in the equation for blood volume or GFR calculation.
Key Words: long-term stability of detectors; standard samples

J Nucl Med Technol 1995; 23:271–274

For the estimation of blood volume and GFR/ERPF by plasma samples, it is necessary to measure administered activity and blood samples in two different devices, a radionuclide calibrator and a well detector. Standard samples are used because the syringe activity is too large for the well detector and the blood samples are too low in activity for the radionuclide calibrator. These standards help to solve a dynamic range mismatch of both devices. Making up standards requires precise meticulous technique, however, and in routine work they are often a source of additional errors.

The long-term stability of nuclear medicine detectors has been substantially improved in the last few years. We decided to measure the long-term mutual stability of our tandem radionuclide calibrator-well detector (CRC 35 R-Captus 2000, Capintec, Inc., Ramsey, New Jersey). Suitable mutual stability would enable us to omit making up standard samples for the estimation of blood volume and GFR, and thus simplify the procedures. We used ^{99m}Tc because it is widely used for GFR calculation and recommended even for RBC volume calculation (*1–4*).

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METHOD

Long-Term Mutual Stability

On each of eight days over period of two months we drew up five syringes of ^{99m}Tc in the range of activity 3.5–4.5 MBq (95–120 μCi) for estimation of the long-term stability of the radionuclide calibrator and well detector. After triple measurements in the calibrator (the residual activity in the syringe was subsequently subtracted), we diluted the syringe contents 1:2750 using a volumetric flask. We then measured 1-ml aliquots in a well detector five times each (at that time approx. 1.5 kBq = 40 nCi), with an energy window of 112–168 keV for 60 sec.

There was one day of measurement per week. The total period from the first to the last (eighth) measurement was more than two months. Before starting the measurements, both detectors were autocalibrated according to the manufacturer's instructions using sealed ^{137}Cs and ^{152}Eu sources. All 200 measurements of the 40 aliquots were immediately recalculated to the correction factor: provisional factor of mutual relation (pFMR), where:

$$\text{pFMR} = \frac{\text{aliquot count rate (kcpm)} \times \text{dilution factor}}{\text{radionuclide calibrator reading (kBq)} \times \text{decay correction}}.$$

The average of all 200 pFMRs was the resulting factor of mutual relation (FMR). The FMR represents how many kcpm in the well detector would be recorded from 1 kBq of ^{99m}Tc measured in a radionuclide calibrator, or simply the count rate per kBq of ^{99m}Tc. The calculation included ^{99m}Tc decay correction. No background correction was done due to the negligible background count rate.

All further statistical calculations were done with the pFMRs, and not the aliquot count rate nor with syringe readings. The syringe activities ranged from 3.5 to 4.5 MBq (95–120 μCi) and the well detector counts depend on decay, while with the pFMRs both variables are corrected.

Mean, standard deviation (s.d.) and coefficient of variation (c.v.) (*5*) were calculated from all pFMRs as follows:

I. Differences between pFMR values obtained from five measurements of each individual aliquot (5 aliquots = 25 measurements daily).
II. Differences between average pFMRs of each of five aliquots done in one day.
III. Differences between average pFMR from different days.
IV. The average of all 200 individual calculations of pFMR resulted in the value of the FMR.

Activity Dependence

For evaluation of dependence of the FMR on the level of measured activity we measured 13 additional aliquots (each 1 ml) over a wide range of activity from 89 Bq (2.4 nCi) to 170 kBq (4.6 μCi). Measurements were done under the same conditions as previously mentioned. Even in the 89-Bq ^{99m}Tc sample the value of the background represented less than 1% of the activity of the aliquot.

Volume Dependence

We made 5 aliquots each of approximately 3 kBq (80 nCi) ^{99m}Tc in a volume of 0.1 ml to verify the dependence of the count rate and the FMR on the volume of the sample. Measurement conditions were the same as above (energy window, time). We increased the volume of each individual sample to 1 ml, then 2 ml, 3 ml, 4 ml, 5 ml, 7.5 ml and 10 ml, and measured each volume five times. The mean count rate at each volume was expressed as a percentage of the 0.1-ml sample.

RESULTS

Long-Term Stability

I. We calculated the standard deviation and coefficient of variation of pFMR from five consecutive measurements for each aliquot. Daily averages of these values (Table 1) ranged from 0.09 to 0.24 kcpm/kBq for s.d. and from 0.19% to 0.52% for c.v. with a total average 0.18 kcpm/kBq s.d. and 0.39% c.v.
II. In the evaluation of differences among mean pFMRs from individual aliquots in one day s.d. and c.v. ranged from 0.73 to 1.2 kcpm/kBq with a mean value of 0.95 kcpm/kBq and from 1.6% to 2.6%, mean 2.1% respectively (Table 1).
III. Differences between the average pFMR for individual days of measurement showed a s.d. of 0.66 kcpm/kBq and a c.v. of 1.3%.
IV. The average of all 200 calculations of pFMR determined the value of our factor of mutual relation:

FMR = 45.676 kcpm/1 kBq (1.69 Mcpm/1 μCi) for ^{99m}Tc

(s.d. = 0.53 kcpm/kBq, c.v. = 1.16%).

The Dependence of FMR on Measured Level of Radioactivity

The values obtained from measurements of 13 aliquots of different activities are listed in Table 2 along with their predicted values according to linear fitting equation y [kcpm] = 45.68 x [kBq] which is derived from the value of the FMR. A graphic presentation is shown in Figure 1. The low-value data points are overlapping each other in Figure 1A. The same values are shown in Figure 1B with log − log axes.

TABLE 1
Results of FMR

Day	Mean values of pFMR in one day	Average differences among repeated calculations of pFMR from individual aliquots		Differences among means of pFMR of individual aliquots done in one day	
		s.d. kcpm/kBq	c.v. %	s.d. kcpm/kBq	c.v. %
1	45.1	0.23	0.51	0.99	2.2
2	45.5	0.22	0.48	0.98	2.1
3	44.4	0.14	0.32	0.95	2.1
4	46.3	0.18	0.39	0.75	1.6
5	46.3	0.09	0.19	1.02	2.2
6	45.7	0.24	0.52	1.20	2.6
7	46.1	0.09	0.19	0.99	2.1
8	46.1	0.23	0.50	0.73	1.6
Mean	45.687	0.178	0.388	0.951	2.062

Volume Dependence

The results are listed in Table 3 and graphically presented in Figure 2.

DISCUSSION

A measurement of radiation is one of the most precise measurements. In some nuclear medicine studies, measurement of radioactivity is combined with other procedures, such

TABLE 2
Activity Dependence

Activity		Measured count rate (kcpm)	Predicted count rate (kcpm)*	Δ kcpm	Δ %
kBq	nCi				
0.09	2.4	4.2	4.1	+0.1	+2.4
0.18	4.9	7.8	8.2	−0.4	−4.9
0.92	24.9	42.1	42.0	+0.1	+0.2
3.66	99.0	169.8	167.2	+2.6	+1.5
9.20	249.0	412.0	420.3	−8.3	−2.0
20.50	554.0	910.0	936.4	−26.4	−2.8
34.10	922.0	1540.0	1558.0	−18	−1.1
45.40	1227.0	1996.0	2074.0	−78	−3.8
68.20	1843.0	2990.0	3115.0	−125	−4.8
92.90	2511.0	3236.0	4244.0	−1008	−23.7
102.30	2765.0	3548.0	4673.0	−1125	−24.1
136.40	3686.0	3990.0	6231.0	−2241	−35.9
170.60	4611.0	4170.0	7793.0	−3623	−46.5

* The predicted kcpm were calculated from activity using the linear fitting equation: y [kcpm] = 45.68 x [kBq].

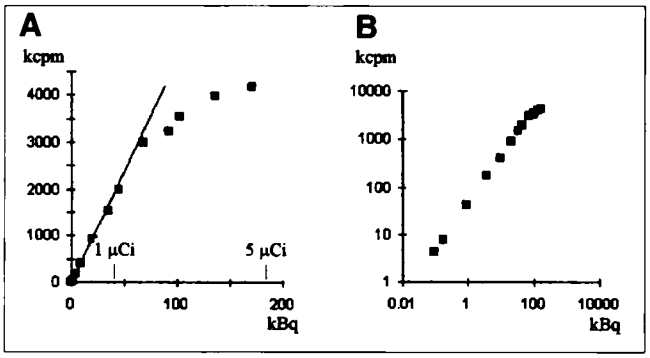

FIGURE 1. Activity dependence. (A) 13 aliquots listed in Table 2. The line represents linear regression: y [kcpm] = 44.9 x [kBq] − 0.14. It is difficult to distinguish it from the y [kcpm] = 45.68 x [kBq] line. (B) The same values in graph with log − log axes.

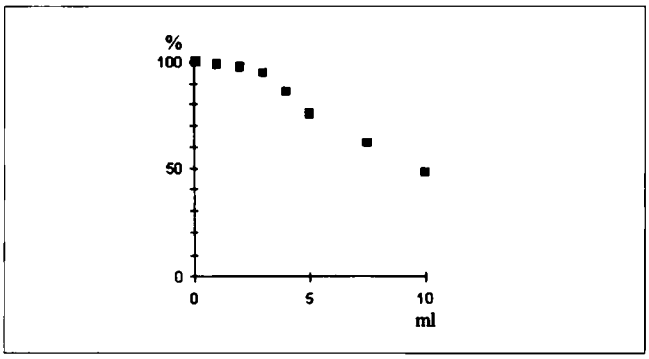

FIGURE 2. The dependence of count rate on the sample volume. Graphic expression of the values listed in Table 3.

as measurement of volume and dilution of the sample, which may cause some error in the final result. Until now nearly all recommended methods for evaluation of blood volume (*6–8*) and GFR/ERPF (plasma sample methods) (*9,10*) require using standard samples. There are only a few methods for determination of GFR/ERPF (*11–13*) which have excluded using calculation of injected activity by standards because they introduce some error, however, they used different techniques than ours.

The smallest differences were found in the repeated measurements of the same aliquot (mean c.v. = 0.39%) which we considered as an error of well counter measurement, or indicator of short-term stability of the detector. The greatest differences were found among averages of pFMRs of individual samples made up in one day where c.v. ranged from 1.6% to 2.6% with a mean of 2.1%. We considered those differences to be errors in the dilution and pipeting of samples. It cannot be explained by the instability of the well detector because all measurements in one day were done within 30 to 40 min. The most important statistic was the evaluation of the pFMRs on different days of measurement, which we considered an indicator of the long-term stability of our tandem radionuclide calibrator-well detector and stability of FMR. As input values, we used daily averages of pFMRs, not individual pFMRs. In this way we partially eliminated error caused by dilution and making of aliquots. The daily averages showed lower differences (c.v. = 1.3%) than within individual days (c.v. = 2.1%) which indicated that the accuracy of calculations with our FMR would be even better than with the use of standard samples. Our aim was to simplify the methods without decreasing the accuracy of the final results.

TABLE 3
Dependence of Sample Activity on Sample Volume*

Sample volume (ml)	%
0.1	100.0
1.0	98.8
2.0	97.6
3.0	94.8
4.0	85.8
5.0	75.5
7.5	61.7
10.0	47.9

*The mean count rate of each volume is expressed as a percentage of the 0.1-ml sample.

Activity Dependence

If we use FMR for prediction of kcpm from kBq we have to postulate linear dependence. In our case y [kcpm] = 45.68 x [kBq]. The linearity is limited by certain levels of activity which we wanted to find. Table 2 shows the differences from predicted values are minimal up to 34.1 kBq, or slightly less than 1 μCi. Count rates above that activity are decreased due to dead time despite the dead time correction done by our computer.

The equation of linear regression of count rate versus activity of the first seven measured aliquots between 0.09 and 34.1 kBq (2.4 nCi − 4.6 μCi) is y [kcpm] = 44.965 [kBq] − 0.139, r = 0.999.

Volume Dependence

There is minimal dependence of the count rate on sample volume up to 2 ml. Starting with 3 ml, the count rate decreases and the error is unacceptable if the FMR is used. Nearly the same dependence is shown by Harbert (*14*).

CONCLUSION

It is possible to avoid making up standards in studies with blood samples. Sufficient mutual stability of our tandem radionuclide calibrator-well detector enables us to use a factor of mutual relation. For our specific setup FMR = 45.68 kcpm/1 kBq = 1.69 Mcpm/1 μCi for ^{99m}Tc. Each nuclear medicine laboratory must calculate the FMR for their particular equipment and for each radionuclide used.

The activity of the syringe is measured directly in the radionuclide calibrator before injection and the residual syringe activity is subtracted afterwards. Then, that value is multiplied by the FMR and the result is used in the equation for RBC or

plasma volume calculation or for GFR/ERPF. Decay correction must be done. This technique simplifies the studies and limits the error caused by making standards.

Limitations include:

I. Activity of the sample. In our laboratory the activity must be ≤ 35 kBq (1 μCi).
II. Volume of the sample. In our laboratory the volume must be ≤ 2 ml.
III. The value of the FMR must be routinely confirmed despite daily autocalibration using ^{137}Cs and ^{152}Eu.

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