

Sensitivity of Radioluminography using ^{14}C -Labeled Tracers in Whole-Body Sections of Rats

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Radioluminography using photostimulated luminescence recently became popular for the quantitation of radioactivity in whole-body sections. In order to estimate the limits of the method, the sensitivity was investigated using routinely prepared blood standards with known amounts of radioactivity. The blood standards underwent the typical steps similar to conventional whole-body autoradiography including freezing, sectioning, and lyophilizing. The limit of detection, defined as 3 times the standard deviation of background values, decreases with exposure time from 18 Bq/g wet tissue for 1 day of exposure to 6 Bq/g for 7 days of exposure. The respective limit of quantitation defined as 10 times the standard deviation of background is 60 to 20 Bq/g. A similar sensitivity is found when the quantitation limit is defined as reproducible measurement with coefficients of variance below 20%: 60 Bq/g can be quantified if the measure area is above 3.5 mm². © 2000 Academic Press

INTRODUCTION

Whole-body autoradiography, introduced by Ullberg (1954), has now found widespread use, particularly in distribution studies on small laboratory animals. Several attempts were made to quantify radioactivity by this method. The quantitative or semiquantitative evaluations practiced successfully make use of densitometrically determined blackening of X-ray film together with reusable polymers or biological standards (blood, tissue) with known radioactivity levels. The process of using the new storage plates based on photostimulated luminescence (PSL) (Hamaoka, 1990) with their large linear range, in combination with digitized data, is referred to as radioluminography (RLG). RLG is designed to make quantification of radioactivity in whole-body sections considerably easier.

The limits of the technique were tested within the framework of validation. In an evaluation of the sensitivity of RLG account must be taken not only of the variable actually being measured—the radioactivity

concentration in tissue sections—but also two groups of influencing factors (covariables):

1. Factors which mainly affect the measured signal include the nature of the tissue, the thickness of the section and the exposure time; and
2. Variables influencing also background levels like shielding, contamination, memory effect of the plates, and flare effect.

The evaluation of sensitivity is addressed by establishing the relationship between factors influencing sensitivity and measured variables (signal and background values) followed by an investigation of the limit of detection (LOD) and the limit of quantitation (LOQ) with standardized parameters.

MATERIALS AND METHODS

Blood standards were prepared from heparinized fresh dog or rat blood by the addition of solutions of various ^{14}C -labeled substances. Serial dilutions were used to obtain several standards. The concentrations of the blood standards were measured by liquid scintillation counting after dissolution with tissue solubilizer and decolorization with hydrogen peroxide or after combustion in a sample oxidizer. For RLG, the blood standards were embedded in holes and drilled into in frozen methylcellulose (2%). The methylcellulose block was then cut into 30- or 40- μm sections. Sections were exposed several times on several imaging plates, for 1, 3, 4, or 7 days.

The equipment consisted of a microtome (Cryomacrocute, Leica, Nußloch Germany), a RLG system (BAS 2000 and imaging plates BASIII, Fuji Photo Film Ltd., Tokyo, Japan), two evaluation systems (Image Analysis (Fuji Photo Film Ltd.) and Tina (Raytest, Straubenhardt, Germany)), and a shielding box (at room temperature 20–22°C or in a cold room at 4–8°C).

Treatment of the Image Plates

Before exposure the image plates are erased for about 1 h. After exposure the plates are rinsed with

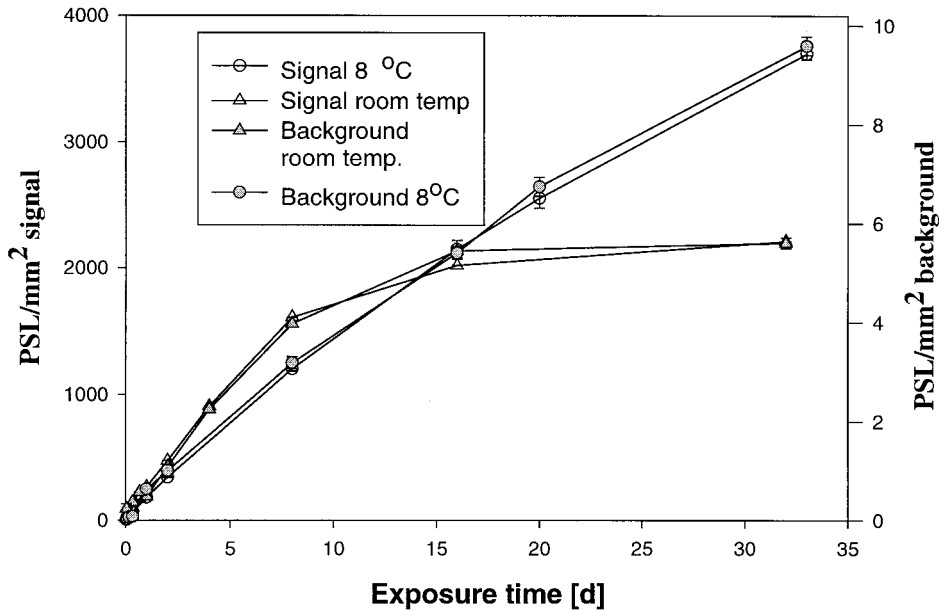


FIG. 1. Dependency of signal and background values on exposure time at room temperature and under cooling conditions ($<8^{\circ}\text{C}$).

ethanol, erased for about 1 h, and stored in the shielding box until they are used again.

Evaluation

The radioactivity concentrations were calculated from the measurements (PSL/mm^2) with the aid of a calibration curve generated by fitting radioactivity concentrations (Bq/g) and relative intensities ($\text{PSL}-\text{bkg}/\text{mm}^2$) using a linear or logarithmic linear regression method. The effective radiation response ($\text{PSL}-\text{bkg}$) was derived by subtraction of background from the PSL value measured.

Different approaches for determining the limit of detection and of quantitation were used. One is based on the standard deviation of the background and the other is based on the coefficient of variation of radioactivity concentrations.

The limit of detection was defined as 3 times the standard deviation of background values. The limit of quantitation was defined either as 10 times the standard deviation of background values or as the radioactivity concentration for which the coefficient of variation was less than 20%.

RESULTS

Influencing Factors

The values obtained in quantitative radioluminography varied with the nature of the tissue, section thickness, area of the section being integrated, the imaging plates, and the conditions of exposure.

Nature of the Tissue and Section Thickness

Thicker sections produce a stronger signal and thus in principle allow higher sensitivity, but the sensitivity improvement is limited by the nonlinearity of this relationship owing to self-absorption (Maas *et al.*, 2000, Klein *et al.*, 2000).

Exposure Time

The measured signal increased proportionally with the increase of duration of exposure from 1 to 7 days, for both room temperature and refrigerated conditions. The measured signal then plateaued after 7 days at room temperature, but continued to increase in the presence of cooling (Fig. 1).

Shielding

Lead shielding reduces the blank value especially for longer exposure times and thus greatly improves the signal/background ratio.

Contamination and Memory Effect

Three kinds of contamination artifacts may be distinguished:

1. Contamination due to carryover: Lyophilized material may adhere to the imaging plates by means of electrostatic charge.
2. Permanent contamination of the imaging plates: Strongly lipophilic compounds may penetrate into the protective layer of the image plates, during previous exposures. Contamination can often be prevented by using a protective film.

TABLE 1
Limit of Detection for Imaging Plates after 1, 3, 4, and 7 Days of Exposure

Unit	Area (mm ²)	1 day	3 days	4 days	7 days
3 times SD of background PSL/mm ²	1.3			0.75	
	10	0.22	0.49	0.34	0.32
	42			0.39	
	66	0.20	0.43	0.32	0.38
Bq/g	42			7	
	10 and 66	18	15	8	6

Note. The limit of detection was defined as 3 times the standard deviation of the background values and is given as PSL/area or as Bq/g of wet tissue.

3. Memory effects: Exposures with high radiation doses can produce long lasting images especially if the plates are not adequately erased.

All three effects can adversely affect the sensitivity of the method by several orders of magnitude. Although contamination can be reduced by standardizing the procedures, a careful evaluation of each individual exposure is necessary, e.g., on the basis of background noise.

Flare Effect

With many scanners (including the Fuji BAS 2000 used here) a flare effect in the scan direction occurs as a result of the scanning mechanism. In flare-affected regions quantitative evaluation is strongly hampered or even impossible.

LOD and LOQ under Selected Conditions

Limit of Detection

Signals are classified as detectable if they can be differentiated from background noise with a sufficient degree of reliability, i.e., if they are at least 3 standard deviations above the background level. Then, the limit of detection was defined as 3 times the standard deviation of the background values. The limit of detection was calculated for each imaging plate, using 10 background values with an area integrated of 10 or 66 mm². The results are presented in the Table 1. There was an increase of the standard deviation of the background values between 1 and 3 days of exposure. Then the standard deviation of the background was relatively stable between 3 and 7 days of exposure. The limit of detection was lower when the exposure time increased (18, 15, 8, and 6 Bq/g after 1, 3, 4, and 7 days of exposure, respectively). The limit of detection was independent of area integrated, when the area was 10,

42, or 66 mm², but dependent on area integrated, when the area was 1.3 mm².

Limit of Quantitation

The limit of quantitation was defined as 10 times the standard deviation of background values or as the radioactivity concentration for which the coefficient of variation was less than 20%.

LOQ defined as 10 times the standard deviation of background values. The limit of quantitation was calculated for each imaging plate, for each exposure using 10 background values with an area integrated of 10 or 66 mm². The limit of quantitation decreased when the exposure time increased (60, 50, 26, and 20 Bq/g after 1, 3, 4, and 7 days of exposure, respectively). The limit of quantitation was independent of area integrated, when the area was 10 or 66 mm² (Table 2).

LOQ defined as the radioactivity concentration for which the coefficient of variation was less than 20%. The limit of quantitation was determined with standard blood calibration curves for each imaging plate, after several exposures by measurements of blood areas of different sizes. Examples of mean, standard deviation, and coefficient of variation of signals or radioactivity concentrations of different blood standard sections of different region sizes exposed several times are presented in Table 3.

The coefficient of variation was less than 20% for all the blood standards, except for the 17 Bq/g blood standard, and the limit of quantitation was equal to 60 Bq/g after 4 days of exposure using an area of integration of 42 or 3.5 mm². For an area of integration of 1.3 mm², the limit of quantitation was equal to 265 Bq/g after 4 days of exposure. In general the limit of quantitation was higher when the area of integration was smaller.

TABLE 2
Limit of Quantitation for Imaging Plates after 1, 3, 4, and 7 Days of Exposure

	Unit	Area (mm ²)	1 day	3 days	4 days	7 days
10 times SD of background	Bq/g	10 and 66	60	50	26	20
	Bq/g	10	64	54	28	18
	Bq/g	66	56	47	26	22
CV < 20%	Bq/g	10 and 66	74	65	33	24
	Bq/g	42 or 3.5			60	
	Bq/g	1.3			264	

Note. The limit of quantitation is given as Bq/g wet tissue and was defined either as 10 times the standard deviation of the background values or as the radioactivity concentration for which the coefficient of variation was less than 20%.

TABLE 3

Mean, Standard Deviation (SD), and Coefficient of Variation (CV) of Measured Signals (PSL/mm²) of Different Blood Standard Sections of Various Region Sizes Exposed Several Times for 4 Days below 8°C

Activity (Bq/g)	Region size 42 mm ²			Region size 3.5 mm ²			Region size 1.3 mm ²		
	Mean (PSL/mm ²)	SD (PSL/mm ²)	CV (%)	Mean (PSL/mm ²)	SD (PSL/mm ²)	CV (%)	Mean (PSL/mm ²)	SD (PSL/mm ²)	CV (%)
17.3	1.4	0.3	22.1	1.5	0.3	22.2	1.7	0.7	39.4
60.1	3.9	0.2	6.0	4.5	0.5	11.5	3.9	0.7	18.4
186.1	11.3	0.4	3.4	11.8	1.6	13.3	12.9	2.8	21.9
264.9	15.2	0.7	4.8	16.0	0.7	4.2	15.5	1.6	10.5
2041.3	116.4	5.9	5.1	125.1	8.0	6.4	124.8	8.9	7.2
6683.7	395.9	10.3	2.6	405.8	9.5	2.3	431.4	55.6	12.9
20246.9	1066	39.7	3.7	1047	37.3	3.6	1109	88.9	8.0

DISCUSSION

The limit of detection and quantitation were determined with fixed parameters as the section thickness (30 or 40 μm) and variable parameters (as area integrated and exposure time). The limit of quantitation was lower when the exposure time increase from 1 to 7 days and was independent of the area integrated from 3.5 to 66 mm². Therefore, to have the highest sensitivity a long exposure time (e.g., 7 days) and an integrated area larger than 3.5 mm² is recommended.

Whatever method was selected to determine the limit of quantitation (defined as 10 times the standard deviation of background values or as the radioactivity concentration for which the coefficient of variation is less than 20%), the results were similar (60, 50, 26, and 20 Bq/g or 74, 65, 33, and 24 Bq/g after 1, 3, 4, and 7 days of exposure, respectively). Nevertheless, to determine the limit of quantitation using the radioactivity concentration for which the coefficient of variation was less than 20%, several exposures of the same imaging plates containing the blood standard sections, with a wide range of concentrations, should be performed. A LOQ, obtained for one imaging plate after several exposures, varied with the blood standard sections used. On the other hand, when using the standard deviation of background values, the LOQ is obtained for each imaging plate after each exposure. Therefore, the latter method to determine the LOQ can be used as an in process control.

CONCLUSION

The sensitivity of RLG is influenced by a large number of factors, some of which like exposure time or temperature can be controlled by standardizing the procedure; others like contamination and flair effect must be carefully estimated for each individual measurement. Under routine conditions of whole-body radioluminography reliable quantification can be performed at least down to 60 Bq/g wet tissue or even below. Concentrations of 6 Bq/g wet tissue can be well detected.

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