

Development and validation of a robust analytical method for the determination of ^{51}Cr in blood samples by liquid scintillation counting (LSC)

Myroslav V. Zoriy¹ · M. Froning¹ · P. Hill¹

Received: 3 May 2016 / Published online: 20 August 2016
© Akadémiai Kiadó, Budapest, Hungary 2016

Abstract A robust analytical method for the measurement of ^{51}Cr in blood samples in routine mode by means of liquid scintillation count was developed and validated with spiked blood samples from different subjects. Prior to the measurements, the samples were microwave digested in order to eliminate the matrix influence. Figures of merits, such as sensitivity, limits of detection, precision and accuracy were studied and confirmed the capability of the method to determine ^{51}Cr in blood samples as low as 0.5 Bq g^{-1} of sample.

Keywords ^{51}Cr · Liquid scintillation counting · Blood samples

Introduction

^{51}Cr is a radioactive isotope of chromium having a half-life of 27.7 days. It decays 91 % of the time by electron capture directly to the ground state of ^{51}V without gamma rays emission. Only 9 % of ^{51}Cr decays directly to the excited state of the daughter ($^{51\text{m}}\text{V}$), which then further decays by isomeric transition to the ground state and emitting a 320 keV gamma ray during the process [1, 2].

At present, ^{51}Cr is used in many application fields, in particular, in medicine [3, 4]. On one side, due to its chemical properties, ^{51}Cr is an excellent tool for the studies

with labeled red blood cells in order to measure their survival time, mass or volume. In sequestration studies labeled platelets are used for the diagnosis of gastrointestinal bleeding [5]. On the other side, gamma emissions from ^{51}Cr present an external dose hazard [6]. The retention of ^{51}Cr in the body (biological half-life) is very dependent on its chemical form (Cr (III) or Cr (IV)). According to ICRP [7], 5 % of the uptake is transferred to bones (and retained with a biological half-life of 1000 days), 30 % is directly excreted and 65 % is distributed to other organs and tissues in the body.

Nowadays, ^{51}Cr activity is usually determined by conventional γ -spectrometry [8–10]. In spite of the fact that the method needs almost no (or minimum) sample preparation steps, careful attention for the calibration geometry should be paid. For instance, the calibration should be performed with a homogenised calibration standard (for blood samples this is not always easy). The limits of detection achieved by γ -spectrometry for ^{51}Cr are normally in the range of 10 Bq g^{-1} depending on the type of detectors. Moreover, for such analysis relatively long counting times are required [11, 12]. However, for some clinical studies, where the incorporated doses are sometimes restricted, a method with better detection capability would be preferable.

Since ^{51}Cr decays by electron capture, it could also be detected using liquid scintillation counting (LSC) [13, 14]. Due to its simplicity, sensitivity, and relative simple sample preparation, this method could be a suitable alternative for γ -spectrometry in order to determine the ^{51}Cr in blood samples.

In the present study we developed a robust analytical method for the measurement of ^{51}Cr in blood samples in routine mode by means of LSC. The method was validated with spiked blood samples from different subjects.

✉ Myroslav V. Zoriy
m.zoriy@fz-juelich.de

¹ Division of Safety and Radiation Protection,
Forschungszentrum Jülich GmbH, 52428 Jülich, Germany

Experimental

Reagents and sample solutions

All acids and reagents used for the development and validation of the current testing method were of analytical grade or better: 65 % v v⁻¹ HNO₃ (Suprapur, Merck, Darmstadt, Germany); 10 % v v⁻¹ H₂O₂ (Suprapur, Merck, Darmstadt, Germany). A standard certified solution of ⁵¹Cr (Cr (III) in 0.2 M HCl) was supplied by Eckert & Ziegler Nuclitec GmbH Braunschweig, Germany (Certificate Nr. 85449-443). For all dilutions, ultrapure water (18.2 MΩ cm) from Merck Millipore (Darmstadt, Germany) water system was used.

For all experiments in this work, blood samples from different subjects spiked with a known amount of ⁵¹Cr standard solution were used.

Instrumentation and methods

In order to reduce the influence of matrix on the LSC measurements as well as to solubilise the Cr, all samples were digested using a micro-wave digestion system MARS (CEM, Germany). one ml of blood sample, one ml of HNO₃ and one ml H₂O₂ were mixed and digested at 180°C for 30 min. Afterwards, the sample solution was neutralised with two ml of NH₃. No residue of the sample was observed.

The measurements in this study have been performed using a LSC HIDEX 300SL (FCI, Mainz, Germany). This instrument incorporates a triple-PMT detector technology facilitating absolute activity counting without external radioactive source using triple-to-double coincidence ratio (TDCR) [15, 16]. For the measurements Ultima Gold LLT cocktail was added to digested blood samples in a sample to cocktail ratio of 5–10. High density polyethylene plastic vials from HDPE of 20 ml were used in order to ensure a low background as well as a high counting efficiency. The measurements were done without quench curve corrections. Typical TDCR values for ⁵¹Cr in blood samples observed in the current study were in the range of 0.5–0.6. The samples were counted 3600 s.

For comparative measurements of ⁵¹Cr activity, γ -spectrometric system equipped with HPGe detectors (Ortec, UK) as well as whole body counter with HPGe detectors (Canberra, USA) installed at Competent Incorporation Monitoring Body Jülich (Jülich, Germany) were used.

Results and discussion

Sensitivity

To study the sensitivity of the developed method laboratory standards were prepared by spiking of one ml aliquots of a

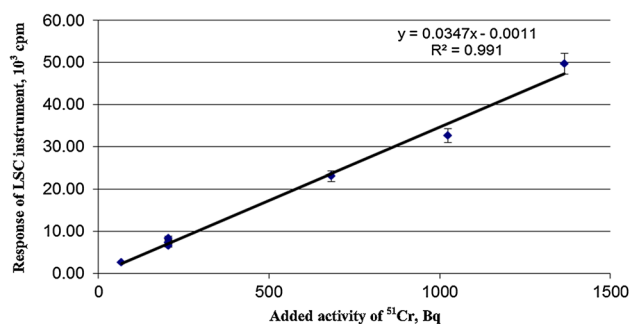


Fig. 1 Sensitivity response for ⁵¹Cr activity obtained with LSC Hidex 300SL

same blood sample with known activities of ⁵¹Cr. In total, eight samples were prepared, comprising of one blank, one sample of 67 ± 4 Bq, three samples of 200 ± 10 Bq, one sample of 670 ± 20 Bq, one sample of 1000 ± 30 Bq and one sample of 1370 ± 35 Bq of ⁵¹Cr. The measured instrument response is shown on the Fig. 1.

A linear function fits the measured activity data with the regression line $y = 0.0347x - 0.0011$ very well and a correlation coefficient of $R^2 = 0.991$ was obtained.

A detection capability of the method was determined in accordance to DIN ISO 11929 (assuming $k_\alpha = 3$ and $k_\beta = 1645$). A detection limit of 0.5 Bq g⁻¹ for ⁵¹Cr in blood samples was achieved with a counting time of 3600 s.

Accuracy

To determine the accuracy, four test samples (*accuracy samples*) with known ⁵¹Cr activities (67 ± 4, 200 ± 10, 670 ± 20, and 1000 ± 30 Bq), prepared independently from the *sensitivity samples*, were measured. For further quality assurance the same experiments were additionally repeated after 1 and 3 days. The results are presented in Table 1.

A very good correlation between the added amount of ⁵¹Cr and the measured values was observed. The difference from the reference value was between -12 and 5 %.

Table 1 Difference in ⁵¹Cr activity from its reference value determined with the developed LSC procedure in spiked blood samples

Expected ⁵¹ Cr activity, Bq/sample	Deviation (%)		
	Day 0	Day +1	Day +3
67 ± 4	-8.9	-11.8	-9.4
200 ± 10	-10.9	-8.4	-8.4
670 ± 20	-2.9	-2.9	-2.7
1000 ± 31	5.0	2.6	4.8

Table 2 Coefficients of variation for the different activities of ⁵¹Cr measured by developed LSC procedure

⁵¹ Cr activity, Bq/sample	CV, % on “day 0”	CV, % on “day + 1”	CV, % on “day + 3”
67 ± 4	5.0	4.4	5.3
200 ± 10	4.4	3.5	3.1
670 ± 20	2.3	2.4	1.8
1000 ± 31	1.3	2.4	2.2

Precision

In order to access the precision of the measurements a coefficient of variation (CV) for the *accuracy samples* was evaluated using Eq. (1):

$$CV = \frac{SD}{MEAN} \times 100 \tag{1}$$

where CV is the coefficient of variation, SD is the standard deviation of the measurements and MEAN is the mean value of the measurements

The same samples were measured 10 times and the coefficient of variation was calculated. The results summarized in Table 2 show that the precisions (CV) of the measurements ranged from 1.3 to 5.3 % and were dependent on the activity of the samples.

To determine a “between run precision” (agreement between individual experimental results obtained with the same method on identical test material or samples, under the same conditions) [17] all the *accuracy samples* were measured again after 1 and 3 days (see Table 2). As shown in the table no significant difference in the precisions was observed.

Stability

The stability (time-resolved) of ⁵¹Cr measurements by means of LSC was determined for two blood samples spiked with the activities of ⁵¹Cr of 775 ± 30 and 1510 ± 70 Bq, respectively. These samples were repeatedly measured during a selected period of time. The observed curves for the two measured samples were corrected for the physical decay of ⁵¹Cr. After the correction (see Fig. 2b) the long term precision of the measurements was calculated to be 0.7 and 0.8 % for the 775 ± 30 and 1510 ± 70 Bq of ⁵¹Cr samples, respectively. This proved an additional insurance of reliable determination of ⁵¹Cr in blood samples.

Comparative measurements

To further validate the developed method comparative determination of ⁵¹Cr activity in two synthetically prepared blood samples using three independent techniques: LSC,

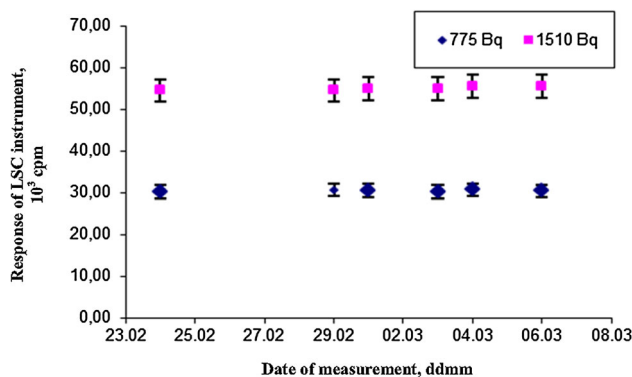


Fig. 2 Response of the LSC instrument for two laboratory-prepared standards with the activity of ⁵¹Cr of 775 ± 30 and 1510 ± 70 Bq, respectively, measured during the selected period of time. The results are corrected with respect to the decay of ⁵¹Cr

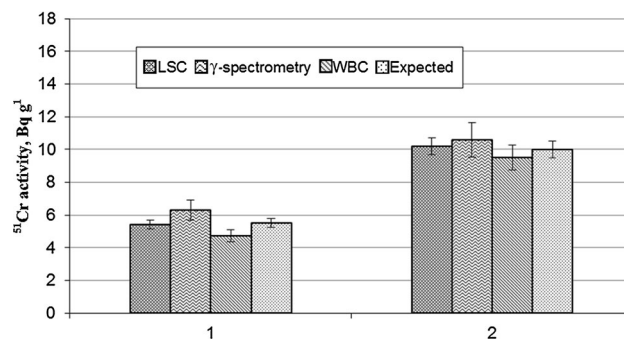


Fig. 3 Comparative measurement of ⁵¹Cr activity in laboratory-prepared blood samples 1 and 2 using three different independent testing methods: LSC, γ -spectrometry and WBC

conventional γ -spectrometry and whole body counter (WBC) were performed. These techniques utilize different analytical principles for ⁵¹Cr analysis (LSC measure the electron capture of ⁵¹Cr, while γ -spectrometry and WBC analyze its γ -emission). The data of these measurements are presented in Fig. 3. A good agreement between the results of all testing methods was obtained. They corresponded to the expected activities of ⁵¹Cr in both measured samples within the measurements error of applied techniques.

Conclusions

In this work we demonstrated the capability of the developed testing procedure for the determination of ^{51}Cr in blood samples by means of LSC. The method meets all quality requirements for the reliable determination of ^{51}Cr in blood samples. Performed studies on sensitivity, accuracy, precision and stability fit the acceptance criteria and confirm the developed procedure being applicable for the measurements of ^{51}Cr in blood samples. Comparative measurements using different (independent) methodologies such as conventional γ -spectrometry as well as WBC additionally prove the competency of developed LSC procedure for the determination of ^{51}Cr in blood samples. The uncertainty for these measurements was not exceeding 12 %. A limit of detection of 0.5 Bq g^{-1} for ^{51}Cr in blood samples was obtained.

Acknowledgments The author would like to thank Dr. Schläger and Dr. Kummerle for the measurements with WBC and γ -spectrometry, respectively, as well as for their valuable discussions. The help of the radioanalytical laboratory staff was also very much appreciated.

References

- Chechev VP, Kuzmenko NK (2016) Decay Data Evaluation Project (DDEP): updated decay data evaluations for Na-24, Sc-46, Cr-51, Mn-54, Co-57, Fe-59, Y-88, Au-198. *Appl Radiat Isot* 109:139–145
- de Almeida MCM, Iwahara A, Poledna R, da Silva CJ, Delgado JU (2007) Absolute disintegration rate and 320 keV gamma-ray emission probability of Cr-51. *Nucl Instrum Methods Phys Res Sect A* 580:165–168
- Fuglsang S, Henriksen U, Hansen H, Bendtsen F, Henriksen J (2016) Gamma-variate plasma clearance versus urinary plasma clearance of ^{51}Cr -EDTA in patients with cirrhosis with and without fluid retention. *Clin Physiol Funct Imaging*. doi:10.1111/cpf.12336
- Delgado R, Sanders TM, Bloor CM (1975) Renal blood flow distribution during steady-state exercise and exhaustion in conscious dogs. *J Appl Physiol* 39:475–478
- Vandermeulen E, De Sadeleer C, Piepsz A, Ham HR, Dobbeleir AA, Vermeire ST, Van Hoek IM, Daminet SV, Slegers G, Peremans KY (2010) Determination of optimal sampling times for a two blood sample clearance method using Cr-51-EDTA in cats. *J Feline Med Surg* 12:577–583
- Shoji M, Kondo T, Honoki H, Nakajima T, Muraguchi A, Saito M (2007) Investigation of monitoring for internal exposure by urine bioassay in a biomedical research facility. *Radiat Prot Dosimetry* 127:456–460
- ICRP Publication 30. Part 2.1980 Limits for Intakes of Radionuclides by Workers Pergamon Press Oxford
- Hermanne A, Takacs S, Adam-Rebeles R, Tarkanyi F, Takacs MP (2013) New measurements and evaluation of database for deuteron induced reaction on Ni up to 50 MeV. *Nucl Instrum Methods Phys Res Sect B* 299:8–23
- Moreira DS, Koskinas MF, Yamazaki IM, Dias MS (2010) Determination of Cr-51 and Am-241 X-ray and gamma-ray emission probabilities per decay. *Appl Radiat Isot* 68:596–599
- Soliman AHM, Al-Abyad M, Ditroi F, Saleh ZA (2016) Experimental and theoretical study for the production of Cr-51 using p, d, He-3 and He-4 projectiles on V, Ti and Cr targets. *Nucl Instrum Methods Phys Res Sect B* 366:19–27
- Kikunaga H, Takamiya K, Hirose K, Ohtsuki T (2015) Comparison of the decay constants of Cr-51 with metal, oxide, and chromate chemical states. *J Radioanal Nucl Chem* 303:1581–1583
- Sakita GZ, Meira DC, Bremer Neto H, Cyrino JEP, Abdalla AL (2015) Chromium oxide ((Cr₂O₃)-Cr-51) used as biological marker was not absorbed by fish. *Arquivo Brasileiro De Medicina Veterinaria E Zootecnia* 67:755–762
- Bobin C, Thiam C, Chauvenet B, Bouchard J (2012) On the stochastic dependence between photomultipliers in the TDCR method. *Appl Radiat Isot* 70:770–780
- Carles AG, Gunther E, Malonda AG (2006) The photoionization-reduced energy in LSC. *Appl Radiat Isot* 64:43–54
- Wanke C, Kossert K, Naehle OJ (2012) Investigations on TDCR measurements with the HIDEX 300 SL using a free parameter model. *Appl Radiat Isot* 70:2176–2183
- Wisser S, Frenzel E, Dittmer M (2006) Innovative procedure for the determination of gross-alpha/gross-beta activities in drinking water. *Appl Radiat Isot* 64:368–372
- Freiser H (1992) Concept calculator in analytical chemistry. CRS Press, Boca Raton