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Original Article

A novel method for blood volume estimation using trivalent chromium in rabbit models

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ABSTRACT

Background: Blood volume measurement though important in management of critically ill-patients is not routinely estimated in clinical practice owing to labour intensive, intricate and time consuming nature of existing methods. **Aims:** The aim was to compare blood volume estimations using trivalent chromium [^{51}Cr(III)] and standard Evans blue dye (EBD) method in New Zealand white rabbit models and establish correction-factor (CF). **Materials and Methods:** Blood volume estimation in 33 rabbits was carried out using EBD method and concentration determined using spectrophotometric assay followed by blood volume estimation using direct injection of ^{51}Cr(III). Twenty out of 33 rabbits were used to find CF by dividing blood volume estimation using EBD with blood volume estimation using ^{51}Cr(III). CF is validated in 13 rabbits by multiplying it with blood volume estimation values obtained using ^{51}Cr(III). **Results:** The mean circulating blood volume of 33 rabbits using EBD was 142.02 ± 22.77 ml or 65.76 ± 9.31 ml/kg and using ^{51}Cr(III) was estimated to be 195.66 ± 47.30 ml or 89.81 ± 17.88 ml/kg. The CF was found to be 0.77. The mean blood volume of 13 rabbits measured using EBD was 139.54 ± 27.19 ml or 66.33 ± 8.26 ml/kg and using ^{51}Cr(III) with CF was 152.73 ± 46.25 ml or 71.87 ± 13.81 ml/kg (P = 0.11). **Conclusions:** The estimation of blood volume using ^{51}Cr(III) was comparable to standard EBD method using CF. With further research in this direction, we envisage human blood volume estimation using ^{51}Cr(III) to find its application in acute clinical settings.

KEY WORDS

Blood volume; Evans blue dye; measurement; trivalent chromium

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INTRODUCTION

Blood volume estimation is one of the cardinal parameter necessary in cases of burns, sepsis, management of critically ill-patients undergoing major surgeries and in intensive care settings.[1,2] Considerable blood volume depletion or hypovolemia leads to clinically identifiable "shock," which requires

emergency intervention. Smaller blood volume depletion leads to normotensive compensated shock associated with vasoconstriction of visceral organs such as gastrointestinal tract, skin, and kidneys. This diminished perfusion to the splanchnic organs results in inadequate oxygenation to the gut mucosa precipitating to a cascade of pathophysiological events proceeding to development of sepsis.[3]

The existing approaches for blood volume estimations are labour intensive, intricate and time consuming. In the last many years, research has brought various methods for blood volume estimation. The widely used methods employ dye dilution and radioisotope techniques. The dye dilution technique using Evans blue dye (EBD) method has limitations due to hypersensitivity reactions in patient and tissue discoloration. The radioisotope labelling methods involving either albumin or red blood cells (RBCs) is time consuming, cumbersome and cannot be repeated on the same patient due to the prolonged stay of radioactive material in the body in the labelled form.[4]

The gold standard method of blood volume estimation using radioisotopes, though accurate has complex procedures, forcing the clinicians to opt and practice on easier and less accurate methods such as history, physical examination, systemic blood pressure, central venous pressure and urine volume.[5-7] It is important to note these methods do not allow for calculation of absolute blood volume. Available reports indicate inaccurate volume assessment holds many risk factors, often leading to inappropriate, unnecessary therapeutic intervention and mortal outcomes.[8]

The aim of this experimental study using New Zealand white rabbit model was to compare blood volume estimation using [^{51}Cr(III)] with that of EBD method and to establish a correction-factor (CF), which can be further related for human application.

MATERIALS AND METHODS

The study was approved by the institutional animal ethics committee. A total of 33 New Zealand white rabbits (*Oryctolagus cuniculus*), which includes 11 males and 22 females weighing approximately 1.5-3.1 kg were used in this experiment. All animals were kept on dietary restriction to stabilise fluctuations in blood volume during the experimental period. Ten minutes before the experiment, rabbits were placed in restrainer and anaesthetised with ketamine hydrochloride (25 mg/kg body weight) injection through the marginal ear vein. The site of injection and blood sample collection was prepared by shaving the area on both ear and gently wiping with 70% alcohol swab.

An intravenous (IV) cannula of 26 GA (BD Neoflon™ Singapore) was inserted to the marginal vein of one of the ear and secured it with an adhesive plaster. This was used for injection of drug or dye in to the vascular system. In to the other ear to withdraw blood a 24 GA (BD Neoflon™ Singapore) IV cannula was placed in the central auricular artery and secured with an adhesive plaster.

All animals were assigned to go through blood volume measurement using EBD method described by Armin *et al*. followed by ^{51}Cr(III).[4,9] An initial (blank) blood sample of 3.2 ml was withdrawn from the auricular artery followed by injection of 1 ml of 0.2% EBD (Himedia Lab. Pvt. Ltd, Mumbai) solution in normal saline through the marginal vein. Subsequently, 0.5 ml saline was used to wash the injected dye. After 8 min, another 2.2 ml blood sample was withdrawn and after this at every 8 min interval three 2.2 ml blood samples were withdrawn.

All samples were collected in heparinised vacutainer and plasma separated from whole blood samples. EBD content was determined by means of a spectrophotometric assay (Thermo scientific GENESYS 10UV, USA). A volume of 1 ml plasma of each blood sample was placed in a 1 ml spectrometer cuvette and the absorbance read at 650 nm. The concentration of dye was calculated from the standard curve of EBD solution in pooled plasma of rabbits.[10]

Plasma volume V, was obtained from $V = \frac{C_{st} \times V}{C_{sa}}$

Where; C_{st} = Dye concentration of standard (2000 μg/ml)
V = Volume of injected EBD
C_{sa} = Dye concentration of samples corrected for dye loss and for spontaneous dilution.

Correction for spontaneous dilution of the blood

Intermittent blood withdrawal from rabbit in a brief time period often leads to slight decrease in levels of plasma proteins, which leads to alteration of plasma EBD concentration during measurements. Noble *et al*. suggested a formula for correction of fluid shift assuming

that the proportion of T-1824 labelled protein to total protein remains constant during the experiment.[11,12]

$$C = \frac{C_n \times A_1}{A_n}$$

Where; C = Corrected concentration of the n^{th} sample of plasma with dye
C_n = The observed concentration of dye in the n^{th} sample
A_1 = The total protein concentration of the 1^{st} plasma sample with dye
A_n = The total protein concentration of the nth dyed sample.

Determination of C_{sa}

C_{sa} is the concentration of dye that would be found if the T-1824 were distributed without loss through circulating plasma. This concentration is required in calculating the plasma volume. Estimation of C_{sa} was done by extrapolation back to zero time.[9]

Determination of haematocrit

Haematocrit was measured by centrifuging 100 mm columns of blood in 3 mm bore Wintrobe tube at 3000 rpm for 30 min in a centrifuge head of 15 cm radius.[13] All packed cell volume measurements were multiplied by 0.95, since 5% of the packed cell volume consists of plasma.

Calculation of blood volume

Estimation of blood volume was calculated from measured plasma volume and haematocrit,[14]

$$\text{Blood volume} = \frac{\text{Plasma volume}}{1 - \text{Hct}}$$

Blood volume estimation using ^{51}Cr(III)

2 mCi of ^{51}Cr in aqueous solution was obtained from Board of Radiation and Isotope Technology, DAE, GOI. Two hours prior to the estimation of blood volume using ^{51}Cr(III), a syringe was loaded with ^{51}Cr(III) by reducing ^{51}Cr in aqueous solution with freshly prepared ascorbic acid (Sisco Research Laboratories Pvt. Ltd, Mumbai.) solution (0.2 mg/ml), which is sufficient to transform entire ^{51}Cr in aqueous solution to ^{51}Cr(III).[15] Activity of the loaded syringe was determined in a calibrated Nucleonix Gamma Ray Spectrometer having NaI (Tl) based well type scintillation detector coupled to single channel analyser.

Approximately, 3000 counts per second (cps) of ^{51}Cr(III) in 1 ml were injected initially to the animal through the IV cannula inserted to the marginal vein followed by 0.5 ml saline to wash the injected ^{51}Cr(III) solution. The syringe used for injection of the dose then collected and measured for the remaining activity. The dose mentioned above is the actual dose that has gone in to the animal by subtracting the left over activity in the syringe. An electronic timer was preset for a minute before the experiment and switched on immediately following injection. After a minute, 1 ml blood sample were obtained after dilution from the central artery and its activity determined in a well type scintillation counter for 360 s.

Blood volume was obtained from the equation,

$$V_2 = \frac{C_1 V_1}{C_2}$$

Where; C_1 = Concentration of tracer injected
C_2 = Concentration of the tracer in the unknown volume
V_1 = Volume of tracer injected
V_2 = Unknown volume to be measured

Development of correction-factor

Entire sample size (n = 33) were randomly divided using statistical package for social sciences (SPSS 15.0, IBM Corporation) in to two groups. The CF was calculated by dividing the estimated blood volume using EBD with blood volume estimated using radioactive ^{51}Cr(III) as discussed by Modi *et al*.[16] Group I, with 60% of the total sample size (n = 20) were used to develop CF and the mean CF obtained. Group II, with 40% of the total sample size (n = 13) were used for validation of CF.

Validation of the correction-factor

The CF obtained from Group I was then applied to Group II by multiplying the CF to each value obtained in Group II. After applying CF, the data were analysed for any significant difference between Group II and blood volume estimation using Evans blue method.

Statistical analysis

Blood volume measurement using EBD and direct injection of ^{51}Cr(III) were analysed using paired *t*-test and by the method of Bland and Altman plot for assessing the agreement between two methods of clinical measurement.[17] All results were expressed in mean ±

standard deviation (SD) and $P < 0.05$ was considered to be statistically significant. SPSS 15.0 was used for the analysis.

RESULTS

The data of 33 rabbits shows a mean body weight of 2.18 ± 0.35 kg, mean arterial packed cell volume of 35.11 ± 3.85%. The mean circulating blood volume (CVB) using EBD was 142.02 ± 22.77 ml or 65.76 ± 9.31 ml/kg. In this group, subsequent determination of the mean CVB by injection of ^{51}Cr(III) was estimated to be 195.66 ± 47.30 ml with the mean CVB being 89.81 ± 17.88 ml. On an average, 53.64 ml (95% confidence interval (CI) - 39.57, 66.86 ml) is the difference in blood volume between the two methods ($P < 0.001$).

A Bland Altman plot of blood volume obtained using EBD and direct injection of ^{51}Cr(III) is shown in Figure 1. The red line indicates the limits of agreement and green line indicates the mean of the two measurements. The limits of agreement were from −23.6 to 130.2 ml.

Development of correction-factor

Owing to the difference in the blood volume values in the two methods of blood volume estimation and a statistically significant difference therein, CF was developed. Among the 33 rabbits, 20 randomly selected rabbits (60% of total number) were used for the development of CF and the remaining 13 rabbits were used for validating the CF. The fourth column in Table 1 gives the ratio of the both methods. The mean and SD of the ratio is 0.77 ± 0.14, which was used as the CF. The values of 20 rabbits selected for CF development is given below in Table 1.

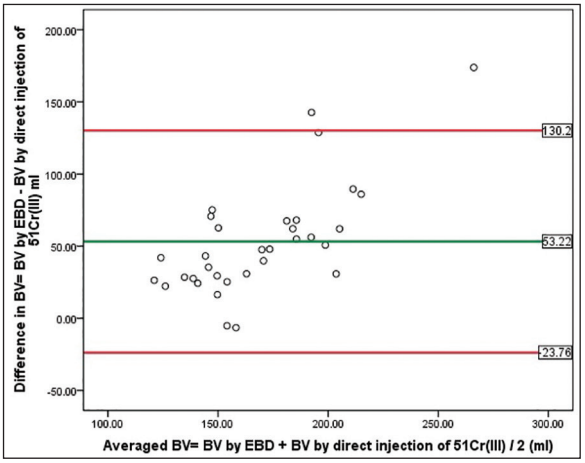

Figure 1: Blood volume obtained using Evans blue dye and direct injection of ^{51}Cr(III) without applying correction-factor (n = 33)

Validation of correction-factor

In the validation group, mean blood volume of 13 rabbits measured using EBD was 139.54 ± 27.19 ml or 66.33 ± 8.26 ml/kg body weight. In the same group, blood volume measurement using ^{51}Cr(III) without applying CF showed a mean blood volume of 199.21 ± 60.33 ml or 93.73 ± 18.01 ml/kg body weight. On an average, 59.67 ml (95% CI - 35.41, 83.94) is the difference between the two methods ($P < 0.001$). In the same group, the mean blood volume measurement by ^{51}Cr(III) with CF was 152.73 ± 46.25 ml or 71.87 ± 13.81 ml/kg body weight. The average difference is 13.19 ml (95% CI - 3.44, 29.83) between the two methods ($P = 0.110$) [Table 2].

A Bland Altman plot for blood volume obtained using EBD and direct injection of ^{51}Cr(III) with CF in the validation group is shown in Figure 2. The plot showed that the limits of agreement were from 68.26 to −41.86. The 95% CI for the lower limits of agreement is −70.68 to −13.04 ml and for the upper limit is 39.44-97.07 ml.

DISCUSSION

Accurate and rapid measurement of blood volume in critical care situations is a challenge for the clinicians in intensive care units. Inability to fix the volume

Table 1: Development of CF

Rabbits serial number	CBV (ml) using EBD (A)	CBV (ml) by injection of ^{51}Cr(III) (B)	A/B
1	134.86	164.27	0.82
2	166.50	256.11	0.65
3	150.73	190.55	0.79
4	131.23	260.00	0.50
6	188.25	219.00	0.86
7	156.64	151.47	1.03
8	152.90	215.00	0.71
13	146.12	193.64	0.75
14	122.68	165.86	0.74
15	120.55	148.99	0.81
18	149.51	197.40	0.76
19	128.02	163.36	0.78
22	174.16	236.20	0.74
23	161.42	154.90	1.04
24	141.51	157.87	0.90
26	158.18	213.03	0.74
27	124.98	152.50	0.82
32	121.12	263.77	0.46
33	147.50	178.35	0.83
34	109.75	184.90	0.59

CBV: Circulating blood volume; CF: Correction-factor; EBD: Evans blue dye

Table 2: Comparison of blood volume obtained by EBD, ^{51}Cr(III) without applying CF and ^{51}Cr(III) with CF

Rabbit serial number	CBV (ml) using EBD	Blood volume (ml/kg)	CBV (ml) by injection of ^{51}Cr(III) without CF	Blood volume (ml/kg)	CBV (ml) by injection of ^{51}Cr(III) with CF	Blood volume (ml/kg)
5	147.42	74.72	214.97	108.96	164.82	83.54
9	151.52	86.98	219.64	126.08	168.40	96.67
10	141.49	73.69	166.72	86.83	127.82	66.58
11	107.85	59.39	134.18	73.89	102.88	56.65
12	103.08	58.84	145.03	82.78	111.19	63.47
16	128.68	60.07	152.91	71.39	117.24	54.73
17	118.80	59.64	181.49	91.11	139.15	69.85
20	172.00	60.99	258.00	91.49	197.81	70.14
21	179.22	63.42	353.04	124.93	270.68	95.78
25	164.20	60.82	220.36	81.61	168.95	62.57
29	114.93	67.37	137.17	80.40	105.17	61.65
30	111.38	66.61	182.11	108.92	139.62	83.51
31	173.39	69.75	224.09	90.14	171.81	69.11
Mean	139.54	66.33	199.21	93.73	152.73	71.87
SD	27.19	8.26	60.33	18.01	46.25	13.81

SD: Standard deviation; EBD: Evans blue dye; CBV: Circulating blood volume; CF: Correction-factor

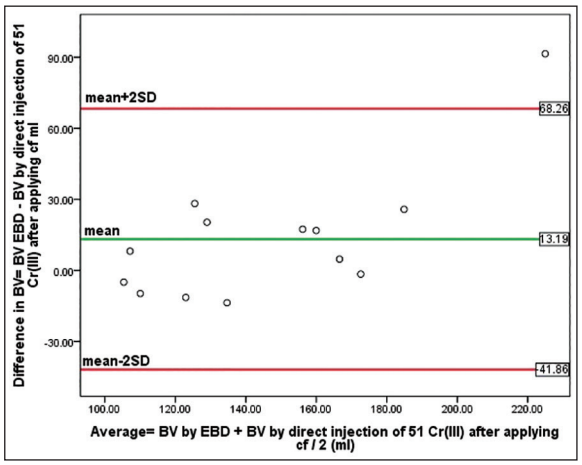

Figure 2: Blood volume obtained using Evans blue dye and direct injection of ^{51}Cr(III) with correction-factor (*n* = 13)

status in hypovolemic situations increases morbidity and mortality in critically ill-patients. Although there are many techniques existing for measurement of CVB, there is dearth for a method, which is easy, reliable and repeatable in nature for use in routine clinical settings. The available methods are inherently complex and time consuming and most dye methods results in unacceptable levels of tissue discoloration and associated hypersensitivity reactions. Moreover, inaccuracies associated with radioactive labelled RBCs or albumin limits their use in blood volume measurement.[18]

This study explores the possibility of using ^{51}Cr(III) to overcome the shortcomings mentioned above. We compared the measurements obtained by trivalent chromium with that of EBD dilution technique. The blood volume values obtained by EBD method were comparable with the existing normal values available for New Zealand white rabbits.[19,20] Initial experiments in rabbits using hexavalent chromium we observed higher blood volume values owing to the fact that during mixing it is permeable through the cell membranes and uptake by various tissues is comparatively higher leading to overestimation of blood volume. Subsequently, we used ^{51}Cr(III), which is not permeable through cell membranes and relatively less up taken by various tissues as established by Gray and Sterling.[21] The blood volume values obtained with ^{51}Cr(III) also showed significant difference with that of EBD method. This difference is attributed to the loss of ^{51}Cr(III) in to the interstitial space, third space loss, excreted in urine and other body secretions. This loss of ^{51}Cr(III) necessitates the development of CF.

After applying CF to blood volume value obtained by 51Cr (III) in the validation group, the two methods were comparable and the mean difference between the two groups was 13.19 ml. Among the measured blood volume values of the 13 rabbits in the validation group, value of one of the rabbits was above two SD and this may be due to an experimental error. After eliminating this value, which was above two SD, the mean difference was found to be only 6.67 ml. In 2007, in a pilot study, we used hexavalent chromium to find a simple, quick and method, which can be used in clinical settings.[4] In this study with better understanding about loss of hexavalent chromium due to its ability to cross cell membranes, we selected trivalent chromium.[21]

Table 3: Cost and time comparison with other human study

Items	ICSH recommended methods	Fluorescein-labelled HES	Trivalent chromium (present study extrapolated to humans)
Man power	National regulatory board certified scientist	State registered medical laboratory scientific officer	National regulatory board certified scientist
Costs of reagents (per patient)	Radiolabeled albumin INR 331.05*, sodium chromate INR 156.98*	INR 35.98*	INR 60†
Procedure and processing time	Automated 1.9 h; manual 2.3 h	Automated 0.7 h; manual 1.9 h	Manual 0.15 h
Costs of infrastructure	Site approval from national regulatory board	Registration not required	Site approval from the national regulatory board
	Conversion of space to allow radioisotope handling INR 16, 93, 248.71*	Conversion not required	Conversion of space to allow radioisotope handling INR 1, 50, 000†
	Laminar flow hood INR 5, 15, 378.60*	Laminar flow hood not necessary	Laminar flow hood not necessary
Instrumentation	Scintillation detector INR 2, 20, 922.42*	Fluorometer with printer INR 2, 94, 563.67*	Scintillation detector INR 2, 00, 000†
	PC/acquisition INR 2, 20, 922.42*	Maintenance INR 1472.65*	Maintenance INR 10, 000†
	Maintenance INR 3682.06*		

*Cost estimate for ICSH recommended methods and fluorescein-labelled HES as on 2004, †Cost estimate for trivalent chromium method as on 2014, ICSH, HES. ICSH: International committee for standardisation in haematology; HES: Hydroxyethyl starch

Time taken for blood volume estimation by EBD method in this study was approximately 1.7 h (average). This method is not used in human studies because of reports of tissue discoloration and suspicion of carcinogenicity.[3]

The present experimental study was conducted in New Zealand white rabbits. Hence, it is not possible to compare cost with other available human studies without any extrapolation. On extrapolation of our experiment to human experiment for cost analysis, we found that total cost for one reference Indian adult will be approximately INR 1000. The time required for estimation was found to be 0.15 h, which is much less, compared to various methods quoted in the literature [Table 3].[22]

CONCLUSION

The method offers an alternative for existing blood volume estimation methods which is easy, quick, can be done in clinical settings and repeatable as ^{51}Cr(III) is not bound to albumin or RBCs. The values obtained are comparable with the standard Evans blue method with the CF. The validity of this technique in situations where cardiac output is very low is uncertain. We need to look at the applicability of this method in acute clinical settings and there is a need to develop CF for it. To conclude estimation of blood volume using ^{51}Cr(III) was comparable to standard EBD method using CF. With further research in this direction we foresee human blood volume estimation using ^{51}Cr(III) to find its application in acute clinical settings.

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REFERENCES

1. Fiddian-Green RG, Haglund U, Gutierrez G, Shoemaker WC. Goals for the resuscitation of shock. Crit Care Med 1993;21: S25-31.
2. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, *et al.* Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med 2001;345:1368-77.
3. Jones JG, Wardrop CA. Measurement of blood volume in surgical and intensive care practice. Br J Anaesth 2000;84:226-35.
4. Kumar P, Kumar R, Pandit N, Aggrawal A, Baby PM. Estimation of blood volume in burns patients using radioisotope chromium-51: A pilot study. Burns 2007;33:1072-3.
5. Cook DJ. Clinical assessment of central venous pressure in the critically ill. Am J Med Sci 1990;299:175-8.
6. McGee S, Abernethy WB 3rd, Simel DL. The rational clinical examination. Is this patient hypovolemic? JAMA 1999;281: 1022-9.
7. Shippy CR, Appel PL, Shoemaker WC. Reliability of clinical monitoring to assess blood volume in critically ill patients. Crit Care Med 1984;12:107-12.
8. Singh S, Kuschner WG, Lighthall G. Perioperative intravascular fluid assessment and monitoring: A narrative review of established and emerging techniques. Anesthesiol Res Pract 2011;2011:231493.
9. Armin J, Grant RT, Pels H, Reeve EB. The plasma, cell and blood volumes of albino rabbits as estimated by the dye (T 1824) and 32P marked cell methods. J Physiol 1952;116:59-73.
10. Jahr JS, Lurie F, Xi S, Golkaryeh M, Kuznetsova O, Kullar R, *et al.* A novel approach to measuring circulating blood volume: The use of a hemoglobin-based oxygen carrier in a rabbit model. Anesth Analg 2001;92:609-14.
11. Noble RP, Gregersen MI, Porter PM, Buckman A. Blood volume in clinical shock. I. Mixing time and disappearance rate of T-1824 in normal subjects and in patients in shock; determination of plasma volume in man from 10-minute sample. J Clin Invest 1946;25:158-71.

12. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem 1949;177:751-66.
13. Leeson D, Reeve EB. The plasma in the packed cell column of the haematocrit. J Physiol 1951;115:129-42.
14. Recommended methods for measurement of red-cell and plasma volume: International Committee for Standardization in Haematology. J Nucl Med 1980;21:793-800.
15. Liu Y, Xu XH, He P. Remediation of Cr(VI) in solution using vitamin C. J Zhejiang Univ Sci B 2005;6:540-2.
16. Modi AC, Guilfoyle SM, Morita DA, Glauser TA. Development and reliability of a correction factor for parent-reported adherence to pediatric antiepileptic drug therapy. Epilepsia 2011;52:370-6.
17. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307-10.
18. Margarson MP, Soni NC. Plasma volume measurement in septic patients using an albumin dilution technique: Comparison with the standard radio-labelled albumin method. Intensive Care Med 2005;31:289-95.
19. Removal of blood from laboratory mammals and birds. First report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Lab Anim 1993;27:1-22.
20. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, *et al.* A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol 2001;21:15-23.
21. Gray SJ, Sterling K. The tagging of red cells and plasma proteins with radioactive chromium. J Clin Invest 1950;29:1604-13.
22. Massey EJ, de Souza P, Findlay G, Smithies M, Shah S, Spark P, *et al.* Clinically practical blood volume assessment with fluorescein-labeled HES. Transfusion 2004;44:151-7.

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