

Erythrocyte and Plasma Volume Measurement

JP Esser, Meander Medical Centre, Amersfoort

NOTE: no changes have been made since the version of 2007

Warning:

¹²⁵I human serum albumin and ¹³¹I human serum albumin are not registered products.

1. Introduction

The isotope dilution technique is used to determine red blood cell and plasma volumes. In order to determine erythrocyte volumes, red blood cells are labelled with ⁵¹Cr sodium chromate. This radiopharmaceutical attaches itself more or less permanently to the erythrocytes and the loss of erythrocytes during the investigation period of 1 h is negligible.

In order to determine plasma volumes, ¹³¹I or ¹²⁵I human serum albumin is used, which contains less than 1% free iodine. Loss of albumin from the blood circulation (transcapillary) is usually less than 4% per hour, which means radioactive washout within one hour of injection is also negligible. The only condition for volume measurement is that the radiopharmaceutical must be well mixed with the circulating blood. The volume is calculated from the quotient of the administered dose and the amount of activity present in the blood sample or plasma sample.

2. Methodology

This guideline is based on available scientific literature on the subject, the previous guideline (Aanbevelingen Nucleaire Geneeskunde 2007), international guidelines from EANM and/or SNMMI if available and applicable to the Dutch situation.

3. Indications

Analysis of polycythaemia; in particular to confirm or rule out polycythaemia vera.

4. Relation to other diagnostic procedures

None.

5. Medical information necessary for planning

- Probability of diagnosis.
- Relevant haematological parameters (Hb, Ht, etc).
- Weight of patient.

6. Radiopharmaceutical

Erythrocyte volume

Preparation: ⁵¹Cr autologous erythrocytes

Nuclide: Chromium-51

Dose: For labelling, 6,0 kBq ⁵¹Cr chromate/kg body weight, of which about 85% is administered

Administration: Intravenous

Plasma volume

Preparation: ¹²⁵I albumin or ¹³¹I albumin

Nuclide: Iodine-125 or Iodine-131

Dose: 1,3 kBq/kg body weight

Administration: Intravenous

7. Radiation safety

a. Pregnancy

Considering the relatively low dose, < 0,5 MBq ⁵¹Cr erythrocytes, the benefits of the diagnostic revenues will quickly outweigh the assumed risk for the unborn child. The estimated dose to the unborn child will be 0,007 mGy for a 0,5 MBq dose. For ¹²⁵I - and ¹³¹I - albumin the dose to the unborn child is 0,02 mGy and 0,05 mGy, respectively. For dosages under 100 mGy no health detriment has been reported for unborn children, therefore a dose less than 0,5 mSv is acceptable.

b. Lactation

Considering the low dose to the woman and the fact that there is no known transport of erythrocytes to the mammary glands, the potential risk of radioactivity in the milk will be minimal. In contrast, with ¹²⁵I and ¹³¹I there is transport of iodine to the mammary glands. Postponing breastfeeding for 8 weeks ensures no activity is transmitted to the baby. Due to the long half-life of ¹²⁵I and ¹³¹I, 60 and 8 days respectively, collected milk should not be kept for feeding.

c. Radiation exposure

The effective dose to the patient from 6 kBq ⁵¹Cr /kg is 1 μSv/ 6 kBq, so the effective dose to a 70 kg patient 0,04 mSv for ⁵¹Cr. For ¹²⁵I and ¹³¹I the dose to a patient of 70 kg will be 0,02 mSv and 0,06 mSv, respectively.

NB. It is assumed the sizes of the relevant organs are independent of the weight of the patient.

8. Patient preparation/essentials for procedure

The patient should lie down for 15 min before the investigation starts (in order to achieve a stable distribution of blood in the body). Then, a venous cannula (venflon) is inserted (so blood can be withdrawn at the required times without tourniquet pressure).

Essentials for procedure:

- A three-way tap allowing the syringe containing the radiopharmaceutical to be flushed three times.
- Saponin to lyse the blood in the standard flask as well as the full blood count samples.
- For erythrocyte labelling: a sterile, pyrogen-free 30 ml siliconized vial containing 2,5 ml ACD solution (0,8% citric acid, 2,45% glucose, 2,2% trisodium citrate).

Procedure:*Conditions*

Good results are obtained if both the erythrocytes and the plasma volume are calculated.

Erythrocyte and plasma volumes can be calculated simultaneously or sequentially. The technique produces good results when the following conditions are met:

- a. Always follow the protocol exactly, when administering activity and withdrawing blood, and when dealing with samples in the laboratory.
- b. If you wish to measure the erythrocyte and plasma volumes simultaneously and immediately following the administration of two tracers (one for the plasma volume and one for the erythrocyte volume), it is important to use radioactive tracers that can be detected independently.

To measure the erythrocyte and plasma volumes sequentially, the erythrocyte volume must be measured first and the plasma volume second. The last plasma sample used for measuring the erythrocyte volume is also used as a blank sample for measuring the plasma volume.

Extrapolation is not necessary because the erythrocyte activity does not decrease significantly in the time it takes to measure plasma volume. There is no spectral interference between the radionuclides, nor is it necessary to use a channel ratio method. ^{131}I albumin can also be used instead of ^{125}I albumin. However, the measuring window of the scintillation counter must be wide enough to detect both peaks (^{51}Cr (320 keV) and ^{131}I (364 keV)).

- c. All of the radioactive tracer must be added to the compartment that is to be measured (the circulating blood volume). In other words, no surplus radioactivity may remain in needle or syringe, the total activity must be administered and no extravasation may occur; any of these factors will result in an overestimated volume. The use of a three way tap is recommended when administering accurate volumes (no less than 10 ml for high accuracy) so the syringe can be flushed three times with a sterile saline solution.
- d. Complete mixing is essential; samples must be taken until the concentrations in the compartment to be measured are stable. Complete mixing of the tracer in the blood circulation usually takes approximately 10 min (up to 30 min for patients with polycythaemia and splenomegaly). In order to check whether this condition has been met, not just one but several samples are taken sequentially (e.g. after 10 and 20 min, or 30 and 60 min for patients with polycythaemia and splenomegaly).
- e. The radioactivity is determined in samples that contain an accurately known volume. The standard and other samples must have the same counting geometry (3,0 ml).
- f. No radioactivity may 'leak' out of the circulatory system; loss of tracer from the blood circulation can be avoided and/or limited by using a suitable radioactive tracer which does not extravasate within the measuring time (approximately 1 h) and which is known to be sufficiently stable for this purpose. Given the extremely small amounts used, it is not necessary to inhibit the uptake of free, radioactive iodine by the thyroid gland.
- g. The blood withdrawn must be anticoagulated and the volume of anticoagulant must be negligible in comparison to the volume of blood collected. Blood must never be taken from the arm into which the radioactive tracer has been administered (to avoid crosscontamination); blood flow may not be restricted (e.g. by using a tourniquet) when blood is being collected.

There is no gold standard available to verify the method and it is therefore essential

that the entire procedure is carried out under conditions that are as reproducible as possible.

Erythrocyte volume

- a. Withdraw 10 ml of blood from the patient, preferably using a 1,1×40 mm needle (0,8×40 mm may also be used), attach a new needle to the syringe and transfer the blood into 2,5 ml ACD solution. Gently invert the container several times in order to mix the solution.
- b. Withdraw 4 ml of blood using an EDTA-tube for haematocrit determination. Flush the system with a heparin solution (3,3 ml heparin/100 ml saline). Flushing is important because the system will be used at a later stage for blood sampling.
- c. Add 6 kBq ⁵¹Cr sodium chromate/kg body weight in no less than 0,2 ml saline solution to the ACD blood solution. Incubate on a rotor at 37°C for 20 min. Add 50 mg vitamin C in order to reduce the surplus Cr⁶⁺ chromate to Cr³⁺, and allow to rotate for a further 3 min on the rotor.
- d. Subsequently administer 10 ml of the ACD solution to the patient in as precise a volume as possible; do not use a smaller volume as this could compromise accuracy! Flush the system three times to ensure quantitative injection of the tracer.
- e. Withdraw blood at 10 and 20 min post injection and also at 60 minutes if necessary (see conditions).
- f. To make the standard, 1 ml labelled ACD blood is pipetted into a test tube and washed twice with saline solution. Every single erythrocyte must be used. The washed erythrocytes are then quantitatively transferred to a 250 ml measuring flask containing approximately 5 ml of saponin. (Saponin is used to lyse the erythrocytes). Homogenise and pipette 3 ml into a standard counting tube.
- g. In order to determine the concentration of radioactivity in the erythrocytes present in the blood samples, a correction is made for radioactivity in the blood plasma thus avoiding the need for these small quantities to be washed. For this purpose, 3 ml of each 10 ml homogenised blood sample is pipetted into counting tubes and lysed with saponin. The remainder of each blood sample is centrifuged, after which 3 ml plasma samples from the same p.i. times are transferred to counting tubes. In this way, corrections can be made for non-radioactive cells in the blood samples.

Plasma volume

- a. The injection fluid must contain 50 mg albumin carrier per ml and 0,13 kBq ¹²⁵I albumin or ¹³¹I albumin per kg body weight per ml.
- b. The patient dose is 1,3 kBq per kg body weight (in 10 ml administered as accurately as possible in as precise a volume as possible; do not use a smaller volume as this could compromise accuracy). Flush the system three times to ensure quantitative injection of the tracer.
- c. Withdraw blood at 10 and 20 min post injection and also at 60 min if necessary (see conditions).
- d. To make the standard, 1 ml of the injection fluid is accurately pipetted into a 250 ml measuring flask containing 5 ml of 20% NaOH and 1 drop of saturated potassium iodide (KI) solution. Fill up to 250 ml, homogenise and pipette 3 ml into a standard counting tube.

For a combination of erythrocyte and plasma volume using a single injection: A single injection solution and a single standard is made using both radiopharmaceuticals when erythrocyte and plasma volume measurement is carried out simultaneously. The albumin radioactivity is added to the previously labelled erythrocyte suspension. It is therefore not necessary to add albumin as a carrier.

9. Acquisition and processing

- All samples are counted for 20 min or a minimum of 10,000 counts in order to achieve the statistical accuracy required. The measuring equipment must be adjusted to the photo peaks of the radionuclides used.
- ^{125}I HSA and ^{51}Cr must be used if two isotopes are to be combined in a single injection. A channel ratio method can then be applied so interference from Compton radiation in the ^{125}I channel can be measured and used to calculate the net ^{125}I counts.
- Photo peaks:
 ^{125}I : 30 keV
 ^{131}I : 364 keV
 ^{51}Cr : 320 keV

10. Interpretation

- In the event of raised Hb levels, determining a patient's erythrocyte volume can help to distinguish between relative polycythaemia and primary polycythaemia (polycythaemia vera), secondary polycythaemia or idiopathic erythrocytosis. Erythrocyte volumes are normal in cases of idiopathic erythrocytosis.
- Theoretically, it should be possible to calculate the erythrocyte volume using the haematocrit and the calculated plasma volume. However, the peripheral haematocrit may not be used as an indicator of the central haematocrit as the latter is always lower than the peripheral value. In other words, both volumes must be determined independently if information about both volumes is required.

11. Report

- The report must contain reference values. The blood volume is given as the sum of the plasma and erythrocyte volumes.
- The International Committee on Standardization in Haematology (ISCH) published recommendations for determining plasma and erythrocyte volumes in 1973 and 1980. The reference values are shown in the table below.

Table 1. Reference values in ml/kg.

	Erythrocyte volume *	Plasma volume
Males	30 ± 5	40
Females	25 ± 5	40

* average ± 2 SD (ml/kg)

- c. Medical literature shows a wide variance of normal values for plasma volume, probably due to the different radiopharmaceuticals used and the natural variance in plasma volume. Therefore, only average values are presented in the table. Volumes are expressed in ml per kg body weight (based on total body weight). It would, of course, be better to use the lean body mass (total body weight minus fat) for calculations, but total body weight is nevertheless recommended as lean body mass values are not generally known in practice.

12. Literature

- Bentley SA, Lewis SM. The relationship between total red cell volume, plasma volume and venous haematocrit. *J Haematology* 1976;33:301-7.
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