# Guidelines for the Measurement of Glomerular Filtration Rate using Plasma Sampling

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## 1. Purpose

The purpose of these guidelines is to assist specialists in Nuclear Medicine in recommending, performing, interpreting and reporting the results of glomerular filtration rate (GFR) studies. It will help individual departments in the development of local protocols

## 2. Background

Glomerular filtration rate is a commonly accepted standard measure of renal function. It is routinely measured using tracers that are cleared exclusively by glomerular filtration, the most common being Cr-51 EDTA and Tc-99m DTPA.

The guidelines are being written specifically in response to the results of an audit carried out during 2001 on the values of GFR calculated at different centres in the UK. This showed that there was considerable variability in the results obtained from the analysis of the same set of experimental data (Cosgriff et al, 2002). The variation is due principally to the different data analysis techniques being used.

The generally accepted gold standard technique for GFR assessment uses inulin infusion. This technique was found to be difficult and time consuming to perform and was therefore considered inappropriate for routine clinical use. Measurement of Cr-

51 EDTA clearance from a single injection emerged as an adequate simpler technique (Garnett et al, 1967, Brochner-Mortensen et al, 1969). GFR was calculated from the area under the plasma clearance curve, which required multiple blood samples to be taken over a period of several hours. Although simpler than inulin infusion, it was still labour intensive and the technique was further simplified by restricting the blood sampling to the second of the two exponential components of clearance (Chantler et al, 1969). This is known as the slope-intercept method. This simplification introduced systematic errors in the values of GFR obtained and various methods of correction have been derived (Chantler et al, 1969, Brochner-Mortensen et al, 1972, Chantler and Barratt, 1972). The methodology underwent even further simplification by reducing the number of samples to one (Fisher and Veall, 1975, Groth, 1984). Empirical relationships between the apparent volume of distribution and GFR were derived which allow GFR to be estimated from a single sample concentration with reasonable precision (Christensen and Groth, 1986, Watson, 1992).

In deciding which method to use as standard, it needs to be considered that several aspects of technical detail may give rise to systematic differences in the GFR value obtained. These include:

(i)      the substance being used

(ii)     whether renal or urinary or plasma clearance is used

(iii)    whether arterial or venous samples are used

It is recommended that the plasma clearance of EDTA from venous samples be taken as the standard measure of GFR. EDTA is the most widely used radiopharmaceutical for GFR measurement in the UK and venous plasma sampling is a precise and convenient technique. If other methods are used then the systematic differences relative to this standard should be noted. DTPA does have some technical advantages over EDTA but normal ranges are not so well established. Small systematic differences have been observed between GFR measurements obtained from EDTA and DTPA (Rehling et al, 1984, Fleming et al, 1991, Biggi et al, 1995). However these are sufficiently small to recommend DTPA as a suitable alternative radiopharmaceutical. It is however recommended that all GFR studies at a given centre should use the same radiopharmaceutical.

It is considered that, among the various methods for measuring EDTA plasma clearance, the slope-intercept method provides the best compromise between accuracy and reliability on the one hand and simplicity on the other hand. The technique involving measurement of the full area under the curve is too time consuming for routine use. The single sample method is recommended by the Radionuclides in Nephrology Committee on Renal Clearance (Blaufox et al, 1996). However, one-sample techniques are generally less precise than the slope-intercept technique (Waller et al, 1987) and the one-sample equation recommended by the committee has been reported as having systematic errors both at low and high GFR (Blaufox et al, 1996, Fleming et al, 2002). There is also no means of quality control for the eventuality of experimental errors such as an extravasated injection. The slope-intercept technique is only a little more complicated and not only provides a significantly improved precision of measurement but also a number of options for quality control checks, which are detailed below.

Various different methods have been described for correcting for the systematic error of the slope-intercept technique. The most commonly used are those of Chantler (Chantler et al, 1969 and Chantler and Barratt, 1972) and Brochner-Mortensen (Brochner-Mortensen, 1972, Brochner-Mortensen et al, 1974). The Brochner-Mortensen technique is preferred as it deals with the variation of percentage error with GFR, which is much larger at higher values. Separate correction equations are described for adults and children, which can be combined using an interpolation according to age. The adult equation has been independently validated for both EDTA and DTPA (Fleming et al, 1991).

By comparison, the Chantler technique tries to correct slope-intercept values of plasma clearance using a single multiplying factor, which does not adequately correct for the variation in percentage error with GFR. Also the Chantler technique tries to correct to inulin urinary clearance, not EDTA plasma clearance, which is the object of the current protocol.

## 3. Common Indications

(i) Monitoring of drugs that might cause nephrotoxicity

(ii) Calculation of dose in chemotherapy (Calvert et al, 1989)

(iii) Detection of renal failure in patients in whom (a) serum creatinine results might be misleading (b) missing a decline in renal function might be disastrous e.g. single kidney, renovascular disease or renal transplant and (c) a 24 h clearance measurement is difficult e.g. elderly or those with learning difficulties.

(iv) Assessment of potential live donors for transplantation e.g. relative of patient on dialysis.

(v) The evaluation and follow up of renal function in chronic glomerulonephropathies such as haemolytic uremic syndrome and diabetes mellitus.

(vi) The evaluation of single kidney function in conjunction with relative renal function measurements from static or dynamic radionuclide imaging.

## 4 Contra-indications

In patients with ascites, oedema or other expanded body space, clearance measurements will be overestimated and urine collection techniques should be used (Blaufox et al, 1996). However it should be noted that clearance estimates using urine samples will be systematically lower than those from plasma sampling alone (Moore et al,2003)

Patients receiving hyperhydration therapy with intravenous fluids

## 5 Radiopharmaceutical

Cr-51 EDTA is generally regarded as the standard radiopharmaceutical for routine GFR measurement in Europe. Tc-99m DTPA is also used particularly in the United

States. Its use had been criticised on the grounds of increased protein binding but recent work (Rehling et al, 2001) has shown that this is not true for many modern commercial preparations. There are some clear advantages of Tc-99m-DTPA (i) measurement of injected activity is easier as it can be assessed with sufficient precision on an isotope calibrator instead of measuring weight, (ii) counting efficiency is higher than for Cr-51 and (iii) it is slightly cheaper. However counting has to be carried out on the same day as the measurement whereas with Cr-51 it can be delayed.

There are indications that DTPA clearance is systematically higher than that of EDTA (Rehling et al, 1984, Fleming et al, 1991, Biggi et al, 1995). Therefore, when obtaining serial measurements on the same patient it is recommended that the same radiopharmaceutical be used, preferably from the same manufacturer.

It should be noted that there are further systematic differences between techniques. EDTA plasma clearance is lower than inulin clearance (Garnett et al, 1967, Chantler et al, 1969). Urine clearance of EDTA is systematically less than that of plasma clearance (Brochner-Mortensen and Rodbro, 1976, Rehling et al, 1984, Moore et al, 2002).

# 6 Procedure

## 6.1 Patient Preparation

To comply with the current legislative regulations ( at the time of writing IR(ME)R 2000), the request card should be completed with all patient details and sufficient clinical details to justify the patient's exposure to radiation

An information sheet about the test should be sent to the patient with the appointment details.

The patient should be adequately hydrated prior to the study. Glomerular filtration (unlike tubular secretion) is not influenced by normal variations in the degree of hydration, because of autoregulation mechanisms. Although specific hydration is not

usually required, a steady intake of fluids over the duration of the study is recommended (approximately 200 ml/h).

Excessive intake of drinks containing caffeine including tea, coffee and Coke should be avoided after 10 pm the night before the test because of their diuretic effect (Clomius et al, 1979).

Protein load may increase GFR (Wilkinson et al, 1991) and it is recommended to avoid high protein meals before the study. Since neither carbohydrate nor fat loads affect GFR, a light meal, low in protein would optimise accuracy and reproducibility. (Levine et al, 1986). A light breakfast is recommended. If the test straddles the lunch period then a light lunch e.g. sandwiches can be taken.

Exercise has been shown to have a variable effect on GFR (Merrill et al, 1948, Kachadorian et al, 1970, Wilkinson et al, 1991). Strict bed rest is unnecessary, but some restriction of activity is necessary for good reproducibility.

Some drugs can reduce renal function e.g. diuretics (frusemide), aminoglycoside antibiotics, penicillins, sulphonamides and aluminium (Blathen et al, 1978) and therefore all medications being taken should be recorded prior to the study being performed.

Patient details should be checked together with an assessment of the possibilities of pregnancy for women of childbearing age (12-55) and of breast-feeding (ARSAC, 1998).

Note patient's height and weight for determination of BSA (Haycock and Schwarz, 1978)

The use of anaesthetic cream or cold spray is optional; not normally needed for adults but useful for children.

The GFR investigation uses very low levels of activity. If other investigations using radionuclides are also to be carried out on the patient such as DMSA or MAG3, the

protocol used will depend on the radiopharmaceutical. With Cr-51 EDTA the tests can be carried out simultaneously and the samples left to decay for 48 h before counting. However, if frusemide is to be administered as part of a MAG3 investigation, the studies will have to be carried out sequentially due to the possible influence of diuretic on GFR. When using Tc-99m DTPA, the GFR should normally be carried out before the imaging procedure. However, when DTPA is being used for both GFR and renography, the tests can be combined, provided that a diuretic is not required. In this case it is necessary to inject an activity higher than that normally required for GFR measurement, and counting should be delayed for 24 hours to avoid count rate loss due to dead time effects.

## 6.2 Syringe preparation

The adult diagnostic reference level for Cr-51 EDTA given by ARSAC (Administration of Radioactive Substances Advisory Committee, 1998) is 3 MBq. For Tc-99m DTPA the suggestion of the current guidelines is for an adult dose of 10 MBq. This is considerably lower than the adult dose of 37 MBq recommended in the European paediatric guidelines (Piepsz et al). The change seems reasonable on the grounds that results with adequate precision can be obtained with a dose of 10 MBq (Fleming et al, 1991).

For Tc-99m DTPA, doses to children should be scaled according to body weight using the table presented in the ARSAC Notes for Guidance (1998). However for Cr-51 EDTA, reducing activity by up to a factor of 10 as suggested by ARSAC (1998) leads to prohibitively long counting times. Therefore these guidelines recommend scaling doses with body weight to a minimum of 1.2 MBq at 3 Kg. This deviation from ARSAC guidelines is considered reasonable in the light of the very low effective dose from administration of 3 MBq Cr-51 EDTA to an adult (0.006 mSv).

A dose vial should be prepared, either for each session of GFR studies or for each individual patient. An appropriately labelled sealed vial (e.g. P6) should be used. The volume of stock solution required for injections and standard should be calculated approximately and the corresponding amount transferred to the dose vial. The calculations should allow for the fact that not all the activity will be removed from the

vial, and, in the case of Tc-99m, also for decay. If required, the solution in the dose vial should be diluted to the required volume, usually with normal saline.

The required activity (+/-10%) should be drawn from the dose vial into a syringe. When using an injection line that requires an interlink rather than a needle, this should be attached prior to dose measurement. The dose is then measured by one of the following methods.

*By weight:* Two possible protocols can be used, (i) *pre and post drawing up the dose -* assessing the difference in weight of the syringe before and after the dose is drawn up and assuming that all the dose is injected and (ii) *pre and post injection -* assessing the difference in weight of the syringe before and after injection. All measurements should be performed using a high precision balance and recorded on the worksheet.

*By activity:* The activity in the syringe is diluted to a fixed volume (typically 1 ml) with 0.9% saline, and measured in a dose calibrator. The background reading on the calibrator is also noted. The background subtracted activity and time of measurement are recorded on the worksheet.

*By volume:* The dose is diluted into a suitable volume using either saline or a solution of non-labelled tracer. It is recommended that prior to implementing this technique, the process of dilution of dose should be verified with the supplier of the radiopharmaceutical. The volume should be large enough to avoid potential errors due to dead space in the syringe (typically 12 ml). A predetermined volume of tracer is then drawn up into a syringe (typically 10 ml for reasonable precision for both the dose and the lower volume standard). To ensure the correct volume is delivered, the syringe should be full right up to the end of the needle or interlink. When injecting, the plunger should never be reversed.

Any of the three methods can be used for DTPA whereas assessment by activity measurement is not possible for EDTA with sufficient precision.

The batch number, expiry date and time of the radiopharmaceutical and saline/hepsal used should also be recorded on the worksheet.

*6.3 Injection technique*

The activity must be injected into the blood stream without extravasation as this will invalidate the result. The possibility of extravasation may be checked using the protocol described in section 6.7. The use of a winged needle infusion set (butterfly gauge 21 or 23) is recommended if no central or peripheral line is available. To eliminate any risk of contamination of the blood samples the tracer must not be injected through the line to be used for blood sampling

Before injecting the tracer, the line should be flushed with 10 ml 0.9% saline to ensure it is working. The tracer is then injected and the line flushed with a further 10ml 0.9% saline. If the line is to remain in-situ, saline/hepsal will be required and this should be administered according to local procedures. Note the time of injection – this is recorded as the midpoint of the administration of the tracer. The clock used should be the same as for the syringe measurement above.

The empty syringe should be assayed for residual activity using the same method used to assess the activity for injection.

*By weight*: (i) *pre and post drawing up the dose method* - the syringe should be thoroughly flushed to ensure that all the activity is injected (ii) *pre and post injection method* – the empty syringe should be weighed and recorded on the worksheet. The syringe should not be flushed when using this method.

*By activity:* The background-subtracted activity remaining in the empty syringe should be measured in the same dose calibrator and recorded on the worksheet. The time of this measurement should also be recorded. This is important, as it is necessary to correct these measurements for decay.

With this technique it is also possible to measure any residual activity in the needle infusion set. Care should be taken when handling to avoid needlestick injury and also radioactive and biological contamination of the calibrator.

*By volume:* It is assumed that all activity is injected.

Techniques that rely on the assumption that all the activity has been injected, may benefit from the use of a 3 way tap to allow the syringe activity to be thoroughly flushed out from the syringe and into the patient

### 6.4 Standard Preparation

The standard should be prepared and measured under the same conditions as the injected dose.

A volumetric flask (typically 1 litre) should be labelled and half-filled with distilled water. An aliquot of the radiopharmaceutical solution is then withdrawn into either a syringe or a pipette. The activity should be such that the corresponding count rate following dilution is in the linear range of the counter. For Tc-99m with a dilution volume of one litre, this may mean using less activity for the standard than for injection.

The standard activity should be measured by the same method used to assess the activity for injection

*By weight:* Again two protocols can be used, (i) *pre and post drawing up the dose -* weighing the syringe before and after the standard dose is drawn up, and assuming that all the dose is emptied into the flask (ii) *pre and post dispensing the dose -* weighing the syringe before and after emptying the standard into the flask.

*By activity:* The activity in the syringe is diluted to the same volume as that used for patient injection with 0.9% saline, and measured in a dose calibrator. The activity and time of measurement should be recorded on the worksheet. The syringe used to prepare the standard should be of the same type and size as that used to draw up the tracer for patient administration.

*By volume:* A predetermined volume of tracer, typically 2 ml, is drawn up using a pipette. A pipette is preferable to a syringe, as it is more precise for the smaller

volume of liquid that is normally used for the standard compared to the injection. The cross calibration of the two methods of volume assessment should be subject to regular quality control using weighing.

The activity should be emptied into the volumetric flask and the volumetric flask filled with water to the mark, capped and the contents thoroughly mixed.

The activity in the empty syringe should be re-measured by the same method as used above and recorded on the worksheet.

*By weight*: (i) *pre and post drawing up the dose method* - the syringe should be thoroughly flushed to ensure that all the activity is injected. (ii) *pre and post dispensing the dose method* - the empty syringe should be weighed and recorded on the worksheet. It should not be flushed if using this technique.

*By activity:* The background-subtracted activity remaining in the empty syringe should be measured in the same dose calibrator and recorded on the worksheet. The time of this measurement should also be recorded. This is important, as it is necessary to correct these measurements for decay.

*By volume:* It is assumed that all the activity is dispensed into the flask

An Eppendorf (or equivalent) should be used to pipette a fixed volume of standard into pre-labelled counting tubes in duplicate. The volume counted will depend upon the counter.

### 6.5 Blood sampling

For routine use two methods are available:

(i) Two, three or four venous blood samples taken at between 2 and 5 hours post injection.
(ii) One sample taken at three or four hours post injection for adults or at two hours for children (Blaufox et al, 1996).

If the full plasma curve is being measured then samples are required at typically 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 min post injection.

To eliminate any risk of contamination, blood samples should not be taken from the site of injection and ideally should come from the contralateral arm. If this is not possible feet can be used. A 21 or 23 gauge needle should be used to withdraw the blood sample to minimise haemolysis. The blood is withdrawn into a syringe and transferred to a collection tube containing dry heparin. Clotting of the blood sample should be avoided but will not affect the measurements. Ideally the volume of each sample should be 10ml for adults and 7 ml for children. A small degree of haemolysis occurs occasionally, giving a slight pink colour to the plasma. This should not unduly affect the GFR result, but marked haemolysis will invalidate the measurement.

If an intravenous line is used to withdraw the blood sample, the line should be cleared of any heparin/saline by withdrawing 5 ml blood and discarding. The sample should then be withdrawn and the line flushed according to local procedures.

The time the blood sample is taken should be recorded on the worksheet. As the procedure may take a minute or two, the time of sampling is taken as the midpoint of the blood collection time and should be recorded to the nearest minute. This should be the same clock as that used to note the time of injection or one synchronised with it.

The blood samples are centrifuged at 1000 g for 10 min to separate the red blood cells from the plasma. This should ideally be done as soon as possible after the samples are taken. If there is any appreciable delay before samples are centrifuged, they should be refrigerated. An Eppendorf (or equivalent) should be used to pipette a fixed volume of plasma (typically 1 ml) into pre-labelled counting tubes in duplicate. Care should be taken to avoid disturbing the interface between the plasma and the red cells. The volume pipetted must be the same as used for the standards above.

If there is insufficient plasma for duplicate plasma samples of the required volume, the maximum volume that can be drawn up, should be determined from the smallest sample. There are then two approaches that might then be used. (i) Use either an adjustable pipette or a fixed volume pipette of appropriate size for both the samples and the standard. (ii) Alternatively a smaller fixed volume pipette can be used for the samples and the counts multiplied up by the ratio of volumes to get the equivalent count rate in the standard volume. In this case the standard sample is prepared as normal. The samples should be made up to the same volume as the standard by adding the appropriate volume of water. This is to ensure the same counting efficiency for both samples and standard. This latter approach is preferred if several studies are being carried out using a single standard.

*6.6 Counting*

After selection of the correct energy peak and window, the plasma samples are counted with appropriate standards and blanks for background in a well counter. Background counts should be subtracted and the duplicate samples and standards averaged to get a final value. This should be done on the day of the test if Tc-99m DTPA is used and a correction should be applied for the decay of Tc-99m during the counting process.

With DTPA, high levels of activity may cause dead time errors. This can be avoided by waiting for 24 h for the activity to decay. High count rates in the standard samples can be avoided by using a larger dilution volume.

Counting times should be long enough to reduce statistical errors. Wherever practical, a minimum of 10 K counts should be recorded for each sample.

If a Tc-99m labelled radiopharmaceutical, such as DMSA or MAG3, has been injected together with Cr-51 EDTA, it is better to wait 48hrs before counting the Cr-51 activity in order to avoid any interference from the Tc-99m activity. However, if an earlier result is required, delayed counting can be avoided by the use of a Cr-51 energy window setting on the gamma counter. In this case it is important to check that the total count rate handled by the detector does not cause dead time problems

and also that there is no contribution from multiple Tc-99m counts (280 keV) occurring in the Cr-51 (320 keV) window. The latter problem can be monitored by taking a baseline plasma sample prior to the Cr-51 EDTA injection.

*6.7 Quality Control*

Quality control must be performed on all equipment (balance, dose calibrator, well counter etc) used in the procedure.

If Tc-99m DTPA is being prepared in house, then regular chromatography on the labelling efficiency should be performed.

It is possible to check for extravasation of the injection using a hand held radiation monitor. The hand or arm used for injection is extended as far as possible away from the body and the count rate over the injection site noted. This is compared to the equivalent site on the contralateral limb.

Errors in sampling are detected by counting in duplicate. A substantial difference (greater than about 3%) in counts between duplicates indicates an error. For the standard this may be either due to pipetting error or in the homogeneity of the standard solution. New samples of the standard should be prepared and re-counted. Significant errors in sample duplicates require the test to be repeated.

If Tc-99m DTPA is being used and the standard is measured in an isotope calibrator then it is possible to calculate the counting efficiency of the gamma counter in count rate per unit activity. This should be a consistent value, typically around 840000 c/s/MBq.

Contamination of the well from samples tubes can be detected by noting the count in background samples placed at the beginning and end of the sample group.

Other quality control checks are covered in the Interpretation and Report section.

## 7 Data analysis

## 7.1 Initial Data Reduction

After the plasma samples and standard have been counted in the gamma counter, the raw data are in the form of counts per minute. However, before the GFR can be calculated it is necessary to interpret the plasma counts in terms of the standard. There are several different conventions for doing this, but one that is convenient and simple to understand is to use the standard to convert the plasma counts into the concentration of tracer in plasma expressed as the percentage of injected dose per litre (%/l). Let $CPM_i$ be the raw counts per minute (c.p.m.) of the $i^{th}$ plasma sample, $CPM_{bck}$ the c.p.m. for the background, and $CPM_{std}$ the c.p.m. for the standard. Let $V_{std}$ be the total volume of water in which the standard dose was diluted, $Q_{pat}$ the activity of tracer used to inject the patient, and $Q_{std}$ the activity of tracer used to make the standard. Then the plasma concentration $P_i$ of the $i^{th}$ plasma sample can be calculated using the following equation:

$$P_i = \frac{Q_{std}}{Q_{pat}} * \frac{(CPM_i - CPM_{bck})}{(CPM_{std} - CPM_{bck})} * \frac{100}{V_{std}} \quad \%/litre \qquad (1)$$

The time interval after injection $t_i$ that the $i^{th}$ plasma sample was taken is found by taking away the time of day for the injection from the time of day that each blood sample was taken. After these calculations the counts data are reduced to a set of plasma concentrations and times ($P_i$, $t_i$).

## 7.2 GFR Calculation using the Bi-exponential Fitting Method

Provided that there is sufficient plasma data available starting at early enough times after injection (say at 5, 10, 15, 30, 45, 60, 90, 120, 180, 240 min after injection) the GFR can be calculated using the fundamental definition (Sapirstein et al, 1955):

$$GFR = Q / \int_0^\infty P(t) \, dt \qquad (2)$$

where $\int_0^\infty P(t)\,dt$ is the area under the plasma concentration curve (AUC) from time zero to infinity.

Although this is a time consuming method, it may be useful for research studies or in circumstances where it is desirable to avoid the assumptions involved in estimating a correction for the AUC when only the terminal exponential is sampled. To use the bi-exponential method the plasma concentration and time data are fitted to the sum of two exponentials:

$$P(t) \; = \; A \exp(-at) \; + \; B \exp(-bt) \tag{3}$$

The fit of the data to Equation 3 can be done using either a least squares curve fitting program or graphically by using the later samples from 2 hours onwards to determine the terminal exponential, and then subtracting the slow exponential from the earlier plasma points and fitting a fast exponential term to the differences. After the four coefficients A, a, B, b are determined the AUC term in the denominator in Equation 2 is calculated as:

$$AUC \; = \; A/a + B/b \tag{4}$$

If the plasma concentration values are expressed as % of injected dose per litre of plasma then Q, the amount of tracer injected in the patient, equals 100%. If time since injection is measured in minutes, then the GFR value calculated using Equation 2 is in units of litres $min^{-1}$. It is therefore necessary to multiply by 1000 to convert the GFR into the conventional units of ml $min^{-1}$.

*The Correction for Body Surface Area*

The value of GFR calculated in the way described above is in absolute units of volume per minute for that individual patient. In order to interpret this result and compare it with the reference range, it is necessary to correct for the effect of body size on GFR. It is conventionally assumed that the normal value of GFR for any individual scales with their body surface area (BSA). It is therefore necessary to

correct the measured GFR to a nominal BSA figure for "standard man", for whom a BSA value of 1.73 m$^2$ is assumed. The GFR value is therefore corrected to the standard BSA using the equation:

$$\text{GFR}_{\text{Corr}} = \text{GFR} * (1.73 / \text{BSA m}^2) \tag{5}$$

Values of BSA may be estimated from the patient's height and weight using the Haycock formula (Haycock et al, 1978):

$$\text{BSA (m}^2) = 0.024265 * \text{Wt}^{0.5378} * \text{Ht}^{0.3964} \tag{6}$$

where Wt is the patient's body weight in kilograms and Ht their height in centimetres.

The BSA corrected GFR can be distinguished by referring to it in units of ml/min/1.73m$^2$.

The Haycock formula is recommended, as it is based on a broad range of subjects and is consistent with the European paediatric guidelines (Piepsz et al). However, the differences resulting from the use of different BSA formulae are relatively small. If an alternative equation such as that due to Du Bois and Du Bois (1916) is already in widespread use in a particular centre, then it seems reasonable to continue using this. However the small systematic differences from the standard approach should be borne in mind when interpreting results.

*7.3 GFR Calculation using the Slope-Intercept Method*

For all routine clinical purposes GFR can be satisfactorily estimated by using 2, 3 or 4 plasma samples to determine the terminal exponential of the plasma clearance curve. The first of these samples should be taken no earlier than 2 hours after injection if a contribution from the fast exponential is to be avoided. In patients with normal GFR the $2^{nd}$, $3^{rd}$ or $4^{th}$ samples should be taken between 3 and 5 hours after injection. In patients with renal failure, later sampling may be worthwhile. The AUC contribution from the terminal exponential dominates the total AUC in Equation 4, and a

correction can be made to allow for the relatively small fraction of the total AUC missed by not including the fast exponential in the measurements.

Because data on only the slow exponential is used, this method is frequently referred to as the slope-intercept method. The 2, 3 or 4 plasma samples are fitted to the single exponential equation:

$$P(t) \ = \ P_0 \exp{(-kt)} \tag{7}$$

The single exponential fit is conveniently done by taking the natural logarithm of the plasma concentrations. If more than two samples are used conventional linear regression analysis of $\log_e P_i$ against $t_i$ is performed to determine the slope, -k, and intercept, $\log_e P_0$. In the case of two samples then the straight line is drawn through the two points. The AUC for the single exponential is given by:

$$AUC_{slow} \ = \ P_0 \ / \ k \tag{8}$$

The value of the slope-intercept GFR (SI-GFR) is now calculated by substituting Equation 8 into Equation 2:

$$SI\text{-}GFR \ = \ 100 * k \ / \ P_0 \tag{9}$$

where we have substituted Q = 100% on the assumption that $P_0$ is in units of % of injected dose per litre of plasma. The ratio $100/P_0$ can be interpreted as the combined volumes of the vascular and extravascular spaces in which the tracer is diluted and is known as the volume of distribution ($V_D$) and is usually measured in litres. With this assumption Equation 9 can be written:

$$SI\text{-}GFR \ = \ V_D * k \tag{10}$$

As noted above, if $V_D$ is measured in litres and k in units of $min^{-1}$, it is necessary to multiply the GFR by a factor of 1000 to express it in units of ml $min^{-1}$.

Because the slope-intercept method ignores the contribution of the fast exponential the total AUC is underestimated, and in consequence the SI-GFR always overestimates the true value of GFR. For patients with low GFR this error is small and may be negligible. But for patients with normal GFR the use of the slope-intercept method can overestimate real GFR by 10 to 20%.

*7.3.1 Correcting the Slope-Intercept GFR*

As with the bi-exponential GFR described above, it is necessary to correct the measured SI-GFR for body surface area before the figure can be compared with reference data. It is also desirable to make a correction for the missing AUC due to the fast exponential. In general it is important that these two corrections are applied in the right order. The BSA correction should be made first:

$$\text{SI-GFR}_{\text{Corr}} = \text{SI-GFR} * (1.73 \,/\text{BSA m}^2) \tag{11}$$

The simplest correction to apply for the missing AUC is the Chantler correction (Chantler et al 1969), which assumes a constant correction factor f:

$$\text{GFR}_{\text{Corr}} = f * \text{SI-GFR}_{\text{Corr}} = f * \text{SI-GFR} * (1.73 \,/\text{BSA m}^2) \tag{12}$$

If the Chantler correction is used it does not strictly matter whether the BSA correction is applied first or second.

In the original paper, the value of f was assessed as 0.93 but a later paper (Chantler and Barratt, 1972) using a combined series of adults and children found f to be 0.87. A recent survey of UK practice revealed that this form of correction was widely used but that different values of the correction constant were being assumed at different centres (Cosgriff et al, 2002).

The Chantler correction amounts to assuming that the missing AUC under the fast exponential scales with AUC under the terminal exponential. This cannot be true, and therefore a more sophisticated correction due to Brochner-Mortensen (BM) is

preferred which uses a quadratic equation (Brochner-Mortensen, 1972). For adults the equation was as follows:

$$\text{BM-GFR}_{\text{Corr}} = 0.9908 * \text{SI-GFR}_{\text{Corr}} - 0.001218 * \text{SI-GFR}_{\text{Corr}}^2 \quad (13)$$

The form of equation was found to be the same for children, but with different coefficients (Brochner-Mortensen et al, 1974).

$$\text{BM-GFR}_{\text{Corr}} = 1.01 * \text{SI-GFR}_{\text{Corr}} - 0.0017 * \text{SI-GFR}_{\text{Corr}}^2 \quad (14)$$

These equations are quite similar and, in the interests of simplicity, it is suggested that a mean correction be applied by averaging the coefficients

$$\text{BM-GFR}_{\text{Corr}} = 1.0004 * \text{SI-GFR}_{\text{Corr}} - 0.00146 * \text{SI-GFR}_{\text{Corr}}^2 \quad (15)$$

If the BM correction is used then it is important that the BSA and AUC corrections are applied in the right order. This is particularly important for results in children when the effects of the BSA correction are greatest.

The absolute GFR is then derived by reversing the BSA correction:

$$\text{BM-GFR} = \text{BM-GFR}_{\text{Corr}} * (\text{BSA m}^2 / 1.73) \quad (16)$$

### 7.4 GFR Estimation using Single-Sample Methods

A large number of different methods have been proposed for estimating GFR from single plasma measurements, usually taken around 3-4 hours after injection for adults (Hamilton et al,1999). These methods work because at any fixed time point there is an inverse relationship between GFR and plasma concentration. It is also important that the errors in estimating the AUC of the terminal exponential are minimised when the sampling time $T \approx 1/k$. It follows that for patients with normal GFR for whom the biological half-life of the terminal exponential is typically 100-120 min, the optimal sampling time is 2.5 to 3 hours. However, unless the sample time is extended in

inverse proportion to GFR, the errors become significantly greater at lower GFR values and may be unacceptably large for GFR values below 30 ml min$^{-1}$.

The single sample method described by the Radionuclides in Nephrology Committee on Renal Clearance (Blaufox et al, 1996) has recently been evaluated (Fleming et al, 2002). This study suggests that the method may give significant systematic errors in GFR of up to 10-15 ml min$^{-1}$ that are GFR dependent. At the present time it is unclear whether this means that improved single-sample algorithms with modified coefficients can be universally applied, or whether the sensitivity to small differences in technique is so great that each Nuclear Medicine Department must calibrate its own algorithm.

Further study is required before any single-sample algorithm can be recommended without the user having to undertake a careful prior evaluation by comparison with a multi-sample method. Users of single-sample methods need to be aware that if a standard sampling time of 3-4 hours is used then measurement errors may become unacceptably large for patients with low GFR. Also, the inherent advantages of the multi-sample methods of several useful internal quality assurance checks are lost with single-sample methods, so that the reliability of the results is critically dependent on avoiding technical errors.

Given the above concerns, we do not recommend the use of the single sample technique. However for users who still wish to use this method, we suggest that, until further information is available, they adopt the algorithm endorsed by the Radionuclides in Nephrology Committee on Renal Clearance (Blaufox et al, 1996). However, it should not be assumed that the method recommended by this group is free from the potential errors associated with single-sample methods.

### 7.5 Implementation

The analysis can be implemented either as a computer program or on a spreadsheet. For departments wishing to change their technique to comply with these recommendations, there are a number of spreadsheets that have been developed for this purpose at centres in the UK.

# 8 Interpretation and Report

## 8.1 Review of output from GFR calculation

Before a clinical report is issued the results of the GFR calculation in each patient should be carefully scrutinised to ensure that all the output data are self-consistent. If 3 or 4 blood samples are used, the goodness of fit to a single exponential should be checked by using either a graphical plot or the value of the correlation coefficient. In patients with a normal GFR, the correlation coefficient $r$ should be greater than 0.985. Experience has shown that only 1% of correct results would trigger this threshold . If the points do not lie on a single exponential curve then applying a single sample GFR formula may help to identify the outlier. If only 2 samples are used then their consistency may be checked using a single sample formula (Fleming et al, 2002). Other quality assurance checks may be made by reviewing the volume of distribution and half-life data. In adults the expected volume of distribution is around 8 times the BSA in $m^2$ with a 2 SD variation of $\pm 25\%$. For women this gives a range 11-17 litres, and in men 13-20 litres. In both sexes the biological half-life in an adult patient with normal GFR is typically in the range 100-120 minutes.

## 8.2 Reference ranges

The normal range for GFR was originally defined by Davis and Shock (1950). This was redefined for Cr-51 EDTA plasma clearance by Granerus and Aurell (1981) for adults between 20 and 75 years. The mean population GFR in young adults was about 105 ml/min/1.73 $m^2$. This fell slowly up to age 50 and then more rapidly. The variation of mean GFR with age can be described by the following equations:

For age (a years) between 20 and 50

$$GFR = 116 - 0.35a$$

For age between 50 and 75

$$GFR = 148 - a$$

The 95 % confidence limits are +/- 25 ml/min.

In children, body surface area corrected GFR increases with age from birth up to two years of age and thereafter remains constant into adulthood (Piepsz et al).

## 8.3 The Clinical Report

It is recommended that the clinical report should at a minimum include the date of the study, the patient's GFR (units: ml/min), the GFR corrected for body surface area (units: ml/min/1.73 $m^2$) and the reference range appropriate for the patient's age. This may be followed by some free text providing a clinical interpretation. The two expressions of GFR are valuable in different contexts and therefore both should be quoted in the standard report format. The report could also clarify which GFR value is to be used in a particular application e.g. if the Calvert formula for adults is to be used then it could be stressed on the report that the absolute GFR should be used (Calvert et al, 1989). In some cases it may be necessary to quote other parameters e.g. biological half-life, which is required by some carboplatin dose formulae in children (Newell et al, 1993).

## 8.4 Repeat Measurements

Variability between repeat measurements in the same subject is probably somewhat larger than the measurement errors. Coefficients of variation (CV) of around 8 to 10% have been reported in the literature (Wilkinson et al, 1991, Blake et al, 1997). It should be noted that the smallest change in GFR that can be regarded as statistically significant with 95% confidence is equal to 2.8 ($= 2\sqrt{2}$) times the CV. This means that a change of at least 20% is required before a measured difference can be regarded as significant.

When the results of repeat measurements are scrutinised, then the follow-up values of the volume of distribution and half-life as well as GFR should be compared. Unless there is ascites or oedema, one would expect real changes in GFR to be reflected in changes in the biological half-life rather than the volume of distribution. It may be preferable to use a single set of height and weight values for the BSA correction for

follow-up measurements. This avoids a potential source of measurement error. Also, any real change in weight is unlikely to affect GFR.

## 9 Pitfalls

Extravasation of injection.

Contamination of blood samples with material from the high specific activity injection.

Incorrect blood sampling technique

Incorrect reporting of sample times, particularly if carried out outside the control of the Nuclear Medicine Department.

Large meal either before or during the study

Heavy exercise during study.

Patients with expanded body space have an overestimated value of GFR.

Patients on drugs which affect renal function, unless these are regular and routine, in which case the GFR measurement reflects the true situation.

Interpretation may be difficult in patients who have had substantial weight fluctuations e.g. due to muscle wasting or are under complex drug regimes.

GFR values are affected both by the radiopharmaceutical and the analysis technique being used. If a department undertakes a change in method, then the systematic differences between the values obtained need to be taken into consideration when interpreting the results.

Interpretation of results in children is difficult as normal ranges are not well defined.

Determination of carboplatin dose using the Calvert formula uses absolute rather than normalised GFR (Calvert et al, 1989). It is important to clarify which GFR result is to be used.

## 10 Controversies

No consensus currently exists on the method of analysis.

The standard equations for calculation of carboplatin dosing for chemotherapy using the Calvert formula (Calvert et al, 1989) are based on GFR values calculated by the Chantler technique (Chantler and Barratt, 1972). Although there will be differences between the values produced by the proposed Brochner Mortensen correction instead of the Chantler method, these are considered unlikely to be clinically significant (Blake et al, 2002).

Recent studies have compared the use of the slope of the second exponential to estimate renal function rather than the GFR but there is no consensus that this provides a more sensitive assessment of changes in renal function (Piepsz and Ham, 1997, Blake et al, 1997, Peters et al, 2001). For example, if volume of distribution changes between sequential studies on a subject for physiological reasons, the slope will change even if GFR remains constant.

One formula for assessing carboplatin dose in children (Newell et al, 1993) is based on GFR measurements in which the one pool correction was not applied. Use of this formula is not to be recommended in view of the potential errors. The authors suggest alternative formulae using measurement of biological half-life, which should be satisfactory.

## 11 Conclusions

These guidelines recommend that for the assessment of glomerular filtration rate using plasma sampling, the following procedure should be used.

(a) Either Cr-51 EDTA or Tc-99m DTPA are suitable radiopharmacueticals for GFR measurement. DTPA gives systematically slightly higher results (Rehling et al, 1984, Fleming et al, 1991 Biggi et al, 1995). However, on average this difference is below 5%, and is considered not to be of clinical relevance. (Rehling et al, 1984)

(b) Two, three or four plasma samples should be taken between 2 and 5 h following injection. Ideally four blood samples should be taken. However very similar results are produced from methods using two or three samples (Waller et al, 1987) and these may be used in the interest of simplicity. Centres choosing this option should however incorporate some method of checking the quality of their technique e.g. by comparing one-samples estimates of GFR from each sample (Fleming et al, 2002).

(c) Methods for assessing administered dose and standard using weight measurements are likely to provide better accuracy and precision. Centres wishing to use other methods for simplicity should ensure that their procedure is validated against a measurement by weight.

(d) Analysis using the slope-intercept method should be used, with the mean Brochner-Mortensen equation described in this report (equation 15) being applied to correct for the one-pool approximation

(e) GFR values should be expressed as both absolute GFR in ml/min and also normalised to body size using body surface area.

## 12 Implementing the Guidelines

Advice regarding the implementation of these guidelines may be obtained either from the local medical physics expert or from the authors.

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