Preparation of Standards for the Calculation of Effective Renal Plasma Flow

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Determination of effective renal plasma flow from single plasma samples requires preparation of quantitative standards for each individual studied. An accurate method that uses one set of standards for many studies is described. Monthly preparation of these standards saves time and money. The use of pipettes rather than syringes to measure volumes minimizes potential errors.

Effective renal plasma flow (ERPF) is an important parameter of renal function that helps clarify the nature of many kidney disorders (*1–7*). Based on the method of Tauxe, et al. (*8*), ERPF can be quite easily and accurately estimated by counting the fraction of the injected dose remaining in a plasma sample drawn 45 min following injection of ^{131}I-orthoiodohippurate (OIH). This procedure is available to any nuclear medicine laboratory that performs OIH renography and is equipped to count radionuclide samples. We have made ERPF determination a routine part of our OIH renogram studies.

The technical aspects of the single plasma sample ERPF determination were described by Kontzen et al. in 1977 (*9*). Because injected counts of OIH must be known in order to calculate theoretical volume of distribution at 45 min, counting of standard solutions of low activity is necessary. We believe the recommended technique (*9*) to prepare these standards has three practical disadvantages:

1. Using injectable OIH as a source for standard solutions adds cost, since OIH is relatively expensive.
2. The time required to make a new set of standards for each patient studied is significant, particularly when the schedule is busy.
3. Significant errors may be introduced when syringes are used to measure volumes accurately as the procedure requires (*9*).

We present an alternative method in which one set of sodium iodide standards are made each month and used for all studies performed that month.

MATERIALS AND METHODS

Preparing Standards

Standard solutions are prepared at the beginning of each month. Starting with a solution of ^{131}I-sodium iodide of approximately 2 mCi in 4 mls, a dose standard (D-STD) and a plasma standard (P-STD) are made.

Using a 1-ml precision pipette, the D-STD is made by pipetting 2 mls of the NaI solution into the barrel of a 3-ml plastic syringe. This is then fitted with a plugged 22-gauge needle, and the plunger is replaced and taped in place. This design matches the geometry of the injected doses of OIH.

To make the P-STD, 1 ml of the ^{131}I-NaI solution is diluted 1:20,000 with water by successive dilutions of 1:100 and 1:200, using a 1-ml precision pipette and volumetric flasks. One milliliter of the last dilution is pipetted into a plastic counting tube. One milliliter of water is then added, and the tube is capped. This matches the geometry of the plasma samples that will be obtained from each patient.

Since both standards derive from the same ^{131}I-NaI solution, there is a constant relationship between the activity of the two standards, such that the P-STD activity is equal to 1 ml/(2 ml × 20,000) or 0.000025 of the D-STD activity.

DETERMINING THE ERPF

We perform the single plasma sample method for determining ERPF in conjunction with ^{131}I-OIH renography using 150–300 μCi of tracer. The dose for the patient is drawn into a 3-ml plastic syringe and is 1.5–2.5 mls in volume. This is measured in a radioisotope dose calibrator,* and the activity of ^{131}I is recorded. Next, the D-STD is measured in the dose calibrator at the same settings and its activity is recorded.

After i.v. injection of ^{131}I-OIH, the syringe, injection lines, and needles are measured in the dose calibrator to determine residual activity. Flushing the injection system with 15–20 mls of saline at the time of injection usually results in a residual activity of < 1 μCi. This is subtracted from the dose activity to calculate the actual dose injected.

At 45 min after injection, a 5-ml blood sample is withdrawn from the opposite arm in a lightly heparinized syringe. This is transferred to a test tube, and spun for 5 min in a high speed centrifuge. Two milliliters of plasma are pipetted into a plastic counting tube identical to that used for the P-STD. The plasma sample and the P-STD are both counted for 3 min in a scintillation well detector. A 3-min background count is subtracted from each, and these counts are then recorded in cts/min/2 mls.

ERPF can be calculated from the apparent volume of distribution of the injected dose at 45 min.

$$V_t, \text{ Volume of distribution (liters)} = I/C_{45}, \qquad \text{Eq. 1}$$

where I is the counts per minute injected and C_{45} is counts

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per minute per liter of plasma at 45 min. This distribution volume (V_t) is then substituted in the exponential equation of ERPF derived by Tauxe et al. (*8*).

$$\text{ERPF (ml/min)} = F_{max}[1 - e^{-\alpha(V_t - V_{lag})}], \quad \text{Eq. 2}$$

where F_{max} is the theoretical ERPF maximum; α, the slope of the curve, V_t, the theoretical volume of distribution at the sampling time; and V_{lag}, the x-axis intercept of the least squares line. For a sampling time of 45 min, F_{max} = 1131.48, α = 0.0078, and V_{lag} = 7.68. In order to calculate volume of distribution, the measured activity of the injected dose and D-STD and the measured counts of the plasma sample and P-STD are used to determine counts per minute injected as follows:

$$\text{I (cpm injected)} = \frac{\mu\text{Ci injected} \times \text{cpm P-STD}}{\mu\text{Ci P-STD}} \quad \text{Eq. 3}$$

and since μCi P-STD = 0.000025 × μCi D-STD,

$$I = \frac{\mu\text{Ci injected} \times \text{cpm P-STD}}{0.000025 \times \mu\text{Ci D-STD}} \quad \text{Eq. 4}$$

Substituting into Eq. 1 gives:

$$V_t = \frac{\mu\text{Ci injected} \times \text{cpm P-STD}}{0.000025 \times \mu\text{Ci D-STD} \times (500 \times \text{cpm/plasma sample})}, \quad \text{Eq. 5}$$

where the 500 in the denominator converts cpm per 2 mls to cpm per liter.

Equation 5 reduces to:

$$V_t = 80 \times \frac{\mu\text{Ci injected} \times \text{cpm P-STD}}{\mu\text{Ci D-STD} \times \text{cpm plasma sample}}. \quad \text{Eq. 6}$$

This value of V_t is then substituted into Eq. 2 to obtain ERPF. A sample calculation follows:

A 32-yr-old renal transplant recipient was studied post-operatively. Creatinine and urine output were both normal.

Syringe before injection (μCi):	166
Syringe after injection:	1
μCi injected:	165
μCi D-STD:	657
cpm background:	73
cpm P-STD:	8075
cpm/2 mls plasma:	3010
Net cpm P-STD:	8002
Net cpm/2 mls plasma:	2937

$$V_t = 80 \times \frac{165 \times 8002}{657 \times 2937} = 54.7 \text{ liters}$$

$$\text{ERPF} = 1131.48\ [1\text{-}e^{-0.0078(54.7-7.68)}] = 347 \text{ ml/min}.$$

TABLE 1. Sample Validation of Standards*

Dose Calibrator Measurements		Three-Minute Counts	
Dose diluted	23.9 μCi	Background	353
D-STD:	806.4 μCi	Net P-STD:	30,696

Aliquot Determinations

Aliquot No.	Net Counts	Net/50	Calculated Volume (liters)
1	30,092	781.84	93.09
2	38,146	762.92	95.40
3	38,267	765.34	95.10
4	37,887	757.74	96.30
5	37,790	755.80	96.05

*Mean Volume = 95.19 ± 1.3 (s.d.) liters; Mean Percent Error = 4.81%; Standards acceptable.

RESULTS AND DISCUSSION

Validating the Standards

Using the same set of standards for each study could result in the introduction of a systematic error into all ERPF determinations. To prevent this, the standards are checked for accuracy after they are made by performing a simple volume of dilution experiment.

A small dose of ^{131}I (~ 25 μCi) in a 3-ml syringe is measured in the dose calibrator and the exact activity recorded. Next, this dose is diluted in exactly 2 liters of tap water, using a volumetric flask. Five 2-ml aliquots are pipetted into counting tubes, each counted for 3 min and background corrected, and the net counts are then divided by 50 to simulate an actual dilution volume of 100 liters. A calculated volume of distribution is then obtained from each aliquot by the method of the previous section using the new standards, with the aliquot equivalent to the plasma sample and the diluted dose equivalent to the injected dose.

Ideally, each aliquot should yield a value of 100 liters. The five actual values are averaged, and the mean subtracted from 100. The difference is the mean percent error. In practice, the standards are considered acceptable if the error is < 5%. If the error is larger, the standards are remade, and then rechecked using the same aliquots. A sample validation is presented in Table 1.

Table 2 shows a comparison between this method of ERPF determination and that of Kontzen et al. (*9*) in five patients with renal transplants. Our values in this comparison tend to be higher than those obtained using Kontzen's method ($p < 0.05$, paired t-test). We believe, however, that our values are reliable since the standards we used were those validated in Table 1. The 4.81 mean percent error cannot account for the differences since correcting our volumes of distribution by this percentage would result in even larger ERPF discrepancies.

SUMMARY

We believe that there are definite advantages to the above

TABLE 2. Comparison of Methods in Renal Transplant Patients

	ERPF (ml/min)	
Patient	Our Method	Kontzen
1	110	98
2	65	59
3	115	107
4	92	93
5	72	60

method of preparing and using standards. Our technologists save time, particularly when many studies are scheduled, since standards need not be made for each study. One additional measurement of the D-STD and 3-min count of the P-STD are all that is necessary. In fact, we have found that by processing all plasma samples at the day's end only a single measurement of the standards is necessary to calculate many ERPFs.

Our standards are made and validated once each month, requiring ~ 60 min of technologist time. They are made from a dose of sodium iodide, rather than injectable OIH. Based on our current radiopharmaceutical prices, we have determined that the minimum cost per study of standard solutions would be $4.03 for the Kontzen method, assuming a maximum number of studies per lot of OIH. Provided we perform at least eight studies per month, the cost per study using our method is $3.88 or less (e.g., for 20 studies per month, the cost is $1.56 per study). This analysis does not consider the cost savings in technologist time and other materials. Other departments must, of course, determine their own potential expenses.

Finally, the Kontzen method uses syringes to make certain volume measurements, whereas we use precision pipettes for all steps. We feel that this, as well as our method of validation, reduces the chance of error in our calculations.

FOOTNOTE

*CRC-10R, Capintec, Inc., Ramsey, NJ.

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